Minireview

Rolling in the clover: trefoil factor family (TFF)-domain peptides, cell migration and cancer

N.A. Wright[a,*], W. Hoffmann[b], W.R. Otto[a], M.-C. Rio[c], L. Thim[d]

[a]*Histopathology Unit, Imperial Cancer Research Fund, London WC2A 3PX, UK*
[b]*Institute for Molecular Biology and Medical Chemistry, University of Magdeburg, Leipziger Strasse 44, Magdeburg D-39120, Germany*
[c]*INSERM U184, Institut de Génetique et de Biologie Moleculaire et Cellulaire, Université Louis Pasteur, Strasbourg, France*
[d]*Department of Protein Chemistry, Novo Nordisk, Bagsvaerd DK-2880, Denmark*

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Abstract Trefoil factor family (TFF)-domain peptides 1–3 are mucin-associated molecules, largely found in epithelia of gastrointestinal tissues. Structurally similar, resistant to enzymatic degradation, they are up-regulated around areas of epithelial damage such as ulcers. Transgenic expression or exogenous peptide ameliorates or prevents gastric mucosal damage due to indomethacin and some are rapidly up-regulated after cryogenic burns. A role in promoting cell migration is strongly suggested. Knockout mice lacking TFF1 or TFF3 show significant pathology, with the former developing gastric tumours. A recent *Conférence Philippe Laudat* agreed upon a new nomenclature for these peptides.

Key words: Trefoil factor family; TFF-domain peptide; Cancer; Cell migration; Ulcer; Invasion

1. Introduction

The finding [1] in 1982 of a peptide abundant in porcine pancreatic extracts during insulin preparation, and the contemporaneous discovery of a cDNA clone during a search for estrogen-induced mRNAs in a breast cancer cell line [2], has developed into the burgeoning field of trefoil/P-domain peptide research, the subject of an excellent Philippe Laudat Conference[1]. Rechristened the Trefoil Factor Family (TFF) [3] domain peptides, these molecules are small, secreted and stable, bear one or more conserved motifs [4], and are very abundantly expressed wherever mucin secretion occurs, most prominently in the mucin-secreting cells of the gastrointestinal mucosa [5]. The TFF domain (Fig. 1) shows a structure tightly held together by three pairs of disulfide bonds [6,7]. These shuffled modules have been highly conserved during evolution; in *Xenopus*, peptides as well as mucins are known that contain multiple TFF domains [8]. Mammalian trefoil factors contain either one or two of these domains, but there is some debate on the necessity of dimerisation of the single domain peptides for function. These peptides are synthesised in mucus-secreting cells, co-packaged with the mucus granules, and are secreted onto epithelial surfaces together with the mucus. It is becoming apparent that TFFs are also a new family of neuropeptides.

2. Different trefoil peptides: new nomenclature

Early work (reviewed in [9]) described the expression and distribution of the molecules. In man there are three known trefoil factors (Fig. 2): TFF1, a single trefoil peptide originally found in breast cancer cell lines but produced mainly in the stomach (originally called pS2 or breast cancer estrogen-inducible: BCEI); TFF2 (formerly spasmolytic peptide: SP) which is abundant in the stomach and duodenal Brunner's glands; and TFF3 (previously called intestinal trefoil factor: ITF) a further single-domain peptide expressed throughout the intestine. For several years after their isolation, no function was ascribable to these molecules, although their increased expression in chronic inflammatory bowel disease and around experimental and peptic ulcers presaged a role in mucosal defence and healing. In experimental gastric damage some are up-regulated very rapidly [10]. However, the advent of recombinant peptides [11] has allowed both in vitro and in vivo studies. It is clear that all TFF peptides thus far tested are *motogens*; they stimulate epithelial cell migration in a variety of test systems. It was reported at the meeting that TFF3, like hepatocyte growth factor (scatter factor) and EGF, both powerful motogens, induces the rapid phosphorylation of β-catenin and the down-regulation of E-cadherin. Interestingly, epithelial cells stably transfected with TFF1 show strikingly diffuse growth patterns in agarose gels, again indicating an action on cell migration.

3. Trefoil peptides and mucosal damage

In vivo TFF domain peptides are heavily overexpressed in the epithelial cells migrating across the base of gastrointestinal ulcers, and when subcutaneously administered, will protect against experimentally induced mucosal damage [12]; larger doses given orally have the same effect [13]. It would thus appear that TFFs have a role in inducing the important phenomenon of *restitution*, in which gastrointestinal epithelial

*Corresponding author. Fax: (44) 181-383-3203.
E-mail: nwright@rpms.ac.uk

Abbreviations: BCEI, breast cancer estrogen-inducible; EGF, epidermal growth factor; ITF, intestinal trefoil factor; SP, spasmolytic polypeptide; TFF, trefoil factor family

[1]Conférence Philippe Laudat: Trefoil/P-domain peptides: from basic research to molecular medicine. Aix-les-Bains, France, 29 September to 3 October 1996. The scientific organising committee (N.A. Wright (President), W. Hoffmann, W.R. Otto, M.-C. Rio and L. Thim) thank the generosity of the Institut National de la Santé et de la Recherche Médicale (INSERM, Paris) for funding this conference.

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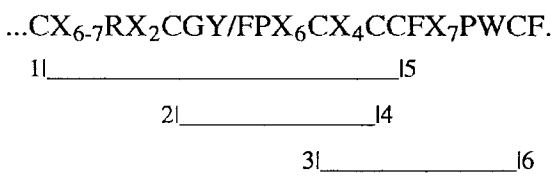

Fig. 1. Consensus sequence of mammalian Trefoil Factor Family domains. A single 'trefoil' motif sequence is shown, with arrangement of disulfide bonds.

cells are able to rapidly migrate across a denuded surface, without dividing, to effect closure. Thus trefoil factors can be regarded as a potentially important mucosal defence mechanism, already present in the visco-elastic mucus layer overlying the epithelium, and rapidly up-regulated after mucosal damage.

How do TFF domain peptides exert this effect? Current opinion seems sharply divided. Because of the close association with mucus, one suggestion is that these peptides interacted with the mucus, possibly to stabilise the mucus gel by binding the long mucus molecules together, either by the oligosaccharide side chains or the core protein at exposed sites; damaging substances such as gastric acid would therefore not reach the epithelial surface so readily. Indeed, the addition of trefoil factors to purified mucin preparations leads to a rapid increase in optical density and viscosity (and presumably therefore on mucus gel formation) and the combination acts synergistically in cell migration assays [13]. However, there is also early evidence for receptor activation after trefoil peptide application to epithelial cells; the stimulation of electrogenic chloride secretion induced by TFF3 only occurs after application to the basolateral surface of human cell lines or rat jejunum [14]. TFF3 appears to bind a protein present in membrane preparations of colorectal epithelial cells which is accompanied by phosphorylation on tyrosine, and the phosphorylation of β-catenin mentioned above occurs within 10 s — redolent of a receptor-mediated response [15]. rTFF3 and EGF act synergistically in stimulating cell migration and preventing mucosal damage [16], and of course recent work has established a link between β-catenin and EGFR in EGF-mediated signal transduction.

Thus trefoil factors appear to be motogens; early work which suggested an action of TFF2 in stimulating epithelial cell proliferation was found to be a conditional response to a reducing (glutathione) environment [17]. Although it has been suggested that trefoil factors may mediate a reduction in apoptosis, mice in which the *TFF3* gene has been knocked out apparently show more proliferating cells in their colonic crypts, despite no change in their crypt height [18].

Initial experience with over- or under-expression by transgenic and knockout mice reinforced the concept that trefoil factors conferred mucosal protection: mice ectopically expressing hTFF1 in the jejunum [19] are resistant to indomethacin-induced damage, and mice in which the *TFF3* gene has been knocked out [18] are very sensitive to dextran sodium sulphate-induced colitis, and do not form a migrating tongue of epithelium across the surface of ulcers. Neither of these mice showed a morphological phenotype nor developed any apparent disease. However, when the *mTFF1* gene was knocked out, significant pathology was found [20]. All of the mice lost the expression of gastric mucus and developed adenomas in the antrum of the stomach, and some of these went on to develop frankly invasive carcinomas. Because hTFF1 expression is lost in some 50% of human gastric carcinomas, Lefebvre and colleagues suggest that TFF1 must therefore be a candidate tumour suppressor gene in the stomach. The mechanism of this merits further investigation but certainly brings these enigmatic peptides sharply into focus.

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Old Locus Name	New Locus Name	Old Peptide Name	New Peptide Name	TFF Domains	Major Sites
BCEI	*TFF1*	pS2/BCEI/pNR-2 pNR-105/Md2	TFF1	1	Stomach
SML1	*TFF2*	Spasmolytic Polypeptide	TFF2	2	Stomach, Duodenum
------	*TFF3*	ITF/P1.B	TFF3	1	Intestine

Fig. 2. New Nomenclature of Mammalian Trefoil Factor Family-Domain Peptides. 'TFF-domain' should be a Keyword for future publications and literature searching. Old locus names may be cited as aliases, but the new names should be used preferentially. A lower-case prefix may indicate species (h, human; m, mouse; r, rat; etc.).

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