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(54) **PRODUCTION OF RECOMBINANT COLLAGEN LIKE PROTEINS**

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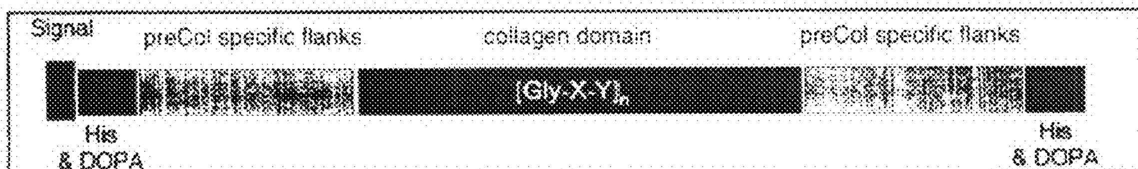
(57) **ABSTRACT**

The present invention is directed to a yeast cell for producing a recombinant collagen like protein. The present invention is further directed to a kit of parts or a co-expression system for use in the production of such a protein and to a method of producing said recombinant protein and a thread made therefrom. Furthermore, the invention pertains to proteins or threads obtainable by these methods as well as their use in various fields of technology and medicine.



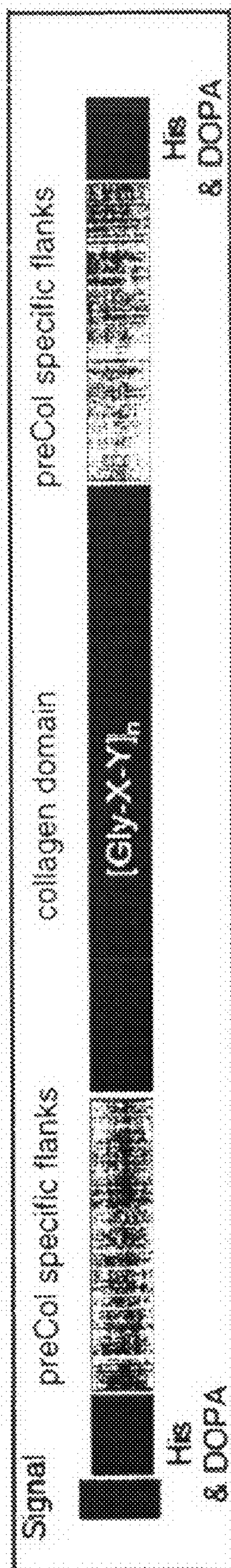


Figure 1

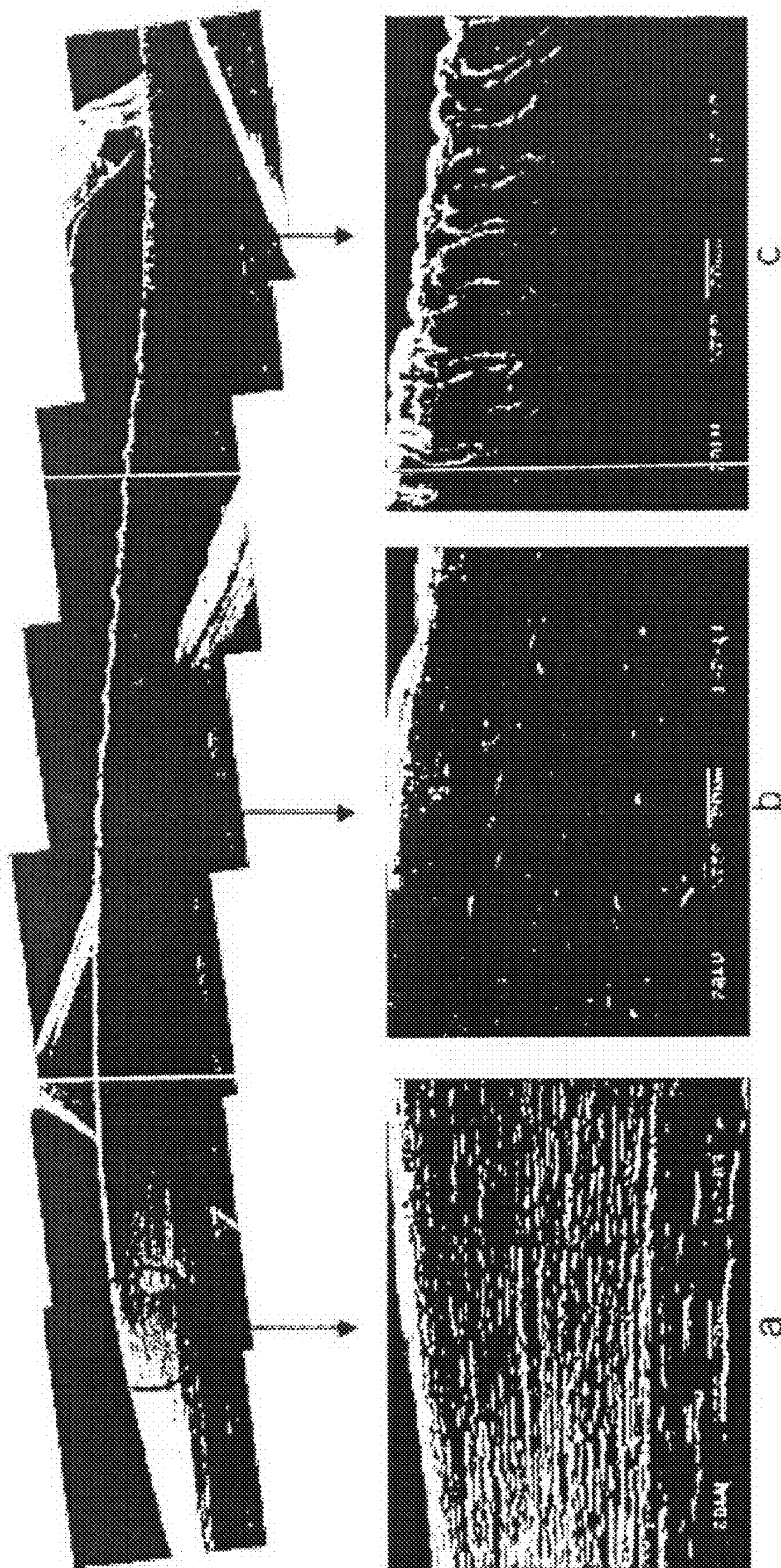


Figure 2

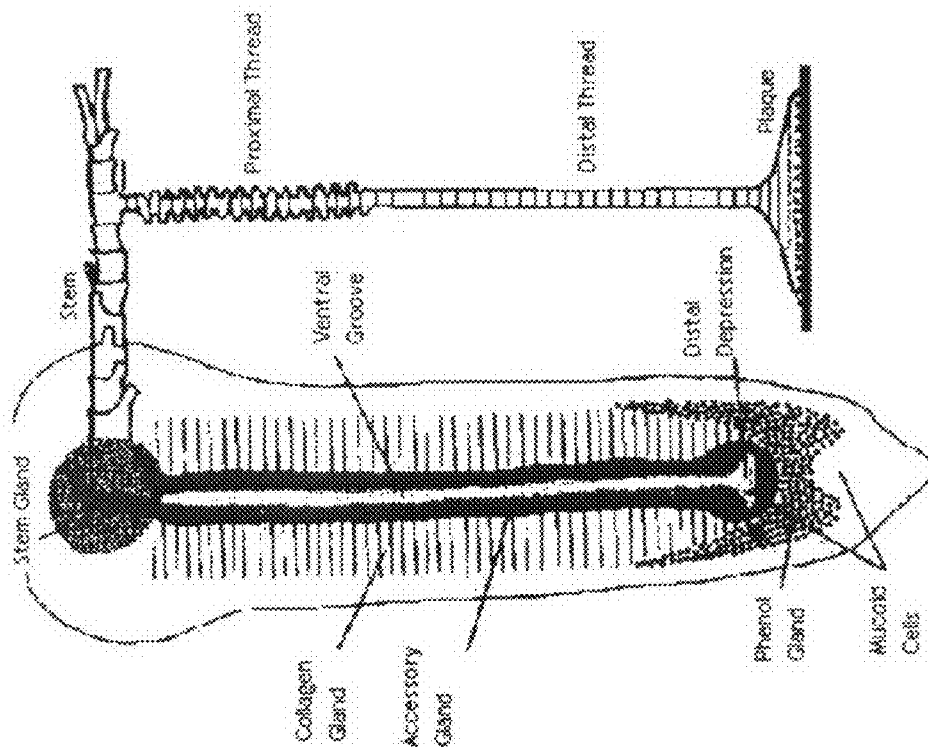


Figure 3

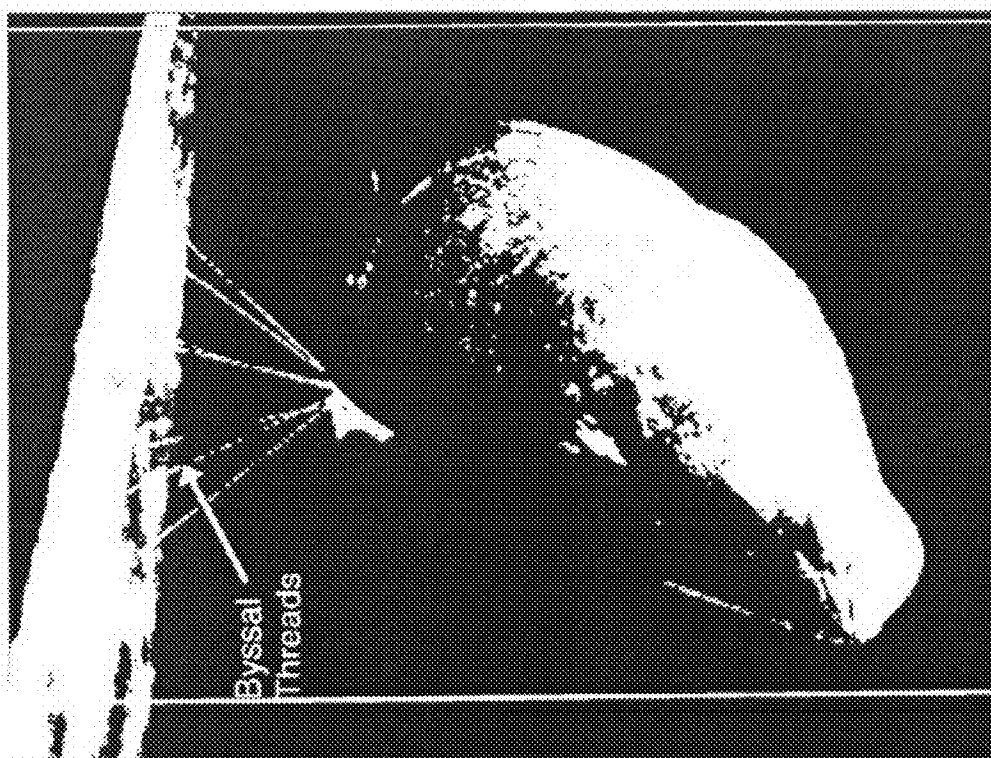


Figure 4

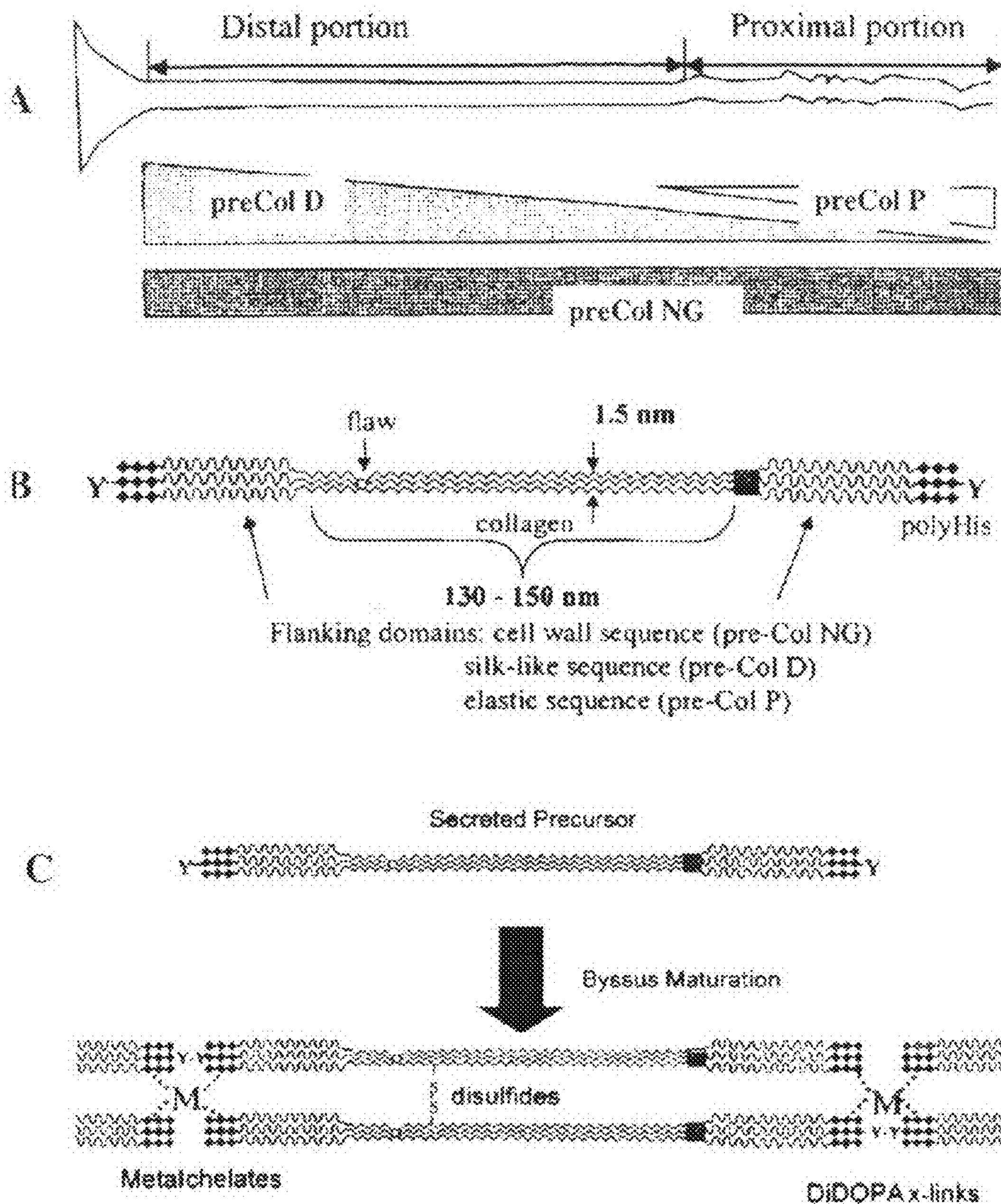


Figure 5

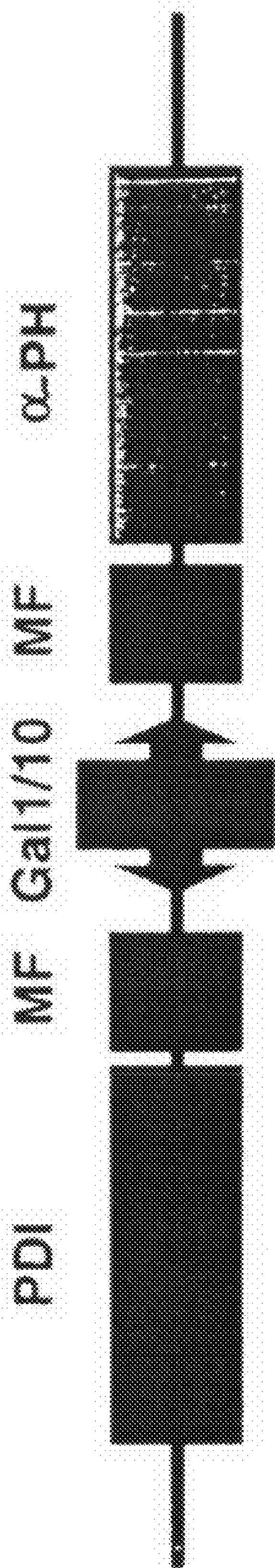


Figure 6

Oligo A:

```
      SalI ──►                                                 NheI  Rndm      ApaI
5´-     ATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCAGCATCCTCCGCGCTAGCAAGGTTCC      -3´
   3´-  TACTCTAAAGGAAGTTAAAAATGACGTCAAAATAAGCGTCGTAGGAGGCGCGATCGTTCCAAGG   -5´
```

Oligo B:

```
      BamHI ──►                                                           BspEI  Rndm     SacII
5´-     ATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCAGCATCCTCCGCATTAGCTGACGCTCCGGATTCCAAGG      -3´
   3´-  TACTCTAAAGGAAGTTAAAAATGACGTCAAAATAAGCGTCGTAGGAGGCGTAATCGACTGCGAGGCCTAAGGTTCC   -5´
```

Figure 7

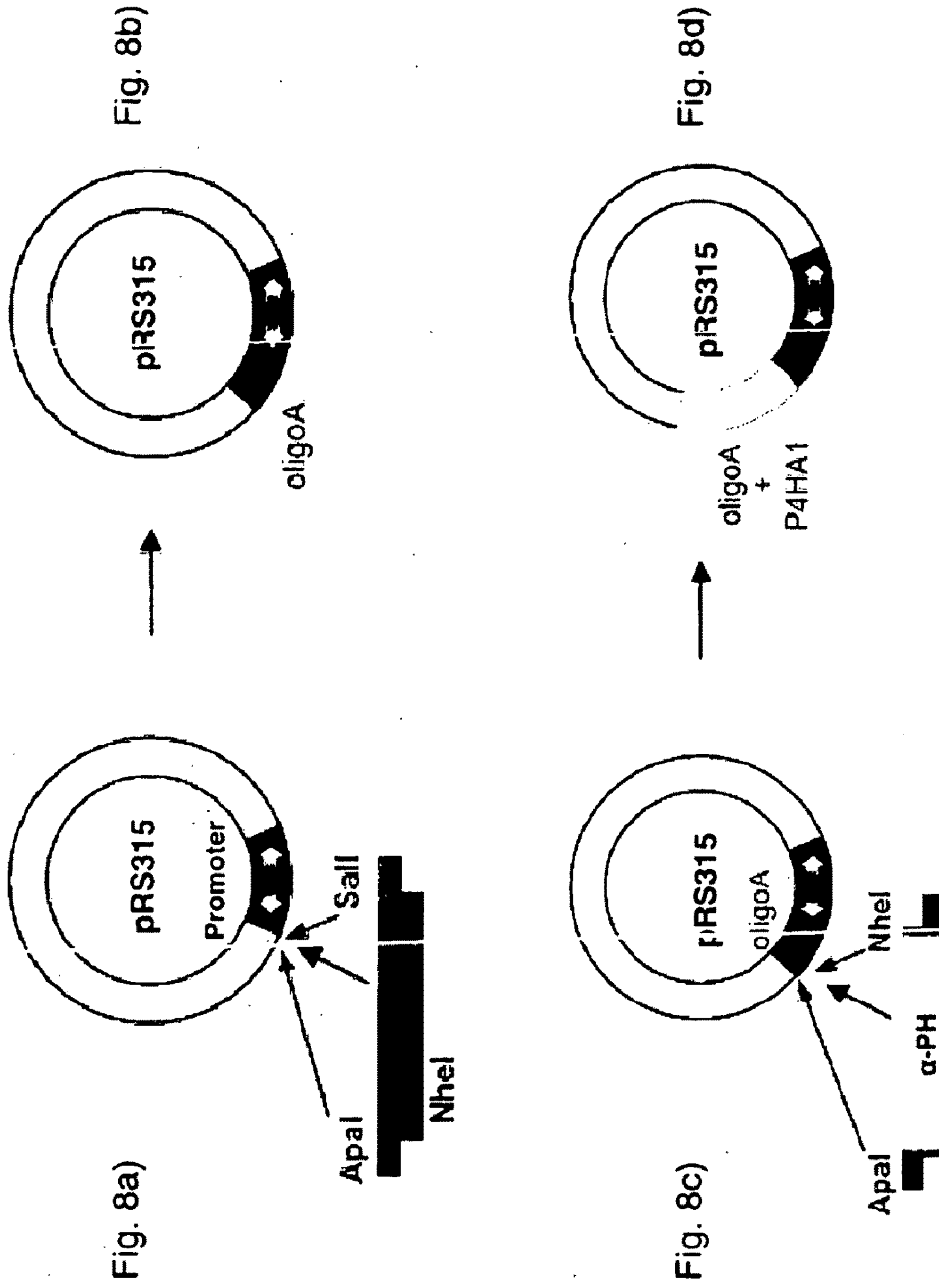


Figure 8

5.1.4 pRS315 und pRS425

gi:984798

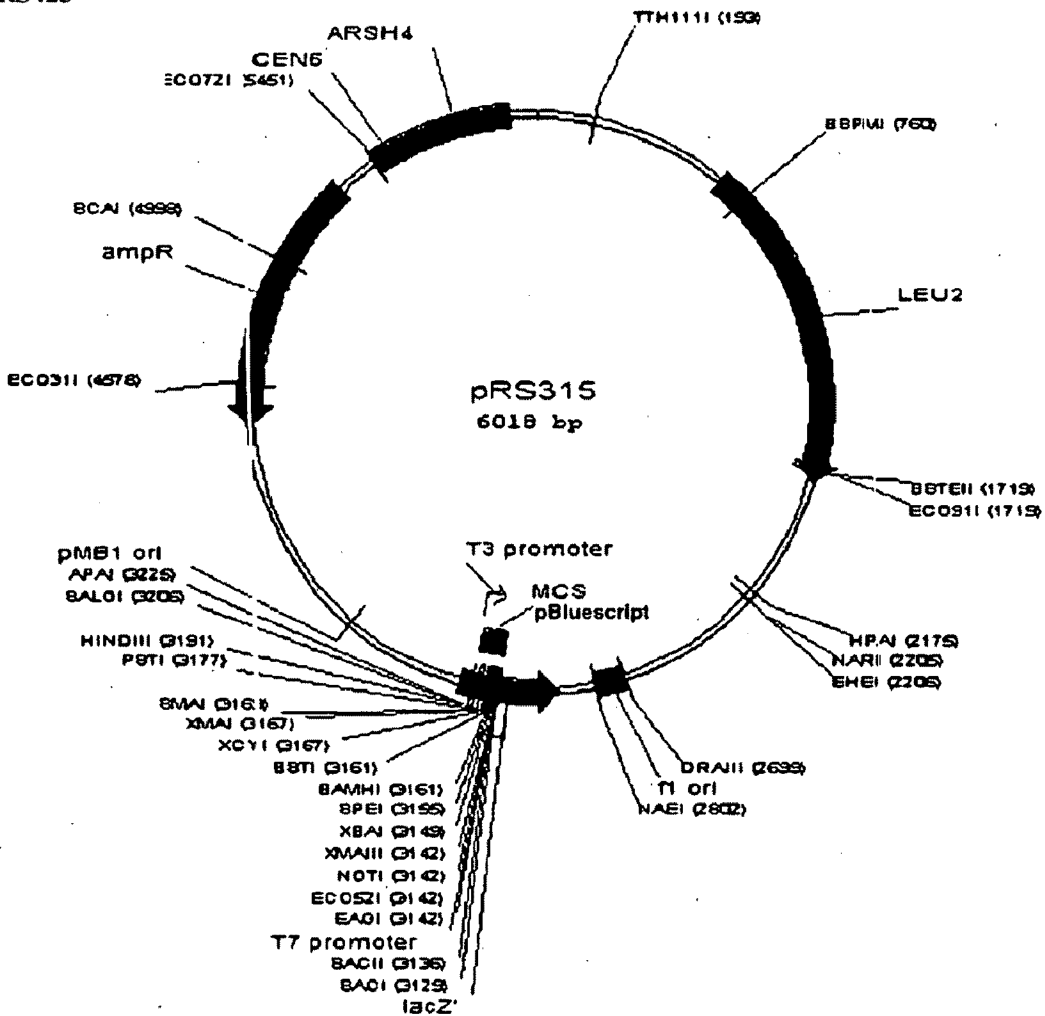


Figure 9a)

5.1.4 pRS315 und pRS425

gi:416323

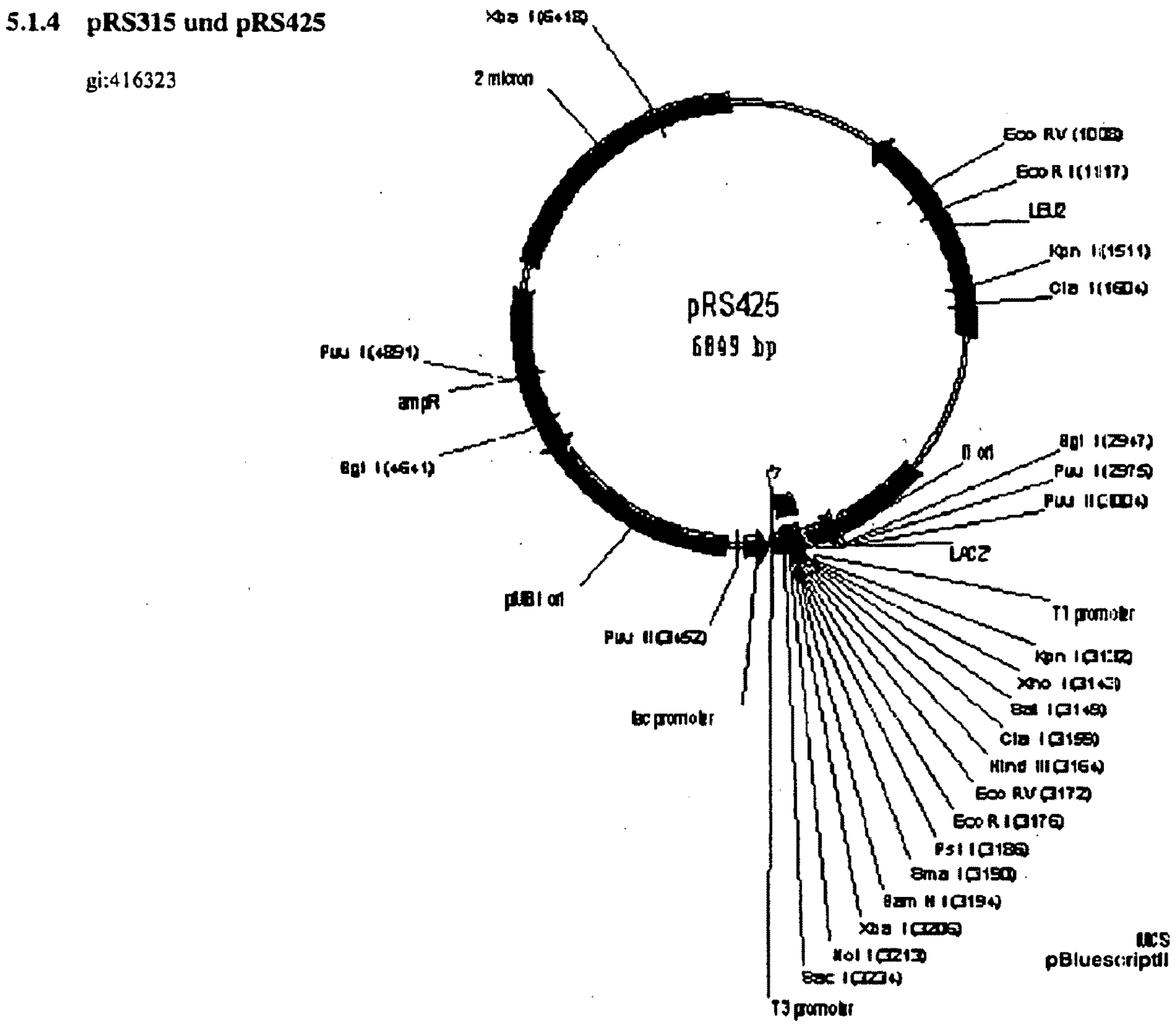


Figure 9b)

5.1.6 pRS315_ und pRS425_Gal1-10+P4H(A1+B)

Fig. 9c)

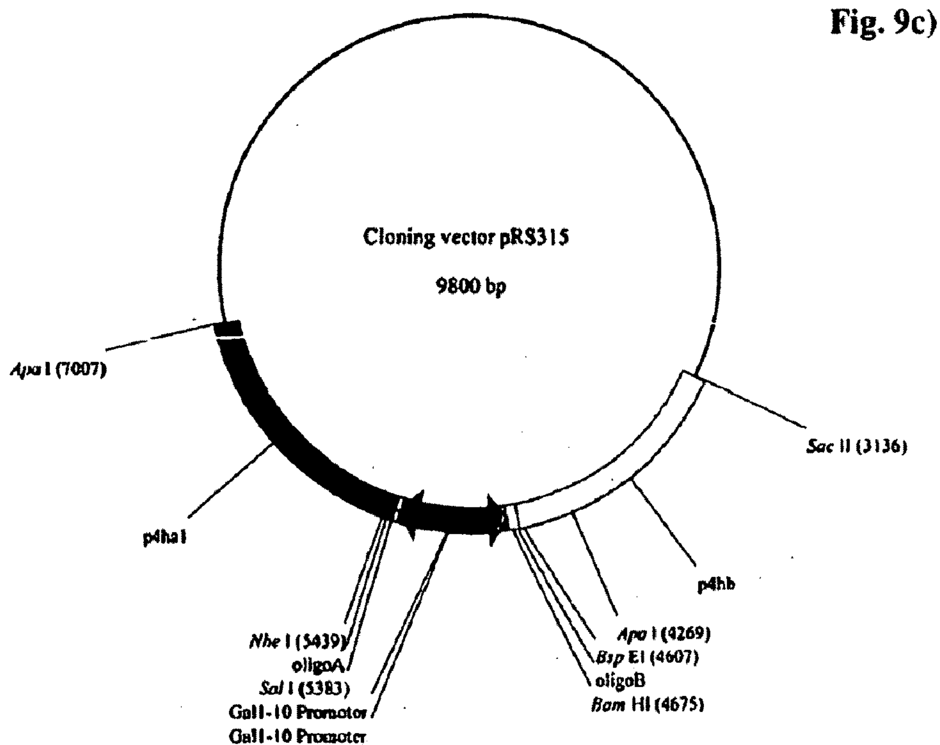
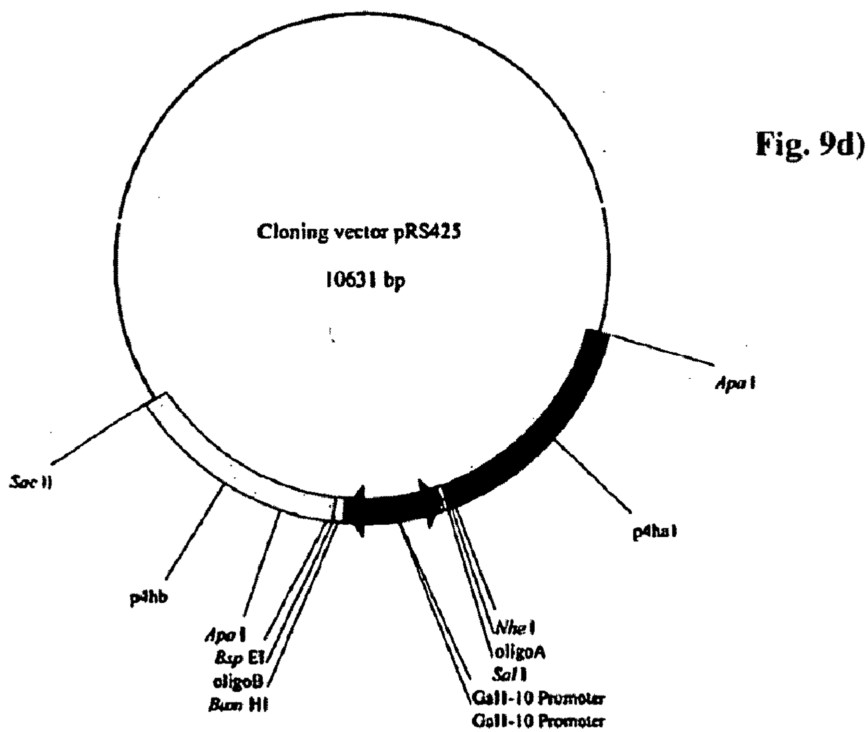


Fig. 9d)



PRODUCTION OF RECOMBINANT COLLAGEN LIKE PROTEINS

[0001] The present invention is directed to a yeast cell for producing a recombinant collagen like protein. The present invention is further directed to a kit of parts or a co-expression system for use in the production of such a protein and to a method of producing said recombinant protein and a thread made therefrom. Furthermore, the invention pertains to proteins or threads obtainable by these methods as well as their use in various fields of technology and medicine.

[0002] Marine mussels are found in the turbulent habitat of the inter-tidal zone and here, marine mussels have been very successful in colonizing rocks, which are exposed to wind and waves. This success is partially due to a unique anchorage by which they fix themselves on the solid surfaces of the rocks. A part of this anchorage is a fibrilar structure, known as "byssus" or also known as "mussel silk". The byssus provides mussels with the necessary tenacity to survive the incessant buffeting of waves by attaching to rocks or hard surfaces.

[0003] The mussel byssus is completely consisting of extra-cellular matrix which is forming a bundle of short threads that resemble tiny tendons [2]. Byssus threads show unusual mechanical properties, since they resemble soft rubber at one end and rigid nylon at the other and these properties are found with a seamless and gradual transition [4]. Byssal threads are also elastomeric: they are able to withstand significant deformations without rupture and can return to their original state, when the stress is removed [5]. At the distal end, the byssus threads are fixed by adhesive plaques at the rock. At the proximal ends, the byssus threads are combined to a so-called byssus stem, which is anchored at the base of the mussel foot (see FIG. **3**).

[0004] The byssus threads of marine mussels are elastomeric fibers with a great capacity of absorbing and dissipating energy. Up to 70% of the total absorbed energy can be dissipated in the byssus. In *Mytilus* species (*M. edulis* and *M. galoprovincialis*), each new thread has dimensions of a few centimeters in length and less than 0.1 cm in diameter and is produced in ca. 5 minutes in the ventral groove of the foot by a process akin to reaction injection molding [3].

[0005] Morphologically, the byssus is divided into four sections (from proximal to distal): root, stem, thread and plaque or pad. Furthermore, the thread is further subdivided into proximal and distal portions according to appearance, i.e. smooth and stiff for the distal, soft and weaker for the proximal portion.

[0006] Byssus threads are elastomeric. The Young's modulus is low (in the range of from 10-500 MPa), the extensibility can be as high as 200% and there is restorative recall. In common with other protein elastomers as elastin, resiline and abductine, byssus threads are quite tough. Thoughness and energy dissipation are both crucial properties for holdfasts. Energy dissipation in fibers subjected to cyclic stress-strain-analysis is frequently normalized with respect to the total absorbed energy and reported as hysteresis or percentage hysteresis.

[0007] The stress-strain cycle for one thread has been dissected into separate mechanical contributions for the distal and proximal portions of the thread. As mentioned above, of these, the distal portion is stronger, stiffer and superior at damping whereas the proximal portion is softer and weaker with a lower, but still significant hysteresis.

[0008] The mechanical properties of byssus threads are further complicated by time- and strain-dependent behavior. It was demonstrated that, when strained beyond its yield point, the distal portion exhibited a schematic stress softening, i.e. the initial modulus of the second cycle was reduced to about 20% of the modulus in the first cycle (500-80 MPa). The complete recovery of the modulus of the first cycle was slow, e.g. longer than 24 h but significant partial recovery can occur within 1 h (30% of the original values). The proximal portion also shows a tendency to change stiffness with cyclic loading. In this case, there is strain-stiffening from an initial modulus of 35 MPa to an asymptotic leveling at 50 MPa, an increase of about 40%.

[0009] MASCOLO and WAITE (1986) first identified chemical gradients in byssus threads in *Mytilus*. After treatment of the threads with pepsin, two pepsin-resistant collagen fragments, called ColP and ColD, having molecular weights of 50 kDa and 60 kDa, respectively were identified. ColP can be found predominantly in the proximal area and is hardly to be found in the distal area. In contrast, the amount of ColD increases in the distal part to approximately 100% (LUCAS et al., 2002; QIN & WAITE, 1995). In the byssus thread as well as in the mussel foot, there is a further collagen-like protein which takes part in the construction of the thread structure. This additional protein is called ColNG (NG=no gradient), and is, in contrast to ColD and ColP, evenly distributed throughout the whole thread. Its physiological function presumably is being an adapter between the two other thread collagens (QIN & WAITE, 1998).

[0010] The Pepsin-cleaved fragments ColD and P originate from the so-called preCollagens P and D. Both preCol's (i.e. D and P) from *M. edulis* are characterized by a common basic structure: a central collagen helix which is flanked by different flanking regions, which are each terminated by a histidine and DOPA rich terminus (see FIG. **1**).

[0011] The mechanism for the assembly of byssus collagens into fibers has been an elusive aspect of the byssus biochemistry. It is well recognized that the collagens undergo stabilization via cross-linking; however the chemistry is still not well understood. There are two distinct cross-linking possibilities: metal complexation and covalent bond formation between collagen units [8, 9]. Metal complexation is suggested by the high levels of iron, copper, nickel and zinc found in byssus and by the occurrence of metal-binding histidine-rich sequences in both terminals of the byssal proteins. Moreover, DOPA is present in both the termini of all PreCol's. Peptidyl-DOPA provides excellent metal binding sites and peptidyl-DOPA-Fe(III) chelates have been reported in the marine adhesive plaque mefp-1 [10]. Further, it has been shown that removal of metal ions from byssal fiber by EDTA reduces the yield strength of the fiber. Covalent cross-links have also been observed. They are generally formed by oxidative coupling between tyrosines, DOPA and cysteines. In a study of byssus stressed by conditions of high flow and aeration, the primary product of oxidation was found to be 5,5'-diDOPA[11]. Other possible coupling products like the Michael-type addition of lysines to oxidized DOPA have not been found [7].

[0012] Like "normal" collagen, each mussel collagen has a signal sequence of 20 amino acids which make sure that the alpha-chains are transported into the endoplasmatic reticulum. There, three identical alpha-chains assemble to a homotrimer. The ColD alpha-chain, which means the pepsin-cleaved preColD, has a molecular mass of 60 kDa by SDS-

PAGE and 47 kDa by MALDI-TOF mass spectometry (QIN et al., 1997). The alpha-chain of ColP, which means the pepsin-cleaved preColP, has a molecular mass of 55 kDa (by SDS-PAGE) and 40 kDa (MALDI-TOF), respectively (COYNE et al., 1997). The precursors of the alpha-chain are named preColD and preColP and have molecular masses of 95 and 97 kDa by means of SDS-PAGE analysis and 75 and 80 kDa respectively by analysis with MALDI-TOF mass spectometry (COYNE et al., 1997; QIN et al., 1997). Both collagens have characteristics which are typical for collagen type I-III. Both have an amount of more than 34% of glycine and show a proline and hydroxyproline content of combined 20% within the collagen domain.

[0013] The flanking regions fully correspond to other structural proteins, namely elastin (preColP) and silk-fibroin (preColD). This structural construction gives an explanation for the mechanical behavior of mussel byssi. For this reason, it would be highly relevant to recombinantly produce the underlying mussel byssus collagens in order to use these extraordinary natural materials as building blocks in new technological applications.

[0014] The development of materials having defined characteristics, in particular of materials which are capable of regenerate themselves following stress or overloading has been of high interest in the material sciences for a long time. Composite structures are of gaining interest in technology, in particular for electronic components and devices, energy converters and other materials. By combination of materials having different mechanical characteristics, structural interfaces will be formed causing new technological problems.

[0015] Thus, for many applications it would highly desirable to provide a graduated structure thereby reducing the overall load of the material.

[0016] Furthermore, the use and application of mussel collagens in medicine is of great interest because of the high potential biocompatibility. Based on this, medical transplants and tissues could be generated having a high degree of immunocompatibility. The production of recombinant mussel collagens is an interesting and important technical problem which has to be solved before technical applications of mussel collagens may be envisioned.

[0017] Therefore it is an object underlying the present invention to provide recombinant mussel byssus proteins having enhanced characteristics as, in particular, improved capability of being expressed in high yield and good strength and flexibility. It is a further object of the present invention to provide recombinant mussel byssus proteins which can be specifically adapted to the required application by specific arrangement of the building blocks on which they are based to provide a graduated structure. Furthermore, it is an object of the present invention to provide expression vectors coding for recombinant mussel byssus proteins, which can be conveniently expressed in already known eucaryotic expression systems. Additionally, it is an object of the present invention to provide improved paper, textile and leather products. Additional objects are to provide new proteins and further materials based on recombinant mussel byssus proteins such as spheres, nanofibrils, hydrogels, threads, foams, films for use in biotechnology, medicine, pharmaceutical and food applications, cosmetics, in electronic devices and for other commercial purposes. It is a still further object of the present invention to provide a host cell, which is capable of expressing collagen like proteins, in particular mussel byssus proteins, in high yield and quality.

[0018] These objects are solved by the subject-matter of the independent claims. Preferred embodiments are set forth in the dependent claims.

[0019] Up to now, the expression of recombinant mussel byssus proteins has never been shown. This might be at least partially due to the complex process of expressing those proteins and threads made therefrom. The complexity of the biosynthesis of collagen leads to a reduced predictability of the outcome of any attempt to express recombinant collagens and, therefore, these attempts might presumably lead to improperly folded proteins, low yield or, in the worst case, to no expression of collagen at all.

[0020] In the present invention, a host cell system is provided which results in high yields of properly folded collagen like proteins, in particular of mussel byssus proteins.

[0021] The present invention in particular is directed to the following aspects and embodiments:

[0022] According to a first aspect, the present invention provides a yeast cell for producing a recombinant collagen like protein, in particular mussel byssus protein, which yeast cell has been transformed with the following elements:

a) a first expression vector which codes for said recombinant collagen like protein; and

b) a second expression vector comprising a nucleic acid coding for prolyl-4-hydroxylase (P4H).

[0023] Due to the complexity of the biosynthesis of collagen, for the recombinant synthesis of collagen-like proteins, the inventors found out that some factors have to be considered, the most important one being the posttranslational modification in the endoplasmatic reticulum (ER) of proline to hydroxyproline by prolyl-4 hydroxylase, a tetrameric enzyme, which is composed of the two sub-units of alpha-PH (=P4HA) and PDI (=P4HB) (BULLEID et al., 2000). For this reason, procaryotic expression systems, for example bacterial expression systems, may not be used in the present invention.

[0024] Yeasts on the one hand offer the cell compartmentation which is required for the synthesis of collagen, on the other hand, however, they are lacking the enzyme prolyl-hydroxylase (P4H) which is required for the synthesis of collagen. Apart therefrom, yeasts would be a desirable expression system for recombinant collagens since their cultivation, also in large scale expression systems, is comparably easy to achieve and the yield of recombinant protein therefrom is superior to other expression systems. Thus, expression in yeast might lead to an efficient (and also cost-effective) production of recombinant collagen like proteins, in particular of mussel byssus proteins. However, as a result of the above drawbacks of yeast cells, an expression of those proteins in yeast cells has not been achieved up to now.

[0025] It could be shown by the inventors for yeast cells, which do not possess P4H, that human P4H subunits can be produced recombinantly and can be correctly folded. Apart therefrom, it could be shown for these yeast strains that by co-expression of both human P4H subunits, the synthesis of mussel byssus collagen is possible and folded, stabile collagen is formed. Interestingly, the co-expression of the genes of both P4H subunits is sufficient for the formation of a stable triple helix in yeast and no further enzymes or folding promoters or chaperones specific for collagen are required, as for example Hsp47, or in other words, the chaperones which are inherent to yeast are sufficiently “active”. Human collagens, recombinantly produced in yeast possessed the same content

of hydroxyproline and, furthermore, are identical in respect to many other characteristics compared to native collagens.

[0026] By efficient transport in the ER of yeast, signal sequences of the co-expressed P4H subunits play an important role. A maximum sufficiency of localization can be achieved by replacing in a preferred embodiment, the human with a yeast signal sequence, for example from the *S. cerevisae* pheromone mating factor alpha1 (MFa). The P4H subunits modified by the MFa signal sequence were effectively transported into the lumen of the ER.

[0027] More preferably, the signal sequence is mating factor alpha 1 (MFa) of *S. cerevisiae* according to SEQ ID NO: 10.

[0028] As a yeast cell, preferably *S. cerevisiae, Schizosaccharomyces pombe, Pichia pastoris, Candida albicans*, or *Hansenula polymorpha* cells might be used.

[0029] The first expression vector preferably further comprises one or more regulatory elements. The expression vector must be suitable for expression in yeast cells.

[0030] Preferably, the regulatory elements contain a promoter selected from constitutive or inducible promoters, more specifically from GPD, GAL4, CUP1, MET25, GAL1 or GAL1-10.

[0031] In a further embodiment, the expression vector is a plasmid.

[0032] Preferably, the recombinant collagen like protein is a recombinant mussel byssus protein comprising or consisting of one or more fragments of a collagen domain flanked by elastin or silk fibroin.

[0033] This recombinant mussel byssus protein is composed of one or more types of building blocks, which provide different characteristics to the protein formed: as mentioned above, elastin and silk-fibroin have certain mechanical characteristics, which can give an explanation for the mechanical behavior of mussel byssi and, thus, also for the design of recombinantly produced mussel byssus proteins.

[0034] Therefore, these fragments can be used as one single type of fragment only, or, as an alternative, the recombinant protein can comprise two or more different fragments. For example, if great elasticity is wanted, the protein may only or predominantly comprise fragments of collagen flanked by elastin. If great stiffness and strength is required, the protein may comprise fragments of collagen flanked by silk-fibroin. As a further and preferred alternative, the protein may comprise a mixture of both types of fragments, for example forming a gradient from one region to the other. Thus, a protein/thread can be formed having specifically adapted configurations, i.e. parts having higher elasticity and parts having higher stiffness etc.

[0035] The term "flanked" means that elastin (or silk-fibroin) is present on both sides of the collagen domain.

[0036] The above fragments may be naturally derived, for example, the fragments may be obtained from *Mytilus* sp., preferably from *M. edulis, M. galloprovincialis, M. californians*, or *Geukeria demissa*.

[0037] According to a preferred embodiment, the recombinant mussel byssus protein of the invention comprises or consists of one or more of the fragments preColP and/or preColD or variants thereof. These fragments have been outlined above. Both preCol's (i.e. D and P) are derived from *M. edulis* and are characterized by a common basic structure: a central collagen helix which is flanked by different flanking regions, which are each terminated by a histidine and DOPA rich terminus (see FIG. **1**). The flanking regions fully correspond to known structural proteins, namely elastin (preColP) and silk-fibroin (preColD).

[0038] The sequences of preColP and preColD are translated from the respective nucleic acids. Therefore, whenever amino acids are recited herein in the following, they are referring to preColP and preColD and these sequences will be used in the various technological applications mentioned hereinabove. The nucleic acid sequences mentioned herein in the first place are directed to preColP and preColD encoding sequences.

[0039] According to a further embodiment, the recombinant protein of the invention comprises or consists of one or more fragments of SEQ ID NO: 3 and/or 4 or variants thereof. The Seq ID No's reflect the sequences of preColP and preColD.

[0040] As mentioned above, the present invention also comprises variants of those amino acid sequences. For example, said variants may contain one or more substitutions, insertions and/or deletions when compared to the amino acid sequences mentioned above.

[0041] In particular variants of the protein, for example deletions, insertions and/or substitutions in the sequence, which cause so-called "silent" changes, are considered to be part of the invention.

[0042] Preferably are such amino acid substitutions the result of substitutions which substitute one amino acid with a similar amino acid with similar structural and/or chemical properties, i.e. conservative amino acid substitutions.

[0043] Amino acid substitutions can be performed on the basis of similarity in polarity, charges, solubility, hydrophobic, hydrophilic, and/or amphipathic (amphiphil) nature of the involved residues. Examples for hydrophobic amino acids are alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. Polar, neutral amino acids include glycine, serine, threonine, cysteine, thyrosine, asparagine and glutamine. Positively (basic) charged amino acids include arginine, lysine and histidine. And negatively charged amino acids include aspartic acid and glutamic acid.

[0044] "Insertions" or "deletions" usually range from one to five amino acids. The allowed degree of variation can be experimentally determined via methodically applied insertions, deletions or substitutions of amino acids in a polypeptide molecule using recombinant DNA methods. The resulting variants can be tested for their characteristics, in particular their mechanical characteristics.

[0045] It is noted that the term "variant" as used herein also comprises the above amino acid sequences of preColP and preColD, wherein the first 19 amino acids constituting the original mussel signal sequence were replaced by other signal sequences. A preferred example hereof is replacement of the mussel signal sequence by signal sequence alpha MF (SEQ ID NO: 10: "MRFPSIFTAV LFAASSALA"). This signal sequence in particular is suitable for expression of the nucleic acids in yeasts.

[0046] The present invention also provides an isolated nucleic acid encoding the recombinant protein as defined above. The term "isolated" as used herein with reference to nucleic acids refers to a naturally-occurring nucleic acid that is not immediately contiguous with both of the sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally-occurring genome of the organism from which it is derived.

[0047] For example, an isolated nucleic acid can be, without limitation, a recombinant DNA molecule of any length, provided one of the nucleic acid sequences normally found immediately flanking that recombinant DNA molecule in a naturally-occurring genome is removed or absent. Thus, an isolated nucleic acid includes, without limitation, a recombinant DNA that exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences as well as recombinant DNA that is incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or into the genomic DNA of a prokaryote or eukaryote. In addition, an isolated nucleic acid can include a recombinant DNA molecule that is part of a hybrid or fusion nucleic acid sequence.

[0048] The term "isolated" also includes any non-naturally-occurring nucleic acid since non-naturally-occurring nucleic acid sequences are not found in nature and do not have immediately contiguous sequences in a naturally-occurring genome. For example, non-naturally-occurring nucleic acid such as an engineered nucleic acid is considered to be isolated nucleic acid. Engineered nucleic acid can be made using common molecular cloning or chemical nucleic acid synthesis techniques. Isolated non-naturally-occurring nucleic acids can be independent of other sequences, or incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or the genomic DNA of a prokaryote or eukaryote. In addition, a non-naturally-occurring nucleic acid can include a nucleic acid molecule that is part of a hybrid or fusion nucleic acid sequence.

[0049] It will be apparent to those of skill in the art that a nucleic acid existing among hundreds to millions of other nucleic acid molecules within, for example, cDNA or genomic libraries, or gel slices containing a genomic DNA restriction digest is not to be considered an isolated nucleic acid.

[0050] A nucleic acid encoding the above amino acids may be a nucleic acid sequence coding for the mature or the immature amino acid sequence of the recombinant mussel byssus protein.

[0051] In a preferred embodiment, the isolated nucleic acid comprises or consists of the nucleic acid of SEQ ID NO: 1 and/or 2 or variants thereof. These variants are each defined as having one or more substitutions, insertions and/or deletions as compared to the sequences of SEQ ID NO: 1 or 2, provided that said variants hybridize under moderately stringent or stringent conditions to a nucleic acid which comprises the sequence of SEQ ID NO: 1 or 2, or provided that said variants comprise nucleic acid changes due to the degeneracy of the genetic code, which code for the same or a functionally equivalent amino acid as the nucleic acid sequence of SEQ ID NO: 1 or 2.

[0052] As mentioned above, the present invention also encompasses a variant of said nucleic acids,

[0053] wherein the nucleic acids coding for the first 19 amino acids (signal sequence) were replaced, preferably by the yeast signal sequence MFa (SEQ ID NO: 10).

[0054] Stringency of hybridization, as used herein, refers to conditions under which polynucleotide duplexes are stable. As known to those of skill in the art, the stability of duplex is a function of sodium ion concentration and temperature (see, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd Ed. (Cold Spring Harbor Laboratory, (1989)). Stringency levels used to hybridize can be readily varied by those of skill in the art.

[0055] Stringent washing conditions mean 0.2×SSC (0.03 M NaCl, 0.003 M sodium citrate, pH 7)/0.1% SDS at 65° C. For shorter fragments, e.g. oligonucleotides up to 30 nucleotides, the hybridization temperature is below 65° C., for example at 50° C., preferably above 55° C., but below 65° C. Stringent hybridization temperatures are dependent on the size or length, respectively of the nucleic acid and their nucleic acid composition and will be experimentally determined by the skilled artisan. Moderate stringent hybridization temperatures are for example 42° C. and washing conditions with 0.2×SSC/0.1% SDS at 42° C.

[0056] The P4H used in the present invention preferably is human or mussel P4H.

[0057] In a second aspect, a kit of parts or a co-expression system comprising the following constituents is provided:

[0058] a) the first expression vector as defined herein; and

[0059] b) the second expression vector as defined above.

[0060] This kit of parts or co-expression system may be efficiently used in expressing the recombinant mussel byssus protein in yeast cells.

[0061] In a still further aspect, a method of producing recombinant collagen like proteins, in particular mussel byssus proteins is disclosed comprising the steps of:

[0062] a) providing a yeast cell as defined hereinabove;

[0063] b) transforming said yeast cell with an expression vector or the co-expression system explained above;

[0064] c) expressing recombinant protein from said host cell under suitable conditions; and

[0065] d) recovering said protein.

[0066] Furthermore, a method for producing threads from recombinant mussel byssus protein is provided, comprising the following steps:

[0067] a) providing recombinant protein produced in accordance with the above method, and

[0068] b) spinning or moulding said protein into threads by a suitable method.

[0069] The spinning may preferably be done by electrospinning. Electrospinning is a fiber formation technique that uses electrostatic forces to create continuous, nanometer diameter fibers. A wide variety of natural and artificial polymers have been electrospun from the solution and melt phase and are of interest for an assortment of application areas that require high surface area materials (filtration membranes and biomedical devices).

[0070] An additional aspect of the invention is a protein or thread obtainable by one of the above methods.

[0071] The proteins/threads of the invention find application preferably in the field of biotechnology and/or medicine.

[0072] For example, they might be used for the manufacture of wound closure or coverage systems or suture materials. Furthermore, the proteins/threads may preferably be used for the manufacture of replacement materials, preferably artificial cartilage or tendon materials.

[0073] Additionally, the threads/proteins of the invention can be used in the manufacture of medical devices such as medical adhesive strips, skin grafts, replacement ligaments, and surgical mesh; and in a wide range of industrial and commercial products, such as clothing fabric, bullet-proof vest lining, container fabric, bag or purse straps, cable, rope, adhesive binding material, non-adhesive binding material, strapping material, automotive covers and parts, aircraft con-

struction material, weatherproofing material, flexible partition material, sports equipment; and, in fact, in nearly any use of fiber or fabric for which high tensile strength and elasticity are desired characteristics. Adaptability and use of the stable fiber product in other forms, such as a dry spray coating, bead-like particles, or use in a mixture with other compositions is also contemplated by the present invention.

[0074] It is explicitely noted that preferred applications of the mussel byssus collagens of the present invention are in the manufacture and processing of clothing fabric (textiles) and leather, automotive covers and parts, aircraft construction materials as well as in the manufacture and processing of paper.

[0075] The recombinant mussel byssus proteins of the present invention may be added to cellulose and keratin and collagen products and thus, the present invention is also directed to a paper or a skin care and hair care product, comprising cellulose and/or keratin and/or collagen and the proteins of the present invention. Papers and skin care and hair care products, in which the proteins of the present invention are incorporated are showing improved characteristics, in particular improved tensile strength or tear strength.

[0076] Furthermore, the recombinant mussel byssus proteins of the invention may be used as a coating for textile and leather products, thereby conferring stability and durability to the coated product. The proteins in particular show applicability for coating leather products, since in this case, tanning and its negative effects for environment can be avoided or at least reduced.

[0077] The invention is also directed to products containing said mussel byssus proteins, for example, wound closure or coverage systems, suture materials, replacement materials, preferably artificial cartilage or tendon materials, cosmetics, drug delivery vehicles, fabrics, textile, paper product, leather product, automotive parts or aircraft parts. In general, it is also directed to materials based on recombinant mussel byssus proteins such as spheres, nanofibrils, hydrogels, foams, films.

[0078] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the examples are illustrative only and not intended to be limiting.

[0079] The invention is now further illustrated by examples and the accompanying drawings, which are showing the following:

[0080] FIG. **1** is illustrating the general structure of mussel byssus collagens;

[0081] FIG. **2** depicts a series of SEM images of byssus threads in direction distal to proximal—the marked portions are each enlarged below. a) distal; b) median; c) proximal;

[0082] FIG. **3** shows the structure of mussel byssus;

[0083] FIG. **4** illustrates a mussel adhered to a solid surface by byssus threads;

[0084] FIG. **5**: (A) Distribution of preCols in the thread. (B) Schematic of a collagenous subunit with flanking domains. Terminal regions denoted by diamonds are His-rich. DOPA is designated by Y. (C) Model of cross-linking interactions between axial and lateral preCols;

[0085] FIG. **6**: Design of the P4H construct;

[0086] FIG. **7**: Design of oligonucleotides to generate α-MF signal sequences ready to be cloned into respective expression plasmids;

[0087] FIG. **8**: Cloning strategy for α-PH;

[0088] FIG. **9**: Vector maps.

EXAMPLES

Expression of Collagen Proteins of Mussel Byssus in Yeast

[0089] Collagen synthesis in general reflects a complex biochemical process. The process requires e.g. post-translational modification of certain prolines of the respective collagens to 4-hydroxyproline in the ER by the enzyme Prolyl 4-hydroxylase (P4H). P4H, an $\alpha_2\beta_2$ tetramer in vertebrates, plays a central role in the synthesis of collagens. 4-hydroxyproline residues, generated by P4H, are essential for the folding of the newly synthesized collagen polypeptide chains into triple-helical collagen molecules [13].

Human Prolyl-4-Hydroxylase Expression Construct

[0090] The construct of P4H requires the cloning of a signal sequence into the yeast vector adjacent to the genes for the two subunits of P4H, α-PH and PDI. Both genes are placed under the control of a bi-directional promotor, which is induced in the presence of Galactose (Gal1/10) (see FIG. **6**). The signal sequence is required for translocation of P4H subunits into the ER, where they can assemble into the native tetramer. Maximum efficiency for localization has been achieved when the human signal sequence is replaced by yeast's own signal sequence of the mating factor α-MF [12]. See FIG. **6** in this context.

[0091] The gene for α-PH (without signal sequence) is amplified by PCR from a c-DNA library from HepG2 liver cells (provided by Professor Adamski, GSF Munich, Germany), while the c-DNA of the beta-subunit (PDI) (without signal sequence) will be amplified from an *E. coli* cloning vector (provided by Professor Neil Bulleid, University of Manchester, UK). For each gene a respective αMF signal sequence will be engineered based on two single stranded oligonucleotides. The oligos A and B are planned in a way (see FIG. **7**) that after annealing the double stranded DNA can be directly cloned into respective vectors.

[0092] The cloning strategy for α-PH is shown as an example (FIG. **8**). Cloning of the cDNA of PDI will be performed in an identical way. Two different yeast vectors will be used: pRS315 (CEN, reflecting a single copy number plasmid) and pRS425 (2μ, reflecting a multi copy plasmid), both containing the bi-directional Gal1/10 promotor, allowing the simultaneous expression of both subunits from one plasmid.

Recombinant Synthesis of PreColD and PreColP

[0093] Recombinant synthesis of preColD and preColP, two major protein components of Mussel Byssus is an example of the present invention. The c-DNA of PreColP and PreColD in *E. coli* cloning vectors has been obtained from Prof. Waite (UCSB, USA). The cDNA is amplified by PCR and cloned into different yeast expression vectors. The vectors differ in copy number per cell, as well as in the choice of the activator (either the constitutive promotor GPD or the inducible promotor GAL4). Also the original signal sequence

will be replaced by the signal sequence of the yeast α-MF for maximum localization efficiency.

Detection of ColP and ColD During Recombinant Synthesis

[0094] The test for the efficient recombinant synthesis of mussel collagen requires availability of polyclonal antibodies against mussel collagen. Preliminary tests with polyclonal antibodies against human collagen type I-III showed a very weak cross-reactivity against chemically denatured collagen from mussel byssus. This cross-reactivity is not sufficient to detect the levels of collagen present during the recombinant synthesis. Hence antibodies need to be raised against mussel collagen. In order to be able to raise antibodies, purified native mussel collagen is required. Byssus will be extracted from fresh mussels and purified using several chromatographic methods (reverse phase chromatography among others).

[0095] The purified protein samples, which contain both preColD and preColP, are used to immunize rabbits and generate antibodies.

Biophysical Studies on Recombinant Collagen

[0096] Various physical methods can be used to characterize the individual proteins preColP/preColD and to evaluate the efficiency of fiber formation on self-assembly. These methods include far- and near-UV circular dichroism (CD), static and dynamic light scattering, fourier transformed infrared spectroscopy (FTIR), electron microscopy (EM), atomic force microscopy (AFM) and field flow fractionation (FFF).

Characterization of Individual PreColP and PreColD

[0097] CD and FTIR will be used to determine the secondary and tertiary structures of preColP and preColD. Their chemical and thermal stability will also be tested under various conditions. Data on the shape of the proteins involved in the collagen formation are provided by light scattering, by AFM and TEM.

Evaluation of Rate and Efficiency of Fiber Formation

[0098] Secondary and tertiary structure of mussel byssus collagen are analyzed by CD and FTIR. AFM and EM will provide information on the quaternary structure and morphology of the assembled aggregates and fibers. FFF, a one-phase matrix-free chromatography, would be used to evaluate the different kinds of species formed during assembly of the collagen. Since FFF is a matrix-free chromatography technique, it can separate different dissolved macromolecules, especially fibers, which can not be separated by other classical chromatographic techniques.

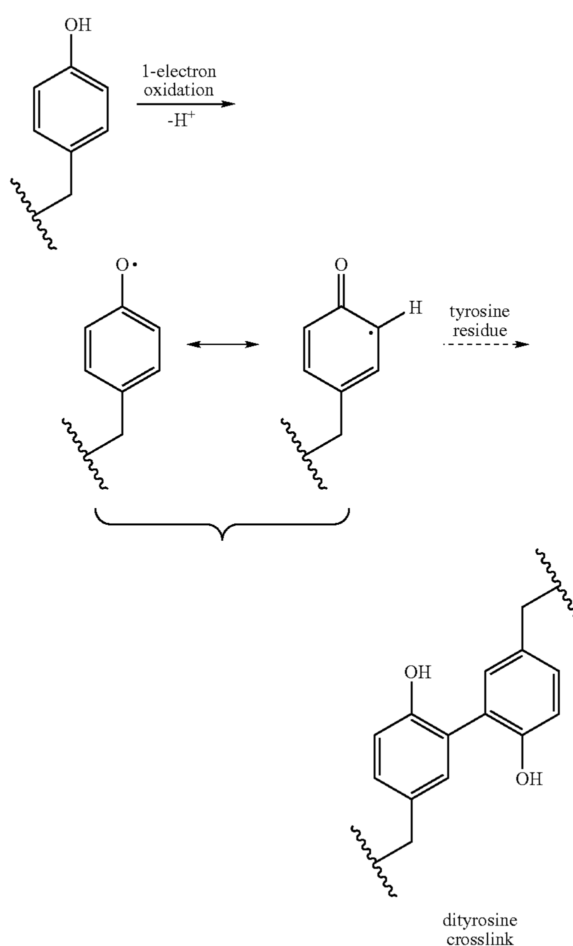
[0099] Kinetics of the assembly process will also be investigated with CD and FTIR, which can be performed as a function of time by monitoring changes in the secondary and tertiary structure during the fiber formation. Further, static light scattering, dynamic light scattering and time-lapse AFM allow to monitor protein assembly in real-time.

[0100] Fluorescent dyes can also be used to investigate the structural changes associated with protein assembly. The fluorescent properties of some dyes, such as the N-benzyl derivatives of 3-chloro-6-methoxy-9 aminoacridine and amino naphthalene sulfonic acids, change with the polarity of the protein environment. Therefore, labeling of collagen with these dyes are used to study its assembly process.

Study of the Role of Metals in the Assembly and Cross-Linking of Collagens

Role of GGH in DOPA and Tyrosine Cross-Links

[0101] The amino acid sequence GGH has been observed at the carboxylterminus of both preColP and preColD. The tripeptide NH2-Gly-Gly-His-COOH(GGH) mediates cross-linking of associated proteins in solution in the presence of nickel acetate [Ni(OAc)2] and oxidant magnesium monoperoxyphthalate (MMPP) [18, 19]. Further, the peptide provides a favorable coordination environment for the nickel center, and a putative Ni(III) intermediate is thought to abstract an electron from the aromatic ring of an accessible tyrosine, leading to a tyrosyl radical after the loss of a proton (see FIG. 5). The highly activated radical intermediate couples to a nearby tyrosine leading to a cross-linked adduct.



Mechanism of Tyrosine Cross-Linking

[0102] A possible role for the GGH in the carboxylterminus of mussel collagens could be to bind Ni(II) in order to form the active catalyst Ni-GGH. This complex can slowly catalyze aerial oxidation of tyrosine and DOPA to form cross-links. The proximity of the catalyst to tyrosine and/or DOPA would significantly increase the oxidation rates. To test this hypothesis, the GGH sequence could be genetically deleted

or modified so that it would not bind nickel. The rate of cross-linking and assembly would be monitored by methods described above.

Chemical Oxidation of Tyrosines to Form Cross-Links in Collagen

[0103] Visible-light irradiation in the presence of ruthenium(II) tris(bipyridyl) dication $[Ru(bpy)_3^{2+}]$ and an electron acceptor such as ammonium persulfate (APS) [18, 20, 21] induces very efficient cross-linking between contacting proteins. This process is highly efficient and the mechanism has been assumed to be similar to that of Ni/GGH/MMPP. The fiber formed from self-assembly of preColP and/or preColD will be subject to irradiation with $[Ru(bpy)_3^{2+}]$ in the presence of APS. This should lead to increased cross-linking of tyrosine/DOPA in byssal collagen and lead to fibers with altered mechanical properties, which will be assessed upon physico-chemical characterization as described above.

Further Examples and Sequences:

[0104] DNA sequences of mussel collagen preColP and proColD are provided in the following. The cDNA of both preCol proteins (P and D) were integrated in the pGEM-T cloning vectors. In order to verify the starting material, both cDNAs were completely sequenced and as standard primers T7 and SP6 were used and as internal primers preCol (P or D)-T7/1 and SP6/1, respectively, were used. The obtained DNA sequences showed differences as regards the published versions of both preCols and were compared accordingly.

preColP

(SEQ ID NO:1)

```
 atggttcg gttctcccta gcatcggtac tattactggc agtcaccagc acagctttcg
ctggaccagt tagtgattat ggtggtggtg gaatcaaagt agtaccctac cacggaggcg
gaggtggaag cggcggcggt ggcggtggag gccatggcgg aagcggtatt ggtggtatcg
gaggaggatc atcacatgca catgcccact cttcagcatc tgcccatgtg caccattttg
gaccaggtgg atcttcacac gcatcagctg gttcatcatc ccatgcatcc gcatcccata
acggtttagg aggtggcagt gctcatgcac atagcagttc cagcgccaac gctcattccg
gtggattcgg tggattcggc ggtattggtg gtattggcgg tattggccca ggaggaagtg
tcggaggcgg tattggccca ggaggaagtg tcggaggcgg cattggcggt attggcggta
ttggcggcgg tggtggacca ggcggtaatg gcggtatcgg attcggacca ggattcggag
gaggattcgg accaggttca tctgctagtg gatccggaag tggcagcgca ttcggtggtc
caggaggttc aagcgcaagc gcaaacgcag ctgcacgtgc aaatgcaaat ggtggtggag
gattcggtgg accaggtacc ccaggaaact caggaccacc aggccaaccc ggactaccag
gagcaccagg ccaaccagga cgtccaggaa gtaccccacc aggtcgacca ggaaaccccg
gaccaccagg tcaaccaggt aacccaggac gtccaggctc ttcaggaaga ccaggaggat
ccggccaacc aggaggtcca ggacgtccag gaaccccegg caaaccagga aaccgaggac
aaccaggaca gccaggcggc ccaggacaac caggtcaccc aggagcagga ggacaaccag
gacgaaacgg aaatccagga aaccccggta aaccaggaac accaggtcac ccaggaacag
caggatcacg aggaatgcca ggaaccccag gaaccccagg acaaccagga attccaggca
ccgtcggagg acgaggacca agaggaccag ctggaatcat cggattaatt ggaccaaaag
gaaatccagg agagccagga aatccaggtg caccaggagg cccaggatct acaggaccac
aaggaccaca aggaccagcc ggaggaccag gagcatcagg cggaccagga gacaaaggcg
caccaggtac accaggagga actggaccaa gaggaccaat cggaccatca ggaccatcag
gagcaccagg ggaccaagga ccacaaggag gtagaggaac accaggactc gcaggcaaac
caggacctaa aggactacaa ggatcaaatg gagaagttgg accccaagga ccatctggac
ccgcaggacc acaaggccca caaggaaaga acggtgtcaa aggagcagca ggagatcaag
gagctagggg accagaagga aaagccggac cagctggacc acaaggagaa acaggaccaa
aaggaccaac aggagcacaa ggaccagccg gtccagccgg accatcagga gaacaaggac
caggagggga aagaggaggc cagggaccac aaggagctga aggaccaagt ggaccagcag
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gaccaagagg accagcagga tcacaaggac caagtggtga acgcggagaa ccaggagcac

caggtaaaaa aggaccaaat ggagaccgag gaaaccaagg atcaccagga gcaccaggca

aaaacggagc acgaggaaat agaggatcaa gaggaagcaa cggatcaccc ggcagatcag

gatcaccagg aagccgagga aaaccaggac cacaaggacc acatggacca agaggagcaa

gaggatcacc aggacaaaaa ggaccacgtg gagaccaagg agcaccaggt gttattcgta

ttgttatcga tgaccagaga acaggaccag aagttgcaga attcccagga tttggtggat

tcggaggagc ttcagctaac gcagcaagtt cagcaaatgc atttgctggt ggacccggtg

gttccgctgg agcaggttca tcatcaggag ctaacgcaaa cgcaggtgga ttcccattcg

gaggaggacc attcggagga gcaggaggtg gtcccggagc agcaggaggc ccaggaggag

caggaggccc aggaggagta ggaggaggag ttggaggtgg accaggagga gtaggaggtg

gagtaggagg tggaccagga ggagtaggag gtggaccagg aggagcagga ccaggaggag

caggaggatt tggaccagga ggagcaggag gatttggtgg atttggagga ggatctagcg

ctggagcatc atcatcagga tcagcatctg catctaacgg tggaccattc ggagtactca

atgtaggacc cggaggtaga atcggtggtg gaagcgcatc agcatctgca gcatctagag

cacatgcaca cgcttttggt ggtctcggag ggggaagtgc ctcagctggt agtcattcct

catctagctc acactcattt ggcggacacg tattccacag tgtgacccat catggaggtc

catcacatgt ttcaagcgga ggtcacggag gtcatggagg aggtccatac aaacctggat attaa
```

[0105] Due to the degeneration of the genetic code, not every base exchange is leading to an amino acid exchange. Therefore, the DNA sequences were translated into the amino acid sequences and were compared. Here, the alignment of the published sequence (COYNE et al., 1997) (variant P38, (COYNE & WAITE, 2000)) with the sequence of preColP obtained by sequencing is shown. The database sequence corresponds to SEQ ID NO: 9, the sequenced preColP sequence is SEQ ID NO:3.

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Database      1 MVRFSLASVLLLAVTSTAFAGPVSDYGGGGIKVVPYHGGGGGGGGGGGGG  50
                ||||||||||||||||||||||||||||||||||||||||||.|||||||
Sequencing    1 MVRFSLASVLLLAVTSTAFAGPVSDYGGGGIKVVPYHGGGGGSGGGGGG  50
Database     51 HGGSFRNGRHGGIGGIGGGSSHAHAHSSASAHVHHFGPGGSSHASAGSSS 100
                ||||       |||||||||||||||||||||||||||||||||||||||
Sequencing   51 HGGS-------GIGGIGGGSSHAHAHSSASAHVHHFGPGGSSHASAGSSS  50
Database    101 HASASHNGLGGGSAHAHSSSSANAHSGGFGGFGGIGGIGGIGPGGSVGGG 150
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing   94 HASASHNGLGGGSAHAHSSSSANAHSGGFGGFGGIGGIGGIGPGGSVGGG 143
Database    151 IGPGGSVGGGIGGIGGIGGGGGPGGNGGIGFGPGFGGGFGPGSSASGSGS 200
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing  144 IGPGGSVGGGIGGIGGIGGGGGPGGNGGIGFGPGFGGGFGPGSSASGSGS 193
Database    201 GSAFGGPGGSSASANAAARANANGGGGFGGPGTPGNSGPPGQPGLPGAPG 250
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing  194 GSAFGGPGGSSASANAAARANANGGGGFGGPGTPGNSGPPGQPGLPGAPG 243
Database    251 QPGRPGSTPPGRLGNPGPPGQPGNPGRPGSSGRPGGSGQPGGPGRPGTPG 300
                ||||||||||||.|||||||||||||||||||||||||||||||||||||
Sequencing  244 QPGRPGSTPPGRPGNPGPPGQPGNPGRPGSSGRPGGSGQPGGPGRPGTPG 293
Database    301 KPGNRGQPGQPGGPGQPGHPGAGGQPGRNGNPGNPGKPGTPGHPGTAGSR 350
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing  294 QPGRPGSTPPGRPGNPGPPGQPGNPGRPGSSGRPGGSGQPGGPGRPGTPG 343
Database    351 GMPGTPGTPGQPGIPGTVGGRGPRGPAGIIGLIGPKGNPGEPGNPGAPGG 400
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing  344 GMPGTPGTPGQPGIPGTVGGRGPRGPAGIIGLIGPKGNPGEPGNPGAPGG 393
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Database   401 PGSTGPQGPQGPAGGPGASGGPGDKGAPGTPGGTGPRGPIGPSGPSGAPG  450
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing 394 PGSTGPQGPQGPAGGPGASGGPGDKGAPGTPGGTGPRGPIGPSGPSGAPG  443
Database   451 DQGPQGGRGTPGLAGKPGPKGLQGSNGEVGPQGPSGPAGPQGPQGKNGVK  500
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing 444 DQDGPGGRGTPGLAGKPGPKGLQGSNGEVGPQGPSGPAGPQGPQGKNGVK  493
Database   501 GAAGDQGARGPEGKAGPAGPQGETGPKGPTGAQGPAGPAGPSGEQGPGGE  550
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing 494 GAAGDQGARGPEGKAGPAGPQGETGPKGPTGAQGPAGPAGPSGEQGPGGE  543
Database   551 RGGQGPQGAEGPSGPAGPRGPAGSQGPSGERGEPGAPGKKGPNGDRGNQG  600
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing 544 RGGQGPQGAEGPSGPAGPRGPAGSQGPSGERGEPGAPGKKGPNGDRGNQG  593
Database   601 SPGAPGKNGARGNRGSRGSNGSPGRSGSPGSRGKPGPQGPHGPRGLRGSP  650
               |||||||||||||||||||||||||||||||||||||||||||||.||||
Sequencing 594 SPGAPGKNGARGNRGSRGSNGSPGRSGSPGSRGKPGPQGPHGPRGARGSP  643
Database   651 GQKGPRGDQGAPGVIRIVIDDQRTGPEVAEFPGFGGFGGASANAASSANA  700
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing 644 GQKGPRGDQGAPGVIRIVIDDQRTGPEVAEFPGFGGFGGASANAASSANA  693
Database   701 FAGGPGGSAGAGSSSGANANAGGF-----PFGGAGGGPGAAGGPGGAGGP  745
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing 694 FAGGPGGSAGAGSSSGANANAGGFPFGGGPFGGAGGGPGAAGGPGGAGGP  743
Database   746 GGVGGGVGGGPGGVGGGVGGGPGGVGGGPGGAGPGGAGGFGPGGAGGFGG  795
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing 744 GGVGGGVGGGPGGVGGGVGGGPGGVGGGPGGAGPGGAGGFGPGGAGGFGG  793
Database   796 FGGGSSAGASSSGSASASNGGPFGVLNVGPGGRIGGGSASASAASRAHAH  845
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing 794 FGGGSSAGASSSGSASASNGGPFGVLNVGPGGRIGGGSASASAASRAHAH  843
Database   846 AFGGLGGGSASAGSHSSSSSHSFGGHVFHSVTHHGGPSHVSSGGHGGHGG  895
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing 844 AFGGLGGGSASAGSHSSSSSHSFGGHVFHSVTHHGGPSHVSSGGHGGHGG  893
Database   896 GPYKPGY  902
               |||||||
Sequencing 894 GPYKPGY  900
```

[0106] COYNE & WAITE already showed the existence of different preColP variants (P22, P33 and P38) in certain partial regions of their cDNA sequence (COYNE & WAITE, 2000). If these short, known sequence regions of variant P22 are compared with the present DNA sequence of preColP, a matching of 100% is achieved.

```
preColD
                                                            (SEQ ID NO:2)
  atggtcta caaactcctg accgtgtgtc ttgtagcatc tcttctagag atttgcttag
ctgactataa cggcaacaaa cagtatggcg gcagatacgg caacagatac ggaaacggtt
taggaggcgg taatggtggt gcaggagccg tagcccatgc ccatgcccat gcccatgcca
gtgccggagc aaacggaaga gcaagagcac atgcacgagc cttggcccat gcacatgccg
gtggtggcgc tgcacatgga cacccaggat tcccagttgg tggtagcgca agcgcagccg
cacgagcagc agcacgagca tcagcaggag gattaggtgg attcggatca gcagcagcca
atgcagcagc agcagcaaga gcaggagcag gatttggtgg attcggtgga ttaggaggat
tcggaggact cggaggagtt ggcggtccag gtcaaccagg acatgccggt aaacacggaa
ccgcaggagc agcaggcaaa gcaggacgtc caggaccatg tggagataga ggggcaccag
gagtaccagg caaacaagga ccagtaggag gacaaggacc agcaggacca cgaggaccac
gaggagatga aggaccagtt ggaccaaagg gcgaaccagg agcaagagga gctgatggta
aaccaggaga caaaggacct gatggagaaa ccggaccaca aggaccagct ggaccaaagg
gacaagtagg agaccaaggc aaaccaggag caaagggaga aaccggagat caaggagcac
gaggtgaagc aggaaaggcc ggcgaacaag gaccaggagg catccaagga ccaaagggac
cagtaggagg acaaggacca gcaggaccag ccggaccact cggaccacaa ggaccaatgg
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gtgaacgagg accacaagga ccaacaggat cagaaggacc agttggagca ccaggaccaa
agggatcagt cggagaccaa ggagcacaag gagaccaagg agcaactggc gctgatggca
aaaagggaga accaggagag agaggacaac aaggagcagc aggaccagtc ggccgaccag
gaccaagagg agatagagga gcaaagggaa ttcaaggaag ccgaggacga ccaggtggta
tgggtagacg aggaaaccgt ggatcccaag gagcagtagg accacgagga gaaactggcc
cagacggtaa ccaaggacaa cgtggagaac aaggagcacc aggagttatc acccttgtca
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caggagcagg aggaaacggt ggagcaggag caggaggagc aggaggaaac ggtggaggat
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cacaagcagc agcacaagca gcagcatcag cagcagcaaa ttcaggactt ggagcaggag
cagcaagagc agcagcatca gcagccgcta gagcaaccgt agcaggacat ggaagtggaa
ccgccgcagc agcagccaac gcagccgcac aagcacatgc agcaacacga ggacaaggag
gatcacacgc acacgctgcc gccgcagctc acgcagccgc aagtagcgta atccatggtg
gtgactatca cggaaacgat gccggctatc acaaaccagg atattaa
```

[0107] In the following, the alignment of the published sequence (QIN et al., 1997) [gi:2772914] with the overall sequence obtained by sequencing of preColD is shown. The DNA sequences were translated into the protein sequence. It is noted that the database sequence is SEQ ID NO: 8 and the sequence obtained by sequencing is SEQ ID NO: 4.

```
Database      1 MVYKLLTVGLVASLLEICLADYNGNKQYGGRYGNRYGNGLGGGNGGAGAV  50
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing    1 MVYKLLTVGLVASLLEICLADYNGNKQYGGRYGNRYGNGLGGGNGGAGAV  50
Database     51 AHAHAHAHASAGANGRARAHARALAHAHAGGGAAHGHPGFPVGGSASAAA 100
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing   51 AHAHAHAHASAGANGRARAHARALAHAHAGGGAAHGHPGFPVGGSASAAA 100
Database    101 RAAARASAGGLGGFGSAAANAAAAARAGAGFGGFGGLGGFGGLGGVGGPG 150
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing  101 RAAARASAGGLGGFGSAAANAAAAARAGAGFGGFGGLGGFGGLGGVGGPG 143
Database    151 QPGGPGGPGGPGGPGGPGMPGGPGGPSGPGTGGPGQPGGPGGPGGPGGPG 200
                |
Sequencing  151 Q------------------------------------------------- 151
Database    201 GPSMPGGPGGPGGPGMPGGPGGPGGPGGAGGIPGMTGPAGPPGPAGPQGP 250

Sequencing  151 -------------------------------------------------- 151
Database    251 EGEQGPRGRTPAGTPGPPGNPGEPGQGGAPGAPGAPGHAGKHGTAGAAGK 300
                                                   |||||||||||||||
Sequencing  152 -----------------------------------PGHAGKHGTAGAAGK 166
Database    301 AGRPGPXGQAGASGSSGQHGASGAPGRPGNPGSTGRPGATGDPGRPGATG 350
                ||||
Sequencing  167 AGRP---------------------------------------------- 170
Database    351 TTGRPGPSGAPGNPGAPGALGAPGPRGSPGFVGLPGPRGSPGEPGNQGPI 400

Sequencing  170 -------------------------------------------------- 170
Database    401 GGPGYPGPRGPQGPDGAMGPQGPCGDRGAPGVPGKQGPVGGQGPAGPRGP 450
                                     |||||||||||||||||||||||||||||
Sequencing  171 ---------------------GPCGDRGAPGVPGKQGPVGGQGPAGPRGP 199
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Database    451 RGDEGPVGPKGEPGARGADGKPGDKGPDGETGPQGPAGPKGQVGDQGKPG 500
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing  200 RGDEGPVGPKGEPGARGADGKPGDKGPDGETGPQGPAGPKGQVGDQGKPG 249
Database    501 AKGETGDQGARGEAGKAGEQGPGGIQGPKGPVGGQGPAGPAGPLGPQGPM 550
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing  250 AKGETGDQGARGEAGKAGEQGPGGIQGPKGPVGGQGPAGPAGPLGPQGPM 299
Database    551 GERGPQGPTGSEGPVGAPGPKGSVGDQGAQGDQGATGADGKKGEPGERGQ 600
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing  300 GERGPQGPTGSEGPVGAPGPKGSVGDQGAQGDQGATGADGKKGEPGERGQ 349
Database    601 QGAAGPVGRPGPRGDRGAKGIQGSRGRPGGMGRRGNRGSQGAVGPRGETG 650
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing  350 QGAAGPVGRPGPRGDRGAKGIQGSRGRPGGMGRRGNRGSQGAVGPRGETG 399
Database    651 PDGNQGQRGEQGAPGVITLVIEDLRTAGVESPVETFDAGAGTGGPAPGVG 700
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing  400 PDGNQGQRGEQGAPGVITLVIEDLRTAGVESPVETFDAGAGTGGPAPGVG 449
Database    701 AAATAGAFAGAGPGGANAGGNAAAGAGPGVGPGGLGGLGGLGAGGLGGGL 750
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing  450 AAATAGAFAGAGPGGANAGGNAAAGAGPGVGPGGLGGLGGLGAGGLGGGL 499
Database    751 GGGLGGLGGAGGLGGGLGGLGGGLGGGLGGLG---GGAGGAGAGGNGGAG 797
                ||||||||||||||||||||||||||||||||   |||||||||||||||
Sequencing  500 GGGLGGLGGAGGLGGGLGGLGGGLGGGLGGLGGGAGGAGGAGAGGNGGAG 549
Database    798 AGGAGGNGGGSAAARAAAQAAAAAGGNGGAAQAAAQAAASAAANSGLGAG 847
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sequencing  550 AGGAGGNGGGSAAARAAAQAAAAAGGNGGAAQAAAQAAASAAANSGLGAG 599
Database    848 AARAAASAAARATVTGHGSGTAAAAANAAAQAHAATRGQGGSHAHAAAAA 897
                ||||||||||||||.|||||||||||||||||||||||||||||||||||
Sequencing  600 AARAAASAAARATVAGHGSGTAAAAANAAAQAHAATRGQGGSHAHAAAAA 649
Database    898 HAAASSVIHGGDYHGNDAGYHKPGY 922
                |||||||||||||||||||||||||
Sequencing  650 HAAASSVIHGGDYHGNDAGYHKPGY 674
```

[0108] There are significant differences in both sequences: The preColD sequence used is by 250 amino acids shorter than the published sequence. The major part of the amino acids in the collagen domain is missing. Therefore, the presently disclosed preColD gene is an up to now unpublished and unknown version of the preColD gene. It is noted that the truncation of the collagen domain increases the amount of silk fibroin domains in the whole protein and therefore, the behavior of the overall protein will be different.

Expression Construct of P4H

[0109] In the following, the DNA sequence after expression plasmid for P4H in the region of SacII to ApaI is shown as double strand. The beginning and the end of MFa/P4H fusion constructs are both printed. The used restriction sites are underligned.

```
                                                   SEQ ID NO: 7
  1 ccgcggtcat tacagttcat ctttcacagc tttctgatca tcgtcttcct ccatgtctgg
  1 ggcgccagta atgtcaagta gaaagtgtcg aaagactagt agcagaagga ggtacagacc
 SacII 2 x Stop

 61 ctcctctgct tcttccaggt cctcgagatc gtcatcatcc cctgccccat cctggccacc
 61 gaggagacga agaaggtcca ggagctctag cagtagtagg ggacggggta ggaccggtgg

121 cgagaggtcc ttaaagaatt ttggtaggtc gcacgcaagg ggcaacatta gttactggca
121 gctctccagg aatttcttaa aaccatccag cgtgcgttcc ccgttgtaat caatgaccgt

181 cctgtcggca ctggcaggaa agaacttgag tgtggggaag ctgtgcactt tgacggcctc
181 ggacagccgt gaccgtcctt tcttgaactc acaccccttc gacacgtgaa actgccggag

241 cacctcgttg gcagtcgagt ccatcttggc gatgacgatg ttctcatggt ccttgtacgt
241 gtggagcaac cgtcagctca ggtagaaccg ctactgctac aagagtacca ggaacatgca
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 301 ctctcccagt ttatcccaaa tgggagccaa ctgtttgcag tgaccacacc atggggcata
 301 gagagggtca aatagggttt accctcggtt gacaaacgtc actggtgtgg taccccgtat

 361 gaactccaca aagacgtttt ttttctcatc aaaagccacg tcttcaaagt tcttccccaac
 361 cttgaggtgt ttctgcaaaa aaaagagtag ttttcggtgc agaagtttca agaagggttg

 421 aagcaccttg acaggctgct tgtcccagtc ctccggcagc tcctggctca tcaggtgggg
 421 ttcgtggaac tgtccgacga acagggtcag gaggccgtcg aggaccgagt agtccacccc

 481 cttgattttg ccctccagga agcggtggca gaactctgtg atcctctctg ccgtcagctc
 481 gaactaaaac gggaggtcct tcgccaccgt cctgagacac taggagagac ggcagtcgag

 541 ctccgattcg ggcttgtact tggtcatctc ctcctccagg gtgatgaggc gcacggccgg
 541 gaggctaagc ccgaacatga accagtagag gaggaggtcc cactactccg cgtgccggcc

 601 gcactcttcc ttcttcaggc caaagaactc gaggatgcgc tggttgtcgg tgtggtcgct
 601 cgtgagaagg aagaagtccg gtttcttgag ctcctacgcg accaacagcc acaccagcga

 661 gtcgatgaag atgaacagga tcttgccctt gaagctctcg gctgctgttt tgaagttgct
 661 cagctacttc tacttgtcct agaacgggaa cttcgagagc cgacgacaaa acttcaacga

 721 cagtttgccg tcatagtcag acacactctt gggcaagaac agcaggatgt gagtcttgat
 721 gtcaaacggc agtatcagtc tgtgtgagaa cccgttcttg tcgtcctaca ctcagaacta

 781 ttcacctcca aaaatcttcg ggctgtctg ctcggtgaac tcgatgacaa ggggcagctg
 781 aagtggaggt ttttagaagc cccgacagac gagccacttg agctactgtt ccccgtcgac

 841 gttgtgtttg ataaagtcca gcaggttctc cttggtgacc tccccttcaa agttgttccg
 841 caacacaaac tatttcaggt cgtccaagag gaaccactgg aggggaagtt tcaacaaggc

 901 gccttcatca aacttcttaa agaggacaac ccatctttg tcgagctggt atttggagaa
 901 cggaagtagt ttgaagaatt tctcctgttg ggtagaaac agctcgacca taaacctctt

 961 cacgtcactg ttggaagtga tcccaaatgg tatgtcatcg atggcctctg ctgcctgcaa
 961 gtgcagtgac aaccttcact agggtttacc atacagtagc taccggagac gacggacgtt

1021 aaactgcttg gcagagtccg actccacgtc cttgaagaag ccgatgacag ccacctcgct
1021 tttgacgaac cgtctcaggc tgaggtgcag gaacttcttc ggctactgtc ggtggagcga

1081 ggactccacc aaggactctg cagctgcgcc gtcaggcagg gtggtggcag ccgggcccgt
1081 cctgaggtgg ttcctgagac gtcgacgcgg cagtccgtcc caccaccgtc ggcccgggca
                                ApaI

1141 gcgcttcttc agccagttca cgatgtcatc agcctctctg ccagctgtat attccttggg
1141 cgcgaagaag tcggtcaagt gctacagtag tcggagagac ggtcgacata taaggaaccc

1201 ggaagccgtg tctccattcc tgaagaactt gatggtggga tagccgcgca cgccgtactg
1201 ccttcggcac agaggtaagg acttcttgaa ctaccaccct atcggcgcgt gcggcatgac

1261 ctgggccagg tcagactcct ccgtggcgtc caccttggcc aacctgatct cggaaccttc
1261 gacccggtcc agtctgagga ggcaccgcag gtggaaccgg ttggactaga gccttggaag

1321 tgccttcagc ttcccagcgg ctttggcata ctcaggggcc agagccttgc agtggccaca
1321 acggaagtcg aagggtcgcc gaaaccgtat gagtccccgg tctcggaacg tcaccggtgt

1381 ggttccccgt atcttgaggt ggtcgtccat gaacacccgg cggtcgcgga ggcgcttcaa
1381 ccaaggggca tagaactcca ccagcaggta cttgtgggcc gccagcgcct ccgcgaagtt

1441 gcttttccgc agcaccagga cgtggtcctc ctcctccgga gcgtcagcta atgcggagga
1441 cgaaaaggcg tcgtggtcct gcaccaggag gaggaggcct cgcagtcgat tacgcctcct
                        BspEI

1501 tgctgcgaat aaaactgcag taaaaattga aggaaatctc atggatccgg ggtttttct
1501 acgacgctta ttttgacgtc atttttaact cctttagag tacctaggcc ccaaaaaaga
                          Start BamHI

1561 ccttgacgtt aaagtataga ggtatattaa caatttttg ttgatacttt tattacattt
1561 ggaactgcaa tttcatatct ccatataatt gttaaaaaac aactatgaaa ataatgtaaa

1621 gaataagaag taatacaaac cgaaaatgtt gaaagtatta gttaaagtgg ttatgcagtt
1621 cttattcttc attatgtttg gcttttacaa ctttcataat caatttcacc aatacgtcaa

1681 tttgcattta tatatctgtt aatagatcaa aatcatcgc ttcgctgatt aattacccca
1681 aaacgtaaat atatagacaa ttatctagtt tttagtagcg aagcgactaa ttaatggggt
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1741 gaaataaggc taaaaaacta atcgcattat catcctatgg ttgttaattt gattcgttca
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1801 tttgaaggtt tgtggggcca ggttactgcc aatttttcct cttcataacc ataaaagcta
1801 aaacttccaa acaccccggt ccaatgacgg ttaaaaagga gaagtattgg tattttcgat

1861 gtattgtaga atctttattg ttcggagcag tgcggcgcga ggcacatctg cgtttcagga
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1921 acgcgaccgg tgaagacgag gacgcacgga ggagagtctt ccttcggagg gctgtcaccc
1921 tgcgctggcc acttctgctc ctgcgtgcct cctctcagaa ggaagcctcc cgacagtggg

1981 gctcggcggc ttctaatccg tacttcaata tagcaatgag cagttaagcg tattactgaa
1981 cgagccgccg aagattaggc atgaagttat atcgttactc gtcaattcgc ataatgactt

2041 agttccaaag agaaggtttt tttaggctaa gataatgggg ctctttacat ttccacaaca
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2101 tataagtaag attagatatg gatatgtata tggatatgta tatggtggta atgccatgta
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2161 atatgattat taaacttctt tgcgtccatc caaaaaaaaa gtaagaattt ttgaaaattc
2161 tatactaata atttgaagaa acgcaggtag gttttttttt cattcttaaa aacttttaag

2221 aaggaattcg atatcaagct tatcgatacc gtcgacatga gatttccttc aatttttact
2221 ttccttaagc tatagttcga atagctatgg cagctgtact ctaaaggaag ttaaaaatga
     EcoRI             SalI Start

2281 gcagttttat tcgcagcatc ctccgcgcta gctcatccag gctttttttac ttcaattggt
2281 cgtcaaaata agcgtcgtag gaggcgcgat cgagtaggtc cgaaaaaatg aagttaacca
                NheI

2341 cagatgactg atttgatcca tactgagaaa gatctggtga cttctctgaa agattatatt
2341 gtctactgac taaactaggt atgactcttt ctagaccact gaagagactt tctaatataa

2401 aaggcagaag aggacaagtt agaacaaata aaaaaatggg cagagaagtt agatcggcta
2401 ttccgtcttc tcctgttcaa tcttgtttat tttttaccc gtctcttcaa tctagccgat

2461 actagtacag cgacaaaaga tccagaagga tttgttgggc atccagtaaa tgcattcaaa
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2521 ttaatgaaac gtctgaatac tgagtggagt gagttggaga atctggtcct taaggatatg
2521 aattactttg cagacttatg actcacctca ctcaacctct tagaccagga attcctatac

2581 tcagatggct ttatctctaa cctaaccatt cagagaccag tactttctaa tgatgaagat
2581 agtctaccga aatagagatt ggattggtaa gtctctggtc atgaaagatt actacttcta

2641 caggttgggg cagccaaagc tctgttacgt ctccaggata cctacaattt ggatacagat
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2701 accatctcaa agggtaatct tccaggagtg aaacacaaat cttttctaac ggctgaggac
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3181 aaactctttt gccgctacca tgatggaaac cgtaatccta aatttattct ggctccagct
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3361 gaccctgaga ctggaaaatt gaccacagca cagtacagag tatctaagag tgcctggctc
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3541 gaaccccatt ttgactttgc acggaaagat gagccagatg ctttcaaaga gctggggaca
3541 cttggggtaa aactgaaacg tgcctttcta ctcggtctac gaaagtttct cgacccctgt

3601 ggaaatagaa ttgctacatg gctgttttat atgagtgatg tgtctgcagg aggagccact
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3721 aatctgtttg ccagtggaga aggagattat agtacacggc atgcagcctg tccagtgcta
3721 ttagacaaac ggtcacctct tcctctaata tcatgtgccg tacgtcggac aggtcacgat

3781 gttggcaaca aatgggtatc caataaatgg ctccatgaac gtggacaaga atttcgaaga
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3841 ggaacatgca acagtcttaa ccttactact cccggg
               2 × Stop ApaI
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Protein Sequences

[0110]

MFa-P4HA

(SEQ ID NO:5)

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MRFPSIFTAV LFAASSALAH PGFFTSIGQM TDLIHTEKDL
VTSLKDYIKA EEDKLEQIKK WAEKLDRLTS TATKDPEGFV
GHPVNAFKLM KRLNTEWSEL ENLVLKDMSD GFISNLTIQR
PVLSNDEDQV GAAKALLRLQ DTYNLDTDTI SKGNLPGVKH
KSFLTAEDCF ELGKVAYTEA DYYHTELWME QALRQLDEGE
ISTIDKVSVL DYLSYAVYQQ GDLDKALLLT KKLLELDPEH
QRANGNLKYF EYIMAKEKDV NKSASDDQSD QKTTPKKKGV
AVDYLPERQK YEMLCRGEGI KMTPRRQKKL FCRYHDGNRN
PKFILAPAKQ EDEWDKPRII RFHDIISDAE IEIVKDLAKP
RLSRATVHDP ETGKLTTAQY RVSKSAWLSG YENPVVSRIN
MRIQDLTGLD VSTAEELQVA NYGVGGQYEP HFDFARKDEP
DAFKELGTGN RIATWLFYMS DVSAGGATVF PEVGASVWPK
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ERGQEFRRPC TLSELE
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MFa-P4HB

(SEQ ID NO:6)

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MRFPSIFTAV LFAASSALAD APEEEDHVLV LRKSNFAEAL
AAHKYLLVEF YAPWCGHCKA LAPEYAKAAG KLKAEGSEIR
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LAKVDATEES DLAQQYGVRG YPTIKFFRNG DTASPKEYTA
GREADDIVNW LKKRTGPAAT TLPDGAAAES LVESSEVAVI
GFFKDVESDS AKQFLQAAEA IDDIPFGITS NSDVFSKYQL
DKDGVVLFKK FDEGRNNFEG EVTKENLLDF IKHNQLPLVI
EFTEQTAPKI FGGEIKTHIL LFLPKSVSDY DGKLSNFKTA
AESFKGKILF IFIDSDHTDN QRILEFFGLK KEECPAVRLI
TLEEEMTKYK PESEELTAER ITEFCHRFLE GKIKPHLMSQ
ELPEDWDKQP VKVLVGKNFE DVAFDEKKNV FVEFYAPWCG
HCKQLAPIWD KLGETYKDHE NIVIAKMDST ANEVEAVKVH
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GDDDDLEDLE EAEEPDMEED DDQKAVKDEL
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Sequence of MFa

(SEQ ID NO:10)

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MRFPSIFTAV LFAASSALA
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SEQUENCE LISTING

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ggtggaagcg gcggcggtgg cggtggaggc catggcggaa gcggtattgg tggtatcgga     180

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ccaggtggat cttcacatgc atcagctggt tcatcatccc atgcatccgc atcccataac     300

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ggaggcggta ttggcccagg aggaagtgtc ggaggcggca ttggcggtat tggcggtatt     480

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tctagctcac actcatttgg cggacacgta ttccacagtg tgacccatca tggaggtcca   2640

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35                  40                  45

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50                  55                  60

His Ala His Ala His Ser Ser Ala Ser Ala His Val His His Phe Gly
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Pro Gly Gly Ser Ser His Ala Ser Ala Gly Ser Ser Ser His Ala Ser
85                  90                  95

Ala Ser His Asn Gly Leu Gly Gly Gly Ser Ala His Ala His Ser Ser
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Ser Ser Ala Asn Ala His Ser Gly Gly Phe Gly Gly Phe Gly Gly Ile
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130                 135                 140

Gly Pro Gly Gly Ser Val Gly Gly Gly Ile Gly Gly Ile Gly Gly Ile
145                 150                 155                 160

Gly Gly Gly Gly Gly Pro Gly Gly Asn Gly Gly Ile Gly Phe Gly Pro
165                 170                 175

Gly Phe Gly Gly Gly Phe Gly Pro Gly Ser Ser Ala Ser Gly Ser Gly
180                 185                 190

Ser Gly Ser Ala Phe Gly Gly Pro Gly Gly Ser Ser Ala Ser Ala Asn
195                 200                 205

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245                 250                 255

Gly Asn Pro Gly Pro Pro Gly Gln Pro Gly Asn Pro Gly Arg Pro Gly
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290                 295                 300

Gly Gly Pro Gly Gln Pro Gly His Pro Gly Ala Gly Gly Gln Pro Gly
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Arg Asn Gly Asn Pro Gly Asn Pro Gly Lys Pro Gly Thr Pro Gly His
325                 330                 335

Pro Gly Thr Ala Gly Ser Arg Gly Met Pro Gly Thr Pro Gly Thr Pro
340                 345                 350

Gly Gln Pro Gly Ile Pro Gly Thr Val Gly Gly Arg Gly Pro Arg Gly
355                 360                 365

Pro Ala Gly Ile Ile Gly Leu Ile Gly Pro Lys Gly Asn Pro Gly Glu
370                 375                 380

Pro Gly Asn Pro Gly Ala Pro Gly Gly Pro Gly Ser Thr Gly Pro Gln
385                 390                 395                 400

Gly Pro Gln Gly Pro Ala Gly Gly Pro Gly Ala Ser Gly Gly Pro Gly
405                 410                 415

Asp Lys Gly Ala Pro Gly Thr Pro Gly Gly Thr Gly Pro Arg Gly Pro
420                 425                 430

Ile Gly Pro Ser Gly Pro Ser Gly Ala Pro Gly Asp Gln Gly Pro Gln
435                 440                 445

Gly Gly Arg Gly Thr Pro Gly Leu Ala Gly Lys Pro Gly Pro Lys Gly
450                 455                 460

Leu Gln Gly Ser Asn Gly Glu Val Gly Pro Gln Gly Pro Ser Gly Pro
465                 470                 475                 480

Ala Gly Pro Gln Gly Pro Gln Gly Lys Asn Gly Val Lys Gly Ala Ala
485                 490                 495

Gly Asp Gln Gly Ala Arg Gly Pro Glu Gly Lys Ala Gly Pro Ala Gly
500                 505                 510

Pro Gln Gly Glu Thr Gly Pro Lys Gly Pro Thr Gly Ala Gln Gly Pro
515                 520                 525

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530                 535                 540

Gly Gly Gln Gly Pro Gln Gly Ala Glu Gly Pro Ser Gly Pro Ala Gly
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Pro Arg Gly Pro Ala Gly Ser Gln Gly Pro Ser Gly Glu Arg Gly Glu
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Pro Gly Ala Pro Gly Lys Lys Gly Pro Asn Gly Asp Arg Gly Asn Gln
580                 585                 590

Gly Ser Pro Gly Ala Pro Gly Lys Asn Gly Ala Arg Gly Asn Arg Gly
595                 600                 605

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610                 615                 620
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645 650 655

Val Ile Arg Ile Val Ile Asp Asp Gln Arg Thr Gly Pro Glu Val Ala
660 665 670

Glu Phe Pro Gly Phe Gly Gly Phe Gly Gly Ala Ser Ala Asn Ala Ala
675 680 685

Ser Ser Ala Asn Ala Phe Ala Gly Gly Pro Gly Gly Ser Ala Gly Ala
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725 730 735

Pro Gly Gly Ala Gly Gly Pro Gly Gly Val Gly Gly Gly Val Gly Gly
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755 760 765

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770 775 780

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785 790 795 800

Gly Ala Ser Ser Ser Gly Ser Ala Ser Ala Ser Asn Gly Gly Pro Phe
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Gly Val Leu Asn Val Gly Pro Gly Gly Arg Ile Gly Gly Gly Ser Ala
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Ser Ala Ser Ala Ala Ser Arg Ala His Ala His Ala Phe Gly Gly Leu
835 840 845

Gly Gly Gly Ser Ala Ser Ala Gly Ser His Ser Ser Ser Ser Ser His
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Ser Phe Gly Gly His Val Phe His Ser Val Thr His His Gly Gly Pro
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Gly Arg Ala Arg Ala His Ala Arg Ala Leu Ala His Ala His Ala Gly

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100                 105                 110

Gly Phe Gly Ser Ala Ala Ala Asn Ala Ala Ala Ala Ala Arg Ala Gly
115                 120                 125

Ala Gly Phe Gly Gly Phe Gly Gly Leu Gly Gly Phe Gly Gly Leu Gly
130                 135                 140

Gly Val Gly Gly Pro Gly Gln Pro Gly His Ala Gly Lys His Gly Thr
145                 150                 155                 160

Ala Gly Ala Ala Gly Lys Ala Gly Arg Pro Gly Pro Cys Gly Asp Arg
165                 170                 175

Gly Ala Pro Gly Val Pro Gly Lys Gln Gly Pro Val Gly Gly Gln Gly
180                 185                 190

Pro Ala Gly Pro Arg Gly Pro Arg Gly Asp Glu Gly Pro Val Gly Pro
195                 200                 205

Lys Gly Glu Pro Gly Ala Arg Gly Ala Asp Gly Lys Pro Gly Asp Lys
210                 215                 220

Gly Pro Asp Gly Glu Thr Gly Pro Gln Gly Pro Ala Gly Pro Lys Gly
225                 230                 235                 240

Gln Val Gly Asp Gln Gly Lys Pro Gly Ala Lys Gly Glu Thr Gly Asp
245                 250                 255

Gln Gly Ala Arg Gly Glu Ala Gly Lys Ala Gly Glu Gln Gly Pro Gly
260                 265                 270

Gly Ile Gln Gly Pro Lys Gly Pro Val Gly Gly Gln Gly Pro Ala Gly
275                 280                 285

Pro Ala Gly Pro Leu Gly Pro Gln Gly Pro Met Gly Glu Arg Gly Pro
290                 295                 300

Gln Gly Pro Thr Gly Ser Glu Gly Pro Val Gly Ala Pro Gly Pro Lys
305                 310                 315                 320

Gly Ser Val Gly Asp Gln Gly Ala Gln Gly Asp Gln Gly Ala Thr Gly
325                 330                 335

Ala Asp Gly Lys Lys Gly Glu Pro Gly Glu Arg Gly Gln Gln Gly Ala
340                 345                 350

Ala Gly Pro Val Gly Arg Pro Gly Pro Arg Gly Asp Arg Gly Ala Lys
355                 360                 365

Gly Ile Gln Gly Ser Arg Gly Arg Pro Gly Gly Met Gly Arg Arg Gly
370                 375                 380

Asn Arg Gly Ser Gln Gly Ala Val Gly Pro Arg Gly Glu Thr Gly Pro
385                 390                 395                 400

Asp Gly Asn Gln Gly Gln Arg Gly Glu Gln Gly Ala Pro Gly Val Ile
405                 410                 415

Thr Leu Val Ile Glu Asp Leu Arg Thr Ala Gly Val Glu Ser Pro Val
420                 425                 430

Glu Thr Phe Asp Ala Gly Ala Gly Thr Gly Gly Pro Ala Pro Gly Val
435                 440                 445

Gly Ala Ala Ala Thr Ala Gly Ala Phe Ala Gly Ala Gly Pro Gly Gly
450                 455                 460

Ala Asn Ala Gly Gly Asn Ala Ala Ala Gly Ala Gly Pro Gly Val Gly
465                 470                 475                 480
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Gly Gly Leu Gly Gly Gly Leu Gly Gly Leu Gly Gly Ala Gly Gly Leu
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Gly Gly Gly Leu Gly Gly Leu Gly Gly Gly Leu Gly Gly Gly Leu Gly
515                 520                 525

Gly Leu Gly Gly Gly Ala Gly Gly Ala Gly Gly Ala Gly Ala Gly Gly
530                 535                 540

Asn Gly Gly Ala Gly Ala Gly Gly Ala Gly Gly Asn Gly Gly Gly Ser
545                 550                 555                 560

Ala Ala Ala Arg Ala Ala Ala Gln Ala Ala Ala Ala Ala Gly Gly Asn
565                 570                 575

Gly Gly Ala Ala Gln Ala Ala Ala Gln Ala Ala Ala Ser Ala Ala Ala
580                 585                 590

Asn Ser Gly Leu Gly Ala Gly Ala Ala Arg Ala Ala Ala Ser Ala Ala
595                 600                 605

Ala Arg Ala Thr Val Ala Gly His Gly Ser Gly Thr Ala Ala Ala Ala
610                 615                 620

Ala Asn Ala Ala Ala Gln Ala His Ala Ala Thr Arg Gly Gln Gly Gly
625                 630                 635                 640

Ser His Ala His Ala Ala Ala Ala Ala His Ala Ala Ala Ser Ser Val
645                 650                 655

Ile His Gly Gly Asp Tyr His Gly Asn Asp Ala Gly Tyr His Lys Pro
660                 665                 670

Gly Tyr


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Leu Ile His Thr Glu Lys Asp Leu Val Thr Ser Leu Lys Asp Tyr Ile
35                  40                  45

Lys Ala Glu Glu Asp Lys Leu Glu Gln Ile Lys Lys Trp Ala Glu Lys
50                  55                  60

Leu Asp Arg Leu Thr Ser Thr Ala Thr Lys Asp Pro Glu Gly Phe Val
65                  70                  75                  80

Gly His Pro Val Asn Ala Phe Lys Leu Met Lys Arg Leu Asn Thr Glu
85                  90                  95

Trp Ser Glu Leu Glu Asn Leu Val Leu Lys Asp Met Ser Asp Gly Phe
100                 105                 110

Ile Ser Asn Leu Thr Ile Gln Arg Pro Val Leu Ser Asn Asp Glu Asp
115                 120                 125

Gln Val Gly Ala Ala Lys Ala Leu Leu Arg Leu Gln Asp Thr Tyr Asn
130                 135                 140

Leu Asp Thr Asp Thr Ile Ser Lys Gly Asn Leu Pro Gly Val Lys His
145                 150                 155                 160
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Lys Ser Phe Leu Thr Ala Glu Asp Cys Phe Glu Leu Gly Lys Val Ala
165                 170                 175

Tyr Thr Glu Ala Asp Tyr Tyr His Thr Glu Leu Trp Met Glu Gln Ala
180                 185                 190

Leu Arg Gln Leu Asp Glu Gly Glu Ile Ser Thr Ile Asp Lys Val Ser
195                 200                 205

Val Leu Asp Tyr Leu Ser Tyr Ala Val Tyr Gln Gln Gly Asp Leu Asp
210                 215                 220

Lys Ala Leu Leu Leu Thr Lys Lys Leu Leu Glu Leu Asp Pro Glu His
225                 230                 235                 240

Gln Arg Ala Asn Gly Asn Leu Lys Tyr Phe Glu Tyr Ile Met Ala Lys
245                 250                 255

Glu Lys Asp Val Asn Lys Ser Ala Ser Asp Asp Gln Ser Asp Gln Lys
260                 265                 270

Thr Thr Pro Lys Lys Lys Gly Val Ala Val Asp Tyr Leu Pro Glu Arg
275                 280                 285

Gln Lys Tyr Glu Met Leu Cys Arg Gly Glu Gly Ile Lys Met Thr Pro
290                 295                 300

Arg Arg Gln Lys Lys Leu Phe Cys Arg Tyr His Asp Gly Asn Arg Asn
305                 310                 315                 320

Pro Lys Phe Ile Leu Ala Pro Ala Lys Gln Glu Asp Glu Trp Asp Lys
325                 330                 335

Pro Arg Ile Ile Arg Phe His Asp Ile Ile Ser Asp Ala Glu Ile Glu
340                 345                 350

Ile Val Lys Asp Leu Ala Lys Pro Arg Leu Ser Arg Ala Thr Val His
355                 360                 365

Asp Pro Glu Thr Gly Lys Leu Thr Thr Ala Gln Tyr Arg Val Ser Lys
370                 375                 380

Ser Ala Trp Leu Ser Gly Tyr Glu Asn Pro Val Val Ser Arg Ile Asn
385                 390                 395                 400

Met Arg Ile Gln Asp Leu Thr Gly Leu Asp Val Ser Thr Ala Glu Glu
405                 410                 415

Leu Gln Val Ala Asn Tyr Gly Val Gly Gly Gln Tyr Glu Pro His Phe
420                 425                 430

Asp Phe Ala Arg Lys Asp Glu Pro Asp Ala Phe Lys Glu Leu Gly Thr
435                 440                 445

Gly Asn Arg Ile Ala Thr Trp Leu Phe Tyr Met Ser Asp Val Ser Ala
450                 455                 460

Gly Gly Ala Thr Val Phe Pro Glu Val Gly Ala Ser Val Trp Pro Lys
465                 470                 475                 480

Lys Gly Thr Ala Val Phe Trp Tyr Asn Leu Phe Ala Ser Gly Glu Gly
485                 490                 495

Asp Tyr Ser Thr Arg His Ala Ala Cys Pro Val Leu Val Gly Asn Lys
500                 505                 510

Trp Val Ser Asn Lys Trp Leu His Glu Arg Gly Gln Glu Phe Arg Arg
515                 520                 525

Pro Cys Thr Leu Ser Glu Leu Glu
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Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
1               5                   10                  15

Ala Leu Ala Asp Ala Pro Glu Glu Glu Asp His Val Leu Val Leu Arg
20                  25                  30

Lys Ser Asn Phe Ala Glu Ala Leu Ala Ala His Lys Tyr Leu Leu Val
35                  40                  45

Glu Phe Tyr Ala Pro Trp Cys Gly His Cys Lys Ala Leu Ala Pro Glu
50                  55                  60

Tyr Ala Lys Ala Ala Gly Lys Leu Lys Ala Glu Gly Ser Glu Ile Arg
65                  70                  75                  80

Leu Ala Lys Val Asp Ala Thr Glu Glu Ser Asp Leu Ala Gln Gln Tyr
85                  90                  95

Gly Val Arg Gly Tyr Pro Thr Ile Lys Phe Phe Arg Asn Gly Asp Thr
100                 105                 110

Ala Ser Pro Lys Glu Tyr Thr Ala Gly Arg Glu Ala Asp Asp Ile Val
115                 120                 125

Asn Trp Leu Lys Lys Arg Thr Gly Pro Ala Ala Thr Thr Leu Pro Asp
130                 135                 140

Gly Ala Ala Ala Glu Ser Leu Val Glu Ser Ser Glu Val Ala Val Ile
145                 150                 155                 160

Gly Phe Phe Lys Asp Val Glu Ser Asp Ser Ala Lys Gln Phe Leu Gln
165                 170                 175

Ala Ala Glu Ala Ile Asp Asp Ile Pro Phe Gly Ile Thr Ser Asn Ser
180                 185                 190

Asp Val Phe Ser Lys Tyr Gln Leu Asp Lys Asp Gly Val Val Leu Phe
195                 200                 205

Lys Lys Phe Asp Glu Gly Arg Asn Asn Phe Glu Gly Glu Val Thr Lys
210                 215                 220

Glu Asn Leu Leu Asp Phe Ile Lys His Asn Gln Leu Pro Leu Val Ile
225                 230                 235                 240

Glu Phe Thr Glu Gln Thr Ala Pro Lys Ile Phe Gly Gly Glu Ile Lys
245                 250                 255

Thr His Ile Leu Leu Phe Leu Pro Lys Ser Val Ser Asp Tyr Asp Gly
260                 265                 270

Lys Leu Ser Asn Phe Lys Thr Ala Ala Glu Ser Phe Lys Gly Lys Ile
275                 280                 285

Leu Phe Ile Phe Ile Asp Ser Asp His Thr Asp Asn Gln Arg Ile Leu
290                 295                 300

Glu Phe Phe Gly Leu Lys Lys Glu Glu Cys Pro Ala Val Arg Leu Ile
305                 310                 315                 320

Thr Leu Glu Glu Glu Met Thr Lys Tyr Lys Pro Glu Ser Glu Glu Leu
325                 330                 335

Thr Ala Glu Arg Ile Thr Glu Phe Cys His Arg Phe Leu Glu Gly Lys
340                 345                 350

Ile Lys Pro His Leu Met Ser Gln Glu Leu Pro Glu Asp Trp Asp Lys
355                 360                 365

Gln Pro Val Lys Val Leu Val Gly Lys Asn Phe Glu Asp Val Ala Phe
370                 375                 380
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Asp Glu Lys Lys Asn Val Phe Val Glu Phe Tyr Ala Pro Trp Cys Gly
385                 390                 395                 400

His Cys Lys Gln Leu Ala Pro Ile Trp Asp Lys Leu Gly Glu Thr Tyr
405                 410                 415

Lys Asp His Glu Asn Ile Val Ile Ala Lys Met Asp Ser Thr Ala Asn
420                 425                 430

Glu Val Glu Ala Val Lys Val His Ser Phe Pro Thr Leu Lys Phe Phe
435                 440                 445

Pro Ala Ser Ala Asp Arg Thr Val Ile Asp Tyr Asn Gly Glu Arg Thr
450                 455                 460

Leu Asp Gly Phe Lys Lys Phe Leu Glu Ser Gly Gly Gln Asp Gly Ala
465                 470                 475                 480

Gly Asp Asp Asp Asp Leu Glu Asp Leu Glu Glu Ala Glu Glu Pro Asp
485                 490                 495

Met Glu Glu Asp Asp Asp Gln Lys Ala Val Lys Asp Glu Leu
500                 505                 510


<210> SEQ ID NO 7
<211> LENGTH: 3876
<212> TYPE: DNA
<213> ORGANISM: Mytilus edulis

<400> SEQUENCE: 7

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ctcctctgct tcttccaggt cctcgagatc gtcatcatcc cctgccccat cctggccacc     120

cgagaggtcc ttaaagaatt ttggtaggtc gcacgcaagg ggcaacatta gttactggca     180

cctgtcggca ctggcaggaa agaacttgag tgtggggaag ctgtgcactt tgacggcctc     240

cacctcgttg gcagtcgagt ccatcttggc gatgacgatg ttctcatggt ccttgtacgt     300

ctctcccagt ttatcccaaa tgggagccaa ctgtttgcag tgaccacacc atggggcata     360

gaactccaca aagacgtttt ttttctcatc aaaagccacg tcttcaaagt tcttcccaac     420

aagcaccttg acaggctgct tgtcccagtc ctccggcagc tcctggctca tcaggtgggg     480

cttgattttg ccctccagga agcggtggca gaactctgtg atcctctctg ccgtcagctc     540

ctccgattcg ggcttgtact tggtcatctc ctcctccagg gtgatgaggc gcacggccgg     600

gcactcttcc ttcttcaggc caaagaactc gaggatgcgc tggttgtcgg tgtggtcgct     660

gtcgatgaag atgaacagga tcttgccctt gaagctctcg gctgctgttt tgaagttgct     720

cagtttgccg tcatagtcag acacactctt gggcaagaac agcaggatgt gagtcttgat     780

ttcacctcca aaaatcttcg gggctgtctg ctcggtgaac tcgatgacaa ggggcagctg     840

gttgtgtttg ataaagtcca gcaggttctc cttggtgacc tccccttcaa agttgttccg     900

gccttcatca aacttcttaa agaggacaac cccatctttg tcgagctggt atttggagaa     960

cacgtcactg ttggaagtga tcccaaatgg tatgtcatcg atggcctctg ctgcctgcaa    1020

aaactgcttg gcagagtccg actccacgtc cttgaagaag ccgatgacag ccacctcgct    1080

ggactccacc aaggactctg cagctgcgcc gtcaggcagg gtggtggcag ccgggcccgt    1140

gcgcttcttc agccagttca cgatgtcatc agcctctctg ccagctgtat attccttggg    1200

ggaagccgtg tctccattcc tgaagaactt gatggtggga tagccgcgca cgccgtactg    1260

ctgggccagg tcagactcct ccgtggcgtc caccttggcc aacctgatct cggaaccttc    1320
```

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tgccttcagc ttcccagcgg ctttggcata ctcaggggcc agagccttgc agtggccaca   1380
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gcttttccgc agcaccagga cgtggtcctc ctcctccgga gcgtcagcta atgcggagga   1500
tgctgcgaat aaaactgcag taaaaattga aggaaatctc atggatccgg ggtttttct   1560
ccttgacgtt aaagtataga ggtatattaa caattttttg ttgatacttt tattacattt   1620
gaataagaag taatacaaac cgaaaatgtt gaaagtatta gttaaagtgg ttatgcagtt   1680
tttgcattta tatatctgtt aatagatcaa aaatcatcgc ttcgctgatt aattacccca   1740
gaaataaggc taaaaaacta atcgcattat catcctatgg ttgttaattt gattcgttca   1800
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acgcgaccgg tgaagacgag gacgcacgga ggagagtctt ccttcggagg gctgtcaccc   1980
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atatgattat taaacttctt tgcgtccatc caaaaaaaaa gtaagaattt ttgaaaattc   2220
aaggaattcg atatcaagct tatcgatacc gtcgacatga gatttccttc aatttttact   2280
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cagatgactg atttgatcca tactgagaaa gatctggtga cttctctgaa agattatatt   2400
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actagtacag cgacaaaaga tccagaagga tttgttgggc atccagtaaa tgcattcaaa   2520
ttaatgaaac gtctgaatac tgagtggagt gagttggaga atctggtcct taaggatatg   2580
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caggttgggg cagccaaagc tctgttacgt ctccaggata cctacaattt ggatacagat   2700
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gcagaaattg aaatcgtcaa agacctagca aaaccaaggc tgagccgagc tacagtacat   3360
gaccctgaga ctggaaaatt gaccacagca cagtacagag tatctaagag tgcctggctc   3420
tctggctatg aaaatcctgt ggtgtctcga attaatatga gaatacaaga tctaacagga   3480
ctagatgttt ccacagcaga ggaattacag gtagcaaatt atggagttgg aggacagtat   3540
gaaccccatt ttgactttgc acggaaagat gagccagatg ctttcaaaga gctggggaca   3600
```

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ggaaatagaa ttgctacatg gctgttttat atgagtgatg tgtctgcagg aggagccact 3660

gtttttcctg aagttggagc tagtgtttgg cccaaaaaag gaactgctgt tttctggtat 3720

aatctgtttg ccagtggaga aggagattat agtacacggc atgcagcctg tccagtgcta 3780

gttggcaaca aatgggtatc caataaatgg ctccatgaac gtggacaaga atttcgaaga 3840

ccttgtacgt tgtcagaatt ggaatgatga gggccc 3876

<210> SEQ ID NO 8
<211> LENGTH: 922
<212> TYPE: PRT
<213> ORGANISM: Mytilus edulis
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (307)..(307)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 8

Met Val Tyr Lys Leu Leu Thr Val Cys Leu Val Ala Ser Leu Leu Glu
1 5 10 15

Ile Cys Leu Ala Asp Tyr Asn Gly Asn Lys Gln Tyr Gly Gly Arg Tyr
20 25 30

Gly Asn Arg Tyr Gly Asn Gly Leu Gly Gly Gly Asn Gly Gly Ala Gly
35 40 45

Ala Val Ala His Ala His Ala His Ala His Ala Ser Ala Gly Ala Asn
50 55 60

Gly Arg Ala Arg Ala His Ala Arg Ala Leu Ala His Ala His Ala Gly
65 70 75 80

Gly Gly Ala Ala His Gly His Pro Gly Phe Pro Val Gly Gly Ser Ala
85 90 95

Ser Ala Ala Ala Arg Ala Ala Ala Arg Ala Ser Ala Gly Gly Leu Gly
100 105 110

Gly Phe Gly Ser Ala Ala Ala Asn Ala Ala Ala Ala Ala Arg Ala Gly
115 120 125

Ala Gly Phe Gly Gly Phe Gly Gly Leu Gly Gly Phe Gly Gly Leu Gly
130 135 140

Gly Val Gly Gly Pro Gly Gln Pro Gly Gly Pro Gly Gly Pro Gly Gly
145 150 155 160

Pro Gly Gly Pro Gly Gly Pro Gly Met Pro Gly Gly Pro Gly Gly Pro
165 170 175

Ser Gly Pro Gly Thr Gly Gly Pro Gly Gln Pro Gly Gly Pro Gly Gly
180 185 190

Pro Gly Gly Pro Gly Gly Pro Gly Gly Pro Ser Met Pro Gly Gly Pro
195 200 205

Gly Gly Pro Gly Gly Pro Gly Met Pro Gly Gly Pro Gly Gly Pro Gly
210 215 220

Gly Pro Gly Gly Ala Gly Gly Ile Pro Gly Met Thr Gly Pro Ala Gly
225 230 235 240

Pro Pro Gly Pro Ala Gly Pro Gln Gly Pro Glu Gly Glu Gln Gly Pro
245 250 255

Arg Gly Arg Thr Pro Ala Gly Thr Pro Gly Pro Pro Gly Asn Pro Gly
260 265 270

Glu Pro Gly Gln Gly Gly Ala Pro Gly Ala Pro Gly Ala Pro Gly His
275 280 285

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Ala Gly Lys His Gly Thr Ala Gly Ala Ala Gly Lys Ala Gly Arg Pro
290                 295                 300

Gly Pro Xaa Gly Gln Ala Gly Ala Ser Gly Ser Ser Gly Gln His Gly
305                 310                 315                 320

Ala Ser Gly Ala Pro Gly Arg Pro Gly Asn Pro Gly Ser Thr Gly Arg
325                 330                 335

Pro Gly Ala Thr Gly Asp Pro Gly Arg Pro Gly Ala Thr Gly Thr Thr
340                 345                 350

Gly Arg Pro Gly Pro Ser Gly Ala Pro Gly Asn Pro Gly Ala Pro Gly
355                 360                 365

Ala Leu Gly Ala Pro Gly Pro Arg Gly Ser Pro Gly Phe Val Gly Leu
370                 375                 380

Pro Gly Pro Arg Gly Ser Pro Gly Glu Pro Gly Asn Gln Gly Pro Ile
385                 390                 395                 400

Gly Gly Pro Gly Tyr Pro Gly Pro Arg Gly Pro Gln Gly Pro Asp Gly
405                 410                 415

Ala Met Gly Pro Gln Gly Pro Cys Gly Asp Arg Gly Ala Pro Gly Val
420                 425                 430

Pro Gly Lys Gln Gly Pro Val Gly Gly Gln Gly Pro Ala Gly Pro Arg
435                 440                 445

Gly Pro Arg Gly Asp Glu Gly Pro Val Gly Pro Lys Gly Glu Pro Gly
450                 455                 460

Ala Arg Gly Ala Asp Gly Lys Pro Gly Asp Lys Gly Pro Asp Gly Glu
465                 470                 475                 480

Thr Gly Pro Gln Gly Pro Ala Gly Pro Lys Gly Gln Val Gly Asp Gln
485                 490                 495

Gly Lys Pro Gly Ala Lys Gly Glu Thr Gly Asp Gln Gly Ala Arg Gly
500                 505                 510

Glu Ala Gly Lys Ala Gly Glu Gln Gly Pro Gly Gly Ile Gln Gly Pro
515                 520                 525

Lys Gly Pro Val Gly Gly Gln Gly Pro Ala Gly Pro Ala Gly Pro Leu
530                 535                 540

Gly Pro Gln Gly Pro Met Gly Glu Arg Gly Pro Gln Gly Pro Thr Gly
545                 550                 555                 560

Ser Glu Gly Pro Val Gly Ala Pro Gly Pro Lys Gly Ser Val Gly Asp
565                 570                 575

Gln Gly Ala Gln Gly Asp Gln Gly Ala Thr Gly Ala Asp Gly Lys Lys
580                 585                 590

Gly Glu Pro Gly Glu Arg Gly Gln Gln Gly Ala Ala Gly Pro Val Gly
595                 600                 605

Arg Pro Gly Pro Arg Gly Asp Arg Gly Ala Lys Gly Ile Gln Gly Ser
610                 615                 620

Arg Gly Arg Pro Gly Gly Met Gly Arg Arg Gly Asn Arg Gly Ser Gln
625                 630                 635                 640

Gly Ala Val Gly Pro Arg Gly Glu Thr Gly Pro Asp Gly Asn Gln Gly
645                 650                 655

Gln Arg Gly Glu Gln Gly Ala Pro Gly Val Ile Thr Leu Val Ile Glu
660                 665                 670

Asp Leu Arg Thr Ala Gly Val Glu Ser Pro Val Glu Thr Phe Asp Ala
675                 680                 685
```

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Gly Ala Gly Thr Gly Gly Pro Ala Pro Gly Val Gly Ala Ala Ala Thr
690   695   700

Ala Gly Ala Phe Ala Gly Ala Gly Pro Gly Gly Ala Asn Ala Gly Gly
705   710   715   720

Asn Ala Ala Ala Gly Ala Gly Pro Gly Val Gly Pro Gly Gly Leu Gly
725   730   735

Gly Leu Gly Gly Leu Gly Ala Gly Gly Leu Gly Gly Gly Leu Gly Gly
740   745   750

Gly Leu Gly Gly Leu Gly Gly Ala Gly Gly Leu Gly Gly Gly Leu Gly
755   760   765

Gly Leu Gly Gly Gly Leu Gly Gly Gly Leu Gly Gly Leu Gly Gly Gly
770   775   780

Ala Gly Gly Ala Gly Ala Gly Gly Asn Gly Gly Ala Gly Ala Gly Gly
785   790   795   800

Ala Gly Gly Asn Gly Gly Gly Ser Ala Ala Ala Arg Ala Ala Ala Gln
805   810   815

Ala Ala Ala Ala Ala Gly Gly Asn Gly Gly Ala Ala Gln Ala Ala Ala
820   825   830

Gln Ala Ala Ala Ser Ala Ala Ala Asn Ser Gly Leu Gly Ala Gly Ala
835   840   845

Ala Arg Ala Ala Ala Ser Ala Ala Ala Arg Ala Thr Val Thr Gly His
850   855   860

Gly Ser Gly Thr Ala Ala Ala Ala Ala Asn Ala Ala Ala Gln Ala His
865   870   875   880

Ala Ala Thr Arg Gly Gln Gly Gly Ser His Ala His Ala Ala Ala Ala
885   890   895

Ala His Ala Ala Ala Ser Ser Val Ile His Gly Gly Asp Tyr His Gly
900   905   910

Asn Asp Ala Gly Tyr His Lys Pro Gly Tyr
915   920

<210> SEQ ID NO 9
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 9

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
1   5   10   15

Ala Leu Ala

<210> SEQ ID NO 10
<211> LENGTH: 952
<212> TYPE: PRT
<213> ORGANISM: Mytilus edulis

<400> SEQUENCE: 10

Met Val Arg Phe Ser Leu Ala Ser Val Leu Leu Leu Ala Val Thr Ser
1   5   10   15

Thr Ala Phe Ala Gly Pro Val Ser Asp Tyr Gly Gly Gly Gly Ile Lys
20   25   30

Val Val Pro Tyr His Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
35   40   45

Gly Gly His Gly Gly Ser Phe Arg Asn Gly Arg His Gly Gly Ile Gly

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```
50                  55                  60

Gly Ile Gly Gly Gly Ser Ser His Ala His Ala His Ser Ser Ala Ser
65                  70                  75                  80

Ala His Val His His Phe Gly Pro Gly Gly Ser Ser His Ala Ser Ala
                85                  90                  95

Gly Ser Ser Ser His Ala Ser Ala Ser His Asn Gly Leu Gly Gly Gly
            100                 105                 110

Ser Ala His Ala His Ser Ser Ser Ser Ala Asn Ala His Ser Gly Gly
        115                 120                 125

Phe Gly Gly Phe Gly Gly Ile Gly Gly Ile Gly Gly Ile Gly Pro Gly
    130                 135                 140

Gly Ser Val Gly Gly Gly Ile Gly Pro Gly Gly Ser Val Gly Gly Gly
145                 150                 155                 160

Ile Gly Gly Ile Gly Gly Ile Gly Gly Gly Gly Gly Pro Gly Gly Asn
                165                 170                 175

Gly Gly Ile Gly Phe Gly Pro Gly Phe Gly Gly Gly Phe Gly Pro Gly
            180                 185                 190

Ser Ser Ala Ser Gly Ser Gly Ser Gly Ser Ala Phe Gly Gly Pro Gly
        195                 200                 205

Gly Ser Ser Ala Ser Ala Asn Ala Ala Ala Arg Ala Asn Ala Asn Gly
    210                 215                 220

Gly Gly Gly Phe Gly Gly Pro Gly Thr Pro Gly Asn Ser Gly Pro Pro
225                 230                 235                 240

Gly Gln Pro Gly Leu Pro Gly Ala Pro Gly Gln Pro Gly Arg Pro Gly
                245                 250                 255

Ser Thr Pro Pro Gly Arg Leu Gly Asn Pro Gly Pro Pro Gly Gln Pro
            260                 265                 270

Gly Asn Pro Gly Arg Pro Gly Ser Ser Gly Arg Pro Gly Gly Ser Gly
        275                 280                 285

Gln Pro Gly Gly Pro Gly Arg Pro Gly Thr Pro Gly Lys Pro Gly Asn
    290                 295                 300

Arg Gly Gln Pro Gly Gln Pro Gly Gly Pro Gly Gln Pro Gly His Pro
305                 310                 315                 320

Gly Ala Gly Gly Gln Pro Gly Arg Asn Gly Asn Pro Gly Asn Pro Gly
                325                 330                 335

Lys Pro Gly Thr Pro Gly His Pro Gly Thr Ala Gly Ser Arg Gly Met
            340                 345                 350

Pro Gly Thr Pro Gly Thr Pro Gly Gln Pro Gly Ile Pro Gly Thr Val
        355                 360                 365

Gly Gly Arg Gly Pro Arg Gly Pro Ala Gly Ile Ile Gly Leu Ile Gly
    370                 375                 380

Pro Lys Gly Asn Pro Gly Glu Pro Gly Asn Pro Gly Ala Pro Gly Gly
385                 390                 395                 400

Pro Gly Ser Thr Gly Pro Gln Gly Pro Gln Gly Pro Ala Gly Gly Pro
                405                 410                 415

Gly Ala Ser Gly Gly Pro Gly Asp Lys Gly Ala Pro Gly Thr Pro Gly
            420                 425                 430

Gly Thr Gly Pro Arg Gly Pro Ile Gly Pro Ser Gly Pro Ser Gly Ala
        435                 440                 445

Pro Gly Asp Gln Gly Pro Gln Gly Gly Arg Gly Thr Pro Gly Leu Ala
    450                 455                 460
```

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```
Gly Lys Pro Gly Pro Lys Gly Leu Gln Gly Ser Asn Gly Glu Val Gly
465                 470                 475                 480

Pro Gln Gly Pro Ser Gly Pro Ala Gly Pro Gln Gly Pro Gln Gly Lys
485                 490                 495

Asn Gly Val Lys Gly Ala Ala Gly Asp Gln Gly Ala Arg Gly Pro Glu
500                 505                 510

Gly Lys Ala Gly Pro Ala Gly Pro Gln Gly Glu Thr Gly Pro Lys Gly
515                 520                 525

Pro Thr Gly Ala Gln Gly Pro Ala Gly Pro Ala Gly Pro Ser Gly Glu
530                 535                 540

Gln Gly Pro Gly Gly Glu Arg Gly Gly Gln Gly Pro Gln Gly Ala Glu
545                 550                 555                 560

Gly Pro Ser Gly Pro Ala Gly Pro Arg Gly Pro Ala Gly Ser Gln Gly
565                 570                 575

Pro Ser Gly Glu Arg Gly Glu Pro Gly Ala Pro Gly Lys Lys Gly Pro
580                 585                 590

Asn Gly Asp Arg Gly Asn Gln Gly Ser Pro Gly Ala Pro Gly Lys Asn
595                 600                 605

Gly Ala Arg Gly Asn Arg Gly Ser Arg Gly Ser Asn Gly Ser Pro Gly
610                 615                 620

Arg Ser Gly Ser Pro Gly Ser Arg Gly Lys Pro Gly Pro Gln Gly Pro
625                 630                 635                 640

His Gly Pro Arg Gly Leu Arg Gly Ser Pro Gly Gln Lys Gly Pro Arg
645                 650                 655

Gly Asp Gln Gly Ala Pro Gly Val Ile Arg Ile Val Ile Asp Asp Gln
660                 665                 670

Arg Thr Gly Pro Glu Val Ala Glu Phe Pro Gly Phe Gly Gly Phe Gly
675                 680                 685

Gly Ala Ser Ala Asn Ala Ala Ser Ser Ala Asn Ala Phe Ala Gly Gly
690                 695                 700

Pro Gly Gly Ser Ala Gly Ala Gly Ser Ser Ser Gly Ala Asn Ala Asn
705                 710                 715                 720

Ala Gly Gly Phe Pro Phe Gly Gly Ala Gly Gly Gly Pro Gly Ala Ala
725                 730                 735

Gly Gly Pro Gly Gly Ala Gly Gly Pro Gly Gly Val Gly Gly Gly Val
740                 745                 750

Gly Gly Gly Pro Gly Gly Val Gly Gly Gly Val Gly Gly Gly Pro Gly
755                 760                 765

Gly Val Gly Gly Gly Pro Gly Gly Ala Gly Pro Gly Gly Ala Gly Gly
770                 775                 780

Phe Gly Pro Gly Gly Ala Gly Gly Phe Gly Gly Phe Gly Gly Gly Ser
785                 790                 795                 800

Ser Ala Gly Ala Ser Ser Ser Gly Ser Ala Ser Ala Ser Asn Gly Gly
805                 810                 815

Pro Phe Gly Val Leu Asn Val Gly Pro Gly Gly Arg Ile Gly Gly Gly
820                 825                 830

Ser Ala Ser Ala Ser Ala Ala Ser Arg Ala His Ala His Phe Gly Gly
835                 840                 845

Gly Ser Ser Ala Gly Ala Ser Ser Ser Gly Ser Ala Ser Ala Ser Asn
850                 855                 860
```

-continued

```
Gly Gly Pro Phe Gly Val Leu Asn Val Gly Pro Gly Gly Arg Ile Gly
865                 870                 875                 880

Gly Gly Ser Ala Ser Ala Ser Ala Ala Ser Arg Ala His Ala His Ala
885                 890                 895

Phe Gly Gly Leu Gly Gly Gly Ser Ala Ser Ala Gly Ser His Ser Ser
900                 905                 910

Ser Ser Ser His Ser Phe Gly Gly His Val Phe His Ser Val Thr His
915                 920                 925

His Gly Gly Pro Ser His Val Ser Ser Gly Gly His Gly Gly His Gly
930                 935                 940

Gly Gly Pro Tyr Lys Pro Gly Tyr
945                 950
```

1. A yeast cell for producing a recombinant collagen like protein, preferably a recombinant mussel byssus protein, which yeast cell has been transformed with the following elements:

a) a first expression vector which codes for said recombinant collagen like protein; and

b) a second expression vector comprising a nucleic acid coding for prolyl-4-hydroxylase (P4H).

2. The yeast cell of claim **1**, wherein the P4H sequence is linked to a signal sequence for efficient transport of said sequence to the ER of said yeast cell.

3. The yeast of claim **2**, wherein the signal sequence is mating factor alpha 1 (MFa) of *S. cerevisiae* (SEQ ID NO: 10).

4. The yeast cell of one or more of claims **1-3**, wherein the yeast cell, preferably is a *S. cerevisiae, Schizosaccharomyces pombe, Pichia pastoris, Candida albicans*, or *Hansenula polymorpha* cell.

5. The yeast cell of one or more of claims **1-4**, wherein the first expression vector further comprises one or more regulatory elements.

6. The yeast cell of claim **5**, wherein the regulatory elements contain a promoter selected from constitutive or inducible promoters.

7. The yeast cell of claim **6**, wherein the promoter is selected from GPD, GAL4, CUP1, MET25, GAL1 or GAL1-10.

8. The yeast cell of one or more of the preceding claims, wherein the expression vectors are plasmids.

9. The yeast cell of one or more of the preceding claims, wherein the recombinant collagen like protein is a recombinant mussel byssus protein comprising or consisting of one or more fragments of a collagen domain flanked by elastin or silk fibroin.

10. The yeast cell of one or more of the preceding claims, wherein the fragments are derived from *Mytilus* sp., preferably *M. edulis, M. galloprovincialis, M. californians*, or *Geukeria demissa.*

11. The yeast cell of one or more of the preceding claims, wherein the recombinant mussel byssus protein comprises or consists of one or more of the fragments preColP and/or preColD or variants thereof.

12. The yeast cell of one or more of the preceding claims, wherein the recombinant protein comprises or consists of the amino acid sequence of SEQ ID NO: 3 and/or 4 or variants thereof.

13. The yeast cell of one or more of the preceding claims, wherein in the recombinant protein the signal sequence of the respective amino acid sequence is replaced by yeast specific signal sequence, preferably by mating factor alpha 1 (MFa) of *S. cerevisiae.*

14. The yeast cell of one or more of the preceding claims, wherein P4H is human or mussel P4H.

15. A kit of parts or a co-expression system for use in the production of recombinant collagen comprising proteins comprising the following constituents:

a) the first expression vector as defined in one or more of claims **1-14**; and

b) the second expression vector as defined in one or more of claims **1-14**;

16. A method of producing a recombinant collagen like protein, preferably mussel byssus protein, comprising the steps of:

a) providing a yeast cell;

b) transforming said yeast cell with a first and second expression vector as defined in one or more of claims **1-14** or with the co-expression system of claim **15**;

c) expressing the recombinant collagen like protein, preferably recombinant mussel byssus protein, from said yeast cell under suitable conditions; and

d) recovering said recombinant protein.

17. A method for producing threads from recombinant mussel byssus protein, comprising the following steps:

a) providing recombinant protein as produced in claim **16**, and

b) (electro)spinning or molding said protein into threads by a suitable method.

18. A protein obtainable by the method of claim **16** or a thread obtainable by the method of claim **17**.

19. Use of the proteins/threads of claim **18** in the field of biotechnology and/or medicine.

20. Use of the proteins/threads of claim **18** for the manufacture of wound closure or coverage systems.

21. The use of claim **20** for the manufacture of suture materials.

22. The use of claim **21**, wherein the suture material is intended for use in neurosurgery or ophthalmic surgery.

23. Use of the proteins/threads of claim **18** for the manufacture of replacement materials, preferably artificial cartilage or tendon materials.

24. Wound closure or coverage systems, suture materials, replacement materials, preferably artificial cartilage or tendon materials, which are obtainable using proteins/threads of claim **18**.

25. Cosmetics, drug delivery vehicles, fabrics, textile, paper product, leather product, automotive parts or aircraft parts, which contain proteins/threads of claim **18**.

* * * * *