Molecular Cloning of a Mammalian Hyaluronidase Reveals Identity with Hemopexin, a Serum Heme-binding Protein*

Li Zhu‡, Thomas J. Hope§, Jackson Hall, Anthony Davies, Michael Stern¶, Ursula Muller-Eberhard**, Robert Stern, and Tristram G. Parslow†‡

From the Departments of Pathology and of Microbiology and Immunology, and the Department of Oral and Maxillofacial Surgery, University of California, San Francisco, California 94143-0506 and the **Departments of Pediatrics, Biochemistry, and Pharmacology, Cornell University Medical School, New York, New York 10021

Hyaluronan is the most abundant glycosaminoglycan of the extracellular matrix and is a critical substrate for cellular attachment and locomotion. Little is known about the class of enzymes, termed hyaluronidases, that are responsible for hyaluronan catabolism in mammals. We have determined a partial amino acid sequence from a purified preparation of porcine liver hyaluronidase and have used this information as the basis for cloning complementary DNA that encodes the corresponding protein. When expressed in a recombinant baculovirus system, the protein exhibited hyaluronidase activity in a substrate-gel assay. The deduced sequence of this mammalian hyaluronidase is that of a 459-amino-acid polypeptide bearing four potential N-glycosylation sites as well as a copy of a proposed hyaluronan binding motif. Remarkably, amino acid sequence comparisons and immunologic cross-reactivities strongly suggest that the cloned protein is identical to hemopexin, an abundant, heme-binding serum protein. Although hemopexin has not previously been reported to possess any enzymatic activity, it includes a conserved domain found in collagens, proteoglycans, and other enzymes that metabolize the extracellular matrix. We conclude that hemopexin is the predominant hyaluronidase expressed in mammalian liver.

Hyaluronan (hyaluronic acid, HA), a high molecular weight glycosaminoglycan composed of repeating N-acetylglucosamine and D-glucuronic acid subunits, is one of the most abundant constituents of the vertebrate extracellular matrix (ECM) (for review see Ref. 1). Through its interactions with CD44 and with other specific cell-surface receptors (2-5), HA serves as a substrate for cellular attachment and locomotion and appears in some instances to provide signals that modulate the function or differentiation of mammalian cells (1, 4-7). Alterations in the rate of enzymatic synthesis or breakdown of HA, and in expression of cellular HA receptors, are commonly observed at sites of wound repair, tumor invasion, immune reactions, angiogenesis, or embryogenesis, implying that HA may contribute to a wide range of physiologic phenomena that involve cell migration (e.g. Refs. 3-11). Little is known, however, about the enzymes that mediate HA production and catabolism. Endoglucosidases that specifically hydrolyze HA (i.e. hyaluronidases) have been isolated or molecularly cloned from honeybee venom (12), from a streptococcal bacteriophage (19), and from Streptomyces hyalurolyticus (14), where they serve as virulence factors. But the enzymes that metabolize HA in vertebrates are less well characterized: hyaluronidases from a variety of mammalian tissues have been studied enzymologically (8, 10, 11, 15-19), but none of these has yet been cloned, and few have been characterized at the molecular level.

One rich source of hyaluronidase activity in mammals is the liver, which serves as the predominant site for removal and degradation of HA fragments from the blood (1, 17-19). Stern et al. recently purified the major hyaluronidase activity from porcine liver, a glycoprotein with an apparent molecular mass of 55-70 kDa (19). Beginning with partial amino acid sequences from this purified material, we have now isolated and cloned complementary DNA (cDNA) that encodes the porcine liver hyaluronidase. The mRNA specifying this protein is expressed at high levels in the liver but is not detectable in various other tissues. We have confirmed that the cloned protein is recognized by an antiserum raised against the purified enzyme and that it exhibits hyaluronolitic activity when expressed from a recombinant baculovirus. Surprisingly, the predicted amino acid sequence and serologic reactivities of the cloned protein strongly suggest that it is identical to hemopexin, an abundant, liver-derived serum protein whose only previously identified functions are to bind and detoxify heme. Our findings represent the first molecular identification of a mammalian hyaluronidase and are also the first to impute enzymatic activity to hemopexin or to suggest a role for hemopexin in metabolism of the ECM.

**MATERIALS AND METHODS**

Amino Acid Sequence Determination—Automated protein sequence analysis was carried out by Edman degradation using both intact pig liver hyaluronidase, which had been purified as described, and an internal peptide derived from this material by cyanogen bromide cleavage and reverse-phase high performance liquid chromatography (HPLC).

cDNA Cloning and Sequence Analysis—Degenerate oligonucleotide primers encoding the amino-terminal sequence and an antisense form

*This work was supported in part by Grants GM43574, GM46765, DK30203, and CA44768 from the National Institutes of Health and by funds from the Robert Leet and Clara Guthrie Patterson Trust. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U14751.

‡ Supported in part by a Cheng Scholar Award.
§ Current address: Infectious Diseases Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92186-5800.
¶ Recipient of National Institutes of Health Dentist Scientist Award DE07204.

To whom correspondence should be addressed: Dept. of Pathology, University of California, San Francisco, CA 94143-0506; Tel: 415-476-1015; Fax: 415-476-9672; E-mail: parslow@ccgl.ucsf.edu.

The abbreviations used are: HA, hyaluronan (hyaluronic acid); ECM, extracellular matrix; HPLC, high performance liquid chromatography; kb, kilobase(s).

2 M. Stern, A. Chang, and R. Stern, submitted for publication.
of the internal peptide sequence from hyaluronidase (with flanking BamHI or BglII restriction sites) were prepared by chemical synthesis. The sequences were obtained using these primers in conjunction with primers derived from the EB1 sequence; these were then cloned using a TA-cloning kit (Invitrogen). The complete sequences of all three informative cDNA clones were determined on both strands using the dideoxy chain termination method.

**Sequence Data Base Searches**—Homology searches of the Brookhaven Protein Data Bank, GenBank, and EMBL Data Library protein sequence databases (April 1994 releases) were conducted using the BLAST network service of the NIH (21).

**RNA Blot Analysis**—Total cellular RNA was extracted from various adult pig tissues using guanidinium thiocyanate, and 10-μg aliquots were then heat denatured and fractionated electrophoretically on a 1% agarose, 2.2 M formaldehyde gel. The RNA was transferred to a nylon/ nitrocellulose composite membrane (Hybond TM-C Extra, Amersham Corp.), which was then prehybridized, for 2 h at 42 °C with 100 μg/ml sonicated salmon sperm DNA in 50% formamide, 5 × SSC (1 × SSC is 150 mM NaCl, 15 mM sodium citrate), 0.02% (w/v) bovine serum albumin, 0.02% (w/v) polyvinylpyrrolidone, 0.02% (w/v) Ficoll, 50 mM sodium phosphate, pH 6.5. Hybridization was performed in the same solution at 42 °C overnight in the presence of 5 × 106 cpm/ml [32P]deoxyctydylate-labeled EB1 DNA prepared by nick translation. The membrane was then washed three times for 15 min each at room temperature in 2 × SSC, 0.1% (w/v) sodium dodecyl sulfate (SDS), and twice for 30 min each at 65 °C in 0.1 × SSC, 0.1% (w/v) SDS, then dried and examined by autoradiography.

**cDNA Expression in Recombinant Baculoviruses**—To achieve high level expression of the cloned cDNA sequence, we subcloned it into the baculovirus transfer vector pBacPAK-9 (Clontech), which was then used to drive recombinant baculovirus expression. The AcMNPV strain of baculovirus (22). As these studies were begun before sequences 5′ to EB1 had been cloned, synthetic oligonucleotides encoding the leader (amino acids 1-24) of rat hemopexin were ligated onto the 5′ end of the then-available porcine cDNA (codons 29-459) to create a hybrid that combined the full-length mature porcine protein with a functional leader peptide. Baculovirus infections, plaque assays, and virion purification were carried out using standard methods (23), except for reverse transcription and polymerase chain reaction amplification of unenriched pig liver RNA, which yielded a unique 1.1-kb amplified DNA product. Combined BamHI and BglII digestion of this product generated a 0.6-kb fragment which we cloned and designated EB1. Nucleotide sequencing revealed a single uninterrupted translational reading frame that extended across the full length of EB1; neither the start nor stop codons of this reading frame were present in the clone. We therefore utilized sequences from within EB1 as primers for reverse transcription and cloning of two additional cDNA clones that were partially overlapping EB1 but extended farther 5′ and 3′, respectively, along the RNA template (Fig. 1). The composite nucleotide sequence of the hyaluronidase cDNA shown in Fig. 1c. It encompasses the major open reading frame along with 30 base pairs of 5′-untranslated sequence, 117 base pairs of 3′-untranslated sequence that includes a polyadenylation signal, and a portion of the polyadenylate tail. The sequence is predicted to encode a 459-amino acid polypeptide that contains both of the peptide sequences we obtained from pig liver hyaluronidase, including residues that were not encoded by the original polymerase chain reaction primers. Presuming that the experimentally determined amino-terminal sequence corresponds to the amino terminus of the mature protein, the cDNA sequence indicates the presence of a 28-amino acid leader peptide in the initial translation product, as expected for a secretory protein. The predicted protein includes four potential sites for N-glycosylation (asterisks), all of which are in the amino-terminal half. In addition, it includes a single copy of a consensual motif, first identified in CD44 and other HA-binding proteins, that has been proposed to be necessary and sufficient for HA binding (29). This motif is defined as a pair of arginine or lysine residues separated from each other by 7 non-acidic amino acids, at least one of which is basic (29).

**Immunoblot Analysis**—Immunoblots were performed using peroxidase-labeled goat anti-rabbit antisemur as the secondary antibody.

**RESULTS**

The protocol employed by Stern et al.2 to purify pig liver hyaluronidase involved a sequence of gradient chromatography on DEAE-cellulose, lectin affinity chromatography on concanavalin A-Sepharose, and HPLC fractionation on sequential Mono Q and Superose-12 columns. Purification at each step was monitored using a substrate gel assay which specifically detects hyaluronolytic activity (25). This approach yielded a biologically active fraction that contained only a single protein species (about 60 kDa) detectable by silver staining in denaturing or nondenaturing polyacrylamide gels. This homogeneous fraction was used as an immunogen to prepare a polyclonal antiserum in rabbits, here designated anti-hyaluronidase serum. The intact protein fraction, as well as an internal peptide derived from it by cyanogen bromide cleavage, were also subjected to protein sequence determination. Each yielded an initial uninterrupted sequence of 8-10 amino acids followed by several individual unambiguous residues (Fig. 1a). Comparison with the GenBank data base revealed no convincing matches for the amino-terminal sequence. By contrast, the internal sequence strongly resembled a sequence termed the hemopexin domain, which is found in hemopexins, vitronectin, and certain metalloproteases that act on the ECM (26-28).

We synthesized a pair of degenerate DNA oligonucleotides that comprised all possible coding sequences for the two hyaluronidase peptides, with flanking BglII and BamHI restriction sites to facilitate cloning. These oligonucleotides were used as primers for reverse transcription and polymerase chain reaction amplification of unenriched pig liver RNA, which yielded a unique 1.1-kb amplified DNA product. Combined BamHI and BglII digestion of this product generated a 0.6-kb fragment which we cloned and designated EB1. Nucleotide sequencing revealed a single uninterrupted translational reading frame that extended across the full length of EB1; neither the start nor stop codons of this reading frame were present in the clone. We therefore utilized sequences from within EB1 as primers for reverse transcription and cloning of two additional cDNA clones that partially overlapped EB1 but extended farther 5′ and 3′, respectively, along the RNA template (Fig. 1a).

The composite nucleotide sequence of the hyaluronidase cDNA shown in Fig. 1c. It encompasses the major open reading frame along with 30 base pairs of 5′-untranslated sequence, 117 base pairs of 3′-untranslated sequence that includes a polyadenylation signal, and a portion of the polyadenylate tail. The sequence is predicted to encode a 459-amino acid polypeptide that contains both of the peptide sequences we obtained from pig liver hyaluronidase, including residues that were not encoded by the original polymerase chain reaction primers. Presuming that the experimentally determined amino-terminal sequence corresponds to the amino terminus of the mature protein, the cDNA sequence indicates the presence of a 28-amino acid leader peptide in the initial translation product, as expected for a secretory protein. The predicted protein includes four potential sites for N-glycosylation (asterisks), all of which are in the amino-terminal half. In addition, it includes a single copy of a consensual motif, first identified in CD44 and other HA-binding proteins, that has been proposed to be necessary and sufficient for HA binding (29). This motif is defined as a pair of arginine or lysine residues separated from each other by 7 non-acidic amino acids, at least one of which is basic (29).
Hyaluronidase Activity of Hemopexin

**a**

| Thr | Gyl | Pro | LyS | Gyl | Ala | Glu | --- | Arg | --- | Glu | --- | Lys | Pro | Asp | Pro | Val |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

**b**

**c**

---

**Fig. 1.** Isolation and sequence of a cDNA encoding the porcine liver hyaluronidase. Panel a, amino acid sequences determined from the amino terminus of the hyaluronidase preparation and from an internal cyanogen bromide fragment. These were later found to correspond to amino acids 2948 and 369-388, respectively, of the cloned hyaluronidase sequence (see panel c). Sequences used to design the degenerate polymerase...
To examine the tissue-distribution of mRNA sequences encoding this protein, RNA samples from various adult pig tissues were analyzed electrophoretically and probed for sequences complementary to EB1. As shown in Fig. 2, this probe detected relatively large amounts of a single RNA species, approximately 1.6 kb in length, which were present in the liver but not in any of the other tissues we examined. This suggested that the cDNA encodes a relatively abundant, liver-specific protein.

A search of the GenBank data base revealed that our predicted sequence for porcine hyaluronidase bore a compelling resemblance to those reported previously for the human, rabbit, and rat hemopexin proteins (data not shown). The hyaluronidase sequence exhibited more than 60% amino acid identity and over 70% similarity (i.e., including conservative substitutions) to each of these hemopexins, with an essentially uniform degree of similarity extending throughout the length of the proteins. This strongly suggested that the cloned protein might be porcine hemopexin. As no independent sequence data were available for porcine hemopexin, we tested for serologic cross-reactivity of our hyaluronidase preparation with hemopexins from other species (Fig. 3). We found that our anti-hyaluronidase serum did not recognize purified human, chicken, or bovine hemopexins but reacted with a sample of purified pig hemopexin (a gift from D. R. Babin, Creighton University). Conversely, our hyaluronidase preparation, as well as the authentic porcine hemopexin, cross-reacted weakly but detectably with an antisera raised against human hemopexin. These data strongly indicate that the hyaluronidase protein we cloned is identical to porcine hemopexin.

To assess the properties of this cloned protein, we prepared a recombinant baculovirus that would direct synthesis and secretion of the cloned hyaluronidase/hemopexin protein in an infected insect cell host. We chose this expression system in part because baculoviral vectors can direct copious production and secretion of exogenous proteins and also in the hope that the insect cells might glycosylate the cloned protein with sufficient authenticity to retain its biological properties (22, 23, 30). For these studies, Sf9 insect cells were infected either with the recombinant baculovirus (AcHA) or with the parental wild-type virus, which expresses similarly large amounts of viral polyhedrin protein. Because the fetal calf serum in which Sf9 cells typically are grown would be expected to contain endogenous hemopexin and hyaluronidase activity (15, 31), a separate plate of cells infected with wild-type virus was washed thoroughly 24 h after infection and thereafter was maintained for 48 h in serum-free medium.

Supernatants from the infected cells were first examined by immunoblot using the anti-hyaluronidase serum. As shown in Fig. 4A, abundant immunoreactive protein of approximately 65–70 kDa was present in the supernatants of cells infected with the recombinant AcHA virus but not in supernatants from uninfected cells or from cells infected with the wild-type baculovirus. The molecular mass of this protein on denaturing gels was in agreement with the value of 67 kDa reported for authentic pig hemopexin (32). When examined using the substrate gel assay (Fig. 4B), supernatants from cells infected with either of two independent clones of AcHA virus were found to contain hyaluronidase activity (arrows) that was not present in the controls. The active species migrated relatively slowly in the HA-impregnated substrate gel, as is typical of proteins that have an affinity for HA (25). At least two discrete but relatively diffuse bands of activity were discernible on substrate gels, which are run under non-denaturing conditions; this contrasted with the single immunoreactive band seen on conventional denaturing gels (Fig. 4A). However, when substrate gels were blotted onto nitrocellulose membranes and then probed using the anti-hyaluronidase serum, both bands of hyaluronidase activity were found to be immunoreactive (Fig. 5). This may indicate that the single polypeptide expressed by AcHA gives rise to two or more alternatively processed hyaluronidase isoforms.

Fig. 2. Liver-specific expression of the hyaluronidase mRNA.
Equal amounts of total cellular RNA from the indicated adult pig tissues were probed for EB1 sequences. Northern blot (top panel) detected a 1.6-kb RNA species in the liver sample only. These same RNAs are also shown in the ethidium bromide-stained gel (bottom panel) prior to Northern blotting.

Fig. 3. Immunologic cross-reactivity of the porcine hyaluronidase with hemopexin (Hpx) proteins from various species. Purified hemopexins from the indicated species were fractionated on three identical denaturing polyacrylamide gels, along with a sample of the purified liver hyaluronidase preparation. One gel was silver stained; the two others were immunoblotted using antisera raised either against hyaluronidase or human hemopexin.
Hyaluronidase Activity of Hemopexin

**Fig. 4.** The cloned protein has hyaluronolytic activity when expressed from a recombinant baculovirus. Panel A, immunoblot detection of hyaluronidase protein secreted from insect cells infected with various independent isolates of the AcHA viral strain grown in medium containing 5% serum but not from cells infected with the parental (wild-type) virus and grown in serum-free (wild-type) or 5% serum-enriched (wild-type + serum) media. Samples were fractionated on a denaturing 12.5% polyacrylamide gel in the presence of SDS and then were probed using the anti-hyaluronidase antiserum. Panel B, hyaluronolytic activity of the virally expressed protein. The non-denaturing substrate gel (left) reveals bands of hyaluronidase activity (arrows) in supernatants from cells infected with two independent clones of AcHA virus and grown in medium containing 5% serum. A sample of Streptomyces hyaluronidase (Strep HAase) and supernatants from cells infected with wild-type virus grown in serum-free (wild-type) or 5% serum-enriched (wild-type + serum) media, are also shown. At right is an identical gel stained with Coomassie Blue.

with different affinities for HA, perhaps corresponding to the known isoforms of porcine hemopexin (32). Together, these findings demonstrate that the cloned protein binds HA, is recognized by our nominal anti-hyaluronidase antiserum, and has intrinsic hyaluronidase activity.

**DISCUSSION**

Hemopexin is a serum β-glycoprotein that is constitutively synthesized and secreted predominantly by the liver and whose production is markedly enhanced during the hepatic acute-phase response (for review see 33). Long recognized as the preeminent heme-binding protein in mammalian plasma, hemopexin has the highest affinity for heme of any known protein. Hemopexin serves a critical function in scavenging free heme from the blood and also in limiting heme-catalyzed free radical toxicity at sites of hemorrhage into tissues (34). In addition, by facilitating cellular uptake of exogenous heme via receptor-mediated endocytosis of the heme-hemopexin complex, hemopexin is thought to contribute to heme- and iron-regulated expression of several mammalian genes (35-37). Each hemopexin protein is organized in two structurally related but functionally nonequivalent halves: the amino-terminal half is sufficient for heme binding and for interaction with cellular hemopexin receptors, whereas the function of the carboxyl-terminal half is unknown (38-43).

Our findings now provide evidence of an additional, unanticipated, role for hemopexin as an enzyme that catalyzes HA. This conclusion is supported in part by direct amino acid sequence data we obtained from purified pig liver hyaluronidase, which suggest that the active protein fraction showed homology to hemopexin. The resemblance was confirmed when we exploited those peptide sequence data to isolate a cDNA, which was found to encode a protein with extensive, colinear sequence identity to the hemopexin proteins of three other mammalian species. Moreover, antibodies raised against the hyaluronidase cross-react with porcine hemopexin (Fig. 3). The hyaluronidase preparation appears to have been relatively pure, as judged by the criteria of silver-stained gels and peptide sequencing. The cDNA we cloned codes for a protein that matches that sequence and which is recognized by an antibody raised against the original enzyme preparation (Figs. 4A and 5). This implies that we have cloned cDNA corresponding to the most abundant protein in the hyaluronidase fraction but does not exclude the possibility that enzymatic activity in that fraction was due to a minor contaminant. More compelling evidence, however, comes from our finding that expression of the cloned cDNA in recombinant baculoviruses yields protein that specifically cleaves HA in a substrate gel assay (Figs. 4B and 5).

Taken together, these findings indicate that the protein we cloned is indeed porcine hemopexin and that this hemopexin possesses hyaluronidase activity. This interpretation would further imply that hemopexin is one of the many hyaluronidases normally found in mammalian serum (15, 25), although it is not the most abundant of these (31). Although it is possible that liver hyaluronidase is related to, but distinct from, hemopexin, we view this as unlikely in light of the degree of sequence homology. The levels of hyaluronidase activity in normal serum seem disproportionately low in comparison with the abundance of hemopexin protein (31), and this observation may suggest that only a fraction of hemopexin molecules are enzymatically active. However, multiple electrophoretic isoforms of hemopexin are known to occur in the liver and serum (31),
The human, rabbit, and rat hemopexins. The single copy of this motif in the porcine hemopexin/hyaluronidase sequence (top) is shown in alignment with corresponding portions of the human, rabbit, and rat hemopexins. Basic amino acids are indicated by white lettering.

perhaps resulting from alternative glycosylation, and it is possible that only one minor isoformal possesses hyaluronidase activity. Although we have thus far been unable to demonstrate hyaluronidase activity in hemopexin preparations isolated from blood (data not shown), we note that these were not purified with a view to preserving enzymatic activity and may have become inactivated during isolation.

The protein we cloned exhibits retarded electrophoretic mobility in HA-impregnated gels, a property characteristic of HA-binding proteins (25). Moreover, the cloned protein contains a consensus amino acid motif (Fig. 6) which has been shown to mediate specific HA binding and which is also found in other HA-binding proteins such as CD44, link protein, and RHAMM (29). This putative HA binding motif is located in the carboxy-terminal half of the hemopexin protein, a region that is not required for heme scavenging (38–41), although its sequence is evolutionarily conserved. This raises the possibility that the carboxy-terminal half of porcine hemopexin is the region primarily responsible for hyaluronolytic activity. We note that the putative HA binding motif is also present at the same location in all other hemopexins sequenced to date (Fig. 6). Moreover, human hemopexin isolated using a modified procedure has recently been found to bind HA in vitro.3

The possible physiological role of hemopexin as a hyaluronidase remains a matter of conjecture. In addition to clearing HA and its hyaluronolytic activity, in addition to its ability to sequester heme iron. All of these hypothetically beneficial effects would be enhanced by the rise in hemopexin production which occurs once in each half of the hemopexin protein (Fig. 1c, dashed boxes). This sequence first came to light as a region of homology between hemopexin and the serum-derived ECM component vitronectin, and it has since been found in collagens, gelatinases, stromelysins, and nearly all other members of the matrix metalloprotease family of ECM-associated enzymes (26–28). The hemopexin domains found in the matrix metalloproteases appear to contribute to recognition of specific protease substrates such as collagen and to serve as binding sites for other host proteins that inhibit protease activity (28, 45). Such findings have shed no light, however, on the role that domains of this type might play in the biology of hemopexin. Our findings now raise the possibility that the prototypical hemopexin domains found in hemopexin itself might serve either to promote recognition of an ECM substrate or to modulate intrinsic enzymatic activity. The hemopexin domains of hemopexin/hyaluronidase may thus be viewed as emblematic of its relationship to other enzymes that metabolize the ECM.

REFERENCES

1. Laurent, T. C., and Fraser, J. R. E. (1992) FASEB J. 6, 2397–2404
2. Stamenkovic, I., Amiot, M., Pesant, J., and Seed, B. (1989) Cell 56, 1057–1062
3. Lesley, J., Hyman, R., and Kincade, P. W. (1983) Adv. Immunol. 34, 271–335
4. Hittick, C., Hoare, K., Owens, R., Hohn, H. P., Hook, M., Moore, D., Crippa, V., Austen, L., Nance, D. M., and Turley, E. A. (1992) J. Cell Biol. 117, 1343–1350
5. Bader, S. D., and Toole, B. P. (1990) J. Cell Biol. 119, 643–652
6. Lamberg, J., Yusa, S. H., and Hascall, V. C. (1986) J. Invest. Dermatol. 86, 659–667
7. Kujoth, G., Pechak, D. G., Finkman, M. Y., and Caplan, A. I. (1986) Dev. Biol. 113, 10–16
8. Bartoloni, C. N., and Donof, R. B. (1982) J. Invest. Dermatol. 79, 417–421
9. West, D. C., Hampson, I. N., Arnold, F., and Kumar, S. (1985) Science 228, 1324–1326
10. Kulyk, W. M., and Kosher, R. A. (1987) Dev. Biol. 120, 353–541
11. Orkin, R. W., and Toole, B. P. (1988) J. Biol. Chem. 263, 1036–1042
12. Gmachl, M., and Kreil, U. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3560–3573
13. Hynes, W. L., and Ferretti, J. J. (1989) Infect. Immun. 57, 533–539
14. Ohyu, T., and Kinne, Y. (1978) Biochim. Biophys. Acta 190, 607–609
15. De Salguy, M., and Pignam, W. (1987) Arch. Biochem. Biophys. 250, 60–67
16. Polansky, J. R., and Toole, B. P., and Gros, J. (1973) Science 183, 862–863
17. Gold, E. W. (1982) Biochem. J. 205, 69–74
18. Aronson, N. N., Jr., and Davidson, E. A. (1967) J. Biol. Chem. 242, 440–447
19. Joy, M. B., Dodgson, K. S., Olaveen, A. H., and Gaeae, P. (1985) Biochim. Biophys. Acta 838, 257–263
20. Prohmnan, M. A. (1993) Methods Enzymol. 218, 340–356
21. Atchison, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 415–410
22. Dubuse, P. (1993) Bio/Technology 12, 47–50
23. King, L. A., and Possen, R. D. (1992) The Baculovirus Expression System: A Laboratory Guide, Chapman and Hall, London
24. Kitts, P. A., and Possee, R. D. (1993) BioTechniques 14, 810–817
25. Guntenhoener, M. W., Pogrel, M. A., and Stern, R. (1992) Matrix 12, 388–396
26. Stanley, K. R. (1986) FBSLS Lett. 199, 249–253
27. Hanks, S., Barker, W. C., and Chen, H. R. (1985) Prot. Seq. Data Anal. 1, 21–26
28. Matrisian, I. M. (1992) BioEssays 14, 455–463
29. Yang, B., Yang, B. L., Savani, R. C., and Turley, E. A. (1994) EMBO J. 13, 296–296
30. Sato, T., Hiyoryuki, S, Shin-shiro, I., Hirakl, Z., Feyton, D. H., and Muller-Eberhard, U. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8425–8427
31. Alyes, A. H., Stern, M., Guntenhoener, M., and Stern, R. (1993) Arch. Biochem. Biophys. 305, 434–441
32. Spencer, H., Prote, M. J., and Babin, D. R. (1990) Int. J. Biochem. 22, 367–377
33. Hidler-Eberhard, U. (1988) Methods Enzymol. 163, 56–65
34. Muller-Eberhard, U., and Prigl, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 629–632
35. Alam, J., and Smith, A. (1989) J. Biol. Chem. 264, 17639–17640
36. Takakushi, S., Kohno, H., Sawamura, T., and Turkawa, T. (1990) J. Biol. Chem. 265, 13981–13985
37. Alam, J., and Smith, A. (1992) J. Biol. Chem. 267, 16379–16384
38. Morgan, W., and Smith, A. (1984) J. Biol. Chem. 259, 12001–12006
39. Takakushi, S., Takakushi, Y., and Putnam, F. W. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 73–77
40. Arlendra, P., Path, V., Bostogra, G., and Silono, L. (1988) J. Mol. Evol. 27, 102–108
41. Muster, P., Tatum, F., Smith, A., and Morgan, W. F. (1991) J. Protein Chem. 10, 123–128
42. Nikkala, H., Gitlin, J. D., and Muller-Eberhard, U. (1991) Biochemistry 30, 829–833
43. Morgan, W. E. M., Muster, P., Tatum, S., Kao, S., Alam, J., and Smith, A. (1993) J. Biol. Chem. 268, 6256–6262
44. Wessels, M. R., Mone, A. E., Goldberg, J. B., and DiCesare, T. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8317–8321
45. Sanchez-Lopez, R., Alexander, C. M., Behrendska, O., Breitbach, R., and Werb, Z. (1993) J. Biol. Chem. 268, 7238–7247