

# Trypsin and host defence: a new role for an old enzyme

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By acting as a pro-defensin convertase in human Paneth cells, trypsin is involved in the regulation of innate immunity in the small intestine

Limited proteolysis is a highly specific irreversible process which serves to initiate physiological function by converting precursor proteins into biologically active forms. It can also be a mechanism for terminating biological activity. Limited proteolysis is used in a wide variety of biological systems, including blood coagulation, hormone regulation, and host defence.

Alpha defensins are small (~29–35 amino acids) cationic disulphide linked peptides that are expressed mainly in neutrophils<sup>1,2</sup> and Paneth cells (PCs) of the gastrointestinal tract.<sup>3–6</sup> In mouse small intestine, multiple  $\alpha$ -defensins (known as cryptidins) have been identified that constitute 70% of the released bactericidal activity.<sup>7</sup> Human PCS express only two members (human defensins 5 (HD-5) and 6 (HD-6)) of this family of antimicrobial peptides.<sup>4,5</sup> Functions attributed to PC derived defensins include regulation of the microbial density of the small intestine, protection of neighbouring stem cells in the crypt, and defence against pathogens.<sup>7–9</sup> Defensins are synthesised as larger precursor molecules (~100 amino acids) with a putative amino terminal signal sequence (which targets the peptide to the endoplasmic reticulum for eventual export), followed by a middle propeptide segment which is likely to be crucial for correct folding and/or in stabilising charge interactions during synthesis and storage.<sup>10,11</sup> The bioactive antimicrobial peptide resides in the final carboxy terminal sequence of the precursor. There is increasing evidence to suggest that processing of  $\alpha$ -defensins during storage and subsequent secretion may be species and cell specific.<sup>10,12,13</sup> Why such a variation in defensin storage form exists in nature is unclear.

To date, studies of the regulation of mouse cryptidins have been particularly fruitful in providing greater understanding of the proteolytic processes involved in the release of active defensin peptide. In 1999, Wilson *et al* showed that the enzyme matrilysin, a matrix metalloproteinase (MMP-7), was responsible for the processing of mouse cryptidins.<sup>8</sup> The human small intestine does not express MMP-7 and the identity of the human counterpart has to date remained elusive. However, recent studies by Ghosh *et al* reveal that in addition to its known digestive properties, the serine protease trypsin is the prodefensin-5 processing enzyme in the human small intestine.<sup>14</sup>

**Ghosh D, Porter E, Shen B, *et al*. Paneth cell trypsin is the processing enzyme for human defensin-5. *Nat Immunol* 2002;3:583–90.**

**The antimicrobial peptide human alpha-defensin 5 (HD5) is expressed in Paneth cells, secretory epithelial cells in the small intestine. Unlike other characterized defensins, HD5 is stored in secretory vesicles as a propeptide. The storage quantities of HD5 are approximately 90 450 microg per cm<sup>2</sup> of mucosal surface area, which is sufficient to generate microbicidal concentrations in the intestinal lumen. HD5 peptides isolated from the intestinal lumen are proteolytically processed forms—HD5(56–94) and HD5(63–94)—that are cleaved at the Arg55-Ala56 and Arg62-Thr63 sites, respectively. We show here that a specific pattern of trypsin isozymes is expressed in Paneth cells, that trypsin colocalizes with HD5 and that this protease can efficiently cleave HD5 propeptide to forms identical to those isolated in vivo. By acting as a prodefensin convertase in human Paneth cells, trypsin is involved in the regulation of innate immunity in the small intestine.**

The role of pancreatic trypsin in digestive processes is well established, so the identification of a new role in the regulation of gastrointestinal innate immune responses is initially surprising. The presence of trypsin inhibitors in PCS has been reported previously<sup>15,16</sup> but their exact role and contribution to gastrointestinal homeostasis has remained unclear. Ghosh and colleagues<sup>14</sup> have now extended these earlier observations and demonstrated PC specific expression of two isoforms of trypsin and the presence of two trypsin inhibitors, the  $\alpha$ -1 antitrypsin and pancreatic secretory trypsin inhibitor. Both in vitro and in vivo biochemical analyses (including inhibitor studies, mass spectroscopy, and N terminal peptide sequencing) have allowed the establishment of exact cleavage sites of pro-HD-5 that are susceptible to trypsin. Finally, colocalisation of intestinal trypsinogen, its inhibitors, and human pro-HD-5 in secretory granules of PCs suggest that taut interactions are involved in the regulation of mature HD-5 liberation.

The identity of intestinal trypsin as the pro-HD-5 processing enzyme raises several possible scenarios. Local production of the enzyme and its inhibitors in PC secretory granules suggests a greater role in PC biology is likely. The presence of active trypsin in the crypt lumen may further modulate intestinal function. The ability of trypsin to activate members of other protease families<sup>17</sup> and membrane bound receptors such as proteinase activated receptor 2<sup>18</sup> suggests the enzyme may have a potential role in gut inflammation and tissue remodelling. The mo-

lecular mechanism(s) involved in the activation of trypsinogen and subsequent release of active HD-5 needs to be clarified.  $\beta$ -Defensins are a closely related family of antimicrobial peptides expressed by enterocytes where their expression is augmented by infection and inflammation.<sup>19</sup> At present, regulation of  $\beta$ -defensin peptide secretion is unknown. As Ghosh *et al* observed intestinal trypsin expression to be confined to PCs, elucidation of the role of trypsin, if any, in  $\beta$ -defensin regulation is eagerly awaited.

The concept of precursor activation is well documented for the mammalian serine proteinase family as a self protective and rapid response mechanism to stimuli, properties that may place them central in the regulation of host innate immune responses. For example, there is increasing evidence for such a role as members of this family are implicated in complement activation by the classical and mannan binding lectin pathway.<sup>20,21</sup> Human neutrophil granules contain several neutral serine proteases, including elastase, which theoretically can cleave the N terminal of bactericidal/permeability increasing factor and also participate in proteolytic processing of hCAP-18 to generate the active antimicrobial peptide from the precursor molecule.<sup>22</sup> How trypsin weaves into innate immune functions in the intestine, and whether such pathways may be exploited therapeutically to modulate innate immunity, offer new avenues for future investigation.

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