Multi-author Review
Trefoil factors

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Cytoprotective trefoil peptides abound in new functions

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Trefoil peptides entered the scientific stage more than 20 years ago when in the early 1980s Jorgensen and coworkers [1] reported their PSP (pancreatic spasmolytic peptide) and Westley and Rochefort [2] published an unknown 46-kD estrogen-inducible protein for which Masiakowski et al. [3] isolated and characterised the corresponding complementary DNA (cDNA) sequence (pS2). Due to the different laboratories and experimental methods involved a heterogeneous collection of names (some of them still lingering today) was introduced for the three mammalian genes and their peptides. Since some names caused for confusion (hSP resembles heat shock proteins, pS2 is similar to presenilin 2 etc.), a homogeneous assignment approved by the gene nomenclature committee was introduced at the First Trefoil Peptide Meeting in Aix-les-Bains organised in1996 by the Conférences Philippe Laudat [4]. Thus, the family of TFF1, TFF2 and TFF3 came into existence based on the observation of the particular clover-like folding (trefoil domain, fig. 1) of the peptides [5]. The trefoil motif (C-X_{9-10}-C-X_9-C-X_4-C-C-X_{10}-C) is the hallmark of these vertebrate peptides; if somewhat modified (C-$X_{5,6}$-[ST]-X_3-C-$X_{4,5}$-C-C-[FYWH]-X_{2-24}-C-[FY]), it can be traced back to primitive chordates such as tunicates or even non-vertebrates. While *Saccharomyces* lacks such sequences the evolutionary dawn of this motif seemingly dates back to an open reading frame (ORF) in *Caenorhabditis elegans* [6]. It is not restricted to peptides secreted by epithelia; it also occurs in eggshell and *zona pelucida* peptides [7, 8].

Understanding the function of TFFs proved a much more laborious task. First, observations linked them with mucin expression (reviewed in [9]), and solid experimental evidence still supports the notion of a physical TFF-mucin interaction [10]. Further data also point to a cytoprotective function in the digestive tract [11–13]. Data from many additional studies, however, led to a much more complex picture: TFFs can serve as motogenes [14, 15]; are linked to anti-apoptosis [16 ,17]; their absence, e. g. via methylation of the gene's promoter [18] or experimental impairment (knockout mice [19]) is connected with neoplastic development; they participate in the immune response [20, 21], can trigger chemotaxis [22] and

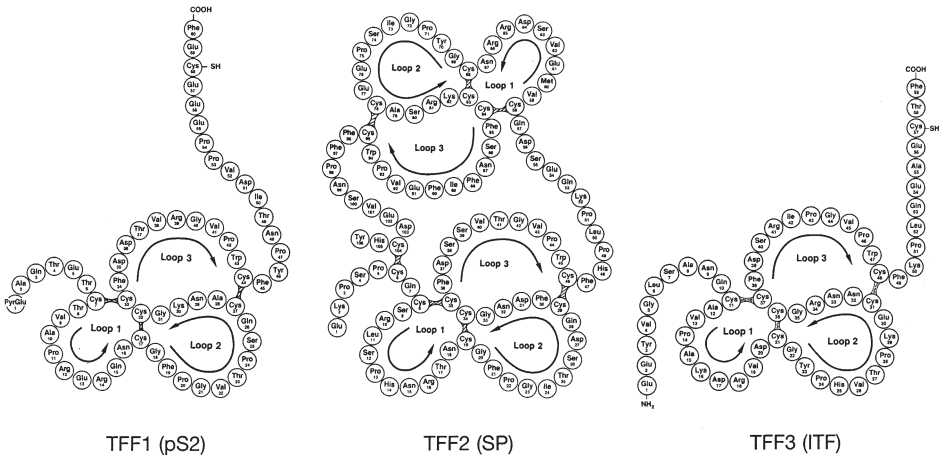

Figure 1. Trefoil structure of the three human TFF peptides, suggested by Thim [5].

are possibly involved in neural development [23]. In mammals all three TFF genes are clustered within a close distance, in humans within 50 kb of the chromosomal band 21q22.3 [24]. They share similar cis-regulatory motifs suggestive of co-ordinated control of expression, but they also display specific signalling sequences allowing for unique regulation [25]. Therefore, each TFF peptide shows a particular expression pattern, and different mucin-producing cells are characterised by their specific TFF peptide/secretory mucin combinations. This TFF specificity extends to other cell types and organs that lack mucin expression [26].

In the early days, sufficient evidence was accumulated to imply that TFFs were strongly connected with tumorigenesis by changing their expression pattern in a multitude of tumours [27], and TFF1, e. g., was suggested as a new marker for breast tumour prognosis [28] or listed as a tumour suppressor gene [19]. One of the following reviews will present interesting data along this line (Tomasetto and Rio). TFF involvement in dysplasia and neoplasia of the digestive tract will be revisited (Regalo et al.). Our increasing knowledge of expanding functions assigned to TFFs is summarised in several reviews, also discussing regulatory pathways and TFF-triggered signals (Baus and Giraud; Hoffmann). Such new tasks within and outside the cell require additional interacting partners (structural peptides, receptors, co-ligands), and surprisingly, no convincing candidate for a receptor has been found as yet. But binding motifs (such as von Willebrand factor) [29] and peptides appear on the scene (reviewed by Otto and Thim). Structural elucidation of the TFFs will surely aid in this quest (see Thim and May). Moreover, since genes for all three TFFs were successfully knocked out in mouse models [19, 30, 31], we are able to study such effects in vivo. Evidently, TFF2 deficiency, not exerting a drastic phenotype in the $Tff2^{-/-}$ animals [21, 31] is, however, connected with a significant response of the innate immune defence (reviewed by Baus-Loncar et al.). Due to mostly preliminary data the only field remaining not discussed here is the presence of TFFs in neural cells or their possible function as neuropeptides. Some data indicate that the protein coded by the *deleted in the malignant brain tumor-1* gene (*DMBT1*) may to interact with TFFs [32, 33]; newer findings point to the presence of TFFs in specialised cells of the neurosensory system [our unpublished data]. In the context of neural cells, the TFFs thus still await a thorough functional description and were only briefly reviewed by Hoffmann and Jagla [34].

Where will our increasing understanding of TFF function take us considering that, originally, TFF2 (as PSP, porcine spasmolytic peptide) was fortuitously discovered as a side fraction during insulin purification [1]? This fact in no way diminishes the significance of these peptides when one remembers that many currently important proteins (e. g. restriction endonucleases, monoclonal antibodies) were also detected more or less by chance. Interestingly, the participation of the TFFs in dysplasia and neoplasia implied possible diagnostic or prognostic applications; their cytoprotective function even suggested clinical benefits. Thus, not surprisingly, about 10 US and European patents were filed starting in 1983 and continuing until today. They cover various peptide forms, a screen for gastric adenocarcinoma and mucosal repair (http://ep.espacenet.com; www.uspto.gov). Delivering TFF as a drug in isolated form seems to encounter problems [35]; thus new ways of application with microbial help are being tested, as exemplified by the ingenious use of TFF-synthesising *Lactobacillus* [36]. While commercial gains, speculative at the present, will require the test of time, the scientific part of the trefoil peptide story is already now full of highly interesting developments. The following Reviews prove this fact.

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