Homology cloning of rat 72 kDa type IV collagenase: cytokine and second-messenger inducibility in glomerular mesangial cells

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Glomerular mesangial cells (MC) play a central role in the synthesis and turnover of the glomerular extracellular matrix. Prior studies [Davies, Thomas, Martin and Lovett (1988) Biochem. J. **251**, 419–425; Martin, Davies, Thomas and Lovett (1989) Kidney Int. **36**, 790–801] have characterized at the protein level a 72 kDa type IV collagenase that is secreted by cultured human and rat MC. While exposure of most cell types to interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α) or phorbol ester has little or even an inhibitory effect on 72 kDa type IV collagenase secretion, these factors significantly increased the synthesis of this enzyme by rat MC. Given this divergent pattern of expression, a homology-based PCR cloning strategy using rat MC cDNA templates was employed to define at the molecular level the structure of the mesangial 72 kDa type IV collagenase. The nucleotide sequence within the open reading frame of the rat mesangial 72 kDa type IV collagenase cDNA diverges from the sequence of the human homologue by approx. 9%. The divergence in the 3′ untranslated region was much more extensive. Steady-state levels of the 3.1 kb transcript of the 72 kDa type IV collagenase were low or undetectable in resting MC, but were greatly stimulated following incubation with IL-β, TNF-α or phorbol ester. None of these factors induced synthesis by MC of the closely related 92 kDa type IV collagenase. Synthesis by MC of the 72 kDa type IV collagenase was also induced by second-messenger analogues, including 8-bromo-cyclic AMP and forskolin. It is concluded that MC regulate the expression of this enzyme in an unusual, tissue-specific fashion. Cytokine and second-messenger inducibility may contribute to the enhanced expression of the enzyme during glomerular inflammatory disorders.

INTRODUCTION

The extracellular matrix (ECM), which includes the glomerular basement membrane and the mesangial matrix, fulfils an important structural and regulatory function in the renal glomerulus. This highly specialized matrix forms a negatively charged, permselective filtration barrier and provides structural support against the high intraglomerular hydrostatic pressure. A common feature of most chronic forms of glomerulonephritis is the excessive accumulation of multiple glomerular ECM proteins, a process leading to glomerulosclerosis and loss of filtration function [1,2]. To date, the processes involved in the catabolism of this matrix compartment under basal or pathological conditions remain incompletely understood. As a consequence of its central location within the glomerulus, the intrinsic mesangial cell (MC) plays a key role in both the synthesis and the degradation of the glomerular ECM [3]. Numerous studies have documented the ability of cultured MC of either human or rodent origin to synthesize a broad variety of intrinsic glomerular ECM proteins, including type IV and V collagens, interstitial collagens I and III, fibronectin, laminin, thrombospondin and sulphated proteoglycans [3–7]. Considering the diverse compositional nature of the glomerular mesangial ECM, we have postulated that a parallel group of matrix-degrading enzymes must exist to provide for the turnover of these proteins. Given their ability to act extracellularly, the activities of the matrix metalloproteinases have received particular attention.

Prior protein purification and characterization studies from our laboratories have documented the synthesis of a specific 72 kDa type IV collagenase by cultured rat and human MC lines [8–10]. Amino acid sequence analysis of tryptic peptides derived from the purified human MC 72 kDa type IV collagenase suggested structural identity with the 72 kDa type IV collagenase recently cloned from H-*ras*-transformed tracheal bronchial epithelial cells [11,12]. In almost all evaluated cell lines of either malignant or non-malignant nature, the synthesis of the 72 kDa type IV collagenase is constitutive and exhibits little, if any, response to inflammatory cytokines or phorbol esters [12–14]. In contrast, our earlier studies with cultured rat MC suggested that the synthesis of the MC type IV collagenase exhibited certain tissue-specific features, including stimulation by monocyte supernatants or interleukin-1β (IL-1β) [15]. Given these differences of potential pathophysiological significance, a PCR-based homology cloning strategy was devised to define at the nucleic acid level the precise molecular and structural characteristics of the rat MC 72 kDa type IV collagenase. In this paper the molecular structure of the rat 72 kDa type IV collagenase is defined and the patterns of response to cytokines, phorbol ester and second messenger analogues are detailed.

MATERIALS AND METHODS

MC cultures

The methods for the establishment, characterization and maintenance of homogenous cultures of rat glomerular MC have been reported in detail [9,15]. Experiments were undertaken with cells in passages 8–10 and results were obtained with three independent MC lines. For preparation of conditioned media, subconfluent cultures were washed three times with PBS and cultured for 3 days in a rest medium consisting of RPMI 1640 medium

Abbreviations used: MC, mesangial cell(s); IL-1β, interleukin-1β; TNF-α, tumour necrosis factor-α; ECM, extracellular matrix; PMA, phorbol myristate acetate; cAMP, cyclic AMP; RT-PCR, reverse transcription PCR; MMLV, Moloney murine leukaemia virus.

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supplemented with 1% non-essential amino acids, 2 mM glutamine, 100 μg/ml streptomycin, 100 units/ml penicillin and 0.1% fetal bovine serum (culture reagents obtained from Gibco, Grand Island, NY, U.S.A.). Subsequently, the MC were incubated for 48 h in fresh rest medium supplemented as indicated with recombinant human IL-1β (25 units/ml; Genzyme, Boston, MA, U.S.A.) or IL-1β plus recombinant human tumour necrosis factor-α (TNF-α; 25 ng/ml; Genzyme). Phorbol myristate acetate (PMA; 10 nM), forskolin (50 μM) or 8-bromo-cyclic AMP (cAMP) (0.5 mM; Sigma Chemical Co., St. Louis, MO, U.S.A.) were used in a similar fashion for the times indicated. After harvesting of the conditioned media, the cell layers were washed twice in PBS at 4 °C and twice in 5% trichloroacetic acid at 4 °C, followed by protein determination as reported [9]. The conditioned media were stored at −80 °C prior to assay or use in SDS/gelatin substrate gels (see below). [^{3}H]Gelatin was used to quantify type IV collagenase activity as reported in detail previously [9,10]. The assay incubation mixture included 0.7 mM *p*-aminophenylmercuric acetate to permit complete activation of any latent enzyme. One unit of type IV collagenase activity is defined as that amount which degrades 1 μg of substrate/h under the given assay conditions; units are expressed in relation to 100 μg of cellular protein.

Electrophoretic procedures

SDS/PAGE gelatin substrate gels were prepared by including 1 mg/ml gelatin (from rat tail type I collagen) in the polymerization mixture. Enzyme samples were solubilized in non-reducing sample buffer, incubated at 37 °C for 5 min and electrophoresed at 7 °C at 25 mA/gel. Following electrophoresis, the gels were incubated at room temperature in 2.5% Triton X-100 and 50 mM Tris/HCl, pH 8.0, for 30 min, and then overnight at 37 °C in 50 mM Tris/HCl, pH 5.0, 5 mM $CaCl_2$ and 1 μM $ZnCl_2$. Thereafter, the gels were stained with Coomassie Blue and zones of lysis were visualized. Standard proteins were utilized for assignment of molecular mass.

cDNA library construction and structural homology cloning

Oligo(dT)-selected poly(A)$^+$ RNA was extracted from subconfluent cultures of rat MC stimulated for 48 h with a combination of IL-1β (25 units/ml) and TNF-α (25 ng/ml). A homology-based reverse transcription PCR (RT-PCR) was performed in two steps. For the RT part of the protocol, 600 ng of poly(A)$^+$ RNA template in 5 μl of water was combined with 3 μl of random hexamers, 2 μL of 0.1 M dithiothreitol, 5 μl of 2 mM dNTPs, 1 μl of Moloney murine leukaemia virus (MMLV) reverse transcriptase (200 units/μl; Superscript, Gibco–BLR) and 4 μl of 5 × 250 mM Tris/HCl, pH 8.3, 375 mM KCl, 15 mM $MgCl_2$ and 10 mM dithiothreitol. For the subsequent PCR, 25% of the RT reaction mixture was used as the starting DNA template, along with dNTPs (200 μM each) and 2.5 units of Taq polymerase (Perkin–Elmer Cetus). There were a total of 31 amplification cycles consisting of 30 s at 94 °C, 30 s at 52 °C and 90 s at 72 °C. A final extension time of 10 min was used in all cases. PCR priming pairs were based on the nucleotide sequences for the human 72 kDa type IV collagenase [12]. The sequence encoding the highly conserved activation locus (residues P^{71}RCGNPDVAN) was used as the 5′ primer (5′-CCA-CGC-TGC-GGC-AAC-CCA-GAT-GTG-GCC-AAC-3′) and the sequence encoding the conserved zinc-binding domain (residues V^{371}AAHEFGHA) as the 3′ primer (5′-GTG-GCA-GCC-CAC-GAG-TTT-GGC-CAC-GCC-3′, used in the reverse complement form). The PCR product was electrophoresed on a 1% agarose gel and eluted by repeated freeze–thawing. For ligation into vectors, the recovered PCR product was end-polished with T4 polymerase, ligated with *Eco*RI adaptors, phosphorylated and subcloned into the plasmid vector pGem-7Zf(+) (Promega) using standard procedures [16].

A non-amplified λ gt10 cDNA library containing 1.2×10^6 primary recombinants was prepared using 5 μg of oligo(dT)-selected poly(A)$^+$ RNA from rat MC stimulated for 48 h with 10 nM PMA. To obtain more complete 72 kDa type IV collagenase cDNA inserts, 7.5×10^4 primary recombinants from the unamplified λgt10 cDNA library were screened using the 900 bp activation locus–zinc-binding domain PCR product as a probe. The probe was labelled by the random hexamer method with [^{32}P]dCTP (NEN). Library screening, identification of positive clones and subcloning into pGem-7Zf(+) were performed using standard methodology [16]. Sequencing of double-stranded plasmid DNA was performed according to Sanger et al. [17], using Sequenase II (USB). Both strands of the mesangial type IV collagenase 2.7 kb cDNA insert obtained from the rat MC λ gt10 library were sequenced in their entirety using a series of synthetic oligonucleotide primers.

Northern blot analysis

Poly(A)$^+$ RNA (2.5 μg/lane) from quiescent rat MC and from cultures incubated for 48 h with recombinant IL-1β (25 units/ml), IL-1β (25 units/ml) plus TNF-α (25 ng/ml) or PMA (10 nM) was electrophoresed on 1.2% denaturing agarose gels and transferred to nylon membranes. Poly(A)$^+$ RNA (2.5 μg/lane) from quiescent controls and cultures incubated for 24 h with 10 nM PMA, 50 μM forskolin or 0.5 mM 8-bromo-cAMP (or combinations thereof) were processed in an identical manner. The blots were probed with the 900 bp cDNA activation locus/zinc-binding domain probe and with a full-length β-actin cDNA probe. Hybridizations were performed under standard conditions, followed by washing at high stringency in 1% SDS/0.1 × SSC at 65 °C for 45–60 min. Densitometry and normalization of the exposed films to β-actin mRNA abundance were performed as reported [11].

RESULTS

Cytokine and phorbol ester stimulation of MC type IV collagenase secretion

Non-stimulated cultures of rat MC constitutively release low levels of type IV collagenase activity (Table 1). Incubation for 48 h with recombinant human IL-1β (25 units/ml) led to an

Table 1 Induction of synthesis of rat MC type IV collagenase by IL-1β and TNF-α

Culture supernatants from rat MC incubated for 48 h with either rest medium (Control), IL-1β (25 units/ml), TNF-α (25 ng/ml) or IL-1β plus TNF-α were assayed for enzyme activity using a [^{3}H]gelatin assay. Results are expressed as units of enzyme activity/100 μg of cell protein (means ± S.D.; $n = 6$); $^*P < 0.05$; $^{**}P < 0.01$ compared with control.

	Type IV collagenase activity (units/100 μg of protein)
Control	9.7 ± 1.1
IL-1β	22.3 ± 1.7*
TNF-α	40.1 ± 3.4**
IL-1β plus TNF-α	55.4 ± 5.7**

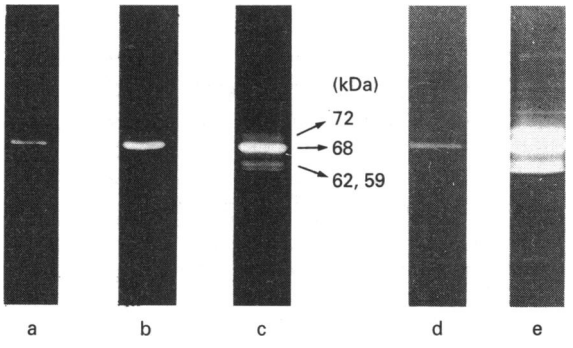

Figure 1 Gelatin substrate SDS/PAGE of MC culture supernatants following stimulation with cytokines or phorbol ester

Culture supernatants from MC incubated for 48 h with rest medium (control), IL-1β (25 units/ml), TNF-α (25 ng/ml) or IL-1β plus TNF-α were analysed by SDS/PAGE containing 1 mg/ml gelatin. Lane a, culture medium from unstimulated rat MC; lane b, medium from cells stimulated with IL-1β; lane c, cells stimulated with IL-1β plus TNF-α; lane d, culture medium from unstimulated cells; lane e, culture medium from cells stimulated with PMA (10 nM). The molecular masses of the proteolytically active proteins are denoted for lane c.

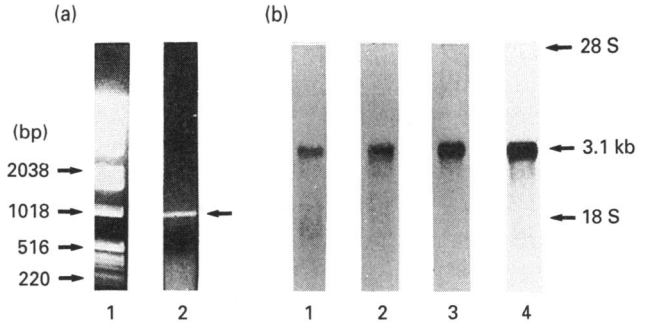

Figure 2 Amplification by RT-PCR of the 72 kDa type IV collagenase and Northern blot analysis of rat MC mRNA

(**a**) cDNA templates were prepared by RT with mRNA from rat MC incubated for 48 h with IL-1β (25 units/ml) plus TNF-α (25 ng/ml). Following 31 cycles of PCR amplification using priming pairs specific for the activation locus and the zinc-binding domain of the 72 kDa type IV collagenase, the products were separated on 1% agarose gels. The closed arrow beside lane 2 indicates the predicted 900 bp PCR product; lane 1 contains an appropriate ethidium bromide-stained DNA reference ladder. (**b**) Northern blot analysis of MC poly(A)$^+$-selected RNA (2.5 μg/lane) following hybridization at high stringency with a labelled 900 bp activation locus/zinc-binding domain 72 kDa type IV collagenase cDNA probe. Lane 1, control rat MC. Cells with stimulated for 48 h with IL-1β (25 units/ml; lane 2), IL-1β plus TNF-α (25 ng/ml, lane 3), or PMA (10 nM, lane 4).

approx. 2-fold increase in secreted enzymic activity, while incubation with TNF-α (25 ng/ml) induced a nearly 4-fold increase. The combination of IL-1β and TNF-α resulted in an additive increase in type IV collagenase secretion (Table 1). In a previous study [9] with rat MC we found that the control type IV collagenase activity was accounted for exclusively by the 72 kDa enzyme. The present work confirms this and further demonstrates that the cytokine-stimulated activity was the consequence of an exclusive increase in 72 kDa type IV collagenase synthesis, as demonstrated by SDS/gelatin zymograms (Figure 1). Culture supernatants of rat MC incubated with a combination of IL-1β and TNF-α also exhibited an increased abundance of the catalytically active 62 and 59 kDa forms of the enzyme (Figure 1, lane c), indicative of an increased conversion of latent to active enzyme under these conditions. Incubation with 10 nM PMA for 48 h also increased the synthesis of the 72 kDa type IV collagenase by cultured rat MC (Figure 1, lanes d and e). Interestingly, rat MC did not respond to any of these agents by synthesizing significant levels of the 92 kDa type IV collagenase, an enzyme which is induced by these factors in many cell types [18–21].

Molecular characterization of the mesengial 72 kDa type IV collagenase

The induction of the MC 72 kDa type IV collagenase by IL-1β, TNF-α or phorbol ester is at variance with experience obtained with multiple cell types, in which synthesis of the enzyme is either constitutive or even inhibited by cytokines such as IL-1β [12–14]. To evaluate this issue in more detail, we utilized a homology-based RT-PCR protocol to amplify rat MC 72 kDa type IV collagenase transcripts. Using PCR priming pairs encoding the conserved activation locus and the zinc-binding domain of the human cDNA, a prominent 900 bp product was obtained with RNA templates prepared from cells incubated with a combination of IL-1β and TNF-α (Figure 2a). The product size is consistent with the distance between these priming pairs in either the human 72 kDa or the 92 kDa type IV collagenase cDNAs [12,22]. However, when used as a cDNA probe for Northern analysis under conditions of high stringency, this RT-PCR product yielded a single hybridizing 3.1 kb transcript (Figure 2b). The size of this transcript is identical to that found within human cells expressing the 72 kDa type IV collagenase. Probing of the same Northern blot with the cDNA of the human 92 kDa collagenase did not yield hybridizing bands (results not shown). Under similar hybridization conditions this human probe readily hybridized with 92 kDa type IV collagenase mRNA obtained from rat leucocytes. The abundance of the 3.1 kb 72 kDa type IV collagenase transcript was significantly and specifically enhanced in rat MC by incubation with IL-1β or IL-1β plus TNF-α (1.7- and 3.9-fold increases respectively, as measured by normalized densitometry). Northern blot analysis of RNA extracted from PMA-stimulated cells also revealed a significant increase in steady-state 72 kDa type IV collagenase mRNA levels (approx. 6-fold by normalized densitometry; Figure 2b).

Because of the unexpected pattern of cytokine responses manifested by MC, the possibility of a variably regulated second isoform of the 72 kDa type IV collagenase was considered. Therefore, to unequivocally identify this transcript, we determined the nucleotide sequence of the 900 bp activation locus/zinc-binding site PCR product following subcloning into a plasmid vector. Sequence analysis of multiple clones of this product on both strands demonstrated minimal nucleotide divergence (9%) from the human 72 kDa type IV collagenase cDNA sequence [12]. In no case were clones obtained containing cDNA inserts encoding the 92 kDa type IV collagenase. Thereafter, the 900 bp cDNA activation locus/zinc-binding domain fragment was used to screen a non-amplified rat MC cDNA λ gt10 library prepared from PMA-stimulated cells. There were two immediate reasons for this step. First, we wished to obtain a more extensive cDNA sequence containing the complete open reading frame. Secondly, it was important to confirm the pattern of minimal sequence divergence of the rat mesengial type IV collagenase using a cDNA obtained independently of a PCR reaction. Screening of 1×10^6 primary recombinants yielded a series of individual clones, of which one, pTIVSF, was the largest, containing a 2681 bp insert. The sequence of pTIVSF is shown in Figure 3 and includes the complete coding sequence for a 627-amino-acid pro-type IV collagenase protein, as well as 754 bp of 3′ untranslated region. At the extreme 5′ end of the cDNA clone, 13 nucleotides encoding a partial signal peptide were obtained. Repeated screening of the PMA-stimulated λ

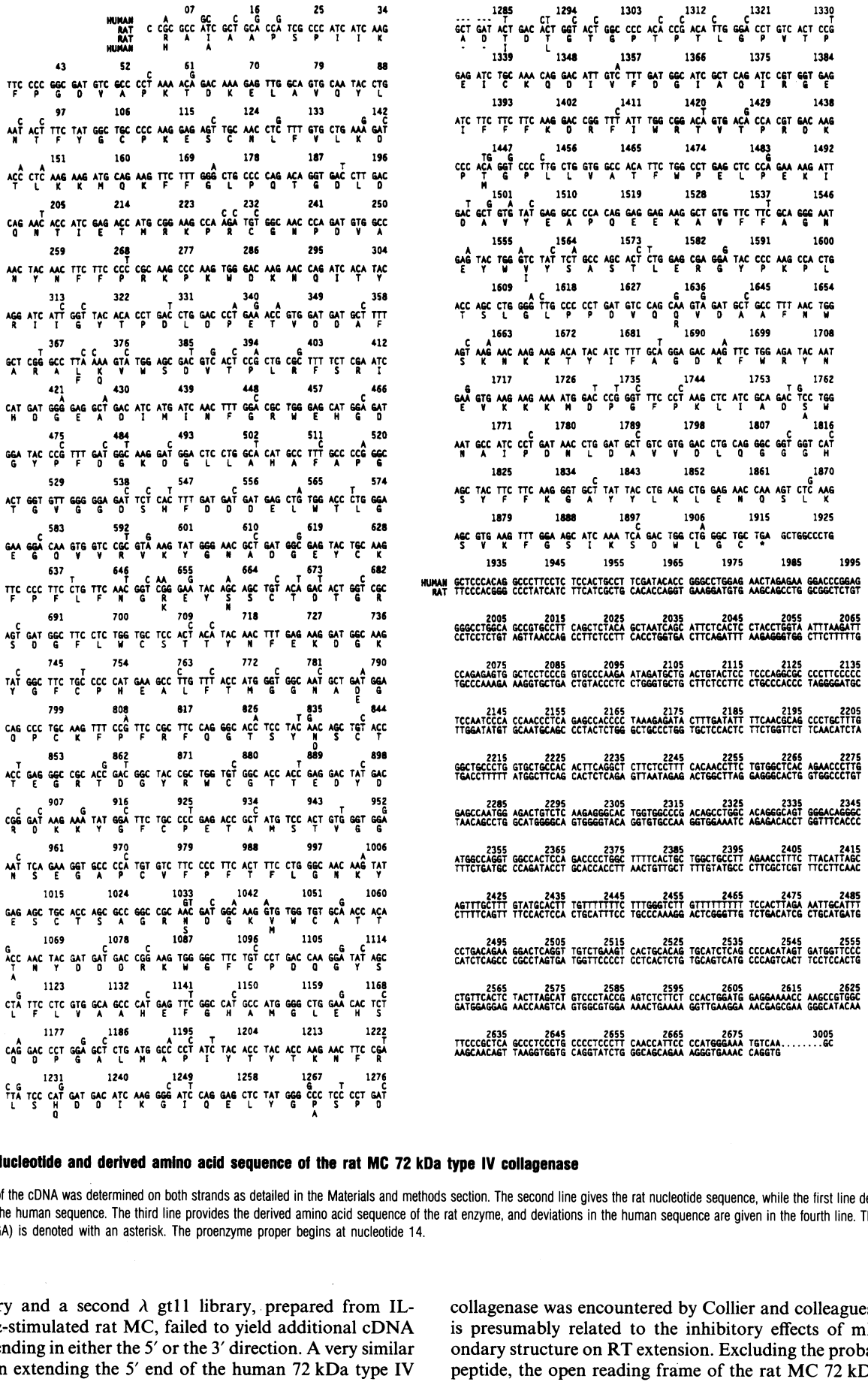

Figure 3 Nucleotide and derived amino acid sequence of the rat MC 72 kDa type IV collagenase

The sequence of the cDNA was determined on both strands as detailed in the Materials and methods section. The second line gives the rat nucleotide sequence, while the first line denotes differing nucleotides in the human sequence. The third line provides the derived amino acid sequence of the rat enzyme, and deviations in the human sequence are given in the fourth line. The translational stop codon (TGA) is denoted with an asterisk. The proenzyme proper begins at nucleotide 14.

gt10 library and a second λ gt11 library, prepared from IL-1β/TNF-α-stimulated rat MC, failed to yield additional cDNA clones extending in either the 5′ or the 3′ direction. A very similar difficulty in extending the 5′ end of the human 72 kDa type IV collagenase was encountered by Collier and colleagues [12], and is presumably related to the inhibitory effects of mRNA secondary structure on RT extension. Excluding the probable signal peptide, the open reading frame of the rat MC 72 kDa type IV

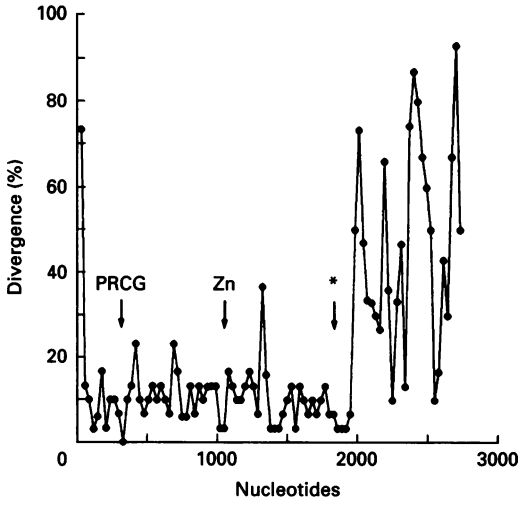

Figure 4 Nucleotide divergence of rat MC 72 kDa type IV collagenase from the human sequence

The percentage divergence of sequential 30 bp segnments of the nucleotide sequence of the rat enzyme from the human enzyme sequence is depicted. PRCG and Zn denote the locations of the respective activation locus and zinc-binding domains within the cDNA sequence. The asterisk denotes the location of the translational stop codon.

collagenase encodes a proenzyme with a molecular mass of 71101 Da. The nucleotide sequence within the open reading frame of the rat cDNA diverges from the human type IV collagenase cDNA sequence by only 9.91%. The degree of nucleotide divergence was much greater within the immediate sequence encoding the partial signal peptide and the 3′ untranslated region (Figure 4). The majority of the nucleotide substitutions within the open reading frame were silent or neutral in nature, with a total of 21 amino acid substitutions. These amino acid substitutions are diffusely distributed, with the exception of a cluster of five amino acid substitutions near residue 410. Most amino acid substitutions are conservative in nature, and analysis of predicted secondary structure and hydrophobicity profiles did not demonstrate any significant deviation from the predicted structure or profiles of the human type IV collagenase protein. The rat 72 kDa type IV collagenase cDNA reveals extensive similarity with the recently published murine sequence [23], with 100% nucleotide identity within the open reading frame and divergence only developing within the 3′ end of the sequence (rat nucleotide 2581 and beyond).

Modulation of mesangial expression of 72 kDa type IV collagenase by second-messenger analogues

The structure of the human 72 kDa type IV collagenase gene has been defined [24]. Sequence analysis of the immediate 5′ flanking region (415 bp) is notable for the absence of an AP-1 element, a cAMP-responsive element or the serum-response element, among others. The AP-1 sequence confers induction by phorbol ester and is present within the immediate 5′ flanking regions of the phorbol-inducible interstitial collagenase, stromelysin and 92 kDa type IV collagenase genes [18–21]. However, phorbol ester can also induce transcriptional activation, in co-operation with cAMP, via the AP-2 factor [25,26]. We postulated, in the absence of a consensus AP-1 sequence, that the induction of 72 kDa type IV collagenase transcription in MC could be mediated through an AP-2-like pathway. To examine this possibility, cultured MC were incubated with the cAMP analogue, 8-bromo-cAMP, or with forskolin, an activator of adenylate cyclase. The potential additive effects of phorbol ester and forskolin were also considered. The results of these studies are summarized in Table 2. Incubation for 24 h with 50 μM forskolin induced a greater than 2-fold increase in 72 kDa type IV collagenase activity present in the culture supernatants, while incubation with 0.5 mM 8-bromo-cAMP yielded a nearly 4-fold increase. Combination of phorbol ester (10 nM) with forskolin resulted in an additive increase in enzyme secretion (approx. 6-fold). A similar additive response was observed with the combination of phorbol ester and 8-bromo-cAMP (results not shown). The increases in enzyme secretion were matched by corresponding increments in steady-state 72 kDa type IV collagenase mRNA levels (Table 2). Thus the pattern of response by rat MC to phorbol ester and cAMP elevation is consistent with the activation of a tissue-specific AP-2-like pathway.

Table 2 Type IV collagenase activity from Northern blotting of RNA extracted from rat MC treated with various second-messenger analogues

Confluent rat MC were cultured for 24 h in rest medium alone (Control) or treated with forskolin (50 μM), 8-bromo-cAMP (0.5 mM) or forskolin (50 μM) plus PMA (10 nM), and the culture medium assayed for type IV collagenase activity. Results are expressed as units of enzyme activity/100 μg of cell protein (means ± S.D.; $n = 4$); $^*P < 0.05$; $^{**}P < 0.01$ compared with control. Poly(A)$^+$-selected RNA (2.5 μg/lane) from the control and analogue-treated cells was electrophoresed, transferred to nitrocellulose membranes and probed as described in the legend to Figure 2. The mRNA levels (relative to β-actin) are expressed in relation to the controls assigned an abundance level of 1 ($n = 1$).

	Type IV collagenase activity (units/ 100 μg of protein)	Type IV collagenase mRNA (relative units)
Control	12.1 ± 2.1	1.0
Forskolin	27.5 ± 3.6	4.1
8-Bromo-cAMP	44.3 ± 2.2	5.8
Forskolin plus PMA	67.1 ± 4.3	8.1

DISCUSSION

In this paper, evidence is presented for the tissue-specific regulation of the 72 kDa type IV collagenase by rat glomerular MC. The pattern of mesangial response to incubation with inflammatory cytokines, phorbol ester or second-messenger analogues is distinctive. For example, Collier and colleagues [12] were unable to induce expression of 72 kDa type IV collagenase with phorbol ester in any strain examined, including skin fibroblasts, endothelial cells, keratinocytes, melanoma cells and fibrosarcoma cells. Brown and colleagues [13] documented the constitutive expression of the 72 kDa type IV collagenase by a number of tumour-derived cell lines, and noted in some cases an inhibitory effect of IL-1β or phorbol ester. Fini and Gerard [27] have also reported an inhibitory effect of phorbol ester on secretion of the 72 kDa type IV collagenase by passaged corneal fibroblasts. The divergent pattern of 72 kDa type IV collagenase synthesis by MC initially raised the possibility of second, variably regulated isoform of the enzyme. A homology cloning strategy permitted the rapid isolation and identification of the cytokine- and phorbol ester-induced rat 72 kDa type IV collagenase as the homologue of the cDNAs from human and murine sources. Further evidence against the existence of a second 72 kDa type IV collagenase isoform has been provided by the mapping of the human genomic sequence, whereby a single gene copy was localized to chromo-

some 11 [28]. Sequence analysis of the rat cDNA demonstrated a very high degree of conservation when compared with the open reading frames of the human (91%) and murine (100%) sequences. This degree of sequence similarity is significantly higher than the approx. 40–50% conservation maintained between the rat and human forms of stromelysin-1 and interstitial collagenase [29].

In most cell types examined, exposure to inflammatory cytokines or phorbol esters induces synthesis of the 92 kDa type IV collagenase, an event attributed to the presence of AP-1-binding sites within the 5′ flanking region of the gene [18]. Notably, rat MC failed to respond to these factors with an induction of 92 kDa type IV collagenase synthesis. Thus MC appear to regulate the expression of the type IV collagenases in a pattern which is the functional inverse of that exhibited by most cell types studied. The additive response in terms of 72 kDa type IV collagenase synthesis by rat MC to the combination of phorbol ester and cAMP+elevation is compatible with the activation of an AP-2-like pathway. Sequencing of the immediate 5′ flanking region of a genomic clone of the rat 72 kDa type IV collagenase did not reveal a consensus AP-1-binding site, but did reveal an AP-2-like consensus sequence (D. H. Lovett, unpublished work). While sequence analysis of the human 72 kDa type IV collagenase gene reveals a potential AP-2-binding site within the first exon [24], transcriptional regulation studies by Frisch and Morisaki [30] have defined an AP-2-binding enhancer region located 1650 bp upstream of the transcriptional start site. Significantly, a potent cell-type-specific silencer element was located immediately adjacent to the AP-2 enhancer region, suggesting that it may be responsible for a limited range of tissue expression.

While a functional AP-2 enhancer region could explain the MC responses to phorbol ester and cAMP elevation, it is likely that other elements are involved in the response to IL-1β and TNF-α. Elevation of intracellular cAMP has not been convincingly demonstrated in response to IL-1β binding, nor is the classical polyphosphoinositide/diacylglycerol/protein kinase C pathway commonly involved [31]. Recent studies from our laboratory have defined an alternative IL-1 (and TNF) signalling pathway in MC resulting from the rapid activation of lysophosphatidate acyltransferase and phosphatidate phosphohydrolyase [32]. The consequence of this activation is a rapid stimulation of phosphatidic acid and diacylglycerol synthesis. The fatty acyl profile of the resultant diacylglycerol differs radically from that of phosphoinositide-derived diacylglycerol, suggesting that alternative isoforms of protein kinase C may be involved. Diaz-Meco and colleagues have recently proposed a similar pathway for the activation of stromelysin transcription, in which distinctive species of diacylglycerol derived from phospholipase C-mediated hydrolysis of phosphatidylcholine are generated [33]. Future studies utilizing transcriptional analysis constructs within the context of MC may be expected to provide further insights into the unusual regulation of the 72 kDa type IV collagenase by this cell type and to define the regulatory regions involved in this process.

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