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(57) Abrégé/Abstract:
Glycosylated, partially glycosylated and non-glycosylated polypeptides which have the amino-acid sequences indicated in the sequence listing and in which up to 10 amino-acid residues can be replaced by residues of other natural amino acids are described. The peptides are suitable for controlling diseases.

Abstract of the Disclosure: Glycosylated, partially glycosylated and non-glycosylated polypeptides which have the amino-acid sequences indicated in the sequence listing and in which up to 10 amino-acid residues can be replaced by residues of other natural amino acids are described. The peptides are suitable for controlling diseases.

RECOMBINANT FIBRINOGENASES, PREPARATION AND USE THEREOF

The present invention relates to novel proteins with fibrinogenolytic properties, called fibrinogenases, the preparation and use thereof for the prophylaxis and therapy of diseases.

To date it has been possible to isolate from the venom of the Malayan pit viper (Agkistrodon rhodostoma) only one fibrinogen-cleaving enzyme having anticoagulant properties (Biochem. J. 131 (1973) 799). This protein is called Arvin, Arwin or ancrod in the literature.

The possible uses of this protein are limited because signs of resistance may appear after 6 to 8 weeks and are presumably attributable to the production of ancrod-neutralizing antibodies. Hemorrhagic complications also occur in a few cases.

We have now found, and prepared pure, other proteins with fibrinogenolytic properties.

The present invention relates to glycosylated, partially glycosylated or non-glycosylated polypeptides with the amino-acid sequences 1, 2, 3, 4 and 5 given in the sequence listing, where Xaa and Xab are residues of natural α-amino acids, and to the allelic variants thereof which are identical in more than 95% of the amino-acid positions to the indicated sequences.

The residue Xaa is Asn, Gln, Ser, Thr, Gly, Asp, Glu, Lys, Arg or Pro, but preferably Asn, Gln, Ser and Thr and, in particular, Asn and Gln. Xab is preferably Phe, Tyr, Leu, Ile, Ala, Val, Thr or Ser.

The present invention also relates to DNA sequences which code for the abovementioned proteins, and to vectors which contain these DNA sequences. Preferred DNA sequences are depicted as sequences Nos. 6 to 9 in the sequence listing.

The proteins according to the invention can be prepared by known methods of genetic manipulation.

Thus, it is possible to isolate from the glandular tissue of a Malayan pit viper (Agkistrodon

rhodostoma) mRNA and to convert it into double-stranded cDNA. A cDNA library is set up after insertion of this cDNA into a commercial cloning vector, eg. λ gt 10. The methods used for this can be found, for example, in Maniatis et al., Molecular Cloning, CSH Press (1982). The screening of such gene banks with radiolabeled oligonucleotide probes or radiolabeled DNA fragments is also now a widely used and described method. This method can be used to isolate and characterize a cDNA clone which has homology with the oligonucleotide probe or with radiolabeled DNA fragments, and is described in DNA cloning, Vol. I, IRL Press, 1985.

The cDNA which has been characterized in this way can easily be obtained using restriction enzymes. The fragments resulting from this can be used, where appropriate in combination with chemically synthesized oligonucleotides, adaptors or gene fragments, to clone the sequences coding for the protein. The gene fragments or synthetic DNA sequences are incorporated into cloning vectors, eg. the commercial plasmids M13mp or pkk-223-3, in a conventional manner. The genes or gene fragments can also be provided with suitable control regions which have been chemically synthesized or isolated from bacteria, phages, eukaryotic cells or viruses thereof and which make expression of the proteins possible.

The transformation or transfection of suitable host organisms with the hybrid plasmids obtained in this way is likewise known and described in detail (M. Wigler et al., Cell 16 (1979) 777-785; F.L. Graham and A.J. van der Eb, Virology 52 (1973) 456-467). The hybrid plasmids can also be provided with appropriate signal sequences to allow the polypeptides to be secreted into the medium.

Vectors which can be used for expression in mammalian cells are those which place the gene to be expressed, in this case the cDNA which codes for one of the fibrinogenases described in the sequence listing, under the control of the mouse metallothionein or viral

SV40 promoter (J. Page Martin, Gene, 37 (1985) 139-144). The presence of the methionine start codon and of the leader/prosequence of the gene for the appropriate protein is necessary for expression. Clones which contain copies of these vectors as episomes or integrated in the genome are then isolated. Integration and expression of the foreign gene on the basis of the bovine papilloma virus are particularly advantageous. It is possible to construct shuttle vectors in conjunction with prokaryotic sequences which code for replication in bacterial cells and for antibiotic resistance. The plasmid is initially constructed and multiplied in bacterial cells and is then transferred into the eukaryotic cells, eg. into the mouse fibroblast cell line c127.

It is also possible to use other cell systems, eg. yeast and other fungi, insect cells and animal and human cells such as CHO, COS, L and 293 cells, in conjunction with suitable expression vectors for the expression of the cloned cDNA.

These eukaryotic expression systems have the advantage that they are able to secrete their products efficiently and usually in native form. They also have the ability to carry out post-translational modification on their products.

Thus, on expression in eukaryotic cells, the described fibrinogenases acquire glycoside side-chains. These side-chains are absent in the polypeptides produced in bacteria. The glycoside side-chains can also be removed completely or partially using appropriate glycosidases. Most eukaryotic proteins expressed in bacteria, result as denatured inclusion bodies in the cell and must be renatured by appropriate methods. In addition, bacteria are often incapable of eliminating the initiator amino acid methionine from the finished protein. These difficulties can be avoided by using secretion systems (Donald Oliver, Ann. Rev. Microbiol. 39, (1985) 615-48; John Ghrayeb et al. The EMBO Journal 3 (1984) 2437-2442.

However, because of the degeneracy of the genetic code, it is also possible to use other DNA sequences, eg. chemically synthesized genes with different DNA sequences, for the expression of the described fibrinogenases. Application of established methods of mutagenesis to the cloned genes allows production of variants of these fibrinogenases with a similar action.

The resulting polypeptides are purified from the culture medium by chromatography, eg. affinity chromatography on arginine-Sepharose®, Matrex-RedA-Sepharose®, heparin-Sepharose® or ion exchange materials in a conventional manner (Lit.: Guide to Protein Purification, Murray P. Deutscher [ed], Academic Press 1990).

The purification of the fibrinogenases can likewise be purified [sic] directly from the venom of A. rhodostoma by a combination of suitable chromatographic methods, preferably using Matrex-redA-Sepharose®, heparin-Sepharose®, arginine-Sepharose®, conA-Sepharose®, Q-Sepharose®, S-Sepharose® and chromatofocussing as described in Example 5. Particularly suitable for the final purification are HPLC methods.

The present invention also relates to drugs which contain the proteins prepared according to the invention, where appropriate in a pharmaceutically tolerated carrier or excipient. The drugs can also contain combinations of the proteins prepared according to the invention with other pharmacologically active substances such as thrombolytics (tPA, streptokinase), hirudin or thromboxane receptor antagonists.

Further embodiments of the invention are described in detail in the Examples.

For methods of genetic manipulation, reference may be made to, for example, the handbook by Maniatis et al., Molecular Cloning, Cold Spring Harbor Laboratory, 1982 or DNA cloning Vol. I - III, IRI [sic] Press 1985 - 87, edited by D.M. Glover.

The polypeptides according to the invention are

suitable for the treatment of glomerulonephritis, myocardial infarct, non-ischemic stroke, disturbances of peripheral arterial blood flow (especially atherosclerosis obliterans, thrombangitis obliterans, diabetic microangiopathy and Raynaud's disease), unstable angina pectoris, deep vein thrombosis and other thromboses, rethrombosis after thrombolysis or vascular surgery, such as angioplasty, and for preventing thromboses in extracorporeal circulations.

EXAMPLE 1

Isolation of a fibrinogenase cDNA clone from the Malayan pit viper (Agkistrodon rhodostoma)

1 g of venom gland tissue from a 5-year old snake of the genus [sic] Agkistrodon rhodostoma was disrupted in 6 M guanidinium thiocyanate, 5 mM sodium citrate (pH 7.0), 0.1 M 2-mercaptoethanol, 0.5% sarkosyl in an ULTRA-TURRAX®. Large cell detritus was removed by centrifugation at 3000 rpm. The RNA was removed by centrifugation through a 5.7 M CsCl cushion at 45,000 rpm overnight. The polyA$^+$-containing RNA fraction was then isolated by affinity chromatography on oligo(dT)-cellulose.

The polyA$^+$ RNA was converted into single-stranded cDNA using AMV reverse transcriptase and oligo(dT)12-18 as primer. The second strand was synthesized using E.coli DNA polymerase I. An EcoRI adaptor of the sequence 5'AATT CCATGG ATG CATGC 3' was attached to the double-stranded cDNA using T4-DNA ligase. The commercial phage vector λ gt 10 (Fig. 1a, 1b) was linearized with the restriction enzyme EcoRI. The two DNAs were ligated together and packaged with the commercial packaging extract to give infectious phages. The recombinant phages were plated out with E.coli C 600 Hfl on NZYDT plates and incubated at 37°C overnight. The resulting cDNA library contained 2×10^6 independent clones. Amplification of the cDNA library by conventional methods was followed by plating out of 500,000 phages with C 600 Hfl cells. The phages were transferred to nitrocellulose filters, lyzed with 0.5 N

NaOH/1.5 M NaCl, and the denatured DNA was firmly bound to the filter by baking at 80°C for 2 hours. The filters were prehybridized in 6 x SET buffer (1 x SET = 0.15 M NaCl, 15 mM tris/HCl, pH 7.4, 1 mM EDTA), 0.1% SDS and 5 x Denhardt's solution (100 x Denhardt = 1 g of Ficoll, 1 g of polyvinylpyrrolidone, 1 g of BSA per 50 ml) at 68°C for 4 h.

Hybridization was carried out with a nick-translated cDNA (Fig. 2) which codes for ancrod protein.

The filters were incubated in a solution which contained 2 x SET, 0.1% SDS, 30% formamide, 5 x Denhardt's and 10% dextran sulfate at 42°C overnight while shaking gently. They were then washed several times with 2 x SET/0.1% SDS at 42°C, dried and exposed to an X-ray film. Clones which gave a radioactive response in the screening were isolated and cultured further in order to obtain the corresponding phage DNA.

Phage DNA was prepared by incubating the purified phages with protenase [sic] K (ad 60 μg/ml) at 55°C for 1 h and subsequent phenol/chloroform extraction. Addition of 3 volumes of ethanol (-20°C) resulted in precipitation of the phage DNA, which was transferred with a sterile injection needle into 70% ethanol, washed and briefly sedimented. The pellet was briefly dried in air and then suspended in TE buffer.

The purified phage DNA was transferred to nitrocellulose filters, renatured, reneutralized, baked and prehybridized as described above. Hybridization was then carried out under stringent conditions, using a radiolabeled oligonucleotide probe which was homologous to the ancrod-encoded [sic] cDNA:

5' GTC TAC GAT TAT CGT GAC TGG GTC AA 3'

The filters were incubated in a solution which contained 2 x SET, 0.1% SDS, 30% formamide, 5 x Denhardt's and 10% dextran sulfate at 42°C overnight while shaking gently. They were then washed several times in 2 x SET/0.1% SDS at 60°C, dried and exposed to an

X-ray film.

The DNA which did not hybridize under these conditions was subconed [sic] in the single-stranded phage MB for further analysis.

EXAMPLE 2

Preparation of single-stranded DNA which codes for ancrod

The starting point were [sic] the phage DNA which did not hybridize with the ancrod-specific oligonucleotide as described in Example 1. They were each separately cut preparatively with the restriction enzyme Eco RI. The Eco RI fragments which contained the cDNA inserts were eluted from the gel by electrophoresis. 30 ng of each of these fragments were ligated at 4°C for 12 h with 100 ng of the commercial cloning vector M13mp18 or M13mp19 (Fig. 3) which had been cut with Eco RI. The volume of the ligation mixture was 10 µl. Ligation was stopped by heating at 80°C for 5 min.

1/10 of the volume of each ligation mixture was employed to transform 100 µl of competent SR 101 cells. After the transformation was complete, 60 µl of 0.2 M IPTG solution and 120 µl of XGal (20 mg/ml) were added to the transformation mixture. The resulting mixture was plated out in NZYDT top agar on NZYDT agar plates containing 200 µl of SR 101 cells (OD_{600}=1). The NZYDT medium is commercially available (GIBCO-BRL). Clones which contained cDNA inserts were identifiable because the plaques were not stained blue. DNA sequence analysis (Sanger et al., Proc. Natl. Acad. Sci. USA 74, (1977) 5463-67) was used to elucidate the sequence of this cDNA insert (sequence listing, Nos. 6 to 9).

EXAMPLE 3

Construction of vectors for the expression of ancrod in eukaryotic cells

SV40 DNA was cut with the restriction enzymes BamHI and BclI, and the 0.24 kb fragment was prepared by gel electrophoresis (Fig. 4). The ends were filled in with the Klenow fragment in the presence of the four

deoxynucleotide triphosphates dATP, dCTP, dGTP and dTTP. XhoI linkers were then ligated on.

In parallel the commercial vector pUC18 was linearized with SmaI. XhoI linkers were then likewise attached. The DNA of this vector (puC18Xho) was linearized with XhoI, treated with alkaline phosphatase and ligated to the 0.24 kb XholI [sic] SV40 fragment (see above). The result was pSVpA.

pSVpA DNA was cleaved preparatively with XhoI and incubated with Klenow polymerase in the presence of the four dNTPs as above. The 0.24 kb fragment was isolated from the gel.

At the same time, the eukaryotic expression vector CL28XhoBPV, produced by ligation of CL28x and pB2-2 (Reddy et al. DNA 6, (1987) 461-72) was partially cut with the restriction enzyme XbaI, ie. the incubation time was restricted so as to result in molecules cleaved at only one of the two XbaI recognition sequences, ie. linearized (Fig. 5). The mixture was then reacted with Klenow polymerase and dNTPs as described. The linear molecules were subsequently isolated by gel electrophoresis.

The linear pCL28XhoBPV fragments were then ligated with the pretreated 0.24 kb SV40 fragment. After transformation and screening of minilysates, a clone which carried the SV40 fragment in the former XbaI site located about 0.15 kB [sic] 3′ of the XhoI site was isolated; this DNA (pCL28XhoBPV-SVpolyA) carried the SV40 transcription stop signals of the early genes.

Plasmid DNA from pCL28XhoBPV-SVpolyA was linearized with XhoI and treated with alkaline phosphatase. At the same time, the cDNA inserts which did not hybridize with the Ancrod-specific oligonucleotide as described in Example 2 were provided with Xho linkers using T4 ligase. The two fragments were connected together using T4 ligase. After transformation and analysis of minilysates, a clone which contained the cDNA inserts singly and in

the correct orientation was isolated: pCL28BPV-fibrogenase [sic] I-IV.

EXAMPLE 4

Transfection and establishment of cell lines

C127I cells (J. Virol. 26 (1978) 292; ATCC catalog of cell lines and hybridomas 5th edition, 1985, p.142) were transfected with BPV expression plasmids using the calcium phosphate coprecipitation method (Virology 52 (1973), 456, DNA cloning; Volume II, ed. D.M. Glover IRL Press, (1985) 143ff and 213).

DMEM (Dulbeccos's Modified Eagles Medium) + 10% FCS (fetal calf serum) in 60 mm Petri dishes was inoculated with 5 x 10^5 C127I cells. The next day the medium was changed to MEM (Modified Eagles Medium) containing 25 nM Hepes + 10% FCS. A Ca phosphate coprecipitate was formed with 10^{-5} g of CsCl-purified plasmid DNA and was cautiously placed on the C172I cells. The cells were incubated at 37°C, 7% CO_2 for 4 h. A subsequent glycerol shock treatment considerably increased the efficiency of transfection. For this, 4 h after addition of the precipitate the medium was aspirated off from the cells. The cells were incubated with 2 ml each [sic] of 15% glycerol/HBS (DNA cloning Vol. II, page 152) in a 60 mm Petri dish at room temperature for 3 min. The glycerol/HBS solution was aspirated off and the cell lawn was washed with 3 ml of DMEM + 10% FCS. The cells were incubated with DMEM + 10% FCS at 37°C, 7% CO_2. The DMEM + 10% FCS was aspirated off and replaced by fresh three times a week. After 2 - 3 weeks, the transfected cells which contain the BPV genome were evident as collections of transformed cells, called foci.

After the foci had been subcloned, the medium supernatants from the individual subclones were tested for fibrinogen-cleaving activity by conventional methods.

For production, after the cell lines had reached confluence they were maintained in serum-free DMEM. The novel fibrinogenases can be purified by conventional

methods from the serum-free cell culture supernatant obtained in this way and used for pharmacological and chemical analyses.

EXAMPLE 5

Isolation and purification of a fibrinogen-cleaving enzyme (sequence listing, No. 5) from the venom of Agkistrodon rhodostoma

550 mg of crude venom (dry substance) from Agkistrodon rhodostoma were taken up in 20 ml of 20 mM Na_2HPO_4, 0.01% Tween® 80, 500 mM NaCl, pH 7.0 (= buffer A).

a) Chromatography on Matrex Red A-Sepharose®:

A chromatography column (diameter 2.5 cm, length 5.1 cm) was packed with 25 ml of Matrex red A-Sepharose® (from Amicon). The column was equilibrated with 100 ml of buffer A and then loaded with the dissolved crude venom. The column was washed with 45 ml of buffer A (flow rate 120 ml/h) and then eluted with 85 ml of buffer B, which was composed of 20 mM Na_2HPO_4, 2 M NaCl, 0.01% Tween, pH 7.0. The UV-active fraction (280 nm) was collected.

The eluate was dialyzed twice against 2.5 l of 20 mM Na_2HPO_4, 0.01% Tween® 80, pH 7.0 (= buffer C) in a dialysis tube (Visking size 8.32/32) for 2 h each time. The conductivity of the dialyzed tubes [sic] was about 2.2 mS/cm (4°C).

b) Chromatography on arginine-Sepharose®:

A chromatography column (diameter 2.5 cm, length 10 cm) was packed with arginine-Sepharose® (from Pharmacia) and equilibrated with 200 ml of buffer C.

The dialyzed eluate (vol. about 140 ml) from the Matrex red A-Sepharose® was loaded on the column with a flow rate of 120 ml/h.

The flow-through from the column (about 180 ml) was collected and processed further. Still bound to the column was, inter alia, ancrod which can be obtained by elution with arginine salts.

c) Chromatofocussing

A chromatography column (diameter 0.5 cm, length 5 cm) was packed with 1 ml of PBE® 94 gel material (from Pharmacia). The column was equilibrated with 5 column volumes of 20 mM tris/HCl, 0.01% Tween® 80, pH 8.0 (= buffer D).

The column was loaded with 20 ml of the flow-through from the arginine-Sepharose®.

The chromatography was carried out with a linear gradient from buffer D to 20 mM acetic acid/HCl, 0.01% Tween® 80, pH 2.0 (= buffer E) in 25 min with a flow rate of 1 ml/min. After this time, the column was eluted with buffer E for a further 13 min. The UV-active fraction (280 nm) eluted during this was collected. About 1 ml of a protein solution which contained, according to protein determination (method: Anal. Biochem. 153, 267-271), about 0.04 mg/ml was obtained.

d) Characterization of the purified fibrinogenase V

d1) SDS gel electrophoresis

Comparing with standard proteins, the protein solution showed a main band (about 70 to 90%) at ~ 42000 Dalton.

d2) N-terminal sequencing

The N-terminal sequence of the purified protein solution was determined (see sequence listing, sequence No. 5).

d3) Fibrinogenase assay

Fibrinogenase activities were determined by converting fibrinogen with the enzyme to be assayed into deAA fibrinogen.

This reaction was associated with an increase in turbidity which was followed by photometry (DD [sic] 340 nm).

The activity was quantified by calibration with an ancrod standard (Arwin®) of 3000 U/mg.

The fibrinogenase activity of the purified enzyme was about 500 U/mg.

Sequence listing

Sequence No. 1: 234 amino-acid sequence

Val Ile Gly Gly Asp Glu Cys Asn Ile Asn Glu His Arg Phe Leu Val
5 10 15

Ala Leu Tyr Asp Ser Thr Thr Arg Asn Phe Leu Cys Gly Gly Val Leu
20 25 30

Ile His Pro Glu Trp Val Ile Thr Ala Lys His Cys Asn Lys Lys Ser
35 40 45

Met Val Leu Tyr Leu Gly Lys His Lys Gln Ser Val Lys Phe Asp Asp
50 55 60

Glu Gln Glu Arg Phe Pro Lys Glu Lys His Phe Ile Arg Cys Asn Lys
65 70 75 80

Pro Arg Thr Arg Trp Gly Glu Asp Ile Met Leu Ile Arg Leu Asn Lys
85 90 95

Pro Val Xaa Asn Ser Glu His Ile Ala Pro Leu Ser Leu Pro Ser Gly
100 105 110

Pro Pro Ile Val Gly Ser Val Cys Arg Val Met Gly Trp Gly Ser Ile
115 120 125

Asn Lys Tyr Ile Asp Val Leu Pro Asp Glu Pro Arg Cys Ala Asn Ile
130 135 140

Asn Leu Tyr Xaa Tyr Thr Val Cys Arg Gly Val Phe Pro Arg Ile Gly
145 150 155 160

Lys Lys Ser Lys Ile Leu Cys Ala Gly Asp Leu Gln Gly Arg Leu Asp
165 170 175

Ser Cys His Cys Asp Ser Gly Gly Pro Leu Ile Cys Ser Glu Glu Phe
180 185 190

His Gly Ile Val Tyr Arg Gly Pro Asn Pro Cys Ala Gln Pro Asp Lys
195 200 205

Pro Ala Leu Tyr Thr Asn Ile Phe Asp His Leu His Trp Ile Leu Ser
210 215 220

Ile Val Ala Gly Xaa Ala Thr Cys Tyr Pro
225 230

Sequence listing

Sequence No. 2: 236 amino-acid sequence

Val Val Gly Gly Asp Glu Cys Asn Ile Asn Glu His Arg Phe Leu Ala
5 10 15

Leu Val Tyr Ile Thr Ser Gly Phe Leu Cys Gly Gly Thr Leu Xab His
20 25 30

Pro Glu Trp Val Val Ser Ala Ala His Cys Ala Arg Gly Glu Ile Glu
35 40 45

Val Phe Phe Gly Val His Ser Leu Lys Asp Ile Arg Thr Asn Lys Asp
50 55 60

Val Gln Lys Arg Val Ala Lys Glu Met Phe Phe Cys Leu Ser Ser Lys
65 70 75 80

Xaa Tyr Thr Lys Trp Asp Lys Asp Ile Met Leu Ile Lys Leu Asp Ser
85 90 95

Pro Val Xaa Asn Ser Thr His Ile Ala Pro Ile Ser Leu Pro Ser Ser
100 105 110

Pro Pro Ser Val Gly Ser Val Cys Arg Val Met Gly Trp Gly Val Thr
115 120 125

Thr Ser Pro Xaa Gly Thr Xab Pro Ser Val Pro His Cys Ala Asn Ile
130 135 140

Asn Ile Leu Asp Tyr Xab Val Cys Arg Ala Ala Arg Pro Lys Leu Pro
145 150 155 160

Ala Lys Ser Arg Thr Leu Cys Ala Gly Ile Leu Glu Gly Gly Lys Ser
165 170 175

Ala Cys Asp Gly Asp Ser Gly Gly Pro Leu Asn Cys Asn Gly Glu Ile
180 185 190

Gln Gly Ile Val Ser Trp Gly Gly Asn Ile Cys Ala Gln Pro Arg Lys
195 200 205

Pro Ala His Tyr Xab Lys Val Ala Asp Tyr Thr Asp Trp Ile Lys Ser
210 215 220

Ile Ile Ala Gly Xaa Thr Thr Ala Thr Cys Pro Pro
225 230 235

Sequence listing

Sequence No. 3: 236 amino-acid sequence

Val Ile Gly Gly Ala Glu Cys Asn Val Asn Glu His Arg Phe Leu Val
5 10 15

Ala Leu Tyr Asp Xaa Leu Thr Gly Thr Leu Gln Cys Gly Gly Thr Leu
20 25 30

Ile His Pro Glu Trp Val Leu Thr Ala Ala His Cys Asp Arg Lys Ser
35 40 45

Met Val Ile Tyr Leu Gly Met His Xaa Lys Ser Val Asn Asn Asp Asp
50 55 60

Gln Gln Arg Arg Ser Ala Lys Glu Lys Tyr Phe Phe Ser Cys Ser Lys
65 70 75 80

Ser Ile Ala Ala Trp Glu Lys Asp Ile Met Leu Ile Arg Leu Asp Ser
85 90 95

Pro Val Xaa Asn Ser Thr His Ile Ala Pro Leu Ser Leu Pro Ser Arg
100 105 110

Pro Pro Thr Val Gly Ser Val Cys Arg Val Met Gly Trp Gly Ala Ile
115 120 125

Thr Ser Pro Lys Glu Thr Tyr Pro Glu Val Pro His Cys Thr Asp Ile
130 135 140

Asn Leu Leu Xaa Tyr Ser Glu Cys His Gly Asp Phe Pro Arg Leu Arg
145 150 155 160

Ala Thr Ser Arg Ile Leu Cys Ala Gly Val Leu Gln Gly Gly Ile Asp
165 170 175

Thr Cys Asn His Asp Ser Gly Gly Pro Leu Ile Cys Asp Glu Gln Phe
180 185 190

Gln Gly Ile Val Ser Trp Gly Pro Tyr Pro Cys Ala Gln Pro Arg Asn
195 200 205

Ala Ala Ile Tyr Thr Lys Val Phe Asn Tyr Leu Val Trp Val Trp Ser
210 215 220

Thr Ile Ala Gly Xaa Thr Thr Val Thr Cys Pro Pro
225 230 235

Sequence listing

Sequence No. 4: 234 amino-acid sequence

Val Val Gly Gly Asn Glu Cys Asn Ile Asn Glu His Arg Phe Leu Val
5 10 15

Ala Ile Phe Xab Ser Thr Gly Phe Val Cys Ala Gly Thr Leu Ile His
20 25 30

Pro Glu Trp Val Val Thr Ala Ala His Cys Glu Ser Thr Asp Leu Lys
35 40 45

Met Lys Phe Gly Met His Ser Lys Lys Val Gln Asn Glu Asp Glu Gln
50 55 60

Thr Arg Asn Ala Lys Glu Lys Phe Ile Cys Pro Asn Lys Lys Asn Asp
65 70 75 80

Glu Val Leu Asp Lys Asp Ile Met Leu Ile Lys Leu Asn His Pro Val
85 90 95

Ser Asn Ser Glu His Ile Ala Pro Leu Ser Leu Pro Ser Ser Pro Pro
100 105 110

Ser Val Gly Ser Phe Cys His Ile Met Gly Trp Gly Ser Ile Thr Pro
115 120 125

Val Lys Val Thr Phe Pro Asp Val Pro His Cys Ala Asn Ile Asn Leu
130 135 140

Leu Glu Glu Ala Glu Cys His Ala Gly Tyr Pro Glu Val Leu Ala Glu
145 150 155 160

Tyr Arg Thr Leu Cys Ala Gly Ile Val Gln Gly Gly Lys Asp Thr Cys
165 170 175

Met Tyr Asp Ser Gly Gly Pro Leu Ile Cys Asn Glu Gln Val Gln Gly
180 185 190

Ile Val Ser Tyr Gly Ala His Pro Cys Gly Gln Pro Leu Lys Pro Gly
195 200 205

Ile Tyr Thr Arg Leu His Asp Tyr Asn Asp Trp Ile Asn Ser Ile Met
210 215 220

Ala Gly Asn Thr Ala Val Thr Cys Pro Pro
225 230

Sequence listing

Sequence No. 5: 25 amino-acid sequence

Val Ile Gly Gly Asp Glu Cys Asn Ile Asn Glu His Pro Phe Leu Val
5 10 15

Ala Val Tyr Glu Glu Thr Ala Gly Ala
20 25

Sequence listing

Sequence No. 6: 1096 nucleotide sequence corresponding to amino-acid sequence No. 1

Strandedness : double-stranded

Topology: linear

Molecule type : cDNA to mRNA

Original source: Agkistrodon rhodostoma

The region coding for the protein of sequence No. 1 starts at base 144 and terminates at base 841.

```
GAATTCCATG GATGCATGCG TTTGGGACTG GGATCTTACA GGCAAAGAGC TTTCTGTGCA     60
GAGTTGAAGC TATGGTGCTG ATCAGAGTGC TAGCAAACCT TGTGATACTA CAGCTTTCTT    120
ACGCACAAAA GTCTTCTGAA CTGGTCATTG GAGGTGATGA ATGTAACATA AATGAACATC    180
GTTTCCTTGT AGCCTTGTAT GACAGTACGA CTCGGAATTT TCTCTGTGGT GGGGTTTTGA    240
TCCATCCGGA ATGGGTGATC ACTGCTAAAC ACTGCAACAA GAAAAGTATG GTCCTATACC    300
TTGGTAAGCA TAAACAAAGT GTAAAATTTG ACGATGAGCA GGAAAGATTC CCAAAGGAGA    360
AGCACTTTAT TCGCTGTAAC AAACCCCGTA CCAGATGGGG CGAGGACATC ATGTTGATCA    420
GGCTGAACAA ACCTGTTAAC AACAGTGAAC ACATCGCTCC TCTCAGCTTG CCTTCCGGCC    480
CTCCCATTGT GGGCTCAGTT TGCCGTGTTA TGGGATGGGG CTCAATCAAT AAATATATAG    540
ACGTTTTGCC CGATGAACCT CGTTGTGCTA ATATTAACCT GTACAATTAC ACGGTGTGTC    600
GTGGAGTTTT TCCAAGGATA GGAAAGAAAA GCAAAATATT GTGTGCAGGT GACCTGCAAG    660
GACGCCTAGA TTCATGTCAC TGTGACTCTG GGGGACCTCT CATTTGTAGT GAAGAATTCC    720
ATGGCATTGT ATATCGGGGA CCCAATCCTT GTGCCCAACC AGATAAGCCT GCCCTCTACA    780
CCAACATCTT CGATCATCTT CACTGGATCC TTAGCATTGT GGCAGGAAAT GCAACTTGCT    840
ATCCATAAAA CCTTTTGAAA TAGTTAAGTG GAGAAAATGT AACATATTAG TAAATCTCTT    900
CTATATCCTT GCATTGGAAC ATATTCCCAG GCTGTAAGCT TTTTAGACTC AAATAGGACT    960
ACCTTTGGAG TAAGAAGTGC TCAAAATAGT GCTGCAGGGA TCATGTCCCA TTTAATTTCA   1020
GTTTAAAACA GTCTCCATAG ATTGGAGGCC TGTTTAGGGT TAGGTGCAAA TTTCTGACTC   1080
TAAATGGACC ATTCCC
```

Sequence listing

Sequence No. 7: 1333 nucleotide sequence corresponding to amino-acid sequence No. 2

Strandedness: double-stranded

Topology: linear

Molecule type: cDNA to mRNA

Original source: Agkistrodon rhodostoma

The region coding for the protein of sequence No. 2 starts at base 231 and terminates at base 935.

```
ANCCCCCTTT NNNGGNGGGG GGGGNCCAGA AGTTNCCCAG ATTNCTTGGC CACCCCGGTT    60
GCTTAATTTG ATCAAATAAA GTGCTGCTTG ATCCAAGAAA TTCTCCGCTT GGGTTATCTG   120
ATTAGGCAAA CAGCTTGCCA CGCAGAGTTG AAGCTATGGT GCTGATCAGA GTGCTAGCAA   180
ACCTTCTGAT ACTACAACTT TCTNACGCAC AAAAGTCATC TGAACNGGNC GTTGGAGGTG   240
ATGAATGTAA CATAAATGAA CATCGTTTCC TTGCACTCGT GTATATCACT AGTGGTTTTC   300
TCTGCGGTGG GACTTTGANC CACCCGGAAT GGGTGGTCAG TGCTGCACAT TGCGCTAGGG   360
GAGAAATAGA GGTATTCTTT GGTGTGCATA GCCTAAAGGA TATACGGACA AATAAGGATG   420
TGCAGAAAAG AGTCGCAAAG GAGATGTTCT TTTGCCTCAG TAGCAAAAAC TATACCAAAT   480
GGGACAAGGA CATCATGTTA ATCAAGCTGG ACAGTCCTGT TAACAACAGT ACTCACATCG   540
CGCCTATCAG CTTGCCTTCC AGCCCTCCCA GTGTGGGCTC AGTTTGCCGT GTTATGGGAT   600
GGGGCGTAAC CACATCTCCT AATGGGACTA TNCCCAGTGT NCCTCACTGT GCTAACATTA   660
ACATACTCGA TTATNCGGTG TGTCGAGCAG CTAGGCCAAA GTTGCCGGCG AAAAGCAGAA   720
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TTATTGCAGG AAATACAACT GCAACTTGCC CCCCGTGAAA ATTTTTGAAA AACTTAAGAG   960
GAGAAAATAC ATCTCTTCTA TATCCCTAGC CATATTCAAT TACATTGGAA TATATTCCCA  1020
AGTTAACTCT ACATCAACAA AAAATCCTAC NAAACAACAA CAGAGAAGGA GCAGATAAAA  1080
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ACCATGATTT AATCCTTGTG GGGGGGGAAA TCACAAGAAT TGGAAAAAAA CAACTTATCC  1200
CTTAGACAGC AAACTAAATC TGAGGACAAG AAAACAGATT GGATAAAATG GACTGTAGAA  1260
ATGTCAGGAA CATCGGAGAG AAAGGAAATA ATAAGAGAAG CAAAAAAAAA AAAAGCATGC  1320
ATCCATGGAA TTC
```

Sequence listing

Sequence No. 8: 988 nucleotide sequence corresponding to amino-acid sequence No. 3

Strandedness: double-stranded

Topology: linear

Molecule type: cDNA to mRNA

Original source: Agkistrodon rhodostoma

The region coding for the protein of sequence No. 3 starts at base 197 and terminates at base 904.

AACAATAAAG	NCTGCNTGAN	CAAGAAGCNN	CTGCTTAGCT	TATCTGATAA	GATTGACATG	60
TATCTCAAGC	TTAAGTTGGG	ACTGGGATCT	TACAGCAAAG	AGCTTTCCAC	GCAGAGTTGA	120
AGCTATGGTG	CTGATCAGAG	TGCTAGCAAA	CCTTCTGATA	CTACAGCTTT	CTTACGCACA	180
AAAGTCTTCT	GAACTGGTCA	TTGGAGGTGC	TGAATGTAAC	GTAAATGAAC	ATCGTTTCCT	240
TGTAGCCTTG	TATGACAATT	TGACTGGGAC	TTTGCAGTGT	GGTGGGACTT	TGATCCACCC	300
GGAATGGGTG	CTCACTGCTG	CGCACTGCGA	CAGGAAAAGT	ATGGTCATAT	ACCTTGGTAT	360
GCATAACAAA	AGTGTAAACA	ATGACGATCA	GCAGAGAAGA	TCCGCAAAGG	AGAAGTACTT	420
TTTTAGCTGT	AGCAAAAGCA	TTGCCGCATG	GGAAAAGGAC	ATCATGTTGA	TCAGGCTGGA	480
CAGTCCTGTT	AACAACAGTA	CACACATCGC	CCCTCTCAGC	TTGCCTTCCA	GACCTCCCAC	540
TGTGGGCTCA	GTTTGCCGTG	TTATGGGATG	GGGCGCAATC	ACATCTCCTA	AAGAGACTTA	600
TCCTGAGGTC	CCTCATTGTA	CTGACATTAA	CCTGTTAAAT	TATTCGGAGT	GTCATGGAGA	660
TTTCCCACGG	TTGCGGGCGA	CAAGCAGAAT	ATTGTGTGCA	GGTGTCCTGC	AAGGAGGCAT	720
AGATACATGT	AATCATGACT	CTGGGGGACC	TCTCATCTGT	GATGAACAAT	TCCAGGGCAT	780
TGTATCTTGG	GGACCCTATC	CTTGTGCCCA	ACCGCGTAAC	GCTGCCATCT	ACACCAAAGT	840
CTTCAATTAT	CTTGTCTGGG	TCTGGAGCAC	TATTGCAGGA	AATACAACTG	TGACTTGCCC	900
CCCATGAAAA	CATTTTTATT	TCCACAAAGG	AGTTTCCAAA	GGAATTAAAA	CTAAATAATG	960
TGGTAAAAAA	AAAAAAAAAA	AAAAAAAA				

Sequence listing

Sequence No. 9: 957 nucleotide sequence corresponding to amino-acid sequence No. 4

Strandedness: double-stranded

Topology: linear

Molecule type: cDNA to mRNA

Original source: Agkistrodon rhodostoma

The region coding for the protein of sequence No. 4 starts at base 210 and terminates at base 911.

CTNAATTNNA	CAAAAAAAGT	GCTGCTTGGT	CAAGAGGTNC	TCCGCTTCGG	TTATCTGATT	60
AGATTGATAC	GTATCTCAAG	TATAAGTTTG	GGACTGGGAT	CTTACAGGAA	AACAGCTTTC	120
CGTGCAGAGT	TGAAGTTATG	GTACTGATCA	GAGTGCTAGC	AAACCTTCTG	ATACTACAGC	180
TTTCTTACGC	ACAAAAGTCA	TCTGAACTGG	TCGTTGGAGG	TAATGAATGT	AACATAAATG	240
AACATCGTTT	CCTTGTAGCC	ATCTTTAACT	CTACTGGGTT	TGTCTGCGCT	GGGACTTTGA	300
TCCACCCAGA	ATGGGTGGTC	ACTGCTGCAC	ACTGCGAGAG	TACGGATCTC	AAGATGAAGT	360
TTGGTATGCA	TAGCAAAAAG	GTACAAAATG	AGGATGAGCA	GACAAGAAAC	GCAAAGGAAA	420
AGTTCATTTG	TCCCAATAAG	AAAAACGATG	AAGTACTGGA	CAAGGACATT	ATGTTGATCA	480
AGCTGAACCA	TCCTGTTAGC	AATAGTGAAC	ACATCGCGCC	TCTCAGCTTG	CCTTCCAGCC	540
CTCCCAGTGT	GGGCTCATTT	TGCCATATTA	TGGGATGGGG	CTCAATCACA	CCTGTTAAAG	600
TGACTTTCCC	CGATGTCCCT	CATTGTGCTA	ACATTAACCT	ACTCGATGAT	GCAGAGTGTC	660
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CCAGGCTCCA	TGATTATAAT	GACTGGATCA	ACAGCATTAT	GGCAGGAAAT	ACAGCTGTGA	900
CTTGCCCCCC	GTGAAAACTT	TAGTATCAGA	AGGTTTGCTG	CATGCATCCA	TGAATTC	

We claim:

1. A glycosylated, partially glycosylated or non-glycosylated polypeptide with amino-acid sequence 1, 2, 3 or 4, which is indicated in the sequence listing and in which Xaa and Xab are each residues of natural α-amino acids, as well as the allelic variants thereof which are identical to the stated sequences in more than 95% of the amino-acid positions.

2. A polypeptide obtainable from the crude venom of Agkistrodon rhodostoma by chromatography on matrex red A-Sepharose® and arginine-Sepharose® and subsequent chromatofocusing with the gel material PBE® 94, which exhibits a main band with a molecular weight of about 42,000 dalton, has a fibrinogenase activity of about 500 U/mg and has the N-terminal amino-acid sequence 5.

3. A DNA sequence which codes for a polypeptide as claimed in claim 1 or 2.

4. A recombinant DNA which contains a DNA sequence as claimed in claim 3.

5. A recombinant DNA molecule as claimed in claim 4, which contains a DNA sequence as claimed in claim 3, which is functionally connected to an expression control system which makes expression possible in suitable host systems.

6. A recombinant DNA molecule as claimed in claim 5, wherein the expression control sequence is an E.-coli promoter system, a promoter system of an E.-coli bacteriophage, a yeast or fungus expression control sequence or another eukaryotic expression control sequence.

7. A host cell which contains at least one recombinant DNA molecule as claimed in claim 4, 5 or 6.

8. A host cell as claimed in claim 7, which is a bacterium, a yeast, a fungus, an animal or a human cell.

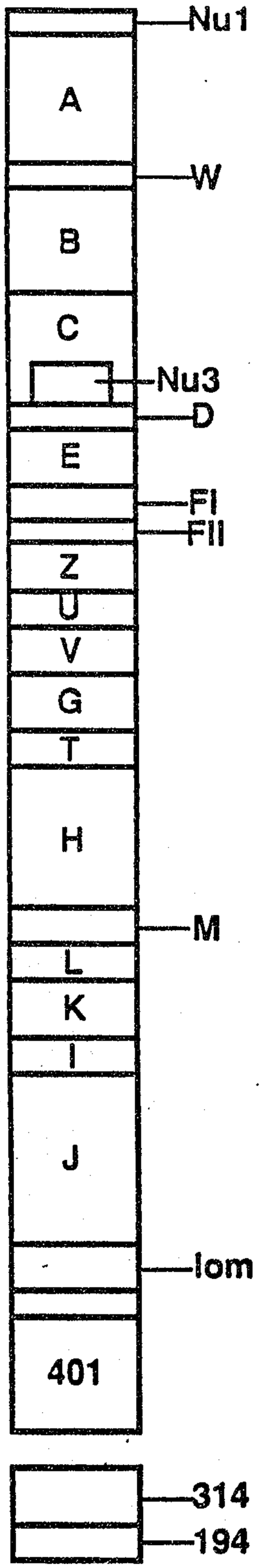
9. A genetic engineering process for preparing a polypeptide as claimed in claim 1 or 2, which comprises bringing about the expression in a suitable host cell

of DNA sequences which code for peptide sequences as claimed in claim 1.

10. A polypeptide as claimed in claim 1 or 2 for use for preventing and controlling diseases.

11. The use of a polypeptide as claimed in claim 1 or 2 for producing drugs for the treatment and prophylaxis of thromboembolic disorders and glomerulonephritis.

Fig. 1 a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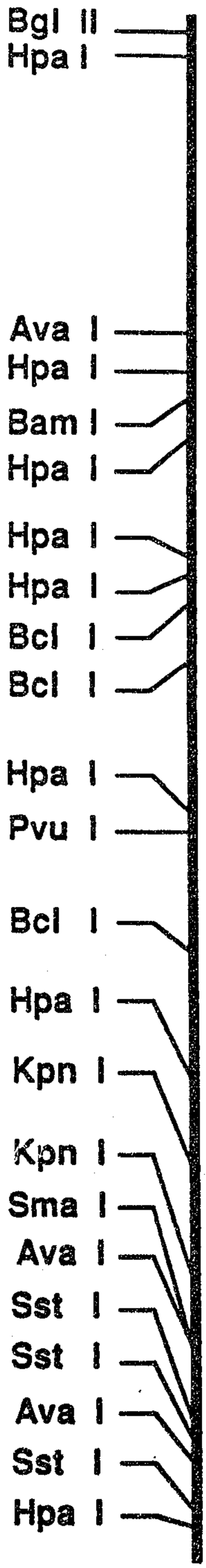
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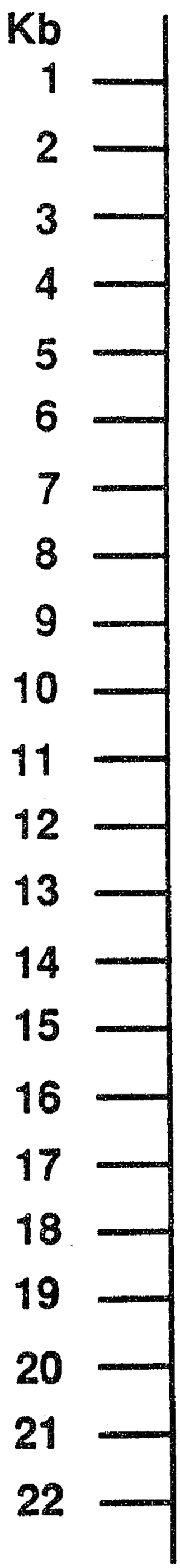
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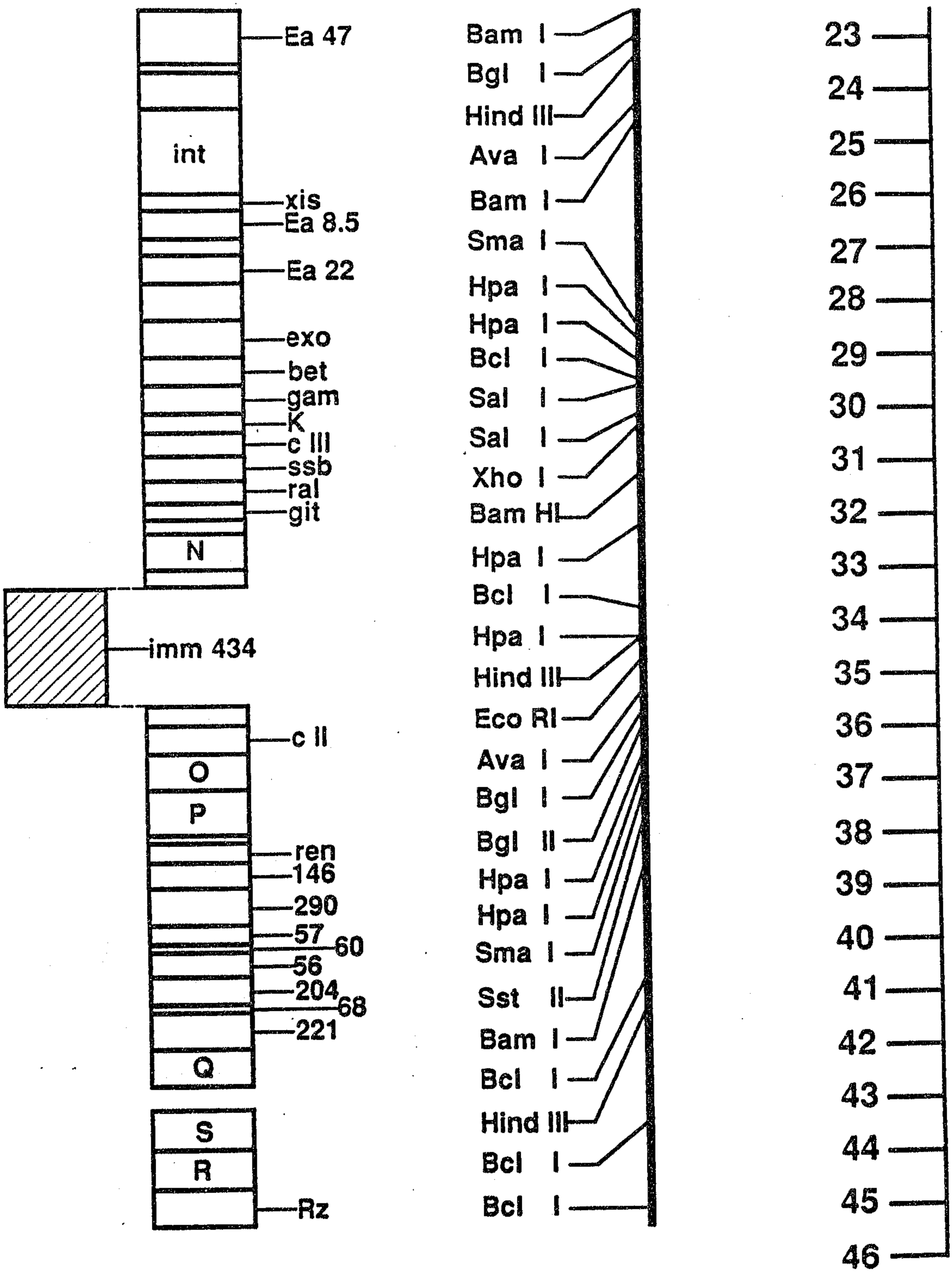
Fig. 1 b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O
P
ren
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290
57
60
56
204
68
221
Q
S
R
Rz
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Bgl I
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Bam I
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Hpa I
Hpa I
Bcl I
Sal I
Sal I
Xho I
Bam HI
Hpa I
Bcl I
Hpa I
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Fig. 2

cDNA coding for ancord (sic)

```
CCATGGATGC ATGCGGCAAA GAGCTTCTGC GCAGAGTTGA AGCTATGATG CTGATCAGAG    60
TGCTAGCAAA CCTTCTGATA CTACAGCTTT CTTATGCACA AAAGTCTTCT GAACTGGTCA   120
TTGGAGGTGA TGAATGTAAC ATAAATGAAC ATCGTTTCCT TGTAGCCGTG TATGAAGGTA   180
CAAATTGGAC TTTTATCTGC GGTGGGGTTT TGATCCACCC GGAATGGGTG ATCACCGCTG   240
AACACTGTGC CAGGAGACGT ATGAACCTAG TCTTTGGTAT GCATAGAAAA AGTGAAAAAT   300
TTGACGATGA GCAGGAAAGA TACCCAAAGA AAAGGTACTT TATTCGCTGC AACAAAACCC   360
GTACCAGTTG GGACGAGGAC ATCATGTTGA TCAGGCTGAA CAAACCTGTT AACAACAGTG   420
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CTGGGGGACC TCTCATTTGT AATGAAGAAC TCCATGGCAT TGTAGCTAGG GGACCCAATC   720
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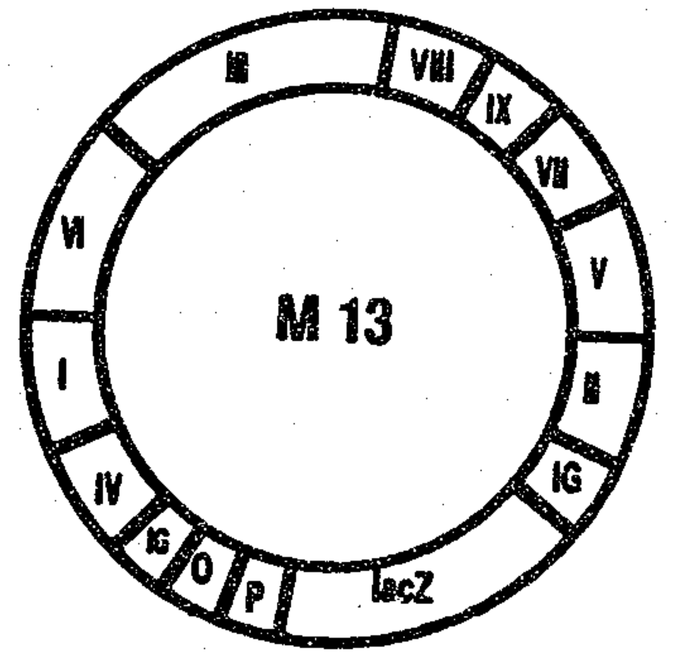
20833286

Fig. 3



(+) STRAND SYNTHESIS

(-) STRAND SYNTHESIS

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M 13 mp 18

A T G A T T A CGCCAAGCTTGCATGCCTGCAGGCTGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCA C T G G C C

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Fig. 4

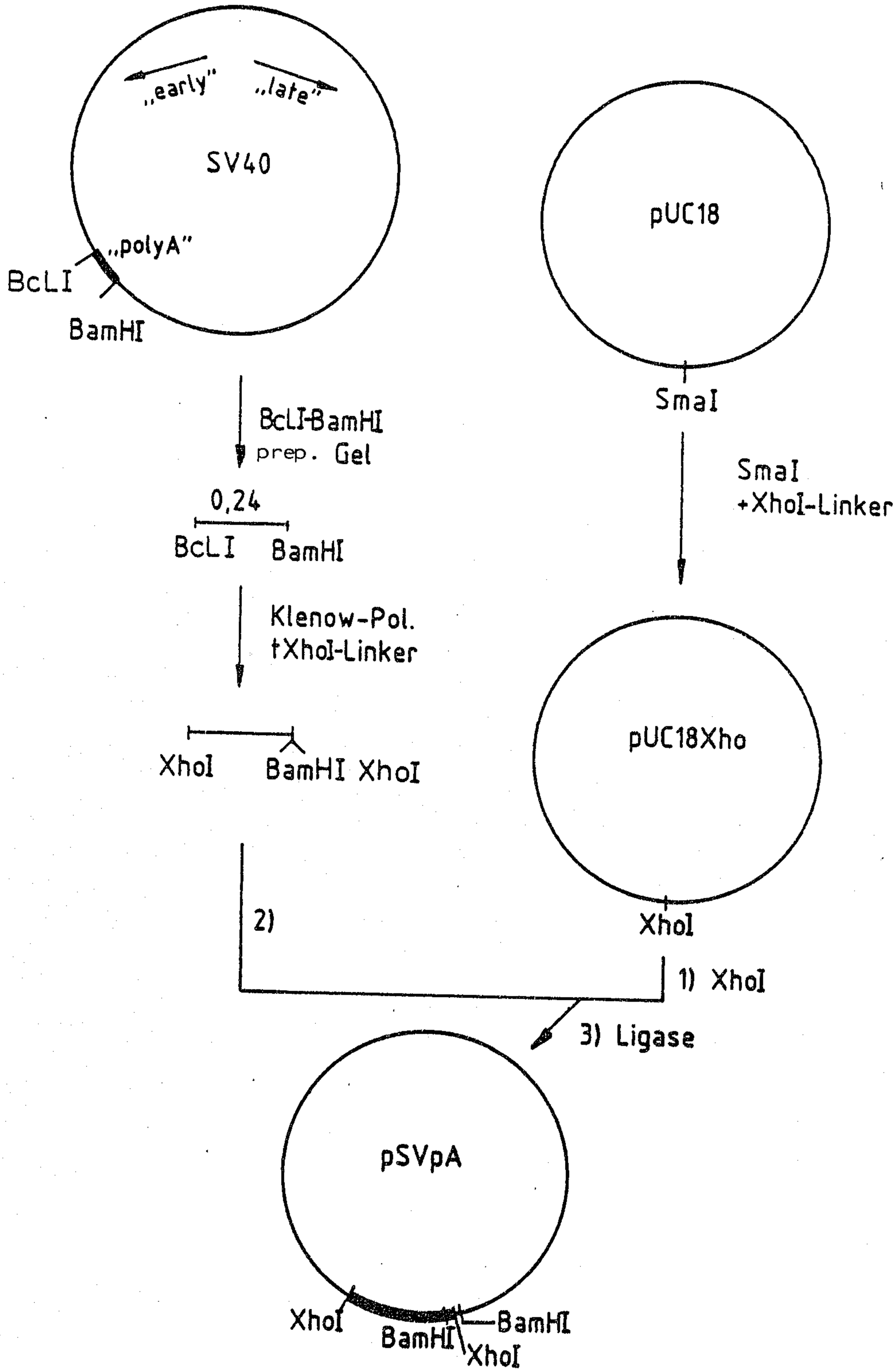
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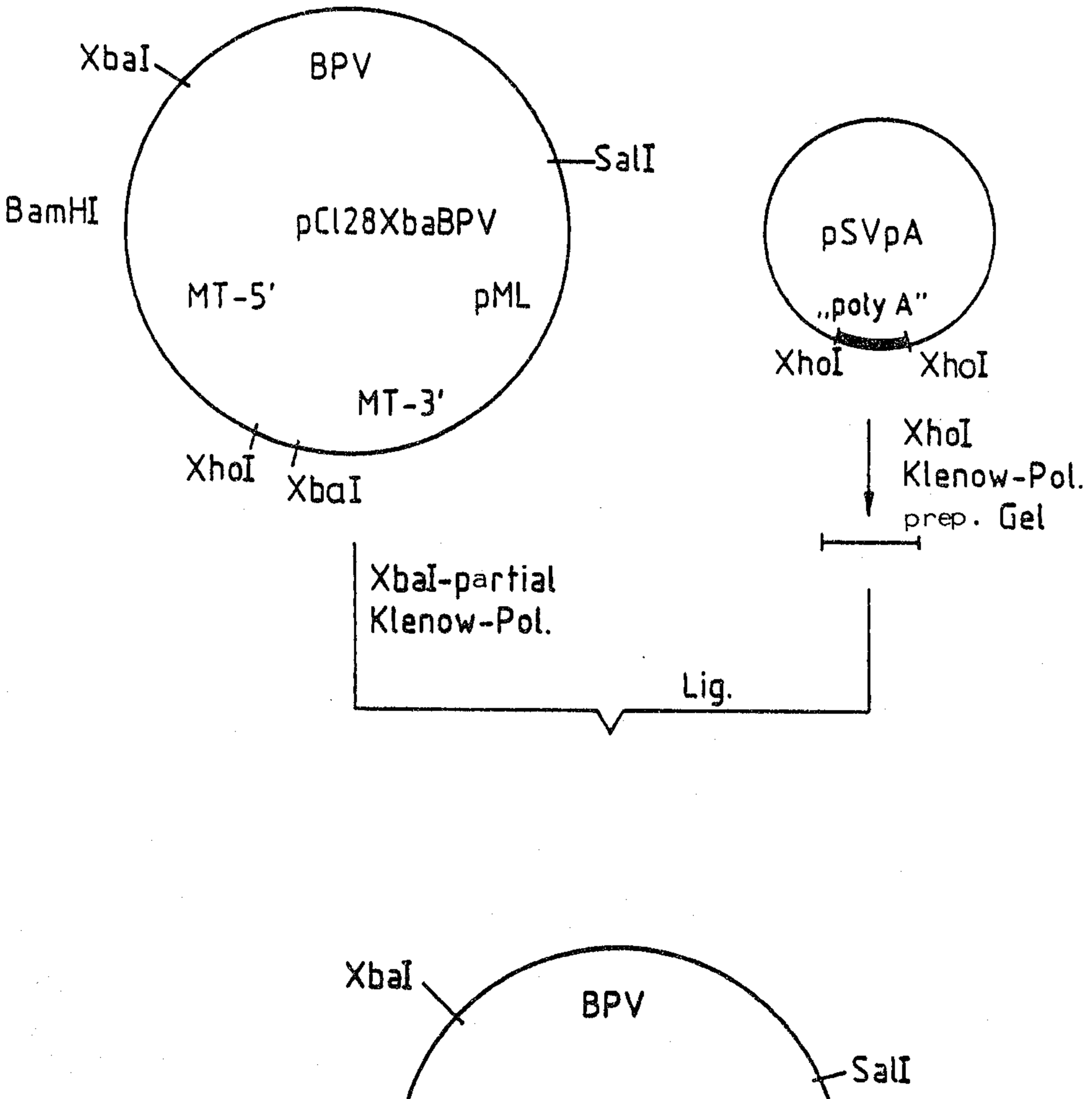
Fig. 5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