Cell. Mol. Life Sci. 62 (2005) 2939–2946
1420-682X/05/242939-8
DOI 10.1007/s00018-005-5482-8

Cellular and Molecular Life Sciences

Trefoil factor family-interacting proteins

W. R. Otto[a] and L. Thim[b,*]

[a] Histopathology Unit, Cancer Research UK, 44, Lincoln's Inn Fields, London WC2A 3PX (United Kingdom)
[b] Protein Engineering, Novo Nordisk A/S, 2760 Måløv (Denmark), e-mail: lth@novonordisk.com

Online First 5 December 2005

Abstract. Trefoil factor family (TFF) peptides have many in vivo and in vitro effects on restitution, wound healing, apoptosis, cell motility, adhesion and vectorial ion pumping, amongst others. ^{125}I-TFF peptides bind to cell membranes with classical saturable ability. It would be surprising if there were not TFF-protein interactions that would explain these actions, but to date no convincing TFF-binding partner has been shown which unambiguously takes part in any of these functions. Nevertheless, several TFF-binding proteins exist, including the small intestinal CRP-ductin (muclin), which binds TFF2, and the recently described gastric foveolar proteins TFIZ1 (TFF1-binding) and blottin (TFF2-binding), any of which may yet interact in novel ways to elicit TFF-mediated events. This review describes the expression and, where known, the functions of such proteins.

Key words. Trefoil peptides; binding protein; receptor; mucin; vWF domain C; CRP-ductin; TFIZ1; blottin; Brichos domain.

Introduction

Trefoil factor family (TFF) peptides have long been orphan ligands in search of a receptor. This family of peptides was discovered by several routes; in mammals as an oestrogen-inducible peptide (TFF1, formerly pS2) in MCF-7 cells [1], as a side fraction from porcine pancreatic insulin production (TFF2, formerly pancreatic spasmolytic polypeptide, PSP) [2] and from rat intestine (TFF3, formerly intestinal trefoil factor, ITF) [3], as well as from frog skin and intestines [4, 5]. The molecules have been extensively studied in vivo and in vitro, and reviews exist on their structure [6] and biological functions (this issue). Surprisingly, despite many attempts to find them, to date no convincing TFF receptor has been found, although several candidates exist, which we describe in this review.

* Corresponding author.

Evidence suggesting receptor-mediated, TFF peptide-dependent events

Many reports suggest strongly that TFF peptides act via some form of receptor-ligand interaction. Thus whole rat small intestinal preparations and epithelial monolayers both exhibit a basolateral preference for rTFF3 to elicit a luminal chloride secretory event when placed in an Ussing chamber [7]. In contrast, the ability of hTFF2 to produce a restitution response after indomethacin-induced gastric injury to rats in vivo was without effect when applied luminally, rather than parenterally [8]. The in vivo wound healing response of rats to gastric cryoprobe ulceration results in a rapid induction of TFF2 messenger RNA (mRNA) within 30 min, followed by the other TFFs and transforming growth factor (TGF)-α up to 3 days later [9]. In vitro cell motility is likewise a rapid event after TFF stimulation of gastrointestinal cells [10], an event which synergises with epidermal growth factor (EGF) [11]. Beta-catenin phosphorylation is stimulated within 1 min of TFF3 application to HT29 cells [12], followed by adenomatous polyposis coli (APC) translocation to the nucleus and downregulation of E-cadherin

[13]. Several other cellular responses to TFF peptides exist, including apoptosis, morphogenesis, angiogenesis and transcriptional regulation, events which have been reviewed recently [14]. All these events triggered by TFF peptides are likely to be mediated by a receptor-ligand interaction.

Evidence for TFF-binding proteins

^{125}I-pTFF2 binding to rat small intestinal cell membranes

Purified or cloned TFF peptides have been used as radioligands and fusion proteins to try to elucidate their binding sites. Early work from Frandsen and colleagues suggested that porcine TFF2 (formerly PSP) bound to rat intestinal mucosal cell membranes with dissociation constants in the 10^{-6} to 10^{-7} molar range, and could inhibit the basal or vasoconstrictive intestinal polypeptide (VIP) and secretin-stimulated activity of adenyl cyclase with half-maximal effects at 3 x 10^{-5} M (Table 1). VIP was not affected by, or did not affect, the binding of pTFF2 [15]. ^{125}I-labelled pTFF2 binding to intestinal cells or their membranes were identical and curvilinear, and revealed two classes of complex formation with a fast dissociation (half-life 1.5 min) and a slower one (38 min). No protein was isolated in these studies that accounted for these observations. The dissociation constant of 10^{-6} to 10^{-7} M found in these initial studies did not point to a peptide ligand-receptor interaction which is normally in the range of 10^{-9} to 10^{-12} M. The observed binding could, however, represent interaction between porcine TFF2 and some membrane-bound components (e. g. intestinal mucins).

rTFF3 and hTFF2 binding to rat small intestinal and HT29 cells

Chinery and Cox [7] reported the existence of a binding partner for rTFF3 and hTFF2 peptides in rat intestinal epithelial cell membranes and HT-29 colorectal cells, respectively, by using a cross-linking method. Immunoblot analyses of immunoprecipitates revealed two bands corresponding to a 45-kDa unreduced protein and 28 kDa when reduced. Neither band was further characterised. ^{125}I-TFF3 probing of frozen tissue sections showed autoradiographic signals in rat stomach, jejunum and colon, which was partially inhibitable by cold hTFF2 (10^{-6} M) [16]. This complemented their earlier findings of specific β-gal-rTFF3 binding to the foveolar and surface epithelium of rat stomach, as well as kidney collecting ducts and colon [17]. In view of more recent findings of CRP-ductin in the small intestine (see below), it may be possible that the β-gal-rTFF3 bound to that, or a related, protein.

Table 1. TFF-binding proteins.

						TFF binding			
Author	Protein	Source	MW	pI	mono/dimer	1	2	3	Comment
Frandsen [15]		r SI memb					Y		K_d 10^{-6}-10^{-7}M
Chinery [16]		r St/r SI	28R/45		dimer?	Y	Y		
Tan [18]		r SI	50					Y	WGA binding
Poulsen [20–22]		r TFF2+ cells				Y	Y	Y	
Taupin [63]	CD71 (transferrin-R)	AGS/HT29	95	6.2				Y?	IHC colocalisation only
Thim [24]	CRP-ductin	p St	224	4.7	mono (multidomain)		Y		
	β-FN-R	p St	140	(5.3, 5.7)*			Y		
Tomasetto [42]	mMUC2 vWFC1	m St/Duo			di/oligo	Y			yeast 2-hybrid screen
	mMUC5AC vWFC2	m St/Duo			di/oligo	Y			yeast 2-hybrid screen
Otto [44]	blottin	m St	18	6.9 (obs)	mono		Y		
Westley [43]	TFIZ1	h St	18	6.6 (theor)	mono+TFF1	Y			covalently bonded TFF1
Zhang [62]	Bm-TFF2	frog skin							binds human platelets

MW, molecular weight (kDa); pI, isoelectric point; K_d, dissociation constant (M); r, rat; SI, small intestine; St, stomach; R, reduced; Y, yes; WGA, wheat germ agglutinin; kid, kidney; m, mouse; duo, duodenum; p, pig/porcine; FN-R, fibronectin receptor; * pI, values for human and mouse β-FN-R, respectively; obs, observed; theor, theoretical; vWFC, von Willebrand Factor domain C; Bm, *Bombina maxima*.

rTFF3 binding to rat small intestinal cell membranes

Tan and colleagues [18] used a biotinylated rat TFF3 recombinant protein to find binding sites in gastric mucous cells and small intestinal crypts. Ligand blots of small intestinal cell membranes, but not cytosol, revealed a glycosylated protein of 50 kDa [18]. The glycosylation status was shown by binding to wheat germ agglutinin-conjugated beads, which react with N-acetyl glucosamine moieties. Further glycosylation analysis was not undertaken. Unlike Chinery et al. [16], these workers did not find colonic binding of biotin-rTFF3, though this may have been a sensitivity issue, rather than true negativity. There were several overlaps in the data of Tan and Chinery, which suggest they may have both obtained a similar, if not the same, protein. However, Chinery et al. performed reduced and non-reduced analyses, obtaining bands at 28 and 45 kDa respectively, whereas only reduced cell membrane proteins were analysed by Tan et al., and a single band of 50 kDa was revealed. These discrepancies remain unexplained. The molecular weight of the reduced band of Chinery et al. (28 kDa) was close to that found later by Westley et al. for TFIZ1 covalently bound to TFF1 (see below).

bt-rTFF3 binding to IEC18 cells augmented by mucin

Tan and colleagues reported further studies using biotinylated rTFF3 as a probe for IEC-18 rat intestinal epithelial cells grown in vitro. Fluorescence-activated cell sorting (FACS) analysis of non-enzymatically detached cells incubated at 4 °C indicated that fluorescein isothiocyanate (FITC)- or phycoerythin-conjugated mouse anti-biotin antibody bound to the outer surface of these cells in a specific manner [19], and that the binding was increased by preincubating in mucin (5 mg/ml for 90 min), a situation that resulted in augmented nitric oxide production upon stimulation with rTFF2 (2.5 μM, 18 h). These data were interpreted as showing that the interaction of rTFF3 with IEC-18 cells was upregulated by mucin. However, it is possible that the increase in bound rTFF3 was a reflection of the mucin binding events, as suggested by in vitro experiments using purified or cloned TFFs and mucins (see below).

^{125}I-TFF binding in rats

A series of in vivo binding and distribution studies using the ^{125}I-labelled peptides pTFF2 and recombinant hTFF1 and hTFF3 were performed by Poulsen and colleagues, using rats as recipients [20–23]. The administration of intravenous ^{125}I-pTFF2 was found specifically in several stomach compartments (surface mucus, mucous neck cells, parietal cell, and pyloric glands) as well as duodenal Brunner's glands and small intestinal Paneth cells, and the lower portion of the colonic crypts. The binding was displacable by unlabelled pTFF2, and this was interpreted as demonstrating the presence of a receptor [20]. Oral ^{125}I-pTFF2 was found on the surface mucous of the stomach and small intestine, but not much reached the colon intact [21]. The same group reported intravenous (iv) administrations of recombinant ^{125}I-hTFF1 and ^{125}I-hTFF3 monomer and dimer. ^{125}I-hTFF3 dimer was found at sites of TFF2 expression. ^{125}I-hTFF1 and ^{125}I-hTFF3 monomers both bound to the stomach in a similar manner, but each was far less (15%) than the dimeric hTFF3. Interestingly, neither radioligand monomer bound outside the gastrointesetinal (GI) tract, nor to cells expressing either TFF peptide. They concluded that TFF2-expressing cells possess a basolateral receptor for all TFF peptides, and that mucous neck cells preferentially take up TFFs that have two TFF domains [22].

pTFF2 binding to CRP-ductin and possibly its splice variants

A biochemical approach to finding TFF binding proteins was used by Thim and co-workers [24]. They obtained a porcine stomach mucosal extract and applied it to an affinity column containing immobilised pTFF2. After high-strength salt washes (0.1, 0.4 M), four proteins were eluted by 1 mg/ml pTFF2. Two bands were discounted due to contaminating human keratins, leaving two of interest, and peptide sequencing revealed them to be CRP-ductin (also known as muclin) and the β subunit of the fibronectin receptor, with molecular weights (MWs) of 224 kDa and 140 kDa, respectively. Two further bands which eluted with pTFF2 at MWs of around 50–55 kDa were not further characterised.

The discovery of CRP-ductin as a pTFF2 binding protein has cast some light on the field, but raised more questions than it has answered. The gene encoding CRP-ductin is probably the same as that encoding several proteins that differ by their subunit splicing patterns and include hensin and deleted in malignant brain tumours-1 (DMBT1) [25], ebnerin [26] and vomeroglandin [27] (Fig. 1, Tables 1 and 2).

The literature to date does not reveal a function for CRP-ductin, though the related protein hensin regulates cell polarity and differentiation, certainly in the collecting ducts of the kidney [28] and developing embryos [29]. In the kidney collecting duct, the β intercalated cell is dependent on polymerised hensin within the basement membrane for its columnar phenotype and the polarity of acid-base homeostatic pumps, which regulate the pH of urine. Soluble hensin and antibodies against an extracellular domain revert the cells to an α-cell phenotype and reverse the acid pumping. In developing embryonic stem (ES) cells hensin is required to maintain a columnar phenotype, while laminin induces a squamous change

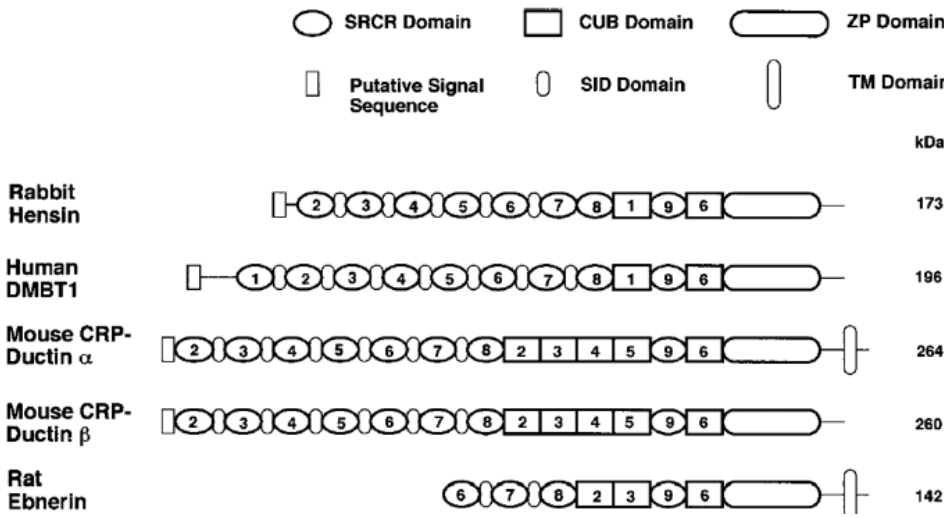

Figure 1. Domain structures of CRP-ductin and related proteins.
SRCR, scavenger receptor cysteine-rich; CUB, C1r/C1s Uegf Bmp1; ZP, zona pelucida; SID, SRCR interspersed domains; TM, transmembrane; DMBT, deleted in malignant brain tumours. Reproduced from [26].

Table 2. CRP-ductin/hensin/DMBT-1/ebnerin family proteins.

			Functional Domains					
Author	Protein	Source	SRCR	CUB	ZP	TM	MW	Comment
Takito [25]	CRP-ductinα	m SI	8	5	1	1	264	apical expression; binds TFF2
	CRP-ductinβ		8	5	1	–	260	
Takito [25]	hensin	rab kid	8	2	1	–	230/173*	BM/polarity agonist, apical + basolateral
Takito [25]	DMBT-1	h brain	9	2	1	–	196	
Li [26]	ebnerin	r taste bud	4	3	1	1	220/142*	
Matsushita [27]	vomeroglandin	mVNO	7	3	1	1	235/210*	also in SI, LI; N-linked ConA binding

SRCR, scavenger receptor cysteine rich; CUB, C1r/C1s Uegf Bmp1; ZP, zona pelucida; TM, transmembrane; MW, molecular weight in kDa; m, mouse; SI, small intestine; rab, rabbit; *, deglycosylated molecular weight; BM, basement membrane; DMBT1, deleted in malignant brain tumours-1; h, human; r, rat; VNO, vomeronasal organ; LI, large intestine; Con A, concanavalin A agglutinin.

[29]. Is it possible that hensin is also a putative TFF-binding protein? There appears to be a gradient of hensin expression in the small intestinal crypts, there being less towards the stem cell region [30]. It may be that a TFF peptide could promote cell flattening in the intestine in a wound situation when luminal TFFs would suddenly have access to the basolateral surface of live cells at an ulcer edge, and may inhibit the polarising action of hensin/CRP-ductin and allow cell flattening to occur, as seen in the well-known phenomenon of epithelial restitution. TFF peptides are well known to accelerate this process (see Taupin and Podolsky for review [14]).

A synonym for CRP-ductin is muclin, and this was found apically in the small intestinal and pancreatic acinar and ductal cell membranes of the cystic fibrosis (CF) mouse [31]. The normal role and site of expression of this protein is thought to be packaging zymogen granules in the trans-Golgi apparatus prior to secretion [32]. In this, muclin has a possible role in mucin secretion as well. The CF mouse has intestinal crypts that are plugged with immobile mucus from postnatal day P16 onwards, and this mucous is periodic acid-Schiff (PAS) positive, in contrast to normal littermates where the crypts are clear. Muclin expression also increased with postnatal age after P16 when compared with normal littermates. No study of TFF peptides has been performed in this model to ascertain their role in the condition. It would be expected that the TFF-mucin thickening properties (see below) would exacerbate the generalised mucinopathy of CF. How this relates to any muclin-TFF interactions, which would be expected to inhibit this effect, is unknown.

The related DMBT-1 protein is known to be deleted in glioblastoma multiforme and in medulloblastoma, in four of five brain tumour cell lines, and may be a tumour suppressor gene [33]. Its role in normal brain is unknown, as is its relationship to TFF peptides in that organ. Of interest are reports that TFF3 is expressed in the brain, in particular in the oxytocinergic neurons of the paraventricular and supraoptic nuclei of the hypothalamus, as well as in the amygdala (reviewed by Hoffmann [34]). After

direct injection into the rat amygdale, TFF3 resulted in anxiolytic effects at low doses and anxiogenic effects at high doses [35]. Whether an interaction occurs with the DMBT1 family of splice variants is not known, but this may be a fruitful area to explore.

DMBT-1 is also reported to be downregulated in several other cancers, including skin [36], breast [37] and hepatic cholangiocarcinomas [38], to be variable, as were TFF peptide expressions, in bile duct disease [39], but upregulated in colorectal cancer [40] and biliary hepatolithiasis [41]. Such data suggest a complex interrelationship between tumorigenesis and the DMBT-1 expression patterns of any one cell type, and warrant much further investigation, including the interplay of TFF peptides.

The protein ebnerin was first described in von Ebner's glands from the rat, and has also been found in circumvallate papillae, lateral nasal glands and the olfactory epithelium [27], suggesting a much more restricted function than the other splice variants of the CRP-ductin/hensin family. No indications exist to date whether ebnerin may associate with any TFF peptide.

mTFF1 binding to von Willebrand Factor C domains

Tomasetto and colleagues [42] used a yeast two-hybrid method to determine binding partner proteins for mTFF1 from a mouse stomach-duodenal complementary DNA (cDNA) library. Two candidates were identified in the MUC2 and MUC5AC mucins, being the von Willebrand Factor (vWF) C1 and C2 domains, located towards the C-termini of both mucins. Both mMUC2 and mMUC5AC C domains share 100% conservation of 10 cysteine residues, though the patterns in each C1 or C2 subtype are slightly different. Binding of TFF1 to one C domain was lower than to both C domains of the same mucin, suggesting a degree of co-operativity. Further mutational analyses were not performed to elucidate the actual mTFF1 binding site(s).

TFF1 and TFF2 bind homologous gastric proteins: hTFIZ1 and mBlottin

Recent data suggest that CRP-ductin interacts with the pulmonary surfactant SP-D in a calcium-dependent manner, and is able to agglutinate invading bacteria in the lung [32], implying a role for this putative TFF2 binding partner in host defence. Westley et al. reported that TFF1 specifically and covalently binds to TFIZ1, a newly described 18 kDa secreted protein isolated from human stomach epithelium [43]. A homologous protein was found in mouse stomach, where it was isolated as a mTFF2-alkaline phosphatase fusion protein binding partner, and named blottin [44]. Both proteins are found in gastric foveolar cells and the secreted mucus layer, but there was no evidence of basolateral cell membrane insertion at the electron microscope level [45]. The expression of these proteins is thought to be downregulated in gastric cancer, as suggested by the original reports [46, 47], and subsequently corroborated [45]. A related protein, gastrokine-1 (formerly foveolin) is co-expressed with TFIZ1/blottin in the stomach foveolar and surface mucus cells, but does not appear to have TFF-binding activity [48]. It is of great interest that human TFIZ1 binds to hTFF1 monomer by a covalent bond and that the murine homologue blottin binds to mouse or human TFF2 directly. TFF1 uses its free sulphydryl (Cys 58) in covalently bonding to TFIZ1, whereas TFF2, under non-reducing conditions, has its N- and C-terminal cysteines covalently linked, and so presumably any binding to blottin occurs by salt bridges and/or hydrophobic interactions. The N/C terminal disulphide bond (Cys6–Cys104) of pTFF2 is solvent exposed, unlike the six structural disulphide bonds of the two trefoil domains [49], and is thus labile in the presence of reduced glutathione (GSH). This property may be involved in the possible control of cell proliferation, since pTFF2-stimulated HT-29 cells grew faster in a GSH-containing medium, but not one without [50]. So, the cell biological and biochemical relationships among the TFFs, the CRP-ductin family and TFIZ1/blottin in reducing and non-reducing conditions should be well worth exploring.

Has the brichos domain a role to play in TFF binding?

There are probably over 34 known brichos domains, which have been conserved over evolutionary time from worms and flies to mammals (reviewed by Sanchez-Pulido [51]). TFIZ1/blottin is another protein with this approximately 100 amino acid brichos domain, and it is similar to those contained in the surfactant proteins (SPs) SP-C and SP-D, both of which are expressed in the lung. Recent evidence suggests that mutations in exon 4 of the brichos domain of SP-C may lead to annexin V binding and apoptosis by activating caspase 3 [52]. Since it is well accepted that TFF peptides possess anti-apoptotic activity in several cell types in vitro and in vivo [53–56], this suggests that there may be a novel interaction between these peptides and the brichos domain that negatively regulates the apoptotic pathway. It has been recently reported that antisense TFF3 mRNA can induce apoptosis in the gastric cell line SNU-1 [57], and it would be interesting to see whether those cells also express the putative TFF1 or TFF2-binding protein TFIZ1/blottin.

Mucin binding of TFF peptides

Thim and colleagues [58] mixed purified TFFs with mucin and measured the subsequent changes in viscosity by rheometry. This parameter was raised 10-fold

when TFF2 was added to stomach mucin, forming an elastic gel, while dimers of TFF3 were less effective. The monomers TFF1 and TFF3 did not alter viscosity by much. Kindon et al. [59] reported that human T84 colon cancer cell monolayers could be protected from several insults if incubated with hTFF3 (monomer), as measured by a transwell ^{3}H-mannitol penetration assay. When precoated with hTFF3 mixed with human colonic mucin (but not with mucin from rat colon or stomach), this protection was enhanced almost 2-fold. Similar results were obtained with hTFF2 and rTFF3. While not formally proving the presence of binding phenomena, it would be surprising if significant ones were not involved in explaining these results.

A recent report has indicated that the human mucin MUC5AC binds to TFF1 in gastric mucosa [60]. These two molecules are co-packaged in the surface mucous and foveolar cells and thus are co-secreted onto the stomach surface where mutual binding would raise the mucous gel viscosity and enhance the protective effects needed to guard against acid or food-induced damage to the epithelium. These researchers showed that TFF1 co-segregates with MUC5AC on gel filtration, and that the interaction is predominantly that of the TFF1 homodimer. These interactions were disrupted by EDTA and EGTA, whereas calcium increased the amount of bound TFF1. MUC6 was not involved in TFF1 binding.

Helicobacter pylori binds to hTFF1 dimers

An allied report to the MUC5AC story is that *Helicobacter pylori*, a well-established human gastric carcinogenic organism, binds avidly to the dimeric form of hTFF1 and uses this interaction to further bind to porcine gastric mucin, to which it cannot bind without TFF1 being present [61]. Other organisms, such as *Campylobacter jejuni* and *Escherichia coli*, did not bind TFF1. The TFF1-*H. pylori* binding was found to be highly specific in competition assays on a Biacore surface plasmon resonance chip, in which covalently bound TFF1 captured the organisms, which could be released using excess soluble TFF1 or a monoclonal antibody to TFF1. Neither monomeric TFF1 and TFF3 nor TFF2 were studied in this report.

Platelet binding by frog skin BmTFF2

A newly described TFF peptide extracted from the skin of the frog *Bombina maxima* possesses two TFF domains, in which 12 trefoil domain cysteine residues seen in human TFF2 are identically conserved [62]. The peptide is not homologous to that encoded by the frog *Xenopus laevis* skin trefoil xP2 gene. BmTFF2 has the property of agglutinating human platelets in a dose-dependent manner. This interaction can be inhibited by a monoclonal antibody to the β3 integrin subunit, a component of the fibronectin receptor. This result confirms the previous report of Thim et al. [25] that the β subunit of the fibronectin receptor bound to porcine TFF2. It will be interesting to see which other TFF peptides can agglutinate platelets – it maybe a property of dimeric or two-domain TFFs rather than the monomers – and whether this property is involved in disorders of blood clotting or inflammatory conditions such as inflammatory bowel disease (IBD).

Concluding remarks

It is probably true to say that the TFF-binding proteins thus discovered have not been shown to possess that singular property of a receptor, the ability to transduce a signal to a responsive cell in a recognisable manner. It may be that no single molecule transduces a TFF signal, and that an unrecognised multimer of other transmembrane proteins is responsible, or that the TFF peptides augment or inhibit existing pathways in undiscovered ways. It seems likely that several gastrointestinal proteins expressed in close proximity to one another (CRP-ductin, hensin, TFIZ1/blottin, mucins with vWF domains) can all contribute to TFF-signalling events, and the resultant effect(s) may depend on context, such as gut growth and development, epithelial sheet breakdown, inflammation and so on. It will be fruitful to investigate a range of models with the expression of these proteins under conditional knockout or knockin control. Such studies would greatly enhance our understanding of their roles. Research in the next few years will no doubt illuminate these matters and lead to a fuller understanding of TFF peptide biology.

1 Jakowlew S. B., Breathnach R., Jeltsch J. M., Masiakowski P. and Chambon P. (1984) Sequence of the pS2 mRNA induced by estrogen in the human breast cancer cell line MCF-7. Nucleic Acids Res. **12:** 2861–2878
2 Jørgensen K. H., Thim L. and Jacobsen H. E. (1982) Pancreatic spasmolytic polypeptide (PSP): I. Preparation and initial chemical characterization of a new polypeptide from porcine pancreas. Regul. Pept. **3:** 207–219
3 Suemori S., Lynch-Devaney K. and Podolsky D. K. (1991) Identification and characterization of rat intestinal trefoil factor: tissue- and cell-specific member of the trefoil protein family. Proc. Natl. Acad. Sci. USA **88:** 11017–11021
4 Hoffmann W. (1988) A new repetitive protein from Xenopus laevis skin highly homologous to pancreatic spasmolytic polypeptide. J. Biol. Chem. **263:** 7686–7690
5 Hauser F. and Hoffmann W. (1991) xP1 and xP4. P-domain peptides expressed in Xenopus laevis stomach mucosa. J. Biol. Chem. **266:** 21306–21309
6 Thim L. and May F. E. B. (2005) Cell. Mol. Life Sci. **(this issue)**.
7 Chinery R. and Cox H. M. (1995) Modulation of epidermal growth factor effects on epithelial ion transport by intestinal trefoil factor. Br. J. Pharmacol. **115:** 77–80
8 Playford R. J., Marchbank T., Chinery R., Evison R., Pignatelli M., Boulton R. A. et al. (1995) Human spasmolytic polypeptide is a cytoprotective agent that stimulates cell migration. Gastroenterology **108:** 108–116

9 Alison M. R., Chinery R., Poulsom R., Ashwood P., Longcroft J. M. and Wright N. A. (1995) Experimental ulceration leads to sequential expression of spasmolytic polypeptide, intestinal trefoil factor, epidermal growth factor and transforming growth factor alpha mRNAs in rat stomach. J. Pathol. **175:** 405–414

10 Dignass A., Lynch-Devaney K., Kindon H., Thim L. and Podolsky D. K. (1994) Trefoil peptides promote epithelial migration through a transforming growth factor beta-independent pathway. J. Clin. Invest. **94:** 376–383

11 Chinery R. and Playford R. J. (1995) Combined intestinal trefoil factor and epidermal growth factor is prophylactic against indomethacin-induced gastric damage in the rat. Clin. Sci. **88:** 401–403

12 Liu D., el-Hariry I., Karayiannakis A. J., Wilding J., Chinery R., Kmiot W. et al. (1997) Phosphorylation of beta-catenin and epidermal growth factor receptor by intestinal trefoil factor. Lab. Invest. **77:** 557–563

13 Efstathiou J. A., Noda M., Rowan A., Dixon C., Chinery R., Jawhari A. et al. (1998) Intestinal trefoil factor controls the expression of the adenomatous polyposis coli-catenin and the E-cadherin-catenin complexes in human colon carcinoma cells. Proc. Natl. Acad. Sci. USA **95:** 3122–3127

14 Taupin D. and Podolsky D. K. (2003) Trefoil factors: initiators of mucosal healing. Nat. Rev. Mol. Cell. Biol. **4:** 721–732

15 Frandsen E. K., Jørgensen K. H. and Thim L. (1986) Receptor binding of pancreatic spasmolytic polypeptide (PSP) in rat intestinal mucosal cell membranes inhibits the adenylate cyclase activity. Regul. Pept. **16:** 291–297

16 Chinery R. and Cox H. M. (1995) Immunoprecipitation and characterization of a binding protein specific for the peptide, intestinal trefoil factor. Peptides **16:** 749–755

17 Chinery R., Poulsom R., Elia G., Hanby A. M. and Wright N. A. (1993) Expression and purification of a trefoil peptide motif in a beta-galactosidase fusion protein and its use to search for trefoil-binding sites. Eur. J. Biochem. **212:** 557–563

18 Tan X. D., Hsueh W., Chang H., Wei K. R. and Gonzalez-Crussi F. (1997) Characterization of a putative receptor for intestinal trefoil factor in rat small intestine: identification by in situ binding and ligand blotting. Biochem. Biophys. Res. Commun. **237:** 673–677

19 Tan X. D., Liu Q. P., Hsueh W., Chen Y. H., Chang H. and Gonzalez-Crussi F. (1999) Intestinal trefoil factor binds to intestinal epithelial cells and induces nitric oxide production: priming and enhancing effects of mucin. Biochem. J. **338 (Pt. 3):** 745–751

20 Poulsen S. S., Thulesen J., Nexo E. and Thim L. (1998) Distribution and metabolism of intravenously administered trefoil factor 2/porcine spasmolytic polypeptide in the rat. Gut **43:** 240–247

21 Poulsen S. S., Thulesen J., Christensen L., Nexø E. and Thim L. (1999) Metabolism of oral trefoil factor 2 (TFF2) and the effect of oral and parenteral TFF2 on gastric and duodenal ulcer healing in the rat. Gut **45:** 516–522

22 Poulsen S. S., Thulesen J., Hartmann B., Kissow H. L., Nexø E. and Thim L. (2003) Injected TFF1 and TFF3 bind to TFF2-immunoreactive cells in the gastrointestinal tract in rats. Regul. Pept. **115:** 91–99

23 Poulsen S. S., Kissow H., Hare K., Hartmann B. and Thim L. (2005) Luminal and parenteral TFF2 and TFF3 dimer and monomer in two models of experimental colitis in the rat. Regul. Pept. **126:** 163–171

24 Thim L. and Mortz E. (2000) Isolation and characterization of putative trefoil peptide receptors. Regul. Pept. **90:** 61–68

25 Takito J., Yan L., Ma J., Hikita C., Vijayakumar S., Warburton D. and Al-Awqati Q. (1999) Hensin, the polarity reversal protein, is encoded by DMBT1, a gene frequently deleted in malignant gliomas. Am. J. Physiol. **277:** F277–289

26 Li X. J. and Snyder S. H. (1995) Molecular cloning of Ebnerin, a von Ebner's gland protein associated with taste buds. J. Biol. Chem. **270:** 17674–17679

27 Matsushita F., Miyawaki A. and Mikoshiba K. (2000) Vomeroglandin/CRP-Ductin is strongly expressed in the glands associated with the mouse vomeronasal organ: identification and characterization of mouse vomeroglandin. Biochem. Biophys. Res. Commun. **268:** 275–281

28 Takito J., Hikita C. and Al-Awqati Q. (1996) Hensin, a new collecting duct protein involved in the in vitro plasticity of intercalated cell polarity. J. Clin. Invest. **98:** 2324–2331

29 Takito J. and Al-Awqati Q. (2004) Conversion of ES cells to columnar epithelia by hensin and to squamous epithelia by laminin. J. Cell Biol. **166:** 1093–1102

30 Vijayakumar S., Takito J., Hikita C. and Al-Awqati Q. (1999) Hensin remodels the apical cytoskeleton and induces columnarization of intercalated epithelial cells: processes that resemble terminal differentiation. J. Cell Biol. **144:** 1057–1067

31 Lisle R. C. de, Petitt M., Isom K. S. and Ziemer D. (1998) Developmental expression of a mucinlike glycoprotein (MUCLIN) in pancreas and small intestine of CF mice. Am. J. Physiol. **275:** G219–227

32 Lisle R. C. de and Ziemer D. (2000) Processing of pro-Muclin and divergent trafficking of its products to zymogen granules and the apical plasma membrane of pancreatic acinar cells. Eur. J. Cell Biol. **79:** 892–904

33 Mollenhauer J., Wiemann S., Scheurlen W., Korn B., Hayashi Y., Wilgenbus K. K. et al. (1997) DMBT1, a new member of the SRCR superfamily, on chromosome 10q25.3–26.1 is deleted in malignant brain tumours. Nat. Genet. **17:** 32–39

34 Hoffmann W. and Jagla W. (2002) Cell type specific expression of secretory TFF peptides: colocalization with mucins and synthesis in the brain. Int. Rev. Cytol. **213:** 147–181

35 Schwarzberg H., Kalbacher H. and Hoffmann W. (1999) Differential behavioral effects of TFF peptides: injections of synthetic TFF3 into the rat amygdala. Pharmacol. Biochem. Behav. **62:** 173–178

36 Mollenhauer J., Deichmann M., Helmke B., Muller H., Kollender G., Holmskov U. et al. (2003) Frequent downregulation of DMBT1 and galectin-3 in epithelial skin cancer. Int. J. Cancer **105:** 149–157

37 Braidotti P., Nuciforo P. G., Mollenhauer J., Poustka A., Pellegrini C., Moro A.et al. (2004) DMBT1 expression is downregulated in breast cancer. BMC Cancer **4:** 46

38 Sasaki M., Huang S. F., Chen M. F., Jan Y. Y., Yeh T. S., Ishikawa A. et al. (2003) Decrease of deleted in malignant brain tumour-1 (DMBT-1) expression is a crucial late event in intrahepatic cholangiocarcinoma. Histopathology **43:** 340–346

39 Sasaki M., Tsuneyama K., Saito T., Kataoka H., Mollenhauer J., Poustka A. et al. (2004) Site-characteristic expression and induction of trefoil factor family 1, 2 and 3 and malignant brain tumor-1 in normal and diseased intrahepatic bile ducts relates to biliary pathophysiology. Liver Int. **24:** 29–37

40 Robbe C., Paraskeva C., Mollenhauer J., Michalski J. C., Sergi C. and Corfield A. (2005) DMBT1 expression and glycosylation during the adenoma-carcinoma sequence in colorectal cancer. Biochem. Soc. Trans. **33:** 730–732

41 Sasaki M., Huang S. F., Chen M. F., Jan Y. Y., Yeh T. S., Ishikawa A. et al. (2003) Expression of deleted in malignant brain tumor-1 (DMBT1) molecule in biliary epithelium is augmented in hepatolithiasis: possible participation in lithogenesis. Dig. Dis. Sci. **48:** 1234–1240

42 Tomasetto C., Masson R., Linares J. L., Wendling C., Lefebvre O., Chenard M. P. et al. (2000) pS2/TFF1 interacts directly with the VWFC cysteine-rich domains of mucins. Gastroenterology **118:** 70–80

43 Westley B. R., Griffin S. M. and May F. E. (2005) Interaction between TFF1, a gastric tumor suppressor trefoil protein, and TFIZ1, a brichos domain-containing protein with homology to SP-C. Biochemistry **44:** 7967–7975

44 Otto W. R., McKinnell I., Lee C.-Y., Frith D., Hanrahan S., Blin N. et al. (2004) Blottin: a novel TFF2-binding protein. Gut **53:** A15–A16

45 Otto W. R., Patel K., McKinnell I., Evans M. D., Lee C.-Y., Frith D. et al. (2005) Identification of blottin : a novel trefoil factor family-2 binding protein. unpublished observations
46 Du J. J., Dou K. F., Peng S. Y., Wang W. Z., Wang Z. H., Xiao H. S. et al. (2003) [Down-regulated full-length novel gene GDDR and its effect on gastric cancer]. Zhonghua Yi Xue Za Zhi **83:** 1166–1168
47 Du J. J., Dou K. F., Peng S. Y., Li J. T., Wang W. Z., Guan W. X. et al. (2005) [Study on novel gene GDDR related to gastric cancer]. Zhonghua Wai Ke Za Zhi **43:** 10–13
48 Oien K. A., McGregor F., Butler S., Ferrier R. K., Downie I., Bryce S. et al. (2004) Gastrokine 1 is abundantly and specifically expressed in superficial gastric epithelium, down-regulated in gastric carcinoma and shows high evolutionary conservation. J. Pathol. **203:** 789–797
49 De A., Brown D. G., Gorman M. A., Carr M., Sanderson M. R. and Freemont P. S. (1994) Crystal structure of a disulfide-linked 'trefoil' motif found in a large family of putative growth factors. Proc. Natl. Acad. Sci. USA **91:** 1084–1088
50 Otto W. R., Rao J., Cox H. M., Kotzian E., Lee C. Y., Goodlad R. A. et al. (1996) Effects of pancreatic spasmolytic polypeptide (PSP) on epithelial cell function. Eur. J. Biochem. **235:** 64–72
51 Sanchez-Pulido L., Devos D. and Valencia A. (2002) BRICHOS: a conserved domain in proteins associated with dementia, respiratory distress and cancer. Trends Biochem. Sci. **27:** 329–332
52 Mulugeta S., Nguyen V., Russo S. J., Muniswamy M. and Beers M. F. (2005) A surfactant protein C precursor protein BRICHOS domain mutation causes endoplasmic reticulum stress, proteasome dysfunction and caspase 3 activation. Am. J. Respir. Cell Mol. Biol. **32:** 521–530
53 Chen Y. H., Lu Y., De Plaen I. G., Wang L. Y. and Tan X. D. (2000) Transcription factor NF-kappaB signals antianoikic function of trefoil factor 3 on intestinal epithelial cells. Biochem. Biophys. Res. Commun. **274:** 576–582
54 Siu L. S., Romanska H., Abel P. D., Baus-Loncar M., Kayademir T., Stamp G. W. et al. (2004) TFF2 (trefoil family factor2) inhibits apoptosis in breast and colorectal cancer cell lines. Peptides **25:** 855–863
55 Taupin D. R., Kinoshita K. and Podolsky D. K. (2000) Intestinal trefoil factor confers colonic epithelial resistance to apoptosis. Proc. Natl. Acad. Sci. USA **97:** 799–804
56 Kinoshita K., Taupin D. R., Itoh H. and Podolsky D. K. (2000) Distinct pathways of cell migration and antiapoptotic response to epithelial injury: structure-function analysis of human intestinal trefoil factor. Mol. Cell. Biol. **20:** 4680–4690
57 Chan M. W., Chan V. Y., Leung W. K., Chan K. K., To K. F., Sung J. J. et al. (2005) Anti-sense trefoil factor family-3 (intestinal trefoil factor) inhibits cell growth and induces chemosensitivity to adriamycin in human gastric cancer cells. Life Sci. **76:** 2581–2592
58 Thim L., Madsen F. and Poulsen S. S. (2002) Effect of trefoil factors on the viscoelastic properties of mucus gels. Eur. J. Clin. Invest. **32:** 519–527
59 Kindon H., Pothoulakis C., Thim L., Lynch-Devaney K. and Podolsky D. K. (1995) Trefoil peptide protection of intestinal epithelial barrier function: cooperative interaction with mucin glycoprotein. Gastroenterology **109:** 516–523
60 Ruchaud-Sparagano M. H., Westley B. R. and May F. E. (2004) The trefoil protein TFF1 is bound to MUC5AC in human gastric mucosa. Cell. Mol. Life Sci. **61:** 1946–1954
61 Clyne M., Dillon P., Daly S., O'Kennedy R., May F. E., Westley B. R. et al. (2004) Helicobacter pylori interacts with the human single-domain trefoil protein TFF1. Proc. Natl. Acad. Sci. USA **101:** 7409–7414
62 Zhang J., Zhang Y., Wan S. G., Wei S. S. and Lee W. H. (2005) Bm-TFF2, a trefoil factor protein with platelet activation activity from frog Bombina maxima skin secretions. Biochem. Biophys. Res. Commun. **330:** 1027–1033
63 Taupin D., Wu D. C., Jeon W. K., Devaney K., Wang T. C. and Podolsky D. K. (1999) The trefoil gene family are coordinately expressed immediate-early genes: EGF receptor- and MAP kinase-dependent interregulation. J. Clin. Invest. **103:** R31–8.