REVIEW ARTICLE

The trappin gene family: proteins defined by an N-terminal transglutaminase substrate domain and a C-terminal four-disulphide core

Joost SCHALKWIJK^{*1}, Oliver WIEDOW[†] and Shigehisa HIROSE[‡]

^{*}Department of Dermatology, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands, [†]Department of Dermatology, University of Kiel, Schittenhelmstr. 7, D-24105 Kiel, Germany, and [‡]Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226, Japan

Recently, several new genes have been discovered in various species which are homologous to the well-characterized human epithelial proteinase inhibitor elafin/SKALP (skin-derived anti-leukoproteinase). Because of the high degree of conservation and the similarities in genomic organization, we propose that these genes belong to a novel gene family. At the protein level, the family members are defined by: (1) an N-terminal domain consisting of a variable number of repeats with the consensus sequence Gly-Gln-Asp-Pro-Val-Lys that can act as an anchoring motif by transglutaminase cross-linking, and (2) a C-terminal four-disulphide core or whey acidic protein (WAP) domain, which harbours a functional motif involved in binding of proteinases and possibly other proteins. We have proposed the name trappin gene family as a unifying nomenclature for this

group of proteins (trappin is an acronym for **TR**ansglutaminase substrate and **wAP** domain containing **ProteIN**, and refers to its functional property of 'getting trapped' in tissues by covalent cross-linking). Analysis of the trappin family members shows extensive diversification in bovidae and suidae, whereas the number of primate trappins is probably limited. Recent biochemical and cell biological data on the human trappin family member elafin/SKALP suggest that this molecule is induced in epidermis by cellular stress. We hypothesize that trappins play an important role in the regulation of inflammation and in protection against tissue damage in stratified epithelia.

Key words: epithelia, proteinase inhibitors, transglutaminases.

HISTORY

In 1989, Araki et al. [1] described a protein from pig duodenum that had inhibitory activity towards Na⁺/K⁺-ATPase, and was named sodium/potassium ATPase inhibitor-2 (SPAI-2). SPAI-2 had structural similarity to the serine proteinase inhibitor secretory leucocyte proteinase inhibitor (SLPI) [2] (also known as anti-leucoprotease [3] and human seminal fluid inhibitor [4]), which is a member of the four-disulphide core protein or whey acidic protein (WAP) family [5]. In 1990 two groups independently described an elastase inhibitor purified from psoriatic scales. Wiedow et al. [6] coined the name elafin (referring to its function of inhibiting elastase), whereas Schalkwijk et al. [7] used the name skin-derived anti-leucoproteinase (SKALP), which referred to its similarity to anti-leucoprotease/SLPI and its source. The amino acid sequences of elafin and SKALP indicated that they represented the same molecule [8], and that this protein was homologous to SLPI. Subsequently, Sallenave et al. [9] isolated an elastase-specific inhibitor (ESI) from human lung sputum, and N-terminal sequencing indicated that it was identical to elafin/SKALP. Cloning of the elafin/SKALP/ESI cDNA and gene revealed that the molecule was larger than previously anticipated [10–12]. In addition to the proteinase-inhibiting domain, which was given the name elafin in the original paper by Wiedow et al. [6], an N-terminal domain was found that had no structural similarity to SLPI, or to any of the WAP family members. This domain was similar to the guinea pig seminal

vesicle protein, which was known to be a transglutaminase substrate, involved in formation of the vaginal plug. The name cementoin was coined by Nara et al. [13] to denote this N-terminal anchoring motif. The genes for pig SPAI-2, pig elafin/SKALP and a new pig protein (pWAP-3) have now been cloned, and it was shown that these proteins are homologous and have similar genomic organization [14,15]. Zeeuwen et al. [16] cloned partial cDNAs from bovine tongue which contained the transglutaminase substrate domain and the WAP domain, and are probably also new SPAI-2/elafin/SKALP family members.

NOMENCLATURE

We have proposed the name 'trappin' gene family as a unifying nomenclature for this group of proteins [16,17], trappin being an acronym for **TR**ansglutaminase substrate and **wAP** domain containing **ProteIN**, referring to its property of getting 'trapped' in a tissue and thereby functioning as an anchored protein. Trappin family members are defined by an N-terminal transglutaminase substrate domain consisting of hexapeptide repeats with the consensus sequence Gly-Gln-Asp-Pro-Val-Lys (GQDPVK), and a C-terminal four-disulphide core. Numbering of the trappins will be according to their order of identification. SPAI-2 will therefore be designated trappin-1, and elafin/SKALP/ESI as trappin-2. Tentative numbering for recently described trappins is given in Zeeuwen et al. [16] and Furutani et al. [17]. Apparent orthologues will be given the same number, e.g. trappin-2 has now been identified in human, monkey, pig,

Abbreviations used: SKALP, skin-derived anti-leukoproteinase; SLPI, secretory leucocyte proteinase inhibitor; WAP, whey acidic protein; ESI, elastase-specific inhibitor; SPAI, sodium/potassium ATPase inhibitor; REST genes, rapidly evolving seminal vesicle transcribed genes; TNF- α , tumour necrosis factor- α ; AP-1, activator protein-1.

¹ To whom correspondence should be addressed (e-mail j.schalkwijk@derma.azn.nl).

Figure 1 Structural features of trappins

(a) Covalent cross-linking of trappins to other proteins through the transglutaminase substrate domain. (b) Domain structure of the trappin precursor. (c) Mechanism of action of transglutaminase, which catalyses the formation of the isopeptide bond. A glutamine residue (Q) acts as an acyl donor and a lysine residue (K) serves as an acceptor. The catalytic thiol group involved in the formation of an acyl-enzyme intermediate is also shown (for reviews, see [62,63]). (d) Three-dimensional structure of human trappin-2 (SKALP/elafin) determined by X-ray crystallography [21] and NMR spectroscopy [64]. The four disulphide bonds are indicated by broken lines. (e) Amino acid sequence comparison of the WAP motif of trappins [1,6,15,16] and closely related proteins: caltrin-like protein (EMBL/GenBank accession no. P22075) and SLPI (EMBL/GenBank accession nos. X04470 and M57446). An arrow indicates the cleavage site in human trappin-2 that generates a small 6 kDa species that lacks the transglutaminase substrate domain; (d) shows this small species of 57 amino acid residues. The boxed region (region V) represents highly variable sequences that interact with the target enzymes.

wart-hog and cow. We propose that, for historical reasons, the old names such as SPAI-2 and elafin/SKALP/ESI can still be used, but that new members will be given systematic names (trappin followed by a number), to avoid further confusion.

In this article both the names trappin-2 and elafin will be used. The reason for this is that many biochemical studies were performed with purified or recombinant material which consisted of the 57 C-terminal amino acids of trappin-2 (the four-disulphide

core), which was originally described as elafin. Hence when the name elafin is used in this review, it refers to studies with the 57-amino-acid C-terminal domain.

STRUCTURAL ASPECTS OF TRAPPIN FAMILY MEMBERS

Protein structure

Members of the trappin family contain two functional domains (Figure 1b), a transglutaminase substrate domain composed of multiple hexapeptide repeats with the consensus sequence GQDPVK, and a C-terminal inhibitor domain that folds into the four-disulphide core structure (Figure 1d). As the term four-disulphide core implies, the C-terminal domain contains eight conserved cysteine residues that form four disulphide bonds and stabilize the inhibitor domain (Figure 1e). The resulting compact structure is also called the WAP motif [18]. The name WAP derives from the well-characterized member of the protein family with the four-disulphide core, whey acidic protein, which is the most abundant protein in rodent milk. The four-disulphide core structure is found in a wide variety of proteins, including whey proteins, proteinase inhibitors, neurophysins, plant agglutinin, adhesion molecules, scorpion toxins, bactericidal peptides and pollen proteins. The disulphide-bonding pattern depicted in

Figure 1(e) has been established by X-ray crystallography [19–21] and chemical synthesis of trappin-1 [1] and elafin [22].

The N-terminal transglutaminase substrate domain is composed of unique repetitive sequences rich in glutamine and lysine residues, which serve as acyl donor and acceptor sites respectively in transglutaminase-mediated isopeptide formation [ϵ -(γ -glutamyl)lysine cross-links] (Figure 1c). The length of the transglutaminase substrate domain varies considerably among species and family members; for example, pig trappins-1, -2, and -3 have 17, 12 and 10 repeats respectively, whereas human, monkey and bovine trappin-2 have five, five and 12 repeats respectively. Sequence similarities among the known members of the trappin family are illustrated in Figure 1(e). The highly variable region V (Figure 1e) of elafin has been demonstrated by X-ray crystallography to interact with the active site of elastase (Figures 1d and 2) [21], suggesting that the corresponding regions of other trappins also serve as specificity determinants for proteinases.

cDNA and gene structure

cDNAs for pig trappin-1 [14] and human trappin-2 [12] have been cloned and characterized. The cDNA of human trappin-2, for example, codes for a putative precursor of 117 amino acids, comprising a signal peptide of 22 amino acids at the N-terminus followed by the above-mentioned transglutaminase substrate

Figure 2 Interaction between elastase and the inhibitory domain of trappin-2 (SKALP/elafin)

(a) Backbone drawing of the inhibitory domain of human trappin-2 (SKALP/elafin). The domain indicated (Thr¹¹–Gln⁵⁷) corresponds to residues 71–117 of the trappin-2 precursor. Residues involved in the interaction with elastase are labelled. Ala²⁴, Met²⁵ and Leu²⁶ represent the variable region indicated in Figure 1(e). (b) Backbone drawing of pig pancreatic elastase (residues Val¹⁶–Asn²⁴⁵). The catalytic triad, comprising Asp¹⁰², His⁵⁶ and Ser¹⁹⁵, in the active centre is highlighted. (c) Structure of the trappin-2–elastase complex determined by X-ray crystallography [56].

Figure 3 Structure and evolution of trappin genes

(a) Structure of the gene and biosynthetic processes of human trappin-2. The untranslated regions are depicted in white, the presequence in grey, the transglutaminase (TGase) substrate domain in pink and the WAP motif in black. (b) Evolutionary history of trappin genes.

domain and WAP motif (Figure 1). The presence of signal sequences or presequences indicates that trappins are secreted proteins, and can be cross-linked to extracellular-matrix proteins by transglutaminase (Figures 1a and 1c). Partial cDNA sequences that correspond to the mature portions have also been obtained by PCR for monkey trappin-2 and bovine trappins-2, -4 and -5 [16].

Trappins are encoded by 2 kb single-copy genes composed of three exons (Figure 3). The first exon codes for the 5'-untranslated region, the signal peptide and the first few amino acid residues of the mature protein; the second exon encodes most of the mature protein; and the third exon encodes the 3'-untranslated region (Figure 3). This genomic organization is shared by all members of the trappin family whose gene structures have been determined. So far, the genes for pig trappins-1 [15], -2 [15] and -3 [15], and human trappin-2 [10,11], have been isolated and characterized. The most striking feature of the trappin genes is a surprisingly high degree of conservation of intron sequences (93–98%) compared with that of the exon 2 sequences (60–77%) [15]. This feature will be discussed in detail below. The human trappin-2 gene is located on chromosome 20 at 20q12–13 [23,24].

Molecular evolution of the domain structure

The two-domain structure of trappins is considered to have evolved by exon shuffling. With regard to the WAP motif region,

there are, as mentioned above, many proteins that have this motif, constituting the WAP superfamily. In particular, the occurrence of a member, SLPI (see below), that is composed of two tandemly repeated WAP motifs supports the idea that gene multiplication and exon shuffling were the major driving force in the evolution of trappins. Concerning the evolution of the transglutaminase substrate domain, however, little information was available until the recent identification of a close similarity between the trappin and REST genes [25,26]. The REST genes encode a group of seminal-vesicle transglutaminase substrates and were named 'REST' for 'rapidly evolving seminal vesicle transcribed'. They have a common three-exon/two-intron organization; the first and third exons, like those of the trappin genes, encode almost exclusively signal peptides and 3'-untranslated regions respectively [27–29]. Furthermore, the REST and trappin genes share considerable similarities even in the non-coding and intron sequences. It is therefore conceivable that the trappin gene family is derived from exon shuffling and gene duplication between the ancestral REST gene and the SLPI gene (Figure 3). The location of the human trappin-2 gene in region 20q12–13 of chromosome 20, where the related genes {i.e. the SLPI gene (STS marker WI-6969) [30] and semenogelin genes [27] that are members of the REST gene family} are all co-localized, provides strong support for this assumption.

Analysis of pig trappin genes has revealed a tremendous diversification in the WAP motif coding region in exon 2

Figure 4 Two possible mechanisms for apparent accelerated evolution of the exon 2 sequences of trappin genes

See the text for details.

compared with the surrounding regions, including introns 1 and 2 [15]. Speculatively, this wide divergence in the inhibitor domain could have arisen as an evolutionary adaptation to environments that contain a wide variety of invasive proteinases. The molecular mechanism underlying the divergence requires further study. Although the high frequency of mutations in the WAP motif coding region is most easily explained by a mechanism of error-prone repair (Figure 4a), the gene conversion mechanism should also be considered. In this, the targeted sequences (that flank exon 2) undergo periodic homogenization (Figure 4b). This type of gene conversion erases the molecular evolutionary records accumulated before the last round of homogenization and contributes to maintaining the target site sequence identical with, or very similar to, that of the donor sequence.

BIOCHEMICAL ASPECTS

Properties of the four-disulphide core domain of trappin-2

Elafin (the four-disulphide core domain of trappin-2) and the C-terminal four-disulphide core domain of SLPI carry the inhibitory active centre for serine proteinase inhibition. For both molecules, the three-dimensional structures have been solved by X-ray crystallography in complex with serine proteinases [19,21]. The two domains share the same distribution of disulphide bridges, and a methionine residue in the P_1' position of the inhibitory active centre. On comparing the inhibitory specificity of the two inhibitors, it is clear that elafin is a potent and specific inhibitor of elastin-degrading serine proteinases (human leucocyte elastase, proteinase 3), whereas SLPI inhibits most of the serine proteinases with a broad spectrum of substrates, with the exception of a weak inhibitory activity for proteinase 3 (Table 1).

The inhibitory activity of elafin for the human epidermis-derived chymotryptic serine proteinase called 'stratum corneum chymotryptic enzyme' shows a relatively high K_i of $1.6 \mu\text{M}$, which makes it unlikely that elafin is a physiologically relevant inhibitor of this enzyme. Nevertheless, elafin could be shown to inhibit the release of human corneocytes from callus *in vitro* [31], which is thought to be mediated by stratum corneum enzyme activity. Other chymotrypsin-like enzymes, such as cathepsin G, have been shown to be less than 50% inhibited by a more than

Table 1 Dissociation constants of trappin-2 and SLPI for inhibition of serine proteinases

Abbreviations: n.i., no inhibition with a 3-fold molar excess of inhibitor; n.d., no kinetic data available.

Proteinase	K_i (M)	
	Trappin-2	SLPI
Human leucocyte elastase	1.7×10^{-10} [32]	6.3×10^{-11} [65]
Pig pancreatic elastase	1×10^{-9} [66]	n.d.
Human proteinase 3	9.5×10^{-9} [66]	10^{-6} [66]
Stratum corneum chymotryptic enzyme	1.6×10^{-6} [67]	6.3×10^{-8} [31]
Human mast cell chymase	n.i.	5×10^{-8} [68]
Human cathepsin G	n.i.	4.2×10^{-9} [69]
Bovine chymotrypsin	n.i.	2.2×10^{-10} [65]
Pig trypsin	n.i.	8.3×10^{-9} [69]

1000-fold molar excess of elafin, which is clearly not of biological significance (O. Wiedow, unpublished work). Elafin was found to be highly cationic, with a pI of 9.7 [6], and has a molecular mass of 5999 Da [22]. The dissociation constant for elafin in complex with human leucocyte elastase shows a pH-dependence that is most pronounced between pH 5.5 and 7.0. This points towards the fact that protonization of His-57 in the catalytic triad of human leucocyte elastase is involved in formation of the enzyme-inhibitor complex [32]. At pH 8.0, values of $k_1 = 3.6 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$ and $k_{-1} = 5.8 \times 10^{-4} \text{ s}^{-1}$ have been determined. The kinetic data are consistent with the assumption that elafin is a potent substrate-like and fully reversible inhibitor of human leucocyte elastase.

The biochemical properties of the four-disulphide core domain of other trappin family members are largely unknown. However, it was shown that chemically synthesized pig trappin-1 and trappin-3 did not inhibit leucocyte elastase [15]. However, it was speculated that all trappins are likely to be proteinase inhibitors, possibly directed against microbial proteinases, in view of their expression in cells that are exposed to microbial stimuli.

Properties of the transglutaminase substrate domain

The N-terminal domain of the trappins is very similar to guinea pig seminal-vesicle protein, a known transglutaminase substrate. Molhuizen et al. [12] showed that full-length trappin-2 and a 14-amino-acid N-terminal peptide of trappin-2 could be used as an acyl acceptor or donor. Nara et al. [13] coined the name 'cementoin' for the transglutaminase substrate domain of trappin-2, and they showed that the cementoin moiety was readily cross-linked to other proteins by tissue transglutaminase. A number of trappin family members have now been identified in various species, and comparison of the N-terminal domains revealed that these domains are composed of variable numbers of repeats conforming to the consensus sequence GQDPVK [15,16]. These repetitive sequences are similar to that of seminal vesicle protein, but clearly different from other known repetitive transglutaminase substrate sequences, such as in involucrin and in cornifin. Not only these repeats, but also the single hexapeptide repetitive unit (GQDPVK) [16], have been demonstrated to be good substrates for transglutaminase, suggesting that there is no strict secondary or tertiary structural requirement for recognition by transglutaminase. In fact, the recombinant transglutaminase substrate domain of pig trappin-1 was suggested to assume a random coil structure by CD measurements (M. A. Ghoneim and S. Hirose, unpublished work). Zeeuwen et al. [16] made a

Figure 5 Immunohistochemical staining of psoriatic human epidermis (a and b) and normal human oesophagus (c and d) with antisera against trappin-2 (a and c) and transglutaminase type 1 (b and d)

Trappin-2 and transglutaminase co-localize in the suprabasal differentiated cells of these stratified epithelia.

detailed analysis of the biochemical properties of the GQDPVK repeat. Using amino acid substitutions in biotinylated peptide probes, it was demonstrated that both the lysine and the glutamine could be used in cross-linking reactions by transglutaminases. The cross-linking reaction was sequence-specific, as scrambling or inversion of the GQDPVK motif greatly decreased the incorporation of the hexapeptide in stratum corneum proteins.

A study by Steinert and Marekov [33] described the isolation of cross-linked proteins from human stratum corneum derived from foreskin. Cross-links were purified and subjected to N-terminal sequencing, which revealed several proteins covalently

attached to trappin-2, including keratin 1 and loricrin. Evidence that transglutaminase type 1 is a likely mediator of cross-linking of trappin-2 to other cell-envelope components is witnessed by the co-localization of these two proteins in stratified epithelia (see Figure 5).

The presence of a signal sequence strongly suggests that the trappins are secreted proteins. Indeed, it was found that trappin-2 is secreted in large quantities into the medium of cultured keratinocytes [34,35]. *In vivo* it is found in plasma and urine, suggesting that it is secreted into the extracellular space and cleared via blood and urine [36–38]. This finding raises the question of how and when trappin-2 is incorporated into the cornified envelope of human epidermis. The formation of the cornified envelope is thought to occur in phases, whereby involucrin acts as an initial scaffold for the subsequent cross-linking of other proteins (cornifins, loricrin, keratins) by the action of transglutaminase type 1, to form the insoluble structure of the cornified envelope [39]. Since trappin-2 is a secreted protein, but is actually found cross-linked to other stratum corneum proteins, this would imply that trappin-2 is incorporated from the outside rather than from the inside of the membrane. The fact that soluble transglutaminase activity is found in epidermal scales and is not strictly membrane-bound indicates that this is a likely scenario.

Part of the cross-linked trappin-2 is released from the stratum corneum by hitherto unknown proteinases. This process generates the C-terminal WAP domain, which contains the anti-proteinase activity, that can now diffuse freely to the interstitial fluid. Indeed, in urine only small fragments of trappin-2 are found, consisting of C-terminal sequences, as determined by N-terminal amino acid sequencing [40]. When psoriatic scales are extracted with acetic acid, only the C-terminal half of trappin-2 is found, suggesting that the N-terminal domain remains cross-linked to the stratum corneum proteins. N-terminal sequencing of trappin-2 purified from psoriatic scales revealed several potential cleavage sites, although the peptide starting with Ala⁶¹ (the molecule originally described as elafin) was most frequently found. When trappin-2 was purified from cultured keratinocytes and subjected to N-terminal sequencing, an intact molecule was found starting at Ala²³, indicating that processing of the signal peptide occurs between Ala²² and Ala²³, giving rise to a mature protein of 9.9 kDa. This suggests that the subsequent clipping of mature trappin-2 into two domains, as found *in vivo*, occurs extracellularly.

CELL BIOLOGICAL ASPECTS

Tissue distribution and subcellular localization of trappins

The proteinase inhibitor trappin-2 was originally discovered in the skin of patients with psoriasis, where it is present at high levels in the differentiated compartment (see Figure 5). This was shown at both the protein and mRNA levels. The presence of trappin-2 in normal skin is somewhat controversial [13,35]. The studies of Nara et al. [13] and Pfundt et al. [35] using Northern blot analysis and immunohistochemistry showed that trappin-2 is constitutively expressed in a number of normal human epithelia (Table 2). Mesenchymal and neural tissues were always negative for the protein. At the ultrastructural level, trappin-2 was found in lamellar granules and non-lamellar secretory vesicles in the stratum spinosum of psoriatic epidermis, indicating that it is secreted into the extracellular space during terminal differentiation [35]. Sallenave et al. [41] purified trappin-2, then designated ESI, from the sputum of a patient with bronchial carcinoma, and found it to be present in bronchoalveolar lavage fluid from normal subjects. Although trappin-2 is present in the

Table 2 Properties of trappin family members and SLPI

Abbreviation: HUSI, human seminal fluid inhibitor.

Name	Synonym	Biological properties	Regulation of expression	Site(s) of expression
Trappin-1	SPAI-2	Na ⁺ /K ⁺ -ATPase inhibitor	Unknown	Small intestine [42]
Trappin-2	SKALP, elafin, ESI	Inhibitor of leucocyte elastase and proteinase-3; substrate for transglutaminases	Induction by TNF- α and interferon- γ ; involvement of AP-1	Inflamed epidermis, oesophagus, vagina, oral epithelia [13,35]
Trappin-3	WAP-3	Unknown	Unknown	Trachea [15]
Trappin-4	—	Unknown	Unknown	Tongue, trachea [16]
Trappin-5	—	Unknown	Unknown	Tongue, small intestine [16]
SLPI	ALP, HUSI-1	Inhibitor of leucocyte elastase and cathepsin G; anti-viral and anti-microbial properties	Induction by TNF- α , interleukin-1, elastase and PMA	Epidermis, seminal vesicles, oral epithelia, glandular epithelia, bronchial epithelium [70]

trachea, the exact cellular source in the lower respiratory tract has not yet been identified. Some species differences appear to exist in expression patterns, as the putative pig trappin-2 orthologue was identified in the trachea and large intestine by RNase protection assay [42], whereas human trappin-2 was not detected in the small intestine [35]. Although trappin-2 expression is low or absent in normal human epidermis, it is readily induced upon trauma or irritation. It was shown that disruption of skin barrier function by tape stripping, application of detergent or wounding induced trappin-2 expression within 24 h, at both the protein and mRNA levels [34,43]. Teleologically this makes sense, as during wound healing the keratinocytes are migrating through a hostile environment consisting of large numbers of activated neutrophils within the wound bed which are secreting proteinases, including elastase and proteinase 3. In this way the migrating epidermis is protected against self-proteinases, which could otherwise degrade structural proteins and adhesion molecules involved in wound closure.

Trappin-1, originally described as a Na⁺/K⁺-ATPase inhibitor, has so far only been described in the pig, and was found to be almost expressed exclusively in the enteroendocrine cells of the lower crypt in the small intestine, as shown by immunohistochemistry and mRNA *in situ* hybridization [42]. Significant amounts of trappin-1 were also found in pig plasma.

The other known trappins (-3, -4 and -5) are as yet poorly characterized. Their function is unknown, although it is likely that they are proteinase inhibitors. These trappins are expressed in a number of epithelia (see Table 2).

Induction and regulation *in vitro*

Only trappin-2 has been studied in some detail in cultured cells, such as epidermal keratinocytes, mammary epithelial cells and airway epithelial cell lines [35,44–46]. The synthesis and secretion of trappin-2 by human epidermal keratinocytes is strongly dependent on the culture conditions, and appears to be stimulated by fetal-calf serum and tumour necrosis factor- α (TNF- α) [35]. Expression of trappin-2 has been used as a model system to mimic normal and psoriatic differentiation *in vitro* [44]. Similarly, in airway epithelial cell lines with features of Clara cells or type II alveolar cells, trappin-2 was up-regulated by TNF- α and interleukin-1 [46].

In mammary epithelial cells and breast carcinoma cells, trappin-2 expression was examined at the mRNA level [45,47]. The trappin-2 gene was active in mammary epithelial cells, but not in the carcinoma cell lines. The promoter region of the trappin-2 gene was examined using reporter constructs transiently

transfected in mammary epithelial and carcinoma cells [47]. Deletion analysis indicated a positively regulatory element between positions –575 and –434, and a negatively regulatory element between –611 and –571 (positions relative to the translation start site) [45]. Further analysis and site-directed mutagenesis identified an AP-1 (activator protein-1) site at –543 as a *cis*-element conferring high transcription rates in normal mammary epithelial cells, and PMA-inducible transcription in carcinoma cell lines [47].

Trappin-2 gene regulation in epidermal keratinocytes appears to be distinct from that in mammary epithelial cells. Using deletion constructs in transiently transfected keratinocytes, strong positively regulating *cis*-elements were found between –440 and –290 (positions relative to the translation start site), a region that contains a potential AP-1 site and two nuclear factor interleukin-6 sites (R. Pfundt, P. Zeeuwen and J. Schalkwijk, unpublished work). Determination of the transcription start site showed an unusual short 5'-untranslated region, and revealed multiple transcription start sites at positions –16, –17 and –18 relative to the translation start site. Speculatively, an imperfect TATA box (the TAAGA sequence at –48) may be used rather than the TATA box at –110, which could explain the presence of multiple start points.

The findings that AP-1 sites may be of importance in regulating trappin-2 activity, the induction of gene expression *in vitro* by TNF- α , and the induction in skin by exogenous stress factors (such as detergent, tape stripping and UV-irradiation) *in vivo* suggests that cellular stress response pathways may be involved in the induction of gene expression. Because trappin-2 is constitutively expressed in various non-keratinizing epithelia that are continuously exposed to microbial and inflammatory stimuli, we would speculate that in these epithelia the stress response pathways are permanently switched on, whereas in epidermis this pathway is only active following injury or skin barrier disruption.

CLINICAL ASPECTS

So far, no genetic deficiency of trappin-2 has been reported in humans. We have learned that trappin-2 expression is restricted to the epithelia of various organs. The anti-proteolytic properties of trappin-2, mainly in inhibiting elastase and proteinase 3 derived from neutrophils, make it likely that it has the capacity to inhibit neutrophil-mediated proteolytic tissue damage. A limited number of investigations have been conducted with recombinant elafin [48], but ample information has been obtained from investigations with other elastase inhibitors. The first idea

that has been thoroughly investigated was the potential of elastase inhibitors to prevent lung emphysema [49]. In animal models, such as elastase-induced lung emphysema in hamsters, systemically administered synthetic elastase inhibitors have been proven to be useful [50]. Also, application of SLPI as an aerosol has been proven to be effective in animals [51]. In the special case of genetic deficiency of α_1 -proteinase inhibitor, substitution with this protein is under investigation in humans [52]. Trappin-2 is one of the options for current gene therapy approaches for the treatment of lung emphysema and cystic fibrosis [53]. Successful gene transfer and expression of trappin-2 in rat lung tissue has been reported [53].

Reperfusion injury is caused mainly by neutrophil-mediated damage after pronounced ischaemia. In two different models, recombinant elafin has been shown to be effective. Inhibition of elastase by recombinant elafin improved myocardial function after repetitive ischaemia and reduced myocardial infarction in the rat heart [54]. Elafin attenuated post-cardiac transplant coronary arteriopathy and reduced myocardial necrosis in rabbits after heterotopic cardiac transplantation [55]. In all these studies, recombinant elafin was used [48], consisting of the 57 C-terminal amino acids, indicating that the transglutaminase substrate domain is not required for efficacy *in vivo*. Recombinant elafin has the advantages over other natural elastase inhibitors (α_1 -antitrypsin, SLPI) that it is smaller and unusually resistant to extreme pH, heat and oxidation.

For inflammatory diseases of the human epidermis, therapeutic application of recombinant elafin is still under consideration. In psoriasis, neutrophils invade the epidermis, and active human leucocyte elastase can be demonstrated on the skin surface [56,57]. Neutrophils have been shown to detach keratinocytes from cover slips, a phenomenon that can be antagonized by recombinant elafin [35]. This observation is in line with the finding of Kuijpers et al. [58] that pustule formation in patients with pustular psoriasis could be mediated by local deficiencies of trappin-2. This provides evidence that trappin-2 is a component that maintains epidermal integrity. Currently it is not clear whether topical or systemic application of elafin is therapeutically useful for psoriasis. We would speculate that the constitutive expression of SLPI in human epidermis and the inducible production of trappin-2 in psoriasis [37,59,60] lead to the production of elastase inhibitors in sufficient amounts, but in skin diseases with altered barrier function of the stratum corneum these water-soluble compounds might be lost. It appears likely that reconstitution of barrier function by topical application of lipids prevents the loss of elastase inhibitors, thereby explaining the effectiveness of occlusion or 'placebo cream treatment' frequently observed in psoriasis and eczema.

Finally, a variety of inflammatory skin diseases involving the dermis appear to be candidates for elastase inhibitor therapies. Elastase-specific inhibitors have been demonstrated to prevent the development of solar elastosis [61] and UV-induced carcinoma development in the skin [61] in mice.

PERSPECTIVES

In recent years, a vast number of new trappin family members have been identified in Suidae and Bovidae. Whether the trappin family has diversified in primates remains to be investigated. At present we have not been able to detect other potential members in humans, apart from trappin-2, which remains the best characterized family member up to now. Many questions still remain with respect to the function of the newly discovered trappins. We would anticipate that most of them are proteinase inhibitors, although the target specificity is unknown. Specu-

latively, the trappins could be directed against self-proteinases, but may also act against microbial proteinases, given that they are expressed at the interface between the organism and the external environment.

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REFERENCES

- Araki, K., Kuroki, J., Ito, O., Kuwada, M. and Tachibana, S. (1989) *Biochem. Biophys. Res. Commun.* **164**, 496–502
- Thompson, R. C. and Ohlsson, K. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6692–6696
- Klasen, E. C. and Kramps, J. A. (1985) *Biochem. Biophys. Res. Commun.* **128**, 285–289
- Schiessler, H., Arnhold, M., Ohlsson, K. and Fritz, H. (1976) *Hoppe-Seyler's Z. Physiol. Chem.* **357**, 1251–1260
- Drenth, J., Low, B. W., Richardson, J. S. and Wright, C. S. (1980) *J. Biol. Chem.* **255**, 2652–2655
- Wiedow, O., Schroder, J. M., Gregory, H., Young, J. A. and Christophers, E. (1990) *J. Biol. Chem.* **265**, 14791–14795
- Schalkwijk, J., Chang, A., Janssen, P., de Jongh, G. J. and Mier, P. D. (1990) *Br. J. Dermatol.* **122**, 631–641
- Schalkwijk, J., de Roo, C. and de Jongh, G. J. (1991) *Biochim. Biophys. Acta* **1096**, 148–154
- Sallenave, J. M. and Ryle, A. P. (1991) *Biol. Chem. Hoppe-Seyler* **372**, 13–21
- Saheki, T., Ito, F., Hagiwara, H., Saito, Y., Kuroki, J., Tachibana, S. and Hirose, S. (1992) *Biochem. Biophys. Res. Commun.* **185**, 240–245
- Sallenave, J. M. and Silva, A. (1993) *Am. J. Respir. Cell Mol. Biol.* **8**, 439–445
- Molhuizen, H. O., Alkemade, H. A., Zeeuwen, P. L., de Jongh, G. J., Wieringa, B. and Schalkwijk, J. (1993) *J. Biol. Chem.* **268**, 12028–12032
- Nara, K., Ito, S., Ito, T., Suzuki, Y., Ghoneim, M. A., Tachibana, S. and Hirose, S. (1994) *J. Biochem. (Tokyo)* **115**, 441–448
- Kuroki, J., Hosoya, T., Itakura, M., Hirose, S., Tamechika, I., Yoshimoto, T., Ghoneim, M. A., Nara, K., Kato, A., Suzuki, Y. et al. (1995) *J. Biol. Chem.* **270**, 22428–22433
- Tamechika, I., Itakura, M., Saruta, Y., Furukawa, M., Kato, A., Tachibana, S. and Hirose, S. (1996) *J. Biol. Chem.* **271**, 7012–7018
- Zeeuwen, P. L., Hendriks, W., de Jong, W. W. and Schalkwijk, J. (1997) *J. Biol. Chem.* **272**, 20471–20478
- Furutani, Y., Kato, A., Yasue, H., Alexander, L. J., Beattie, C. W. and Hirose, S. (1998) *J. Biochem. (Tokyo)* **124**, 491–502
- Bairoch, A., Bucher, P. and Hofmann, K. (1997) *Nucleic Acids Res.* **25**, 217–221
- Grutter, M. G., Fendrich, G., Huber, R. and Bode, W. (1988) *EMBO J.* **7**, 345–351
- Tsunemi, M., Matsuura, Y., Sakakibara, S. and Katsube, Y. (1993) *J. Mol. Biol.* **232**, 310–311
- Tsunemi, M., Matsuura, Y., Sakakibara, S. and Katsube, Y. (1996) *Biochemistry* **35**, 11570–11576
- Tsunemi, M., Kato, H., Nishiuchi, Y., Kumagaye, S. and Sakakibara, S. (1992) *Biochem. Biophys. Res. Commun.* **185**, 967–973
- Molhuizen, H. O., Zeeuwen, P. L., Olde Weghuis, D., Geurts van Kessel, A. and Schalkwijk, J. (1994) *Cytogenet. Cell Genet.* **66**, 129–131
- Robinson, P. A., Leek, J. P., Carr, I. M., Bailey, A., Lench, N. J., Morrison, J., Hume, W. J., High, A. S. and Markham, A. F. (1996) *Arch. Oral Biol.* **41**, 445–452
- Lundwall, A. and Ulvsback, M. (1996) *Biochem. Biophys. Res. Commun.* **221**, 323–327
- Hagstrom, J. E., Fautsch, M. P., Perdok, M., Vrbel, A. and Wieben, E. D. (1996) *J. Biol. Chem.* **271**, 21114–21119
- Ulvsback, M., Lazure, C., Lilja, H., Spurr, N. K., Rao, V. V., Loffler, C., Hansmann, I. and Lundwall, A. (1992) *J. Biol. Chem.* **267**, 18080–18084
- Lundwall, A. (1996) *Eur. J. Biochem.* **235**, 424–430
- Lundwall, A. and Lazure, C. (1995) *FEBS Lett.* **374**, 53–56
- Schuler, G. D., Boguski, M. S., Stewart, E. A., Stein, L. D., Gyapay, G., Rice, K., White, R. E., Rodriguez Tome, P., Aggarwal, A., Bajorek, E. et al. (1996) *Science* **274**, 540–546
- Franzke, C. W., Baici, A., Bartels, J., Christophers, E. and Wiedow, O. (1996) *J. Biol. Chem.* **271**, 21886–21890
- Ying, Q. L. and Simon, S. R. (1993) *Biochemistry* **32**, 1866–1874
- Steinert, P. M. and Marekov, L. N. (1995) *J. Biol. Chem.* **270**, 17702–17711
- Alkemade, J. A., Molhuizen, H. O., Ponc, M., Kempenaar, J. A., Zeeuwen, P. L., de Jongh, G. J., van Vlijmen Willems, I. M., van Erp, P. E., van de Kerkhof, P. C. and Schalkwijk, J. (1994) *J. Cell Sci.* **107**, 2335–2342

- 35 Pfundt, R., van Ruissen, F., van Vlijmen Willems, I. M., Alkemade, H. A., Zeeuwen, P. L., Jap, P. H., Dijkman, H., Fransen, J., Croes, H., van Erp, P. E. and Schalkwijk, J. (1996) *J. Clin. Invest.* **98**, 1389–1399
- 36 Alkemade, H., van de Kerkhof, P. and Schalkwijk, J. (1992) *J. Invest. Dermatol.* **99**, 3–7
- 37 Alkemade, H. A., de Jongh, G. J., Arnold, W. P., van de Kerkhof, P. C. and Schalkwijk, J. (1995) *J. Invest. Dermatol.* **104**, 189–193
- 38 Streit, V., Wiedow, O., Bartels, J. and Christophers, E. (1995) *J. Invest. Dermatol.* **105**, 562–566
- 39 Eckert, R. L., Crish, J. F. and Robinson, N. A. (1997) *Physiol. Rev.* **77**, 397–424
- 40 Molhuizen, H. O. and Schalkwijk, J. (1995) *Biol. Chem. Hoppe-Seyler* **376**, 1–7
- 41 Sallenave, J. M., Marsden, M. D. and Ryle, A. P. (1992) *Biol. Chem. Hoppe-Seyler* **373**, 27–33
- 42 Furukawa, M., Suzuki, Y., Ghoneim, M. A., Tachibana, S. and Hirose, S. (1996) *J. Biol. Chem.* **271**, 29517–29520
- 43 van Bergen, B. H., Andriessen, M. P., Spruijt, K. I., van de Kerkhof, P. C. and Schalkwijk, J. (1996) *Arch. Dermatol. Res.* **288**, 458–462
- 44 van Ruissen, F., de Jongh, G. J., Zeeuwen, P. L., van Erp, P. E., Madsen, P. and Schalkwijk, J. (1996) *J. Cell. Physiol.* **168**, 442–452
- 45 Zhang, M., Zou, Z., Maass, N. and Sager, R. (1995) *Cancer Res.* **55**, 2537–2541
- 46 Sallenave, J. M., Shulmann, J., Crossley, J., Jordana, M. and Gauldie, J. (1994) *Am. J. Respir. Cell Mol. Biol.* **11**, 733–741
- 47 Zhang, M., Magit, D., Pardee, A. B. and Sager, R. (1997) *Cancer Res.* **57**, 4631–4636
- 48 Chen, Y., Pioli, D. and Piper, P. W. (1994) *Bio/Technology* **12**, 819–823
- 49 Powers, J. C. and Bengali, Z. H. (1986) *Am. Rev. Respir. Dis.* **134**, 1097–1100
- 50 Kennedy, A. J., Cline, A., Ney, U. M., Johnson, W. H. and Roberts, N. A. (1987) *Eur. J. Respir. Dis.* **71**, 472–478
- 51 Rudolphus, A., Kramps, J. A., Mauve, I. and Dijkman, J. H. (1994) *Histochem. J.* **26**, 817–824
- 52 Gadek, J. E. and Pacht, E. R. (1996) *Chest* **110**, 273S–277S
- 53 Sallenave, J. M., Xing, Z., Graham, F. and Gauldie, J. (1997) *Chest* **111**, 128S–129S
- 54 Tiefenbacher, C. P., Ebert, M., Niroomand, F., Batkai, S., Tillmanns, H., Zimmermann, R. and Kubler, W. (1997) *Pflügers Arch.* **433**, 563–570
- 55 Cowan, B., Baron, O., Crack, J., Coulber, C., Wilson, G. J. and Rabinovitch, M. (1996) *J. Clin. Invest.* **97**, 2452–2468
- 56 Wiedow, O., Wiese, F. and Christophers, E. (1995) *Arch. Dermatol. Res.* **287**, 632–635
- 57 Wiedow, O., Wiese, F., Streit, V., Kalm, C. and Christophers, E. (1992) *J. Invest. Dermatol.* **99**, 306–309
- 58 Kuijpers, A. L., Zeeuwen, P. L., de Jongh, G. J., van de Kerkhof, P. C., Alkemade, H. A. and Schalkwijk, J. (1996) *Arch. Dermatol. Res.* **288**, 641–647
- 59 Schalkwijk, J., van Vlijmen, I. M., Alkemade, J. A. and de Jongh, G. J. (1993) *J. Invest. Dermatol.* **100**, 390–393
- 60 Nonomura, K., Yamanishi, K., Yasuno, H., Nara, K. and Hirose, S. (1994) *J. Invest. Dermatol.* **103**, 88–91
- 61 Starcher, B., O'Neal, P., Granstein, R. D. and Beissert, S. (1996) *J. Invest. Dermatol.* **107**, 159–163
- 62 Lorand, L. and Conrad, S. M. (1984) *Mol. Cell. Biochem.* **58**, 9–35
- 63 Greenberg, C. S., Birckbichler, P. J. and Rice, R. H. (1991) *FASEB J.* **5**, 3071–3077
- 64 Francart, C., Dauchez, M., Alix, A. J. and Lippens, G. (1997) *J. Mol. Biol.* **268**, 666–677
- 65 Ying, Q. L., Kemme, M. and Simon, S. R. (1994) *Biochemistry* **33**, 5445–5450
- 66 Wiedow, O., Ludemann, J., Utecht, B. and Christophers, E. (1993) *Adv. Exp. Med. Biol.* **336**, 61–64
- 67 Franzke, C. W., Baici, A., Christophers, E. and Wiedow, O. (1997) in *Proteolysis in Cell Functions* (Hopsu-Havu, V. K., Jarvinen, M. and Kirschke, K., eds.), pp. 438–446, IOS Press, Amsterdam
- 68 Walter, M., Plotnick, M. and Schechter, N. M. (1996) *Arch. Biochem. Biophys.* **327**, 81–88
- 69 Smith, C. E. and Johnson, D. A. (1985) *Biochem. J.* **225**, 463–472
- 70 Willems, L. N., Otto Verberne, C. J., Kramps, J. A., ten Have Opbroek, A. A. and Dijkman, J. H. (1986) *Histochemistry* **86**, 165–168