Expression of Matrix Metalloproteinases and Inhibitor by Human Retinal Pigment Epithelium

J. Preston Alexander,* John M. B. Bradley,* John D. Gabourel,† and Ted S. Acott*‡

Extracellular matrix turnover is initiated, at least in part, by the regulated secretion of members of a family of matrix metalloproteinases. The authors show that interstitial collagenase, stromelysin, two gelatinases (the 72-kD and 92-kD type IV collagenases), and the tissue inhibitor of metalloproteinases (TIMP) are secreted into the culture medium of human retinal pigment epithelium (RPE). These enzymes and their inhibitor were identified by probing immunoblots of western transfers with specific polyclonal antibodies that were made against these proteins or against peptides containing unique sequences from these proteins. Stromelysin and the gelatinases are also active against substrates that are polymerized into polyacrylamide gels before electrophoresis and require metal ions (probably zinc and/or calcium) for activity. The phorbol mitogen, 12-tetradecanoylphorbol-13-acetate, differentially increases the levels of these metalloproteinases and TIMP found in retinal pigment epithelium culture medium with stromelysin and the 92-kD type IV collagenase responding most strongly and TIMP actually decreasing in certain cases. Additional changes in metalloproteinase profiles are observed after approximately 20 passage of several RPE lines in culture. Modulation of extracellular matrix turnover by changing RPE secretion of these matrix metalloproteinases and their TIMP, may play a central role in the normal function and in the pathology of the retina. Invest Ophthalmol Vis Sci 31:2520-2528, 1990

A family of secreted matrix metalloproteinases (matrix metalloproteinases and inhibitors have been called a number of different and confusing names. Those which have been sequenced and identified as discrete gene products include: (1) MMP-1, interstitial collagenase; (2) MMP-2, 72-kD gelatinases, and 72-kD type IV collagenase; (3) MMP-3, stromelysin, proteoglycanases, transin, and procollagenase activating enzyme; (4) MMP-9, 92-kD gelatinase, type V collagenase, and 92-kD type IV collagenase; (5) stromelysin-2; (6) tissue inhibitor of metalloproteinases (TIMP), TIMP-1, human collagenase inhibitor, and erythroid-potentiating activity; and (7) TIMP-2 and CSC-21K is thought to play a central role in initiating the turnover of a broad spectrum of extracellular matrix (ECM) components. This family includes interstitial collagenase,1-3 gelatinases (including two forms of type IV collagenase),3-5 and stromelysin(s).6-8 They are active at neutral pH, appear to be dependent on zinc and/or calcium for activity, and are secreted by diverse cell types. Interstitial collagenase cleaves native collagens (types I, II, and III) into two fragments, one fourth and three fourths of their original size.3,9,10 Gelatinases, including two type IV collagenase11,12 (72- and 92-kD type), are active against denatured interstitial collagens (gelatins), laminin, fibronectin, and types IV and V collagens.3-5,10 Stromelysin cleaves proteoglycans into two or more fragments and is active against laminin, fibronectin, type IV collagen, and a number of globular proteins.4,6-8 The exact number of members of this family is uncertain. Interstitial collagenase,13-15 the 72- and the 92-kD type IV collagenases,11,12 and stromelysin14,16,17 have been cloned and sequenced. They contain typical hydrophobic signal sequences, are secreted in latent proenzyme forms, and can be activated by proteinase treatment,1,2 by mercurial treatment with p-aminophenylmercuric acetate,1,2 or by incubation with sodium dodecyl sulfate (SDS);18 their physiologic activation is apparently proteolytic. This proteinase family shares considerable sequence similarity,11-17 including a putative zinc-binding site, one or more apparent disulfide bridges, and secondary structure similarity;11 they also have domains with differences in structure, which are thought to impart substrate specificity.11

Matrix metalloproteinase secretion is increased by various growth factors, mitogens, cellular transfor...
mation, and oncogene activation. Their activities are also regulated by TIMPs, which are also secreted in a regulated manner by several types of cells. The relative concentration and temporal-release patterns of these proteinases and TIMPs are thought to control the extent of ECM turnover. Two TIMPs have been cloned and sequenced.

The retinal pigment epithelium (RPE), sandwiched between the choroidal blood supply and the neural retina, supplies the rods and cones with nutrients and retinol for the visual cycle and removes wastes and the photodamaged membranes that are shed periodically by the photoreceptor cells. The RPE is partially responsible for the synthesis and maintenance of Bruch’s membrane, found at its basal surface on the choroidal side, and of the interphotoreceptor matrix, found at its apical surface on the photoreceptor side. The regulation of RPE cellular functions is not well understood; the manner of interactive signaling between RPE and adjacent cells and of the RPE’s control of its ECMs are even less clear.

Because of the pivotal role of the RPE and its ECMs in supporting the visual process and the purported complicity of RPE defects in various retinal pathologies, we conducted studies to identify members of this family of matrix metalloproteinases and their inhibitor, TIMP, in the conditioned medium of cultured human RPE. In addition, we investigated the regulation of cultured RPE secretion of these enzymes and their tissue inhibitors by 12-decanoylphorbol-13-acetate (TPA), a broad-spectrum cellular mitogen.

Materials and Methods

Cell Culture and Treatments

Human eyes from donors, which were free from ocular or complicating diseases, were obtained from the Oregon Lion’s Eye Bank (Portland, OR). The RPE was cultured as previously described, maintained at 37°C in an humidified 95% air:5% CO2 atmosphere using Dulbecco’s modified Eagle medium, supplemented with 1000 units of penicillin, 100 μg of streptomycin, and 0.25 μg of amphotericin B per ml medium, 1.1 mg sodium pyruvate per ml, 1% L-glutamine, 24 mM sodium bicarbonate, 50 mM Hepes, and 10% fetal calf serum (all from Gibco, Santa Clara, CA). Except as specifically indicated, all cultures were used by the fifth passage with a one to three split at passage.

Before experimenting, densely confluent cultures were maintained serum free in the same medium supplemented with 5 μg of insulin per ml, 5 μg of transferrin per ml, 5 μg of iron-loaded transferrin per ml, and 5 ng of selenious acid per ml (Collaborative Research, Bedford, MA) for 24 hr, before exposure to TPA (Sigma, St. Louis, MO) at 100 ng/ml in the same medium. Human fetal lung fibroblasts, IMR-90 (#CCL186; American Type Tissue Collection, Rockville, MD), maintained and treated similarly, were used as indicated. We should note that although the data presented are from cultures subjected to serum-free conditions, similar results were obtained with cultures which were not subjected to serum-free culture. Serum proteins, particularly in concentrated conditioned medium samples, distort the proteinases’ electrophoretic migration pattern and make molecular-weight (Mr) analysis difficult.

SDS-Polyacrylamide Gel Electrophoresis and Substrate Gels

We conducted SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on vertical slab gels with sample reduction (100 mM dithiothreitol, DTT). Substrate SDS-PAGE gels were used to evaluate proteinase activities; gelatin or β-casein (0.15%; Sigma) was mixed with the acrylamide (9 or 11%) before standard SDS-PAGE gels were polymerized. For substrate gels, the samples were not reduced (no DTT) but were incubated at 95°C for 5 min before electrophoresis. After electrophoresis, SDS was removed from the substrate gels by incubation at room temperature for two 10-min periods with 2.5% Triton X-100 (Bio-Rad, Richmond, CA), and proteinase reactions were done for 18 hr at 37°C with gentle shaking in buffer containing 150 mM NaCl, 50 mM Tris (pH 8), 10 mM CaCl2, and 1 μM ZnCl2. Gels were then stained with Coomassie blue, destained, dried, and photographed. Prestained and unstained molecular weight marker kits (Bio-Rad, Sigma, and BRL, Gaithersberg, MD) were used to estimate the Mr of protein bands.

In the inhibitor studies, phenylmethylsulfonyl fluoride (PMSF, Boehringer-Mannheim, Indianapolis, IN) was incubated at 1 mM with the samples before electrophoresis and during activity incubations. Metal chelators (10 mM; Sigma), including 1, 10-phenanthroline (preferential zinc chelator), ethylenediaminetetraacetic acid (EDTA, general divalent cation chelator), and ethylene glycol-bis(β-aminoethyl ether)N,N,N',N'-tetra acetic acid (EGTA) (preferential calcium chelator), were included in the SDS/Triton exchange and the proteinase activation buffers. Trypsin (Sigma) was also added as a control. Gels were then processed as described.

Peptides and Antibodies

Fifteen-mer peptides, selected from the sequences of human interstitial collagenase (aa R262-P275+C), stromelysin (aa P276-N290+C), 72-kD type IV collagenase (aa V468-T482+C), or TIMP-1 (aa P104-
K118+C), were synthesized and purified by reverse-phase high-performance liquid chromatography on a C-18 column by Dr. Eckard Weber (Portland, OR) or by Multiple Peptide Systems (San Diego, CA). As indicated, a cysteine was added to the carboxyl terminal of the peptides for ease of coupling to the carrier protein. The peptides were conjugated to keyhole limpet hemocyanin (Sigma) using sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Pierce, Rockford, IL) and following the manufacturer's instructions. Polyclonal antipeptide antibodies were produced in rabbits using MPL/TDM/CWS adjuvant (RIBI Immunochem, Hamilton, MT) according to the manufacturer’s instructions. Antibody production protocols were approved by the Oregon Health Sciences University Animal Welfare Committee and were in accordance with the guidelines of the Public Health Service and with the ARVO Resolution on the Use of Animals in Research. The antisera were tested by enzyme-linked immunosorbent assay using the respective peptides as substrates and by immunoblots of western transfers from SDS-PAGE gels. An immunoglobulin G (IgG) fraction was isolated from each antipeptide antiserum using DEAE-cellulose (Bio-Rad); this fraction was then immunoaffinity purified with the respective peptide antigen coupled to Affi-gel 10 (Bio-Rad) according to the manufacturer's instructions. Polyclonal rabbit antibodies to human skin fibroblast interstitial collagenase and TIMP were gifts from Dr. G. P. Stricklin, and a rabbit polyclonal antibody to human neutrophil gelatinase was a gift from Dr. M. S. Hibbs.

Immunoblots of Western Transfers from SDS-PAGE

After traditional SDS-PAGE under reducing conditions, proteins were transferred electrophoretically to nitrocellulose sheets and probed by immunoblot with the individual antibodies. Secondary antibody (goat anti-rabbit IgG; Sigma) conjugated to alkaline phosphatase was then incubated with the blots, and bands were visualized by incubation with the 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium substrate system (Sigma) as recommended by the manufacturer.

Results

Basal and TPA-Stimulated Proteinases Activities

The conditioned medium from serum-free RPE or IMR-90 cultures contains a major band of gelatinase activity (Fig. 1A) with Mr ~ 72 kD and bands of caseinase activity (Fig. 1B) with the major band at Mr ~ 49 kD, as determined using substrate SDS-PAGE. Incubation of these cells with TPA increases the activity observed at these Mr values for both enzymes and from both cell lines; TPA also induces secretion of gelatinase activity at Mr ~ 92 kD from RPE but not from IMR-90 cells. Cultures of IMR-90 and RPE both secrete a minor additional gelatinase activity at Mr 67 kD and caseinase activities with apparent Mr 55 kD, 52 kD, and 44 kD. Note the light band of 49-kD stromelysin activity on the gelatin gel and the light band of 92-kD gelatinase activity on the casein gel detectable with TPA-treatment.
**Metal Dependence of Metalloproteinase Activities**

The effects of proteinase inhibitors on these activities in substrate gels (Fig. 2) allows the identification of all of these activities as metalloproteinases. Trypsin, a serine proteinase which was added as a control, is inhibited by PMSF (Figs. 2B, G), but the gelatinase (Fig. 2B) and caseinase (Fig. 2G) activities are not affected. However, treatment with the metal chelators EDTA (Figs. 2C, H), EGTA (Figs. 2D, I) or 1,10-phenanthroline (Figs. 2E, J) completely eliminates the measurable secreted RPE proteinase activities without inhibiting the trypsin activity. Treatment with reducing agents before electrophoresis also eliminates all secreted proteinase activity, as detected with either of the substrates (data not shown).

**Immunologic Identification of Proteinases and TIMPs**

When western transfers of SDS-PAGE separations of conditioned culture medium from RPE with or without TPA-treatment are probed with the specific polyclonal antibodies against either the purified proteins or against peptide sequences from these proteins (Fig. 3), bands of interstitial collagenase, gelatinase, stromelysin, and TIMP are observed. Interstitial collagenase reactivity is observed (Fig. 3A) primarily at Mr \(~\approx\) 49 kD without TPA by both the antifibroblast interstitial collagenase antibody and the antinterstitial collagenase peptide antibody (lanes 1 and 3, respectively). The TPA treatment increases the amount of this 49-kD band; additional light bands of similar size are also detectable with both of these both antibodies, particularly after TPA treatment.

Probing similar blots with an antibody made to human neutrophil gelatinase allows detection of a single band at Mr \(~\approx\) 95 kD, the level of which is increased significantly by TPA treatment (Fig. 3B, lanes 1 and 2 respectively). The anti-72-kD type IV collagenase peptide antibody recognizes a band at Mr \(~\approx\) 76 kD, which is only very slightly increased by TPA treatment (Fig. 3B, lanes 3 and 4, respectively). Cross reactivity is not apparent with either antibody for the other gelatinase.

An antipeptide antibody made to a unique sequence selected from human stromelysin (Fig. 3C) is reactive against a pair of bands at Mr \(~\approx\) 57 and 59 kD after TPA treatment (lane 2); essentially no reactivity is apparent without TPA (lane 1). Occasionally, at very high concentrations of medium from RPE without TPA treatment, a band at 59 kD is detectable (data not shown).

When western blots of medium from cultured RPE treated for 48 hr without or with TPA are probed with the antifibroblast TIMP protein antibody (Fig. 3D, lanes 1 and 2, respectively) or with an antibody made against a peptide from the sequence for TIMP-1 (data not shown, but identical to lanes 1 and 2), immunoreactivity is observed at Mr \(~\approx\) 29 kD. Treatment with TPA increases the amount of this band significantly. With both antibodies, we infrequently observed a light band at Mr \(~\approx\) 18 kD (data not shown).

On several Immunoblots (four of 20) probing with either TIMP antibody, we observed less immunoreactivity at Mr \(~\approx\) 29 kD after TPA treatment (Fig. 3D, lanes 3–6 for an example probed with the anti-TIMP peptide antibody). To evaluate this contradictory observation, we collected medium after treatment with TPA for 8 (lanes 3 and 4) and 48 hr (lanes 5 and 6). After 8 hr, no significant TPA effect was observed; however, after 48 hr the TIMP immunoreactivity in control medium gradually increased (lane 5), and TIMP immunoreactivity in the medium from TPA-treated cells did not increase (lane 6) beyond that observed at 8 hr (lanes 3 and 4). This pattern, less TIMP after TPA treatment, was more pronounced...
Immunological identification of the matrix metalloproteinases and TIMP secreted by RPE. Western transfers of proteins from SDS-PAGE gels to nitrocellulose were immunoblotted with monospecific polyclonal antibodies. For each pair of lanes, identical volumes of concentrated culture medium from RPE cells, which had been plated at identical densities, was collected after 48 hr untreated (odd-numbered lanes) or TPA-treated (even-numbered lanes). Immunoblots used: (A) anti-fibroblast interstitial collagenase antibody (lanes 1 and 2, arrow is Mr $\approx 49$ kD); (B) anti-neutrophil gelatinase antibody (lanes 1 and 2) or anti-peptide interstitial collagenase antibody (lanes 3 and 4, arrow is Mr $\approx 95$ and 76 kD); (C) anti-stromelysin peptide antibody (lanes 1 and 2, arrow is Mr $\approx 57$ kD); and (D) anti-fibroblast TIMP antibody (lanes 1 and 2) or anti-TIMP-1 peptide antibody (lanes 3 and 4, medium collected after 8 hr, and lanes 5 and 6, medium collected after 48 hr, arrow indicates Mr $\approx 29$ kD). Cell passage numbers were less than 5, except lanes 3-6 of D at passage 21.

The immunoreactivity was increased with longer medium conditioning times (eg, 48–72 hr) during which TIMP continues to accumulate in control medium but not in TPA-treatment medium (data not shown). The only unifying variable resolving the difference between these two patterns of TIMP responsiveness to TPA treatment, was the passage number of the cell lines. Cells at low (0–5) or intermediate (6–20) passage number respond to TPA treatment with an increased medium TIMP level compared with controls; cells passages greater than 20 times often have less TIMP immunoreactivity after TPA treatment than they do without treatment. Additional changes in RPE responses at higher passage numbers are shown.

Gelatinase Activities From Different RPE Lines and Passage Numbers

Comparisons of the metalloproteinases secreted by several RPE cell lines at various passage numbers and media concentrations provide additional interesting information (Fig. 4). When the gelatinase activity secreted by numerous cell lines of cultured RPE, which are between passage 2 and approximately 20, was analyzed by substrate gel electrophoresis, the basic secretion pattern without and with TPA treatment was similar (eg, Fig. 1, lanes 10 and 13 compared with Fig. 4, lanes 1 and 2). This pattern was observed for all of the normal RPE lines we studied, with only small differences in the levels of the major bands of gelatinase activity. However, when RPE cultures were passaged more than approximately 20 times, changes in this pattern of basal and TPA-stimulated secretion were observed. Comparison of an RPE cell line at passage 11, without and with TPA treatment (Fig. 4, lanes 1 and 2, respectively), and the same RPE line at passage 26, without and with TPA treatment (Fig. 4, lanes 3 and 4), shows a loss of the 72-kD gelatinase without or with TPA treatment and a slight increase in the 92-kD gelatinase without TPA treatment. This passage-dependent pattern change is shown with another cell line at passage 3 with TPA treatment (lane 7) compared with the same line at passage 24 without and with TPA treatment (lanes 5 and 6, respectively).

Several other RPE cell lines at passage number 23, 16, 22, 17, 22, 27, and 24, all stimulated with TPA, are also shown (lanes 8–14, respectively). Some exhibit the same loss or reduction of the 72-kD gelatinase (lane 8), but others show a different pattern in which the 92-kD gelatinase is lost or diminished but the 72-kD gelatinase is still expressed (lanes 10, 12, 13, and 14). Morphologic and growth rate changes in the RPE cells was observed with several of these lines at high passage numbers. Diminished secretion of stromelysin, both with and without TPA treatment, is also frequently seen above passage 20 (data not shown).

Note the light bands of gelatinase activity at high Mr ($\approx 84, 120, 160,$ and 220 kD) predominately on several of these lanes, particularly lanes 15 and 16.
Fig. 4. Survey of secreted gelatinase activity from several cell lines at different passage numbers. Conditioned medium from RPE cultures, collected after 48-hr exposure to cells, was subjected to gelatin substrate SDS-PAGE. Medium from one RPE cell line at passage 11, without and with TPA treatment (lanes 1 and 2, respectively), is compared with medium from the same cell line at passage 26, without and with TPA treatment (lanes 3 and 4). Identical volumes of medium from plates passed at the same density were applied to each lane. Another RPE cell line at passage 24, without (lane 5) or with TPA treatment (lane 6) and the same cell line at passage 3 with TPA treatment (lane 7) are compared; volumes and cell densities were also the same. Medium from different RPE cell lines, all treated with TPA, at passages 23, 16, 22, 17, 22, 27, and 24 are shown in lanes 8–14, respectively; volumes applied to lanes 8–14 were adjusted to show the bands and are not necessarily equivalent. Medium from passage 19 retinitis pigmentosa RPE cell line without and with TPA treatment (lanes 15 and 16, respectively) are shown. Arrows indicate Mr = 92 and 72 kD.

Discussion

We showed that human RPE in cell culture secretes several members of the family of matrix metalloproteinases and their tissue inhibitor. Specifically identified were interstitial collagenase, both the 72- and the 92-kD type IV collagenases, stromelysin, and TIMP. The combination of methods and probes that we used and these proteins' specific characteristics makes their identification relatively certain. Their characteristics, including their TPA responsiveness, are similar to those secreted by various other cell lines.1–17 The same individual proteinases and inhibitor were identified in culture media from the trabecular meshwork,45 and interstitial collagenase has been studied in some detail in the cornea.46–49 Based on observations in other tissues, we assume that the appearance of multiple forms of these proteins on our gels is due to: (1) variable glycosylation,1,16,27 (2) proteolytic degradation to lower Mr active and eventually to small inactive forms;2,3,5,10 and (3) their secretion as inactive proenzymes, which are activated by proteolytic cleavage removing a significant N-terminus prosequence.1–3,5,10–18 Our observations of RPE TIMP require additional comment. The TIMP-1 and TIMP-2 transcript cDNAs that have been sequenced code for ~20-kD proteins,28–30 which have 41% sequence identity and an additional 29% conservative substitutions.30 The 29-kD TIMP form is due to glycosylation.28–30 Our antipeptide TIMP antibody was made to a TIMP-1 sequence, which has only slight sequence similarity to TIMP-2,28–30 and the anti-TIMP protein antibody was made to TIMP-1; therefore, it seems that RPE produce TIMP-1. We cannot exclude the additional presence of TIMP-2 in RPE medium. Our observations indicate that TIMP-1 production by RPE, which has undergone less than 20 passages, is increased in response to TPA treatment. The observed reversal of this response may be due to reduced synthesis or increased degradation of TIMP immunore-
activity in the medium during the TPA-treatment period. Clarification of this observation and of the occasional presence of an 18-kD TIMP immunoreactivity observed by us and others will require detailed studies of TIMP transcription, translation, posttranslational modification, and secretion.

The absence of proteinase activities on substrate gels from other than this family of matrix metalloproteinases is not evidence against their presence in RPE-conditioned culture medium. The substrates that we used and the conditions of the electrophoresis and assay are such that only very "robust" proteinases with the appropriate substrate specificity are detected. Tissue plasminogen activator, as an example of another class of proteinases, was not detectable when added to these substrate gels (data not shown). Our studies do not exclude the presence of other families of secreted RPE proteinases, which could play additional roles in RPE ECM turnover.

Comparison of a number of RPE lines at passage numbers between 2 and approximately 20, without and with TPA treatment, shows that similar metalloproteinase secretion patterns are a general property of human RPE in cell culture. At passage numbers above 20, several cell lines showed significant changes, particularly in the ratio and in TPA responsiveness of the 72- and 92-kD gelatinases and of TIMP levels. Different specific changes were observed with different RPE cell lines. Stromelysin production is often diminished after approximately 20 passages (data not shown). The exact passage number for this change is somewhat variable between RPE lines. We could detect no changes in RPE morphology or growth patterns before this metalloproteinase change, but we could detect changes soon afterwards. The variable presence of small amounts of larger Mr gelatinase activities (Mr = 120, 160, and 220 kD) has been reported in other tissues but not studied in detail. Their increased levels in one retinitis pigmentosa RPE line is intriguing, but we have not demonstrated any cause-and-effect relationship. Although this pattern was observed in two retinitis pigmentosa RPE lines, it was not observed in several others (data not shown). Additional studies will be necessary to determine the significance, if any, of this observation.

The most likely first step in ECM turnover is the secretion of specific proteinases, presumably including members of this family of metalloproteinases. Because of the complex and relatively strong interactions of ECM components with cells and with each other, it seems unlikely that regions of the ECM could be endocytosed en bloc without prior disruption by proteinases. It is equally unlikely that appreciable degradation of ECM components occurs extracellularly. The matrix metalloproteinases, working in concert, are ideally suited to disrupt regions of ECM before endocytosis and degradation. Since we, and others, observed a basal level of secretion of these metalloproteinases, the normal "housekeeping" of the RPE's ECMs, both Bruch's membrane and the interphotoreceptor matrix, could be completed by this family of metalloproteinases. The increased secretion of these proteinases in response to TPA or to other more physiologic signals for cell division or migration would provide enhanced ECM processing under special conditions. The activation of the proenzyme forms by stromelysin or other secretory proteinases is apparently one mode of their extracellular activation, although our understanding of this process is still incomplete. Roles for other proteinases in ECM turnover are also a possibility. Because of the central role of the RPE and its ECMs in the visual process, regulation of these ECMs is probably critical to normal retinal function. Disruption of this proteinase to inhibitor ratio is a likely candidate to explain several forms of retinal pathology.

**Key words:** collagenase, gelatinase, stromelysin, TIMP, extracellular matrix turnover, retinal pigment epithelium, matrix metalloproteinase

### Acknowledgments

The authors thank the Oregon Lion's Eye Bank for providing donor eyes; Dr. Margaret Hibbs for the antigelatinase antibody; Dr. George Stricklin for the anticollagenase and anti-TIMP antibodies; Dr. M. DelMonte, Dr. R. Weleber, and the Retinitis Pigmentosa Center of Oregon for providing some of the RPE lines; Dr. Zena Werb for suggestions regarding the stromelysin studies; and Barry Anderson, Barbara Beaucage, and Curtis Sweatt for technical assistance with the cell culture. Brief accounts of portions of this work have appeared in abstract form.

### References

6. Chin JR, Murphy G, and Werb Z: Stromelysin, a connective tissue-degrading metalloendopeptidase secreted by stimulated...