Interaction of trefoil family factors with mucins: clues to their mechanism of action?


Abstract
The trefoil peptides or trefoil factor family (TFFs) are a group of secreted proteins found predominantly in mucus-secreting cells in the gastrointestinal mucosa. TFFs have raised considerable interest because of their possible role in healing of the epithelium after damage. However, there is no general agreement concerning their mode of action—whether they act through interaction with secreted mucins or via interaction with a specific receptor—or indeed interaction with the EGF receptor. Using the yeast two-hybrid system, pS2/TFF1 interacting proteins were sought in the stomach and duodenum of the mouse. Clones were isolated which corresponded to the murine counterpart of fragments of MUC2 and MUC5AC mucins. Mutagenesis studies showed that pS2/TFF1 interacts by binding to the von Willebrand Factor (VWBF)C1 and VWBFC2 domains of the mucins, which are also cysteine-rich. These results indicate that the protective effect of TFFs may operate by organising the mucin layer which protects the mucosa from damage.

Comment
In recent years there have been numerous reviews and articles describing the distribution of a group of molecules known as the trefoil peptides or trefoil family factors (TFFs). In mammals, the family is represented by three members, pS2/TFF1, hSP/TFF2, and ITF/TFF3. They are characterised by possession of the TFF motif, a three looped structure held tightly together by disulphide bonds based on six cysteine residues—one such motif in pS2/TFF1 and ITF/TFF3, and two in hSP/TFF2. These molecules are found in the mammalian gastrointestinal tract in more or less defined sites—for example, pS2/TFF1 is located mainly in the foveolar cells of the gastric mucosa, hSP/TFF2 is seen in mucous neck cells, deep pyloric glands, and Brunner’s glands, while ITF/TFF3 is expressed predominately in the goblet cells of the small and large intestine. In abnormal situations, as in the ulcer associated cell lineage (UACL) in Crohn’s disease, or in the epithelium seen migrating over the surface of healing peptic ulcers, all three TFFs can be expressed. Indeed, there is evidence for coordinated expression of TFFs, at least in transsected cell lines.

Experiments in polarised epithelial monolayers in vitro show that TFFs promote cell migration and are therefore thought to promote epithelial restitution after damage. Mice over expressing pS2/TFF1 in the small intestine are more resistant to induced damage whereas mice in which ITF/TFF3 has been knocked out by gene targeting show reduced resistance. When the pS2/TFF1 gene was deleted, mice showed pronounced gastric and small intestinal mucosal inflammation, with the development of adenomas and occasional intramucosal carcinomas in the antrum.

But there is no general agreement about the mode of action of trefoil factors on epithelial cells: some reports have claimed that gastrointestinal epithelial cells have binding sites for TFFs—putative receptors. Moreover, TFFs have actions in vitro very redolent of a receptor:ligand interaction—application of ITF/TFF3 to epithelial cells leads to phosphorylation of β-catenin on tyrosine within 10 seconds. Other well known motogens, such as hepatocyte growth factor and epidermal growth factor (EGF), also phosphorylate β-catenin, and indeed during this process ITF/TFF3 also activates the EGF receptor itself, a finding which has been confirmed. But this activation does not appear to be direct. Moreover, ITF/TFF3, again on intestinal cell lines, inhibits both the ERK (extracellular signal related kinase) and the MAPK (mitogen activated protein kinase) pathways, probably through induction of a tyrosine phosphatase.

Possibly central to TFF action is the observation that they can form both homo and heterodimers via a free cysteine residue present in the conserved acidic carboxy terminal domain and when this dimerisation is prevented, both the migration inducing and cytoprotective properties of pS2/TFF1 are compromised. However, the significance of this potentially important point is not yet clear.

In this article, Tomasetto et al made use of the yeast two hybrid system to search directly for interacting proteins, employing a cDNA expression library from the mouse stomach and duodenum. In these experiments, a plasmid encoding the secreted form of mouse pS2/TFF1 was introduced into an appropriate “reporter” yeast strain to act as the “bait”, and this strain was then transformed with the mouse stomach and duodenum cDNA library. A number of clones then grew on selective media; these were isolated and the plasmids isolated and retransformed into the reporter strain. The complete cDNA sequences of four clones which were isolated by this procedure were determined: three of the clones encoded overlapping cDNA fragments of the same gene and showed considerable homology with rat MUC5AC and the carboxy terminal region of human MUC2. These findings indicated that proteins which interacted with pS2/TFF1 included the murine homologue of mucin proteins, corresponding to MUC2 and MUC5AC. In situ hybridisation with probes derived from these cDNAs showed patterns consistent with the distribution of MUC2 and MUC5AC in the intestine and stomach.

It was noted that two of the clones shared copies of the cysteine rich von Willebrand factor (VWF) domain common to all mucins, and originally found in pre-pro-VWF. There are two such domains, VWFC1 and VWFC2. A strategy involving site directed mutagenesis was used to confirm that the common VWFC sequences were respon-
sible for the interaction: deletion of the VWFC domains largely prevented interaction. Tomasetto et al concluded that VWFCs were the domains of interaction with pS2/TFF1, and for at least one clone, both of the domains were necessary for obtaining maximal interaction.

Mucins are extremely important molecules as far as the gastrointestinal tract is concerned: they are heavily glycosylated and are found in membrane bound or secretory forms throughout the gut epithelium. There are presently nine human epithelial mucins, several of which are found at more or less specific locations in the gastrointestinal tract. Thus MUC2 is found in intestinal goblet cells and MUC5AC in the foveolar cells of the stomach. Mucin molecules such as MUC2 have a central region containing repeated sequences rich in serine and threonine, and here the molecule is extensively glycosylated. There are three types of cysteine rich domains, similar to several domains present within the pre-pro-VWF protein—namely D, C, and CTCK (C terminal cysteine knot). The D domain is considered to be responsible for multimerisation while the C domain is some 70 amino acid residues long and contains 10 cysteines and is probably involved in oligomerisation, although not initial dimerisation; dimerisation is thought to be the function of the CTCK domain.

It has been known for some time that TFFs and mucins are closely connected. In amphibians, multiple copies of the TFF domain are interspersed with the threonine rich domains. Moreover, in the human gut, TFFs and mucins are often coexpressed in mucous cells in a closely related manner—pS2/TFF1 with MUC5AC, hSP/TFF2 with MUC6, and ITF/TFF3 with MUC2, suggesting their involvement in mucin processing or mucin structure, a fairly specific interrelationship, at least in the normal state. However, interestingly, pS2/TFF1 seems to also interact with MUC2, possibly providing a molecular reason for the occasional reports which describe pS2/TFF1 in the colon, where MUC2 is expressed. In vitro studies also suggest cooperativity between TFFs and mucins in epithelial protection, and Tomasetto et al's paper now suggests a molecular mechanism for this relationship.

The TFF motif contains a hydrophobic binding pocket which could represent a binding site for a protein side chain, sugar residues, or indeed for a specific receptor. Tomasetto et al's findings indicate that TFFs bind to the VWFC domain of mucins, a domain thought to be involved in the oligomerisation of these large mucin molecules, itself a complex process, the end result of which is the formation of a mucus gel of high viscosity. However, this seminal paper raises even more questions. If the role of TFFs at the apical surface of the epithelial cell is to ensure that the process of multimerisation of mucins proceeds, how is this related to their role as motogens and in cytoprotection? Is this role mediated solely via its interaction with mucins? How do we then explain the very rapid effects on cellular signalling mechanisms, especially when these are mediated via the basolateral surface of gut epithelial cells and appear to involve the EGF receptor? Is there a TFF receptor, and if so, how do we equilibrate these receptor mediated effects with the putative mucin-oligomerisation role? And why does dimerisation appear so important for the single residue TFFs? Intriguing questions, no doubt.