

AUSTRALIA
PATENTS ACT 1990
MICROORGANISM NOTICE OF ENTITLEMENT

We, Ludwig Institute for Cancer Research of 1345 Avenue of the Americas, New York, New York 10105, United States of America, the applicant(s)/Nominated Person(s) in respect of Application No. 34817/93 state the following:-

The Nominated person is the depositor of the deposits listed hereafter:

Accession No.	Depository Institution	Deposit Date
I-1166	Collection Nationale de Cultures de Microorganismes Insitut Pasteur	24 April, 1992

DATED this 23rd day of January, 1995



.....
a member of the firm of
DAVIES COLLISON CAVE
for and on behalf of the
applicant(s)

660213

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PATENTS ACT 1990
NOTICE OF ENTITLEMENT

We, Ludwig Institute for Cancer Research, the applicant/Nominated Person in respect of Application No. 34817/93 state the following:-

The Nominated Person is entitled to the grant of the patent because the Nominated Person derives title to the invention from the inventors by assignment.

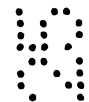
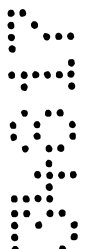
The Nominated Person is entitled to claim priority from the application listed in the declaration under Article 8 of the PCT because the Nominated Person is the assignee of the applicants in respect of the application listed in the declaration under Article 8 of the PCT, and because that application was the first application made in a Convention country in respect of the invention.

DATED this TWENTY FIFTH day of AUGUST 1994



a member of the firm of
DAVIES COLLISON
CAVE for and on behalf
of the applicant(s)

(DCC ref: 1680100)





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<p>(21) International Application Number: PCT/US93/00561 (22) International Filing Date: 19 January 1993 (19.01.93) (30) Priority data: 07/826,577 27 January 1992 (27.01.92) US (71) Applicant: LUDWIG INSTITUTE FOR CANCER RE- SEARCH [US/US]; 1345 Avenue of the Americas, New York, NY 10105 (US). (72) Inventors: MIYAZONO, Kohei ; HELDIN, Carl-Henrik ; OLOFSSON, Anders ; S-751 23 Uppsala (SE). (74) Agent: HANSON, Norman, D.; Felfe & Lynch, 805 Third Avenue, New York, NY 10022 (US).</p>		<p>(81) Designated States: AU, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> 660213</p>
<p>(54) Title: LARGE, LATENT COMPLEXES OF TGF-BETA2 AND TGF-BETA3</p>		
<p>(57) Abstract The invention relates to large latent complexes of TGF-β2 and TGF-β3, and methods for isolating these. The complex consists of a dimerized form of TGF-β2 or TGF-β3, the appropriate latency associated peptide, and the latent TGF-β1 binding protein, referred to as LTBP. Also described is a protein which binds to all of TGF-β1, TGF-β2 and TGF-β3, but is immunologically distinct from LTBP, referred to as LTBP-2.</p>		

Large, Latent Complexes of TGF-beta2 and TGF-beta3

5 FIELD OF THE INVENTION

This invention refers to the family of molecules known as the transforming growth factor betas, or "TGF- β s". More specifically, it refers to new complexes of these molecules, sometimes referred to as "large latent" or "LL" complexes. 10 The invention also relates to a newly recognized component of such "LL" complexes, referred to as "LTBP-2".

BACKGROUND OF THE INVENTION

Transforming growth factor beta, or "TGF- β " as used hereafter, refers to a family of multifunctional, dimeric 15 polypeptides having a molecular weight of about 25000 daltons. See U.S. Patent No. 4,931,548 to Lucas et al., the disclosure of which is incorporated by reference, as well as Lyons et al., Eur. J. Biochem 187: 467-473 (1990); Massagué, Ann. Rev. Cell Biol. 6: 597-641 (1990); Roberts et al., in Peptide 20 Growth Factors And Their Receptors, part 1 (Sporn et al., ed), pp. 419-472 (1990); Sporn et al., Science 233: 532-534 (1986); Massagué, Trends in Biochem. Sci. 10: 239-240 (1985). The TGF- β s have been found to stimulate certain cell types and to inhibit others with respect to cell growth and 25 differentiation. They also influence adipogenesis, myogenesis, chondrogenesis, osteogenesis, epithelial cell differentiation and immune cell function. See Lucas et al., supra.

At least three related isoforms of TGF- β have been 30 identified, i.e., "TGF- β 1, TGF- β 2 and TGF- β 3". Although related, their properties are not identical, as summarized by, e.g., Graycar et al., Mol. Endocrinol 3: 1977-1986 (1989), Cheifetz et al., J. Biol. Chem. 265: 20533-10538 (1990). Promoter regions of the three isoforms vary considerably, and 35 their production is differently regulated, as pointed out by Roberts et al., Ciba Found. Symp. 157: 7-28 (1991).

TGF- β molecules have been observed to be produced in an

inactive, high molecular weight forms. For example, TGF- β 1, isolated from human and rat platelets, have been found as a complex of three components, referred to hereafter as the "large latent complex", the "LL" complex or "LLTGF- β 1". This complex consists of a dimer of the active TGF- β 1 molecule, i.e., the 25 KDa structure referred to supra. It also includes a molecular moiety referred to as the "latency associated peptide" or " β 1-LAP", and a larger molecule, referred to as the latent TGF- β 1 binding protein or "LTBP". As to the high molecular weight forms, see Pircher et al., Canc. Res. 44: 5538-5543 (1984); Wakefield et al., J. Cell Biol. 105: 965-975 (1987). As to β 1-LAP and LTBP, see Miyazono et al., J. Biol. Chem. 263: 6407-6415 (1988); Wakefield et al., J. Biol. Chem. 263: 7646-7654 (1988); and Okada et al., J. Biochem 106: 304-310 (1989). Latent TGF- β 1 can be activated in vitro via various physical and chemical treatments or by exposure to low or high pH (Brown et al., Growth Factors 3: 35-43 (1990)). The activating mechanism in vivo remains unclear, but may involve enzymatic digestion, as suggested by Miyazono et al., Ciba Found. Symp. 57: 81-89 (1991).

The three recognized forms of TGF- β have been produced, in recombinant form, where each form of TGF- β dimer is non covalently associated with the β -LAP. These complexes are inactive, have a molecular mass of about 100 KDa, and are activated to produce the mature and active TGF- β dimer. See Brown, supra; Gentry et al., Mol. Cell Biol. 7: 3418-3427 (1987). The complex of TGF- β and β -LAP is referred to as a "small, latent TGF- β complex".

The role of LTBP in vivo is not completely clear. It has been found to be involved in the manufacture and secretion of TGF- β 1 by a human erythroleukemia cell line. (Miyazono et al., EMBO J 10: 1091-1101 (1991)). The cDNA for the molecule has been cloned, and the protein contains several epidermal growth factor like repeats. See Kanzaki et al., Cell 61: 1051-1061 (1990); Tsuji et al., Proc. Natl. Acad. Sci. USA 87: 8835-8839 (1990). This feature is shared with many other

molecules. An additional repeating structure is also found, which has 8 cysteine residues in one motif.

The structure of the LL-TGF β 1 complex has been analyzed in some detail, and is as described supra; however, the LL
5 complexes of TGF- β 2 and TGF- β 3 have not been studied. Given the fact that the TGF- β 2 and TGF- β 3 molecules differ from TGF- β 1, and that their associated "LAP" proteins differ, it would have been expected that there would be a binding protein specific to each form and differing from that associated with
10 TGF- β 1. Surprisingly, it has been found that the binding protein for both TGF- β 2 and TGF- β 3 is the same as that for TGF- β 1. Isolated large latent complexes are thus described which contain (i) either dimerized TGF- β 2 or TGF- β 3, (ii) the B-LAP for the TGF- β 2 or TGF- β 3 form, and (iii) the LTBP
15 molecule, which was previously associated only with TGF- β 1. The complexes are useful as inactive forms of TGF- β 2 and TGF- β 3, which can be treated to yield the active TGF- β 2 and TGF- β 3 molecules.

The investigations described herein led to a surprising
20 discovery in that an additional binding protein immunologically distinct from LTBP and having a molecular mass of about 150 KDa associates with all of TGF- β 1, TGF- β 2 and TGF- β 3. This is referred to as "LTBP-2" hereafter. Thus, new complexes containing TGF- β 1 are described, as well as a second
25 form of isolated LL-TGF- β 2 and LL-TGF- β 3 complexes. All of the complexes described herein are characterized in preferred embodiments by a molecular weight of about 210 KDa as determined by SDS-Page.

These and other aspects of the invention are elaborated upon
30 in the disclosure which follows.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A, B and C show the results of immunoblot studies on conditioned medium obtained from various human cell lines (glioblastoma and fibroblasts), using antisera to various TGF- β s and LTBP.
35

Figure 2A presents ion exchange chromatography using a Q-Sepharose column and [3 H]thymidine incorporation data for TGF-

β containing fractions of conditioned medium from glioblastoma cells.

Figure 2B depicts immunoblot analysis ion exchange of chromatography eluents.

5 Figure 2C is analysis of flow through fractions of ion exchange chromatography.

Figure 3 presents data secured when a fraction of Sepharose eluent was applied to an anti-LTBP Sepharose column followed by immuno-blotting using anti-LTBP, anti- β 1-LAP, anti β 2-LAP,
10 and anti β 3-LAP.

Figure 4 shows analysis of a fraction of Q-Sepharose eluent which contains a component that is not LTBP, i.e. LTBP-2.

Figure 5A schematically shows the purification protocol described herein.

15 Figure 5B presents molecular models of the small latent TGF- β complex, the large latent TGF- β complex with LTBP, and large latent TGF- β complexes with the non-LTBP model.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Example 1

20 Various cell lines are used in the experiments described infra. This example discusses the various conditions under which these were grown and cultured.

The different human glioblastoma cell lines used were cultured in Dulbecco's modified Eagle's medium, supplemented
25 with 10% fetal bovine serum ("FBS" hereafter, and antibiotics (100 U of penicillin, 50 μ g of streptomycin). The cells were kept in a 5% CO₂ atmosphere at 37°C.

The human foreskin fibroblast cell line AG 1518 is publicly available. This was cultured in Eagle's minimum essential
30 medium supplemented with 10% FBS and the antibiotics listed supra.

Cell line PC-3 is a human prostate carcinoma cell line, and it was cultured in RPM1 1640 supplemented with 10% FBS and antibiotics.

35 To study the complexes, large amounts of conditioned medium from the cell line U-1240 MG, a human glioblastoma, were required. To achieve this, the cells were grown to confluence

in roller bottles. These were then washed, three times, with phosphate buffered saline, and were then incubated in 50 ml of serum free Dulbecco's modified Eagle's medium (DMEM) per bottle. The medium was collected after two days. This procedure was repeated with three days of replenishment of the cells, using DMEM with 10% FBS between each collection. In an alternative protocol, the conditioned medium was harvested every two days over a six day period. The cells were then grown in DMEM with 10% FBS for one week. The collection scheme was repeated three times.

Collected conditioned medium, regardless of how secured, was centrifuged at 2000xg for 10 minutes, and the supernatants were stored at -20°C.

For other cell types, the conditioned media were collected on a much smaller scale. To that end, 175 cm² Falcon flasks were used, and DMEM plus 10% FBS was the medium. The atmosphere was 5% CO₂. For AG 1518 fibroblasts, the medium used was Eagle's minimum essential medium.

Example 2

Experiments were first carried out to determine if the various cell lines cultured were producing latent TGI- β complexes. To do this, conditioned medium was collected from control cell line AG 1518, and four human glioblastoma cell lines (U-1240 MG; U-251MGO; U-251 MGSp; U-343 MGa Cl2:6). Conditioned media (500 ul; 4 ml/lane) was concentrated 50 fold using ultrafiltration in the presence of 0.1% SDS. These media had not been subjected to ammonium sulphate precipitation. The samples were then analyzed via SDS-gel electrophoresis. This analysis involved mixing the samples with SDS-sample buffer (100 mM Tris-HCl, pH 8.8, 0.01% bromophenol blue, 36% sucrose and 4% SDS) without reducing agents, and heating to 95°C for 3-4 minutes. After this, the samples were applied to 5-18% polyacrylamide gels, in accordance with Blobel et al., J. Cell Biol. 67: 835-851 (1975) under non-reducing conditions. Following this, the sample was immunoblotted. First, it was electrophoretically transferred to a nitrocellulose membrane for 12-16 hours in

the presence of 0.02% SDS. Following this, the blotted samples were contacted first with an antiserum against LTBP (Miyazono et al., EMBO J 10: 1091-1101 (1991), and then with antiserum against each of β 1-LAP, β 2-LAP, and LTBP. The antibodies were visualized using ^{125}I labeled protein A followed by autoradio-graphy, in accordance with Miyazono et al., Biochemistry 28: 1704-1710 (1989).

The results of the immunoblotting, presented in Figures 1A, 1B and 1C, show that when LTBP specific antiserum was used, multiple bands corresponding to sizes between 100-200 KDa, and between 210 and 310 KDa₁ were generated. This was true both for the fibroblast line AG1518, as well as for all glioblastoma lines.

When β 1-LAP specific antisera was used, it showed the presence of TGF- β 1 in large complexes of about 220 KDa, as well as small complexes (80-100 KDa), in conditioned media from U-1240MG and U-251 MGsp (panel 1B; lanes b and d, respectively). Panel 1C, which depicts experiments using β 2-LAP antiserum shows that small or large complexes were seen only in U-1240 MG.

Comparable experiments were carried out using β 3-LAP specific antisera. While these results are not shown, faint bands were found in all glioblastomas.

Interpretation of these data indicate that the larger bands in panel 1A most probably represent associations of LTBP with β -LAPs, with the smaller bands representing free LTBP.

Example 3

In order to assess the TGF- β 1 activity of the conditioned media from the tested cell lines, inhibition of growth of mink lung epithelial cells, CCL 664 was tested, using a [^3H]thymidine incorporation assay as described by Miyazono et al., EMBO J 10: 1091-1101 (1991). TGF- β is known to inhibit the growth of these cells.

Samples were concentrated 10 times using ultrafiltration. Total TGF- β activity was determined after heating media to 80°C for 10 minutes to activate any inactive TGF- β . Contribution of inhibitory activity not from TGF- β 1 was estimated by assaying samples in the presence of a TGF-



$\beta 1$ /TGF- $\beta 2$ neutralizing antibody. (It is unknown if this antibody neutralizes TGF- $\beta 3$). The contribution of activity which could not be neutralized with the antibody was about 10%, in both the active and non-active fractions.

5 The results, which are shown in Table 1, show that while U-251 MGO, U-251 MGsp and U-343 MGa Cl2:6 give similar activities, U-1240 MG gave much higher values, although there were large variations from batch to batch. The percent of TGF- β activity in U-1240 MG conditioned medium from active
10 forms was found to be 26% while no active forms were found in the other lines.

15 These data suggested that U-1240 MG should be chosen for further studies of the structural properties of small and large latent TGF- β complexes, and the relationship between LTBP and TGF- $\beta 2$.

Table I

cell line	TGF- β activity	
	(ng/ml)	(% active TGF- β)
U-1240 MG	8 ± 7^a	26 ± 12^b
U-251 MGO	0.5	< 1
U-251 MGsp	0.6	< 1
U-343 MGa Cl 2:6	0.6	< 1

^aData are expressed as means \pm S.D., n = 6.

^bData are expressed as means \pm S.D., n = 4.

30 Example 4

In order to characterize the different TGF- β complexes synthesized and secreted by U-1240 MG, the conditioned medium, collected as described in Example 1, was used. 3350 ml of conditioned medium was obtained, it was thawed, recentrifuged,
35 and passed through siliconized glass wool. Following this, a solution of 95% ammonium sulfate was added, and the mixture was equilibrated at 4°C overnight. This treatment results in

a precipitated protein, which was recovered by centrifuging at 8000xg for 25 minutes. The resulting pellet was dissolved in 220 ml of 50 mM NaCl, 10 mM phosphate buffer, pH 7.4, then dialyzed against the same buffer, followed by filtration through siliconized glass wool and a 0.45 μ m filter. The resulting solution (350 ml) was applied on to a Q-Sepharose column for chromatography. A flow-through portion resulted, as did eluents. The column was equilibrated with 50 mM NaCl, 10 mM phosphate, at pH 7.4, at a flow rate of 4 ml/min at 0°C. Elution was carried out using an NaCl gradient of from 50 mM to 1000 mM in 10 mM phosphate, pH 7.4 at a rate of 4 mM NaCl/ml and a flow rate of 2.5 ml/min. Eluents were collected in 5 ml fractions. The fractions were treated as indicated supra (i.e., subjected to SDS PAGE separation, but using a S-18% gradient gel), and were immunoblotted using the ECL Western blotting system. Antisera against each of β 1-LAP, β 2-LAP, β 3-LAP and LTBP were used, leading to the patterns shown in Figure 2B.

These results show that β 1-LAP was found in fractions 14-22 at a size of about 210 KDa. The β 2-LAP complex was also found in fractions 14-22, also as a large complex of about 210 KDa. A small complex was found in fractions 8-12, and having a mass of about 75 KDa.

As to β 3-LAP, small amounts of 210 KDa large complex were found in fractions 14-20, together with small complexes of 74 KDa in fractions 10-12.

The flow-through portion of the test material was also immunoblotted, and these results are shown in Figure 2C. 80 and 97 KDa forms were identified with anti- β -LAP. The 80 KDa form probably indicates a β 1-LAP dimer. The 97 KDa entity is probably an unprocessed TGF- β 1 precursor dimer, and/or a complex of β 1-LAP and mature TGF- β 1, held together by an anomalous disulphide bond.

With respect to TGF- β 2, a small TGF- β 2 complex of 75 KDa is found in the flow through fraction. Similarly, small TGF- β 3 complexes were found in flow through.

As to LTBP, this was found in fractions 18-22 in 210 KDa

complexes, and in a free form at a size of about 150 KDa in fractions 20-22.

Example 5

For further characterization fractions containing TGF- β activity were divided into four pools denoted A (fractions 8-13), B (fractions 14-16) C (fractions 17-22) and flow through.

Pool B interestingly contained large complexes but no LTBP, suggesting that TGF- β s in these fractions are covalently associated with other molecule(s) of similar size(s) as LTBP. Part C (fractions 17-22) contained large TGF- β complexes with LTBP and LTBP in a free form (see Figure 2B).

Experiments were carried out to determine the activity of the TGF- β material in each pool. The mink lung epithelial cell assay was used, as described supra, and the results are summarized in Table 2, which follows. To summarize, 30% of activity was found in the flow-through fraction, 12% in pool A, 19% in pool B, and 26% in pool C. The total recovery, compared to the medium prior to Q-Sepharose chromatography, is 96%.

Table II

materials	TGF- β		protein (mg)
	(μ g)	(%)	
starting material	53	100	410
Q-Sepharose			
flow-through	20	39	52
pool A	7	12	37
pool B	10	19	74
pool C	14	26	112

Experiments show that all three forms of β -LAP occur in so-called "small forms" of 75-97 KDa, and large forms of 210 KDa. TGF- β activity in conditioned medium is usually latent, suggesting that different forms probably represent small and large latent TGF- β complexes.

Example 6

The observation that large latent complexes did not necessarily contain LTBP merited further experimentation. Further purification of pools B and C by chromatography on a mono Q column followed by chromatography on an alkyl sepharose column showed that it was possible to obtain some further separation of the large latent TGF- β complexes containing LTBP, from those not containing LTBP, but a complete separation could not be obtained.

In order to investigate whether each one of the TGF- β isoforms could form large latent complexes with LTBP as well as with LTBP2 separation using LTBP sepharose was employed. 500 ml of conditioned medium from U-1240 MG was subjected to Q-sepharose chromatography as described supra with the exception that the material was not subjected to ammonium sulphate precipitation. Fractions in the salt gradient were assayed by immunoblotting with antisera against LTBP and β_1 LAP. Fractions which contained large TGF- β complexes with LTBP (corresponding to pool C) and fractions containing large TGF- β complexes without LTBP (corresponding to pool B) were pooled separately.

The pool C was incubated with Sepharose beads, which had been previously coated with anti-LTBP antiserum. To make this material, immunoglobulin fractions of antiserum to LTBP was purified via chromatography on protein A Sepharose. After this, the immunoglobulin fraction was eluted with 100 mM citric acid, pH 3.0. About 50 mg of immunoglobulin was obtained using 10 ml of serum. The immunoglobulin was then dialyzed against phosphate buffered saline, followed by coupling to CNBr activated Sepharose. Approximately 17 mg of immunoglobulin was added per gram of these beads. Medium from pool C described supra was then incubated with 2.5 ml portions of the treated Sepharose. Beads were washed with 0.5 M NaCl, 100 mM Tris·HCl, pH 8.0, and then with 0.15 M NaCl, 10 mM Tris·HCl, pH 8.0. After this, bound protein was eluted by heating to 96°C in the presence of 1% SDS, 20 mM Tris·HCl, pH 8.8. The eluted protein was concentrated via centricon 10, as

described, and elution and immunoblotting as described supra was carried out using antisera to LTBP β 1-LAP β 2-LAP and β 3-LAP. The results shown in Figure 3 indicates that all TGF- β isoforms are present in large latent complexes associated with LTBP.

Pool B was then analyzed for the presence of a large latent complex containing a component distinct from LTBP. To this, pool B was incubated with anti-LTBP Sepharose, prepared as described supra. This absorbed any LTBP from the fraction. The unabsorbed fraction was then applied to SDS-gel electrophoresis using 5-15% gradient gel, followed by immunoblotting, also as described, and using the ECL detection system. As a positive control, free LTBP prepared from PC-3 cells conditioned medium was used.

The results are shown in Figure 4. Lane b shows that anti LTBP serum gave no indication of the molecule being present, while the PC-3 sample clearly shows free LTBP. When anti- β -LAP antisera were used, however, complexes of 205 kd were revealed, showing that each complex does in fact exist as a large latent complex with a molecule which is not LTBP, but which does have a molecular mass of about 150 kd.

A summary of the purification protocols described in these examples is presented in Figure 5a, together with an indication of the species found in each fraction. Figure 5b shows the derived structure of the various forms of TGF- β complexes discussed herein.

Several features of the invention are worth noting and are described here. First, it has unexpectedly been found that eukaryotic cells, such as human cell lines, ~~exist~~^{exist.} which produce large latent complexes of all TGF- β isoforms. "Large latent complex" as defined supra refers to a construct containing three parts: (i) the dimerized form of a TGF- β molecule, such as TGF- β 1, TGF- β 2 or TGF- β 3, (ii) the latency associated protein or "B-LAP" molecule, and (iii) the latent TGF- β 1 binding protein, or "LTBP". These cells and cell lines can also produce constructs where the third element is replaced by another moiety, discussed infra. When the cellular material



is described as producing the stated TGF- β isoforms, such a statement does not preclude its production of complexes where the third moiety is replaced.

Human glioblastoma cell lines are preferred, in particular, cell line U-1240, MG, which has been deposited at the Collection Nationale de Cultures de Microorganismes (CNC M) on 27 January 1992 and then 24 April 1992, Institut Pasteur, 28, rue due Docteur Roux, 75724 Paris Cedex 15, France, under Accession Number I-1166 in accordance with the Budapest Treaty.

The identification of the complexes of β -LAP, TGF- β and LTBP molecules enables the skilled artisan to manufacture isolated complexes containing these. As has been indicated supra, complexes of TGF- β 1, β 1-LAP and LTBP are known, but it was not known, nor was it suggested, that TGF- β 2 and TGF- β 3, individually, associate with their corresponding β -LAP moiety and LTBP, previously believed to associate with the TGF- β 1 form of the TGF- β molecule only.

The experiments have also identified new large latent complexes, wherein a TGF- β molecule, its associated LAP moiety, and a non-LTBP moiety associate. This latter moiety is characterized by a molecular mass of about 150 kd as determined by SDS-Page, and by being immunologically distinct from the recognized LTBP molecule. "Immunologically distinct" means that antibodies which are specific to LTBP do not bind to this non-LTBP molecule. This molecule is referred to as "LTBP-2" hereafter.

One can, of course, produce any of the complexes, as well as the isolated non-LTBP molecule by culturing the cell lines discussed supra, and then purifying the resulting complexes. This can be done via, e.g., contact with antibodies specific for the TGF- β component of the complex.

Other variations and modifications of the invention described herein will be clear to the skilled artisan and need not be elaborated upon herein.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding



or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Isolated large latent TGF- β complex, consisting of:
 - 5 (i) dimerized TGF- β 2 or dimerized TGF- β 3;
 - (ii) a beta latency associated peptide; and
 - (iii) latent, TGF- β 1 binding protein.

2. The isolated large latent TGF- β complex of claim 1, wherein said complex
10 contains dimerized TGF- β 2.

3. The isolated large latent TGF- β complex of claim 1, wherein said complex
contains dimerized TGF- β 3.

- 15 4. Isolated molecular complex, consisting of:
 - (i) a dimerized form of a TGF- β molecule,
 - (ii) a beta latency associated peptide, and
 - (iii) a latent TGF- β binding protein, referred to as LTBP-2 which
is immunologically distinct from LTBP, and has a molecular mass of about 150 KDa
20 as determined by SDS-PAGE.

5. The isolated molecular complex of claim 4, wherein said TGF- β molecule
is TGF- β 1.

- 25 6. The isolated molecular complex of claim 4, wherein said TGF- β molecule
is TGF- β 2.

7. The isolated molecular complex of claim 4, wherein said TGF- β molecule
is TGF- β 3.
30

8. Isolated LTBP-2 protein which binds to TGF- β 1, TGF- β 2, and TGF- β 3, has
a molecular mass of about 150 kd as determined by SDS-Page, and which is



immunologically distinct from LTBP.

Dated this 23rd day of January, 1995

Ludwig Institute for Cancer Research

5 By DAVIES COLLISON CAVE

Patent Attorneys for the Applicant(s)

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FIG. 1A

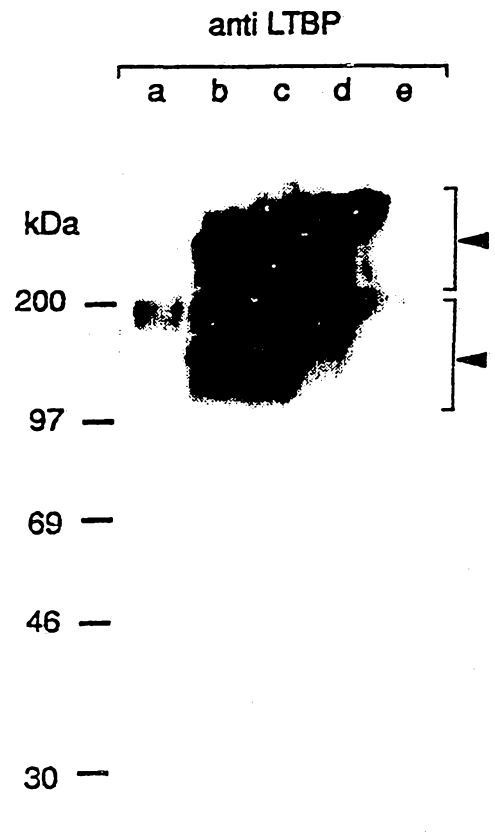


FIG. 1B

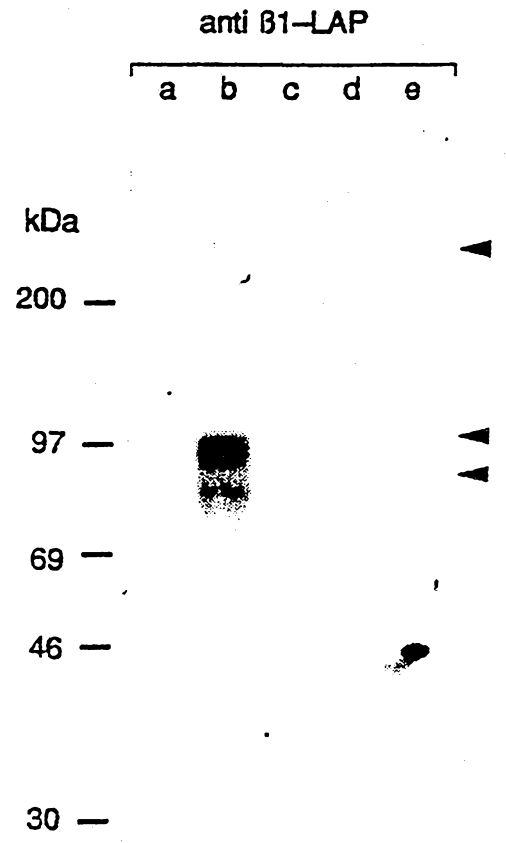
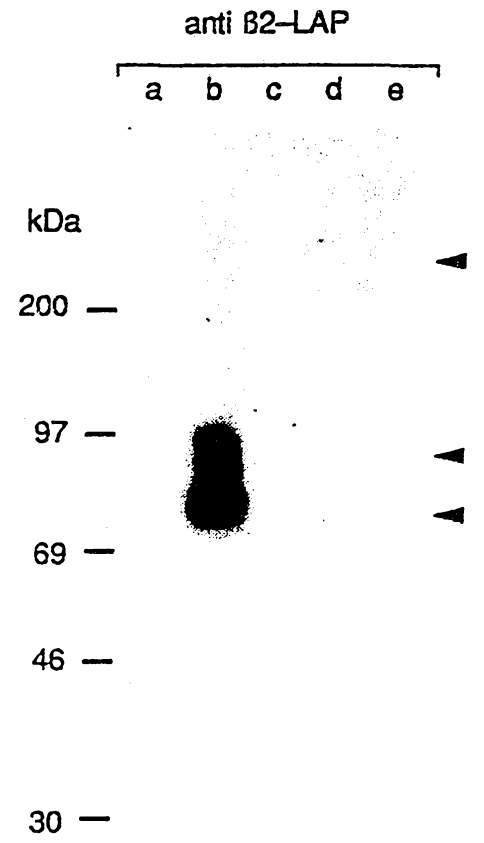


FIG. 1C



SUBSTITUTE SHEET

FIG. 2A

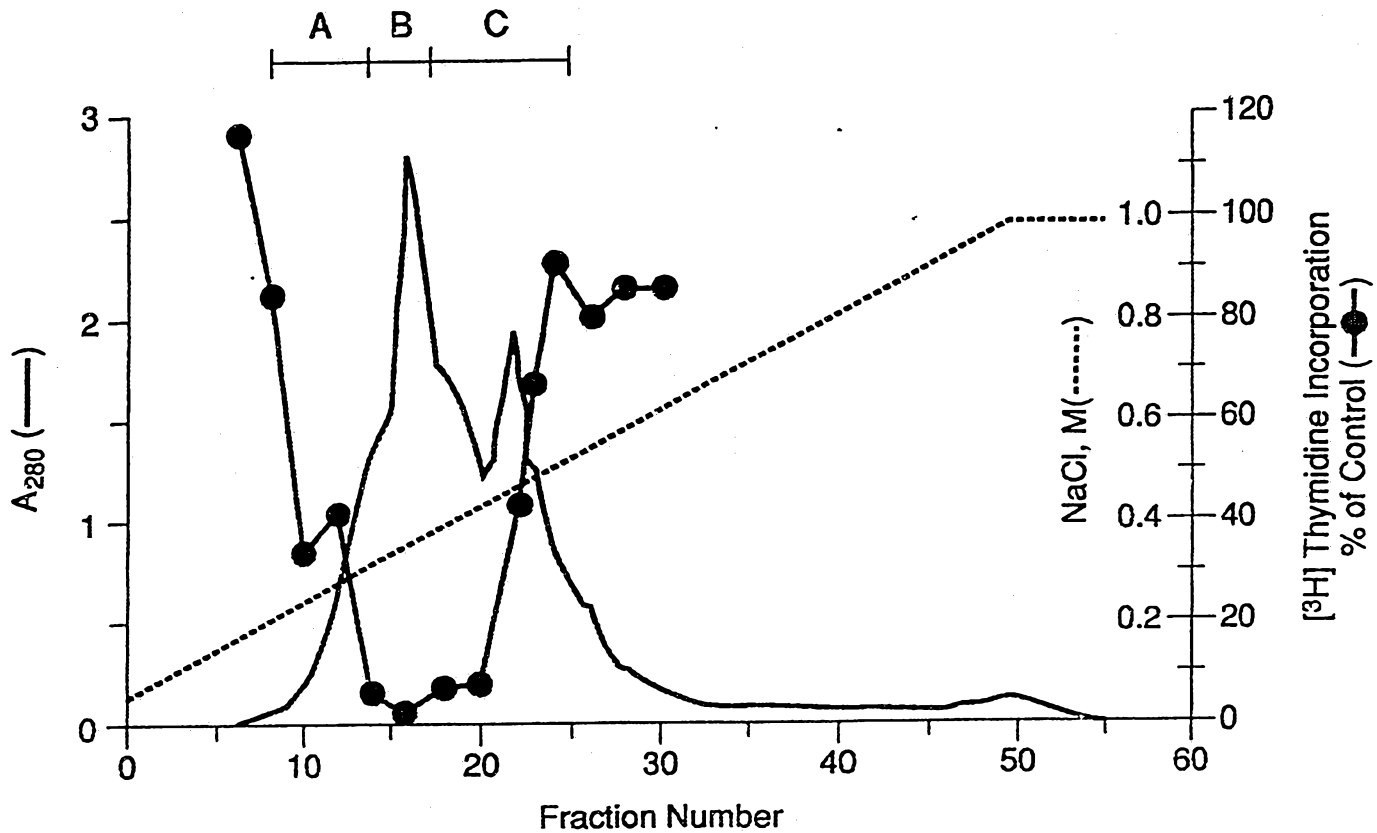


FIG. 2B

fraction 8 10 12 14 16 18 20 22 24 26
anti LTBP

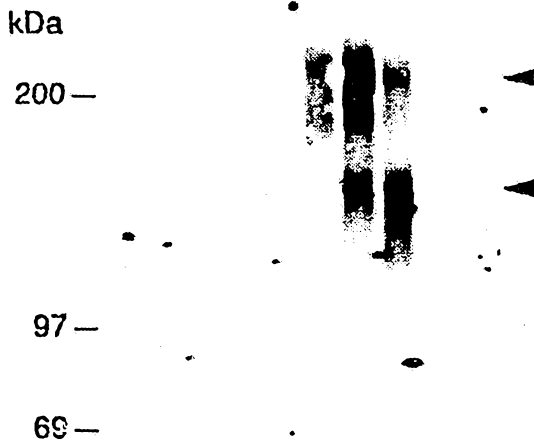


FIG. 2C

fraction 8 10 12 14 16 18 20 22 24 26
anti β 1-LAP

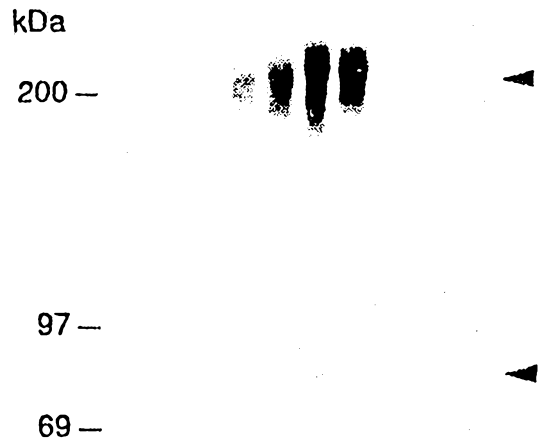


FIG. 2D

fraction 8 10 12 14 16 18 20 22 24 26
anti β 2-LAP

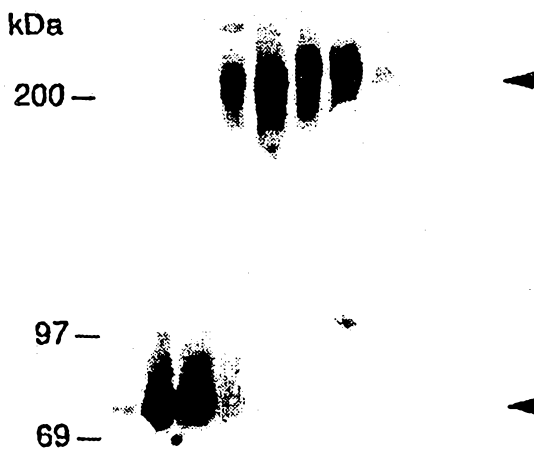
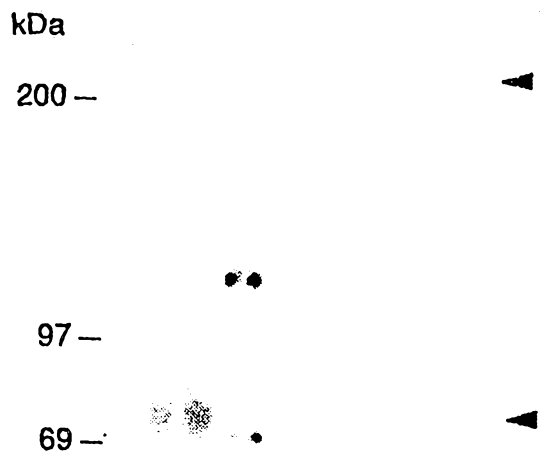


FIG. 2E

fraction 8 10 12 14 16 18 20 22 24 26
anti β 3-LAP



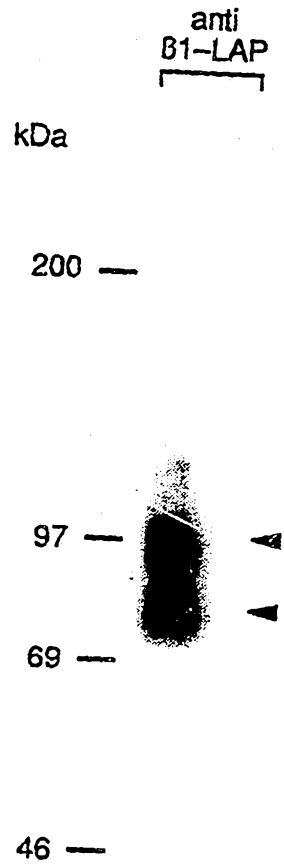


FIG. 2F

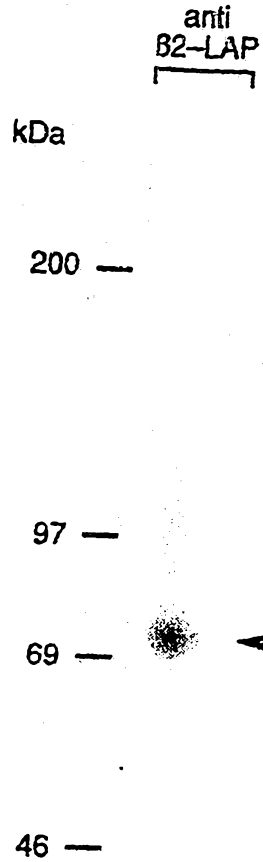


FIG. 2G

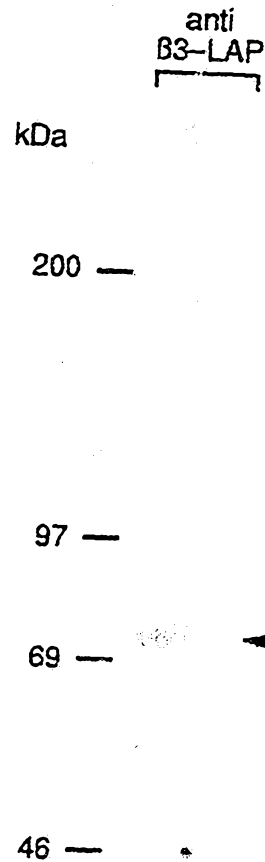


FIG. 2H

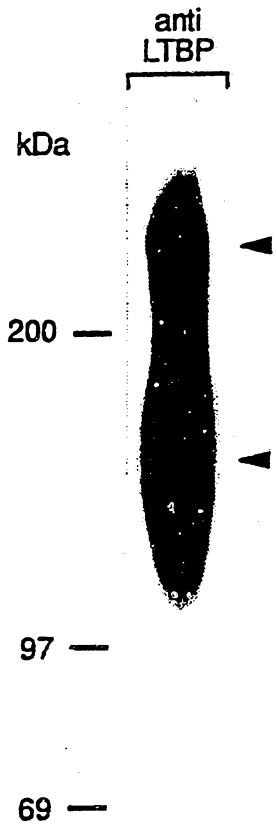


FIG. 3A

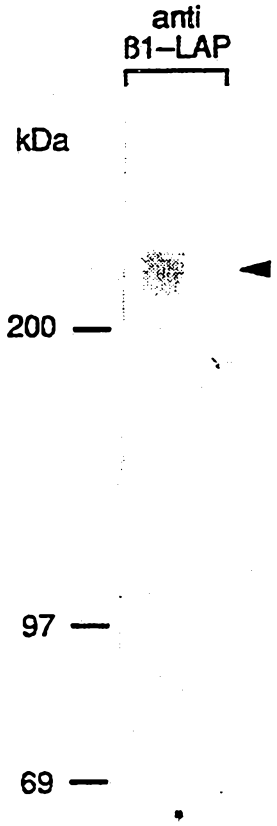


FIG. 3B

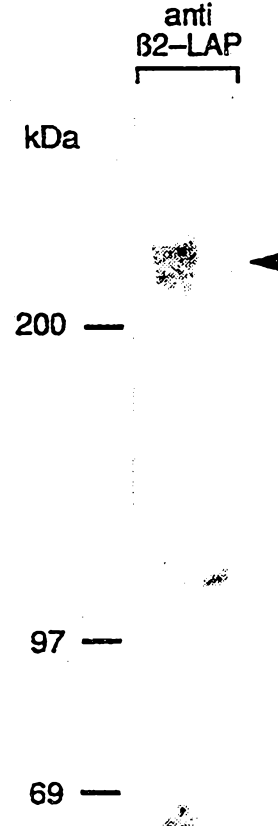


FIG. 3C

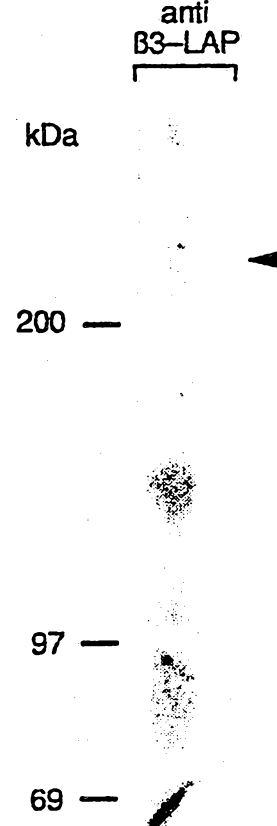
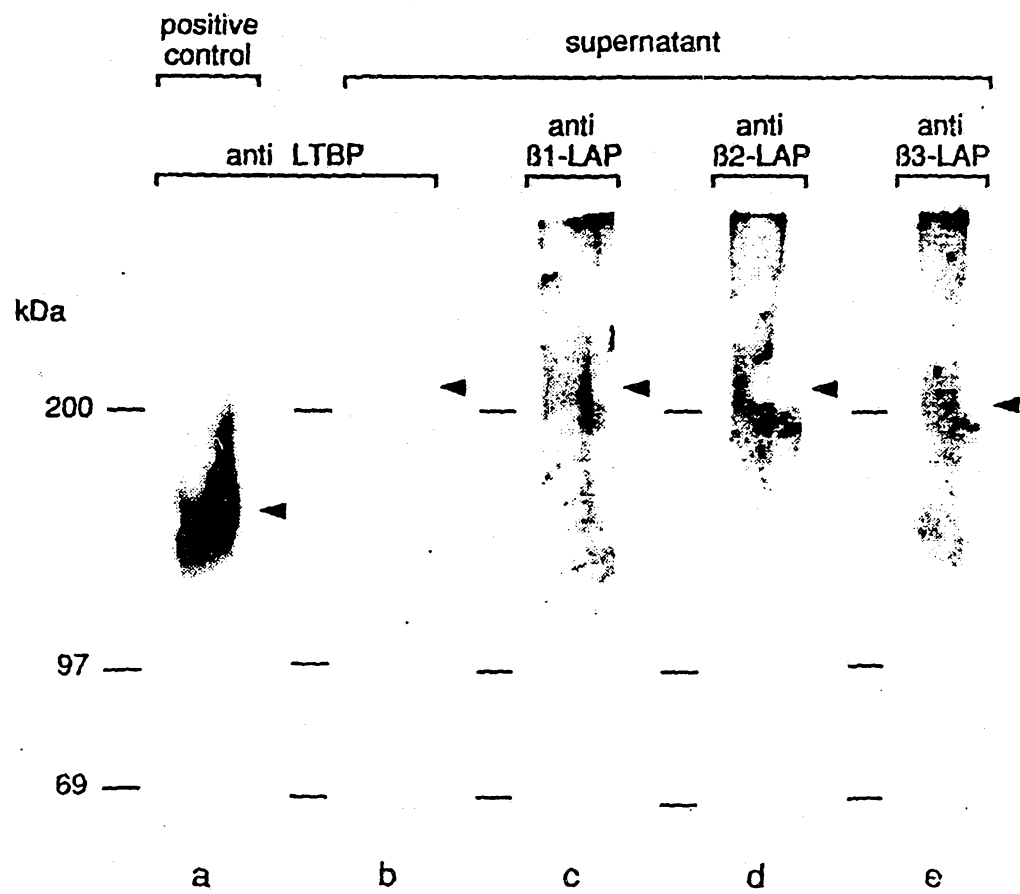


FIG. 3D

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FIG. 4



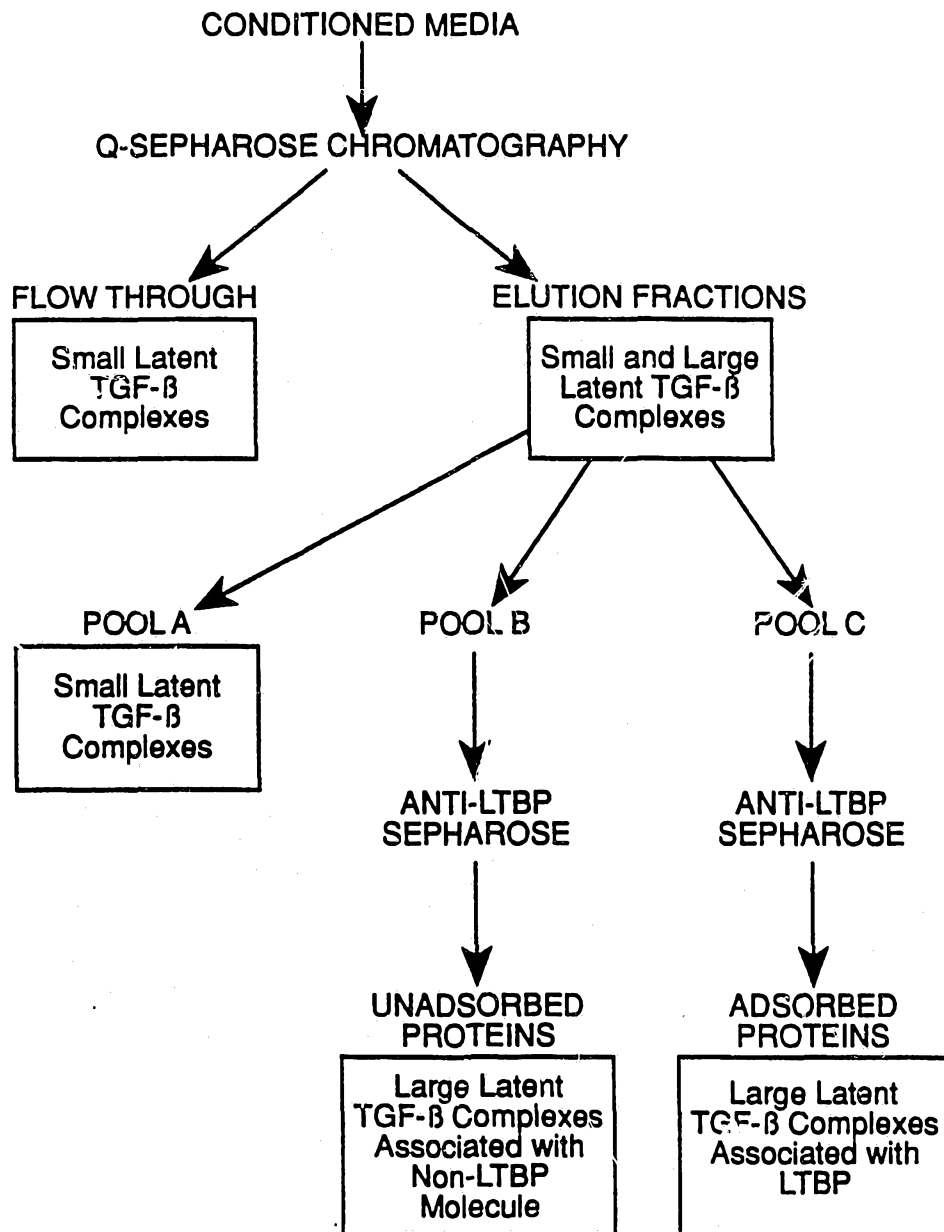
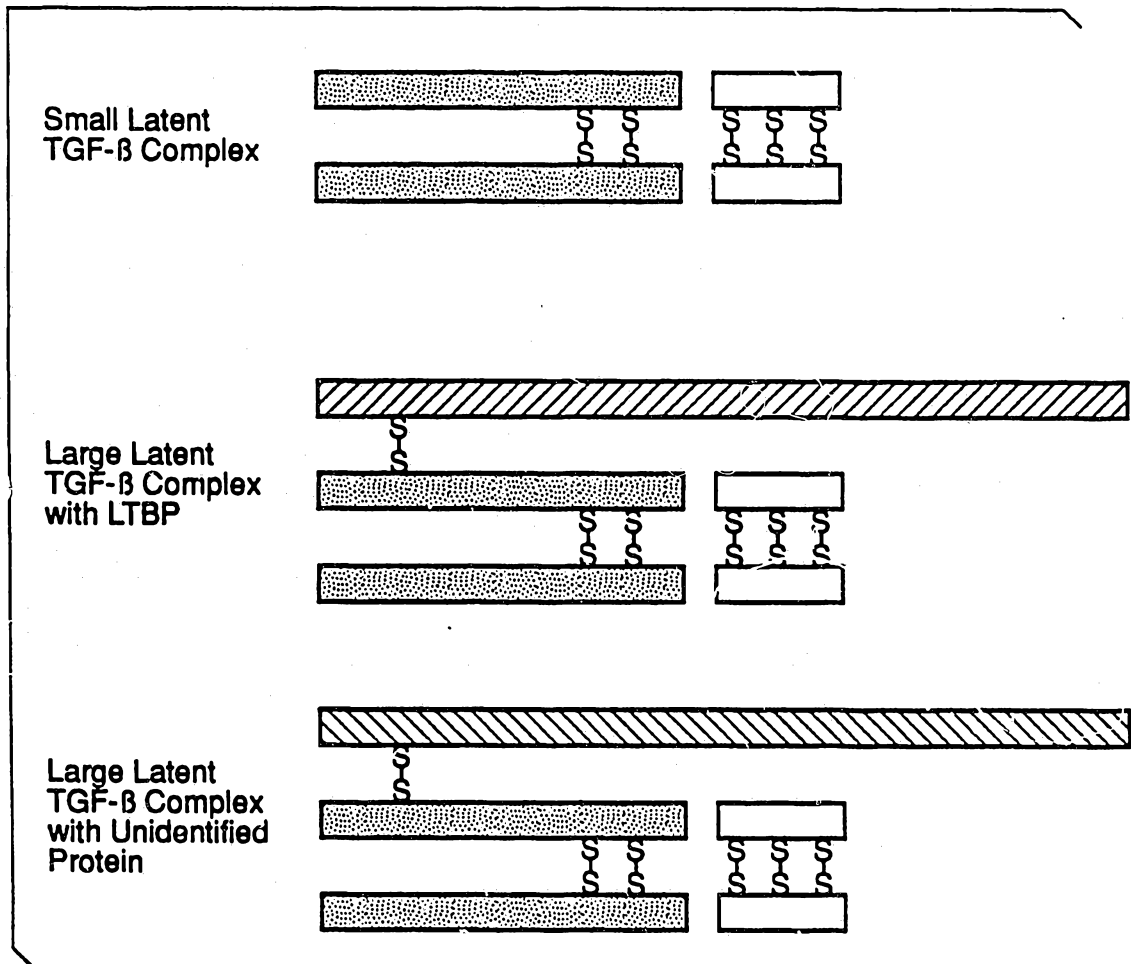


FIG. 5A

FIG. 5B



SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00561

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C07K 13/00

US CL : 30/399, 413; 514/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/399, 413; 514/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog, APS

search terms: transforming growth factor, latency associated peptide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 263, No. 13, issued 05 May 1988, K. Miyazono <u>et al.</u> , "Latent High Molecular Weight Complex of Transforming Growth Factor- β 1", pages 6407-6415, see abstract.	1-10
Y	NATURE, Volume 338, No. 6211, issued 09 March 1989, K. Miyazono <u>et al.</u> , "Role for Carbohydrate Structures in TGF- β 1 Latency," pages 158-160.	1-10
Y	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 263, No. 16, issued 05 June 1988, L.M. Wakefield <u>et al.</u> , "Latent Transforming Growth Factor- β from Human Platelets," pages 7646-7654, see abstract.	1-10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

24 February 1993

Date of mailing of the international search report

20 APR 1993

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00561

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CELL, Volume 48, issued 1987, S. Cheifetz <u>et al.</u> , "A Complex Pattern of Cross-Reactive Ligands and Receptors," pages 409-415, see "Discussion".	1-10
Y	DNA, Volume 7, No. 1, issued 1988, L. Madisen <u>et al.</u> , "Transforming Growth Factor- β 2: cDNA Cloning and Sequence Analysis," pages 1-8, see figure 1.	1-10
A	MOLECULAR AND CELLULAR BIOLOGY, Volume 7, No. 10, issued October 1987, L.E. Gentry <u>et al.</u> , "Type 1 Transforming Growth Factor Beta: Amplified Expression and Secretion of Mature and Precursor Polypeptides in Chinese Hamster Ovary Cells", pages 3418-3427.	1-10