Matrix metalloproteinases: old dogs with new tricks
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Abstract

The matrix metalloproteinase family in humans comprises 23 enzymes, which are involved in many biological processes and diseases. It was previously thought that these enzymes acted only to degrade components of the extracellular matrix, but this view has changed with the discovery that non-extracellular-matrix molecules are also substrates.

Four decades ago a collagenase was discovered that was responsible for involution of the tadpole tail in amphibian morphogenesis [1]. Today, the matrix metalloproteinase (MMP) family, to which this collagenase belongs, has expanded to include 23 gene products in humans (Table 1), which encode zinc-dependent and calcium-dependent pro-teases that cleave within a polypeptide (endopeptidases). There are also two other large families that have major roles in extracellular proteolysis, the ADAM family (A disintegrin and metalloprotease domain, with about 33 members in humans) and the ADAMTS family (A disintegrin-like and metalloprotease domain (reprolysin type) with thrombospondin type I repeats, with 19 members). Traditionally, the MMPs have always been thought to cleave components of the extracellular matrix (ECM). As the ECM was regarded for a long time as nothing more than a passive structure used for cell attachment, mechanical support and force transmission, extracellular proteases such as MMPs were thought to simply remodel the ECM for its homeostasis or to facilitate cell migration. This view has changed, however [2]: the ECM is now known to contain growth factors, their binding proteins, and other bioactive molecules, as well as binding sites for cell-surface molecules, some of which are revealed only after proteolysis. The proteases responsible for turnover of the ECM therefore contribute significantly to its dynamic interactions with cells. More recently, considerable evidence has emerged showing that the role of MMPs goes far beyond that of digesting ECM molecules alone; they are now known to process a number of cell-surface and soluble regulators of cell behavior (summarized in Table 1). Mouse genetic models and human diseases have revealed unexpected biological functions of MMPs (summa-rized in Table 2); many of the phenotypes of these animals are currently unexplained and suggest that there may be as yet unknown substrates for these enzymes that are not ECM components.

One consequence of the previously held view of MMPs as solely ECM-excavating enzymes was the belief that they were uniformly harmful in cancer and should therefore be tar-geted in cancer therapy. This was not entirely without basis, given that many MMPs are highly expressed in various cancers, both by tumor cells and in surrounding stromal cells such as macrophages. As recent reviews [3-6] have pointed out, however, the unexpectedly complex role of these enzymes in cancer is leading to their re-evaluation as drug targets, or at the very least, a pause while some of the evidence is analyzed in more detail. Although this is disappoint-ing from the clinical perspective, the study of these enzymes has never been more exciting than it is today, because the human genome project has led to identification of all MMPs, and genetic alterations in individual MMP genes have provided new insights into their functions. In this article, we summarize some of the essential concepts in the MMP field at present and the new insights that are pouring in from biological studies.
Table 1

Human matrix metalloproteases and their substrates

| Protein name* | Alternative names | Collagenous substrates | Non-collagenous ECM substrates | Non-structural ECM component substrates |
|---------------|-------------------|------------------------|-------------------------------|----------------------------------------|
| MMP-1         | Collagenase-1     | Collagen types I, II, III, VII, VIII, X, and gelatin | Aggrecan, casein, nidogen, serpins, versican, perlecan, proteoglycan link protein, and tenasin-C | α-antichymotrypsin, α-antitrypsin/α1-proteinase inhibitor, IGFBP-3, IGFBP-5, IL-1β, L-selectin, ovostatin, recombinant TNF-α peptide, and SDF-1 |
| MMP-2         | Gelatinase-A      | Collagen types I, IV, V, VII, X, XI, XIV, and gelatin | Aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan link protein, and versican | Active MMP-9, active MMP-13, FGF R1, IGF-BP3, IGFBP5-1, IL-1β, recombinant TNF-α peptide, and TGF-β |
| MMP-3         | Stromelysin-1     | Collagen types II, IV, IX, X, and gelatin | Aggrecan, casein, decorin, elastin, fibronectin, laminin, nidogen, perlecan, proteoglycan, proteoglycan link protein, and versican | α-antichymotrypsin, α1-proteinase inhibitor, antithrombin III, E-cadherin, fibrinogen, IGFBP3, L-selectin, ovostatin, pro-HB-EGF, pro-IL-1β, pro-MMP-1, pro-MMP-8, pro-MMP-9, pro-TNF-α, and SDF-1 |
| MMP-7         | Matrilysin-1, neutrophil collagenase | Collagen types I, II, III, V, IV, and X | Aggrecan, casein, elastin, enactin, laminin, and proteoglycan link protein | β3 integrin, decorin, defensin, E-cadherin, Fas-L, plasminogen, pro-MMP-2, pro-MMP-7, pro-TNF-α, transferrin, and syndecan |
| MMP-8         | Collagenase-2     | Collagen types I, II, III, VII, VIII, X, and gelatin | Aggrecan, laminin, and nidogen | α2-antiplasmin and pro-MMP-8 |
| MMP-9         | Gelatinase-B      | Collagen types IV, V, VII, X, and XIV | Fibronectin, laminin, nidogen, proteoglycan link protein, and versican | CXCL5, IL-1β, IL-2R, plasminogen, pro-TNF-α, SDF-1, and TGF-β |
| MMP-10        | Stromelysin-2     | Collagen types III, IV, V, and gelatin | Fibronectin, laminin, and nidogen | Pro-MMP-1, pro-MMP-8, and pro-MMP-10 |
| MMP-11        | Stromelysin-3     |                        | Laminin | α-antitrypsin, α1-proteinase inhibitor, and IGFBP-1 |
| MMP-12        | Macrophage metalloelastase |                        | Elastin | Plasminogen |
| MMP-13        | Collagenase-3     | Collagen types I, II, III, IV, V, IX, X, XI, and gelatin | Aggrecan, fibronectin, laminin, perlecan, and tenasin | Plasminogen activator 2, pro-MMP-9, pro-MMP-13, and SDF-1 |
| MMP-14        | MT1-MMP           | Collagen types I, II, III, and gelatin | Aggrecan, dermatan sulphate proteoglycan, fibrin, fibronectin, laminin, nidogen, perlecan, tenasin, and vitronectin | αβ3 integrin, CD44, gC1qR, pro-MMP2, pro-MMP-13, pro-TNF-α, SDF-1, and tissue transglutaminase |
| MMP-15        | MT2-MMP           | Collagen types I, II, III, and gelatin | Aggrecan, fibronectin, laminin, nidogen, perlecan, tenasin, and vitronectin | Pro-MMP-2, pro-MMP-13, and tissue transglutaminase |
| MMP-16        | MT3-MMP           | Collagen types I, III, and gelatin | Aggrecan, casein, fibronectin, laminin, perlecan, and vitronectin | Pro-MMP-2 and pro-MMP-13 |
| MMP-17        | MT4-MMP           | Gelatin | Fibrin and fibronectin | |
| MMP-19        | RASI-1            | Collagens types I, IV, and gelatin | Aggrecan, casein, fibronectin, laminin, nidogen, and tenasin | |
| MMP-20        | Enamelysin        |                        | Aggrecan, amelogenin, and cartilage oligomeric protein | |
| MMP-21        |                   |                        |                                 | α-antitrypsin |
| MMP-23        | CA-MMP            | Gelatin | Chondroitin sulfate, dermatan sulfate, and fibronectin | Pro-MMP2 and pro-MMP-13 |
| MMP-24        | MT5-MMP           | Gelatin | Chondroitin sulfate, dermatan sulfate, and fibronectin | Pro-MMP2 and pro-MMP-13 |
| MMP-25        | Leukolysin, MT6-MMP | Collagen type IV and gelatin | Fibrin and fibronectin | Pro-MMP-2 |
| MMP-26        | Matrilysin-2, endometase | Collagen type IV and gelatin | Casein, fibrinogen, and fibronectin | β1-proteinase inhibitor |
| MMP-28        | Epilysin          |                        | Casein |

*Although there are 23 human MMPs, 29 numbers have been used in the literature. The symbols MMP-4, MMP-5, MMP-6 and MMP-29 are redundant in humans and are no longer in use; MMP-18 corresponds to a Xenopus laevis collagenase, for which no human ortholog is known, and a human protein published as MMP-18 is now called MMP-19. Two nearly identical human genes found in a segment of chromosome 1 that is duplicated were called MMP21 and MMP22 but are now referred to as MMP23A and MMP23B.
Classification and evolution

There are 23 human MMP genes (Table 1). Before this genetic diversity was known, it was customary to divide the MMPs into collagens, gelatinases, stromelysins, elastase and others, on the basis of the known substrates, but as some MMPs have overlapping substrate specificities, the boundary between the previously used enzyme classes is now blurred. Nevertheless, the trivial names are often useful, particularly if they reflect a function or a distinct structural feature or location, and they have therefore been retained (and are indicated in Table 1). MMP genes are structurally similar to each other, indicating that they evolved by duplication of a common ancestral gene followed by divergent evolution. A number of MMP genes (MMP1, MMP3, MMP7, MMP8, MMP10, MMP12, MMP13, MMP20, and MMP26) are found in a cluster on human chromosome 11 (11q21-23) [7,8]. The human and mouse genomes appear not to contain an identical complement of MMPs, which may complicate the use of transgenic mice for insights into function of the human genes. For example, human MMP1 does not have a clear-cut ortholog in the mouse but instead is most similar to two genes, mColA and mColB, whose functions are not present in the human genome.

MMP genes have been identified in a number of non-vertebrate species, and the number will undoubtedly increase as more genome projects are completed. The nematode *Caenorhabditis elegans* has a number of MMPs, including C31, H19 and Y19 [10]; there is a *Drosophila* MMP [11]; sea urchins contain an MMP essential for hatching, known as envelysin [12]; and the hydra has an MMP that is required for foot-process development [13]. Interestingly, there is an MMP in green algae [14] as well as in plants such as the soybean [15] and *Arabidopsis thaliana* [16].

Domain organization and protein structure

The close evolutionary relationship between MMPs is further reflected in the conservation of their domain structure (Figure 1) and of their mechanisms of catalysis and regulation. Most MMPs are secreted, although some are anchored to the cell surface by a transmembrane segment (MMP-14, MMP-15, MMP-16 and MMP-24, referred to as membrane-type MMPs or MT-MMPs) [17] or via a glycosylphosphoinositol (GPI) anchor (MMP-17 and MMP-25, sometimes also included in the MT-MMPs) [18]. Most of the MT-MMPs are type I transmembrane proteins - oriented with the amino terminus outside the cell - although one, MMP-23, is unusual in apparently having the amino terminus positioned intracellularly (a type II orientation) [19]. The MT-MMPs have a single transmembrane domain followed by a short, highly conserved cytosolic segment of 20 amino acids. ‘Soluble’ MMPs may also be located at the cell surface via interaction with membrane-bound molecules or complexes such as integrin αβ3 [20], the extracellular matrix metalloproteinase inducer EMMPRIN/CD147 [21], the hyaluronan receptor CD44 [22],...
domain - most commonly a domain similar to sequences in the heme-binding protein hemopexin and the soluble fibronectin-related protein vitronectin [24,25] (see Figure 1).

The protease domain contains a signal peptide, the pro-domain, and the catalytic module, in that order. MMP-2 and MMP-9, which are the main gelatinases, differ from other MMPs in that they have three tandem fibronectin type II cell-surface heparan-sulfate proteoglycans such as syndecans [23], or transmembrane MMPs [17].

All MMPs except MMP-7 (matrilysin-1) and MMP-26 (endometae or matrilysin-2) consist of two domains connected by a flexible proline-rich hinge peptide. The domains comprise a protease domain and an ancillary (helper) domain - most commonly a domain similar to sequences in the heme-binding protein hemopexin and the soluble fibronectin-related protein vitronectin [24,25] (see Figure 1). The protease domain contains a signal peptide, the pro-domain, and the catalytic module, in that order. MMP-2 and MMP-9, which are the main gelatinases, differ from other MMPs in that they have three tandem fibronectin type II 

Figure 1
The domain composition and important structural features of the various subtypes of MMPs.
Regulation of MMPs

In keeping with their potential for tissue destruction, MMPs are stringently regulated at multiple levels, including transcription, activation of the zymogen forms, extracellular inhibitors, location inside or outside the cell and internalization by endocytosis. The pro-domain keeps the enzyme latent using the thiol group of a highly conserved, unpaired cysteine at its carboxyl terminus. This conserved cysteine acts as a fourth inactivating ligand for the catalytic zinc atom in the active site, resulting in the exclusion of water and rendering the enzyme inactive (Figure 2). For the enzyme to be activated, this cysteine-zinc pairing needs to be disrupted by a conformational change or by proteolysis (such as by the protease plasmin or by other MMPs). Once the thiol group is replaced by water, the enzyme is able to hydrolyze the propeptide to complete the activation process and can then cleave the peptide bonds of its substrates. This system of regulation is referred to as the ‘cysteine-switch’ mechanism [33]. Most MMPs are not activated until they are outside the cell, but the MT-MMPs and MMP-11, MMP-23 and MMP-28 are activated by a prorpeptide convertase (such as furin) within the secretory pathway [34-37].

Once activated, there are multiple mechanisms that can inactivate the MMPs [38]. Four classes of metalloproteinase inhibitors are found in extracellular spaces and body fluids that have broad inhibitory activity against many MMPs. One class is the tissue inhibitors of metalloproteinases (TIMPs), which are disulfide-bonded proteins of 20-30 kDa that directly interact with the MMP active site through a small number of their amino acids. An unrelated small inhibitor derived by proteolysis of the procollagen C-proteinase enhancer has structural similarity to TIMPs and may inhibit MMPs through a similar mechanism [39]. Recently, a membrane-anchored molecule, reversion-inducing cysteine-rich protein with Kazal motifs (RECK), has been discovered that appears to regulate MMP-2, MMP-9 and MMP-14 post-translationally by affecting secretion and activation as well as by inhibition of the active site [40]. In the circulation, the protease inhibitor α2-macroglobulin inactivates active MMPs by a ‘bait and trap’ mechanism [38,41]: when protease-sensitive sites within the inhibitor are cleaved, it springs closed around the proteinase and isolates it from potential substrates. MMP zymogens can also be activated by MMPs themselves; for example, MMP-2 is activated by MT-MMPs such as MMP-14 [17]. Paradoxically, the inhibitor TIMP-2 is absolutely required for this process, which requires it to bind to the active site of MMP-14 and also, through its carboxy-terminal domain, to the hemopexin/vitronectin-like domain of MMP-2. The essential role of TIMP-2 is emphasized by the lack of pro-MMP-2 activation in Timp2-null mice [42] as well as by the exquisite co-regulation of the Mmp14 and Timp2 genes (but not other TIMP genes) during mouse development [43]. (MMP-14 also activates MMP-13 at the cell surface, but this does not seem to require TIMP-2 [44].)
The further activation of MMP-2 bound to TIMP-2 probably involves complex but highly regulated repositioning of membrane-bound MMPs through their cytoplasmic tails or transmembrane domains or through interaction with cellular receptors. Lehti et al. [45,46] have suggested that oligomerization may occur between the cytoplasmic domains of the two MMP-14 molecules, an observation that ties in with a report that a conserved cysteine in the MMP-14 cytoplasmic tail is essential for dimerization [47].

In addition to a possible role in oligomerization, the cytoplasmic domain of MT-MMPs is essential for regulating the activity of these enzymes; it acts by altering their spatial distribution in the cell in response to intracellular signaling events. The cytoplasmic sequence targets the MT-MMPs to specific domains within the cell membrane, including invadopodia (surface protrusions of invasive cells) [48] and caveolae (specialized membrane invaginations) [49]. Recently, a role for the cytoplasmic domain has been identified in the cycling of MT-MMPs between an intracellular pool and the cell surface via clathrin-coated pits and vesicles [50,51]; this may be one of several cellular mechanisms by which the amount of enzyme at the cell surface is regulated. Studies of thrombospondin-2 null mice have indicated that thrombospondin-2 has a role in targeting active MMP-2 to the scavenger-receptor pathway [52], and it has been shown that MMP-13 binds to a specific cell-surface receptor before it is internalized via the low-density lipoprotein receptor [53].

Functions of MMPs

The substrates of MMPs are given in Table 1, and the functions of MT-MMPs in particular are illustrated in Figure 3.

Enzyme-substrate co-localization and interactions

The collagensases MMP-1, MMP-8, MMP-13, and MMP-14 are the only MMPs that can efficiently degrade the fibrillar collagens (types I, II and III) in their triple-helical domains [54]. Cleavage by these enzymes renders the collagen molecules thermally unstable, so that they unwind to form gelatin, after which they can be degraded by other members of the MMP family such as the major gelatinases MMP-2 and MMP-9. The collagenase active site is unable to accommodate the entire cross-section of the collagen triple helix, an observation that has generated much interest in how this substrate is actually cleaved.

The two major gelatinases, MMP-2 and MMP-9, have several distinctive features. They can be distinguished by the fact that MMP-2 binds preferentially to TIMP-2, which is required for its activation, whereas MMP-9 is preferentially inhibited by TIMP-1 [38]. MMP-2 becomes located at the cell surface by binding of its carboxyl terminus to the integrin αβ₁ [29] or the MMP-14-TIMP-2 complex; when bound, the catalytic site of MMP-2 is exposed and can be cleaved and activated. The α2 chains of collagen IV bind MMP-9 with a high affinity even when MMP-9 is inactive [55]; this juxtaposition of enzyme and substrate means that a pool of the enzyme is rapidly available upon activation for any remodeling events.

Non-matrix substrates and consequences of MMP activity

The extracellular matrix contains sites that can bind growth factors, either directly and via growth-factor-binding proteins. When bound, growth factors - such as transforming growth factor β (TGF-β), fibroblast growth factor 1 (FGF-1) and insulin-like growth factor 1 (IGF-1), tumor necrosis factor α, and heparin-binding epidermal growth factor-like growth factor (HB-EGF) [56] - are unable to bind their receptors and signal to the nucleus [57-61]. Several MMPs are able to release growth factors by cleaving either the growth-factor-binding protein or the matrix molecule to which these proteins attach. In addition, MMP-3 and MMP-7 can cleave the adherens-junction protein E-cadherin, thus promoting cell invasion by disrupting cell aggregation [62,63]. MMP-3 can release a soluble form of the adhesion molecule L-selectin [64] from leukocytes. It also sheds membrane-bound HB-EGF, so that it can exert signaling functions [56], MMP-7 releases soluble Fas ligand; this occurs during involution of
the prostate after castration and induces apoptosis [65]. In contrast to the activation of growth factors by proteolytic release, many growth factors are proteolytically inactivated by MMPs, including the chemokine connective tissue activating peptide III (CTAP-III), monocyte chemoattractant protein and stromal cell-derived factor 1 (SDF-1) [66,67]. A second mechanism by which growth factors and cytokines are negatively regulated is when MPPs cause the shedding of their receptors from the cell membranes, as in the case of surface FGF receptor 1 [68].

The immune system is also influenced by MMPs. Firstly, the defensins are a family of polar antimicrobial peptides that make up part of the innate immune system of some animals. Defensins are synthesized in an inactive form and activated by the proteolytic removal of the pro-domain by MMP-7, which allows them to insert into the bacterial membrane and disrupt its integrity [69,70]. Secondly, MMP-3 (stromelysin-1) and MMP-7 (matrilysin-1) can cleave all immunoglobulin G proteins; this cleavage is important as it prevents the initiation of the complement cascade and is potentially beneficial in the removal of the immunoglobulin G from damaged or inflamed tissue [71]. And finally, the receptor of the complement component C1q, gC1qR, exists in both a membrane-bound form and a soluble form that may inhibit the hemolytic activity of C1q. By releasing gC1qR, MT1-MMP may help tumor cells to avoid targeted destruction by the complement system and may thus facilitate tumor-cell survival [72-74].

Genetic alterations in human and mouse MMPs

An extensive body of literature indicates an association of MMPs with cancer, arthritis, numerous other inflammatory or autoimmune disorders, cardiovascular and cerebrovascular diseases, and fibrotic diseases. Despite this, there are relatively few instances in which MMPs have proved to be the primary cause of disease. One of these is a rare inherited disorder of bone (nodulosis arthropathy osteolysis syndrome), one of a set of ‘vanishing bone syndromes’, in which there is severe resorption and destruction of bones, primarily those of the hands and feet [75]. The affected individuals also have chronic arthritis and subcutaneous nodules in the hands and feet. The disease is inherited in an autosomally recessive manner, and loss-of-heterozygosity analysis mapped the responsible gene to 16q12-q22, an interval containing the MMP2 gene. Analysis of the serum and fibroblasts of affected individuals showed a complete absence of MMP2. The family was found to have two homoallelic MMP2 mutations, resulting in the ablation of MMP2 activity. The presence of collagenous nodules fits with the role of MMPs in removing excess ECM; the paradox in these patients, however, is that excessive destruction of bones and arthritis are caused by an absence of a destructive enzyme, not an excess as might have been expected. There are a number of mechanisms by which this may occur: there may be compensatory overproduction of another MMP or protease, MMP-2 may be essential for processing of an inductive factor required for the activity of bone-forming cells (osteoblasts), or it may be required to regulate the activity of bone-degrading cells (osteoclasts) in tissues. Interestingly, the Mmp2 knockout mouse is not known to recapitulate the human disease, which resembles more closely the phenotype of Mmp14 knockout mice [76,77]. Given that MMP-14 and TIMP-2 are required for MMP-2 activation, it is possible that mutations in the genes encoding these enzymes may result in a similar human disease.

The chromosome 1p36.3 region that contains the two closely related MMP23 genes (see Table 1) is altered or frequently deleted in neuroblastomas in which the oncogene MYCN has been amplified, in a subset of malignant melanomas and in a 1p35 deletion disorder [78], but the role of the two MMP genes in these disorders has not been investigated.

A polymorphism in the MMP1 promoter can result in one allele having a sequence of either one or two guanines in a particular position. The presence of two guanines results in the de novo appearance of a binding site for the ETS-1 transcription factor that cooperates with an adjacent site for the AP-1 transcription factor to enhance the expression of MMP1 [79-81]. It is interesting to note that cancer patients, on average, have a higher incidence of the two-guanine allele than have people in the unaffected population. A regulatory polymorphism is also present in the MMP3 promoter, leading to the presence of either five or six adenosines [82]. The allele with six (6A) has a reduced transcriptional activity compared to the allele with five. A study on patients with coronary artery atherosclerosis revealed that patients homozygous for the 6A allele showed a more rapid progression of both global and focal atherosclerotic lesions.

The creation of transgenic mice that are null for specific genes has been useful in attributing functions to a number of MMP family members (Table 2). In general, most MMP gene knockouts are viable, with subtle phenotypic differences from their wild-type counterparts [83]. This could reflect either a lack of function in vital developmental processes or a significant amount of redundancy amongst these enzymes for substrates, with the consequence that other family members can be upregulated to compensate for the loss of an individual enzyme. Although the lack of a dramatic phenotype in many of the knockout mice may initially have seemed a disappointing outcome, it has in fact been very valuable for dissecting the role of MMPs in cancer and inflammation. The exception to the rule is the Mmp14-knockout mouse [76,77], which has severe postnatal growth-retardation and skeletal anomalies and dies before the onset of sexual maturity (Figure 4). The severity of the Mmp14 knockout, in contrast to the other MMP knockout animals, may reflect the position of this enzyme as an initiator of a number of proteolytic cascades, as well as its obligate location at the cell surface. (Note that none of the other MT-MMPs has yet been inactivated in mice.) The defects seen in these animals are probably attributable to both deficiencies in the turnover of direct MMP-14 substrates...
The phenotype of Mmp14-null mice, which is the most dramatic developmental phenotype of all MMP null transgenes. A rare example of an Mmp14-null mouse that has survived to 10 weeks of age is shown (front, alongside a normal littermate); note the severe dwarfism and craniofacial anomalies.

The importance of MMPs in physiological processes and human disease is now undisputed. What is not known, however, is the full extent to which they are involved in every process in mammals. The failure of broad-spectrum and even relatively specific MMP inhibitors in cancer therapy is not surprising, because the complete effects for a given inhibitor depend on the functions of its targets, and these are mostly unidentified and unknown. The fact that there are two other large families of related proteases with important physiological functions, the ADAM and ADAMTS families, suggests that additional caution should be taken when using MMP inhibitors to treat disease states. More subtle approaches are going to be required to modulate the functions of these enzymes in a more selective fashion [84]. By understanding the precise mechanisms by which MMPs are regulated and their interactions with various binding partners, it may be possible to block the deleterious functions of these enzymes without the concomitant loss of beneficial functions. This underscores the need for a greater investment than ever before in MMP biology and biochemistry. The next five years are expected to bring many exciting insights into the role of MMPs in development and human disease and may lead to even broader interest in what was once thought to be a highly specialized and restricted field.

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