Editorial

The observations by Sakai et al. (1) in this issue illuminate the once dimly lit path followed by tryptases as they mature into the major secreted proteins of human mast cells. Tryptases are odd and somewhat enigmatic proteases, flouting conventions followed by most trypsin-like enzymes (2). They were first sighted over three decades ago as mast cell trypsin-like activity in tissue sections. This was noteworthy first because the activity survived fixation and sectioning, displaying unusual hardiness. Second, the activity resided in cytosolic granules, distinguishing it from other trypsin-like proteases stored and released as inactive zymogens, to be activated extracellularly by propeptide cleavage. Even today, tryptases stand virtually alone in this regard among tryptic serine proteases, including those of the hemostatic, fibrinolytic, complement-activating, and pancreatic zymogen cascades. The presence of active enzyme inside of cells led to early speculation that tryptases act intracellularly. However, doubts arose from later observations, such as that mast cell granules are acidic. Like other serine proteases, tryptases are active at alkaline pH and much less so below pH 7. Thus, activity in granules is a fraction of that at the higher pH outside of cells. Moreover, tryptases are densely compacted in the granule, which can appear crystalline. During exocytosis this orderliness breaks down, liberating heparin, tryptases, and fellow-travelers. The soluble, secreted complex of heparin and tetrameric tryptase is stable in humans, resisting all circulating protease inhibitors. Data of this sort predict that tryptases, by accident or by design, act extracellularly.

Searches for the presumed extracellular targets of tryptases have rounded up several suspects (2). For example, tryptases inactivate bronchodilating peptides and thereby may increase airway tone in allergic asthma. By degrading procoagulant proteins, tryptases (along with attached heparin) may prevent deposition of fibrin and act as extravascular anticoagulants. Further, by activating urokinase, tryptases may accelerate fibrin lysis. In this fashion, tryptases may ease the ingress of plasma immune proteins and leukocytes recruited in the wake of the mast cell's response to real and perceived threats, such as parasites and pollens. Also, by activating matrix metalloproteinases and by stimulating growth of fibroblasts and other cells, tryptases may extend their influence beyond acute inflammation to include tissue growth and remodeling in response to injury. It should be stressed, however, that tryptases are not take-no-prisoners proteases like trypsin. Their specificity is much more restricted. In some contexts, they may be anti-inflammatory, as when terminating the flare reaction caused by calcitonin gene-related peptide. Nonetheless, suspicion of an inciting role in allergic disease has generated interest in a therapeutic role for tryptase inhibitors.

If tryptases indeed worsen illness, then the question of how they are activated is clinically important. Some clues, as it turns out, are available from studies of mast cell chymases and cathepsin G. These enzymes, like related lymphocyte and neutrophil proteases, e.g., granzyme B and elastase, are activated intracellularly, as are tryptases. However, they differ from tryptases in having an acidic activation dipeptide (Gly-Glu or Glu-Glu), which is removed intracellularly by dipeptidyl peptidase I (DPPI) (3). The propeptides of tryptases are sufficiently odd that those of us reporting the first such sequence predicted a novel mechanism of activation (4). Subsequent cloned tryptase cDNAs and genes from multiple mammals confirmed the presence of a preprosequence consisting of a signal peptide and a 10–12–amino acid propeptide, the latter ending in Gly with a basic Arg or Lys in the "−3" position relative to the -1 Gly. The lone exception is human α-tryptase, which lacks the basic residue and now is suggested to be secreted in an inactive form — stillborn, as it were — because it cannot be autoprocessed at the −3 site. It remains to be seen whether α-tryptase is activated by other pathways (perhaps after release) and whether it has actions that do not require enzymatic activity. In any case, increasing evidence suggests that one or more β-type tryptases (I, II/β, or III) is the major stored form of human mast cell tryptase.

Thus, it appears that preprotryptase processing is indeed unique, involving the sequential actions of signal peptidase, tryptase itself, and DPPI. With β-tryptase cleaving its own proform, it seems that prior speculation regarding an intracellular role was correct. Tryptase in fact may act both inside and outside of the cell. Although the activation path is not used by α-tryptase, it seems a safe bet that it does pertain to other "−3 basic residue" tryptases, including mouse enzymes, although earlier studies of DPPI's activation of murine tryptases seemed contradictory (3, 5). The activation mechanism also may apply to more distantly related enzymes, such as dog mast cell protease-3 (dMCP-3), which has a similar propeptide but distinct structural features (4, 6). However, the mechanism remains perplexing in some aspects. Particularly, it is curious that the β-zymogen cleaves other β-zymogens, a feat with little precedent among serine proteases. Doing so in the acidic environment of the secretory granule is all the more remarkable because of low serine protease activity at low pH. Could it be that acidic pH favors tryptase zymogen activity while discouraging that of the mature enzyme? Or perhaps tryptase is activated in a more neutral pre-secretory organelle, such as the Golgi.

The intriguing dependence of the process on heparin may explain the co-existence of heparin and tryptase in mast cells of humans, dogs and rodents. Mucosal mast cells of rats and mice contain little heparin and little if any tryptase protein or mRNA. In contrast, virtually all human mast cells produce tryptases. However, one must distinguish between a need for heparin during activation and a dependence on heparin following exocytosis. Here there are clear species differences, with dog and human tryptases being stabilized by heparin upon release but with rat tryptase parting from heparin and being unstable. On the other hand, dMCP-3 binds to heparin but is stable without it. These and other idiosyncrasies in

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tryptase expression and behavior continue to challenge the conventional wisdom regarding serine proteases.

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