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REVIEW ARTICLE

Transglutaminases and their substrates in biology and human diseases: 50 years of growing

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Abstract Transglutaminase is an enzyme able to play more than one enzymatic action, acting on a variety of different substrates. The growth of knowledge about the members of the enzyme transglutaminase's family and its substrates since the last 50 years indicates a large interest and curiosity about this protein, whose function and structure was, but still is, an important object of research. On the other hand, the involvement in a number of human diseases together with the lack of knowledge about the biological functions played by some of the most studied members of this family, make this enzyme a fascinating field of study. The history of this enzyme and its substrates, whose cross-linking action was reported for the first time 50 years ago, suggests that an effort to increase knowledge and communication among researchers is required. To achieve this important result, 10 years ago an internet web page called worldwide happening around transglutaminase (WHAT) was created. Driven by these experiences, novel points-of-view to look at Transglutaminase and its substrates may be identified.

Keywords TGase family · Enzyme substrate · G-protein · Crosslink reaction

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Introduction

Transglutaminase was described for the first time in 1957 (Clarke et al. 1957) and 2 years later the name "transglutaminase" was officially used in the title of a scientific report (Mycek et al. 1959). Therefore, we can assume that the birthday of this fascinating family of enzymes within the international scientific community should be between these years and the present year may be considered the 50th "birth-date" of Transglutaminase (TGase). In order to celebrate the scientific importance of this enzyme, it should be taken into account the very large and complex aspects of its study, at structural, evolutionary and functional level. All together, the scientific reports about this family of enzymes depict the absolutely fascinating story of a protein whose functions are still not well elucidated, but, nevertheless, whose applications in biology, chemistry and drug discovery are increasing over the years. We will try to tell part of this story, as others will do in the same issue, hoping to highlight some of the TGase's features less characterized. In particular, we will look at TGase starting from the other side of the moon: the substrates of this enzyme, i.e. the effectors of its function(s).

How much TGase was and is studied?

Many names were used to identify this enzyme or members of this family (e.g. factor XIIIa, fibrin stabilizing factor, fibrinoligase, glutaminylpeptide gamma-glutamyltransferase, glutamyltransferase, glutaminyl-peptide gamma-, Laki-Lorand factor, polyamine transglutaminase, R-glutaminyl-peptide:amine gamma-glutamyl transferase, tissue transglutaminase, transglutaminase, transglutaminase C, TGC, TGase, protein-glutamine gamma-glutamyltransferase, as reported for instance by the BRENDA database http://www.brenda.uni-koeln.de/, and others). Further, more than one enzymatic function was associated to this enzyme family, which represents, on this specific feature, a fantastic example of moonlighting-enzyme, according to the Jeffery's definition (Jeffery 1999). Therefore, since a precise calculation about how many times this enzyme class was cited and studied in literature is difficult, we performed a Medline bibliographic search using the query "transglutaminase" searched as word in the All Fields tag. We performed this search on beginning of March 2008, and this query was found 4,989 times. When "transglutaminase" was searched as a word present in Title field, 2,000 references were found. Since a comprehensive calculations of citations about this enzyme should take into account also the synonymous words and the alternative names currently used, it is noteworthy that the interest about TGase may be considered surely larger than that reported in Table 1, where the results are compared with a number of other well known enzymes or proteins. The percent ratio Title/All Fields was found surprisingly high in the case of "p53" and "transglutaminase", i.e. higher than 40. This means that more than 40% of manuscripts indexed on Medline/PubMed containing those protein names within the text (anywhere in the text), put basically the main scientific focus on those proteins, since their name was present in title too. This may indicate that (at least as far as the literature cited in Medline/PubMed) the efforts in literature and in biomedical research specifically focused on p53 and TGases are presently very high, when compared to other proteins.

Despite the large effort and interest on TGase, many aspects regarding its biological functions are still poorly understood, basically due to:

(a) The physiological mechanisms in which most members of TGase family are involved is still unknown.

(b) Several members of this family play more than one enzymatic function, only partially related. Further, the potential substrates of TGase family members may be proteins, enzymes, mono- and polyamines, nucleotides and drugs.

(c) The TGases-functions may be very roughly exerted in two different biological compartments:

- a high-Ca^{++} compartment (e.g. at extracellular level, characterized by absence/low GTP or other nucleotide concentration);
- a low-Ca^{++} compartments (e.g. at intracellular level, and with higher GTP or ATP levels). This compartmentalization of functions reflects a compartmentalization of isoforms, although this is not always true. Changing of ions and nucleotide availability may trigger the switching of a different action and/or activation. This implies that often the researchers studying TGases' functions may come from different scientific backgrounds and therefore look at the TGase's biological functions as living on two different faces of the moon.

(d) Related to the previous point, the communication and dissemination of knowledge among TGase-workers was often difficult or required a lot of energy.

The "TGase web-community"

To overcome, at least partially, this gap of information, 10 years ago we created an Internet web site (http://crisceb.unina2.it/what/) specifically devoted to improve and spread the knowledge on TGase. It was called WHAT, an acronym standing for worldwide happening around transglutaminase. Until today, more than 27.000 web contacts to the home page confirm a large interest about this enzyme. Presently, the WHAT. Home Page is connected by the worldwide users about 1,600 times each 6 months. This number is only indicative and surely defective, since the contacts on the specific sub-sections are not presently counted. With about 10 subsections, devoted to discussions, meetings reports, useful links and others, available by clicking on a menu at the left side of the home page, the web community using this web site has started an interesting experiment of knowledge exchange with the WHAT web site as reference point.

Different members of TGase's family

Several distinct TGase isoenzymes have been identified in mammals at the genomic level. Eight are structurally and functionally related with the TGase's functions, namely the TGase 1–7 and factor XIII; an additional one, protein band 4.2, highly similar to the others, lacks the catalytic site, therefore it is considered only structurally/evolutively related to the others (Griffin et al. 2002; Fesus and Piacentini 2002). As shown in Table 2, the nine members of this family are widely but differently expressed in cell compartments or tissues, as well as some of their biochemical features (like calcium- or GTP-dependent activity or protease-activation, when this information is available). In addition, other members have been recently identified from lower species, like a bacterial TGase from Streptoverticillium, from the filarial worm *Dirofilaria immitis*, or from *C. Elegans* and *Aplysia*, as well as transglutaminase-like functions have been associated to other bacterial proteins and toxins (Fortin et al. 2007). TGase activity has also been observed in plants, and a first form was characterized in 2004 by *Arabidopsis* (Della Mea et al. 2004).

Table 1 Search of some protein names within Medline: the name of proteins was selected by chance among a pool of protein/enzymes/growth factors with general interest for biology and molecular medicine

Query (listed in alphabetic order)	Title matches	All Fields matches	Percent ratio T/AF (% Title/All Fields)
Immunoglobulin	26,288	628,259	4.2
Albumin	19,733	151,551	13.0
Hemoglobin	19,212	121,928	15.8
Actin	14,704	61,536	23.9
Protein kinase C	15,007	46,476	32.3
p53	19,922	45,259	**44.0**
Calmodulin	8,381	30,579	27.4
Glucagon	9,807	29,664	33.1
VEGF	3,904	22,182	17.6
Casein	4,540	21,830	20.8
Ferritin	4,744	18,461	25.7
Caspase 3	2,141	17,592	12.2
Akt	4,056	15,449	26.3
c-myc	4,300	13,582	31.7
Melanin	2,386	11,008	21.7
Myoglobin	3,252	10,481	31.0
Streptokinase	3,230	10,469	30.9
Thyroglobulin	2,808	9,449	29.7
NGF	1,689	9,582	17.6
PDGF	1,700	9,151	18.6
FGF	2,126	8,793	24.2
Papain	1,694	8,398	20.2
Hexokinase	2,106	7,765	27.1
Rhodopsin	2,723	7,013	38.8
Ceruloplasmin	1,836	6,396	28.7
Crystallin	2,285	5,982	38.2
Transglutaminase	2,000	4,889	**40.9**
Parvalbumin	989	3,109	31.8
Vinculin	399	2,181	18.3
h-ras	633	2,281	27.8
PI3 kinase	337	2,031	16.6
Transducin	504	1,916	26.3
Gelsolin	546	1,491	36.6
GATA	1,285	4,382	29.3
Factor XIII	1,096	3,260	33.6
Caldesmon	470	3,230	14.6
Nestin	326	1,874	17.4
Galactokinase	241	845	28.5
Neuropilin	240	723	33.2
PlGF	52	361	14.4

These values report the number of occurence of the protein name in PubMed, and are only indicative, being the numbers increasing in the time, although the percent ratio should be almost constant, and obviously incomplete because they do not take into account the use of synonymous terms

The most interesting feature coming from the observation of Table 2 is that the members of this enzyme family, although highly similar, are able to catalyze at least 6–7 different reactions depending on how much these reactions are considered "different". In fact, while from a chemical point of view it is clear that the reported enzymatic reactions are different, from a physiological/pathological point of view some of them show interesting similarities. Further, the multi-functionality displayed by different members of TGase's family is intriguingly related to their different involvement in human diseases. In the attempt to "unify" some "different" reactions, we can basically take into account two main functionalities:

Table 2 Classification of TGases: related information about activation, localization, chemical reaction and possible involvement in human diseases, when available on published reports, have been enclosed

Isoenzyme	Activation	Localization	Biological function(s)	Chemical reaction	Related disease
TGase 1	By proteolysis	Keratinocyte TGase, exists as membrane-bound and soluble forms	Cell envelop formation in keratinocytes differentiation	Transamidation Griffin et al. (2002), Fesus and Piacentini (2002), Greenberg et al. (1991)	Congenital autosomal recessive ichthyosis and other human epidermis diseases
TGase 2	By calcium, clostridial toxins Facchiano and Luini (1992)	Ubiquitous tissue, but also in extracellular space and nuclear	Programmed cell death, differentiation, cytoskeleton functions, cell motility and adhesion, signal transduction	Transamidation Griffin et al. (2002), Fesus and Piacentini (2002), Greenberg et al. (1991) [including serotonylation Walther et al. (2003), pegylation Sato (2002)] G-protein/ nucleotide binding/hydrolysing, ATPase Bergamini et al. (1987), Lee et al. (1989), Nakaoka et al. (1994); PDI Hasegawa et al. (2003), Eschenlauer and Page (2003), Knodler et al. (1999) Kinase Mishra and Murphy (2004) deaminase (Fleckenstein et al. (2002), Molberg et al. (1998)	Coeliac disease, neurological disorders, cataract, inflammation, possibly involved in diabetes mellitus and cancer
TGase 3	By calcium, by proteolysis	Keratinocyte and hair follicle	Terminal differentiation of the keratinocyte, hair follicle	Transamidation nucleotide binding/hydrolyzing activity Ahvazi et al. (2004)	Differentiation, human epidermis diseases
TGase 4	By calcium and phosphatidic acid (Esposito et al. 1996)	Prostatic secretory	Reproduction and fertility in rodents (Dubbink et al. 1996)	Transamidation	Infertility
TGase 5	By calcium (Candi et al. 2004)		Epidermal differentiation	Transamidation nucleotide-binding (Candi et al. 2004)	Several human epidermis diseases
Type 6	Not characterized	Not characterized	Not characterized	Transamidation	
Type 7	Not characterized	Not characterized	Not characterized	Transamidation	
Factor XIII	By calcium, by a thrombin-dependent proteolysis	Plasmatic, but also intracellular	Blood coagulation and wound healing	Transamidation	Coagulation disorders
Band 4.2		Erythrocyte membrane	Membrane functions		Spherocytosis

(a) *Protein-structure modifier (PSM)*: TGases may affect protein structure by:

- forming covalent cross-links between protein chains, epsilon-(gamma-glutamyl) lysine isopeptide (Gln-Lys) bonds;
- linking the Gln residues of proteins to the amino group of non-proteic molecules (including the link to monoamines, polyamines, but also the pegylation or serotonylation reactions);
- modifying a Gln residue through its deamination;
- acting as protein-disulphide isomerise.

(b) *Protein signal-transducer (PST)*: TGases may trigger a signal across the membrane and within the cell, acting as:

- a G-protein, including the GTP-binding and GTP-hydrolyzing activities (Bergamini et al. 1987; Lee et al. 1989; Nakaoka et al. 1994);
- a Kinase (Mishra and Murphy 2004);
- related to the previously ones, TGase may also play a role as a modulator of nucleotides or other second messengers availability.

The two unified-functions (PSM and PST) might be, of course, related one to each other or considered two aspects of a single, more complex function which could be the TGase function(s) in the cell fate.

Some of these enzymatic reactions are chemically similar although with differences in terms of substrate specificity and kinetic features. Most of these functions are present in TGase 2. This may reflect a real multi-functionality restricted to this member only, but most probably this is due to the fact that TGase 2, an ubiquitous enzyme, is involved in cellular processes like programmed cell death and differentiation and in human diseases like coeliac disease. Therefore TGase 2 is the most studied one and it is possible that other members of the TGase family, less studied, are multifunctional enzymes too. Another interesting, still not-completely elucidated, physiological role of TGases' family members may be related to their nuclear localization (Singh et al. 1995; Adany et al. 2001; Ballestar et al. 1996) and this will be in the future an interesting field of further study even for the involvement in human diseases like cancer.

The ability of a protein to play a second job, in addition to the canonical one, was called "moonlighting" and several proteins were classified as "moonlighting proteins" (Jeffery 1999), like for instance crystallins, pinin or other proteins (Ouyang 1999, Jeffery 2003). Since TGase 2 is able to play more than two different functions, it can be considered a bright example of moonlighting protein.

TGases substrates

Due the multi-functionality of TGases' family, its substrates could be classified and studied taking into account the different biochemical reaction catalyzed.

TGase is a transamidating enzyme: this is the most known and studied function, consisting of the catalytic post-translational modification of proteins by the formation of covalent bonds. Substrates of this reaction are divided in two main groups: (1) proteins, and (2) other molecules containing primary amino groups reactive as amine group-donors.

Proteins substrates may be divided in two main families:

- protein substrates acting as acyl donor, i.e. possessing the reactive glutamine;
- protein substrates acting as acyl acceptor, i.e. possessing the reactive lysine.

An exception to this rule is the case of human protein synthesis initiation factor 5A, a protein acting as protein substrate of transamidation via the unique amino acid hypusine (Beninati et al. 1995).

Of course, many times, a protein TGase substrate may contain both reactive glutamine(s) and lysine(s) residue. The availability and the number of these reactive residues represent the biochemical features leading to dimer or polymer formation by cross-linking reaction catalyzed by TGase. Protein substrates for transamidating enzymatic reaction are listed in Table 3.

TGase is a G-protein

The TGase type 2 was shown to have GTP-/ATP-binding—hydrolyzing activity (Bergamini et al. 1987; Lee et al. 1989), related to its G-protein function (Nakaoka et al. 1994), therefore nucleotides are the substrates for this reaction.

TGase can deamidate glutamines. This specific function has been observed with both synthetic peptides and natural proteins, in particular gluten proteins (Molberg et al. 1998; Fleckenstein et al. 2002; Mazzeo et al. 2003), thus opening a new view on the role of TGase in the coeliac disease.

Other TGase's functions are: protein serotonylation (Walther et al. 2003), protein disulphide isomerase (PDI) (Hasegawa et al. 2003; Eschenlauer and Page 2003; Knodler et al. 1999), protein pegylation (Sato 2002), intrinsic kinase activity (Mishra and Murphy 2004).

An interesting group of proteins which have been shown to be functionally related to TGase activity are some bacterial toxins (Facchiano and Luini 1990, 1992; Schmidt et al. 1999; Masuda et al. 2000): some of these toxins were useful tools to investigate key cellular functions like signal transduction, neurosecretion, endocytosis and phagocytosis, in which the involvement of TGase was hypothesized or supported by different experimental approaches (Davies et al. 1984, 1980; Facchiano et al. 1993b; Teshigawara et al. 1985; Ashton and Dolly 1997; Abe et al. 2000; Szondy et al. 2003; Sarvary et al. 2004; Balajthy et al. 2006; Akar et al. 2007). Proteins and other molecules undergoing these reactions are listed in Table 3.

A complete classification of all the substrates of TGase's family, including proteins, amines, nucleotides and other molecules, is difficult, since a definitive classification of the above enzymatic functions is still not-available. Nevertheless, in order to simplify a TGase's substrates classification reported in Table 3, we could cluster them according to the PSM and PST functions above mentioned.

Substrate specificity

The substrate specificity represents another debated aspect of TGase activity, largely investigated in the past (Pincus and Waelsch (1968a, b), Lorand et al. 1979; Folk 1983; Bruce et al. 1985; Coussons et al. 1992a; Groenen et al. 1994b; Kim et al. 1994; Hettasch et al. 1997; Nemes et al. 1999; Taguchi et al. 2000), still under investigation. The observation that only specific glutamine and lysine residues

Table 3 Substrates of TGases

Substrates	Isoenzyme	Reactive site	Localization	References
Proteins active as amine donor				
Actin	TGase 2		Intracellular	Takashi (1988), Nemes et al. (1997), Safer et al. (1997)
Aldehyde dehydrogenase	TGase activity of the nematode *C. elegans*			Madi et al. (2004)
Amines (monoamines, diamines, polyamines): cadaverine and monodansilcadaverine, histamine, putrescine, serotonine, spermidine, spermine	Different isoenzymes			Ginsburg et al. (1963), Schrode and Folk (1978), Gorman and Folk (1980a), Lorand and Conrad (1984), Beninati et al. (1988)
Beta amyloid peptide	TGase 2			Rasmussen et al. (1994)
Aspartyl protease	TGase 2 and Factor XIII		Viral protein (HIV-1)	Beninati and Mukherjee (1992)
Bacteriorhodopsin	Bacterial TGase			Seitz et al. (2001)
Calgizzarin—S100C protein—MLN 70—S100A11	TGase 1 and TGase 2		Keratinocyte cornified envelope (CE)	Robinson and Eckert (1998)
Cell adhesion molecule C-CAM	TGase 2			Hunter et al. (1998)
Alpha B-crystallin	TGase 2		Intracellular	Lorand et al. (1992), Groenen et al. (1992)
Cystatin	TGase 2 and possibly others			Zeeuwen et al. (2001)
Beta-endorphin	TGase 2		Endogenous opiates	Pucci et al. (1988)
Fibrinogen A alpha	TGase 2 and factor XIII		Extracellular	Doolittle et al. (1979), Kimura and Aoki (1986), Murthy et al. (2000)
Glutamate dehydrogenase	TGase activity of the nematode Caenorhabditis elegans			Madi et al. (2004)
Glutathione S-transferase	TGase 2		Intracellular	Ikura et al. (1998), Piredda et al. (1999), Taki et al. (2004)
Glyceraldeheyde 3 phosphate dehydrogenase	TGase 2		Intracellular	Cooper et al. (1997), Orru et al. (2002)
gp41	TGase 2		Transmembrane	Mariniello et al. (1993a)
Keratin, type II cytoskeletal 1	TGase 2 and TGase 3			Candi et al. (1998)
Keratin, type II cytoskeletal 2 epidermal	TGase 2 and TGase 3			Candi et al. (1998)
Keratin, type II cytoskeletal 5	TGase 2 and TGase 3			Candi et al. (1998)
Keratin, type II cytoskeletal 6	TGase 2 and TGase 3			Candi et al. (1998)
Alpha ketoglutarate dehydrogenase	TGase 2		Intracellular (Mitocondrial)	Cooper et al. (1997)
Alpha-lactalbumin	TGase 2 and Streptoverticillium TGase (MTGase)		Secretory protein	Lee et al. (2002); Truong et al. (2004), Nieuwenhuizen et al. (2003)
Beta lactoglobulin	TGase 2 (?)		Secretory protein	Coussons et al. (1992b), Nieuwenhuizen et al. (2004)
Laminin	FXIII			Usui et al. (1993)
Loricrin			Cell envelopes	Hohl et al. (1991)
Microtubule-associated protein tau—isoform Tau-F (Tau-4)	TGase 2		Intracellular	Murthy et al. (1998)

Table 3 continued

Substrates	Isoenzyme	Reactive site	Localization	References
Monellin (analog of)	Microbial TGase			Ota et al. (1999)
Root and leaf pea proteins				Lilley et al. (1998)
Protein disulfide isomerase	TGase activity of the nematode Caenorhabditis elegans			Madi et al. (2004)
Seminal vesicle secretory protein IV	TGase 2		Extracellular	Porta et al. (1991)
S-peptide	Microbial TGase (MTG) from Streptomyces mobaraensis			Kamiya et al. (2003)
Thymosin beta 4			Intracellular (cytoplasmatic)	Safer et al. (1997)
Vasoactive intestinal peptide (VIP)	TGase 2			Esposito et al. (1999)
Vimentin	TGase 2			Clement et al. (1998)
Proteins active as amine acceptor (i.e. glutamine donor)				
Acetylcholine esterase	TGase 2		Intracellular	Hand et al. (2000)
Actin	TGase 2		Intracellular (cytoplasmatic)	Takashi (1988), Nemes et al. (1997), Safer et al. (1997)
Beta amyloid peptide	TGase 2			Rasmussen et al. (1994)
Annexin I (lipocortin I)	TGase 2		Intracellular	Ando et al. (1991)
Alpha(2)-antiplasmin	FXIII better than TGase 2		Extracellular	Lee et al. (2000)
Aspartyl protease	TGase 2 and Factor XIII		Viral protein (HIV-1)	Beninati and Mukherjee (1992)
ATP synthase alpha subunit	TGase of the nematode *C. elegans*			Madi et al. (2001)
Bacteriorhodopsin	Bacterial TGase			Seitz et al. (2001)
Calgizzarin—S100C protein—MLN 70—S100A11	TGase 1 and TGase 2		Keratinocyte cornified envelope (CE)	Robinson and Eckert (1998)
Calpactin I light chain (S100A10)	TGase 2			Ruse et al. (2001)
Caraxin-1 (horseshoe crab)				Matsuda et al. (2007)
Beta casein	Factor XIII		Secreted protein	Gorman and Folk (1980a, b)
Chloroplast proteins	Plant TGase(s)			Dondini et al. (2003)
Collagen alpha 1(III)	TGase 2		Extracellular	Bowness et al. (1987), Orban et al. (2004)
Beta A3 crystallin	TGase 2		Intracellular	Berbers et al. (1984), Groenen et al. (1994a)
Beta B3 crystallin			Intracellular	Berbers et al. (1984)
Beta Bp (betaB2) crystallin			Intracellular	
Cytocrome c	TGase 2		Intracellular	Butler and Landon (1981)
Beta-endorphin	TGase 2		Endogenous opiates	Pucci et al. (1988)
Enolase	Transglutaminase of the nematode Caenorhabditis elegans		Intracellular (Cytoplasmic)	Madi et al. (2001)

Table 3 continued

Substrates	Isoenzyme	Reactive site	Localization	References
Erythrocyte anion transporter—band 3 anion transport protein	Intrinsic TGase of human red blood cell		Intracellular	Murthy et al. (1994)
Fibrinogen A alpha	TGase 2 and factor XIII		Extracellular	Doolittle et al. (1979), Kimura and Aoki (1986), Murthy et al. (2000)
Fibrinogen gamma	Factor XIII		Extracellular	Murthy et al. (2000)
FibN (peptide derived from the N-terminal sequence of fibronectin)			Extracellular	Sato et al. (2000)
Glucagon	TGase 2			Folk and Cole (1965)
Glutathione S-transferase	TGase 2		Intracellular	Ikura et al. (1998), Piredda et al. (1999), Taki et al. (2004)
gp41	TGase 2		Transmembrane	Mariniello et al. (1993a, b)
gp120	TGase 2		Viral envelope	
H3 histone	TGase 2		Intracellular	Ballestar et al. (1996)
H4 histone	TGase 2		Intracellular	Ballestar et al. (1996)
H2A histone	TGase 2		Intracellular	Ballestar et al. (1996)
H2B histone	TGase 2		Intracellular	Ballestar et al. (1996)
Hemoglobin (denatured)				Pincus and Waelsch (1968a, b)
Hepatitis C virus core protein	TGase 2		Viral protein	Lu et al. (2001)
Insulin A chain	TGase 2			Folk and Cole (1965)
Insulin B chain	TGase 2			
Insulin-like growth factor-binding protein-1	TGase 2			Sakai et al. (2001)
Interleukin 2	Microbial transglutaminase (M-TGase)			Sato et al. (2001)
Involucrin	TGase 1		Membrane	Simon and Green (1988), Nemes et al. (1999)
Alpha-lactalbumin	TGase 2 and Streptoverticillium TGase (MTGase)		Secretory protein	Lee et al. (2002), Truong et al. (2004), Nieuwenhuizen et al. (2003)
Beta lactoglobulin	TGase 2 (?)		Secretory protein	Coussons et al. (1992b), Nieuwenhuizen et al. (2004)
Laminin	FXIII			Usui et al. (1993)
Loricrin			Cell envelopes	Hohl et al. (1991)
Alpha-2-macroglobulin receptor-associated protein			Extracellular	Rasmussen et al. (1999)
Mellittin	TGase 2			Perez-Paya et al. (1991)
Microtubule-associated protein tau—isoform Tau-F (Tau-4)	TGase 2		Intracellular	Murthy et al. (1998)
Midkine	TGase 2			Mahoney et al. (1996), Kojima et al. (1997), Mahoney et al. (2000)
Monellin (analog of)	Microbial TGase			Ota et al. (1999)
Nidogen (entactin)	TGase 2		Extracellular	Aeschlimann et al. (1992)
Osteonectin	TGase 2		Extracellular	Aeschlimann et al. (1995)
Osteopontin (extracellular matrix cell adhesion protein)	TGase 2		Extracellular	Kaartinen et al. (2002), Prince et al. (1991), Sorensen et al. (1994)
Root and leaf pea proteins				Lilley et al. (1998)
Phosphoglycerate kinase			Intracellular ?	Coussons et al. (1991)

Table 3 continued

Substrates	Isoenzyme	Reactive site	Localization	References
Phospholipase A2	TGase 2		Extracellular	Cordella-Miele et al. (1990)
Alpha2 plasmin inhibitor	FXIII		Extracellular?	Tamaki and Aoki (1982)
Plasminogen-activator inhibitor type-2	TGase 2 and FXIII			Jensen et al. (1993), Ritchie et al. (1999)
Polyglutamine repeats	TGase 2			Violante et al. (2001), Kahlem et al. (1996)
Procarboxypeptidase U (EC 3.4.17.20) plasma procarboxypeptidase B	TGase 2 and factor XIII			Valnickova and Enghild (1998)
S100 calcium-binding protein A7—Psoriasin (S100A7 or PSOR1)	TGase 2			Ruse et al. (2001)
Seminal vesicle secretory protein IV	TGase 2		Extracellular	Porta et al. (1991)
S-peptide	Microbial TGase (MTG) from Streptomyces mobaraensis			Kamiya et al. (2003)
Substance P	TGase 2		Extracellular	Ferrandiz et al. (1994)
Synapsin I	TGase 2		Intracellular	Facchiano et al. (1993a)
Tetanus toxin	TGase 2			Facchiano and Luini (1992), Facchiano and Luini (1990)
Vasoactive intestinal peptide—VIP	TGase 2			Esposito et al. (1999)
Vimentin	TGase 2			Clement et al. (1998)
Vitronectin	Factor XIII		Extracellular	Skorstengaard et al. (1990)
Von Willebrand factor	Factor XIII			Usui et al. (1993)
Unknown acceptor/donor function, non protein substrates, other molecules and activities				
Aldolase			Intracellular	Lee et al. (1992)
Androgen receptor	TGase 2		Intracellular (nuclear receptor)	Mandrusiak et al. (2003)
CD38	TGase 2		Intracellular	Umar et al. (1996)
Clara Cell p10 Kda				Mantile et al. (1993)
eIF5A (initiation factor 5A)	TGase 2 and Factor XIII	hypusine	Intracellular	Beninati et al. (1995)
Drugs (antibiotics)	TGase homologue			Fortin et al. (2007)
Fibronectin	Factor XIII		Extracellular	McDonagh et al. (1981), Mosher and Schad (1979)
Filaggrin linker segment peptide (FLSP)	TGase 3			Takahashi et al. (1996)
Galectin 3	TGase 2			Mehul et al. (1995), Mahoney et al. (2000)
Glutathione S-transferase	TGase 2	Fluorescein is covalently attached only to the N- or C-terminal site	Intracellular	Piredda et al. (1999), Taki et al. (2004)
Gluten proteins (alpha/beta-, gamma-gliadin, and low molecular weight glutenin)	TGase 2	Glutamine deamidation	Extracellular	Vader et al. (2002), Mazzeo et al. (2003); Mamone et al. (2004), Fleckenstein et al. (2004), Piper et al. (2002)
Growth hormone	TGase 2	Glutamine40 and Glutamine141 are PEGylated		Fontana et al. (2008)
Small GTPases		Serotonylation	Intracellular	Walther et al. (2003)

Table 3 continued

Substrates	Isoenzyme	Reactive site	Localization	References
Histidine-rich glycoprotein	FXIII		Expressed by the liver and secreted in plasma	Halkier et al. (1994)
Alpha2 HS-glycoprotein (AHSG)	TGase 2			Kaartinen et al. (2002)
Huntingtin	TGase 2			Zainelli et al. (2004)
Importin alpha3	TGase 2	Interacts with TGase 2	Nuclear transport protein	Peng et al. (1999)
Insulin-like growth factor binding protein-3 (IGFBP-3)	TGase 2	Kinase activity		Mishra and Murphy (2004)
Latent TGF-beta binding protein-1 (LTBP-1)	TGase 2		Extracellular	Verderio et al. (1999)
Lipoprotein a	TGase 2 and FXIII			Borth et al. (1991)
Alpha2 macroglobulin				Mortensen et al. (1981)
Myoglobin	TGase 2	Glutamine91 is PEGylated		Fontana et al. (2008)
Myosin			Intracellular	Eligula et al. (1998)
Nucleotide(s) binding/ hydrolyzing	TGase 2TGase 3TGase 5		Intracellular	Bergamini et al. (1987), Lee et al. (1989), Ahvazi et al. (2004), Candi et al. (2004)
Osteocalcin			Extracellular	Kaartinen et al. (1997)
Periplakin	TGase 2			Aho (2004)
Phosphorylase kinase			Intracellular	Nadeau et al. (1998)
Proapoptotic kinase DLK	TGase 2	oligomerization		Robitaille et al. (2004)
Protein disulfide isomerase	TGase activity of the nematode *Caenorhabditis elegans*	Amine donor		Madi et al. (2004)
Retinoblastoma protein				Oliverio et al. (1997)
Rho A			Intracellular	Singh et al. (2001)
Ribonuclease A	TGase 2	PDI activity		Hasegawa et al. (2003)
Semenogelin I	Factor XIII			Peter et al. (1998)
Semenogelin II	Factor XIII			
Bone sialoprotein (BSP)	TGase 2		Bone matrix	Kaartinen et al. (2002)
Soybean proteins			Extracellular	Larrè et al. (1993)
Alpha-synuclein	TGase 2		Intracellular	Andringa et al. (2004)
Thrombospondin	factor XIII		Extracellular	Lynch et al. (1987)
Troponin T			Intracellular	Gorza et al. (1996)
Tubulin			Intracellular	Maccioni and Seeds (1986)
Uteroglobin	TGase 2 FXIII?		Extracellular	Manjunath et al. (1984)
Vinculin	Factor XIII			Asijee et al. (1988)
Whey proteins	Microbial TGase		Extracellular	Truong et al. (2004)

may act in proteins as acyl donor and acyl acceptor has aimed the researchers to investigate the specificity of these residues within the protein environment. A review published few years ago summarized the state-of-the-art about the need of specific residues in the environment of glutamine residues (Esposito and Caputo 2005). Recent studies have been aimed to explore with phage display random peptide library the preference of the surrounding sequence of the reactive glutamine residues. Different research groups found consensus sequences which include the reactive glutamines. The consensus pQx(P,T,S)l (where x, p and l indicates any amino acid, polar and aliphatic amino acids, respectively) was reported (Keresztessy et al. 2006) by studying the peptides acting as substrate of TGase 2, whereas others (Sugimura et al. 2006) found that TGase 2 catalyzed the reaction for glutamine residues within the

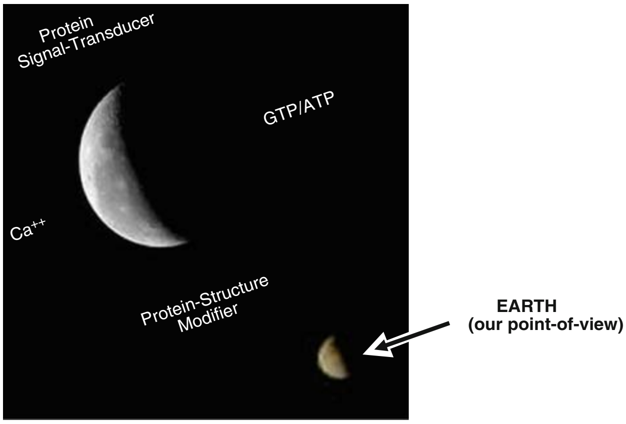

Fig. 1 The other side of the moon

pattern QxPhD(P), QxPh, QxxhDP (where x indicated any amino acid and h indicated an hydrophobic amino acid). On the contrary, Factor XIIIa catalyzed the reaction for the QxxhxWP pattern. These differences in the consensus sequence leaves open the problem. An interesting work (Fontana et al. 2008) suggested that the substrate specificity is not strictly due to the sequence of the substrate segment, while its flexibility is much more relevant to explain its ability to be used for the cross-link reaction. This point of view is based on experimental evidences that reactive sites in myoglobin and growth hormone are located in flexible regions, as well as on a revision of published data on the reactivity of other proteins, peptides and chimera. This finding is also in agreement with the usage of short sequence tags linked to the N-terminus of a protein, which adds to the protein the ability to be cross-linked by TGase at the glutamine in position 4 of the tag (Jäger et al. 2006). The reactivity of this short tag may be due to the used sequence, but also to the predictable high flexibility of a short tag added to the N-terminus of proteins.

A specific database named TRANSIT (TRANsglutamination SITes) was developed (Facchiano et al. 2003) and is still growing, with the specific aim to help the scientific community in deciphering and correctly move in the complex field of TGases' substrates study (Esposito and Caputo 2005; Keresztessy et al. 2006; Sugimura et al. 2006).

Conclusions

The function(s) of TGase(s) and its/their biological role inside and outside the cell is still a fascinating field of study. Probably it is due to both the complexity of the scenario but also to the point-of-view used up to now to look at TGase. In fact, this enzyme family is a moonlighting enzyme, and probably we are trying to investigate its biological function as looking at the Moon (Fig. 1). It is probably required an effort to turn our attention to the other side(s) of the moon, taking into account not only what is detectable when its surface is under the light of our present knowledge, but also the novel findings recently reported about the structural behaviours of TGase substrates. Further we should take in considerations also the additional enzymatic reactions recently shown to be catalyzed by our enzyme, but keeping in memory, as much as possible, previous reports apparently less-relevant when they were published.

It is important to remember that when we see the Moon, we are looking at the face presently under the light, but there is the other side (i.e. the pool of old/pre-existing data to be analyzed under another, novel, light of interpretation and comprehension). It is possible that the "other side of the moon" was not yet discovered in the first 50 years of TGase's story, but our efforts to increase the knowledge and the exchange of information among the researchers may be extremely important to achieve the final result.

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