



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/00	A2	(11) International Publication Number: WO 98/53053 (43) International Publication Date: 26 November 1998 (26.11.98)
(21) International Application Number: PCT/EP98/02970 (22) International Filing Date: 20 May 1998 (20.05.98) (30) Priority Data: 97108240.9 21 May 1997 (21.05.97) EP <i>(34) Countries for which the regional or international application was filed:</i> DE et al. (71) Applicant (for all designated States except US): FOUNDATION FOR RESEARCH AND TECHNOLOGY – HELLAS (FO.R.T.H.) [GR/GR]; Institute of Molecular Biology & Biotechnology, Vassilika Vouton, P.O. Box 1527, GR–711 10 Heraklion (GR). (72) Inventors; and (75) Inventors/Applicants (for US only): THIREOS, George [GR/GR]; Foundation for Research and Technology – Hellas (FO.R.T.H.), Institute of Molecular Biology & Biotechnology, Vassilika Vouton, P.O. Box 1527, GR–711 10 Heraklion (GR). KAFETZOPOULOS, Dimitris [GR/GR]; Foundation for Research and Technology – Hellas (FO.R.T.H.), Institute of Molecular Biology & Biotechnology, Vassilika Vouton, P.O. Box 1527, GR–711 10 Heraklion (GR).		(74) Agent: MÜLLER–BORE & PARTNER; Grafinger Strasse 2, D–81671 München (DE). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: DNA ENCODING AN ARTHROPOD CHITIN SYNTHASE (57) Abstract The present invention relates to nucleic acids comprising a nucleotide sequence encoding at least a portion of an enzyme which catalyzes the synthesis of chitin in arthropods, inhibitors directed to said enzyme, and a method for developing said inhibitors.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

DNA ENCODING AN ARTHROPOD CHITIN SYNTHASE

The present invention relates to nucleic acids comprising a nucleotide sequence encoding at least a portion of an enzyme which catalyzes the synthesis of chitin in arthropods, inhibitors directed to said enzyme, and a method for developing said inhibitors.

Chitin is a carbohydrate homopolymer of $\beta(1 \rightarrow 4)$ linked N-acetylglucosamine. Chitin is a structural polysaccharide occurring mainly in the cell wall of some fungi and in exoskeleton of the arthropods.

Chitin is synthesized by the action of specialized enzymes, referred to as chitin synthases, that catalyze the polymerization of N-acetylglucosaminyl residues into chitin from uridine 5'-diphospho-N-acetylglucosamine.

Chitin synthases belong to a group of enzymes that catalyze the synthesis of linear structural polysaccharides and are collectively termed as processive glycosyltransferases. The primary structures of the known processive glycosyltransferases (i.e. the fungal chitin synthases, the prokaryotic and eukaryotic cellulose synthases, the prokaryotic and eukaryotic hyaluronan synthases, the prokaryotic alginate synthases and the rhizobial chito-oligosaccharide synthases) present some sequence similarities suggesting that these UDP-sugar utilizing enzymes show a similar mode of action. Despite the similarities, the various processive glucosyltransferases display substantial differences mainly in their substrate specificities, their activation and inhibition parameters, the size and conformation of their products.

Since several arthropod species are considered as plant and animal pests, the inhibition of the chitin synthesis machinery has been viewed in particular as an attractive target for the development of pest control agent.

Known insect chitin synthesis inhibitors, either natural or synthetic, fall within two main categories: (a) direct inhibitors of chitin synthesis such as the Streptomyces antibiotics Nikkomycins and Polyoxins and (b) insect growth regulators that interfere indirectly but drastically with the synthesis of chitin in insects,

such as the benzoylphenyl-urea derivatives.

Although the studies for the identification of these compounds have a history of several decades, the action of chitin synthesis inhibitors is still inadequately understood. This is due to both the limited availability of suitable insect chitin synthase enzyme preparations and to the lack of any molecular insight on the chitin synthesis and its regulation in insects.

Thus, the technical problem underlying the present invention is to provide new inhibitors for the chitin synthesis in order to prevent damages caused by arthropods.

The solution to the above technical problem is achieved by providing the embodiments characterized in the claims.

In particular, there is provided a nucleic acid which comprises a nucleotide sequence encoding at least a portion of an enzyme which catalyzes the synthesis of chitin in arthropods.

The terms "nucleic acid" and "nucleotide sequence" refer to endogenously expressed, semisynthetic, synthetic or chemically modified acid molecules of deoxyribonucleotides and/or ribonucleotides. In a preferred embodiment of the present invention the nucleotide sequence include sequences as illustrated in Figures 1 (SEQ ID NO. 1), 2 (SEQ ID NO. 3), and 3 (SEQ ID NO. 5), allelic derivatives of said sequences and DNA-sequences degenerated as a result of the genetic code for said sequences. It also includes DNA sequences hybridizing under stringent conditions with the nucleotide sequence defined above. Although said allelic, degenerate and hybridising sequences may have structural divergences due to (naturally occurring) mutations, such as small deletions or substitutions, they will usually still exhibit essentially the same useful properties, allowing their use in basically the same applications.

In a preferred embodiment the present invention relates to an isolated DNA

sequence encoding a portion of an enzyme which catalyzes the synthesis of chitin in arthropods. Specific embodiments include DNA sequences which are characterized by the ability to hybridize to the DNA sequence represented in Figure 1 at e.g. 55 °C or encode a polypeptide reactive with an antibody against the polypeptide containing the deduced amino acid sequence in figures 1 to 3. The invention also relates to a DNA expression construct containing the isolated DNA sequences and encoding an enzyme having the specificity described above.

The present invention also relates to recombinant molecules comprising the nucleic acid as described above optionally linked to an expression-control sequence. Such vectors may be useful in the production of at least said portion of an enzyme in stable or transiently transformed cells. Several animal, plant, fungal and bacterial systems may be used for the transformation and subsequent cultivation process. Preferably, expression vectors which can be used in the invention contain sequences necessary for the replication in the host cell and are autonomously replicable. It is also preferable to use vectors containing selectable marker genes which can be easily selected for transformed cells. The necessary preparation is well known to those skilled in the art.

It is another object of the invention to provide a host cell transformed by an expression plasmid of the invention and capable of producing at least said portion of an enzyme. Examples of suitable host cells include various eukaryotic and prokaryotic cells, such as E. coli, insect cells, plant cells, mammalian cells, and fungi such as yeast.

Another object of the present invention is to provide a polypeptide comprising at least a portion of an enzyme which catalyzes the synthesis of chitin in arthropods. The term "polypeptide" includes the complete enzyme or a biologically active or inactive fragment thereof encoded by the sequences described above and displaying preferably biological features of said enzyme. The amino acid sequences of specially preferred polypeptides contain the sequences displayed in Figures 1 (SEQ ID NO. 2), 2 (SEQ ID NO. 4) and 3 (SEQ ID NO. 6).

It is a further aspect of the invention to provide a process for the production of the above mentioned polypeptide. Such a process comprises cultivating a host cell being transformed with a nucleic acid sequence of the present invention in a suitable culture medium and purifying the produced polypeptide. Thus, this process allows the production of the sufficient amount of the desired polypeptide for use in applications described herein below. The host cell is obtainable from bacteria such as E. coli, from fungi, such as yeast, from plants such as tobacco, potato, or Arabidopsis, and from animals, in particular vertebrate cells such as the CHO-cells.

The invention also relates to the methods for assaying the effects of various compounds on the expression and the biological activity of arthropod chitin synthases. In these methods the polypeptide in enzymatically active form, preferably the enzyme chitin synthase of an arthropod is produced by recombinant DNA techniques in which an isolated DNA sequence encoding the enzyme is expressed from a DNA expression construct as outlined above.

The figures show:

Figures 1 to 3 show DNA sequences (SEQ ID NOs. 1, 3 and 5) and the deduced amino acid sequences (SEQ ID NOs. 2, 4 and 6) of a portion of chitin synthase of Drosophila melanogaster.

Figures 4 and 5 show multiple protein sequence alignments of the portion of chitin synthase of Drosophila melanogaster with the corresponding regions of a nematode, three yeast chitin synthases and a rhizobial chito-oligosaccharide synthase. In particular, the multiple sequence alignments show the isolated portion of Drosophila melanogaster chitin synthase (Dmechs1 in Figure 4, and Dmechs2 in Figure 5) with the corresponding regions of the nematode chitin synthase (Celchs), the chitin synthase 1 (Scechs1), the chitin synthase 2 (Scechs2) and chitin synthase 3 (Scechs3) of yeast and the rhizobial chito-oligosaccharide synthase (RlenodC). The dots above the alignment indicate similarity and the asterisks identity.

In the following preferred embodiments of the present invention are outlined in more detail.

5 With the isolation of the first insect chitin synthase disclosed here, that of Drosophila melanogaster (fruitfly), recombinant methodology for the expression, purification and identification of chitin synthases can be used. These methods employed in assays for the evaluation and characterization of insect chitin synthase inhibitors can lead to the development of new potent pest control
10 agents.

The present invention was made possible with identification of the DNA sequence shown in Figures 1 to 3, encoding for a portion of chitin synthase of Drosophila melanogaster.

15 A search of the GENBANK databank using the deduced amino acid sequence of Drosophila melanogaster sequence revealed significant similarities with a nematode sequence presumably encoding for a chitin synthase, with several sequences encoding for fungal chitin synthases, and with several rhizobial chito-oligosaccharide synthases. The multiple sequence alignments of the Drosophila melanogaster chitin synthase with these sequences are presented in Figures 4
20 and 5.

25 Despite the sequence similarities of the Drosophila melanogaster chitin synthase with above described enzymes, hybridization of the Drosophila melanogaster DNA to neither the nematode nor the fungal genomic DNA could be observed even at low stringency conditions (0.75M [Na⁺], 55°C).

30 With the isolation of the new insect chitin synthase gene, that of D. melanogaster, the use of recombinant methodology in the production, characterization and identification of arthropod chitin synthases can be employed.

By reference to the DNA sequence listed below, hybridization, immunochemical or polymerase chain reaction amplification methods can be used for the isolation

of either cDNA or genomic DNA of chitin synthase from this species. The same approaches can be used for the identification and isolation of DNA encoding the chitin synthases of other arthropod species.

5 The isolated DNA sequences which fall within the scope of this invention can be used to express the encoded chitin synthases in large quantities in either prokaryotic or eukaryotic host cell.

10 In another aspect, the invention relates to methods for developing pest control agents. Chitin synthases of arthropods can be produced by recombinant DNA methodology as outlined above and used in enzyme assays for the evaluation and characterization of specific chitin synthase inhibitors. These preparations can also be used in the screening for the identification of new chitin synthesis inhibitors.

15 Further, DNA constructs of the arthropod chitin synthases fused with reporter genes (lacZ, GFP, luciferase) can be used to obtain transgenic insects or transformed cell lines as outlined above. These animals and cell lines can be used in the evaluation of effect insect growth regulators have in the biosynthesis of chitin.

20 The present invention will be further illustrated by the following examples.

EXAMPLE 1

25 A DNA fragment containing the isolated DNA sequence and encoding for the insect chitin synthase is inserted in an expression vector containing a lac inducible promoter. The construct is used to transform E. coli cells. Cells are grown in LB medium at half-log phase and the expression of chitin synthase is induced by the addition of 1mM IPTG.

30 After 4 hours the cell are harvested by centrifugation and lysed by sonication. The homogenate is centrifuged at 2000 g for 30 minutes at 4°C and the supernatant of the centrifugation is ultracentrifuged at 100 000 g for one hour. The

pellet containing the membrane fraction were resuspended in a buffer containing 25 mM Tris HCl pH 7.0 and 10 mM Magnesium chloride. This membrane preparation is subsequently used in chitin synthase inhibition assays.

5 The assays are performed by incubating a sample of the membrane fraction with UDP-GlcNAc 5mM and the compounds tested for chitin synthesis inhibition. The activity of chitin synthase is evaluated by either the measurement of incorporation of radiolabelled GlcNAc from UDP-GlcNAc into chitin or by the spectrophotometric estimation of released UDP by the method of the coupled enzyme reactions assay. Comparison of the measured activity to that obtained under control conditions (no compound added) is used for the evaluation of compounds as a chitin synthase inhibitor.

EXAMPLE 2

15 A DNA fragment encoding the promoter and a portion of the coding regions of the insect chitin synthase is fused to lacZ reporter gene. The DNA construct containing the translational fusion of Chitin synthase-lacZ is used for the transformation of Kc insect cells. After the selection and isolation of stable transformants the transformed cell line is used for the testing or screening for compound that affect the transcriptional activation of the chitin synthase gene. In these assays the cell line is incubated with the compounds for 24 hours and the effect of the compounds is evaluated by measuring and comparing the beta-galactosidase activity to that of the control.

EXAMPLE 3

25 100 ng of the DNA fragment, encoding the sequence presented in Figure 2, is used for the incorporation of radioactive nucleotides into the double-stranded DNA according the standard "nick translation" methodology. Radioactive DNA probes generated by this method are used for the identification and isolation of clones encoding for chitin synthases from a genomic DNA cosmid library of D. melanogaster. The DNA of the recombinant cosmids is placed (blotted) on

nitrocellulose filters, denatured and then hybridized with the radioactive DNA probe. Hybridisation is performed at low stringency conditions (0.75M [Na⁺], 55°C). Filters are then washed and exposed for autoradiography.

- 5 Cosmids which hybridized with the probe (positive clones), are isolated and further characterized with restriction enzyme analysis and DNA sequence analysis.

10 From the procedure described above a second gene (Dmechs2) encoding for chitin synthase in this species is identified and partially sequenced. The DNA sequence and the deduced amino acid sequence are presented in Figure 3.

15 Figure 5 shows multiple protein sequence alignment of the portion of Dmechs2 with the corresponding regions of a nematode, three yeast chitin synthases and a rhizobial chito-oligosaccharide synthase.

Claims

1. A nucleic acid which comprises a nucleotide sequence encoding at least a portion of an enzyme which catalyzes the synthesis of chitin in arthropods.
2. The nucleic acid according to claim 1, wherein the nucleotide sequence contains the DNA sequence as shown in SEQ ID NOs. 1, 3 or 5.
3. A recombinant molecule comprising a nucleic acid according to claim 1 or 2.
4. The recombinant molecule according to claim 3, wherein said nucleic acid sequence is functionally linked to an expression-control sequence.
5. A host containing the nucleic acid according to claim 1 or 2, or the recombinant molecule according to claim 3 or 4.
6. A polypeptide comprising at least a portion of an enzyme which catalyzes the synthesis of chitin in arthropods.
7. The polypeptide according to claim 6, wherein the portion contains the amino acid sequence as shown in SEQ ID NOs. 2, 4 or 6.
8. An inhibitor directed to at least the portion as defined in claims 6 or 7.
9. The inhibitor according to claim 8, which is directed to chitin synthase of arthropods.
10. A method for developing chitin synthetase inhibitors, comprising the steps of
 - (a) producing a polypeptide as defined in claims 6 or 7 using recombinant DNA technology, and
 - (b) characterizing specific chitin synthase inhibitors in enzyme assays.

FIGURE 1

GCC	ATT	GGA	CAC	TGG	CTG	CAA	AAG	GCG	ACG
A	I	G	H	W	L	Q	K	A	T
GAG	CAT	GTC	ATT	GGT	TGC	GTC	CTC	TGC	AGT
E	H	V	I	G	C	V	L	C	S
CCG	GGA	TGC	TTC	AGT	CTG	TTC	CGG	GCC	ACC
P	G	C	F	S	L	F	R	A	T
GCC	CTG	ATG	GAG	AAC	AGC	GTG	ATG	AAG	CGG
A	L	M	E	N	S	V	M	K	R
TAC	ACG	ATG	ATC	AGT	TCG	GAG	GCG	ATG	AAG
Y	T	M	I	S	S	E	A	M	K
ATG	GTG	CAG	TAC	GAC	CAG	GGC	GAG	GAC	CGC
M	V	Q	Y	D	Q	G	E	D	R
TGG	CTG	TGT	ACG	CTG	CTC	TTG	AAG	GCC	GGC
W	L	C	T	L	L	L	K	A	G
TTC	CGG	GTG	GAG	TAC	TGG	GGC	GCC	TTG	GAT
F	R	V	E	Y	W	G	A	L	D
GGG	TAT	ACC	CAA	GCG	CCA	GAG	AGC	TTT	AAC
G	Y	T	Q	A	P	E	S	F	N
GAG	TTC	TAC	AAC	CAA	CGA	AGT	CGT	TGG	ATC
E	F	Y	N	Q	R	S	R	W	I

FIGURE 2

GCC	ATT	GGA	CAC	TGG	CTG	CAA	AAG	GCG	ACG
A	I	G	H	W	L	Q	K	A	T
GAG	CAT	GTC	ATT	GGT	TGC	GTC	CTC	TGC	AGT
E	H	V	I	G	C	V	L	C	S
CCG	GGA	TGC	TTC	AGT	CTG	TTC	CGG	GCC	ACC
P	G	C	F	S	L	F	R	A	T
GCC	CTG	ATG	GAG	AAC	AGC	GTG	ATG	AAG	CGG
A	L	M	E	N	S	V	M	K	R
TAC	ACG	ATG	ATC	AGT	TCG	GAG	GCG	ATG	AAG
Y	T	M	I	S	S	E	A	M	K
ATG	GTG	CAG	TAC	GAC	CAG	GGC	GAG	GAC	CGC
M	V	Q	Y	D	Q	G	E	D	R
TGG	CTG	TGT	ACG	CTG	CTC	TTG	AAG	GCC	GGC
W	L	C	T	L	L	L	K	A	G
TTC	CGG	GTG	GAG	TAC	TGC	GGC	GCC	TCG	GAT
F	R	V	E	Y	C	G	A	S	D
GGG	TAT	ACC	CAC	GCG	CCA	GAG	AGC	TTT	AAC
G	Y	T	H	A	P	E	S	F	N
GAG	TTC	TAC	AAC	CAA	CGA	CGC	CGT	TGG	ATC
E	F	Y	N	Q	R	R	R	W	I

Figure 3

GCC CTC ATG GAT GAC AAT GTG ATG AAG AAA
A L M D D N V M K K

TAC ACG ACA CGG TCG GAT GAG GCT CGT CAC
Y T T R S D E A R H

TAC GTG CAG TAC GAT CAG GGC GAG GAT CGT
Y V Q Y D Q G E D R

TGG CTG TGC ACA TTG CTC CTC CAG AGG GGA
W L C T L L L Q R G

TAC CGC GTG GAG TAC TCG GCT GCC AGT GAT
Y R V E Y S A A S D

GCG TAC ACC CAC GCC CCC GAG GGA TTC AAT
A Y T H A P E G F N

GAG TTC TAC AAC CAG CGG CGG AGA TGG GTG
E F Y N Q R R R W V

FIGURE 4

```

      .      .      .      *      .      *      .      .      *
Dmechs  AIGHWLQKATEHVIGCVLCSPGCFSLFR.....
Celchs  AIAHWFQKAAEHVFGCVLCAPGCFSLFR.....
Scechs1 KMSNILDKTTESNFGFITVLPGAFSAYRFEAVRGQ....
Scechs2 KISNILDKPLESVFGYISVLPGALSAYRYRALKNHEDGT
Scechs3 YISHHQAKAFESVFGSVTCLPGCFSMYRIKSPKGS DGYW
Rlenodc WLACNEERAAQARFGAVMCCCCGPCAMYR.....

                        *                        *** *
Dmechs  ATALMENSVMKRYTMISSEAMKMVQ.YD....QGEDRWLC
Celchs  ASALMDDNIMHKYTKTASEPRHYVQ.YD....QGEDRWLS
Scechs1 .P.....LQKYFYGEIMENEGFHFFSSNMYLAEDRILC
Scechs2 GP.....LRSYFLGETQEGRDHDVFTANMYLAEDRILC
Scechs3 VPVLANPDIVERYSDNVTNTLHKKNLLL....LGEDRFLS
Rlenodc RSAML..SLLDQY...ETQLYRGKP.SD....FGEDRHLT

      .      .      .      *      *      .      *      **
Dmechs  .TLLLK..AGFRVEYWGALDGYTQAPESFNEFYNQSRWI
Celchs  .TLLLK..QGYRIEYAAASDAETYAPEGFEEFFNQRRRWT
Scechs1 FEVVTKKNCNWILKYCRSSYASTDVPERVPEFILQRRRWL
Scechs2 WELVAKRDAKWVLKYVKEATGETDVPEDVSEFISQRRRWL
Scechs3 .SLMLKTFPKRKQVFPKAACKTIAPDKFKVLLSQRRRWI
Rlenodc .ILMLS..AGFRTEYVPSAIAATVVPDTMGVYLRQQLRWA

```

```

          *                               *** *
Dmechs2  ALMDDNVMKKYTTRSDEARHYVQ.YD....QGEDRWLC.
  Celchs  ALMDDNIMHKYTKTASEPRHYVQ.YD....QGEDRWLS.
Scechs1  .....LQKYFYGEIMENEGFHHFSSNMYLAEDRILCF
Scechs2  .....LRSYFLGETQEGRDHDVFTANMYLAEDRILCW
Scechs3  VLANPDIVERYSDNVTNTLHKKNLLL.. .LGEDRFLS.
Rlenodc  AML  SLLDQY  ETQLYRGKP SD    FGEDRHLT.

          .           .           *  * .           *  **
Dmechs2  TLLLQ..RGYRVEYSAASDAYTHAPEGFNEFYNQRRRWV
  Celchs  TLLLK..QGYRIEYAAASDAETYAPEGFEEFFNQRRRW
Scechs1  EVVTKKNCNWILKYCRSSYASTDVPERVPEFILQRRRWL
Scechs2  ELVAKRDAKWVLKYVKEATGETDVPEDVSEFISQRRRWL
Scechs3  SLMLKTFPKRKQVFPKAACKTIAPDKFKVLLSQRRRWI
Rlenodc  ILMLS  AGFRTEYVPSAIAATVVPD TMGVYLRQQLRWA

```

FIGURE 5