



(22) Date de dépôt/Filing Date: 2002/01/15
(41) Mise à la disp. pub./Open to Public Insp.: 2002/10/12
(45) Date de délivrance/Issue Date: 2010/05/04
(30) Priorités/Priorities: 2001/04/12 (CA2,342,970);
2001/11/13 (CA2,362,004)

(51) Cl.Int./Int.Cl. *C07K 19/00* (2006.01),
A61K 38/17 (2006.01), *A61K 47/48* (2006.01),
C07K 14/47 (2006.01)
(72) Inventeur/Inventor:
MCKERRACHER, LISA, CA
(73) Propriétaire/Owner:
BIOAXONE THERAPEUTIQUE INC., CA
(74) Agent: OGILVY RENAULT LLP/S.E.N.C.R.L., S.R.L.

(54) Titre : PROTEINES DE FUSION
(54) Title: FUSION PROTEINS

(57) **Abrégé/Abstract:**

The Rho family GTPases regulates axon growth and regeneration. Inactivation of Rho with C3, a toxin from *Clostridium botulinum*, can stimulate regeneration and sprouting of injured axons. The present invention provides novel chimeric C3-like Rho antagonists. These new antagonists are a significant improvement over C3 compounds because they are 3-4 orders of magnitude more potent to stimulate axon growth on inhibitory substrates than recombinant C3. The invention further provides evidence that these compounds promote repair when applied to the injured mammalian central nervous system.



TITLE: FUSION PROTEINS**ABSTRACT**

The Rho family GTPases regulates axon growth and regeneration. Inactivation of Rho with C3, a toxin from *Clostridium botulinum*, can stimulate regeneration and sprouting of injured axons. The present invention provides novel chimeric C3-like Rho antagonists. These new antagonists are a significant improvement over C3 compounds because they are 3-4 orders of magnitude more potent to stimulate axon growth on inhibitory substrates than recombinant C3. The invention further provides evidence that these compounds promote repair when applied to the injured mammalian central nervous system.

10

TITLE: FUSION PROTEINS

FIELD OF THE INVENTION

5

The present invention relates to conjugate or fusion type proteins (polypeptides) comprising, for example, C3 (see below) (i.e., C3-like protein, C3 chimeric proteins). Although, in the following, fusion-type proteins of the present invention, will be particularly discussed in relation to the use to facilitate regeneration of axons and neuroprotection, it is to be understood that the fusion proteins may be exploited in other contexts.

10

The present invention in particular pertains to the field of mammalian nervous system repair (e.g. repair of a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site), axon regeneration and axon sprouting, neurite growth and protection from neurodegeneration and ischemic damage.

15

The Rho family GTPases regulates axon growth and regeneration. Inactivation of Rho with *Clostridium botulinum* C3 exotransferase (hereinafter simply referred to as C3) can stimulate regeneration and sprouting of injured axons; C3 is a toxin purified from *Clostridium botulinum* (see Saito et al., 1995, FEBS Lett 371:105-109; Wilde et al 2000. J. Biol. Chem. 275:16478). Compounds of the C3 family from *Clostridium botulinum* inactivate Rho by ADP-ribosylation and thus act as antagonists of Rho effect or function (Rho antagonists).

20

The present invention in particular relates to a means of intracellular delivery of C3 protein (e.g. C3 itself or other active analogues such as C3-like transferases - see below) or other Rho antagonists to repair damage in the nervous system, to prevent ischemic cell death, and to treat various disease where the inactivation of Rho is required. The means of delivery may take the form of chimeric (i.e. conjugate) C3-like Rho antagonists. These conjugate antagonists provide a significant improvement over C3 compounds (alone) because they are 3 to 4 orders of magnitude more potent with respect to the stimulation of axon growth on inhibitory substrates than recombinant C3 alone. Examples of these Rho antagonists have been made as recombinant proteins created to facilitate penetration of the cell membrane (i.e. to enhance cell uptake of the antagonists), improve dose-response when applied to neurons to

25

30

stimulate growth on growth inhibitory substrates, and to inactivate Rho. Examples of these conjugate Rho antagonists are described below in relation to the designations C3APL, C3APLT, C3APS, C3-TL, C3-TS, C3Basic1, C3Basic2 and C3Basic3.

BACKGROUND OF THE INVENTION

Traumatic injury of the spinal cord results in permanent functional impairment. Most of the deficits associated with spinal cord injury result from the loss of axons that are damaged in the central nervous system (CNS). Similarly, other diseases of the CNS are associated with axonal loss and retraction, such as stroke, human immunodeficiency virus (HIV) dementia, prion diseases, Parkinson's disease, Alzheimer's disease, multiple sclerosis and glaucoma. Common to all of these diseases is the loss of axonal connections with their targets, and cell death. The ability to stimulate growth of axons from the affected or diseased neuronal population would improve recovery of lost neurological functions, and protection from cell death can limit the extent of damage. For example, following a white matter stroke, axons are damaged and lost, even though the neuronal cell bodies are alive, and stroke in grey matter kills many neurons and non-neuronal (glial) cells. Treatments that are effective in eliciting sprouting from injured axons are equally effective in treating some types of stroke (Boston life sciences, Sept. 6, 2000 Press release). Neuroprotective agents often tested as potential compounds that can limit damage after stroke. Compounds which show both growth-promotion and neuroprotection are especially good candidates for treatment of stroke and neurodegenerative diseases. Similarly, although the following discussion will generally relate to delivery of Rho antagonists, etc. to a traumatically damaged nervous system, this invention may also be applied to damage from unknown causes, such as during stroke, multiple sclerosis, HIV dementia, Parkinson's disease, Alzheimer's disease, prion diseases or other diseases of the CNS where axons are damaged in the CNS environment. Also, Rho is an important target for treatment of cancer and metastasis (Clark et al (2000) Nature 406:532-535), and hypertension (Uehata et al. (1997) Nature 389:990) and RhoA is reported to have a cardioprotective role (Lee et al. FASEB J. 15:1886-1884). Therefore, the new C3-like proteins are expected to be useful for a variety of diseases where inhibition of Rho activity is required.

It has been proposed to use various Rho antagonists as agents to stimulate regeneration of (cut) axons, i.e. nerve lesions; please see, for example, Canadian Patent application nos. 2,304,981 (McKerracher et al) and 2,300,878 (Strittmatter). These patent application documents propose the use of known Rho antagonists such as for example C3, chimeric C3 proteins, etc. (see blow) as well as substances selected from among known trans-4-amino(alkyl)-1-pyridylcarbamoylcyclohexane compounds (also see below) or Rho kinase inhibitors for use in the regeneration of axons. C3 inactivates Rho by ADP-ribosylation and is fairly non-toxic to cells (Dillon and Feig (1995) Methods in Enzymology: Small GTPases and their regulators Part. B.256:174-184).

While the following discussion will generally relate or be directed at repair in the CNS, the techniques described herein may be extended to use in many other diseases including, but not restricted to, cancer, metastasis, hypertentension, cardiac disease, stroke, diabetic neuropathy, and neurodegenerative disorders such as stroke, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS). Treatment with Rho antagonists would be used to enhance the rate of axon growth of peripheral nerves and thereby be effective for repair of peripheral nerves after surgery, for example after reattaching severed limbs. Also, treatment with our fusion compounds (proteins) is expected to be effective for the treatment of various peripheral neuropathies because of their axon growth promoting effects.

As mentioned above, traumatic injury of the spinal cord results in permanent functional impairment. Axon regeneration does not occur in the adult mammalian CNS because substrate-bound growth inhibitory proteins block axon growth. Many compounds, such as trophic factors, enhance neuronal differentiation and stimulate axon growth in tissue culture. However, most factors that enhance growth and differentiation are not able to promote axon regenerative growth on inhibitory substrates. To demonstrate that a compound known to stimulate axon growth in tissue culture most accurately reflects the potential for therapeutic use in axon regeneration in the CNS, it is important for the cell culture studies to include the demonstration that a compound can permit axon growth on growth inhibitory substrates. An

example of trophic and differentiation factors that stimulate growth on permissive substrates in tissue culture, are neurotrophins such as nerve growth factor (NGF) and brain-derived growth factor. NGF, however, does not promote growth on inhibitory substrates (Lehmann, et al. (1999) 19: 7537-7547) and it has not been effective in promoting axon regeneration in vivo. Brain derived neurotrophic factor (BDNF) is not effective to promote regeneration in vivo either (Mansour-Robaey, et al. J. Neurosci. (1994) 91: 1632-1636). BDNF does not promote neurite growth on growth inhibitory substrates (Lehmann et al supra).

Targeting intracellular signalling mechanisms involving Rho and the Rho kinase for promoting axon regeneration has been proposed (see, for example, the above mentioned Canadian Patent application nos. 2,304,981 (McKerracher et al)). For demonstration that inactivation of Rho promotes axon regeneration on growth inhibitory substrates, recombinant C3, a protein that inactivates Rho by ADP ribosylation of the effector domain was used. While such a C3 protein can effectively promote regeneration, it has been noted that such a C3 protein does not easily penetrate into cells, and high doses must therefore be applied for it to be effective.

The high dose of recombinant C3 needed to promote functional recovery presents a practical constraint or limitation on the use of C3 in vivo to promote regeneration (Lehmann, et al. (1999) J. Neurosci. 19: 7537-7547; Morii, N and Narumiya, S. (1995) Methods in Enzymology, Vol 256 part B, pg.196-206. In tissue culture studies, it has, for example, been determined that the minimum amount of C3 that can be used to induce growth on inhibitory substrates is 25 ug/ml (Lehmann, et al. (1999) J. Neurosci. 19: 7537-7547; Morii, N and Narumiya, S. (1995) Methods in Enzymology, Vol 256 part B, pg.196-206. If the cells are not triturated, even this dose is ineffective (Figure 1). In the context of the present invention it has been determined, for example, that at least 40 ug/20 g mouse needs to be applied to injured mouse spinal cord or rat optic nerve (McKerracher, Canadian patent application No.: 2,325,842). Calculating doses that would be required to treat an adult human on an equivalent dose per weight scale up used for our rat and mice experiments, it would be necessary to apply 120 mg/kg of C3 (i.e. alone) to the injured human spinal cord. This large amount of recombinant protein creates significant problems for manufacturing, due to the

large scale protein purification and cost. It also limits the dose ranging that can be tested because of the large amount of protein needed for minimal effective doses.

Another related limitation with respect to the use of C3 to promote repair in the injured CNS is that it does not easily penetrate the plasma membrane of living cells. In tissue culture studies when C3 is applied to test biological effects it has been microinjected directly into the cell (Ridley and Hall (1992) *Cell* 70: 389-399), or applied by trituration of the cells to break the plasma membrane (Lehmann, et al. (1999) *J. Neurosci.* 19: 7537-7547, Jin and Strittmatter (1997) *J. Neurosci.* 17: 6256-6263). In the case of axon injury in vivo, the C3 protein is likely able to enter the cell because injured axons readily take up substances from their environment. However, C3-like proteins of the present invention are likely to act also on surrounding undamaged neurons and help them make new connections as well, thus facilitating recovery. After incomplete SCI, there is plasticity of motor systems attributed to cortical and subcortical levels, including spinal cord circuitry (Raineteau, O., and Schwab, M. E. (2001) *Nat Rev Neurosci* 2: 263-73). This plasticity may be attributed to axonal or dendritic sprouting of collaterals and synaptic strengthening or weakening. Additionally, it has been shown that sparing of a few ventrolateral fibers may translate into significant differences in locomotor performance since these fibers are important in the initiation and control of locomotor pattern through spinal central pattern generators (Brustein, E., and Rossignol, S. (1998) *J Neurophysiol* 80: 1245-67). It is well documented that reorganization of spared collateral cortical spinal fibres occurs after spinal cord injury and this contributes to functional recovery (Weidner et al, 2001 *Proc. Natl. Acad. Sci.* 98: 3513-3518). The process of reorganization and sprouting of spared fibers would be enhanced by treatment with C3-like proteins able to enter non-injured neurons. This would enhance spontaneous plasticity of axons and dendritic remodeling known to help functional recovery.

Other methods of delivery of C3 in vitro have been to make a recombinant protein that can be taken up by a receptor-mediated mechanism (Boquet, P. et al. (1995) *Meth. Enzymol.* 256: 297-306). The disadvantage of this method is that the cells needing treatment must express the necessary receptor. Lastly, addition of a C2II binding protein to the tissue culture medium, along with a C21N-C3 fusion toxin allows uptake of C3 by receptor-mediated

endocytosis (Barthe et al. (1998) Infection and Immunity 66:1364). The disadvantage of this system is that much of the C3 in the cell will be restrained within a membrane compartment. More importantly, two different proteins must be added separately for transport to occur (Wahl et al. 2000. J. Cell Biol. 149:263), which make this system difficult to apply to for
5 treatment of disease in vivo.

SUMMARY OF THE INVENTION

In accordance with the present invention a conjugate or fusion protein comprising a therapeutically active agent is provided whereby the active agent may be delivered across a
10 cell wall membrane, the conjugate or fusion protein comprising at least a transport subdomain(s) or moiety(ies) in addition to an active agent moiety(ies). More particularly, as discussed herein, in accordance with the present invention a conjugate or fusion protein is provided wherein the therapeutically active agent is one able to facilitate (for facilitating) axon (or dendrite, or neurite) growth (e.g. regeneration) i.e. a conjugate or fusion protein in the
15 form of a conjugate Rho antagonist.

The present invention in accordance with an aspect thereof provides a drug delivery construct or conjugate [e.g. able to (for) suppress(ing) the inhibition of neuronal axon growth at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion
20 site] comprising at least one transport agent region and an active agent region not naturally associated with the active agent region, wherein the transport agent region is able to facilitate (i.e. facilitates) the uptake of the active agent region into a mammalian (i.e. human or animal) tissue or cell, and wherein the active agent region is an active therapeutic agent region able (i.e. has the capacity or capability) to facilitate axon growth for example on growth inhibitory
25 substrates (e.g. regeneration), either in vivo (in a mammal (e.g., human or animal)) or in vitro (in cell culture), including a derivative or homologue thereof (i.e. pharmaceutically acceptable chemical equivalents thereof - pharmaceutically acceptable derivative or homologue).

In accordance with the present invention the active agent region may be an ADP-ribosyl transferase C3 region. In accordance with the present invention the ADP-ribosyl transferase C3 may be selected from the group consisting of ADP-ribosyl transferase (e.g., ADP-ribosyl transferase C3) derived from *Clostridium botulinum* and a recombinant ADP-ribosyl transferase (e.g., recombinant ADP-ribosyl transferase C3) that includes the entire C3 coding region, or only a part (fragment) of the C3 coding region that retains the ADP-ribosyl transferase activity, or analogues (derivatives) of C3 that retains the ADP-ribosyl transferase activity, or enough of the C3 coding region to be able to effectively inactivate Rho. The active agent could also be selected from other known ADP-ribosyl transferases that act on Rho (Wilde et al. 2000 J. Biol. Chem. 275:16478-16483; Wilde et al 2001. J. Biol. Chem. 276:9537-9542).

In accordance with another aspect the present invention provides a drug conjugate consisting of a transport polypeptide moiety (e.g. rich in basic amino acids e.g. arginine, lysine, histidine, asparagine, glutamine) covalently linked to an active cargo moiety (e.g. by a peptide bond or a labile bond (i.e. a bond readily cleavable or subject to chemical change in the interior target cell environment)) wherein the transport polypeptide moiety is able to or has the capability to facilitate(s) the uptake of the active cargo moiety into a mammalian (e.g. human or animal) tissue or cell (for example, a transport subdomain of HIV Tat protein, a homeoprotein transport sequence (referred also as a transport homeoprotein) (e.g. the homeodomain of antennapedia), a Histidine tag (ranging in length from 4 to 30 histidine repeat) or a variation derivative or homolog thereof, (i.e. pharmaceutically acceptable chemical equivalents thereof)) [by a receptor independent process] and wherein the active cargo moiety is an active therapeutic moiety able (i.e. has the capacity or capability) to facilitate (i.e. for facilitating) axon growth (e.g. regeneration, budding) or neuroprotection (prevention of cell death) either in vivo (in a mammal (e.g., human or animal)) or in vitro (in cell culture).

In accordance with the present invention the transport polypeptide moiety may be selected from the group consisting of SEQ ID NO.: 48, a transport subdomain of HIV Tat protein such as for example SEQ ID NO.: 46, SEQ ID NO.:47, a homeodomain of antennapedia, such as

for example SEQ ID NO.: 44, SEQ ID NO.: 45, a Histidine tag and a functional derivative and analogue thereof (e.g., SEQ ID NO.: 21, SEQ ID NO.: 26, SEQ ID NO.: 31) [i.e. by the addition of polyamine, or any random sequence enriched in basic amino acids] - [i.e. pharmaceutically acceptable chemical equivalents thereof] and wherein the active cargo moiety is selected from the group consisting of C3 protein able (i.e. has the capacity or capability) to facilitate (i.e. for facilitating) axon growth (e.g. regeneration, budding) or neuroprotection (prevention of cell death) either in vivo (in a mammal (e.g., human or animal)) or in vitro (in cell culture).

In accordance with the present invention the C3 protein may be selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogue. In accordance with the present invention the ADP-ribosyl transferase C3 may be selected from the group consisting of ADP-ribosyl transferase (e.g., ADP-ribosyl transferase C3) derived from *Clostridium botulinum* and a recombinant ADP-ribosyl transferase (e.g., recombinant ADP-ribosyl transferase C3). The ADP-ribosyl transferase may be a protein with a C3-like activity, such as that derived from *Staphylococcus aureus* (Wilde et al 2001. J. Biol. Chem. 276:9537-9542). The ADP-ribosyl transferase may be any other transferase that acts to inactivate RhoA, RhoB and/or RhoC such as those derived from *Clostridium limosum*, and *Bacillus cereus* (Wilde et al 2000. J. Biol. Chem. 275:16478-16483). In accordance with the present invention the transport polypeptide moiety may include an active contiguous amino acid sequence as described herein.

In accordance with an additional aspect the present invention provides a fusion protein [e.g. able to (for) suppress(ing) the inhibition of neuronal axon growth at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site] consisting of a carboxy terminal active cargo moiety and an amino terminal transport moiety, wherein the amino terminal transport moiety is selected from the group consisting of a transport subdomain of HIV Tat protein, homeoprotein transport sequence (referred also as a transport homeoprotein) (e.g. the homeodomain of antennapedia), a Histidine tag and a functional derivatives and

analogues thereof (i.e. pharmaceutically acceptable chemical equivalents thereof) and wherein the active cargo moiety consists of a C3 protein.

5 The present invention in particular provides a fusion protein (e.g. able to (for) suppressing the inhibition of neuronal axon growth at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site) consisting of a carboxy terminal active cargo moiety and an amino terminal transport moiety, wherein the amino terminal transport moiety consists of the homeodomain of antennapedia and the active cargo moiety consists of a C3 protein (i.e. as described herein).

10
The present invention also in particular provides a fusion protein (e.g. able to (for) suppressing the inhibition of neuronal axon growth at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site) consisting of a carboxy terminal active cargo moiety and an amino terminal transport moiety, wherein the amino terminal transport
15 moiety consists of a transport subdomain of HIV Tat protein and the active cargo moiety consists of a C3 protein (i.e. as described herein).

In accordance with the present invention the C3 protein may be selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues. In
20 accordance with the present invention the ADP-ribosyl transferase C3 is selected from the group consisting of ADP-ribosyl transferase (e.g., ADP-ribosyl transferase C3) derived from Clostridium botulinum and a recombinant ADP-ribosyl transferase (e.g., recombinant ADP-ribosyl transferase C3).

25 In accordance with an additional aspect the present invention provides a fusion protein [e.g. able to (for) suppress(ing) the inhibition of neuronal axon growth at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site] consisting of an amino terminal active cargo moiety and a carboxy terminal transport moiety, wherein the carboxy terminal transport moiety is selected from the group consisting of a transport subdomain of

HIV Tat protein, a homeoprotein transport sequence (referred also as a transport homeoprotein) (e.g. the homeodomain of antennapedia), a Histidine tag and a functional derivatives and analogues thereof (i.e. pharmaceutically acceptable chemical equivalents thereof) and wherein the active cargo moiety consists of a C3 protein.

5

The present invention in particular provides a fusion protein (e.g. able to (for) suppressing the inhibition of neuronal axon growth at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site) consisting of an amino terminal active cargo moiety and a carboxy terminal transport moiety, wherein the carboxy terminal transport moiety consists of the homeodomain of antennapedia and the active cargo moiety consists of a C3 protein (i.e. as described herein).

10

The present invention also in particular provides a fusion protein (e.g. able to (for) suppressing the inhibition of neuronal axon growth at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site) consisting of an amino terminal active cargo moiety and a carboxy terminal transport moiety, wherein the carboxy terminal transport moiety consists of a transport subdomain of HIV Tat protein and the active cargo moiety consists of a C3 protein (i.e. as described herein).

15

In accordance with the present invention the C3 protein may be selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues. In accordance with the present invention the ADP-ribosyl transferase C3 is selected from the group consisting of ADP-ribosyl transferase C3 derived from Clostridium botulinum and a recombinant ADP-ribosyl transferase C3.

20

25

The present invention in a further aspect provides for the use of a member selected from the group consisting of a drug delivery construct as described herein, a drug conjugate as described herein and a fusion protein as described herein (e.g. including pharmaceutically

acceptable chemical equivalents thereof) for suppressing the inhibition of neuronal axon growth.

5 The present invention in a further aspect relates to a pharmaceutical composition (e.g. for suppressing the inhibition of neuronal axon growth), the pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and an effective amount of an active member selected from the group consisting of a drug delivery construct as described herein, a drug conjugate as described herein, and a fusion protein as described herein (e.g. including pharmaceutically acceptable chemical equivalents thereof).

10 The present invention further provides for the use of a member selected from the group consisting of a drug delivery construct as described herein, a drug conjugate as described herein, and a fusion protein as described herein (e.g. including pharmaceutically acceptable chemical equivalents thereof) for the manufacture of a pharmaceutical composition (e.g. for
15 suppressing the inhibition of neuronal axon growth).

The present invention also relates to a method for preparing a conjugate or fusion protein as defined above comprising

- 20
- cultivating a host cell (bacterial or eukaryotic) under conditions which provide for the expression of the conjugate or fusion protein within the cell; the protein could also be expressed to be produced in an animals, such as, for example, the production of recombinant proteins in the milk of farm animals
 - recovering the conjugate or fusion protein by purification
- 25

The purification of the fusion protein may be by affinity methods, ion exchange chromatography, size exclusion chromatography, hydrophobicity or any other purification technique typically used for protein purification.

The present invention also relates to the expression of the fusion protein in a mammalian cell, which when used with a signal sequence, will allow expression and secretion of the fusion protein into the extracellular milieu.

5

The present invention in particular provides a fusion protein selected from the group consisting of C3APL (SEQ ID NO.: 4), C3APLT (SEQ ID NO.: 37), C3APS (SEQ ID NO.:6), C3-TL (SEQ ID NO.:14), C3-TS (SEQ ID NO.: 18), C3Basic1 (SEQ ID NO.:25), C3Basic2 (SEQ ID NO.: 30), C3Basic3 (SEQ ID NO.:35), SEQ ID NO.: 20, and SEQ ID
10 NO.: 43 and pharmaceutically acceptable chemical equivalents thereof.

15

The present invention in another aspect provides a method of suppressing the inhibition of neuronal axon growth comprising administering (e.g.delivering) a member selected from the group consisting of a drug delivery construct as described herein, a drug conjugate as described herein and a fusion protein as described herein (e.g. including pharmaceutically acceptable chemical equivalents thereof) (e.g. directly) to (at) a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site (of a patient), in an amount effective to counteract said inhibition. Such application could be useful for treatment of a wide variety of peripheral neuropathies, such as diabetic neuropathy.

20

The present invention, for example, provides recombinant Rho antagonists comprising C3 enzymes with basic stretches of amino acids added to the C3 coding sequence to facilitate the uptake thereof into tissue or cells for the repair and/or promotion of repair or promotion of growth in the CNS, even in the lack of traumatic axon damage.

25

In accordance with an additional aspect, the present invention provides a pharmaceutical composition comprising a polypeptide selected from the group consisting of C3APL (SEQ ID NO.:4), C3APLT (SEQ ID NO.:37), C3APS (SEQ ID NO.:6), C3-TL (SEQ ID NO.:14), C3-TS (SEQ ID NO.:18), C3Basic1(SEQ ID NO.:25), C3Basic2 SEQ ID NO.:30), C3Basic3

(SEQ ID NO.:35), SEQ ID NO.: 20 and SEQ ID NO.: 43, and a pharmaceutically acceptable carrier.

5 In accordance with the present invention, the pharmaceutical composition may further comprise a biological adhesive, such as, for example, fibrin (fibrin glue).

10 In accordance with the present the transport agent region may be selected from the group consisting of a basic amino acid rich region (region comprising some basic amino acid (e.g., arginine, lysine, histidine, glutamine, and/or asparagine)) and a proline rich region (e.g. region comprising some proline). A proline rich region may contain for example around 5 % or more of prolines in its amino acid sequence. Prolines may be spaced, for example, 5 to 10 amino acids apart.

15 In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of SEQ ID NO.: 48, a subdomain of HIV Tat protein (e.g., SEQ ID NO.: 46, SEQ ID NO.: 47, or any other subdomain of Tat, that could act as a transport sequence), a homeodomain of antennapedia (e.g., SEQ ID NO.: 44, SEQ ID NO.: 45, or any other domain of antennapedia, that could act as a transport sequence), a homeoprotein transport sequence, a Histidine tag, and analogues thereof (e.g., SEQ ID NO.: 21, SEQ ID
20 NO.: 26, SEQ ID NO.:31).

25 In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

30 In a further aspect the present invention provides a pharmaceutical composition comprising a polypeptide comprising at least one (one or more) transport agent region and an active agent region, said active agent region being selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues, and a pharmaceutically acceptable

carrier.

In accordance with the present invention, the pharmaceutical composition may further comprise a biological adhesive, such as, for example, fibrin (fibrin glue).

5

In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region (region comprising some basic amino acid (e.g., arginine, lysine, histidine, glutamine, and/or asparagine)) and a proline rich region (e.g. region comprising some proline).

10

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of SEQ ID NO.: 48, a subdomain of HIV Tat protein (e.g., SEQ ID NO.: 46, SEQ ID NO.: 47, or any other subdomain of Tat, that could act as a transport sequence), a homeodomain of antennapedia (e.g., SEQ ID NO.: 44, SEQ ID NO.: 45, or any other domain of antennapedia, that could act as a transport sequence), a homeoprotein transport sequence, a Histidine tag, and analogues thereof (e.g., SEQ ID NO.: 21, SEQ ID NO.: 26, SEQ ID NO.:31).

15

In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

20

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

25

In an additional aspect, the present invention provides a polypeptide comprising at least one (one or more) transport agent region and an active agent region, said active agent region being selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues.

30

In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein (e.g., SEQ ID NO.: 46, SEQ ID NO.: 47, or any other subdomain of Tat, that could act as a transport sequence), a
 5 homeodomain of antennapedia and analogues thereof (e.g., SEQ ID NO.: 21, SEQ ID NO.: 26, SEQ ID NO.: 31).

In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31
 10 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof. (e.g., SEQ ID NO.: 44, SEQ ID NO.: 45, SEQ ID NO.: 48, or any other domain of antennapedia, that could act as a transport sequence), a homeoprotein transport sequence, a Histidine tag,

15 In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

In an additional aspect, the present invention provides a polypeptide consisting of a carboxy-terminal active agent moiety and an amino-terminal transport moiety region and wherein said
 20 carboxy-terminal active agent moiety may be selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues thereof.

In accordance with the present invention, the carboxy-terminal transport moiety region may be selected from the group consisting of a basic amino acid rich region and a proline rich
 25 region.

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag and analogues thereof.

30 In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31

(Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

In a further aspect, the present invention relates to a polypeptide consisting of an amino-terminal active agent moiety and a carboxy-terminal transport moiety region, wherein said amino-terminal active agent moiety may be selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues thereof.

In accordance with the present invention, the carboxy-terminal transport moiety region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag and analogues thereof.

In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

In yet a further aspect, the present invention relates to a conjugate comprising at least one transport agent region (including one, two, three or more transport agent region) and an active agent region, said active agent region being selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues, wherein said transport agent region is covalently linked to said active agent region.

In accordance with the present invention, the transport agent region may be cross-linked (e.g., chemically cross-linked, UV cross-linked) to the active agent region (C3-like proteins of the present invention and analogues thereof).

5 In accordance with the present invention, the transport agent region may be fused to ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues according to recombinant DNA technology (e.g., cloning the DNA sequence of the transport agent region in frame with the DNA sequence of the ADP-ribosyl transferase C3 or an ADP-ribosyl transferase C3 analogue comprising or not a spacer DNA sequence (multiple cloning site, linker) or any
10 other DNA sequence that would not interfere with the activity of the C3-like protein once expressed).

In an additional aspect, the present invention relates to the use of a polypeptide selected from the group consisting of C3APL (SEQ ID NO.: 4), C3APLT (SEQ ID NO.:37), C3APS (SEQ
15 ID NO.:6), C3-TL (SEQ ID NO.:14), C3-TS (SEQ ID NO.:18), C3Basic1 (SEQ ID NO.:25), C3Basic2 (SEQ ID NO.:30), C3Basic3 (SEQ ID NO.:35), SEQ ID NO.: 20 and SEQ ID NO.: 43, for the manufacture of a pharmaceutical composition.

In other aspects, the present invention relates to the use of a polypeptide comprising at least
20 one (one or more) transport agent region and an active agent region, for the manufacture of a pharmaceutical composition, or to facilitate (for facilitating) axon growth or for treating (in the treatment of) nerve injury (e.g., nerve injury arising from traumatic nerve injury or nerve injury caused by disease), or for preventing (diminishing, inhibiting (partially or totally)) cell apoptosis (cell death, such as following ischemia in the CNS), or for suppressing the
25 inhibition of neuronal axon growth, or for the treatment of ischemic damage related to stroke, or for suppressing Rho activity, or to regenerate (for regenerating) injured axon (helping injured axon to recover, partially or totally, their function), or to help (for helping) neurons to make new connections (developing axon, dendrite, neurite) with other (surrounding) cells, in a mammal, (e.g., human, animal), wherein said active agent region being selected from the
30 group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues.

In accordance with the present invention, the transport agent region may be at the amino-terminal end of the protein and the ADP-ribosyl transferase C3 or ADP-ribosyl transferase C3 analogue may be at the carboxy-terminal end of the protein.

5 In accordance with the present invention, the transport agent region may be at the carboxy-terminal end of the protein and the ADP-ribosyl transferase C3 or ADP-ribosyl transferase C3 analogue may be at the amino-terminal end of the protein.

10 In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

15 In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

20 In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

25 In a further aspect, the present invention provides a method of (for) suppressing the inhibition of neuronal axon growth (e.g., in a mammal, (e.g., human, animal)) comprising delivering a polypeptide or conjugate comprising at least one (one or more) transport agent region and an active agent region selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues directly at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site, in an amount effective to counteract said
30 inhibition.

In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

5

In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21 (Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

10

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

15

In yet a further aspect, the present invention provides a method of (for) facilitating axon growth (e.g., in a mammal, (e.g., human, animal)) comprising delivering a polypeptide or conjugate comprising at least one transport agent region and an active agent region selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues directly at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site, in an amount effective to facilitate said growth.

20

In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

25

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

30

In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

In an additional aspect, the present invention provides a method of (for) treating nerve injury (e.g., in a mammal, (e.g., human, animal)) comprising delivering a polypeptide or conjugate comprising at least one transport agent region and an active agent region selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues directly at (to) a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site.

In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(basic 1), SEQ ID NO.: 26 (basic 2), SEQ ID NO.: 31 (basic 3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (Tat long), SEQ ID NO.: 47 (Tat short), and analogues thereof.

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

In yet an additional aspect, the present invention provides a method of (for) preventing cell apoptosis (e.g., in a mammal, (e.g., human, animal)) comprising delivering a polypeptide or conjugate comprising at least one transport agent region and an active agent region selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues directly at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site.

In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

5 In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

10 In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

15 In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

20 In another aspect, the present invention provides a method of (for) treating ischemic damage related to stroke (e.g., in a mammal, (e.g., human, animal)) comprising delivering a polypeptide or conjugate comprising at least one transport agent region and an active agent region selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues directly at a central nervous system (CNS) lesion site (to said mammal).

25 In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

30 In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

5 In yet another aspect, the present invention provides a method of (for) suppressing Rho activity comprising delivering a polypeptide or conjugate comprising at least one transport agent region and an active agent region selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues directly at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site, in an amount effective to
10 suppress said activity.

In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

15 In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

In accordance with the present invention, the basic amino acid region may be selected from
20 the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

In accordance with the present invention, the proline rich region may be selected from the
25 group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

In accordance with an additional aspect, the present invention provides a method of (for) regenerating injured axon (e.g., in a mammal, (e.g., human, animal)) comprising delivering a polypeptide or conjugate comprising at least one transport agent region and an active agent
30 region selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues directly at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site (e.g., in a mammal), in an amount effective to regenerate said injured axon.

In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

5 In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

10 In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

15 In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

20 In accordance with a further aspect, the present invention provides a method of (for) helping neurones to make new cell connection (developing axon, dendrite, neurite with other (surrounding) cells) comprising delivering a polypeptide or conjugate comprising at least one transport agent region and an active agent region selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues directly at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site.

25 In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

30 In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31

(Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

5 In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

10 In another aspect, the present invention, provides a polypeptide consisting of a basic amino acid rich region and an active agent region, wherein, amino acids from said basic rich region comprises amino acids selected from the group consisting of Histidine, Asparagine, Glutamine, Lysine and Arginine and wherein the active agent region is ADP-ribosyl transferase C3.

15 In yet another aspect, the present invention relates to the use of a polypeptide comprising at least one transport agent region and an active agent region, said active agent region being selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues for the manufacture of a medicament for suppressing the inhibition of neuronal axon growth.

20 In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

25 In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

30 In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

In a further aspect, the present invention relates to the use of a polypeptide comprising at least one transport agent region and an active agent region, said active agent region being selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues for the manufacture of a medicament for facilitating axon growth.

5

In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

10

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

15

In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

20

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

25

In yet a further aspect the present invention relates to the use of a polypeptide comprising at least one (one or more) transport agent region and an active agent region, said active agent region being selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues for the manufacture of a medicament for treating nerve injury (e.g., in a mammal, (e.g., human, animal)).

30

In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

5

In accordance with the present invention, the proline rich region may selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

10

In accordance with another aspect, the present invention relates to the use of a polypeptide selected from the group consisting of C3APL (SEQ ID NO.: 4), C3APLT (SEQ ID NO.:37), C3APS (SEQ ID NO.:6), C3-TL (SEQ ID NO.:14), C3-TS (SEQ ID NO.:18), C3Basic1 (SEQ ID NO.:25), C3Basic2 (SEQ ID NO.:30), C3Basic3 (SEQ ID NO.:35), SEQ ID NO.: 20 and SEQ ID NO.: 43 for the manufacture of a pharmaceutical composition for suppressing the inhibition of neuronal axon growth (e.g., in a mammal, (e.g., human, animal)).

15

In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

20

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

25

In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

30

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

In yet another aspect, the present invention relates to the use of a polypeptide selected from the group consisting of C3APL (SEQ ID NO.:4), C3APLT (SEQ ID NO.:37), C3APS (SEQ ID NO.:6), C3-TL (SEQ ID NO.:14), C3-TS (SEQ ID NO.:18), C3Basic1 (SEQ ID NO.:25),

C3Basic2 (SEQ ID NO.:30), C3Basic3 (SEQ ID NO.:35), SEQ ID NO.: 20 and SEQ ID NO.: 43 for the manufacture of a pharmaceutical composition for facilitating axon growth (e.g., in a mammal, (e.g., human, animal)).

5 In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

In accordance with the present invention, the basic amino acid rich region may be selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of
10 antennapedia, a Histidine tag, and analogues thereof.

In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID
15 NO.: 47 (TS), and analogues thereof.

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

20 In a further aspect, the present invention relates to the use of a polypeptide selected from the group consisting of C3APL (SEQ ID NO.: 4), C3APLT (SEQ ID NO.:37), C3APS (SEQ ID NO.:6), C3-TL (SEQ ID NO.:14), C3-TS (SEQ ID NO.:18), C3Basic1 (SEQ ID NO.:25), C3Basic2 (SEQ ID NO.:30), C3Basic3 (SEQ ID NO.:35), SEQ ID NO.: 20 and SEQ ID NO.: 43 for the manufacture of a pharmaceutical composition for treating nerve injury.

25 In accordance with the present invention, the transport agent region may be selected from the group consisting of a basic amino acid rich region and a proline rich region.

In accordance with the present invention, the basic amino acid rich region may be selected
30 from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, a Histidine tag, and analogues thereof.

In accordance with the present invention, the basic amino acid region may be selected from the group consisting of SEQ ID NO.: 21(Basic1), SEQ ID NO.: 26 (Basic2), SEQ ID NO.: 31 (Basic3), SEQ ID NO.: 44 (APL), SEQ ID NO.: 45 (APS) SEQ ID NO.: 46 (TL), SEQ ID NO.: 47 (TS), and analogues thereof.

5

In accordance with the present invention, the proline rich region may be selected from the group consisting of SEQ ID NO.: 48 (APLT) and analogues thereof.

10

In another aspect, the present invention provides an isolated polynucleotide comprising at least the polynucleotide sequence (for example the polynucleotide sequence disclosed herein in addition with (or in some cases without) a suitable backbone (e.g., plasmid, viral vector)) selected from the group consisting of SEQ ID NO.: 3, SEQ ID NO.: 5, SEQ ID NO.: 13, SEQ ID NO.: 17, SEQ ID NO.: 19, SEQ ID NO.: 24, SEQ ID NO.: 29, SEQ ID NO.: 34, SEQ ID NO.: 36, and SEQ ID NO.: 42.

15

In yet another aspect, the present invention provides a cell transformed (transfected, transduced, infected, electroporated, micro-injected, etc.) with an isolated polynucleotide comprising at least the polynucleotide sequence (for example the polynucleotide sequence disclosed herein in addition with (or in some cases without) a suitable backbone (e.g., plasmid, viral vector)) selected from the group consisting of SEQ ID NO.: 3, SEQ ID NO.: 5, SEQ ID NO.: 13, SEQ ID NO.: 17, SEQ ID NO.: 19, SEQ ID NO.: 24, SEQ ID NO.: 29, SEQ ID NO.: 34, SEQ ID NO.: 36, and SEQ ID NO.: 42.

20

In a further aspect, the present invention provides a delivery agent consisting of a cargo moiety in combination with a transport moiety, wherein the transport moiety is selected from the group consisting of SEQ ID NO: 48 and analogues thereof. SEQ ID NO: 48 and analogues thereof act as a transport moiety which facilitate penetration of the cell membrane. Any cargo moiety (e.g., protein, chemicals) linked (e.g. attached) to SEQ ID NO: 48 or to some analogues thereof are encompassed by the present invention. For example, SEQ ID NO: 48 and analogues thereof may be fused to an anticancer agent, a therapeutic agent, an apoptotic agent, an anti-apoptotic agent, a reporter protein, an antibody, an antibody fragment, a dye, a probe, a marker etc.

25

30

In accordance with the present invention, the cargo moiety may retain biological activity following transport moiety-dependent intracellular delivery. Biological activity may include for example, biological properties (e.g. enzymatic activity) as well as its immunological properties. The cargo moiety may have a direct biological effect on the cell, such as for example killing the cell following its internalization or may have an indirect biological effect, for example, the cargo moiety may be a pro-drug that is inactive by itself but becomes active following modification (e.g., cleavage, phosphorylation, etc.) or when a second molecule is introduced inside the cell. The cargo moiety may also be a biologically inactive (i.e., inert) compound such as a labelling molecule (e.g., chemicals, proteins), an imaging molecule etc..

In accordance with the present invention, the agent may be a fusion protein having an amino-terminal that is the cargo moiety and having a carboxy-terminal that is the transport moiety.

In accordance with the present invention the cargo moiety may be selected from the group consisting of analytical molecules (e.g., molecules used in tissue culture experiments, markers, probes, dyes, reporter proteins) therapeutic molecules (e.g., toxin, drug, pro-drug), prophylactic molecules and diagnostic molecules (i.e., molecules used in *in vivo* or *in vitro* detection of a specific condition, metabolite, other molecule). Examples of analytical molecules, therapeutic molecules, prophylactic molecules and diagnostic molecules includes proteins (e.g., enzymes (e.g., nucleases, proteases, kinases, etc.), cytokines, chemokines, antigen, antibodies, antibody fragments, reporter proteins such as horseradish peroxidase, beta-galactosidase, fluorescent proteins (e.g., green fluorescent protein)), nucleic acids, polysaccharides, dyes, isotopes (e.g., radioisotope), markers, probes, and other types of chemicals. Transport polypeptides of the present invention may be advantageously attached to cargo molecules by chemical cross-linking or by genetic fusion.

In accordance with the present invention the cargo moiety may be selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues thereof.

In an additional aspect, the present invention relates to the polypeptide set forth in SEQ ID NO: 48 and analogues thereof.

In yet an additional aspect, the present invention provides a polypeptide as set forth in SEQ

ID NO: 48 and analogues thereof, wherein said polypeptide and analogues may be able to act as a transport agent for the intracellular delivery (delivery across the cellular membranes (e.g., cytoplasmic membrane, nuclear membrane) of a cargo agent selected from the group consisting of analytical molecules, therapeutic molecules, prophylactic molecules, and
5 diagnostic molecules.

In accordance with the present invention, the cargo agent may be selected from the group consisting of ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues thereof.

10 The transport of a cargo moiety across the cellular membrane (intracellular delivery) may be facilitated (increased) when linked (e.g., genetically fused, chemically cross-linked, etc.) to SEQ ID NO: 48 and analogues thereof. Therefore it is an object of the present invention to provide a method for the intracellular delivery of a cargo moiety, the method comprising exposing the cell to a delivery agent comprising a cargo moiety and a transport moiety, said
15 transport moiety being selected from the group consisting of SEQ ID NO: 48 and analogues thereof and wherein said transport moiety enables the delivery agent to be delivered inside the cell (i.e., across cellular membranes). An example of a cargo moiety that may be delivered across the cell membrane is ADP-ribosyl transferase C3 and analogues thereof. Other examples of a cargo moiety are mentioned herein. The method also comprise
20 bringing the delivery agent comprising a cargo moiety and a transport moiety (SEQ ID NO: 48 and analogues thereof) in the surrounding of a target cell in a manner (e.g., concentration) sufficient to permit the uptake of the delivery agent by the cell. For example, in the case of in vitro (e.g., cell culture) delivery, the delivery agent (in a pharmaceutically acceptable carrier, diluent, excipient, etc.) may be added directly to the extracellular milieu (e.g., cell culture
25 media) of adherent cells (i.e., cell lines or primary cells) or cells in suspension. Alternatively, cells may be harvested and concentrated before being put in contact with the delivery agent. Intracellular delivery may be monitored by techniques known in the art, such as for example, immunofluorescence, immunohistochemistry or by the intrinsic properties of the cargo moiety (e.g., its enzymatic activity).

30 In vivo delivery (in a mammal) may be performed for example, by exposing (i.e., contacting) a tissue, a nerve injury site, an open wound, etc. with the delivery agent (in a pharmaceutically acceptable carrier, diluent, excipient, fibrin gel etc.) of the present invention

in an amount sufficient to promote the biological effect of the cargo moiety (e.g., recovery, healing of the wounded tissue, etc.). In addition, in vivo delivery may be performed by other methods known in the art such as for example, injection via the intra-muscular (IM), subcutaneous (SC), intra-dermal (ID), intra-venous (IV) or intra-peritoneal (IP) routes or administration at the mucosal membranes including the oral and nasal cavity membranes using any suitable means. Alternatively, cells may be isolated from a mammal and treated (exposed) ex-vivo (e.g., in gene therapy techniques) with the delivery agent of the present invention before being re-infused in the same individual or in a compatible individual.

The invention in particular provides C3-like proteins, which may have additional amino acids added to the carboxy terminal end of the C3 proteins. Examples of such proteins includes:

The term "Rho antagonists" as used herein includes, but is not restricted to, (known) C3, including C3 chimeric proteins, and like Rho antagonists.

The term "C3 protein" refers to ADP-ribosyl transferase C3 isolated from Clostridium botulinum or a recombinant ADP-ribosyl transferase.

The term "C3-like protein", "ADP-ribosyl transferase C3-like protein", "ADP-ribosyl transferase C3 analogue", "C3-like transferase" or "C3 chimeric proteins" as used herein refers to any protein (polypeptide) having a biological activity similar (e.g., the same, substantially similar), to ADP-ribosyl transferase C3. Examples of such C3-like protein include, for example, but are not restricted to C3APL, C3APLT, C3APS, C3-TL, C3-TS, C3Basic1, C3Basic2 and C3Basic3 and the protein defined in SEQ ID NO.: 20.

The term "nerve injury site" refers to a site of traumatic nerve injury or nerve injury caused by disease. The nerve injury site may be a single nerve (eg sciatic nerve) or a nerve tract comprised of many nerves (eg. damaged region of the spinal cord). The nerve injury site may be in the central nervous system or peripheral nervous system or in any region needing repair.

The nerve injury site may form as a result of damage caused by stroke. The nerve injury site may be in the brain as a result of surgery, brain tumour removal or therapy following a cancerous lesion. The nerve injury site may result from stroke, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), diabetes or any other type of neurodegenerative disease.

The term "cargo" refers to a molecule other than the transport moiety and that is either (1) not inherently capable of entering a cell (e.g., cell compartment) or (2) not inherently capable of entering a cell (e.g., cell compartment) at a useful rate. The term "cargo" as used herein refers either to a molecule per se, i.e., before conjugation, or to the cargo moiety of a transport polypeptide-cargo conjugate. Examples of "cargo" include, but are not limited to, small molecules and macromolecules such as polypeptides, nucleic acids (polynucleotides), polysaccharides and chemicals.

As used herein, the term "delivery agent" relates to an agent comprising a cargo moiety and a transport moiety. Examples of cargo moiety are discussed above and includes for example ADP-ribosyl transferase C3 and ADP-ribosyl transferase C3 analogues. Examples of transport moiety comprise for example SEQ ID NO: 48 and analogues thereof.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA, or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA

and RNA; thus "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" includes but is not limited to linear and end-closed molecules. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptides" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds (i.e., peptide isosteres).

"Polypeptide" refers to both short chains, commonly referred as peptides, oligopeptides or oligomers, and to longer chains generally referred to as proteins. As described above, polypeptides may contain amino acids other than the 20 gene-encoded amino acids.

As used herein the term "analogues" relates to mutants, variants, chimeras, fusions, deletions, additions and any other type of modifications made relative to a given polypeptide. The term "analogue" is synonym of homologue, derivative and chemical equivalent or biological equivalent.

As used herein, the term "homologous" sequence relates to nucleotide or amino acid sequence derived from the DNA sequence or polypeptide sequence of C3APL, C3APLT, C3APS, C3-TL, C3-TS, C3Basic1, C3Basic2 and C3Basic3.

As used herein, the term "heterologous" sequence relates to DNA sequence or amino acid sequence of a heterologous polypeptide and includes sequence other than that of C3APL, C3APLT, C3APS, C3-TL, C3-TS, C3Basic1, C3Basic2 and C3Basic3.

As used herein the term "basic amino acid rich region" relates to a region of a protein with a high content of the basic amino acids such as Arginine, Histidine, Asparagine, Glutamine, Lysine (Lys). "Basic amino acid rich region" may have, for example 15% or more (up to 100%) of basic amino acids. In some instance, a "basic amino acid rich region" may have less than 15% of basic amino acids and still function as a transport agent region.

As used herein the term "proline rich region" refers to a region of a protein having 5 % or more (up to 100%) of the amino acid proline in its sequence. In some instance a "proline rich

region" may have between 5% and 15% of proline. "Proline rich region" of the present invention function as a transport agent region.

As used herein the term "to help neuron make new connections with other cells" or "helping neurones to make new cell connection" means that upon treatment of cells (e.g., neuron(s)) or tissue with a drug delivery construct, a conjugate, a fusion-protein, a polypeptide or a pharmaceutical compositions of the present invention, neurons may grow (develop) for example new dendrite, new axon or new neurite (i.e., cell bud), or already existing dendrite(s), axon or neurite (i.e., cell bud) are induce to grow to a greater extent.

As used herein, the term "vector" refers to an autonomously replicating DNA or RNA molecule into which foreign DNA or RNA fragments are inserted and then propagated in a host cell for either expression or amplification of the foreign DNA or RNA molecule. The term "vector" comprises and is not limited to a plasmid (e.g., linearized or not) that can be used to transfer DNA sequences from one organism to another.

The term "pharmaceutically acceptable carrier" or "adjuvant" and "physiologically acceptable vehicle" and the like are to be understood as referring to an acceptable carrier or adjuvant that may be administered to a patient, together with a compound of this invention, and which does not destroy the pharmacological activity thereof. Further, as used herein "pharmaceutically acceptable carrier" or "pharmaceutical carrier" are known in the art and include, but are not limited to, 0.01-0.1 M and preferably 0.05 M phosphate buffer or 0.8 % saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, collating agents, inert gases and the like.

As used herein, "pharmaceutical composition" means therapeutically effective amounts of the agent together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvant and/or carriers. A "therapeutically effective amount" as used herein
5 refers to that amount which provides a therapeutic effect for a given condition and administration regimen. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts). Solubilizing
10 agents (e.g., glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the protein, complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polylactic acid,
15 polyglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance. Controlled or sustained release compositions include formulation in lipophilic depots (e.g., fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions
20 coated with polymers (e.g., poloxamers or poloxamines). Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral routes. In one embodiment the pharmaceutical composition is administered parenterally, paracancerally, transmucosally, transdermally, intramuscularly,
25 intravenously, intradermally, subcutaneously, intraperitoneally, intraventricularly, intracranially intratumorally or more preferably, directly at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site.

As may be appreciated, a number of modifications may be made to the polypeptides of the
30 present invention, such as for example the active agent region (e.g., ADP-ribosyl transferase C3 or ADP-ribosyl transferase C3 analogue) or the transport agent region (e.g., a subdomain of HIV Tat protein, or a homeodomain of antennapedia) and fragments thereof without

deleteriously affecting the biological activity of the polypeptides or fragments. Polypeptides of the present invention comprises for example, those containing amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are known in the art. Modifications may occur anywhere in a polypeptide including the polypeptide backbone, the amino acid side-chains and the amino or carboxy termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslational natural processes or may be made by synthetic methods. Modifications comprise for example, without limitation, acetylation, acylation, addition of acetamidomethyl (Acm) group, ADP-ribosylation, amidation, covalent attachment to flavin, covalent attachment to a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation and ubiquitination (for reference see, Protein-structure and molecular properties, 2nd Ed., T.E. Creighton, W.H. Freeman and Company, New-York, 1993).

Other type of polypeptide modification may comprises for example, amino acid insertion (i.e., addition), deletion and substitution (i.e., replacement), either conservative or non-conservative (e.g., D-amino acids, desamino acids) in the polypeptide sequence where such changes do not substantially alter the overall biological activity of the polypeptide. Polypeptides of the present invention comprise for example, biologically active mutants, variants, fragments, chimeras, and analogues; fragments encompass amino acid sequences having truncations of one or more amino acids, wherein the truncation may originate from the amino terminus (N-terminus), carboxy terminus (C-terminus), or from the interior of the protein. Analogues of the invention involve an insertion or a substitution of one or more amino acids. Variants, mutants, fragments, chimeras and analogues may have the biological

properties of polypeptides of the present invention which comprise for example (without being restricted to the present examples) to facilitate neuronal axon growth, to suppress the inhibition of neuronal axon growth, to facilitate neurite growth, to inhibit apoptosis, to treat nerve injury, to regenerate injured axon and/or to act as a Rho antagonist.

As it may be exemplified (Example 13: reverse Tat sequence), in some instance, the order of the amino acids in a particular polypeptide is not critical. As for the transport agent region described herein, the transport function of this region may be preserved even if the amino acids are not in their original (as it is found in nature) order (sequence).

Example of substitutions may be those, which are conservative (i.e., wherein a residue is replaced by another of the same general type). As is understood, naturally occurring amino acids may be sub-classified as acidic, basic, neutral and polar, or neutral and non-polar. Furthermore, three of the encoded amino acids are aromatic. It may be of use that encoded polypeptides differing from the determined polypeptide of the present invention contain substituted codons for amino acids, which are from the same group as that of the amino acid be replaced. Thus, in some cases, the basic amino acids Lys, Arg and His may be interchangeable; the acidic amino acids Asp and Glu may be interchangeable; the neutral polar amino acids Ser, Thr, Cys, Gln, and Asn may be interchangeable; the non-polar aliphatic amino acids Gly, Ala, Val, Ile, and Leu are interchangeable but because of size Gly and Ala are more closely related and Val, Ile and Leu are more closely related to each other, and the aromatic amino acids Phe, Trp and Tyr may be interchangeable.

It should be further noted that if the polypeptides are made synthetically, substitutions by amino acids, which are not naturally encoded by DNA may also be made. For example, alternative residues include the omega amino acids of the formula $\text{NH}_2(\text{CH}_2)_n\text{COOH}$ wherein n is 2-6. These are neutral nonpolar amino acids, as are sarcosine, t-butyl alanine, t-butyl glycine, N-methyl isoleucine, and norleucine. Phenylglycine may substitute for Trp, Tyr or Phe; citrulline and methionine sulfoxide are neutral nonpolar, cysteic acid is acidic, and ornithine is basic. Proline may be substituted with hydroxyproline and retain the conformation conferring properties.

It is known in the art that mutants or variants may be generated by substitutional mutagenesis

and retain the biological activity of the polypeptides of the present invention. These variants have at least one amino acid residue in the protein molecule removed and a different residue inserted in its place (one or more nucleotide in the DNA sequence is changed for a different one using known molecular biology techniques, giving a different amino acid upon translation of the corresponding messenger RNA to a polypeptide). For example, one site of interest for substitutional mutagenesis may include but are not restricted to sites identified as the active site(s), or immunological site(s). Other sites of interest may be those, for example, in which particular residues obtained from various species are identical. These positions may be important for biological activity. Examples of substitutions identified as “conservative substitutions” are shown in Table 1. If such substitutions result in a change not desired, then other type of substitutions, denominated “exemplary substitutions” in Table 1, or as further described herein in reference to amino acid classes, are introduced and the products screened.

In some cases it may be of interest to modify the biological activity of a polypeptide by amino acid substitution, insertion, or deletion. For example, modification of a polypeptide may result in an increase in the polypeptide’s biological activity, may modulate its toxicity, may result in changes in bioavailability or in stability, or may modulate its immunological activity or immunological identity. Substantial modifications in function or immunological identity are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation. (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side chain properties:

- (1) hydrophobic: norleucine, methionine (Met), Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (Ile)
- (2) neutral hydrophilic: Cysteine (Cys), Serine (Ser), Threonine (Thr)
- (3) acidic: Aspartic acid (Asp), Glutamic acid (Glu)
- (4) basic: Asparagine (Asn), Glutamine (Gln), Histidine (His), Lysine (Lys), Arginine (Arg)
- (5) residues that influence chain orientation: Glycine (Gly), Proline (Pro); and
- (6) aromatic: Tryptophan (Trp), Tyrosine (Tyr), Phenylalanine (Phe)

Non-conservative substitutions will entail exchanging a member of one of these classes for another.

TABLE 1. Preferred amino acid substitution

Original residue	Exemplary substitution	Conservative substitution
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln, His, Lys, Arg	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro	Pro
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala	Leu
Pro (P)	Gly	Gly
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Leu, Met, Phe, Ala, norleucine	Leu

5

Amino acids sequence insertions (e.g., additions) include amino and/or carboxyl-terminal fusions ranging in length from one residues to polypeptides containing a hundred or more

residues, as well as intrasequence insertions of single or multiple amino acid residues. Other insertional variants include the fusion of the N- or C-terminus of the protein to a homologous or heterologous polypeptide forming a chimera. Chimeric polypeptides (i.e., chimeras, polypeptide analogue) comprise sequence of the polypeptides of the present invention fused to homologous or heterologous sequence. Said homologous or heterologous sequence encompass those which, when formed into a chimera with the polypeptides of the present invention retain one or more biological or immunological properties.

Other type of chimera generated by homologous fusion includes new polypeptides formed by the repetition of two or more polypeptides of the present invention. The number of repeat may be, for example, between 2 and 50 units (i.e., repeats). In some instance, it may be useful to have a new polypeptide with a number of repeat greater than 50. For example, it may be useful to fuse (using cross-linking techniques or recombinant DNA technology techniques) polypeptides such as C3APL, C3APLT, C3APS, C3-TL, C3-TS, C3Basic1, C3Basic2 and C3Basic3 either to themselves (e.g., C3APLT fused to C3APLT) or to another polypeptide of the present invention (e.g., C3APLT fused to C3APL).

In addition, a transport agent such as for example, a subdomain of HIV Tat protein, and a homeodomain of antennapedia may be repeated more than one time in a polypeptide comprising the ADP-ribosyl transferase C3 or ADP-ribosyl transferase C3 analogues. The transport agent region may be either at the amino-terminal region of an ADP-ribosyl transferase C3 or ADP-ribosyl transferase C3 analogues or at its carboxy-terminal region or at both regions. The repetition of a transport agent region may affect (increase) the uptake of the ADP-ribosyl transferase C3 or ADP-ribosyl transferase C3 analogues by a desired cell.

Heterologous fusion includes new polypeptides made by the fusion of polypeptides of the present invention with heterologous polypeptides. Such polypeptides may include but are not limited to bacterial polypeptides (e.g., betalactamase, glutathione-S-transferase, or an enzyme encoded by the E.coli trp locus), yeast protein, viral proteins, phage proteins, bovine serum albumin, chemotactic polypeptides, immunoglobulin constant region (or other immunoglobulin regions), albumin, or ferritin.

Other type of polypeptide modification includes amino acids sequence deletions (e.g.,

truncations). Those generally range from about 1 to 30 residues, more preferably about 1 to 10 residues and typically about 1 to 5 residues.

Mutants, variants and analogues proteins

5 Mutant polypeptides will possess one or more mutations, which are deletions (e.g., truncations), insertions (e.g., additions), or substitutions of amino acid residues. Mutants can be either naturally occurring (that is to say, purified or isolated from a natural source) or synthetic (for example, by performing site-directed mutagenesis on the encoding DNA or made by other synthetic methods such as chemical synthesis). It is thus apparent that the
10 polypeptides of the invention can be either naturally occurring or recombinant (that is to say prepared from the recombinant DNA techniques).

A protein at least 50 % identical, as determined by methods known to those skilled in the art (for example, the methods described by Smith, T.F. and Waterman M.S. (1981) Ad.

15 Appl.Math., 2:482-489, or Needleman, S.B. and Wunsch, C.D. (1970) J.Mol.Biol., 48: 443-453), to those polypeptides of the present invention, for example C3APL, C3APLT, C3APS, C3-TL, C3-TS, C3Basic1, C3Basic2 and C3Basic3 are included in the invention, as are proteins at least 70 % or 80 % and more preferably at least 90 % identical to the protein of the present invention. This will generally be over a region of at least 5, preferably at least 20
20 contiguous amino acids.

“Variant” as the term used herein, is a polynucleotide or polypeptide that differs from reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference
25 polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusion and truncations in the polypeptide encoded by the reference sequence, as discussed herein. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally,
30 differences are limited so that the sequence of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid by one or more substitutions, additions, deletions, or any combination therefore. A substituted or inserted amino acid residue may or may not be one

encoded by the genetic code. A variant polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

5

Amino acid sequence variants may be prepared by introducing appropriate nucleotide changes into DNA, or by in vitro synthesis of the desired polypeptide. Such variant include, for example, deletions, insertions, or substitutions of residues within the amino acid sequence. A combination of deletion, insertion and substitution can be made to arrive at the final construct, provided that the final protein product possesses the desired biological activity, or characteristics. The amino acid changes also may alter posttranslational processes such as changing the number or position of the glycosylation sites, altering the membrane anchoring characteristics, altering the intra-cellular location by inserting, deleting or otherwise affecting the transmembrane sequence of the native protein, or modifying its susceptibility to proteolytic cleavage.

15

Unless otherwise indicated, the recombinant DNA techniques utilized in the present invention are standard procedures, known to those skilled in the art. Example of such techniques are explained in the literature in sources such as J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present).

20

25

It is to be understood herein, that if a "range" or "group of substances" is mentioned with respect to a particular characteristic (e.g. amino acid groups, temperature, pressure, time and the like) of the present invention, the present invention relates to and explicitly incorporates herein each and every specific member and combination of sub-ranges or sub-groups therein whatsoever. Thus, any specified range or group is to be understood as a shorthand way of

30

referring to each and every member of a range or group individually as well as each and every possible sub-ranges or sub-groups encompassed therein; and similarly with respect to any sub-ranges or sub-groups therein. Thus, for example,

- with respect to a sequence comprising up to 50 base units it is to be understood as specifically incorporating herein each and every individual unit, as well as sub-range of units;
- with respect to reaction time, a time of 1 minute or more is to be understood as specifically incorporating herein each and every individual time, as well as sub-range, above 1 minute, such as for example 1 minute, 3 to 15 minutes, 1 minute to 20 hours, 1 to 3 hours, 16 hours, 3 hours to 20 hours etc.;
- with respect to polypeptides, a polypeptide analogue comprising a particular sequence and having an addition of at least one amino acid to its amino-terminus or to its carboxy terminus is to be understood as specifically incorporating each and every individual possibility, such as for example one, two, three, ten, eighteen, forty, etc.;
- with respect to polypeptides, a polypeptide analogue having at least 90 % of its amino acid sequence identical to a particular amino acid sequence is to be understood as specifically incorporating each and every individual possibility (excluding 100 %), such as for example, a polypeptide analogue having 90 %, 90.5%, 91%, 93.7%, 97%, 99%, etc., of its amino acid sequence identical to a particular amino acid sequence.
- with respect to polypeptides, a polypeptide analogue having at least 70 % of its amino acid sequence identical to a particular amino acid sequence is to be understood as specifically incorporating each and every individual possibility (excluding 100 %), such as for example, a polypeptide analogue having 70 %, 72.3%, 73%, 88.6%, 98% etc., of its amino acid sequence identical to a particular amino acid sequence.
- with respect to polypeptides, a polypeptide analogue having at least 50 % of its

amino acid sequence identical to a particular amino acid sequence is to be understood as specifically incorporating each and every individual possibility (excluding 100 %), such as for example, a polypeptide analogue having 50 %, 54%, 66.7%, 70.2%, 84%, 93% etc., of its amino acid sequence identical to that particular amino acid sequence.

- with respect to polypeptide, a polypeptide comprising at least one transport agent region is to be understood as specifically incorporating each and every individual possibility, such as for example a polypeptide having one, two, five, ten, etc., transport agent region.
- and similarly with respect to other parameters such as low pressures, concentrations, elements, etc...

It is also to be understood herein that "g" or "gm" is a reference to the gram weight unit; that "C", or " °C " is a reference to the Celsius temperature unit; and "psig" is a reference to "pounds per square inch guage".

Table 2: Abbreviations

Abbreviation	Full name
C3	ADP-ribosyl transferase C3
NGF	Nerve growth factor
BDNF	Brain-derived neurotrophic factor
C or °C	Degree Celcius
ml	milliliter
μl or ul	microliter
μM or uM	micromolar
mM	millimolar
M	molar
N	normal
CNS	Central nervous system
PNS	Peripheral nervous system
HIV	Human immunodeficiency virus
kDa	kilodalton
GST	Glutathione S-transferase
MTS	Membrane transport sequence
SDS-PAGE	Sodium dodecyl sulfte polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
U	unit
BBB	Basso, Beattie Breshnahan behavior recovery scale
IPTG	Isopropyl β-D-thiogalactopyranoside
rpm	Rotation per minutes
DTT	dithiothreitol
PMSF	Phenylmethylsulfonyl fluoride
NaCl	Sodium chloride

Table 2: Abbreviations; continued

Abbreviation	Full name
MgCl ₂	Magnesium chloride
HBSS	Hank's balanced salt solution
NaOH	Sodium hydroxide
CSPG	chondroitin sulfate proteoglycan
PKN	Protein kinase N
RSV	Rous sarcoma virus
MMTV	Mouse mammary tumor virus
LTR	Long terminal repeat
HL	Hind limb
FL	Fore limb
neo	neomycin
hygro	hygromycin
IN-1	monoclonal antibody called IN-1
ADP	Adenosine di-phosphate
ATP	Adenosine tri-phosphate
³² P	Isotope 32 of phosphorus
DHFR	Dihydrofolate reductase
PCR	Polymerase chain reaction

- 5 C3APL: (C3 antennapedia –long) created by annealing sequences from the antennapedia transcription factor to the 3' end of the sequence encoding C3 cDNA. The long antennapedia sequence of 60 amino acids containing the homeodomain of antennapedia, was used;

C3APLT: (C3 antenpedia -truncated) created by annealing sequences from the antenpedia transcription factor to the 3' end of the sequence encoding C3 cDNA. This clone with a frameshift mutation gives a proline-rich transport peptide with good transport activity. This sequence is tuncated ie shorter than C3APL.

5 C3APS: A short 11 amino acid sequence of antenpedia that has transmembrane transport properties was fused to the carboxy terminal of C3 to create C3APS;

10 C3-TL: C3 Tat-long created by fusing amino acids 27 to 72 of Tat to the carboxy terminal of C3 protein;

C3-TS: C3 Tat-short created by fusing the amino acids YGRKRRQRRR to the C3 protein;

15 C3Basic1 : a random basic charge sequence added to the C-terminal of C3;

C3Basic2 : a random basic charge sequence added to the C-terminal of C3;

20 C3Basic3: C3 Tat-short created by fusing the reverse sequence of Tat amino acids RRQRRKKR to the C3 protein.

It has been found that conjugates or fusion proteins (C3-like proteins) Rho antagonists of the present invention are effective to stimulate repair in the CNS after spinal cord injury. The increased cell permeability of new Rho antagonist (new chimeric C3) would now allow treatment of victims of stroke and neurodegenerative disease because Rho signalling pathway is important in repair after stroke (Hitomi, et al. (2000) 67: 1929-39. Trapp et al 2001. Mol.Cell. Neurosci. 17: 883-84). Treatment with Rho antagonists in the adhesive delivery system could be used to enhance the rate of axon growth in the PNS. Also, evidence in the literature now links Rho signalling with formation of Alzheimer's disease tangles through its ability to activate PKN which then phosphorylates tau and neurofilaments (Morissette, et al. (2000) 278: H1769-74., Kawamata, et al. (1998) 18: 7402-10., Amano, et al. (1996) 271: 648-50., Watanabe, et al. (1996) 271: 645-8.). Therefore, Rho antagonists are expected to be useful in the treatment of Alzheimer's disease. The new chimeric C3 drugs should be able to

diffuse readily and therefore can promote repair for diseases that are neurodegenerative. Examples include, but are not limited to stroke, traumatic brain injury, Parkinson's disease, Alzheimer's disease and ALS. Moreover, it is now well established that Rho signalling antagonists are effective in the treatment of other diseases. These include, but are not limited to eye diseases such as glaucoma (Honjo, et al. (2001) 42: 137-44., Rao, et al. (2001) 42: 1029-1037.), cancer cell migration and metastasis (Sahai, et al. (1999) 9: 136-45., Takamura, et al. (2001) 33: 577-81., Imamura, et al. (2000) 91: 811-6.). The effect of the Rho signalling pathway on smooth muscle relaxation are well established. This has led to the identification of Rho signalling antagonists as effective in treatment of hypertension (Chitaley, et al. (2001) 3: 139-144., Somlyo (1997) 389: 908-911, Uehata, et al. (1997) 389: 990-994), asthma (Nakahara, et al. (2000) 389: 103-6., Ishizaki, et al. (2000) 57: 976-83), and vascular disease (Miyata, et al. (2000) 20: 2351-8., Robertson, et al. (2000) 131: 5-9.) as well as penile erectile dysfunction (Chitaley, et al. (2001) 7: 119-22.). Rho is also important as a cardioprotective protein (Lee et al. 2001. FASEB J. 15:1886-1894).

Rho GTPases include members of the Rho, Rac and Cdc42 family of proteins. Our invention concerns Rho family members of the Rho class. Rho proteins consist of different variants encoded by different genes. For example, PC-12 cells express RhoA, RhoB and RhoC (Lehmann et al 1999 supra); PC-12 cells: Pheochromocytom cell line (Greene A and Tischler, A S PNAS 73:2424 (1976). To inactivate Rho proteins inside cells, Rho antagonists of the C3 family type are effective because they inactivate all forms of Rho (eg. RhoA, Rho B etc). In contrast, gene therapy techniques, such as introduction of a dominant negative RhoA family member into a diseased cell, will only inactivate that specific RhoA family member.

Recombinant C3 proteins, or C3 proteins that retain the ribosylation activity are also effective in our delivery system and are covered by this invention. In addition, Rho kinase is a well-known target for active Rho, and inactivating Rho kinase has the same effect as inactivating Rho, at least in terms of neurite or axon growth (Kimura and Schubert (1992) Journal of Cell Biology.116:777-783, Keino-Masu, et al. (1996)Cell.87:175-185, Matsui, et al. (1996)EMBO

J.15:2208-2216, Matsui, et al. (1998)J. Cell Biol.140:647-657, Ishizaki (1997)FEBS Lett.404:118-124), the biological activity that concerns this invention.

The C3 polypeptides of the present invention include biologically active fragments and analogues of C3; fragments encompass amino acid sequences having truncations of one or more amino acids , wherein the truncation may originate from the amino terminus, carboxy terminus, or from the interior of the protein. Fragments containing Glu(173) of C3 are included in this invention (Saito et al. 1995. FEBS Lett. 371-105). Analogues of the invention involve an insertion or a substitution of one or more amino acids. Fragments and analogues will have the biological property of C3 that is capable of inactivating Rho GTPase on Asn(41) on Rho. Also encompassed by the invention are chimeric polypeptides comprising C3 amino acid sequences fused to heterologous amino acid sequences. Said heterologous sequences encompass those which, when formed into a chimera with C3 retain one or more biological or immunological properties of C3. A host cell transformed or transfected with nucleic acids encoding C3 protein or C3 chimeric protein are also encompassed by the invention. Any host cell which produces a polypeptide having at least one of the biological properties of C3 may be used. Specific examples include bacterial, yeast, plant, insect or mammalian cells. In addition, C3 protein may be produced in transgenic animals. Transformed or transfected host cells and transgenic animals are obtained using materials and methods that are routinely available to one skilled in the art. Host cells may contain nucleic acid sequences having the full-length gene for C3 protein including a leader sequence and a C-terminal membrane anchor sequence (see below) or, alternatively, may contain nucleic acid sequences lacking one or both of the leader sequence and the C-terminal membrane anchor sequence. In addition, nucleic acid fragments, variants and analogues which encode a polypeptide capable of retaining the biological activity of C3 may also be resident in host expression systems.

C3 is produced as a 26 kDa protein. The full length C3 protein inactivates Rho by ADP-ribosylating asparagine 41 of Rho A (Han et al. (2001) J. Mol. Biol. 305: 95). Truncated, elongated or altered C3 proteins or C3-derived peptides that retain the ability to ribosylate Rho are included in this invention and can be used to make fusion proteins. The crystal

structure of C3 has been determined giving insight to elements of the C3 protein that could be changed without affecting ribosylating activity (Han et al. (2001) J. Mol. Biol. 305: 95).

5 The Rho antagonist that is a recombinant proteins can be made according to methods present in the art. The proteins of the present invention may be prepared from bacterial cell extracts, or through the use of recombinant techniques. In general, C3 proteins according to the invention can be produced by transformation (transfection, transduction, or infection) of a host cell with all or part of a C3-encoding DNA fragment in a suitable expression vehicle. Suitable expression vehicles include: plasmids, viral particles, and phages. For insect cells,
10 baculovirus expression vectors are suitable. The entire expression vehicle, or a part thereof, can be integrated into the host cell genome. In some circumstances, it is desirable to employ an inducible expression vector.

15 Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems can be used to provide the recombinant protein. The precise host cell used is not critical to the invention. The C3 and C3-like proteins may be produced in a prokaryotic host (e.g., *E. coli* or *B. subtilis*) or in a eukaryotic host (e.g., *Saccharomyces* or *Pichia*; mammalian cells, e.g., COS, NIH 3T3, CHO, BHK, 293, or HeLa cells; or insect cells).

20 Proteins and polypeptides may also be produced by plant cells. For plant cells viral expression vectors (e.g., cauliflower mosaic virus and tobacco mosaic virus) and plasmid expression vectors (e.g., Ti plasmid) are suitable. Such cells are available from a wide range of sources (e.g., the American Type Culture Collection, Rockland, Md.). The methods of transformation or transfection and the choice of expression vehicle will depend on the host
25 system selected.

The host cells harbouring the expression vehicle can be cultured in conventional nutrient media adapted as need for activation of a chosen gene, repression of a chosen gene, selection of transformants, or amplification of a chosen gene. One expression system is the mouse 3T3

fibroblast host cell transfected with a pMAMneo expression vector (Clontech, Palo Alto, Calif.). pMAMneo provides an RSV-LTR enhancer linked to a dexamethasone-inducible MMTV-LTR promotor, an SV40 origin of replication which allows replication in mammalian systems, a selectable neomycin gene, and SV40 splicing and polyadenylation sites. DNA
5 encoding a C3 or C3-like protein would be inserted into the pMAMneo vector in an orientation designed to allow expression. The recombinant C3 or C3-like protein would be isolated as described below. Other preferable host cells that can be used in conjunction with the pMAMneo expression vehicle include COS cells and CHO cells (ATCC Accession Nos. CRL 1650 and CCL 61, respectively).

10 C3 polypeptides can be produced as fusion proteins. For example, expression vectors may be used to create lacZ fusion proteins. The pGEX vectors can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can be easily purified from lysed cells by adsorption to glutathione-
15 agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety. Another strategy to make fusion proteins is to use the His tag system.

20 In an insect cell expression system, Autographa californica nuclear polyhedrosis virus (AcNPV), which grows in *Spodoptera frugiperda* cells, is used as a vector to express foreign genes. A C3 coding sequence can be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter, e.g., the polyhedrin promoter. Successful insertion of a gene encoding a C3 or C3-like
25 protein (polypeptide) will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat encoded by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed (see, Lehmann et al for an example of making recombinant MAG protein).

In mammalian host cells, a number of viral-based expression systems can be utilised. In cases where an adenovirus is used as an expression vector, the C3 nucleic acid sequence can be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene can then be inserted into the adenovirus genome by in vitro or in vivo recombination. Insertion into a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a C3 gene product in infected hosts.

Specific initiation signals may also be required for efficient translation of inserted nucleic acid sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire native C3 gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. In other cases, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators.

In addition, a host cell may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in a specific, desired fashion. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such mammalian host

cells include, but are not limited to, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, choroid plexus cell lines.

Alternatively, a C3 protein can be produced by a stably-transfected mammalian cell line. A number of vectors suitable for stable transfection of mammalian cells are available to the public; methods for constructing such cell lines are also publicly available. In one example, cDNA encoding the C3 protein may be cloned into an expression vector that includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, the C3 or C3-like protein-encoding gene into the host cell chromosome is selected for by including 0.01-300 μ M methotrexate in the cell culture medium (as described in Ausubel et al., supra). This dominant selection can be accomplished in most cell types. Recombinant protein expression may be increased by DHFR-mediated amplification of the transfected gene. Methods for selecting cell lines bearing gene amplifications are known in the art; such methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. DHFR-containing expression vectors commonly used for this purpose include pCVSEII-DHFR and pAdD26SV(A). Any of the host cells described above or, preferably, a DHFR-deficient CHO cell line (e.g., CHO DHFR cells, ATCC Accession No. CRL 9096) are among the host cells preferred for DHFR selection of a stably-transfected cell line or DHFR-mediated gene amplification.

A number of other selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase, and adenine phosphoribosyltransferase genes can be employed in tk, hgprt, or aprt cells, respectively. In addition, gpt, which confers resistance to mycophenolic acid; neo, which confers resistance to the aminoglycoside G-418; and hygromycin may be used.

Alternatively, any fusion protein can be readily purified by utilising an antibody specific for the fusion protein being expressed. For example, a system described in Janknecht et al. (1981) Proc. Natl. Acad. Sci. USA 88, 8972, allows for the ready purification of non-

denatured fusion proteins expressed in human cell lines. In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni²⁺ nitriloacetic acid-
5 agarose columns, and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

Alternatively, C3, C3-like protein or a portion (fragment) thereof, can be fused to an immunoglobulin Fc domain. Such a fusion protein can be readily purified using a protein A
10 column.

To test Rho antagonists for activity, a tissue culture bioassay system was used. This bioassay is used to define activity of Rho antagonists that will be effective in promoting axon regeneration in spinal cord injury, stroke or neurodegenerative disease.

15
Neurons do not grow neurites on inhibitory myelin substrates. When neurons are placed on inhibitory substrates in tissue culture, they remain rounded. When an effective Rho antagonist is added, the neurons are able to grow neurites on myelin substrates. The time that it takes for neurons to growth neurites upon the addition of a Rho antagonist is the same as if
20 neurons had been plated on growth permissive substrate such as laminin or polylysine, typically 1 to 2 days in cell culture. The results can be scored visually. If needed, a quantitative assessment of neurite growth can be performed. This involved measuring the neurite length in a) control cultures where neurons are plated on myelin substrates and left untreated b) in positive control cultures, such as neurons plated on polylysine c) or treating
25 cultures with different concentrations of the test antagonist.

To test C3 in tissue culture, it has been found that the best concentration is 25-50 ug/ml. (Lehmann et al, 1999. J.Neurosci. 19: 7537-7547; Jin & Strittmatter, 1997. J. Neurosci. 17: 6256-6263). Thus, high concentrations of this Rho antagonist are needed as compared to the

growth factors used to stimulate neurite outgrowth. Growth factors, such as nerve growth factor (NGF) are used at concentrations of 1- 100 ng/ml in tissue culture. However, growth factors are not able to overcome growth inhibition by myelin. Our tissue culture experiments are all performed in the presence of the growth factor BDNF for retinal ganglion cells, or
5 NGF for PC-12 cells. When growth factors have been tested in vivo, typically the highest concentrations possible are used, in the ug/ml range. Also they are often added to the CNS with the use of pumps for prolonged delivery (eg. Ramer et al, supra). For in vivo experiments the highest concentrations possible was used when working with C3 stored as a frozen 1 mg/ml solution.

10 The Rho antagonist C3 is stable at 37 °C for at least 24 hours. The stability of C3 was tested in tissue culture with the following experiment. The C3 was diluted in tissue culture medium, left in the incubator at 37 °C for 24 hours, then added to the bioassay system described above, using retinal ganglion cells as the test cell type. These cells were able to extend neurites on
15 inhibitory substrates when treated with C3 stored for 24 hours at 37 °C. Therefore , the minimum stability is 24 hours. This is in keeping with the stability projection based on amino acid composition (see sequence data, below).

A compound can be confirmed as a Rho antagonist in one of the following ways:

- 20 a) Cells are cultured on a growth inhibitory substrate as above, and exposed to the candidate Rho antagonist;
- b) Cells of step a) are homogenized and a pull-down assay is performed. This assay is based on the capability of GST-Rhotekin to bind to GTP-bound Rho. Recombinant GST-Rhotekin or GST rhotekin binding domain (GST-RBD) is
25 added to the cell homogenate made from cells cultured as in a). It has been found that inhibitory substrates activate Rho, and that this activated Rho is pulled down by GST-RBD. Rho antagonists will block activation of Rho, and therefore, an effective Rho antagonist will block the detection of Rho when cell are cultured as described by a) above;

- c) An alternate method for this pull-down assay would be to use the GTPase activating protein, Rho-GAP as bait in the assay to pull down activated Rho, as described (Diekmann and Hall, 1995. In Methods in Enzymology Vol. 256 part B 207-215).

5

Another method to confirm that a compound is a Rho antagonist is as follows: When added to living cells antagonists that inactivate Rho by ADP-ribosylation of the effector domain can be identified by detecting a molecular weight shift in Rho (Lehmann et al, 1999 supra). The molecular weight shift can be detected after treatment of cells with Rho antagonist by
10 homogenizing the cells, separating the proteins in the cellular homogenate by SDS polyacrylamide gel electrophoresis. The proteins are transferred to nitrocellulose paper, then Rho is detected with Rho-specific antibodies by a Western blotting technique.

Another method to confirm that compound is a Rho-kinase antagonist is as follows:

15

- a) Recombinant Rho kinase tagged with myc epitope tag, or a GST tag or any suitable tag is expressed in Hela cells or another suitable cell type by transfection;
- b) The kinase is purified from cell homogenates by immunoprecipitation using
20 antibodies directed against the specific tag (e.g., myc tag or the GST tag);
- c) The recovered immunoprecipitates from b) are incubated with [³²P] ATP and histone type 2 as a substrate in the presence or absence of the Rho kinase inhibitor. In the absence of Rho kinase inhibitor activity, the Rho kinase phosphorylated histone. In the presence of Rho kinase inhibitor the
25 phosphorylation activity of Rho kinase (i.e. phosphorylation of histone) is blocked, and as such identified the compound as a Rho kinase antagonist.

Turning now to the transport side of the conjugates of the present invention, known methods are available to add transport sequences that allow proteins to penetrate into the cell; examples include membrane translocating sequence (Rojas (1998) 16: 370-375), Tat-mediated protein delivery (Vives (1997) 272: 16010-16017), polyargine sequences (Wender et al. 2000, PNAS 24: 13003-13008) and antennapedia (Derossi (1996) 271: 18188-18193). Examples of known transport agents, moieties, subdomains and the like are also shown for example in Canadian patent document no. 2,301,157 (conjugates containing homeodomain of antennapedia) as well as in U.S. patent 5,652,122, 5,670,617, 5,674,980, 5,747,641, and 5,804,604 (conjugates containing amino acids of Tat HIV protein (hereinafter Tat HIV protein is sometimes simply referred to as Tat).

Several receptor-mediated transport strategies have been used to try and improve function of ADP ribosylases: these methods include fusing C2 and C3 sequences (Wilde, et al. (2001) 276: 9537-9542.) and use of receptor-mediated transport with the diphtheria toxin receptor (Aullo, et al. (1993) 12: 921-31 ; Boquet, P. et al. (1995) Meth. Enzymol. 256: 297-306). These methods have not been demonstrated to dramatically increase the potency of C3. Moreover, these proteins require receptor-mediated transport. This means that the cells must express the receptor, and must express sufficient quantities of the receptor to significantly improve transport. Moreover, when C3 enters the cell by endocytosis, it will be locked within a membrane compartment, and therefore most of it will not be available to inactivate Rho. In the case of diphtheria toxin, not all cells express the appropriate receptor, limiting its potential use. The clinical importance for any of these has not been tested or shown. A C2/C3 fusion protein has also been made to try and improve the effectiveness of C3. In this case, the addition of a C2II binding protein to the tissue culture medium is needed, along with the C2-C3 fusion toxin to allow uptake of C3 by receptor-mediated endocytosis (Barthe et al. (1998) Infection and Immunity 66:1364). The disadvantage of this system is that much of the C3 in the cell will be restrained within a membrane compartment. More importantly, two different proteins must be added separately for transport to occur (Wahl et al. 2000. J. Cell Biol. 149:263), which make this system difficult to apply to in vivo for treatment of disease. Moreover, none of the methods to inactivate Rho with C3 or C3 analogues (C3-like protein)

have been demonstrated to be sufficient to overcome growth inhibition in tissue culture, or to promote recovery after CNS damage in vivo.

One strategy which may be used in accordance with the present invention is to exploit the antennapedia homeodomain that is able to transport proteins across the plasma membrane by a receptor-independent mechanism (Derossi (1996) 271: 18188-18193); an alternate strategy is to exploit Tat-mediated delivery (Vives (1997) 272: 16010-16017, Fawell (1994) 91: 664-668, Frankel (1988) 55: 1189-1193).

The Antennapedia strategy has been used for protein translocation into neurons (Derossi (1996) 271: 18188-18193). Antennapedia has, for example, been used to transport biotin-labelled peptides in order to demonstrate the efficacy of the technique; see U.S. Patent No. 6,080,724.

Antennapedia enhances growth and branching of neurons in vitro (Bloch-Gallego (1993) 120: 485-492). Homeoproteins are transcription factors that regulate development of body organization, and antennapedia is a *Drosophila* homeoprotein. Tat on the other hand is a regulatory protein from human immunodeficiency virus (HIV). It is a highly basic protein that is found in the nucleus and can transport reporter genes into cell. Moreover, Tat-linked proteins can penetrate cells after intraperitoneal injection, and it can even cross the blood brain barrier to enter cells within the brain (Schwarze, et al. (1999) 285: 1569-72).

Other transport sequences that have been tested in other contexts, (i.e., to show that they work through the use of reporter sequences), are known. One transport peptide, a 12 mer, AAVLLPVLLAAP, is rich in proline. It was made as a GST-MTS fusion protein and is derived from the h region of the Kaposi FGF signal sequence (Royas et al. 1998 Nature Biotech. 16: 370-375. Another example is the sperm fertilin alpha peptide, HPIQIAAFLARIPPISSIGTCILK (This is reviewed in Pécheur, J. Sainte-Marie, A. Bienvenue, D. Hoekstra. 1999. J. Membrane Biol. 167: 1-17). It must be noted however that the alpha helix-breaking propensity of proline (Pro) residues is not a general rule, since the putative fusion peptide of sperm fertilin alpha displays a high alpha helical content in the

presence of liposomes. However, the Pro-Pro sequence is required for efficient fusion properties of fertilin. The C3APLT fusion protein that we tested fits the requirement of having a two prolines for making an effective transport peptide. Therefore, proline-rich sequences and random sequences that have helix-breaking propensity that act as effective transporters would also be effective if fused to C3.

In the context of axon growth on inhibitory substrates, axon regeneration after injury, or axon regeneration in the brain or spinal cord, no method using these transport sequences has been devised. In particular, it should be noted that the ability of antennapedia to enhance growth was tested with neurons placed on laminin-coated coverslips. Laminin supports axon growth and overrides growth inhibition (David, et al. (1995) 42: 594-602) thus, it is not a suitable substrate to test the potential for regeneration. There is an enormous wealth of literature over the last 20 years on substances that promote axon growth under such favourable tissue culture conditions, but none of these has lead to clinical advances in the treatment of spinal cord injury. The effect of antennapedia was shown to act as similar to a growth factors. Growth factors do not overcome growth inhibition by CNS growth inhibitory substrates (Lehmann, et al. (1999) 19: 7537-7547, Cai, et al. (1999) 22: 89-101). Growth factors applied in vivo do not support regeneration, only sprouting (Schnell, et al. (1994) 367: 170-173).

The transport sequence may be added to the N-terminal (amino-terminal) sequence of the C3 protein. Alternatively, the transport sequence may be added on the C-terminal (carboxy-terminal) end of the C3 protein; because the C-terminal is already quite basic, this should enhance further the transport properties. This is likely one of the reasons that C3APLT shows activity in addition to its basic charge and the proline-rich sequences.

The new chimeric C3 may be used to treat spinal cord injury to promote functional repair. We have demonstrated that both C3APLT and C3APS can overcome growth inhibition on complex inhibitory substrates that include myelin and mixed chondroitin sulfate proteoglycans. Further, we demonstrate that C3APLT can promote functional recovery after application to injured spinal cord in adult mice. The new chimeric protein may be used to

promote axon regeneration and reduce scarring after CNS injury. Scarring is a barrier to nerve regeneration.

5 The advantage of the new chimeric C3 is the ability to treat the injured axons after a significant delay between the injury and the treatment. Also, the new recombinant protein may be useful in the treatment of chronic injury. The chimeric C3 can also be used to treat neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease where penetration of the Rho antagonist to the affected neuronal population is required for effective treatment. The chimeric C3 (fusion proteins) will also be of benefit for the treatment of
10 stroke and traumatic brain injury. Moreover, much evidence suggests efficacy in the treatment of cancer cell migration. Rho antagonists are also useful in the treatment of disease involving smooth muscle, such as vascular disease, hypertension, asthma, and penile dysfunction.

15 For treatment of spinal cord injury, the conjugate Rho antagonists of the present invention may be used in conjunction with cell transplantation. Many different cell transplants have been extensively tested for their potential to promote regeneration and repair, including , but not restricted to, Schwann cells, fibroblasts modified to express growth factors, fetal spinal cord transplants, macrophages, embryonic or adult stem cells, and olfactory ensheathing glia.
20 C3 fusion proteins may be used in conjunction with neurotrophins, apoptosis inhibitors, or other agents that prevent cell death. They may be used in conjunction with cell adhesion molecules such as L1, laminin, and artificial growth matrices that promote axon growth. The chimeric C3 constructs of the present invention may also be used in conjunction with the use of antibodies that block growth inhibitory protein substrates to promote axon growth.
25 Examples of such antibody methods are the use of IN-1 or related antibodies (Schnell and Schwab (1990) 343: 269-272) or through the use of therapeutic vaccine approaches (Huang (1999) 24: 639-647).

BRIEF DESCRIPTION OF THE FIGURES

In drawings which illustrate example embodiments of the present invention:

- 5 Figure 1 illustrates the dose response of normal C3 with and without trituration;
- Figure 2 illustrates ADP ribosylation by C3APLT and C3APS, but not C3 after passively adding the compounds to PC-12 cells;
- Figure 3A illustrates that C3APLT penetrates cells;
- Figure 3B illustrates a lower level of cell penetration by C3 as compared to Figure 3A;
- 10 Figure 4 illustrates the effectiveness of C3APLT and C3APS at low doses;
- Figure 5 illustrates the effectiveness of C3APLT and C3APS at low doses;
- Figure 6 illustrates the effectiveness of C3APLT to stimulate axon regeneration of primary neurons;
- Figure 7 illustrates the effectiveness of C3APLT to promote functional recovery after spinal
15 cord injury;
- Figure 8 illustrates effectiveness of Tat transport sequences to enhance growth as C3-Tat (C3-TL and C3-TS) chimeras;
- Figures 9A and 9B illustrate axon regeneration after spinal cord injury and treatment with C3APLT;
- 20 Figure 10 illustrates effectiveness of C3APLT to prevent cell death after spinal cord injury, thereby showing that it is neuroprotective,
- Figure 11 illustrates a comparison of C3APLT and C3Basic3 to promote neurite outgrowth: and,
- 25 Figure 12 illustrates that C3APLT promotes neurite outgrowth from retinal neurons plated on inhibitory myelin or CSPG substrates. Retinal neurons plated on myelin (*dark bars*) or CSPG (*dotted bars*) substrates and treated with C3-05. Figure 12 A illustrates the percentage

of cells with neurites neurites longer than 1 cell body diameter (neurite outgrowth); Figure 12 B illustrates the length of the longest neurite per cell (neurite length).

Referring to Figure 1, PC-12 cells were plated on inhibitory myelin substrates (0).

5 Unmodified C3 added to the tissue culture medium at concentration from 0.00025 – 50 ug/ml did not significantly improve neurite outgrowth over the untreated control (grey bars). C3 was only effective in stimulating neurite outgrowth for cells plated on myelin substrates after scrape –loading (black bars). This Figure demonstrates the limited or no penetration in cells when passively added to the tissue culture medium. Please see Example 4 below for
10 techniques.

Referring to Figure 2, this Figure provides a demonstration that C3APLT and C3APS, ADP ribosylate Rho. Western blot showing RhoA in untreated cells (lane 1), and cells treated with C3APLT (lane 2) or C3APS (lane 3). When Rho is ADP ribosylated by C3 it undergoes a
15 molecular weight shift (Lehmann et al supra), as observed for lanes 2 and 3. Please see Example 4 below for techniques.

Referring to Figure 3, this Figure shows intracellular activity after treatment with C3APLT. Detection that the new fusion C3 penetrates into the cells. Immunocytochemistry with anti-
20 C3 antibody of PC-12 cells plated on myelin and treated with C3 (A) or C3APLT (B). Cells in A (Figure 3A) are not immunoreactive because C3 has not penetrated into the cells. Cells in B (Figure 3B) are immunoreactive and they are able to extend neurites on myelin substrates. Please see Example 4 below for techniques.

25 Turning to Figure 4, this Figure shows that C3-antennapedia fusion proteins promote growth on inhibitory substrates. The percent of neurons that grow neurites was counted for each treatment. The dose response experiment shows that C3APLT and C3APS promote more neurite growth per cell than control PC-12 cells plated on myelin (0). PC-12 cells were plated on myelin and either scrape loaded with unmodified C3 (C3 50) left untreated (0) or

treated with various concentrations of C3APLT. Compared to C3 used at 25 ug/ml, C3APS is effective at stimulating more cells to grow neurites at 0.0025 ug/ml, a dose 10,000 X less. Please see Example 4 below for techniques.

5 Figure 5 shows a dose-response experiment showing that C3APLT and C3APS elicit long neurites to grow when cells are plated on inhibitory substrates. The length of neurites was measured for each treatment. PC-12 cells were plated on myelin and either scrape loaded with unmodified C3 (C3 50) left untreated (0) or treated with various concentrations of C3APLT. Compared to C3 used at 25 ug/ml, C3APS is effective at stimulating more cells to
10 longer neurite growth at 0.0025 ug/ml, a dose 10,000 X less. Please see Example 4 below for techniques.

As may be seen Figure 6 shows primary neurons growing on inhibitory substrates after treatment with C3APLT. Rat retinal ganglion cells were plated on myelin substrates and
15 treated with different concentrations of C3APLT. Concentrations of 0.025 ug/ml and above promoted significantly longer neurites. This dose is 1000X lower than that of C3 needed to promote growth on myelin.

Referring to Figure 7, this Figure shows behavioral recovery after treatment of adult mice
20 with C3APLT in a dose-response experiment. Mice received a dorsal hemisection of the spinal cord and were left untreated (transection), were treated with fibrin alone (fibrin) or were treated with fibrin plus C3APLT at the indicated concentrations given in ug/mouse. Each point represents one animal. The BBB score (see Example 6 for details) was assessed 24 hours after treatment. Animals treated with C3APLT exhibited a significant improvement
25 in behavioural recovery compared to untreated animals. The effective dose of 0.5 ug is 100X less than unmodified C3 used (see previous experiment shown in Canadian patent application 2,325,842). Please see Example 6.

Referring to Figure 8, this Figure shows promotion of axon growth by C3-Tat chimeric proteins. The dose-response experiment shows that C3-TS and C3-TL promote more neurite growth per cell than control PC-12 cells plated on myelin. PC-12 cells were plated on myelin and either scrape loaded with unmodified C3 (scrape load) left untreated (myelin) or treated with various concentrations of C3-TS (grey bars) or C3-TL (black bars). Compared to C3 used at 25 ug/ml, C3-TL is effective at stimulating more cells to grow neurites at 0.0025 ug/ml, a dose 10,000 X less than C3.

Referring to Figure 9A and 9B, these Figures show axon regeneration in injured spinal cord, i.e. anatomical regeneration after treatment with C3APLT. Section of the spinal cord after anterograde labeling with horseradish peroxidase conjugated to wheat germ agglutinin (WGA-HRP). A) Sprouting of cut axons into the dorsal white matter. Arrows show regenerating axons distal to the lesion. B) Same section 3 mm from the lesion site. Arrows show regenerating axons.

Referring to Figure 10, this Figure shows that C3-APLT protected neurons from cell death following spinal cord injury. Apoptotic (dying) cells were counted following TUNEL labelling (see Example 16) 2mm rostral to the lesion (Rostral) at the lesion site (lesion) and 2 mm caudal to the lesion site (caudal). Bars show average counts of Tunel positive cells from 4 animals treated with fibrin only after spinal cord injury as control (white bars), or with C3APLT in fibrin at 1ug (black bars). Treatment with C3APLT show significantly reduced numbers of Tunel-labeled cells (dying cells). Non-injured spinal cord samples were also processed and these spinal cords did not show Tunel labelling, as expected.

Referring to Figure 11, this Figure shows that C3APLT and C3Basic3 promote rapid neurite outgrowth compared to untreated cells when cells are plated on plastic as part of a rapid bioassay (see Example 4).

Referring to Figure 12A and 12B, to further support the ability of C3-like chimeric proteins

to promote neurite outgrowth on inhibitory substrates, we examined the response of primary cultures plated on inhibitory substrates to C3APLT treatment. Purified retinal ganglion cells (RGCs) were plated on myelin, or CSPG substrates and treated with varying concentrations of C3APLT. During the RGC dissection great care was taken in order to try to limit the amount of mechanical manipulation of the cells, however, the isolation protocol requires that some triturating take place in order to dissociate and separate the cells. When RGCs are plated on inhibitory substrates, they maintained a similar round appearance to PC-12 cells plated on myelin. Treatment of RGCs with C3APLT promoted neurite outgrowth and increased neurite length on both myelin and CSPG substrates. In contrast to the wide range of concentrations shown to be effective in other PC-12 experiments a narrower range of C3APLT treatment, 0.025 ug/ml to 50 ug/ml promoted neurite outgrowth and increased neurite length on myelin. In the case of RGCs plated on CSPG substrates, effective concentration ranges of 0.0025ug/ml to 50ug/ml were observed.

DETAILED DESCRIPTION

Method for making the C3APL, C3APLT, and C3APS

C3APL is the name given to the protein made by ligating a cDNA encoding C3 (Dillon and Feig (1995) 256: 174-184) with cDNA encoding the antennapedia homeodomain (Bloch-Gallego (1993) 120: 485-492). The stop codon at the 3' end of the DNA was replaced with an EcoR I site by polymerase chain reaction (PCR) using the primers 5'GAA TTC TTT AGG ATT GAT AGC TGT GCC 3' (SEQ ID NO: 1) and 5'GGT GGC GAC CAT CCT CCA AAA 3' (SEQ ID NO: 2). The PCR product was sub-cloned into a pSTBlue-1 vector (Novagen, city), then cloned into a pGEX-4T vector using BamH I and Not I restriction site. This vector was called pGEX-4T/C3. The antennapedia sequence used to add to the 3' end of C3 in pGEX-4T/C3 was created by PCR from the pET-3a vector (Bloch-Gallego (1993) 120: 485-492, Derossi (1994) 269: 10444-10450), subcloned into a pSTBlue-1 blunt vector, then cloned into the pGEX-4T/C3, using the restriction sites EcoR I and Sal I, creating pGEX-

4T/C3APL. Another clone (C3APLT) with a frameshift mutation was selected, and the protein made and tested. When the cultures tested positive despite the mutation, the clone was resequenced by another company to confirm the mutation, and this clone was called C3APLT. To confirm the sequence of C3APLT, the coding sequence from both strands was sequenced. The sequence for this clone is given in Examples 16 and 17 (nucleotide sequence of C3APLT; SEQ ID NO: 42, amino acid sequence of C3APLT; SEQ ID NO:43).

A shorter version of the Antennapedia (pGEX-4T/C3APS) was also made. This chimeric sequence was made by ligating oligonucleotides encoding the short antennapedia peptide (Maizel (1999) 126: 3183-3190) into the pGEX-4T/C3 vector cut with EcoR I and Sal I. The recombinant C3APLT and C3APS cDNAs were separately transformed into bacteria, and after the recombinant proteins were produced, a bacterial homogenate was obtained by sonication, and the homogenate cleared by centrifugation. Glutathione-agarose beads (Sigma) were added to the cleared lysate and placed on a rotating plate for 2-3 hours, then washed extensively. To remove the glutathione S transferase sequence from the recombinant protein, 20U (unit) of Thrombin was added, the beads were left on a rotator overnight at 4°C. After cleavage with thrombin, the beads were loaded into an empty 20ml column, and the proteins eluted with PBS (phosphate buffered saline). Aliquots containing recombinant protein were pooled and 100µl p-aminobenzamidine agarose beads (Sigma) were added and left mixing for 45 minutes at 4°C to remove thrombin, then recombinant protein was isolated from the beads by centrifugation. Purity of the sample was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and bioactivity bioassay with PC-12 cells was performed (See Lehmann et al supra).

Other possible methods for making bioactive chimeric proteins include anion exchange chromatography. For this, the GST tag is not required and can be removed. The cDNA can then be cloned into a high expression bacterial vector, such as pET, as given in Example 16.

The Rho antagonist is a recombinant protein and can be made according to methods present in the art. The proteins of the present invention may be prepared from bacterial cell extracts, or through the use of recombinant techniques by transformation, transfection, or infection of a host cell with all or part of a C3-encoding DNA fragment with an antennapedia-derived transport sequence in a suitable expression vehicle. Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems can be used to provide the recombinant protein. The precise host cell used is not critical to the invention.

Any fusion protein can be readily purified by utilising either affinity purification techniques or more traditional column chromatography. Affinity techniques include, but are not restricted to GST (glutathione-S-transferase), or the use of an antibody specific for the fusion protein being expressed, or the use of a histidine tag. Alternatively, recombinant protein can be fused to an immunoglobulin Fc domain. Such a fusion protein can be readily purified using a protein A column. It is envisioned that small molecule mimetics of the above described antagonists are also encompassed by the invention.

Testing the bioactivity of C3APLT, C3APS, C3-TL and C3-TS

To test the efficacy of C3APLT, C3APS, C3-TL and C3-TS a number of experiments were performed with PC-12 cells, a neural cell line, grown on growth inhibitory substrates (see Lehmann et al supra). PC-12 cells were plated on myelin substrates as described (Lehmann et al, supra). C3, C3APLT, C3APS, C3-TL or C3-TS were added at different concentrations without trituration (please refer to Figures 4, 5 and 8 for concentrations used). C3 added passively to the culture medium in this way was not able to promote neurite growth in the growth inhibitory substrates because cells must be triturated for C3 to enter the cells and be active (Figure 1). Both C3APLT and C3APS were able to ADP ribosylate Rho to cause a shift in the molecular weight of RhoA (Figure 2). Both C3APLT and C3APS were able to promote neurite growth and enter neurons after being added passively to the culture medium (Figure 3, Figures 4 and 5). Dose-response experiment where concentrations of 0.25ng/ml, 2.5 ng/ml, 25 ng/ml, 250 ng/ml and 2.5 µg/ml and 25 µg/ml were tested and showed that C3APLT and C3APS helped more neurons differentiate neurites at doses 10,000 fold less

than C3 (Figure 4). Dose response experiments where concentrations of 0.25ng/ml, 2.5 ng/ml, 25 ng/ml, 250 ng/ml and 2.5 µg/ml and 25 µg/ml were tested and showed that C3APLT was able to promote long neurite growth when added at a minimum concentration of 0.0025 ug/ml (Figure 5). These concentrations of 2.5 ng/ml and 25 ng/ml for C3APLT and C3APS, represent 10,000 and 1,000 times less than the dose needed with C3, respectively. Moreover, at the highest concentration tested, 50 ug/ml, these two new Rho antagonists did not exhibit toxic effects on PC-12 cells, and were able to stimulate neurite outgrowth on growth inhibitory substrates.

C3-TL and C3-TS also were tested at concentrations of 0.25ng/ml, 2.5 ng/ml, 25 ng/ml, 250 ng/ml and 2.5 µg/ml and 25 µg/ml and were found to be able to promote neurite growth on myelin substrates at doses significantly less than C3 (Figure 8). C3Basic3 was tested at 50 ug/ml in a fast growth assay (Figure 11).

To verify the ability of C3APLT and C3APS to promote growth from primary neurons, primary retinal cultures were prepared, and the neurons were plated on myelin substrates as described with respect to Example 5. In the absence of treatment with C3APLT or C3APS, the cells remained round and were not able to grow neurites. When treated with C3APLT or C3APS, retinal neurons were able to extend long neurites on inhibitory myelin substrates (Figure 6).

Next, was tested the ability of C3APLT and C3APS to promote growth on a different type of growth inhibitory substrate relevant to the type of growth inhibitory proteins found at glial scars. Chamber slides were coated with a mixture of chondroitin sulfate proteoglycans (Chemicon), and then plated with retinal neurons (results presented in Figure 12). The neurons were not able to extend neurites on the proteoglycan substrates, but when treated with C3APLT or C3APS, they extended long neurites. These studies demonstrate that

C3APLT and C3APS can be used to promote neurite growth on myelin and on proteoglycans, the major classes of inhibitory substrates that prevent repair after injury in the CNS.

Testing ability of C3APLT to promote regeneration and functional recovery after spinal cord injury

To test if C3APLT could promote repair after spinal cord injury, fully adult mice were used (as described with respect to Example 6). A dorsal hemisection was made at T8 (thoracic spinal level 8), and mice were treated with different amounts (Figure 7) of C3APLT in a fibrin glue as described (McKerracher, US patent pending (delivery patent)). In previous known experiments with C3, it was found that 40-50 ug was needed to promote anatomical regeneration in optic nerve (Lehmann et al supra). We tested different doses (see Figure 7) of C3APLT ranging from 1 ug to 50 ug and assessed animals for behavioural recovery according the BBB scale (Basso (1995) 12: 1-21)

The day following surgery and application of C3APLT, behavioural testing began. The animals were placed in an open field environment that consisted of a rubber mat approximately 4' X 3' in size. The animals were left to move randomly, the movement of the animals were videotaped. For each test two observers scored the animals for ability to move ankle, knee and hip joints in the early phase of recovery. Previously C3 treatment of mice was seen to lead to functional recovery observable 24 hours after treatment. In mice treated with C3APLT, functional recovery could be observed as early as 24 hours after spinal cord injury (Figure 7). Untreated mice exhibit a function recovery score according to the BBB scale averaging 0, whereas mice treated with C3 are able to walk and have a BBB score averaging 8 (Figure 7). At higher concentrations of 50 ug, about 50 % of the mice treated with C3APLT died within 24 hours. However, of the mice that survived, they exhibited good long-term functional recovery. These results demonstrate that C3APLT effectively promotes functional recovery early after spinal cord injury, and that it is effective at much lower doses than C3. However, at high concentrations, C3APLT appears to exhibit toxicity, and therefore careful doing will be required for clinical use.

Qualitative observations of the video tapes showed that only animals that received C3APLT reached the late phase of recovery after 30 days of treatment. Untreated control animals did not typically pass beyond the early phase of recovery. These results indicate that the application of C3APLT improved long-term functional recovery after spinal cord injury compared to no treatment, injury alone, or fibrin adhesive alone.

To test if the early recovery was due to neuroprotection, spinal cord sections were examined for apoptosis by Tunel labelling following manufacturer's instruction (Roche Diagnostic).

C3APLT was able to reduce the number of dying cells observed at the lesion site. Therefore, C3APLT should be an effective neuroprotective agent for treatment of ischemia, such as follows stroke.

EXAMPLE 1. DNA AND PROTEIN SEQUENCE DETAILS OF C3APL

Nucleotide sequence of C3APL.

It has been reported that the long version of antennapedia transport sequence can enhance neurite growth (Bloch-Gallego,E.,LeRoux,I., Joliot,A.H., Volovitch,M., Henderson,C.E., Prochiantz,A. 1993. J. Cell Biol. 120:485). Therefore, this sequence is expected to enhance neurite growth. For the sequence given below, the start site, is in the GST sequence of the plasmid (not shown). The vector with the GST sequence is commercially available and thus the entire GST sequence including the start was not sequenced. It was desired to determine only the sequence located 3' to the thrombin cleavage site which releases C3 conjugate from the GST sequence. The GST sequence is cleaved with thrombin.

The APL transport sequence (SEQ ID NO.: 44) is as follows:

VMESRKRARQTYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRR
MKWKKEN

Nucleotide sequence of C3APL (SEQ ID NO: 3)

5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
 CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
 5 GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
 TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
 AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
 AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
 AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
 10 TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
 AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
 AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA
 TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA
 AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
 15 ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
 TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
 GCT ATC AAT CCT AAA GAA TTC GTG ATG GAA TCC CGC AAA CGC GCA AGG
 CAG ACA TAC ACC CGG TAC CAG ACT CTA GAG CTA GAG AAG GAG TTT CAC
 TTC AAT CGC TAC TTG ACC CGT CGG CGA AGG ATC GAG ATC GCC CAC GCC
 20 CTG TGC CTC ACG GAG CGC CAG ATA AAG ATT TGG TTC CAG AAT CGG CGC
 ATG AAG TGG AAG AAG GAG AAC TGA 3'

Amino acid sequence of C3APL (SEQ ID NO: 4)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
 25 YTKSASEINGKLRQNKG VINGFPSNLIKQVELLDKSFNKMKTPENIMLFRGDDPAYL
 GTEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITQFK
 VAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIITATMMGTAINPK
 EFVMESRKRRARQTYTRYQTLELEKEFHFNRYLTRRRRIELAHALCLTERQIKIWFQNR
 RMKWKKEN

Physical characteristics of C3APL

Molecular Weight 34098.03 Daltons

295 Amino Acids

5 48 Strongly Basic(+) Amino Acids (K,R)

28 Strongly Acidic(-) Amino Acids (D,E)

89 Hydrophobic Amino Acids (A,I,L,F,W,V)

94 Polar Amino Acids (N,C,Q,S,T,Y)

9.847 Isoelectric Point

10 20.524 Charge at PH 7.0

Davis,Botstein,Roth Melting Temp C. 79.48

EXAMPLE 2. DNA AND PROTEIN SEQUENCE DETAILS OF C3APS

15 Nucleotide sequence of C3APS (SEQ ID NO: 5). The start site, is in the GST sequence of the plasmid, not shown here.

5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
20 AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT

AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA
 TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA
 AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
 ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
 5 TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
 GCT ATC AAT CCT AAA GAA TTC CGC CAG ATC AAG ATT TGG TTC CAG AAT
 CGT CGC ATG AAG TGG AAG AAG GTC GAC TCG AGC GGC CGC ATC GTG ACT
 GAC TGA 3'

The APS transport sequence (SEQ ID NO.: 45) is as follows:

10 RQIKIWFQNRRMKWKKVDS

Amino acid sequence for C3APS (SEQ ID NO: 6)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
 15 YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKT PENIMLFRGDDPAYL
 GTEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITQFK
 VAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPK
 EFRQIKIWFQNRRMKWKKVDSSGRIVTD

20 Physical characteristics of C3APS

Molecular Weight 29088.22 Daltons

257 Amino Acids

38 Strongly Basic(+) Amino Acids (K,R)

23 Strongly Acidic(-) Amino Acids (D,E)

79 Hydrophobic Amino Acids (A,I,L,F,W,V)

83 Polar Amino Acids (N,C,Q,S,T,Y)

9.745 Isoelectric Point

15.211 Charge at PH 7.0

5 Davis,Botstein,Roth Melting Temp C. 78.34

EXAMPLE 3. METHOD FOR MAKING THE C3APLT AND C3APS PROTEINS

C3APL (amino acid sequence: SEQ ID NO.: 4) and C3APLT (amino acid sequence; SEQ ID NO: 37) are the names given to the proteins encoded by cDNAs made by ligating the functional domain of C3 transferase and the homeobox region of the transcription factor called antenpedia (Bloch-Gallego (1993) 120: 485-492) in the following way. A cDNA encoding C3 (Dillon and Feig (1995) 256: 174-184) cloned in the plasmid vector pGEX-2T was used for the C3 portion of the chimeric protein. The stop codon at the 3' end of the DNA was replaced with an EcoR I site by polymerase chain reaction using the primers 5'GAA TTC TTT AGG ATT GAT AGC TGT GCC 3' (SEQ ID NO: 1) and 5'GGT GGC GAC CAT CCT CCA AAA 3' (SEQ ID NO: 2). The PCR product was sub-cloned into a pSTBlue-1 vector (Novagen, city), then cloned into a pGEX-4T vector using BamH I and Not I restriction site. This vector was called pGEX-4T/C3. The pGEX-4T vector has a 5' glutathione S transferase (GST) sequence for use in affinity purification. The antenpedia sequence used to add to the 3' end of C3 in pGEX-4T/C3 was created by PCR from the pET-3a vector (Bloch-Gallego (1993) 120: 485-492, Derossi (1994) 269: 10444-10450). The primers used were 5'GAA TCC CGC AAA CGC GCA AGG CAG 3' (SEQ ID NO: 7) and 5'TCA GTT CTC CTT CTT CCA CTT CAT GCG 3' (SEQ ID NO: 8). The PCR product obtained from the reaction was subcloned into a pSTBlue-1 blunt vector, then cloned into the pGEX-4T/C3, using the restriction sites EcoR I and Sal I, creating pGEX-4T/C3APL and C3APLT. C3APLT was selected for the presence of a frameshift mutation giving a transport region moiety rich in prolines.

A shorter version of the antennapedia (pGEX-4T/C3AP-short) (amino acid sequence of C3APS; SEQ ID NO.: 6) was also made. This chimeric sequence was made by ligating oligonucleotides encoding the short antennapedia peptide (Maizel (1999) 126: 3183-3190) into the pGEX-4T/C3 vector cut with EcoR I and Sal I. For pGEX-4T/C3AP-short the sequences of the oligos made were 5' AAT TCC GCC AGA TCA AGA TTT GGT TCC AGA ATC GTC GCA TGA AGT GGA AGA AGG 3' (SEQ ID NO: 9) and 5' GGC GGT CTA GTT CTA AAC CAA GCT CTT AGC AGC GTA GTT CAC CTT CTT CCA GCT 3' (SEQ ID NO: 10). The two strands were annealed together by mixing equal amounts of the oligonucleotides, heating at 72 °C for 5 minutes and then leaving them at room temperature for 15 minutes. The oligonucleotides were ligated into the pGEX4T/C3 vector and clones were picked and analyzed.

To prepare recombinant C3APLT (SEQ ID NO.: 37) and C3APS (SEQ ID NO.: 6) proteins, the plasmids containing the corresponding cDNAs (pGEX-4T/C3APLT and pGEX-4T/C3AP-short) were transformed into bacteria, strain XL-1 blue competent *E. coli*. The bacteria were grown in L-broth (10g/L Bacto-Tryptone, 5g/L Yeast Extract, 10g/L NaCl) with ampicillin at 50 ug/ml (BMC-Roche), in a shaking incubator for 1 hr at 37 °C and 300 rpm. Isopropyl β -D-thiogalactopyranoside (IPTG), (Gibco) was added to a final concentration of 0.5 mM to induce the production of recombinant protein and the culture was grown for a further 6 hours at 37 °C and 250 rpm. Bacteria pellets were obtained by centrifugation in 250 ml centrifuge bottles at 7000 rpm for 6 minutes at 4 °C. Each pellet was re-suspended in 10 ml of Buffer A (50mM Tris, pH 7.5, 50 mM NaCl, 5mM MgCl₂, 1mM DTT) plus 1mM PMSF. All re-suspended pellets were pooled and transferred to a 100 ml plastic beaker on ice. The remaining Buffer A with PMSF was added to the pooled sample. The bacteria sample was sonicated 6 x 20 seconds using a Branson Sonifier 450 probe sonicator. Both the bacteria and probe were cooled on ice 1 minute between sonications. The sonicate was centrifuged in a Sorvall SS-34 rotor at 16,000 rpm for 12 minutes at 4 °C to clarify the supernatant. The supernatant was transferred into fresh SS-34 tubes and re-spun at 12,000 rpm for 12 minutes at 4 °C. Up to 20 ml of Glutathione-agarose beads (Sigma) were added to the cleared lysate and placed on a rotating plate for 2-3 hours.

The beads were washed 4 times with buffer B, (Buffer A, NaCl is 150 mM, no PSMF) then 2 times with Buffer C (Buffer B + 2.5 mM CaCl_2). The final wash was poured out till the beads created a thick slurry. To remove the glutathione S transferase sequence from the recombinant protein, 20U of Thrombin (Bovine, Plasminogen-free, Calbiochem) was added, the beads were left on a rotator overnight at 4 °C. After cleavage with thrombin the beads were loaded into an empty 20 ml column. Approximately 20 aliquots of 1 ml were collected by elution with PBS. Samples of each aliquot of 0.5 ul were spotted on nitrocellulose and stained with Amido Black to determine the protein peak. Aliquots containing fusion proteins were pooled and 100 μl p-aminobenzamidine agarose beads (Sigma) were added and left mixing for 45 minutes at 4 °C. This last step removed the thrombin from the recombinant protein sample. The recombinant protein was centrifuged to remove the beads and then concentrated using a centrprep-10 concentrator (Amicon). The concentrated recombinant protein was desalted with a PD-10 column (Pharmacia, containing Sephadex* G-25M) and ten 0.5 ml aliquots were collected. A dot-blot was done on these samples to determine the protein peak, and the appropriate aliquots pooled, filter-sterilized, and stored at -80°C. A protein assay (DC assay, Biorad) was used to determine the concentration of recombinant protein. Purity of the sample was determined by SDS-PAGE, and bioactivity bioassay with PC-12 cells.

EXAMPLE 4: TESTING OF EFFICACY OF C3APLT AND C3APS IN TISSUE CULTURE

To test the ability of C3APLT and C3APS to overcome growth inhibition, PC-12 cells were plated on myelin, a growth inhibitory substrate. The myelin was purified from bovine brain (Norton and Poduslo (1973) 21: 749-757). In some other experiments chondroitin sulfate proteoglycan (CSPG) substrates were made from a purchased protein composition (Chemicon). Before coating coverslips or wells of a 96 well plate, they were coated with poly-L-lysine (0.025 $\mu\text{g}/\text{ml}$) (Sigma, St. Louis, MO), washed with water and allowed to dry. Myelin stored as a 1mg/ml solution at -80 °C was thawed at 37 °C, and vortexed. The myelin was plated at 8 $\mu\text{g}/\text{well}$ in a 8 well chamber Lab-Tek slides (Nuc, Naperville, IL). The myelin solution was left to dry overnight in a sterile tissue culture hood. The next morning the substrate was washed gently with phosphate buffered saline, and then cells in media were added to the substrate. PC-12 cells (Lehmann et al., 1999) were grown in DMEM with 10%

horse serum (HS) and 5% fetal bovine serum (FBS). Two days prior to use the PC-12 cells were differentiated by 50 ng/ml of nerve growth factor (NGF). After the cells were primed, 5ml of trypsin was added to the culture dish to detach the cells, the cells were pelleted and resuspended in 2 ml of DMEM with 1% HS and 50 ng/ml of nerve growth factor.

5 Approximately, 5000 to 7000 cells were then plated on 8 well chamber Lab-Tek slides (Nuc, Naperville, IL) coated myelin. The cells were placed on the test substrates at 37 °C for 3-4 hours to allow the cells to settle. The original media was carefully removed by aspiration, taking care not to disrupt the cells and replaced with DMEM with 1% HS, 50ng/ml of NGF and varying amounts of the C3, C3APLT, or C3APS, depending on the dose desired. After
10 two days, the cells were fixed (4% paraformaldehyde and 0.5% glutaraldehyde). For control experiments with unmodified C3, NGF primed PC-12 cells were trypsinized to detach them from the culture dish, the cells were washed once with scrape loading buffer (114 mM KCL, 15 mM NaCl, 5.5 mM MgCl₂, and 10 mM Tris-HCL) and then the cells were scraped with a rubber policeman into 0.5 ml of scraping buffer in the presence of 25 or 50 µg/ml of C3. The
15 cells were pelleted and resuspended in 2ml of DMEM, 1% HS and 50 ng/ml nerve growth factor before plating. At least four experiments were analyzed for each treatment. For each well, twelve images were collected with a 20X objective using a Zeiss Axiovert microscope. For each image, the numbers of cells with and without neurites were counted and the lengths of the neurites were determined. Since myelin is phase dense, cells plated on myelin
20 substrates were immuno-stained with anti-βIII tubulin antibody before analysis. Quantitative analysis of neurite outgrowth was with the aid of Northern Eclipse software (Empix Imaging, Mississauga, Ontario, Canada). Data analysis and statistics were with Microsoft Excel.

For a fast bioassay, the compounds were tested in tissue culture as described above, except
25 that the cells were plated on the tissue culture plastic rather than on inhibitory substrates. For these experiments the plates were fixed and the neurites counted five hours after plating the cells. The test compounds (C3APLT and C3Basic3) were able to promote faster growth on tissue culture plastic than cells plated without treatment (Figure 11).

To examine ADP ribosylation by C3, C3APLT, and C3APS, the compounds were added to PC-12 cell cultures, as described above. The cells were harvested by centrifugation, cell homogenates prepared and the proteins separated by SDS polyacrylamide gel electrophoresis. The proteins were then transferred to nitrocellulose and the Western blots probed with anti-
5 RhoA antibody (Santa Cruz).

EXAMPLE 5: TESTING ABILITY OF C3APLT AND C3APS TO OVERRIDE INHIBITION OF MULTIPLE GROWTH INHIBITORY PROTEINS.

Myelin substrates were made as described in Example 4 and plated on tissue culture chamber
10 slides. P1 to P3 rat pups were decapitated, the heads washed in ethanol and the eye removed and placed in a petri dish with Hanks buffered saline solution (HBSS, from Gibco). A hole was cut in the cornea, the lens removed, and the retina squeezed out. Typically, four retinas per preparation were used. The retinas were removed to a 15 ml tube and the volume brought to 7 ml. A further 7 ml of dissociation enzymes and papain were added. The dissociation
15 enzyme solution was made as follows: 30 mg DL cysteine was added to a 15 ml tube (Sigma DL cystein hydrochloride), and 70 ml HBSS, 280 ul of 10mg/ml bovine serum albumin were added and the solution mixed and pH adjusted to 7 with 0.3 N NaOH. The dissociate solution was filter-sterilized and kept frozen in 7 ml aliquots, and before use 12.5 units papain per ml (Worthington) was added. After adding the dissociation solution to the retina, the tube was
20 incubated for 30 minutes on a rocking tray at 37 °C. The retinas were then gently triturated, centrifuged and washed with HBSS. The HBSS was replaced with growth medium (DMEM (Gibco), 10 % fetal bovine serum, and 50 ng/ml brain derived neurotrophic factor (BDNF) vitamins, penicillin-streptomycin, in the presence or absence of C3APLT or C3APS. Cells were plated on test substrates of myelin or CSPG in chamber slides prepared as described in
25 Example 4, above. A quantitative analysis was completed as described for Example 4 above. Neurons were visualized by fluorescent microscopy with anti- β III tubulin antibody, which detects growing retinal ganglion cells (RGCs). Results are presented in Figure 6.

EXAMPLE 6. TREATMENT OF INJURED MOUSE SPINAL CORD WITH C3APLT AND MEASUREMENT OF RECOVERY OF MOTOR FUNCTION IN TREATED MICE.

Adult Balb-c mice were anaesthetized with 0.6 ml/kg hypnorm, 2.5 mg/kg diazepam and 35 mg/kg ketamine. This does gives about 30 minutes of anaesthetic, which is sufficient for the entire operation. A segment of the thoracic spinal column was exposed by removing the vertebrae and spinus process with microrongeurs (Fine Science Tools). A spinal cord lesion was then made dorsally, extending past the central canal with fine scissors, and the lesion was recut with a fine knife. This lesion renders all of the control animals paraplegic. The paravertebral muscle were closed with reabsorbable sutures, and the skin was closed with 2.0 silk sutures. After surgery, the bladder was manually voided every 8-10 hours until the animals regained control, typically 2-3 days. Food was placed in the cage for easy access, and sponge-water used for easy accessibility of water after surgery. Also, animals received subcutaneous injection Buprenorphine (0.05 à 0.1 mg/kg) every 8-12 hours for the first 3 days. Any animals that lost 15-20% of body weight were killed.

Rho antagonists (C3 or C3-like proteins) were delivered locally to the site of the lesion by a fibrin-based tissue adhesive delivery system (McKerracher, Canadian patent application No. 2,325,842). Recombinant C3APLT was mixed with fibrinogen and thrombin in the presence of CaCl_2 . Fibrinogen is cleaved by thrombin, and the resulting fibrin monomers polymerize into a three-dimensional matrix. We added C3APLT as part of a fibrin adhesive, which polymerized within about 10 seconds after being placed in the injured spinal cord. We tested C3APLT applied to the spinal cord lesion site after the lesion was made. For control we injected fibrin adhesive alone, or transected the cord without further treatment. For behavioural testing, the BBB scoring method was used to examine locomotion in an open field environment (Basso (1995) 12: 1-21). Results are presented in Figure 7. The environment was a rubber mat approximately 4' X 3' in size, and animals were placed on the mat and videotaped for about 4 minutes. Care was taken not to stimulate the peroneal region or touch the animals excessively during the taping session. The video tapes were digitized and observed by two observers to assign BBB scores. The BBB score, modified for mice, was as follows:

Score Description

- | | | |
|----|----|--|
| | 1 | No observable hindlimb (HL) movement. |
| | 2 | Slight movement of one or two joints. |
| | 3 | Extensive movement of one joint and /or slight movement of one other joint.. |
| 5 | 4 | Extensive movement of two joints. |
| | 5 | Slight movement of all three joints of the HL. |
| | 6 | Slight movement of two joints and extensive movement of the third. |
| | 7 | Extensive movement of two joints and slight movement of the third. |
| | 8 | Extensive movement of all three joints of the HL walking with no weight support. |
| 10 | 9 | Extensive movement of all three joints, walking with weight support. |
| | 10 | Frequent to consistent dorsal stepping with weight support. |
| | 11 | Frequent plantar stepping with weight support. |
| | 12 | Consistent plantar stepping with weight support, no coordination. |
| 15 | 13 | Consistent plantar stepping with consistent weight support, occasional FL-HL coordination. |
| | 14 | Consistent plantar stepping with consistent weight support, frequent FL-HL coordination. |
| 20 | 15 | Consistent plantar stepping with consistent weight support, consistent FL-HL coordination; predominant paw position during locomotion is rotated internally or externally, or consistent FL-HL coordination with occasional dorsal stepping. |
| | 16 | Consistent plantar stepping with consistent weight support, consistent FL-HL coordination; predominant paw position is parallel to the body; frequent to consistent toe drag, or curled toes, trunk instability. |
| 25 | 17 | Consistent plantar stepping with consistent weight support, consistent FL-HL coordination; predominant paw position is parallel to the body, no toe drag, some trunk instability. |
| 30 | 18 | Consistent plantar stepping with consistent weight support, consistent FL-HL coordination; predominant paw position is parallel to the body, no toe drag and consistent stability in the locomotion. |

EXAMPLE . 7 TREATMENT OF INJURED MOUSE SPINAL CORD WITH C3APLT AND ASSESSMENT OF ANATOMICAL RECOVERY.

Mice that received a spinal cord injury and treated as controls or with C3APLT, as described for Example 6 were assessed for morphological changes to the scar and for axon regeneration. To study axon regeneration, the corticospinal axons were identified by anterograde labeling. For anterograde labeling studies, the animals were anaesthetized as above, and the cranium over the motor cortex was removed. With the fine glass micropipetter (about 100 um in diameter) the cerebral cortex was injected with 2-4 ul of horse radish peroxidase conjugated to wheat germ agglutinin (2%), a marker that is taken up by nerve cells and transported anterogradely into the axon that extends into the spinal cord. After injection of the anterograde tracer, the cranium was replaced, and the skin closed with 5-0 silk sutures. The animals were sacrificed with chloral hydrate (4.9 mg/10 g) after 48 hours, and perfused with 4% paraformaldehyde in phosphate buffer as a fixative. The spinal cord was removed, cryoprotected with sucrose and cryostat sections placed on slides for histological examination.

EXAMPLE 8. DNA AND PROTEIN SEQUENCE DETAILS OF C3-TL.

The Tat coding sequence was obtained by polymerase chain reaction of the plasmid SVCMV-TAT (obtained from Dr. Eric Cohen, Universite de Montreal) that contains the entire Tat coding sequence. To isolate the transport sequence of the Tat protein, PCR was used. The first primer (5'GAATCCAAGCACCAAGGAGTCAGCC 3' (SEQ ID NO.: 11)) and the second primer (5' ACC AGCCACCACCTTCTGATA 3' (SEQ ID NO.: 12)) used corresponded to amino acids 27 to 72 of the HIV Tat protein. Upon verification and purification, the PCR product was sub cloned into a pSTBlue-1 blunt vector. This transport segment of the Tat protein was then cloned into pGEX-4T/C3 at the 3' end of C3, using the restriction sites EcoR I and Sac I. The new C3-Tat fusion protein was called C3-TL. Recombinant protein was made as described in Example 3.

DNA sequence of C3-TL (SEQ ID NO.: 13)

5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
 CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
 5 GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
 TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
 AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
 AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
 AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
 10 TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
 AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
 AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA
 TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA
 AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
 15 ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
 TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
 GCT ATC AAT CCT AAA GAA TTC AAG CAT CCA GGA AGT CAG CCT AAA ACT
 GCT TGT ACC AAT TGC TAT TGT AAA AAG TGT TGC TTT CAT TGC CAA GTT
 TGT TTC ATA ACA AAA GCC TTA GGC ATC TCC TAT GGC AGG AAG CGG AGA
 20 CAG CGA CGA AGA GCT CAT CAG AAC AGT CAG ACT CAT CAA GCT TCT CTA
 TCA AAG CAG TAA 3'

The TL transport peptide sequence by itself is as follows:(SEQ ID NO.: 46)

25 KHPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKRRQRRRAHQNSQTHQAS
 LSKQ.

The protein sequence of C3-TL (SEQ ID NO.: 14)

30 GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
 YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKT PENIMLFRGDDPAYL
 GTEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITQFK

VAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPK
EFKHPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKRRQRRRAHQNSQTHQ
ASLSKQ.

5 Molecular Weight 32721.40 Daltons

291 Amino Acids

43 Strongly Basic(+) Amino Acids (K,R)

21 Strongly Acidic(-) Amino Acids (D,E)

82 Hydrophobic Amino Acids (A,I,L,F,W,V)

10 104 Polar Amino Acids (N,C,Q,S,T,Y)

9.688 Isoelectric Point

22.655 Charge at PH 7.0

Total number of bases translated is 876

15 % A = 37.44 [328]

% G = 17.58 [154]

% T = 28.31 [248]

% C = 16.67 [146]

20

EXAMPLE 9. DNA AND PROTEIN SEQUENCE DETAILS OF C3-TS.

A shorter Tat construct was also made (C3-TS). To make the shorter C3 Tat fusion protein the following oligonucleotides were 5' AAT TCT ATG GTC GTA AAA AAC GTC GTC AAC GTC GTC GTG 3' (SEQ ID NO.: 15) and 5' GAT ACC AGC ATT TTT TGC AGC AGT TGC AGC AGC ACA GCT 3' (SEQ ID NO.: 16). The two oligonucleotide strands were annealed together by combining equal amounts of the oligonucleotides, heating at 72 °C for 5 minutes and then letting the oligonucleotide solution cool at room temperature for 15 minutes. The oligonucleotides were ligated into the pGEX4T/C3 vector at the 3' end of C3. The construct was sequenced. All plasmids were transformed into XL-1 blue competent cells. Recombinant protein was made as described in Example 3.

Nucleotide sequence of C3-TS (SEQ ID NO.: 17)

5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
 CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
 GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
 5 TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
 AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
 AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
 AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
 TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
 10 AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
 AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA
 TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA
 AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
 ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
 15 TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
 GCT ATC AAT CCT AAA GAA TTC TAT GGT GCT AAA AAA CGT CGT CAA CGT
 CGT CGT GTC GAC TCG AGC GGC CCG CAT CGT GAC TGA 3'

20 The TS transport peptide sequence by itself is as follows: (SEQ ID NO.: 47)

YGAKKRRQRRRVDSSGPHRD

The protein sequence of C3-TS (SEQ ID NO.: 18)

25 GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
 YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKT PENIMLFRGDDPAYL
 GTEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITQFK
 VAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPK
 EFYGAKKRRQRRRVDSSGPHRD

30

Molecular Weight 26866.62 Daltons

238 Amino Acids

36 Strongly Basic(+) Amino Acids (K,R)

21 Strongly Acidic(-) Amino Acids (D,E)

71 Hydrophobic Amino Acids (A,I,L,F,W,V)

5 78 Polar Amino Acids (N,C,Q,S,T,Y)

9.802 Isoelectric Point

15.212 Charge at PH 7.0

10 Total number of bases translated is 717

% A = 38.91 [279]

% G = 17.43 [125]

% T = 28.45 [204]

% C = 15.20 [109]

15

EXAMPLE 10.

The following example illustrates how a coding sequence can be modified without affecting the efficacy of the translated protein. The example shows modifications to C3Basic3 that would not affect the activity. Sequences may include the entire GST sequence, as shown here that includes the start site, which would not be removed enzymatically. Also, the transport sequence shown in this example has changes in amino acid composition surrounding the active sequence due to a difference in the cloning strategy, and the His tag has been omitted. However, the active region is : R R K Q R R K R R. This sequence is contained in the C3Basic3, and is the active transport sequence in the sequence below. Also note that the C-terminal region of the protein after this active region differs from C3Basic3. That is because the cloning strategy was changed, the restriction sites differ, and therefore non-essential amino acids 3' terminal to the transport sequence are transplanted and included in the protein.

Nucleic acid sequence: (SEQ ID NO.: 19)

1413 base pairs

single strand

linear sequence

5' ATG TCC CCT ATA CTA GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC
 ACT CGA CTT CTT TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG
 TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG
 GGT TTG GAG TTT CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA
 TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC AAC
 ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA
 GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA TAT AGT
 AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG CTA CCT GAA
 ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA ACA TAT TTA AAT
 GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT
 GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA
 GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA CAA ATT GAT AAG TAC

TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC
 ACG TTT GGT GGT GGC GAC CAT CCT CCA AAA TCG GAT CTG GTT CCG CGT
 GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
 CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
 5 GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
 TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
 AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
 AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
 AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
 10 TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
 AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
 AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA
 TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA
 AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
 15 ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
 TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
 GCT ATC AAT CCT AAA GAA TTC AGA AGG AAA CAA AGA AGA A AA AGA
 AGA CTG CAG GCG GCC GCA TCG TGA 3'

20

Amino acid sequence (SEQ ID NO: 20)

479 amino acids

linear, single strand

25

30

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPY
 YIDGDVKLTQSMAIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSR IAYSKDF
 ETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCL
 DAFPKLVCFKKRIEAI PQIDKYLKSSKYIAWPLQG WQATFGGGDHPPKSDLVPRGSSR
 VDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVSYTKS
 ASEINGKLRQNKG VINGFPSNLIKQVELLDKSFNKMKT PENIMLFRGDDPAYLGTEFQ
 NTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITKFKVAKGS

KAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFRRK
QRRKRRLQAAAS.

Molecular Weight 53813.02 Daltons

5 470 Amino Acids
 68 Strongly Basic(+) Amino Acids (K,R)
 55 Strongly Acidic(-) Amino Acids (D,E)
 149 Hydrophobic Amino Acids (A,I,L,F,W,V)
 121 Polar Amino Acids (N,C,Q,S,T,Y)

10 9.137 Isoelectric Point
 14.106 Charge at PH 7.0

Total number of bases translated is 1413

15 % A = 34.61 [489]
 % G = 19.75 [279]
 % T = 29.51 [417]
 % C = 15.99 [226]
 % Ambiguous = 0.14 [2]

20 % A+T = 64.12 [906]
 % C+G = 35.74 [505]

25 Davis,Botstein,Roth Melting Temp C. 79.20

**EXAMPLE 11. ADDITIONAL CHIMERIC C3 PROTEINS THAT WOULD BE EFFECTIVE TO
STIMULATE REPAIR IN THE CNS.**

30 The following sequences could be added to the amino terminal or carboxy terminal of C3 or a
truncated C3 that retains its enzymatic activity.

- 1) Sequences of polyarginine as described (Wender, et al. (2000) 97: 13003-8.). These could be from 6 to 9 or more arginines.
- 2) Sequences of poly-lysine
- 3) Sequences of poly-histidine
- 4) Sequences of arginine and lysine mixed.
- 5) Basic stretches of amino acids containing non-basic amino acids stretch where the sequence added retains transport characteristics.
- 6) Sequences of 5- 15 amino acids containing at least 50 % basic amino acids
- 7) Sequences longer than 15 –30 amino acids containing at least 30 % basic amino acids.
- 8) Sequences longer than 50 amino acids containing at least 18 % basic amino acids.
- 9) Any of the above where the amino acids are chemically modified, such as by addition of cyclohexyl side chains, other side chains, different alkyl spacers.
- 10) Sequences that have proline residues with helix-breaking propensity to act as effective transporters.

EXAMPLE 12. ADDITIONAL CHIMERIC C3 PROTEINS THAT WOULD BE EFFECTIVE TO STIMULATE REPAIR IN THE CNS.

C3Basic1: C3 fused to a randomly designed basic tail

C3Basic2: C3 fused to a randomly designed basic tail

C3 Basic3: C3 fused to the reverse Tat sequence

We have designed the following DNA encoding a chimeric C3 with membrane transport properties. The protein is designated C3Basic1. This sequence was designed with C3 fused to a random basic sequence. The construct was made to encode the peptide given below.

K R R R R R P K K R R R A K R R (SEQ ID NO.: 21)

The construct was made by synthesizing the two oligonucleotides given below, annealing them together, and ligating them into the pGEX-4T/C3 vector with an added histidine tag.

5' AAG AGA AGG CGA AGA AGA CCT AAG AAG AGA CGA AGG GCG AAG
AGG AGA 3' (SEQ ID NO.: 22)

5' TTC TCT TCC GCT TCT TCT GGA TTC TTC TCT GCT TCC CGC TTC
5 TCC TCT 3' (SEQ ID NO.: 23)

DNA sequence of C3Basic1 (SEQ ID NO: 24)

10 5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
15 AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA
20 TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA
AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
GCT ATC AAT CCT AAA GAA TTC AAG AGA AGG CGA AGA AGA CCT AAG
25 AAG AGA CGA AGG GCG AAG AGG AGA CAC CAC CAC CAC CAC CAC GTC
GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA 3'

Protein sequence of C3Basic1 (SEQ ID NO: 25)

30 GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
YTKSASEINGKLRQNKG VINGFPSNLIKQVELLDKSFNKMKTPENIMLFRGDDPAYL
GTEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITKFK

VAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIITATMMGTAINPK
EFKRRRRRPPKKRRRAKRRHHHHHHHVDSSGRIVTD.

5 Molecular Weight 29897.03 Daltons
263 Amino Acids
44 Strongly Basic(+) Amino Acids (K,R)
23 Strongly Acidic(-) Amino Acids (D,E)
75 Hydrophobic Amino Acids (A,I,L,F,W,V)
10 79 Polar Amino Acids (N,C,Q,S,T,Y)

10.024 Isoelectric Point
22.209 Charge at PH 7.0
Davis,Botstein,Roth Melting Temp C. 78.56

15

**EXAMPLE 13. ADDITIONAL CHIMERIC C3 PROTEIN THAT WOULD BE EFFECTIVE TO
STIMULATE REPAIR IN THE CNS.**

We have designed the following DNA encoding a chimeric C3 with membrane transport
20 properties. The protein is designated C3Basic2. This sequence was designed with C3 fused
to a random basic sequence. The construct was made to encode the peptide given below.

K R R R R K K R R Q R R R (SEQ ID NO.: 26)

25 The construct was made by synthesizing the two oligonucleotides given below, annealing
them together, and ligating them into the pGEX4T/C3 vector with an added histidine tag.

5' AAG CGT CGA CGT AGA AAG AAA CGT AGA CAG CGT AGA CGT 3' (SEQ
ID NO.: 27)

30

5' TTC GCA GCT GCA TCT TTC TTT GCA TCT GTC GCA TCT GCA 3'
(SEQ ID NO.: 28)

DNA sequence of C3Basic2 (SEQ ID NO.: 29)

5
 5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
 CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
 GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
 TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
 10 AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
 AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
 AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
 TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
 AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
 15 AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA
 TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA
 AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
 ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
 TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
 20 GCT ATC AAT CCT AAA GAA TTC AAG CGT CGA CGT AGA AAG AAA CGT
 AGA CAG CGT AGA CGT CAC CAC CAC CAC CAC CAC GTC GAC TCG AGC
 GGC CGC ATC GTG ACT GAC TGA 3'

25 Protein sequence of C3Basic2 (SEQ ID NO.: 30)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
 YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKT PENIMLFRGDDPAYL
 GTEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITKFK
 30 VAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPK
 EFKRRRRKKRRQRRRHHHHHHVDSSGRIVTD.

Molecular Weight 29572.61 Daltons

260 Amino Acids

42 Strongly Basic(+) Amino Acids (K,R)

23 Strongly Acidic(-) Amino Acids (D,E)

74 Hydrophobic Amino Acids (A,I,L,F,W,V)

5 80 Polar Amino Acids (N,C,Q,S,T,Y)

9.956 Isoelectric Point

20.210 Charge at PH 7.0

Davis,Botstein,Roth Melting Temp C. 78.45

10

EXAMPLE 14. ADDITIONAL CHIMERIC C3 PROTEIN THAT WOULD BE EFFECTIVE TO STIMULATE REPAIR IN THE CNS.

15 We have designed the following DNA encoding a chimeric C3 with membrane transport properties. The protein is designated C3Basic3. This sequence was designed with C3 fused to a reverse Tat sequence. The construct was made to encode the peptide given below

20 R R K Q R R K R R (SEQ ID NO.: 31)

The construct was made by synthesizing the two oligonucleotides given below, annealing them together, and ligating them into the pGEX4T/C3 vector with an added histidine tag,
25 then subcloning inot pGEX-4T/C3.

5' AGA AGG AAA CAA AGA AGA AAA AGA AGA 3' (SEQ ID NO.: 32)

30 5' TCT TCC TTT GTT TCT TCT TTT TCT TCT 3' (SEQ ID NO.: 33)

DNA sequence of C3Basic3 (SEQ ID NO.: 34)

5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
 CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
 5 GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
 TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
 AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
 AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
 AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
 10 TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
 AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
 AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA
 TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA
 AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
 15 ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
 TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
 GCT ATC AAT CCT AAA GAA TTC AGA AGG AAA CAA AGA AGA AAA AGA
 AGA CAC CAC CAC CAC CAC CAC GTC GAC TCG AGC GGC CGC ATC GTG ACT
 GAC TGA 3'

20

Protein sequence of C3Basic3 (SEQ ID NO.: 35)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
 25 YTKSASEINGKLRQNKG VINGFPSNLIKQVELLDKSFNKMKT PENIMLFRGDDPAYL
 GTEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITKFK
 VAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIITATMMGTAINPK
 EFRRKQRRKRRHHHHHHVDSSGRIVTD.

30 Molecular Weight 29441.47 Daltons

260 Amino Acids

39 Strongly Basic(+) Amino Acids (K,R)

23 Strongly Acidic(-) Amino Acids (D,E)

76 Hydrophobic Amino Acids (A,I,L,F,W,V)

80 Polar Amino Acids (N,C,Q,S,T,Y)

9.833 Isoelectric Point

5 17.211 Charge at PH 7.0

Davis,Botstein,Roth Melting Temp C. 78.29

10 **EXAMPLE 15. SEQUENCES FOR C3APLT**

One of the clones that was selected from the subcloning of C3APL into pGEX encoded a protein that was not the expected size but had good biological activity. This clone that had a frameshift mutation leading to a tuncation, and this clone was called C3APLT. The clone was resequenced and the chromatograms analyzed to confirm the sequence. To confirm the
15 sequences of C3APLT, the coding sequence from both strands of pGEX-4T/C3APLT were sequenced by double strand sequencing of the full length of the clone (BioS&T, Montreal, Quebec).

The DNA sequence for C3APLT is as follows: (SEQ ID NO.: 36)

20
GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA AAAGGCTTAT 60
TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA AAGCTTGGGG TAATGCTCAG 120
TATAAAAAGT ATGGACTAAG CAAATCAGAA AAAGAAGCTA TAGTATCATA TACTAAAAGC 180
GCTAGTGAAA TAAATGGAAA GCTAAGACAA AATAAGGGAG TTATCAATGG ATTTCTTCA 240
25 AATTTAATAA AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA 300
AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT TCAAAACACT 360
CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG AAAAGGCTAA AGCTAAGTTT 420
TTAAATAAAG ATAGACTTGA ATATGGATAT ATTAGTACTT CATTAATGAA TGTTTCTCAA 480
TTTGCAGGAA GACCAATTAT TACAAAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT 540
30 ATTGACCCTA TTAGTGCTTT TGCAGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT 600
TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT TACAGCAACA 660
ATGATGGGCA CAGCTATCAA TCCTAAAGAA TTCGTGATGA ATCCCGCAAA CGCGCAAGGC 720
AGACATACAC CCGGTACCAG ACTCTAGAGC TAGAGAAGGA GTTTCCTTC AATCGCTACT 780
TGACCCGTCG GCGAAGGATC GAGATCGCCC ACGCCCTGTG CCTCACGGAG CGCCAGATAA 840
35 AGATTTGGTT CCAGAATCGG CGCATGAAGT GGAAGAAGGA GAACTGA 887

The APLT transport peptide sequence by itself is as follows (SEQ ID NO.: 48):

VMNPANAQGRHTPGTRL

The protein sequence for C3APLT is as follows: (SEQ ID NO.: 37)

5

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
YTKSASEINGKLRQNKG VINGFPSNLIKQVELLDKSFNKMKT PENIMLFRGDDPAYL
GTEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITKFK
VAKGSKAGYIDPISAFAGQLEMLLPRHSTYHIDDMRLSSDGKQIITATMMGTAINPK
10 EFVMNPANAQGRHTPGTRL

Molecular Weight 27574.42 Daltons

248 Amino Acids

15

33 Strongly Basic(+) Amino Acids (K,R)

21 Strongly Acidic(-) Amino Acids (D,E)

76 Hydrophobic Amino Acids (A,I,L,F,W,V)

80 Polar Amino Acids (N,C,Q,S,T,Y)

20

9.636 Isoelectric Point

12.379 Charge at PH 7.0

EXAMPLE 16. SUBCLONING AND SEQUENCES FOR C3APLT IN PET

25 C3 has been reported to be stably expressed in *E. coli* by both pGEX- series and pET-series
vectors (e.g., Dillon and Feig, 1995 Meth. Enzymol. 256: 174-184. Small GTPases and Their
Regulators. Part B. Rho Family. W.E. Balch, C.J. Der, and A. Hall, eds.
; Lehmann et al., 1999 supra; Han et al., 2001. J. Mol. Biol. 395: 95-107). The fusion
proteins were expressed well in the pGEX vector, for synthesis and testing. However, for
30 large-scale production it is more efficient to synthesize recombinant proteins without an
affinity tag that increases the size of the protein produced. Also, it is more economical to
synthesize proteins in large scale by affinity chromatography using automated FPLC systems.

The polymerase chain reaction was used to transfer recombinant construct C3APLT into the pET T7 polymerase based system *E. coli* expression system (reviewed by Studier et al., 1990. Meth. Enzymol. 185: 60-89. Gene Expression Technology. D.V. Goeddel, ed.). A similar PCR approach is suitable for others in the fusion protein series of C3-based constructs with transport sequences. The pET3a vector DNA was obtained from Dr. Jerry Pelletier, McGill University. PCR primers were obtained from Invitrogen. The upper (5') primer was 5'-GGA TCT GGT TCC GCG TCA TAT GTC TAG AGT CGA CCT G-3' (37 b) (SEQ ID NO.:38). Underlined is the Nde I site that was introduced into the primer to replace the *BamHI* site in pGEX4T-C3APLT. The lower primer was 5'-CGC GGA TCC *ATT* AGT TCT CCT TCT TCC ACT TC-3' (32 b) (SEQ ID NO.:39). This primer introduced two changes in the coding strand DNA of pGEX4T-C3APLT, replacing the EcoRI site from pGEX4T- C3APLT with a BamH I site (underlined) and replacing a TGA stop codon with the strong stop sequence TAAT (the italicized ATTA sequence in the complementary primer). Compared to pGEX4T-C3APLT, the predicted N-terminal sequence of pET3a- C3APLT is Met-Ser rather than Gly-Ser-Ser, a loss of one serine and a substitution of Met for Gly. There were no changes in amino acid sequence at the C-terminus of C3APLT.

The target C3APLT gene was amplified using *Pfu* polymerase (Invitrogen/Canadian Life Technologies) with buffer, DNA and deoxyribonucleotide concentrations recommended by the manufacturer. The PCR was carried out as follows : 95 °C for 5 minutes, 10 cycles of 94 °C for 2 minutes followed by 56 °C for 2 minutes then extension at 70 °C for 2 minutes, then 30 cycles of 94 °C for 2 minutes followed by 70 °C. Completed reactions were stored at 4 °C. The QIAEXII kit (Qiagen) was used to purify the agarose gel slice containing DNA band. The purified PCR product DNA and the vector were digested with BamH I and Nde I (both obtained from New England BioLabs) following the instructions of the manufacturer. The digestion products were separated from extraneous DNA by agarose gel electrophoresis and purified with the QIAEXII kit. . The insert and vector DNA were incubated together overnight at 16 °C with T4 DNA ligase according to directions provided by the manufacturer (New England BioLabs). Competent *E. coli* (DH5α, obtained from Invitrogen/Canadian Life Technologies) were transformed with the ligation mixture.

DNA was prepared from purified colonies using the Qiagen plasmid midi kit, and the entire insert and junction sequences were verified by double strand sequencing of the full length of

the clone (BioS&T, Montreal, Quebec) with forward primer 5' AAA TTA ATA CGA CTC
ACT ATA GGG 3' (24 bases) (SEQ ID NO.: 40) and reverse T7 terminator sequencing
primer 5' GCT AGT TAT TGC TCA GCG G 3' (19 bases) (SEQ ID NO.: 41). The sequence
of the C3APLT cDNA in pET is given in SEQ ID NO.: 42. The amino acid sequence is
5 given in SEQ. ID NO.: 43.

EXAMPLE 17: MODIFICATIONS OF SEQUENCES.

Any of sequences given in Examples 1, 2, 8, 9, 10, 11, 12 and 13, 15 and 16 could be
10 modified to retain C3 enzymatic activity and effective transport sequences. For example
amino acids encoded from DNA at the 3' end of the sequence that represents the translation of
the restriction sites used in cloning may be removed without affecting activity. Some of the
amino terminal amino acids may also be removed without affecting activity. The minimal
amount of sequence needed for biological activity of the C3 portion of the fusion protein is
15 not known but could be easily determined by known techniques. For example, increasingly
more of the 5' end of the cDNA encoding C3 could be removed, and the resulting proteins
made and tested for biological activity. Similarly, increasing amounts of the 3' end could be
removed and the fragments tested for biological activity. Next, fragments testing the central
region could be tested for retention of C3 activity. Therefore, the C3 portion of the protein
20 could be truncated to include just the amino acids needed for activity. Alternatively
mutations could be made in the coding regions of C3, and the resulting proteins tested for
activity. The transport sequences could be modified to add or remove one or more amino
acids or to completely change the transport peptide, but retain the transport characteristics in
terms of effective dose compared to C3 in our tissue culture bioassay (Example 4). New
25 transport sequences could be tested for biological activity to improve the efficiency of C3
activity by plating neurons and testing them on inhibitory substrates, as described in Example
4.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT: LISA MCKERRACHER
(ii) TITLE OF INVENTION: FUSION PROTEINS
(iii) NUMBER OF SEQUENCES: 48
(iv) CORRESPONDENCE ADDRESS:
 (A) ADDRESSEE: BROULLETTE KOSIE
10 (B) STREET: 1100 RENE-LEVESQUE BLVD WEST
 (C) PROV/STATE: QUEBEC
 (D) COUNTRY: CANADA
 (E) POSTAL/ZIP CODE: H3B 5C9
(v) COMPUTER READABLE FORM:
15 (A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: ASCII (TEXT)
(vi) CURRENT APPLICATION DATA:
20 (A) APPLICATION NUMBER:
 (B) FILING DATE:
 (C) CLASSIFICATION:
(vii) PRIOR APPLICATION DATA
 (A) APPLICATION NUMBER: 2,342,970
25 (B) FILING DATE: 2001-04-12
 (C) CLASSIFICATION:
(vii) PRIOR APPLICATION DATA
 (A) APPLICATION NUMBER: 2,362,004
 (B) FILING DATE: 2001-11-13
30 (C) CLASSIFICATION:
(viii) ATTORNEY/AGENT INFORMATION:
 (A) NAME: BROULLETTE KOSIE
 (B) REGISTRATION NO.: 3939
 (C) REFERENCE/DOCKET NO.: 06447-004-CA-02
35 (D) TEL. NO.: (514) 397 8500
 (E) FAX NO.: (514) 397 8515

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
40 (A) LENGTH: 27
 (B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

5

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

10

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

15

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

20

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:1

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GAATTCTTTA GGATTGATAG CTGTGCC

27

30

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

35

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

40

GGTGGCGACC ATCCTCCAAA A

21

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 888
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: DOUBLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

10 (vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA AAAGGCTTAT 60
 15 TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA AAGCTTGGGG TAATGCTCAG 120
 TATAAAAAGT ATGGACTAAG CAAATCAGAA AAAGAAGCTA TAGTATCATA TACTAAAAGC 180
 GCTAGTGAAA TAAATGGAAA GCTAAGACAA AATAAGGGAG TTATCAATGG ATTTTCCTTCA 240
 AATTTAATAA AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA 300
 AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT TCAAAACACT 360
 20 CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG AAAAGGCTAA AGCTAAGTTT 420
 TTAAATAAAG ATAGACTTGA ATATGGATAT ATTAGTACTT CATTAATGAA TGTCTCTCAA 480
 TTTGCAGGAA GACCAATTAT TACACAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT 540
 ATTGACCCTA TTAGTGCTTT TCAGGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT 600
 TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT TACAGCAACA 660
 25 ATGATGGGCA CAGCTATCAA TCCTAAAGAA TTCGTGATGG AATCCCGCAA ACGCGCAAGG 720
 CAGACATACA CCCGGTACCA GACTCTAGAG CTAGAGAAGG AGTTTCACTT CAATCGCTAC 780
 TTGACCCGTC GCGGAAGGAT CGAGATCGCC CACGCCCTGT GCCTCACGGA GCGCCAGATA 840
 AAGATTTGGT TCCAGAATCG GCGCATGAAG TGGAAGAAGG AGAACTGA 888

30

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 295
 (B) TYPE: AMINO ACIDS
 (C) STRANDEDNESS SINGLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(v) FRAGMENT TYPE:

40 (vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

	Gly	Ser	Ser	Arg	Val	Asp	Leu	Gln	Ala	Cys	Asn	Ala	Tyr	Ser	Ile
5	1				5					10					15
	Asn	Gln	Lys	Ala	Tyr	Ser	Asn	Thr	Tyr	Gln	Glu	Phe	Thr	Asn	Ile
					20					25					30
	Asp	Gln	Ala	Lys	Ala	Trp	Gly	Asn	Ala	Gln	Tyr	Lys	Lys	Tyr	Gly
					35					40					45
10	Leu	Ser	Lys	Ser	Glu	Lys	Glu	Ala	Ile	Val	Ser	Tyr	Thr	Lys	Ser
					50					55					60
	Ala	Ser	Glu	Ile	Asn	Gly	Lys	Leu	Arg	Gln	Asn	Lys	Gly	Val	Ile
					65					70					75
	Asn	Gly	Phe	Pro	Ser	Asn	Leu	Ile	Lys	Gln	Val	Glu	Leu	Leu	Asp
15					80					85					90
	Lys	Ser	Phe	Asn	Lys	Met	Lys	Thr	Pro	Glu	Asn	Ile	Met	Leu	Phe
					95					100					105
	Arg	Gly	Asp	Asp	Pro	Ala	Tyr	Leu	Gly	Thr	Glu	Phe	Gln	Asn	Thr
					110					115					120
20	Leu	Leu	Asn	Ser	Asn	Gly	Thr	Ile	Asn	Lys	Thr	Ala	Phe	Glu	Lys
					125					130					135
	Ala	Lys	Ala	Lys	Phe	Leu	Asn	Lys	Asp	Arg	Leu	Glu	Tyr	Gly	Tyr
					140					145					150
	Ile	Ser	Thr	Ser	Leu	Met	Asn	Val	Ser	Gln	Phe	Ala	Gly	Arg	Pro
25					155					160					165
	Ile	Ile	Thr	Gln	Phe	Lys	Val	Ala	Lys	Gly	Ser	Lys	Ala	Gly	Tyr
					170					175					180
	Ile	Asp	Pro	Ile	Ser	Ala	Phe	Gln	Gly	Gln	Leu	Glu	Met	Leu	Leu
					185					190					195
30	Pro	Arg	His	Ser	Thr	Tyr	His	Ile	Asp	Asp	Met	Arg	Leu	Ser	Ser
					200					205					210
	Asp	Gly	Lys	Gln	Ile	Ile	Ile	Thr	Ala	Thr	Met	Met	Gly	Thr	Ala
					215					220					225
	Ile	Asn	Pro	Lys	Glu	Phe	Val	Met	Glu	Ser	Arg	Lys	Arg	Ala	Arg
35					230					235					240
	Gln	Thr	Tyr	Thr	Arg	Tyr	Gln	Thr	Leu	Glu	Leu	Glu	Lys	Glu	Phe
					245					250					255
	His	Phe	Asn	Arg	Tyr	Leu	Thr	Arg	Arg	Arg	Arg	Ile	Glu	Ile	Ala
					260					265					270
40	His	Ala	Leu	Cys	Leu	Thr	Glu	Arg	Gln	Ile	Lys	Ile	Trp	Phe	Gln
					275					280					285

Asn Arg Arg Met Lys Trp Lys Lys Glu Asn

290

295

5 (2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 774

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

10 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

15

```

GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA AAAGGCTTAT 60
TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA AAGCTTGGGG TAATGCTCAG 120
TATAAAAAGT ATGGACTAAG CAAATCAGAA AAAGAAGCTA TAGTATCATA TACTAAAAGC 180
GCTAGTGAAA TAAATGGAAA GCTAAGACAA AATAAGGGAG TTATCAATGG ATTCCTTCA 240
20 AATTTAATAA AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA 300
AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT TCAAAACACT 360
CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG AAAAGGCTAA AGCTAAGTTT 420
TTAAATAAAG ATAGACTTGA ATATGGATAT ATTAGTACTT CATTAATGAA TGTCTCTCAA 480
TTTGCAGGAA GACCAATTAT TACACAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT 540
25 ATTGACCCTA TTAGTGCTTT TCAGGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT 600
TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT TACAGCAACA 660
ATGATGGGCA CAGCTATCAA TCCTAAAGAA TTCCGCCAGA TCAAGATTTG GTTCCAGAAT 720
CGTCGCATGA AGTGGAAGAA GGTGCGACTCG AGCGGCCGCA TCGTGACTGA CTGA 774

```

30

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 257

(B) TYPE: AMINO ACID

35 (C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

40

(vii) IMMEDIATE SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6

	Gly	Ser	Ser	Arg	Val	Asp	Leu	Gln	Ala	Cys	Asn	Ala	Tyr	Ser	Ile	
	1				5					10					15	
5	Asn	Gln	Lys	Ala	Tyr	Ser	Asn	Thr	Tyr	Gln	Glu	Phe	Thr	Asn	Ile	
				20						25					30	
	Asp	Gln	Ala	Lys	Ala	Trp	Gly	Asn	Ala	Gln	Tyr	Lys	Lys	Tyr	Gly	
				35						40					45	
	Leu	Ser	Lys	Ser	Glu	Lys	Glu	Ala	Ile	Val	Ser	Tyr	Thr	Lys	Ser	
10				50						55					60	
	Ala	Ser	Glu	Ile	Asn	Gly	Lys	Leu	Arg	Gln	Asn	Lys	Gly	Val	Ile	
				65						70					75	
	Asn	Gly	Phe	Pro	Ser	Asn	Leu	Ile	Lys	Gln	Val	Glu	Leu	Leu	Asp	
				80						85					90	
15	Lys	Ser	Phe	Asn	Lys	Met	Lys	Thr	Pro	Glu	Asn	Ile	Met	Leu	Phe	
				95						100					105	
	Arg	Gly	Asp	Asp	Pro	Ala	Tyr	Leu	Gly	Thr	Glu	Phe	Gln	Asn	Thr	
				110						115					120	
	Leu	Leu	Asn	Ser	Asn	Gly	Thr	Ile	Asn	Lys	Thr	Ala	Phe	Glu	Lys	
20				125						130					135	
	Ala	Lys	Ala	Lys	Phe	Leu	Asn	Lys	Asp	Arg	Leu	Glu	Tyr	Gly	Tyr	
				140						145					150	
	Ile	Ser	Thr	Ser	Leu	Met	Asn	Val	Ser	Gln	Phe	Ala	Gly	Arg	Pro	
				155						160					165	
25	Ile	Ile	Thr	Gln	Phe	Lys	Val	Ala	Lys	Gly	Ser	Lys	Ala	Gly	Tyr	
				170						175					180	
	Ile	Asp	Pro	Ile	Ser	Ala	Phe	Gln	Gly	Gln	Leu	Glu	Met	Leu	Leu	
				185						190					195	
	Pro	Arg	His	Ser	Thr	Tyr	His	Ile	Asp	Asp	Met	Arg	Leu	Ser	Ser	
30				200						205					210	
	Asp	Gly	Lys	Gln	Ile	Ile	Ile	Thr	Ala	Thr	Met	Met	Gly	Thr	Ala	
				215						220					225	
	Ile	Asn	Pro	Lys	Glu	Phe	Arg	Gln	Ile	Lys	Ile	Trp	Phe	Gln	Asn	
				230						235					240	
35	Arg	Arg	Met	Lys	Trp	Lys	Lys	Val	Asp	Ser	Ser	Gly	Arg	Ile	Val	
				245						250					255	
	Thr	Asp														

40 (2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

- 5 (ii) MOLECULE TYPE: DNA
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM:
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

10 GAATCCCGCA AACGCGCAAG GCAG

24

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 27
- (B) TYPE:NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

- 20 (vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

25 TCAGTTCTCC TTCTTCCACT TCATGCG

27

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 54
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY:LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

- 35 (A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

40 AATTCCGCCA GATCAAGATT TGGTTCCAGA ATCGTCGCAT GAAGTGGAAG AAGG

54

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 54

(B) TYPE:NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGCGGTCTAG TTCTAAACCA AGCTCTTAGC AGCGTAGTTC ACCTTCTTCC AGCT

54

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GAATCCAAGC ATCCAGGAAG TCAGCC

26

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

ACCAGCCACC ACCTTCTGAT A

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 876
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: DOUBLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```

GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA AAAGGCTTAT   60
TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA AAGCTTGGGG TAATGCTCAG   120
TATAAAAAGT ATGGACTAAG CAAATCAGAA AAAGAAGCTA TAGTATCATA TACTAAAAGC   180
GCTAGTGAAA TAAATGGAAA GCTAAGACAA AATAAGGGAG TTATCAATGG ATTTCTTCA   240
AATTTAATAA AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA   300
AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT TCAAAACACT   360
CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG AAAAGGCTAA AGCTAAGTTT   420
TTAAATAAAG ATAGACTTGA ATATGGATAT ATTAGTACTT CATTAATGAA TGTCTCTCAA   480
TTTGCAGGAA GACCAATTAT TACACAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT   540
ATTGACCCTA TTAGTGCTTT TCAGGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT   600
TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT TACAGCAACA   660
ATGATGGGCA CAGCTATCAA TCCTAAAGAA TTCAAGCATC CAGGAAGTCA GCCTAAAAC   720
GCTTGTACCA ATTGCTATTG TAAAAAGTGT TGCTTTCATT GCCAAGTTTG TTTCATAACA   780
AAAGCCTTAG GCATCTCCTA TGGCAGGAAG CGGAGACAGC GACGAAGAGC TCATCAGAAC   840
AGTCAGACTC ATCAAGCTTC TCTATCAAAG CAGTAA                               876

```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 291
 (B) TYPE: AMINO ACID
 (C) STRANDEDNESS: SINGLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

	Gly	Ser	Ser	Arg	Val	Asp	Leu	Gln	Ala	Cys	Asn	Ala	Tyr	Ser	Ile	
	1				5					10					15	
	Asn	Gln	Lys	Ala	Tyr	Ser	Asn	Thr	Tyr	Gln	Glu	Phe	Thr	Asn	Ile	
5					20					25					30	
	Asp	Gln	Ala	Lys	Ala	Trp	Gly	Asn	Ala	Gln	Tyr	Lys	Lys	Tyr	Gly	
					35					40					45	
	Leu	Ser	Lys	Ser	Glu	Lys	Glu	Ala	Ile	Val	Ser	Tyr	Thr	Lys	Ser	
					50					55					60	
10	Ala	Ser	Glu	Ile	Asn	Gly	Lys	Leu	Arg	Gln	Asn	Lys	Gly	Val	Ile	
					65					70					75	
	Asn	Gly	Phe	Pro	Ser	Asn	Leu	Ile	Lys	Gln	Val	Glu	Leu	Leu	Asp	
					80					85					90	
	Lys	Ser	Phe	Asn	Lys	Met	Lys	Thr	Pro	Glu	Asn	Ile	Met	Leu	Phe	
15					95					100					105	
	Arg	Gly	Asp	Asp	Pro	Ala	Tyr	Leu	Gly	Thr	Glu	Phe	Gln	Asn	Thr	
					110					115					120	
	Leu	Leu	Asn	Ser	Asn	Gly	Thr	Ile	Asn	Lys	Thr	Ala	Phe	Glu	Lys	
					125					130					135	
20	Ala	Lys	Ala	Lys	Phe	Leu	Asn	Lys	Asp	Arg	Leu	Glu	Tyr	Gly	Tyr	
					140					145					150	
	Ile	Ser	Thr	Ser	Leu	Met	Asn	Val	Ser	Gln	Phe	Ala	Gly	Arg	Pro	
					155					160					165	
	Ile	Ile	Thr	Gln	Phe	Lys	Val	Ala	Lys	Gly	Ser	Lys	Ala	Gly	Tyr	
25					170					175					180	
	Ile	Asp	Pro	Ile	Ser	Ala	Phe	Gln	Gly	Gln	Leu	Glu	Met	Leu	Leu	
					185					190					195	
	Pro	Arg	His	Ser	Thr	Tyr	His	Ile	Asp	Asp	Met	Arg	Leu	Ser	Ser	
					200					205					210	
30	Asp	Gly	Lys	Gln	Ile	Ile	Ile	Thr	Ala	Thr	Met	Met	Gly	Thr	Ala	
					215					220					225	
	Ile	Asn	Pro	Lys	Glu	Phe	Lys	His	Pro	Gly	Ser	Gln	Pro	Lys	Thr	
					230					235					240	
	Ala	Cys	Thr	Asn	Cys	Tyr	Cys	Lys	Lys	Cys	Cys	Phe	His	Cys	Gln	
35					245					250					255	
	Val	Cys	Phe	Ile	Thr	Lys	Ala	Leu	Gly	Ile	Ser	Tyr	Gly	Arg	Lys	
					260					265					270	
	Arg	Arg	Gln	Arg	Arg	Arg	Ala	His	Gln	Asn	Ser	Gln	Thr	His	Gln	
					275					280					285	
40	Ala	Ser	Leu	Ser	Lys	Gln										
					290											

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

AATTCTATGG TCGTAAAAAA CGTCGTCAAC GTCGTCGTG

39

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GATACCAGCA TTTTGTGCAG CAGTTGCAGC AGCACAGCT

39

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 756
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: DOUBLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```

5  GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA AAAGGCTTAT 60
   TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA AAGCTTGGGG TAATGCTCAG 120
   TATAAAAAGT ATGGACTAAG CAAATCAGAA AAAGAAGCTA TAGTATCATA TACTAAAAGC 180
   GCTAGTGAAA TAAATGGAAA GCTAAGACAA AATAAGGGAG TTATCAATGG ATTCCTTCA 240
   AATTTAATAA AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA 300
10 AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT TCAAAACACT 360
   CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG AAAAGGCTAA AGCTAAGTTT 420
   TTAAATAAAG ATAGACTTGA ATATGGATAT ATTAGTACTT CATTAATGAA TGTCTCTCAA 480
   TTTGCAGGAA GACCAATTAT TACACAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT 540
   ATTGACCCTA TTAGTGCTTT TCAGGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT 600
15 TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT TACAGCAACA 660
   ATGATGGGCA CAGCTATCAA TCCTAAAGAA TTCTATGGTG CTAAAAAACG TCGTCAACGT 720
   CGTCGTGTCG ACTCGAGCGG CCCGCATCGT GACTGA 756

```

20 (2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 251

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

25 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

30

```

Gly Ser Ser Arg Val Asp Leu Gln Ala Cys Asn Ala Tyr Ser Ile
  1             5             10             15
Asn Gln Lys Ala Tyr Ser Asn Thr Tyr Gln Glu Phe Thr Asn Ile
35             20             25             30
Asp Gln Ala Lys Ala Trp Gly Asn Ala Gln Tyr Lys Lys Tyr Gly
             35             40             45
Leu Ser Lys Ser Glu Lys Glu Ala Ile Val Ser Tyr Thr Lys Ser
             50             55             60
40 Ala Ser Glu Ile Asn Gly Lys Leu Arg Gln Asn Lys Gly Val Ile
             65             70             75

```

Asn Gly Phe Pro Ser Asn Leu Ile Lys Gln Val Glu Leu Leu Asp
 80 85 90
 Lys Ser Phe Asn Lys Met Lys Thr Pro Glu Asn Ile Met Leu Phe
 95 100 105
 5 Arg Gly Asp Asp Pro Ala Tyr Leu Gly Thr Glu Phe Gln Asn Thr
 110 115 120
 Leu Leu Asn Ser Asn Gly Thr Ile Asn Lys Thr Ala Phe Glu Lys
 125 130 135
 Ala Lys Ala Lys Phe Leu Asn Lys Asp Arg Leu Glu Tyr Gly Tyr
 10 140 145 150
 Ile Ser Thr Ser Leu Met Asn Val Ser Gln Phe Ala Gly Arg Pro
 155 160 165
 Ile Ile Thr Gln Phe Lys Val Ala Lys Gly Ser Lys Ala Gly Tyr
 170 175 180
 15 Ile Asp Pro Ile Ser Ala Phe Gln Gly Gln Leu Glu Met Leu Leu
 185 190 195
 Pro Arg His Ser Thr Tyr His Ile Asp Asp Met Arg Leu Ser Ser
 200 205 210
 Asp Gly Lys Gln Ile Ile Ile Thr Ala Thr Met Met Gly Thr Ala
 20 215 220 225
 Ile Asn Pro Lys Glu Phe Tyr Gly Ala Lys Lys Arg Arg Gln Arg
 230 235 240
 Arg Arg Val Asp Ser Ser Gly Pro His Arg Asp
 245 250
 25

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1413

30 (B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

35 (A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ATGTCCCCTA TACTAGGTTA TTGGAAAATT AAGGGCCTTG TGCAACCCAC TCGACTTCTT 60
 TTGGAATATC TTGAAGAAAA ATATGAAGAG CATTGTGTATG AGCGCGATGA AGGTGATAAA 120
 40 TGGCGAAACA AAAAGTTTGA ATTGGGTTTG GAGTTTCCCA ATCTTCCTTA TTATATTGAT 180
 GGTGATGTTA AATTAACACA GTCTATGGCC ATCATACGTT ATATAGCTGA CAAGCACAAC 240

```

ATGTTGGGTG GTTGTCCAAA AGAGCGTGCA GAGATTTCAA TGCTTGAAGG AGCGGTTTTG 300
GATATTAGAT ACGGTGTTTC GAGAATTGCA TATAGTAAAG ACTTTGAAAC TCTCAAAGTT 360
GATTTTCTTA GCAAGCTACC TGAAATGCTG AAAATGTTCG AAGATCGTTT ATGTCATAAA 420
ACATATTTAA ATGGTGATCA TGTAACCCAT CCTGACTTCA TGTGTATGA CGCTCTTGAT 480
5 GTTGTTTTAT ACATGGACCC AATGTGCCTG GATGCGTTCC CAAAATTAGT TTGTTTTTAA 540
AAACGTATTG AAGCTATCCC ACAAATTGAT AAGTACTTGA AATCCAGCAA GTATATAGCA 600
TGGCCTTTGC AGGGCTGGCA AGCCACGTTT GGTGGTGGCG ACCATCCTCC AAAATCGGAT 660
CTGGTTCCGC GTGGATCCTC TAGAGTCGAC CTGCAGGCAT GCAATGCTTA TTCCATTAAT 720
CAAAAGGCTT ATTCAAATAC TTACCAGGAG TTTACTAATA TTGATCAAGC AAAAGCTTGG 780
10 GGTAATGCTC AGTATAAAAA GTATGGACTA AGCAAATCAG AAAAAGAAGC TATAGTATCA 840
TATACTAAAA GCGCTAGTGA AATAAATGGA AAGCTAAGAC AAAATAAGGG AGTTATCAAT 900
GGATTTCTT CAAATTTAAT AAAACAAGTT GAACTTTTAG ATAAATCTTT TAATAAAATG 960
AAGACCCCTG AAAATATTAT GTTATTTAGA GGCGACGACC CTGCTTATTT AGGAACAGAA 1020
TTTCAAAACA CTCTTCTTAA TTCAAATGGT ACAATTAATA AAACGGCTTT TGAAAAGGCT 1080
15 AAAGCTAAGT TTTTAAATAA AGATAGACTT GAATATGGAT ATATTAGTAC TTCATTAATG 1140
AATGTTTCTC AATTTGCAGG AAGACCAATT ATTACAAAAT TTAAAGTAGC AAAAGGCTCA 1200
AAGGCAGGAT ATATTGACCC TATTAGTGCT TTTCAGGGAC AACTTGAAAT GTTGCTTCCT 1260
AGACATAGTA CTTATCATAT AGACGATATG AGATTGTCTT CTGATGGTAA ACAAATAATA 1320
ATTACAGCAA CAATGATGGG CACAGCTATC AATCCTAAAG AATTCAGAAG GAAACAAAGA 1380
20 AGAAAAAGAA GACTGCAGGC GGCCGCATCG TGA 1413

```

(2) INFORMATION FOR SEQ ID NO: 20:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 470

(B) TYPE: AMINO ACIDS

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

30 (ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

```

Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln
35 1 5 10 15
Pro Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu
20 25 30
His Leu Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys
35 40 45
40 Phe Glu Leu Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp
50 55 60

```

	Gly	Asp	Val	Lys	Leu	Thr	Gln	Ser	Met	Ala	Ile	Ile	Arg	Tyr	Ile	
					65					70					75	
	Ala	Asp	Lys	His	Asn	Met	Leu	Gly	Gly	Cys	Pro	Lys	Glu	Arg	Ala	
					80					85					90	
5	Glu	Ile	Ser	Met	Leu	Glu	Gly	Ala	Val	Leu	Asp	Ile	Arg	Tyr	Gly	
					95					100					105	
	Val	Ser	Arg	Ile	Ala	Tyr	Ser	Lys	Asp	Phe	Glu	Thr	Leu	Lys	Val	
					110					115					120	
	Asp	Phe	Leu	Ser	Lys	Leu	Pro	Glu	Met	Leu	Lys	Met	Phe	Glu	Asp	
10					125					130					135	
	Arg	Leu	Cys	His	Lys	Thr	Tyr	Leu	Asn	Gly	Asp	His	Val	Thr	His	
					140					145					150	
	Pro	Asp	Phe	Met	Leu	Tyr	Asp	Ala	Leu	Asp	Val	Val	Leu	Tyr	Met	
					155					160					165	
15	Asp	Pro	Met	Cys	Leu	Asp	Ala	Phe	Pro	Lys	Leu	Val	Cys	Phe	Lys	
					170					175					180	
	Lys	Arg	Ile	Glu	Ala	Ile	Pro	Gln	Ile	Asp	Lys	Tyr	Leu	Lys	Ser	
					185					190					195	
	Ser	Lys	Tyr	Ile	Ala	Trp	Pro	Leu	Gln	Gly	Trp	Gln	Ala	Thr	Phe	
20					200					205					210	
	Gly	Gly	Gly	Asp	His	Pro	Pro	Lys	Ser	Asp	Leu	Val	Pro	Arg	Gly	
					215					220					225	
	Ser	Ser	Arg	Val	Asp	Leu	Gln	Ala	Cys	Asn	Ala	Tyr	Ser	Ile	Asn	
					230					235					240	
25	Gln	Lys	Ala	Tyr	Ser	Asn	Thr	Tyr	Gln	Glu	Phe	Thr	Asn	Ile	Asp	
					245					250					255	
	Gln	Ala	Lys	Ala	Trp	Gly	Asn	Ala	Gln	Tyr	Lys	Lys	Tyr	Gly	Leu	
					260					265					270	
	Ser	Lys	Ser	Glu	Lys	Glu	Ala	Ile	Val	Ser	Tyr	Thr	Lys	Ser	Ala	
30					275					280					285	
	Ser	Glu	Ile	Asn	Gly	Lys	Leu	Arg	Gln	Asn	Lys	Gly	Val	Ile	Asn	
					290					295					300	
	Gly	Phe	Pro	Ser	Asn	Leu	Ile	Lys	Gln	Val	Glu	Leu	Leu	Asp	Lys	
					305					310					315	
35	Ser	Phe	Asn	Lys	Met	Lys	Thr	Pro	Glu	Asn	Ile	Met	Leu	Phe	Arg	
					320					325					330	
	Gly	Asp	Asp	Pro	Ala	Tyr	Leu	Gly	Thr	Glu	Phe	Gln	Asn	Thr	Leu	
					335					340					345	
	Leu	Asn	Ser	Asn	Gly	Thr	Ile	Asn	Lys	Thr	Ala	Phe	Glu	Lys	Ala	
40					350					355					360	
	Lys	Ala	Lys	Phe	Leu	Asn	Lys	Asp	Arg	Leu	Glu	Tyr	Gly	Tyr	Ile	

365 370 375
 Ser Thr Ser Leu Met Asn Val Ser Gln Phe Ala Gly Arg Pro Ile
 380 385 390
 Ile Thr Lys Phe Lys Val Ala Lys Gly Ser Lys Ala Gly Tyr Ile
 5 395 400 405
 Asp Pro Ile Ser Ala Phe Gln Gly Gln Leu Glu Met Leu Leu Pro
 410 415 420
 Arg His Ser Thr Tyr His Ile Asp Asp Met Arg Leu Ser Ser Asp
 425 430 435
 10 Gly Lys Gln Ile Ile Ile Thr Ala Thr Met Met Gly Thr Ala Ile
 440 445 450
 Asn Pro Lys Glu Phe Arg Arg Lys Gln Arg Arg Lys Arg Arg Leu
 455 460 465
 Gln Ala Ala Ala Ser
 15 470

(2) INFORMATION FOR SEQ ID NO: 21

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:16

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

25 (ii) MOLECULE TYPE: PEPTIDE

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

30 Lys Arg Arg Arg Arg Arg Pro Lys Lys Arg Arg Arg

1 5 10

Ala Lys Arg Arg

15

35

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:48

40 (B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

AAGAGAAGGC GAAGAAGACC TAAGAAGAGA CGAAGGGCGA AGAGGAGA

48

10

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48

(B) TYPE: NUCLEIC ACID

15

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

TTCTCTTCCG CTTCTTCTGG ATTCTTCTCT GCTTCCCGCT TCTCCTCT

48

25

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 792

30

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

35

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA AAAGGCTTAT 60
TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA AAGCTTGGGG TAATGCTCAG 120
40 TATAAAAAGT ATGGACTAAG CAAATCAGAA AAAGAAGCTA TAGTATCATA TACTAAAAGC 180
GCTAGTGAAA TAAATGGAAA GCTAAGACAA AATAAGGGAG TTATCAATGG ATTCCTTCA 240

AATTTAATAA AACAAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA 300
 AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT TCAAAACACT 360
 CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG AAAAGGCTAA AGCTAAGTTT 420
 TTAAATAAAG ATAGACTTGA ATATGGATAT ATTAGTACTT CATTAATGAA TGTTCCTCAA 480
 5 TTTGCAGGAA GACCAATTAT TACAAAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT 540
 ATTGACCCTA TTAGTGCTTT TCAGGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT 600
 TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT TACAGCAACA 660
 ATGATGGGCA CAGCTATCAA TCCTAAAGAA TTCAAGAGAA GCGAAGAAG ACCTAAGAAG 720
 AGACGAAGGG CGAAGAGGAG ACACCACCAC CACCACCACG TCGACTCGAG CGGCCGCATC 780
 10 GTGACTGACT GA 792

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 263
 (B) TYPE: AMINO ACID
 (C) STRANDEDNESS: SINGLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

20 (vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Gly Ser Ser Arg Val Asp Leu Gln Ala Cys Asn Ala Tyr Ser Ile
 25 1 5 10 15
 Asn Gln Lys Ala Tyr Ser Asn Thr Tyr Gln Glu Phe Thr Asn Ile
 20 25 30
 Asp Gln Ala Lys Ala Trp Gly Asn Ala Gln Tyr Lys Lys Tyr Gly
 35 40 45
 30 Leu Ser Lys Ser Glu Lys Glu Ala Ile Val Ser Tyr Thr Lys Ser
 50 55 60
 Ala Ser Glu Ile Asn Gly Lys Leu Arg Gln Asn Lys Gly Val Ile
 65 70 75
 Asn Gly Phe Pro Ser Asn Leu Ile Lys Gln Val Glu Leu Leu Asp
 35 80 85 90
 Lys Ser Phe Asn Lys Met Lys Thr Pro Glu Asn Ile Met Leu Phe
 95 100 105
 Arg Gly Asp Asp Pro Ala Tyr Leu Gly Thr Glu Phe Gln Asn Thr
 110 115 120
 40 Leu Leu Asn Ser Asn Gly Thr Ile Asn Lys Thr Ala Phe Glu Lys
 125 130 135

Ala Lys Ala Lys Phe Leu Asn Lys Asp Arg Leu Glu Tyr Gly Tyr
140 145 150
Ile Ser Thr Ser Leu Met Asn Val Ser Gln Phe Ala Gly Arg Pro
155 160 165
5 Ile Ile Thr Lys Phe Lys Val Ala Lys Gly Ser Lys Ala Gly Tyr
170 175 180
Ile Asp Pro Ile Ser Ala Phe Gln Gly Gln Leu Glu Met Leu Leu
185 190 195
Pro Arg His Ser Thr Tyr His Ile Asp Asp Met Arg Leu Ser Ser
10 200 205 210
Asp Gly Lys Gln Ile Ile Ile Thr Ala Thr Met Met Gly Thr Ala
215 220 225
Ile Asn Pro Lys Glu Phe Lys Arg Arg Arg Arg Arg Pro Lys Lys
230 235 240
15 Arg Arg Arg Ala Lys Arg Arg His His His His His Val Asp
245 250 255
Ser Ser Gly Arg Ile Val Thr Asp
260

20

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13

(B) TYPE: AMINO ACID

25

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PEPTIDE

(vi) ORIGINAL SOURCE:

30

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Lys Arg Arg Arg Arg Lys Lys Arg Arg Gln Arg Arg Arg
1 5 10

35

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

40

(A) LENGTH: 39

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

5 (A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

AAGCGTCGAC GTAGAAAGAA ACGTAGACAG CGTAGACGT

39

10

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39

15

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

20

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

TTCGCAGCTG CATCTTTCTT TGCATCTGTC GCATCTGCA

39

25

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 783

(B) TYPE: NUCLEIC ACID

30

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA AAAGGCTTAT 60
TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA AAGCTTGGGG TAATGCTCAG 120
TATAAAAAGT ATGGACTAAG CAAATCAGAA AAAGAAGCTA TAGTATCATA TACTAAAAGC 180
40 GCTAGTGAAA TAAATGGAAA GCTAAGACAA AATAAGGGAG TTATCAATGG ATTCCTTCA 240
AATTTAATAA AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA 300

AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT TCAAAACACT 360
 CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG AAAAGGCTAA AGCTAAGTTT 420
 TTAAATAAAG ATAGACTTGA ATATGGATAT ATTAGTACTT CATTAATGAA TGTTTCTCAA 480
 TTTGCAGGAA GACCAATTAT TACAAAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT 540
 5 ATTGACCCTA TTAGTGCTTT TCAGGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT 600
 TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT TACAGCAACA 660
 ATGATGGGCA CAGCTATCAA TCCTAAAGAA TTCAAGCGTC GACGTAGAAA GAAACGTAGA 720
 CAGCGTAGAC GTCACCACCA CCACCACCAC GTCGACTCGA GCGGCCGCAT CGTGACTGAC 780
 TGA

10

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

15

(A) LENGTH: 260

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

20

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Gly Ser Ser Arg Val Asp Leu Gln Ala Cys Asn Ala Tyr Ser Ile
 25 1 5 10 15
 Asn Gln Lys Ala Tyr Ser Asn Thr Tyr Gln Glu Phe Thr Asn Ile
 20 25 30
 Asp Gln Ala Lys Ala Trp Gly Asn Ala Gln Tyr Lys Lys Tyr Gly
 35 40 45
 30 Leu Ser Lys Ser Glu Lys Glu Ala Ile Val Ser Tyr Thr Lys Ser
 50 55 60
 Ala Ser Glu Ile Asn Gly Lys Leu Arg Gln Asn Lys Gly Val Ile
 65 70 75
 Asn Gly Phe Pro Ser Asn Leu Ile Lys Gln Val Glu Leu Leu Asp
 35 80 85 90
 Lys Ser Phe Asn Lys Met Lys Thr Pro Glu Asn Ile Met Leu Phe
 95 100 105
 Arg Gly Asp Asp Pro Ala Tyr Leu Gly Thr Glu Phe Gln Asn Thr
 110 115 120
 40 Leu Leu Asn Ser Asn Gly Thr Ile Asn Lys Thr Ala Phe Glu Lys
 125 130 135

120

Ala Lys Ala Lys Phe Leu Asn Lys Asp Arg Leu Glu Tyr Gly Tyr
140 145 150
Ile Ser Thr Ser Leu Met Asn Val Ser Gln Phe Ala Gly Arg Pro
155 160 165
5 Ile Ile Thr Lys Phe Lys Val Ala Lys Gly Ser Lys Ala Gly Tyr
170 175 180
Ile Asp Pro Ile Ser Ala Phe Gln Gly Gln Leu Glu Met Leu Leu
185 190 195
Pro Arg His Ser Thr Tyr His Ile Asp Asp Met Arg Leu Ser Ser
10 200 205 210
Asp Gly Lys Gln Ile Ile Ile Thr Ala Thr Met Met Gly Thr Ala
215 220 225
Ile Asn Pro Lys Glu Phe Lys Arg Arg Arg Arg Lys Lys Arg Arg
230 235 240
15 Gln Arg Arg Arg His His His His His His Val Asp Ser Ser Gly
245 250 255
Arg Ile Val Thr Asp
260

20

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9

(B) TYPE: AMINO ACID

25

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PEPTIDE

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Arg Arg Lys Gln Arg Arg Lys Arg Arg

1

5

35

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

40

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

AGAAGGAAAC AAAGAAGAAA AAGAAGA

27

10

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: NUCLEIC ACID

15

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

TCTTCCTTTG TTTCTTCTTT TTCTTCT

27

25

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 771

(B) TYPE: NUCLEIC ACID

30

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA AAAGGCTTAT 60
TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA AAGCTTGGGG TAATGCTCAG 120
TATAAAAAGT ATGGACTAAG CAAATCAGAA AAAGAAGCTA TAGTATCATA TACTAAAAGC 180
40 GCTAGTGAAA TAAATGGAAA GCTAAGACAA AATAAGGGAG TTATCAATGG ATTCCTTCA 240
AATTTAATAA AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA 300

```

AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT TCAAAACACT 360
CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG AAAAGGCTAA AGCTAAGTTT 420
TTAAATAAAG ATAGACTTGA ATATGGATAT ATTAGTACTT CATTAATGAA TGTTTCTCAA 480
TTTGCAGGAA GACCAATTAT TACAAAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT 540
5 ATTGACCCTA TTAGTGCTTT TCAGGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT 600
TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT TACAGCAACA 660
ATGATGGGCA CAGCTATCAA TCCTAAAGAA TTCAGAAGGA AACAAAGAAG AAAAAGAAGA 720
CACCACCACC ACCACCACGT CGACTCGAGC GGCCGCATCG TGACTGACTG A 771

```

10

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 256

15

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:

20

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

```

Gly Ser Ser Arg Val Asp Leu Gln Ala Cys Asn Ala Tyr Ser Ile
  1              5              10              15
25 Asn Gln Lys Ala Tyr Ser Asn Thr Tyr Gln Glu Phe Thr Asn Ile
    20              25              30
Asp Gln Ala Lys Ala Trp Gly Asn Ala Gln Tyr Lys Lys Tyr Gly
    35              40              45
Leu Ser Lys Ser Glu Lys Glu Ala Ile Val Ser Tyr Thr Lys Ser
30              50              55              60
Ala Ser Glu Ile Asn Gly Lys Leu Arg Gln Asn Lys Gly Val Ile
    65              70              75
Asn Gly Phe Pro Ser Asn Leu Ile Lys Gln Val Glu Leu Leu Asp
    80              85              90
35 Lys Ser Phe Asn Lys Met Lys Thr Pro Glu Asn Ile Met Leu Phe
    95              100             105
Arg Gly Asp Asp Pro Ala Tyr Leu Gly Thr Glu Phe Gln Asn Thr
    110             115             120
Leu Leu Asn Ser Asn Gly Thr Ile Asn Lys Thr Ala Phe Glu Lys
40              125             130             135

```

Ala Lys Ala Lys Phe Leu Asn Lys Asp Arg Leu Glu Tyr Gly Tyr
140 145 150
Ile Ser Thr Ser Leu Met Asn Val Ser Gln Phe Ala Gly Arg Pro
155 160 165
5 Ile Ile Thr Lys Phe Lys Val Ala Lys Gly Ser Lys Ala Gly Tyr
170 175 180
Ile Asp Pro Ile Ser Ala Phe Gln Gly Gln Leu Glu Met Leu Leu
185 190 195
Pro Arg His Ser Thr Tyr His Ile Asp Asp Met Arg Leu Ser Ser
10 200 205 210
Asp Gly Lys Gln Ile Ile Ile Thr Ala Thr Met Met Gly Thr Ala
215 220 225
Ile Asn Pro Lys Glu Phe Arg Arg Lys Gln Arg Arg Lys Arg Arg
230 235 240
15 His His His His His His Val Asp Ser Ser Gly Arg Ile Val Thr
245 250 255
Asp

20 (2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 887

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

25 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

30 GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA AAAGGCTTAT 60
TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA AAGCTTGGGG TAATGCTCAG 120
TATAAAAAGT ATGGACTAAG CAAATCAGAA AAAGAAGCTA TAGTATCATA TACTAAAAGC 180
GCTAGTGAAA TAAATGGAAA GCTAAGACAA AATAAGGGAG TTATCAATGG ATTCCTTCA 240
35 AATTTAATAA AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA 300
AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT TCAAAACACT 360
CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG AAAAGGCTAA AGCTAAGTTT 420
TTAAATAAAG ATAGACTTGA ATATGGATAT ATTAGTACTT CATTAATGAA TGTTTCTCAA 480
TTTGCAGGAA GACCAATTAT TACAAAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT 540
40 ATTGACCCTA TTAGTGCTTT TGCAGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT 600

TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT TACAGCAACA 660
 ATGATGGGCA CAGCTATCAA TCCTAAAGAA TTCGTGATGA ATCCCGCAAA CGCGCAAGGC 720
 AGACATACAC CCGGTACCAG ACTCTAGAGC TAGAGAAGGA GTTTCAC TTC AATCGCTACT 780
 TGACCCGTCG GCGAAGGATC GAGATCGCCC ACGCCCTGTG CCTCACGGAG CGCCAGATAA 840
 5 AGATTGTTT CCAGAATCGG CGCATGAAGT GGAAGAAGGA GAACTGA 887

(2) INFORMATION FOR SEQ ID NO: 37:

10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 248
- (B) TYPE: AMINO ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

15

(ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

20 Gly Ser Ser Arg Val Asp Leu Gln Ala Cys Asn Ala Tyr Ser Ile
 1 5 10 15
 Asn Gln Lys Ala Tyr Ser Asn Thr Tyr Gln Glu Phe Thr Asn Ile
 20 25 30
 Asp Gln Ala Lys Ala Trp Gly Asn Ala Gln Tyr Lys Lys Tyr Gly
 25 35 40 45
 Leu Ser Lys Ser Glu Lys Glu Ala Ile Val Ser Tyr Thr Lys Ser
 50 55 60
 Ala Ser Glu Ile Asn Gly Lys Leu Arg Gln Asn Lys Gly Val Ile
 65 70 75
 30 Asn Gly Phe Pro Ser Asn Leu Ile Lys Gln Val Glu Leu Leu Asp
 80 85 90
 Lys Ser Phe Asn Lys Met Lys Thr Pro Glu Asn Ile Met Leu Phe
 95 100 105
 Arg Gly Asp Asp Pro Ala Tyr Leu Gly Thr Glu Phe Gln Asn Thr
 35 110 115 120
 Leu Leu Asn Ser Asn Gly Thr Ile Asn Lys Thr Ala Phe Glu Lys
 125 130 135
 Ala Lys Ala Lys Phe Leu Asn Lys Asp Arg Leu Glu Tyr Gly Tyr
 140 145 150

Ile Ser Thr Ser Leu Met Asn Val Ser Gln Phe Ala Gly Arg Pro
155 160 165
Ile Ile Thr Gln Phe Lys Val Ala Lys Gly Ser Lys Ala Gly Tyr
170 175 180
5 Ile Asp Pro Ile Ser Ala Phe Gln Gly Gln Leu Glu Met Leu Leu
185 190 195
Pro Arg His Ser Thr Tyr His Ile Asp Asp Met Arg Leu Ser Ser
200 205 210
Asp Gly Lys Gln Ile Ile Ile Thr Ala Thr Met Met Gly Thr Ala
10 215 220 225
Ile Asn Pro Lys Glu Phe Val Met Asn Pro Ala Asn Ala Gln Gly
230 235 240
Arg His Thr Pro Gly Thr Arg Leu
245

15

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH: 37

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

25

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GGATCTGGTT CCGCGTCATA TGTCTAGAGT CGACCTG

37

30

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

35

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CGCGGATCCA TTAGTTCTCC TTCTTCCACT TC

32

(2) INFORMATION FOR SEQ ID NO: 40:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

10 (ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

15 AAATTAATAC GACTCACTAT AGGG

24

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 19

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

25 (vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

GCTAGTTATT GCTCAGCGG

19

30

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 888

35 (B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

5

```

ATGTCTAGAG TCGCACTGCA GGCATGCAAT GCTTATTCCA TTAATCAAAA GGCTTATTCA 60
AATACTTACC AGGAGTTTAC TAATATTGAT CAAGCAAAAG CTTGGGGTAA TGCTCAGTAT 120
AAAAAGTATG GACTAAGCAA ATCAGAAAAA GAAGCTATAG TATCATATAC TAAAAGCGCT 180
AGTGAAATAA ATGGAAAGCT AAGACAAAAT AAGGGAGTTA TCAATGGATT TCCTTCAAAT 240
10 TTAATAAAAC AAGTTGAACT TTTAGATAAA TCTTTTAATA AAATGAAGAC CCCTGAAAAT 300
ATTATGTTAT TTAGAGGCGA CGACCCTGCT TATTTAGGAA CAGAATTTCA AAACACTCTT 360
CTTAATTCAA ATGGTACAAT TAATAAAACG GCTTTTGAAA AGGCTAAAGC TAAGTTTTTA 420
AATAAAGATA GACTTGAATA TGGATATATT AGTACTTCAT TAATGAATGT TTCTCAATTT 480
GCAGGAAGAC CAATTATTAC AAAATTTAAA GTAGCAAAAG GCTCAAAGGC AGGATATATT 540
15 GACCCTATTA GTGCTTTTGC AGGACAACCTT GAAATGTTGC TTCCTAGACA TAGTACTTAT 600
CATATAGACG ATATGAGATT GTCTTCTGAT GGTAACAAA TAATAATTAC AGCAACAATG 660
ATGGGCACAG CTATCAATCC TAAAGAATTC GTGATGAATC CCGCAAACGC GCAAGGCAGA 720
CATAACCCCG GTACCAGACT CTAGAGCTAG AGAAGGAGTT TCACTTCAAT CGCTACTTGA 780
CCCGTCGGCG AAGGATCGAG ATCGCCACG CCCTGTGCCT CACGGAGCGC CAGATAAAGA 840
20 TTTGGTTCCA GAATCGGCGC ATGAAGTGGA AGAAGGAGGA CTAAGTGA 888

```

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

25

(A) LENGTH: 247

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

30

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

```

Met Ser Arg Val Asp Leu Gln Ala Cys Asn Ala Tyr Ser Ile Asn
35   1           5           10           15
Gln Lys Ala Tyr Ser Asn Thr Tyr Gln Glu Phe Thr Asn Ile Asp
           20           25           30
Gln Ala Lys Ala Trp Gly Asn Ala Gln Tyr Lys Lys Tyr Gly Leu
           35           40           45

```

Ser Lys Ser Glu Lys Glu Ala Ile Val Ser Tyr Thr Lys Ser Ala
 50 55 60
 Ser Glu Ile Asn Gly Lys Leu Arg Gln Asn Lys Gly Val Ile Asn
 65 70 75
 5 Gly Phe Pro Ser Asn Leu Ile Lys Gln Val Glu Leu Leu Asp Lys
 80 85 90
 Ser Phe Asn Lys Met Lys Thr Pro Glu Asn Ile Met Leu Phe Arg
 95 100 105
 Gly Asp Asp Pro Ala Tyr Leu Gly Thr Glu Phe Gln Asn Thr Leu
 10 110 115 120
 Leu Asn Ser Asn Gly Thr Ile Asn Lys Thr Ala Phe Glu Lys Ala
 125 130 135
 Lys Ala Lys Phe Leu Asn Lys Asp Arg Leu Glu Tyr Gly Tyr Ile
 140 145 150
 15 Ser Thr Ser Leu Met Asn Val Ser Gln Phe Ala Gly Arg Pro Ile
 155 160 165
 Ile Thr Gln Phe Lys Val Ala Lys Gly Ser Lys Ala Gly Tyr Ile
 170 175 180
 Asp Pro Ile Ser Ala Phe Gln Gly Gln Leu Glu Met Leu Leu Pro
 20 185 190 195
 Arg His Ser Thr Tyr His Ile Asp Asp Met Arg Leu Ser Ser Asp
 200 205 210
 Gly Lys Gln Ile Ile Ile Thr Ala Thr Met Met Gly Thr Ala Ile
 215 220 225
 25 Asn Pro Lys Glu Phe Val Met Asn Pro Ala Asn Ala Gln Gly Arg
 230 235 240
 His Thr Pro Gly Thr Arg Leu
 245

30

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 64

35

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE:

40

(A) ORGANISM:

xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

```

Val Met Glu Ser Arg Lys Arg Ala Arg Gln Thr Tyr Thr Arg Tyr
  1              5              10              15
5  Gln Thr Leu Glu Leu Glu Lys Glu Phe His Phe Asn Arg Tyr Leu
      20              25              30
Thr Arg Arg Arg Arg Ile Glu Ile Ala His Ala Leu Cys Leu Thr
      35              40              45
10 Glu Arg Gln Ile Lys Ile Trp Phe Gln Asn Arg Arg Met Lys Trp
      50              55              60
Lys Lys Glu Asn

```

(2) INFORMATION FOR SEQ ID NO: 45:

15 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

20 (ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

```

25 Arg Gln Ile Lys Ile Trp Phe Gln Asn Arg Arg Met Lys Trp Lys
  1              5              10              15

```

Lys Val Asp Ser

30 (2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

35 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

Lys His Pro Gly Ser Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr
 1 5 10 15
 Cys Lys Lys Cys Cys Phe His Cys Gln Val Cys Phe Ile Thr Lys
 5 20 25 30
 Ala Leu Gly Ile Ser Tyr Gly Arg Lys Arg Arg Gln Arg Arg Arg
 35 40 45
 Ala His Gln Asn Ser Gln Thr His Gln Ala Ser Leu Ser Lys Gln
 50 55 60

10

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: AMINO ACID

15 (C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

Tyr Gly Ala Lys Lys Arg Arg Gln Arg Arg Arg Val Asp Ser Ser
 1 5 10 15
 Gly Pro His Arg Asp
 20

25

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17

30 (B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE:

35 (A) ORGANISM:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Val Met Asn Pro Ala Asn Ala Gln Gly Arg His Thr Pro Gly Thr
 1 5 10 15

40 Arg Leu

Claims

1. A pharmaceutical composition comprising: a) a polypeptide consisting of SEQ ID NO: 43 and b) a pharmaceutically acceptable carrier.
2. The pharmaceutical composition of claim 1, wherein the carrier comprises a biological adhesive.
3. The pharmaceutical composition of claim 1, wherein the carrier comprises fibrin.
4. A pharmaceutical composition comprising: a) a polypeptide comprising an amino acid sequence of a transport agent covalently linked to an amino acid sequence of an active agent, said amino acid sequence of said active agent consisting of ADP-ribosyl transferase C3 or a fragment thereof retaining ADP-ribosyl transferase activity, said amino acid sequence of said transport agent facilitating uptake of the active agent by a receptor-independent mechanism and being selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, and a Histidine tag, and b) a pharmaceutically acceptable carrier.
5. The pharmaceutical composition of claim 4, wherein the carrier comprises a biological adhesive.
6. The pharmaceutical composition of claim 4, wherein the carrier comprises fibrin.
7. A pharmaceutical composition comprising: a) a polypeptide comprising an amino acid sequence of a transport agent covalently linked to an amino acid sequence of an active agent, said amino acid sequence of said active agent consisting of ADP-ribosyl transferase C3 or a fragment thereof retaining ADP-ribosyl transferase activity, said amino acid sequence of said transport agent facilitating uptake of the active agent by a receptor-independent mechanism and being selected from the group consisting of SEQ ID NO:21, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO: 47, and SEQ ID NO: 48 and b) a pharmaceutically acceptable carrier.
8. The pharmaceutical composition of claim 7, wherein the carrier comprises a biological adhesive.
9. The pharmaceutical composition of claim 7, wherein the carrier comprises fibrin.

10. A pharmaceutical composition comprising: a) a polypeptide comprising an amino acid sequence of a transport agent covalently linked to an amino acid sequence of an active agent, said amino acid sequence of said active agent consisting of ADP-ribosyl transferase C3 or an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 43 and retaining ADP-ribosyl transferase activity, said amino acid sequence of said transport agent facilitating uptake of the active agent by a receptor-independent mechanism and being selected from the group consisting of a subdomain of HIV Tat protein, a homeodomain of antennapedia, and a Histidine tag, and b) a pharmaceutically acceptable carrier.
11. The pharmaceutical composition of claim 10, wherein the carrier comprises a biological adhesive.
12. The pharmaceutical composition of claim 10, wherein the carrier comprises fibrin.
13. A pharmaceutical composition comprising: a) a polypeptide comprising an amino acid sequence of a transport agent covalently linked to an amino acid sequence of an active agent, said amino acid sequence of said active agent consisting of ADP-ribosyl transferase C3 or an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 43 and retaining ADP-ribosyl transferase activity, said amino acid sequence of said transport agent facilitating uptake of the active agent by a receptor-independent mechanism and being selected from the group consisting of SEQ ID NO:21, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO: 47, and SEQ ID NO: 48, and b) a pharmaceutically acceptable carrier.
14. The pharmaceutical composition of claim 13, wherein the carrier comprises a biological adhesive.
15. The pharmaceutical composition of claim 13, wherein the carrier comprises fibrin.
16. Use of a pharmaceutical composition according to any one of claims 1, 4, 7, 10, and 13 directly at a central nervous system lesion site or a peripheral nervous system lesion site for facilitating axon growth.

17. Use of a pharmaceutical composition according to any one of claims 1, 4, 7, 10, or 13 directly at a central nervous system lesion site or a peripheral nervous system lesion site for treating nerve injury.

18. A purified polypeptide comprising an amino acid sequence of a transport agent covalently linked to an amino acid sequence of an active agent, said amino acid sequence of said active agent consisting of ADP-ribosyl transferase C3 or a fragment thereof retaining ADP-ribosyl transferase activity, said amino acid sequence of said transport agent facilitating uptake of the active agent by a receptor-independent mechanism and being selected from the group consisting of a homeodomain of antennapedia, and a Histidine tag, said polypeptide having ADP-ribosyl transferase activity.

19. The purified polypeptide of claim 18, wherein the amino acid sequence of the transport agent is at the carboxy-terminal end of said polypeptide and the amino acid sequence of the active agent is at the amino terminal end of said polypeptide.

20. A purified polypeptide comprising an amino acid sequence of a transport agent covalently linked to an amino acid sequence of an active agent, said amino acid sequence of said active agent consisting of ADP-ribosyl transferase C3 or a fragment thereof retaining ADP-ribosyl transferase activity, said amino acid sequence of said transport agent facilitating uptake of the active agent by a receptor-independent mechanism and being selected from the group consisting of SEQ ID NO:21, SEQ ID NO:26, SEQ ID NO:44, SEQ ID NO:45 and SEQ ID NO:48, said polypeptide having ADP-ribosyl transferase activity.

21. The purified polypeptide of claim 20, wherein the amino acid sequence of the transport agent is at the carboxy-terminal end of said polypeptide and the amino acid sequence of the active agent is at the amino terminal end of said polypeptide.

22. A purified polypeptide comprising an amino acid sequence of a transport agent covalently linked to an amino acid sequence of an active agent, said amino acid sequence of said active agent consisting of ADP-ribosyl transferase C3 or an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 43 and retaining ADP-ribosyl transferase activity, said amino acid sequence of said transport agent facilitating uptake of the active agent by a receptor-independent mechanism and being selected from the group

consisting of, a homeodomain of antennapedia, and a Histidine tag, said polypeptide having ADP-ribosyl transferase activity.

23. The purified polypeptide of claim 22, wherein the amino acid sequence of the transport agent is at the carboxy-terminal end of said polypeptide and the amino acid sequence of the active agent is at the amino terminal end of said polypeptide.

24. A purified polypeptide comprising an amino acid sequence of a transport agent covalently linked to an amino acid sequence of an active agent, said amino acid sequence of said active agent consisting of ADP-ribosyl transferase C3 or an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 43 and retaining ADP-ribosyl transferase activity, said amino acid sequence of said transport agent facilitating uptake of the active agent by a receptor-independent mechanism and being selected from the group consisting of SEQ ID NO:21, SEQ ID NO:26, SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO: 48, said polypeptide having ADP-ribosyl transferase activity.

25. The purified polypeptide of claim 24, wherein the amino acid sequence of the transport agent is at the carboxy-terminal end of said polypeptide and the amino acid sequence of the active agent is at the amino terminal end of said polypeptide.

26. The purified polypeptide of claim 19 wherein the amino acid sequence of the transport agent is as set forth in SEQ ID NO: 48.

27. A purified polypeptide having the sequence set forth in SEQ ID NO: 43

28. A polypeptide set forth in SEQ ID NO: 43.

29. A polypeptide set forth in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 25, SEQ ID NO: 30, SEQ ID NO: 35 or SEQ ID NO: 37.

30. Use of a polypeptide as set forth in SEQ ID NO: 43 in the manufacture of a pharmaceutical composition.

31. A polypeptide comprising at least one transport agent region covalently linked to an active agent region, wherein the transport agent region is a proline rich region, wherein the transport agent region is at the carboxy-terminal end of said polypeptide and the active agent

region is at the amino terminal end of said polypeptide, and said active agent region being selected from the group consisting of ADP-ribosyl transferase C3 and a fragment thereof retaining an ADP-ribosyl transferase activity, wherein the proline rich region is SEQ ID NO: 48.

32. The use of a polypeptide as defined in any one of claims 26 – 29 and 31 for the manufacture of a medicament for the treatment of glaucoma.

33. The use of a polypeptide as defined in any one of claims 26 – 29 and 31 for the manufacture of a pharmaceutical composition.

34. The use of a polypeptide according to claim 33 for the manufacture of a pharmaceutical composition for suppressing the inhibition of neuronal axon growth.

35. The use of a polypeptide according to claim 33 for the manufacture of a pharmaceutical composition for facilitating axon growth.

36. The use of a polypeptide according to claim 33 for the manufacture of a pharmaceutical composition for treating nerve injury.

37. A pharmaceutical composition comprising:

- (a) a polypeptide according to any one of claims 26 – 29 and 31, and
- (b) a pharmaceutically acceptable carrier.

38. A pharmaceutical composition according to claim 37 further comprising a biological adhesive.

39. A pharmaceutical composition according to claim 37 further comprising fibrin.

40. The use of claim 17 or 36, wherein the nerve injury is spinal cord injury.

41. The use of a polypeptide as defined in any one of claims 26 – 29 and 31 for the treatment of glaucoma.

42. The use of a polypeptide as defined in any one of claims 26 – 29 and 31 for suppressing the inhibition of neuronal axon growth.

43. The use of a polypeptide as defined in any one of claims 26 – 29 and 31 for facilitating axon growth.
44. The use of a polypeptide as defined in any one of claims 26 – 29 and 31 for treating nerve injury.
45. The use of claim 44, wherein the nerve injury is spinal cord injury.
46. A pharmaceutical composition comprising:
- (a) a polypeptide consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 25, SEQ ID NO: 30, SEQ ID NO: 35 or SEQ ID NO: 37; and
 - (b) a pharmaceutically acceptable carrier.
47. The pharmaceutical composition of claim 46, wherein the carrier comprises a biological adhesive.
48. The pharmaceutical composition of claim 46, wherein the carrier comprises fibrin.
49. A purified polypeptide having the sequence set forth in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 25, SEQ ID NO: 30, SEQ ID NO: 35 or SEQ ID NO: 37.

Application number/numéro de demande: 2.367.636

Figures: 2, 3A, 3B, 9A, 9B

Pages: _____

Unscannable Item(s) received with this application.

To inquire if you can order a copy of the unscannable item(s),

Please visit the CIPO Website at [HTTP://CIPO.GC.CA](http://CIPO.GC.CA)

Item(s) ne pouvant être balayés.

Documents reçus avec cette demande ne pouvant être balayés.

Pour vous renseigner si vous pouvez commander

une copie des items ne pouvant être balayés,

veuillez visiter le site web de l'OPIC au [HTTP://CIPO.GC.CA](http://CIPO.GC.CA)

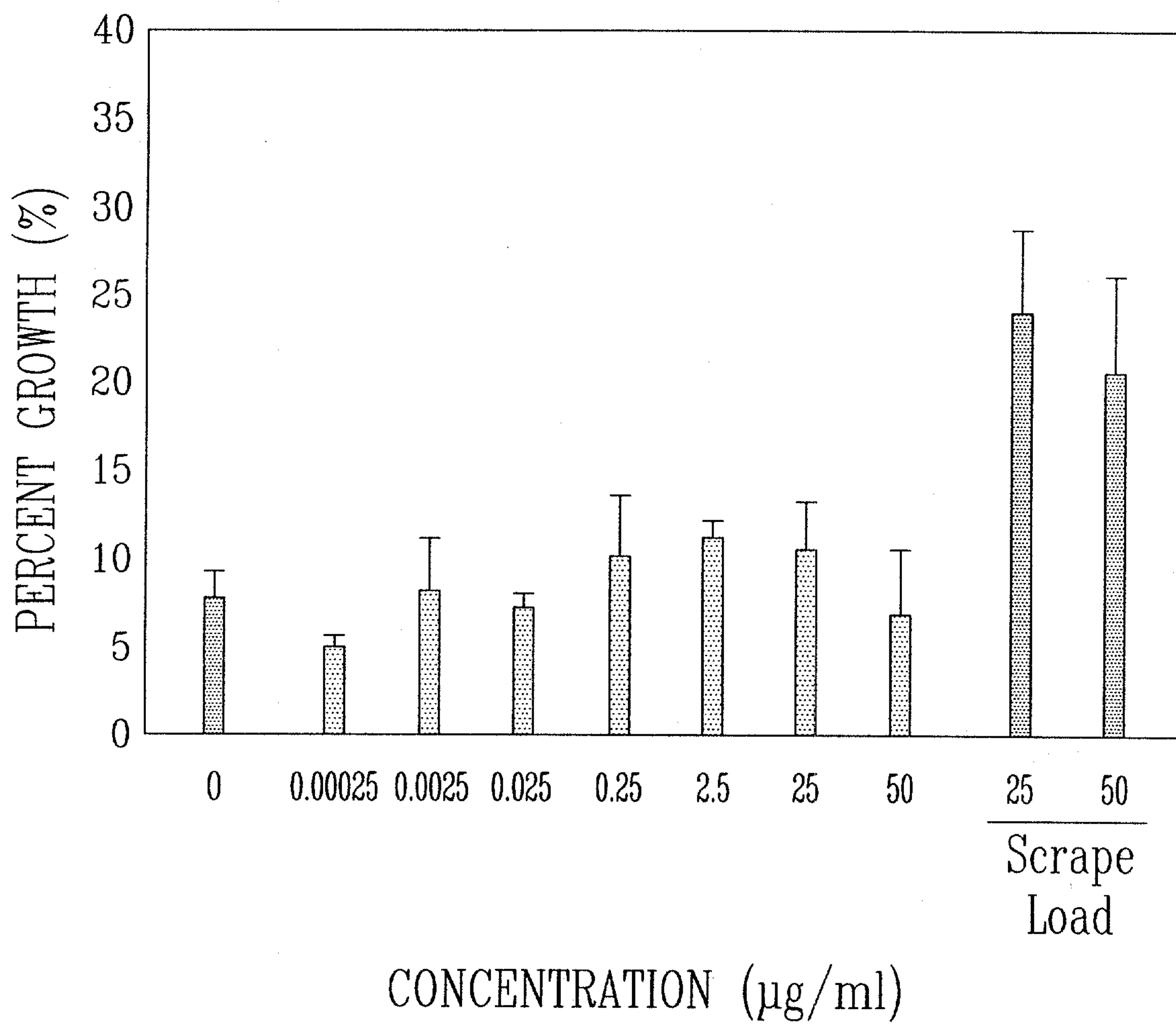
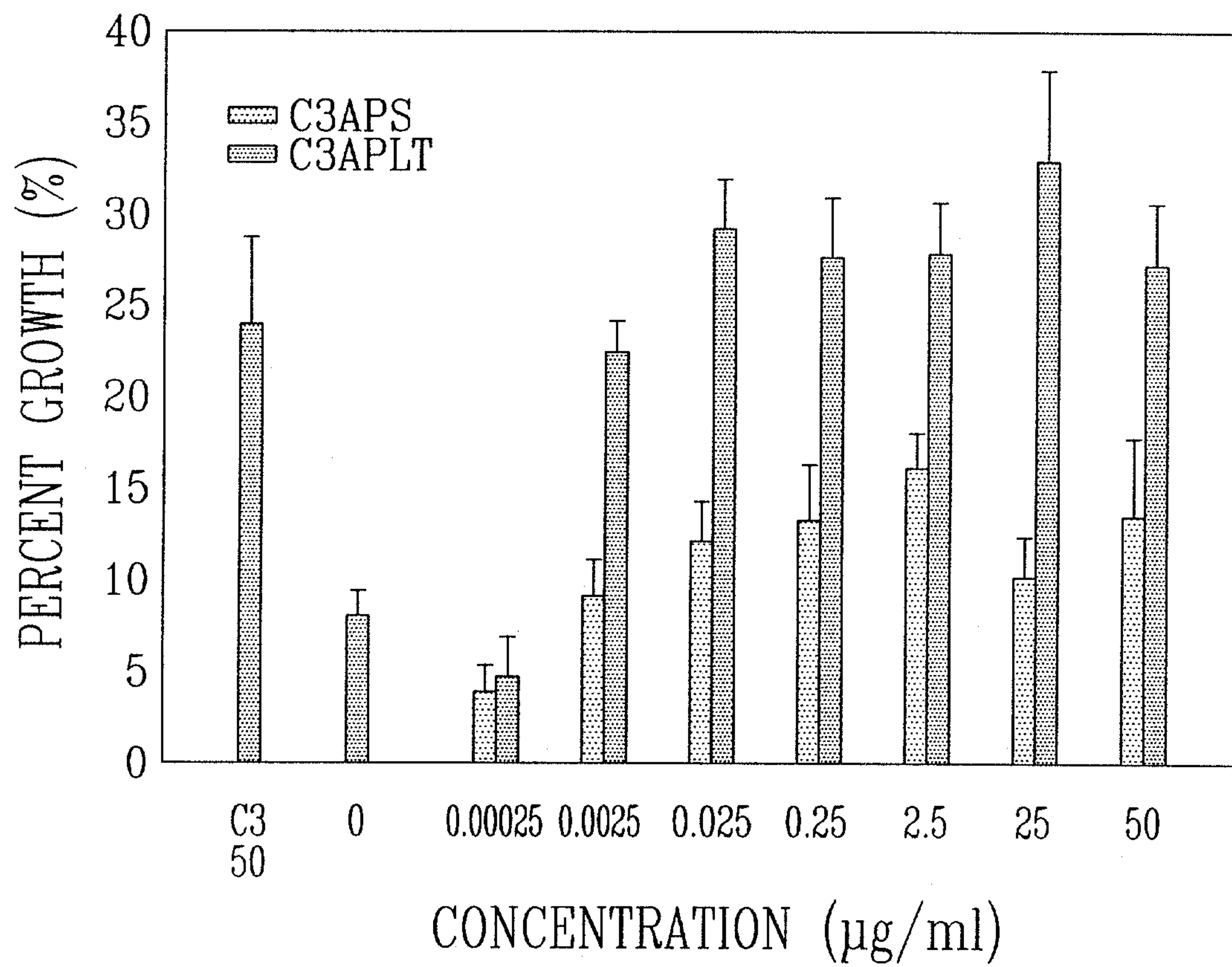
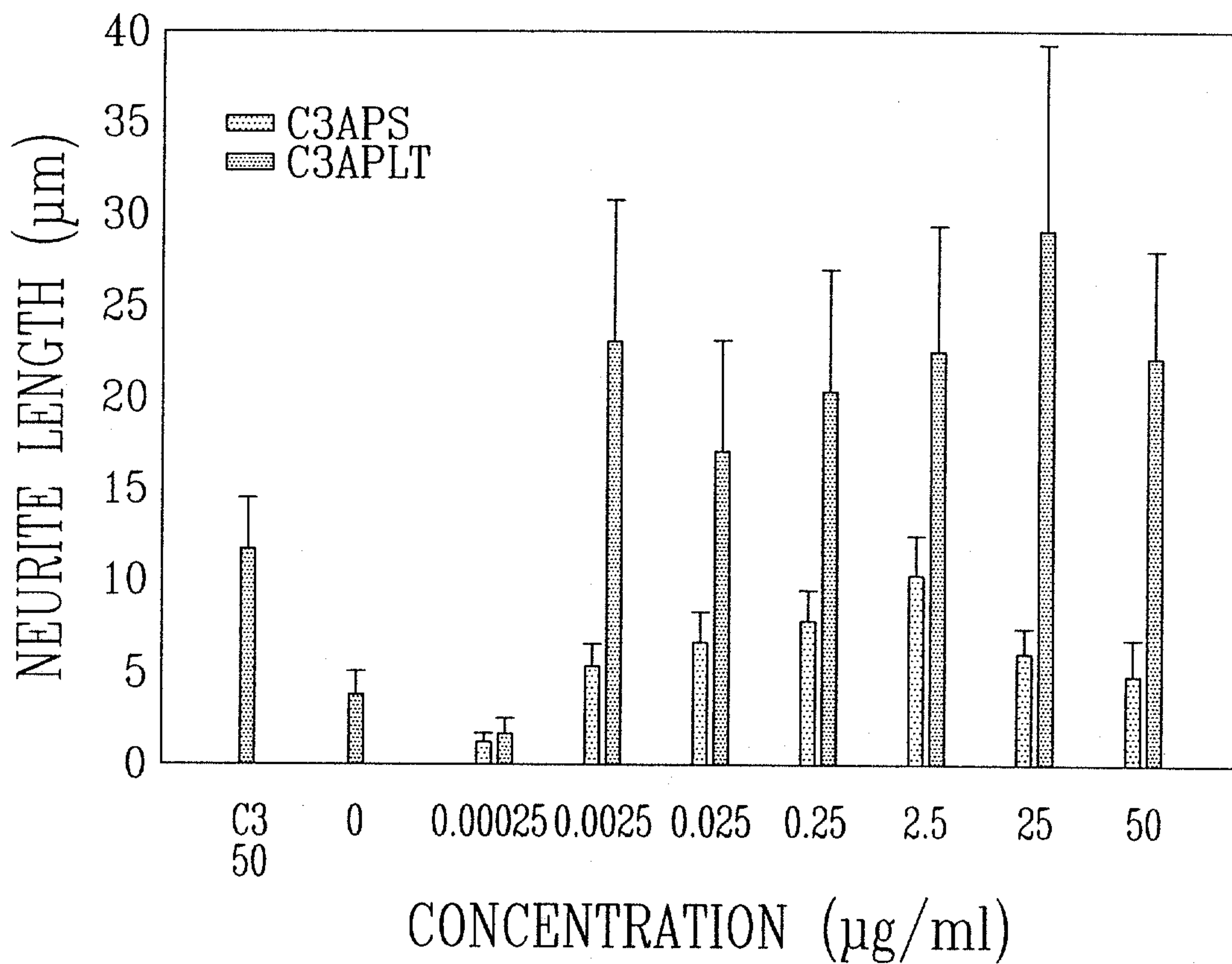
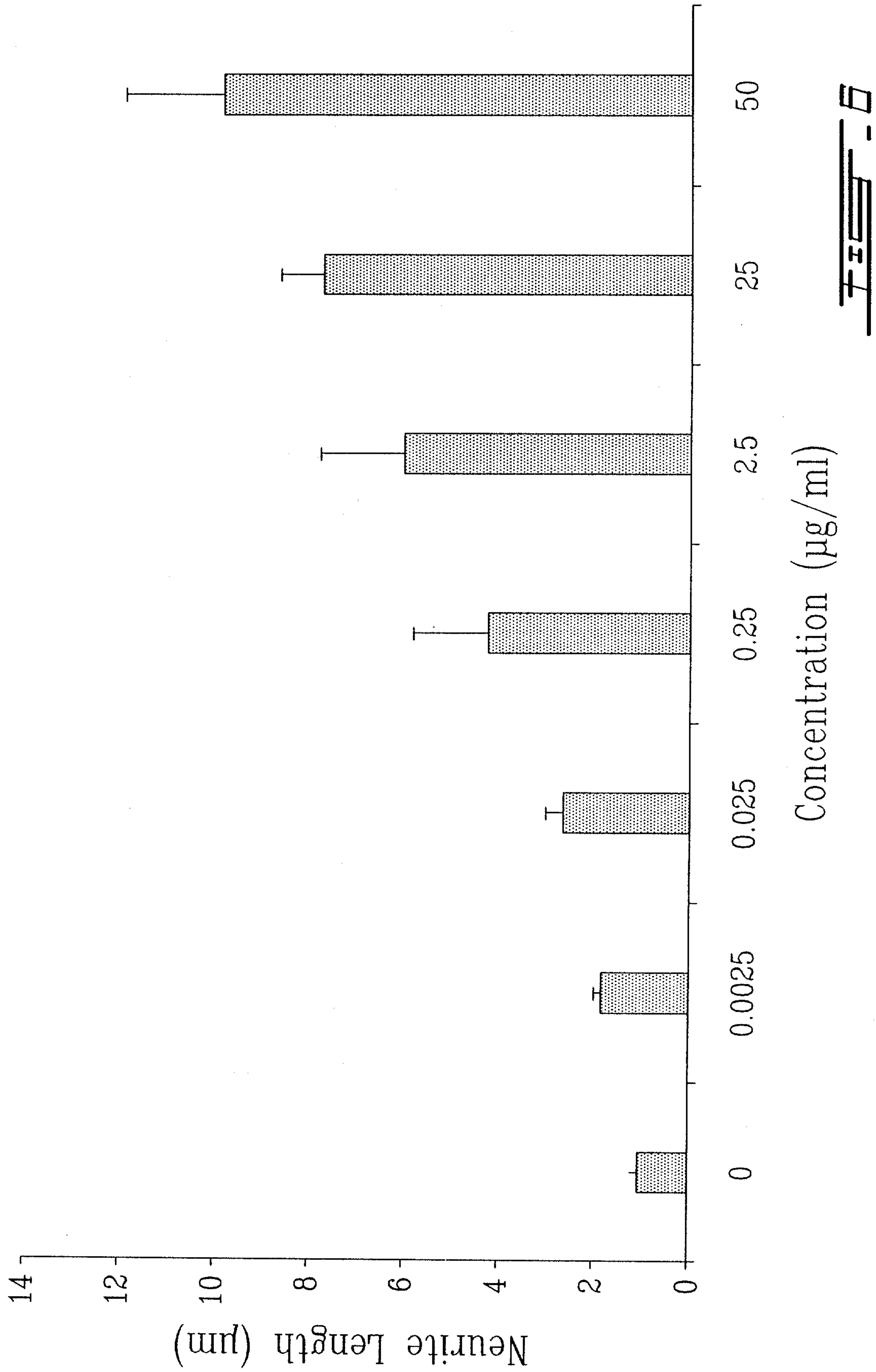
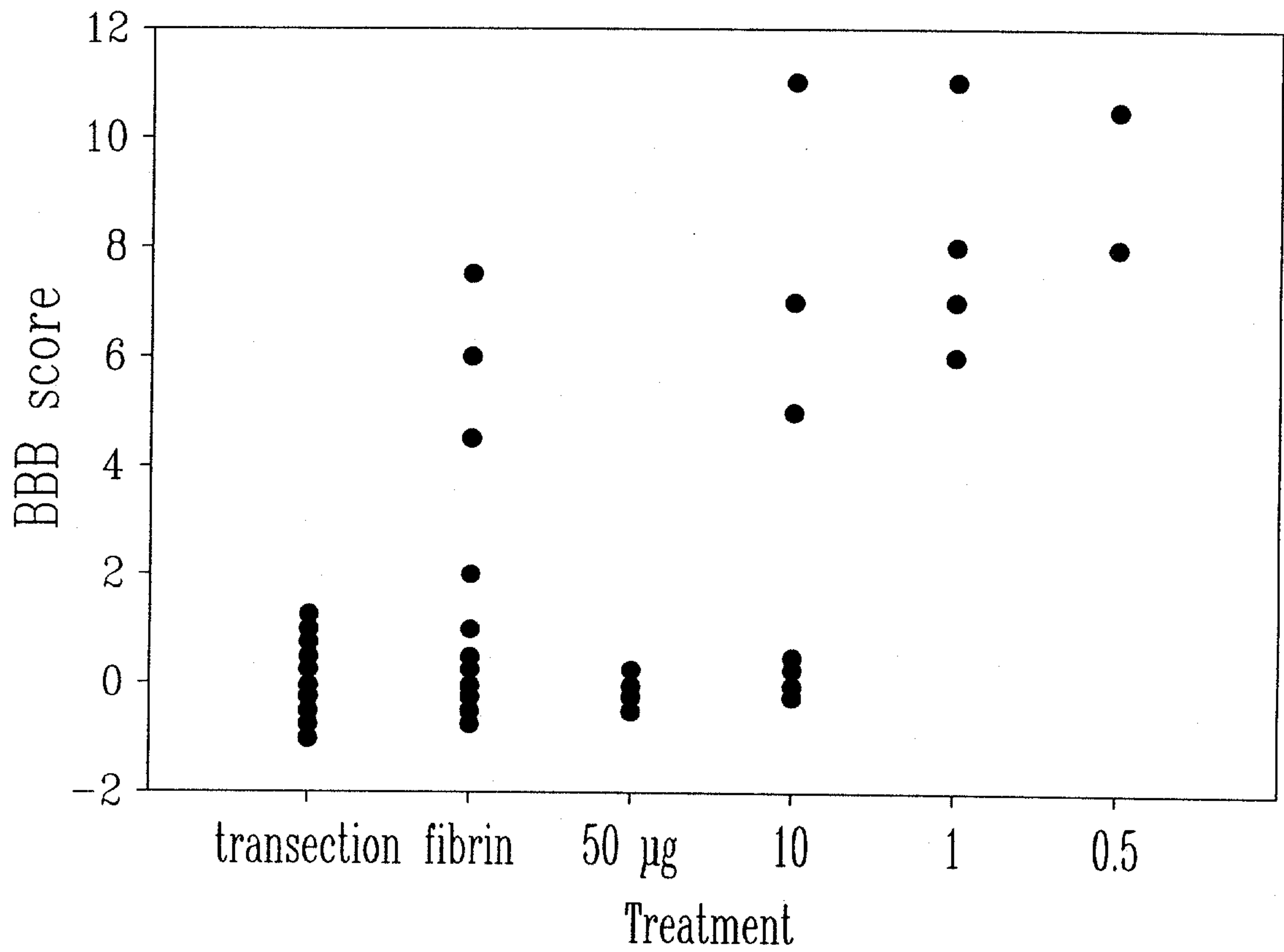
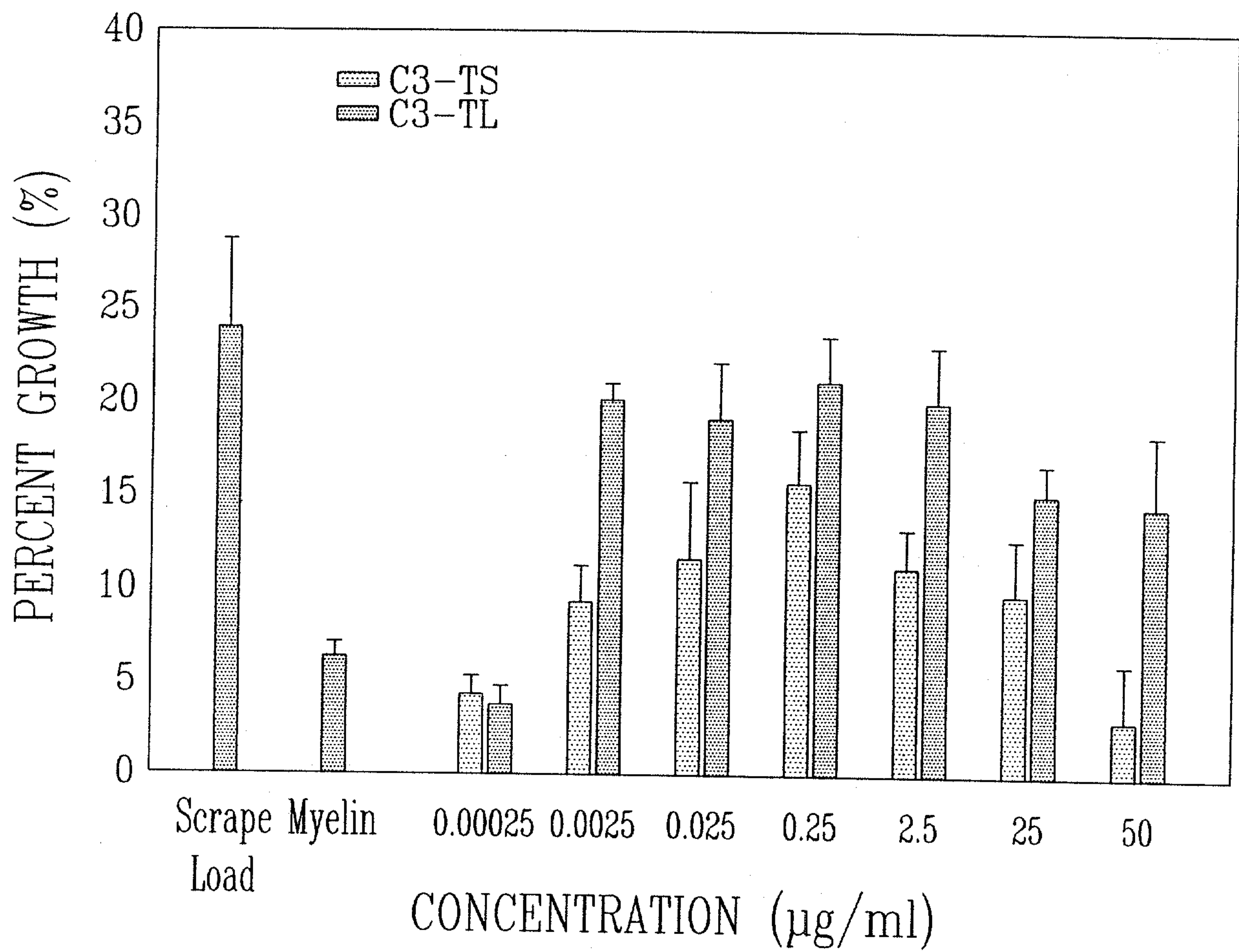


Figure 1

Figure 4Figure 5



Figure 7Figure 8

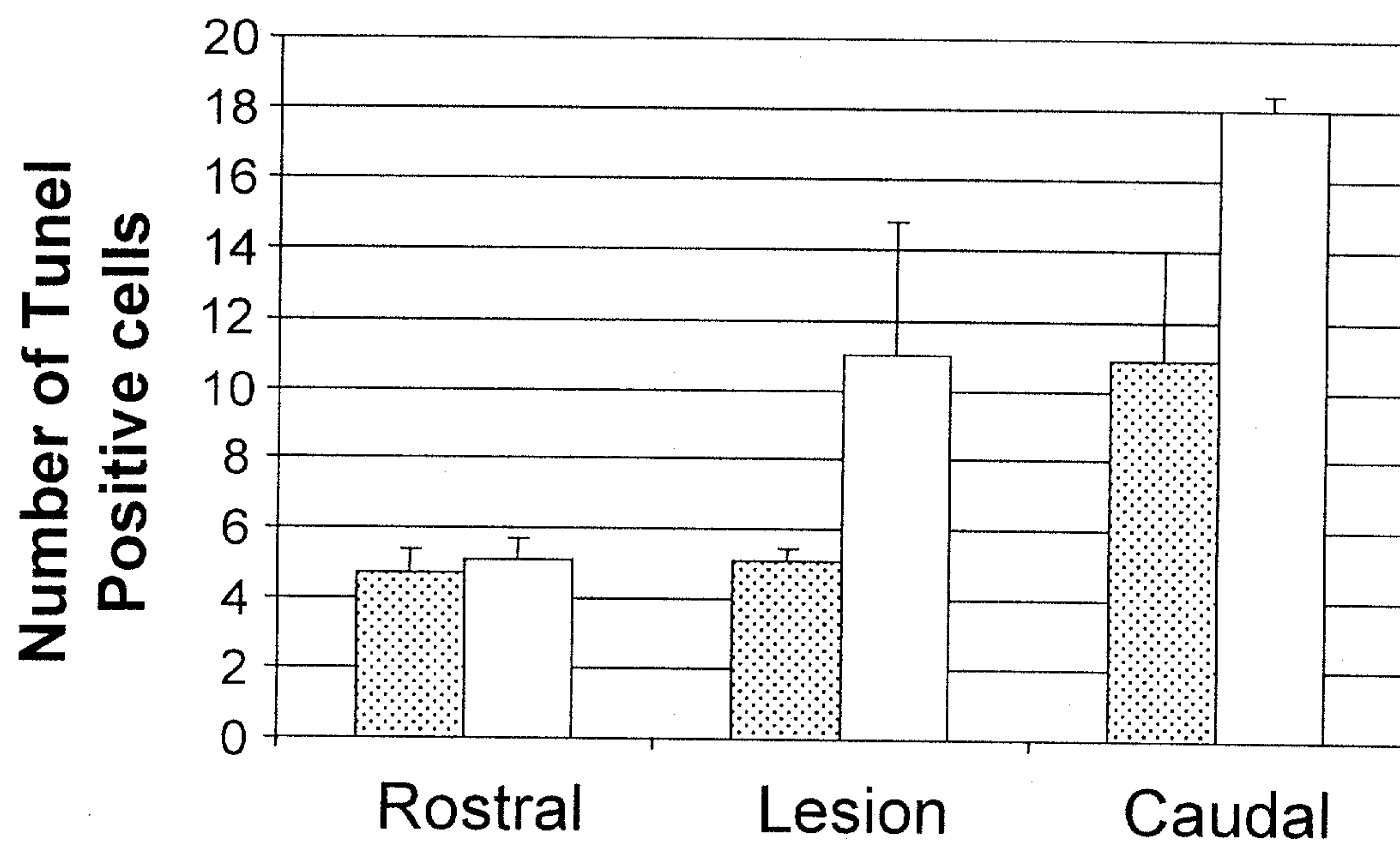


Figure 10

