Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 111, No. 1, February 1999, pp. 197–205

Procollagen C-proteinase and its enhancer protein as regulators of collagen fibril formation and matrix deposition

EFRAT KESSLER
The Maurice and Gabriela Goldschleger Eye Research Institute, Tel-Aviv University Sackler Faculty of Medicine, Sheba Medical Center, Tel-Hashomer 52621, Israel
e-mail: ekessler@post.tau.ac.il

Abstract. Procollagen C-proteinase (PCP) and its enhancer protein (PCPE) are key to collagen fibril-assembly and extracellular matrix formation. PCP cleaves the carboxyl-propeptides of procollagens types I, II, and III and this initiates the self-assembly of collagen fibrils. PCP can also process pro-lysyl oxidase and laminin 5, and it may cleave the type V procollagen N-propeptides. Procollagen processing by PCP is stimulated by PCPE, a glycoprotein that binds to the C-propeptide of type I procollagen through its N-terminal CUB domains. PCPE is also required for normal cell growth and morphology. Two distinct forms of PCP were isolated from mouse and chick sources. These were recently identified as alternatively spliced products of the gene coding for bone morphogenetic protein-1 (BMP-1), a member of a family of Zn-dependent astacin-like metalloendopeptidases implicated in tissue patterning and development. Typically, these are multidomain proteases that, in addition to their catalytic domain, contain a number of EGF-like and CUB protein-protein interaction domains. Recent evidence suggests that PCP/BMP-1 related proteases can activate TGF-β-like growth factors. There is also evidence for additional alternatively spliced PCP variants. Thus, PCPs may have important biological functions in addition to their role in collagen fibril assembly.

Keywords. Procollagen C-proteinase; bone morphogenetic protein-1; procollagen processing; fibril-formation; fibrosis.

1. Introduction

Fibrillar collagens are major components of the extracellular matrix of most connective tissues where they occur as insoluble fibrils with a characteristic axial periodic structure. The fibrils provide the biomechanical scaffold for cell attachment and anchorage of macromolecules, thereby defining and maintaining the shapes and functions of tissues. Type I collagen is most abundant in tendons, ligament, skin, and bone. It is also synthesized in response to injury. Excessive deposition of collagen I occurs in fibrotic disease, and this impairs the normal function of the affected tissue. Type II collagen is found in cartilage and vitreous humor. Type III collagen is abundant in the walls of hollow organs such as arteries, lungs, and intestines, and usually occurs in the same fibril with type I collagen. The minor fibrillar collagen types V and XI are incorporated into the fibrils of collagen types I and II, respectively, and act as regulators of the sizes and shapes of the resultant heterotypic fibrils [1–4].

Fibril-forming collagens are synthesized as soluble precursors, procollagens, in which the central triple-helical, fibril-forming domain is flanked by amino and carboxyl

propeptide domains (figure 1). Proteolytic removal of the propeptides by specific N- and C-proteinases is essential for the formation of the mature collagen monomer that spontaneously self-assembles into fibrils. In particular, failure to remove the C-propeptides of procollagen seems incompatible with fibrillogenesis. Cleavage of the carboxyl propeptides lowers the solubility of procollagen at least 10,000-fold and initiates the self-assembly of fibrils [4,5]. Thus, among the enzymes involved in collagen deposition, procollagen C-proteinase plays a key role.

The first evidence for the existence of a specific procollagen C-proteinase was obtained about 25 years ago from studies with cultures of human and mouse fibroblasts [6,7].

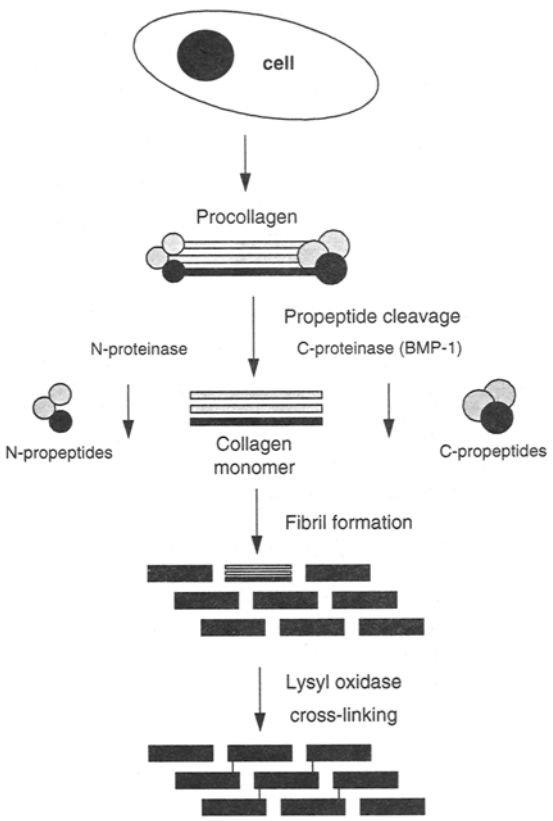

Figure 1. Extracellular events in the biosynthesis of fibrillar collagens: type I collagen as an example. Procollagen type I consists of two pro α1(I) and one pro α2(I) chains (open and solid forms respectively) in which the central triple helical domain (about 300 nm in length; rod-like) is flanked by a trimeric N-propeptide and a larger trimeric globular C-propeptide (represented as triads of small and large circles, respectively). After secretion, the propeptides are removed by specific procollagen N- and C-proteinases, to form the collagen monomer that spontaneously self-assembles into fibrils. The fibrils are stabilized by covalent cross-linking, initiated by oxidative deamination of specific lysine and hydroxylysine residues in collagen by lysyl oxidase. Procollagens II and III have similar structures and undergo the same extracellular modifications but, in contrast to type I procollagen, each consists of three identical polypeptide chains, pro α1(II) and pro α1(III).

Similar activity was documented in cultures of chick embryo calvaria[8,9], chick tendon fibroblasts[10–12], chick embryo chondrocytes[13], and in extracts of chick calvaria[14]. Names used for the enzyme during these early years included procollagen C-peptidase, carboxyl-procollagen peptidase, and carboxy-terminal procollagen peptidase, but the name procollagen C-proteinase (PCP) was subsequently adopted. Initial attempts to isolate the C-proteinase were hampered by the low levels of PCP and the presence of non-specific proteases in all sources which produced conflicting results with regard to the nature of the physiologically relevant enzyme. Isolation of a specific PCP was not achieved until 1985, when Hojima *et al*[15] reported the purification and characterization of a C-proteinase present in media of cultured chick embryo tendons. Later, we isolated a C-proteinase from culture media of mouse fibroblasts that, with the exception of its smaller size, displayed the same properties as those of the chick enzyme[16,17]. Our studies have led to the discovery of the procollagen C-proteinase enhancer (PCPE), a glycoprotein that binds to the C-propeptide of procollagen type I and enhances procollagen processing by PCP dramatically[17,18]. PCPE apparently, is another key player in C-terminal processing of procollagen. In collaboration with Dr Greenspan, we then identified the genes for both PCPE[19] and PCP[20]. These studies revealed partial structural homology between PCP and PCPE and culminated in the unexpected finding that PCP is identical to bone morphogenetic protein-1 (BMP-1)[20], the prototype of a family of proteases implicated in developmental processes and morphogenesis. For the first time, a direct link between an enzyme involved in matrix deposition and genes involved in pattern formation was established. Soon it was recognized that multiple variants of BMP-1 exist[21–23] and can process a number of other matrix proteins[24,25]. PCP has emerged as a potentially multifunctional protease that may play important roles in development and cell signaling in addition to its role in the assembly of collagen fibrils.

2. Procollagen C-proteinase

2.1 Properties and specificity

The molecular masses of the PCPs that were isolated from the culture media of mouse and chick fibroblasts are 80 and 110 kDa respectively[15,17]. It is now known that these represent distinct products of the same gene, referred to as BMP-1 and mTld or pCP1 and pCP2[21,22,26] respectively. Both are secreted N-glycosylated Zn-dependent metallo-proteases that require calcium for activity. The optimal *p*H of both PCPs is 8·6 and both cleave the carboxyl propeptides of procollagens I, II, and III as well as those of the respective pC-collagens (procollagen processing intermediates containing the C- but not the N-propeptides). The cleavage site in the pro- (or pC) α1(I), α2(I), and α1(II) chains is an Ala-Asp bond at the C-telopeptide/C-propeptide junctions in these chains[15,16]. The human and chick pro α1(III) chains are cleaved at the respective Gly-Asp and Arg-Asp bonds. Substitution of the Asp at position P1' in pro α2(I) blocks cleavage[27] but denatured procollagen is processed. PCP does not cleave Ala-Asp or Gly-Asp bonds that occur in the triple helical domain of procollagen but, as was shown for the chick enzyme, it cleaves the Gly-Asp bond at the presumed processing site of prolysyl oxidase[24]. The γ2 chain of laminin 5 is cleaved by human BMP-1 at a Gly-Asp bond[25]. Thus, PCP activity is not restricted to folded procollagen trimers and seems to depend on Asp at the P1' position. An exception to this latter rule is the recent demonstration that BMP-1 processes

the N- (but not the C-) propeptide of pro-α1(V) at a site which lacks the otherwise invariant aspartate at the P1' position [28].

PCP activity towards procollagen type I is stimulated in the presence of PCPE [17,18,29] or when procollagen is aggregated with dextran sulfate or polyethylene glycol [30]. Inhibitors of the enzyme include chelators such as EDTA, EGTA, and 1,10-phenanthroline, basic amino acids (e.g., arginine), dithiothreitol, N-ethylmaleimide, concanavalin-A, and α2-macroglobulin [15,16]. $CdCl_2$, $ZnCl_2$, and $CuCl_2$ are also inhibitory [15,31]. Peptides which mimic the sequences around the cleavage site in proα1(I) and pro-lysyl oxidase are weak inhibitors [14,24]. Natural PCP inhibitors may control PCP activity in the extracellular matrix [15] but these remain to be identified.

2.2 *Structure*

PCP/BMP-1 belongs to the astacin family of metalloendopeptidases, classified as family M12, clan MB of metalloproteases [32–34]. Analyses in several laboratories of cDNAs for PCP indicated that up to six alternatively spliced protein products may exist [21–23]. However, to date, only two of these have been isolated or expressed from the respective cDNAs. These are the short variant that is identical to BMP-1 [17,20,21,35] and the longer one, mTld, has a domain structure identical to that of *Drosophila* tolloid [15,21,22,36]. In common with other family members, the PCPs are synthesized as preproenzymes in which the propeptide is followed by an N-terminal astacin-like zinc metalloproteinase domain that is about 200 amino acid residues long (figure 2). With the exception of astacin, all family members contain additional domains downstream of the protease domain. In BMP-1, the protease domain is followed by two CUB [37] domains, an EGF-like domain, and a third CUB domain. In mTld, the third CUB domain is followed by another EGF-like domain and two additional CUB domains. Both, BMP-1 and mTld, also have short non-homologous sequences at their C-terminus [22]. The CUB domains are implicated in protein-protein interactions and are so called because they were first identified in the Complement components C1r/C1s, sea urchin protein Uegf, and Bone morphogenetic protein-1 [37]. Each CUB domain comprises about 110 residues and 4 conserved cysteine residues whereas the EGF-like domains are ~40 residues long, each containing 6 conserved cysteine residues. The cysteine residues presumably form intra-domain disulfide bonds. The catalytic domain contains an 18-residue signature zinc-binding sequence, **HEXXHXXGFXHEXXRXDR**, and another conserved sequence, SXMHY, that forms a critical 'methionine turn' beneath the active site metal. This places the PCPs in the 'metzincins' family of Zn-metalloendopeptidases that also includes mammalian collagenase and the other MMPs [38]. BMP-1 and mTld contain 5 conserved N-glycosylation sites and both appear to be heavily glycosylated [20,39]. The sequences of mature human and mouse BMP-1 and mTld are 98% identical but those of the prepro regions show only 50% identity. A conserved RSRR sequence at the junction between the pro region and the protease domain suggests intracellular processing by a dibasic peptidase [40].

2.3 *Regulation*

Little is known about the regulation of PCP expression. Recent studies suggested however that BMP-1 expression may be coordinately regulated with that of collagen type I. The

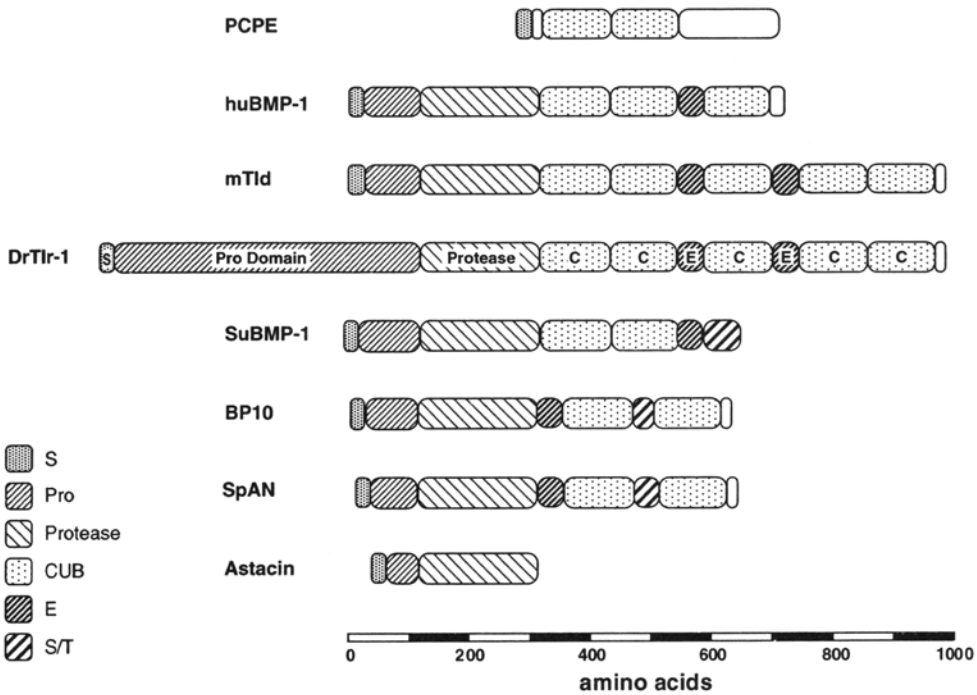

Figure 2. Domain structure of PCPE, BMP-1 and mTld in comparison to other astacin family members. *S*, signal peptide; *Pro*, propeptide domain; *E*, EGF-like domain; *C*, CUB domain; *S/T*, serine/threonine-rich domain; blank boxes, non-homologous regions; hu, human; Su, sea urchin; DrTlr-1, *Drosophila* tolloid-related-1[57]; BP10[58] and SpAN[59], blastula-restricted proteases of sea urchin. The structures of astacin and SuBMP-1 are from Titani *et al*[60] and Hwang *et al*[61] respectively.

addition of TGF-β to cultures of fibrogenic cells (MG63) or keratinocytes was found to increase the levels of BMP-1 and mTld mRNAs up to seven-fold, in parallel to the increase in collagen α1(I) mRNA. This stimulatory effect was better pronounced in the presence of ascorbic acid and the increase in mRNA levels was mirrored by elevated levels of BMP-1 and mTld proteins in the media as well as increased procollagen processing[39]. Similar effects were documented in cultured dermal fibroblasts. In this cell system, exposure to TGF-β or serum, as well as application of mechanical load, increased the levels of BMP-1/mTld and α1(I) mRNAs. Procollagen synthesis and processing into insoluble collagen fibrils were also increased[41]. The significance of these initial observations may be beyond a simple direct correlation between procollagen and PCP expression. TGF-β stimulates the biosynthesis of numerous structural matrix proteins[42]. It also up-regulates lysyl oxidase expression[43]. Since BMP-1 was initially isolated in a complex with other BMPs that are members of the TGF-β superfamily and presumed to have the capacity to induce bone formation, it was hypothesized that the bone-inducing activity of BMP-1 may result from its ability to proteolytically activate latent forms of TGF-β-like BMPs[35]. If PCP/BMP-1 can indeed act as an activator of TGF-β, an important regulatory loop may be envisaged, in which PCPs activate TGF-β and are then induced by TGF-β, to facilitate matrix deposition through procollagen processing activity, lysyl oxidase activation, and biosynthetic processing of laminin 5.

3. Procollagen C-proteinase enhancer (PCPE)

3.1 Properties

PCPE is a 55-kDa glycoprotein, originally discovered in my laboratory by virtue of its ability to stimulate procollagen processing by PCP [17,18]. PCPE binds to the C-propeptide of procollagen type I and while it lacks proteolytic activity of its own, it increases the procollagen processing activity of PCP at least 10-fold with no effect on procollagen N-proteinase activity [17,29]. PCPE enhances the activity of both, the mouse and chick PCPs (BMP-1 and mTld respectively), suggesting that the additional domains in mTld are not essential for PCPE function. In the medium of cultured mouse fibroblasts, PCPE is far more abundant than PCP and is present in both full length (55 kDa) and short (34- and 36-kDa) proteolytic fragments which retain PCP enhancing activity as well as the ability to bind to the C-propeptide. Binding does not seem to induce aggregation of procollagen and when the amount of PCP is adjusted so that the rate of C-terminal processing remains constant, PCPE has no effect on the assembly of collagen or pN collagen *in vitro* following C-terminal processing of the corresponding precursors [29]. PCPE appears to affect both the K_m and V_{max} of the PCP reaction on procollagen [18]. It is conceivable that PCPE interaction with the C-propeptide may induce a conformational change in procollagen that renders it a fitter substrate for cleavage by PCP.

3.2 Structure

In collaboration with Dr Greenspan, we have isolated and sequenced the full length cDNA's for the mouse and human enhancer proteins [19] and, more recently, the nucleotide sequence of the rat enhancer gene has been elucidated [44,45]. The deduced amino acid sequences of the mouse and rat PCPEs are almost identical and highly homologous to that of the human protein. Each contains a putative signal peptide, two N-terminal repeats of the CUB domain, and a non-homologous C-terminal domain (figure 2). Two conserved N-glycosylation sites are located at the extreme ends of the 55-kDa protein. The 36- and 34-kDa forms of PCPE are derived from the N-terminal portion of the intact 55-kDa protein, and are composed almost exclusively of the two CUB domains. PCPE binding to the type I procollagen C-propeptide is mediated by the CUB domains. This is the most conserved region between the rodent and human enhancer proteins. Immediately downstream of the CUB domains in the 55 kDa protein is the least conserved region between the mouse/rat and human proteins. This short region is probably a linker that connects the more highly conserved upstream and downstream domains, in which proteolytic cleavages may occur to produce the 34- and 36-kDa PCPE fragments. Two consensus sequences for RNA binding were noted within the C-terminal domain of PCPE and these are presumed to be involved in the stabilization of mRNAs, recently observed in cultured liver stellate cells [44,46].

3.3 Expression and effect on cell growth

PCPE is abundant in connective tissues rich in collagen type I (e.g., tendon, skin, bone) but is undetectable in brain and liver [19,44,47]. PCPE is expressed however in the fibrotic liver of rats treated with CCl_4 [44]. The expression of PCPE mRNA in cultured liver stellate cells derived from a rat CCl_4-induced cirrhotic liver is up-regulated by TGF-β and down-regulated by tumor necrosis factor α (TNF-α), in parallel to changes in the levels of α1(I)

procollagen mRNA[44]. Similarly, the addition of TGF-β to cultured dermal fibroblasts up-regulates PCPE expression but (in contrast to PCP/BMP-1) no further increase in PCPE mRNA is seen in these cells in response to mechanical load[41]. In cultured fibrogenic cell lines (e.g. MG63), the addition of TGF-β had no effect on PCPE expression[39]. It is possible that other factors are involved in regulation of PCPE that have masked the stimulatory potential of TGF-β in these cultures. Correlation of PCPE expression with that of collagen, observed *in vivo* with respect to normal tissue distribution and induction in the fibrotic liver, along with the coordinated up-regulation of both proteins in response to growth factors *in vitro*, highlight the importance of PCPE in regulation of collagen deposition.

PCPE expression seems to affect cell growth properties. Rat fibroblasts expressing decreased amounts of PCPE as a result of retroviral vector integration into the PCPE gene display a phenotype similar to that of malignantly transformed cells. They lose contact inhibition, show altered morphology, and can grow in an anchorage-independent manner[45]. The normal phenotype is restored upon transfection of the mutant cells with a PCPE expression plasmid. The mechanism by which PCPE affects cellular growth is unclear. Decreased type I collagen synthesis is known to be associated with malignant cellular transformation[48] and restoration of collagen synthesis can suppress the malignant phenotype[49]. It is possible that PCPE alters cellular morphology and growth properties through its stimulation of PCP/BMP-1 activity, which facilitates normal extracellular matrix formation required for normal cell growth and morphology.

4. Concluding remarks

The importance of PCP in regulation of collagen deposition has recently been highlighted by the defects seen in mouse embryos lacking the BMP-1 gene[50]. However, the phenotype of these BMP-1 null mice was not as dramatic as was expected for a complete lack of C-terminal procollagen processing. It is therefore likely that other proteases may compensate for PCP in its absence. The most likely candidate is the product of the mammalian *Tolloid-like* (*Tll*) gene that shares 76% identity in amino acid sequence with that of mTld[51]. However, the question of whether or not Tll has PCP activity remains open. Other enzymes that may substitute for PCP are cathepsin D that can cleave the C-propeptides of procollagen at a slightly acidic *p*H in cultured fibroblasts[52] and a cell-associated protease (telopeptidase) that apparently can process the C-propeptide at a nearby site located within the C-telopeptide[27]. It is unclear whether cleavages by these non-specific proteases produce fully functional collagen monomers. More intriguing is the unexpected finding that PCP is related to proteases involved in patterning. It has been shown that the rate of C-terminal procollagen processing by PCP has a marked effect on the morphology of collagen fibrils assembled *de novo* from pC-collagen[53]. The question arises of whether collagen fibril assembly is directly involved in gene regulation, cytokine induction, and cell-signaling events that are major factors in tissue patterning, or, perhaps, PCP/BMP-1 can regulate developmental processes indirectly, by proteolytic activation of latent growth factors. Given the fact that many growth factors, in particular, members of the TGF-β superfamily, are ECM-associated, this latter possibility is most appealing and there is now evidence to support such roles for BMP-1-related proteases. In *Drosophila*, tolloid was recently shown to activate decapentaplegic (dpp), a functional analog of BMP2/4 involved in dorso–ventral patterning, by cleaving SOG (short gastrulation), a protein that forms an inhibitory complex with dpp. A gradient of active dpp is formed that

directs dorso–ventral patterning[54]. A similar mechanism was also demonstrated in *Xenopus*[55] and zebrafish[56], where xolloid, a metalloproteinase related to PCP/BMP-1, and the zebrafish tolloid, respectively, were shown to activate a BMP pathway by cleaving chordin, an inhibitory protein homologous to SOG. Thus, PCPs are likely to govern matrix deposition at several levels: through activation of TGF-β-like molecules they may stimulate matrix production, and then, facilitate matrix deposition by processing precursors of various matrix molecules, including procollagens. The expression of a particular PCP variant during development and adult life appears to be regulated at the gene level[22]. Cleavage by PCPs of a specific substrate in the matrix may be determined by the unique combination of the CUB and EGF-like domains and availability in the vicinity of a particular protein substrate (e.g., procollagen, laminin 5, lysyl oxidase) for interaction and processing. Elucidating the determinants of PCP regulation and function is a major objective of future research in this new and exciting area. Understanding the biological role(s) of PCPE is yet another important goal. Current knowledge supports PCPE as a key regulator of collagen fibril formation, but it may have additional functions. It is not known whether PCPE interacts with other matrix components and whether it can modulate the activity of PCP on substrates other than procollagen. Also intriguing is the mechanism by which PCPE affects cell-growth properties and whether, as was recently suggested[46], it also fulfils intracellular functions. Because PCP and PCPE are key regulators of collagen deposition, understanding the molecular basis for their mutual interactions is of importance for the development of new approaches to control excessive collagen deposition in fibrotic disease and may provide a basis to evaluate pathogenesis of other connective tissue diseases.

References

1. van der Rest M and Garrone R 1991 *FASEB J.* **5** 2814
2. Hulmes D J S 1992 *Essays Biochem.* **27** 49
3. Prockop D J and Kivirikko K I 1995 *Annu. Rev. Biochem.* **64** 403
4. Prockop D J and Hulmes D J S 1994 In *Extracellular matrix assembly and structure* (eds) P D Yurchenco, D E Birk and R P Mecham (New York: Academic Press) pp. 47–90
5. Kadler K E and Watson R B 1995 *Methods Enzymol.* **248** 771
6. Goldberg B, Taubman M B and Radin A 1975 *Cell* **4** 45
7. Kessler E and Goldberg B 1978 *Anal. Biochem.* **86** 463
8. Fessler L I, Morris N P and Fessler J H 1975 *Proc. Natl. Acad. Sci., USA* **72** 4905
9. Davidson J M, McEneany L S and Bornstein P 1977 *Eur. J. Biochem.* **81** 349
10. Uitto J and Lichtenstein J R 1976 *Biochem. Biophys. Res. Commun.* **71** 60
11. Duksin D, Davidson J M and Bornstein P 1978 *Arch. Biochem. Biophys.* **185** 326
12. Leung M K K, Fessler L I, Greenberg D B and Fessler J H 1979 *J. Biol. Chem.* **254** 224
13. Uitto J 1977 *Biochemistry* **16** 3421
14. Njieha F K, Morikawa T, Tuderman L and Prockop D J 1982 *Biochemistry* **21** 757
15. Hojima Y, van der Rest M and Prockop D J 1985 *J. Biol. Chem.* **260** 15996
16. Kessler E, Adar R, Goldberg B and Niece R 1986 *Collagen Rel. Res.* **6** 249
17. Kessler E and Adar R 1989 *Eur. J. Biochem.* **186** 115
18. Adar R, Kessler E and Goldberg B 1986 *Collagen Rel. Res.* **6** 267
19. Takahara K, Kessler E, Biniaminov L, Brusel M, Eddy R L, Jani-Sait S, Shows T B and Greenspan D S 1994 *J. Biol. Chem.* **269** 26280
20. Kessler E, Takahara K, Biniaminov L, Brusel M and Greenspan D S 1996 *Science* **271** 360
21. Li S-W, Sieron A L, Fertala A, Hojima Y, Arnold W V and Prockop D J 1996 *Proc. Natl. Acad. Sci., USA* **93** 5127
22. Takahara K. Lyons G E and Greenspan D S 1994 *J. Biol. Chem.* **269** 32572
23. Janitz M, Heiser V, Böttcher U, Landt O and Lauster R 1998 *J. Mol. Med.* **76** 141

24. Panchenko M V, Stetler-Stevenson W G, Trubetskoy O V, Gacheru S N and Kagan H M 1996 *J. Biol. Chem.* **271** 7113
25. Amano S, Takahara K, Gerecke D, Nishiyama T, Lee S, Greenspan D S and Burgeson R E 1996 *Mol. Biol. Cell* **7** (suppl.) 58a (Abstr. 338)
26. Prockop D J, Sieron A L and Li S-W 1997/98 *Matrix Biol.* **16** 399
27. Lee S-T, Kessler E and Greenspan D S 1990 *J. Biol. Chem.* **265** 21992
28. Imamura Y, Steiglitz B M and Greenspan D S 1998 *J. Biol. Chem.* **273** 27511
29. Hulmes D J S, Mould A P and Kessler E 1997 *Matrix Biol.* **16** 41
30. Hojima Y, Behta B, Romanic A M and Prockop D J 1994 *Anal. Biochem.* **223** 173
31. Hojima Y, Behta B, Romanic A M and Prockop D J 1994 *Matrix Biol.* **14** 113
32. Bond J S and Beynon R J 1995 *Protein Sci.* **4** 1247
33. Rawlings N D and Barrett A J 1995 *Methods Enzymol.* **248** 183
34. Kessler E 1998 In *Handbook of proteolytic enzymes* (eds) A J Barrett, N D Rawlings and F J Woessner Jr (London: Academic Press) pp. 1236-1240.
35. Wozney J M, Rosen V, Celeste A J, Mitsock L M, Whitters M J, Kriz R W, Hewick R M and Wang E A 1988 *Science* **242** 1528
36. Shimell M J, Ferguson E L, Childs S R and O'Connor M B 1991 *Cell* **67** 469
37. Bork P and Beckmann G 1993 *J. Mol. Biol.* **231** 539
38. Stöcker W, Grams F, Baumann U, Reinemer P, Gomis-Rüth F-X, McKay D B and Bode W 1995 *Protein Sci.* **4** 823
39. Lee S, Solow-Cordero D E, Kessler E, Takahara K and Greenspan D 1997 *J. Biol. Chem.* **272** 19059
40. Barr P J 1991 *Cell* **66** 1
41. Parsons M, Laurant G F, Brown R A and Bishop J E 1998 *FASEB J.* **12** 4256 (Suppl.) (Abstract)
42. Massague J 1990 *Annu. Rev. Cell. Biol.* **6** 597
43. Feres-Filho E J, Jin Choi Y, Han X, Takala T E S and Trackman P C 1995 *J. Biol. Chem.* **270** 30797
44. Ogata I, Auster, A S, Matsui A, Greenwell P, Geerts A, D'Amico T, Fujiwara K, Kessler E and Rojkind M 1997 *Hepatology* **26** 611
45. Masuda M, Igarashi H, Kano M and Yoshikura H 1998 *Cell Growth Differ.* **9** 381
46. Matsui A, Ogata I, Yanase M, Tomiya T, Ikeda H, Fujiwara K and Rojkind M 1998 *Hepatology* **28** A301 (Abstract no. 553)
47. Kessler E, Mould P A and Hulmes D J S 1990 *Biochem. Biophys. Res. Commun.* **173** 81
48. Arbogast B W, Yoshimura M, Kefalides N A, Holtzer H and Kaji A 1977 *J. Biol. Chem.* **252** 8863
49. Travers H, French N S and Norton J D 1996 *Cell Growth Differ.* **7** 1353
50. Suzuki N, Labosky P A, Furuta Y, Hargett L, Dunn R, Fogo A B, Takahara K, Peters D M P, Greenspan D S and Hogan B L M 1996 *Development* **122** 3587
51. Takahara K, Brevard R, Hoffman G G, Suzuki N and Greenspan D S 1996 *Genomics* **34** 157
52. Helseth D L Jr and Veis A 1984 *Proc. Natl. Acad. Sci., USA* **81** 3302
53. Holmes D F, Watson R B, Chapman J A and Kadler K E 1996 *J. Mol. Biol.* **261** 93
54. Marqués G, Musacchio M, Shimell M J, Wünneberg-Stapelton K, Cho K W Y and O'Connor M B 1997 *Cell* **91** 417
55. Piccolo S, Agius E, Lu B, Goodman S, Dale L and De Roberts E M 1997 *Cell* **91** 407
56. Blader P, Rastegar S, Fischer N and Strähle U 1997 *Science* **278** 1937
57. Nguyen T, Jamal J, Shimell M J, Arora K and O'Connor M B 1994 *Dev. Biol.* **166** 569
58. Lepage T, Ghiglione C and Gasche C 1992 *Development* **114** 147
59. Reynolds S D, Angerer L M, Palis J, Nasir A and Angerer R C 1992 *Development* **114** 769
60. Titani K, Torff H J, Hormel S, Kumar S, Walsh K A, Rodl J, Neurath H and Zwilling R 1987 *Biochemistry* **26** 222
61. Hwang S, Partin J S and Lennarz W J 1994 *Development* **120** 559