REVIEW

Regulation of the activities of the mammalian transglutaminase family of enzymes

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Abstract: Mammalian transglutaminases catalyze post-translational modifications of glutamine residues on proteins and peptides through transamidation or deamidation reactions. Their catalytic mechanism resembles that of cysteine proteases. In virtually every case, their enzymatic activity is modulated by elaborate strategies including controlled gene expression, allostery, covalent modification, and proteolysis. In this review, we focus on our current knowledge of post-translational regulation of transglutaminase activity by physiological as well as synthetic allosteric agents. Our discussion will primarily focus on transglutaminase 2, but will also compare and contrast its regulation with Factor XIIIa as well as transglutaminases 1 and 3. Potential structure–function relationships of known mutations in human transglutaminases are analyzed.

Keywords: transglutaminase; post-translational modification; allostery; disulfide redox switch; human mutations

Introduction

The human genome encodes nine members of the transglutaminase (TG) family, although one of them (Band 4.2) lacks enzymatic activity.¹ The catalytic mechanism of all other transglutaminases is highly conserved and is related to that of cysteine proteases (Fig. 1). It centers on a nucleophilic Cys residue (Cys277 in human TG2), which attacks γ -glutamyl residues of proteins and peptides to form a thioester intermediate. The acylated enzyme can then either react with an amine donor, which could be an ϵ -lysine residue from another protein or peptide substrate or a small molecule amine. This results in the formation of

an intermolecular isopeptide bond. Alternatively, in the absence of a suitable amine donor, the thioester can be hydrolyzed to the free (glutamic) acid, which corresponds to a net deamidation of the substrate.

The literature on the biology of mammalian transglutaminases is vast and is beyond the scope of this review and extensively covered elsewhere.^{1–5} We simply note that some transglutaminases are ubiquitous and have both catalytic as well as noncatalytic functions (e.g., TG2), whereas others have more well-defined locations and roles (e.g., TG4 is exclusively expressed in the prostate and is involved in semen coagulation). Like most other proteins encoded by mammalian genomes, the expression of each transglutaminase gene is subject to elaborate regulation by spatial and temporal cues; this too is beyond the scope of our review. Instead, we focus on the post-translational mechanisms by which the

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Figure 1. Catalytic activity of transglutaminase (TGase) enzymes: Reaction of a TGase with a glutamine-bearing substrate results in the formation of an acyl-enzyme intermediate, which in turn can react with either an aliphatic amine (typically lysine sidechains in protein or peptide substrates or small molecules like serotonin) to form an isopeptide bond or with water to transform the glutamine side-chain into a glutamate.

catalytic activity of transglutaminases is regulated. In recent years, it has become abundantly clear that nature has evolved exquisite and diverse strategies for reversibly or irreversibly switching transglutaminase activity on and off. An understanding of these mechanisms could provide a foundation for interrogating the role of individual transglutaminases in health and disease. We start with a review of post-translational regulation of TG2 activity, and then compare and contrast our knowledge of TG2 regulation to that of Factor XIII (FXIII), TG1, and TG3. Our review of each of these transglutaminases includes a discussion of the potential structural and mechanistic implications of known human mutations.

Transglutaminase 2 (TG2)

Structure

TG2 is a 79 kDa protein that consists of four domains; an N-terminal β -sandwich, a catalytic domain harboring the active site Cys277 and two C-terminal β -barrels (Fig. 4).⁶ The spatial arrangement of these domains varies considerably with the binding of cofactors. In a catalytically inactive, nucleotide bound conformation, TG2 adopts a compact ("closed") state, where access to Cys277 is obscured by two loops of the first β -barrel domain.⁶ Upon activation, however, this structure undergoes a large conformational movement to an elongated ("open") conformation that exposes the active site to substrate molecules.⁷

Regulation by physiological mechanisms

As a relatively nonspecific cross-linking enzyme that is abundant in intracellular as well as extracellular environments, TG2 activity must be tightly regulated. The three most well understood physiological regulators of its enzymatic activity are Ca^{2+} ions, guanine nucleotides, and the redox potential.

Binding of Ca^{2+} ions is essential for TG2 catalytic activity. One molecule of TG2 can bind up to

six Ca^{2+} ions with an apparent overall dissociation constant of 90 μM , as demonstrated by equilibrium dialysis.⁸ Although most of these binding sites have not been directly visualized, mutagenesis studies suggest that they are distributed across the protein.⁹ In contrast, GTP or GDP binds TG2 at a unique, structurally defined site with a dissociation constant of 1.6 μM .^{10,11} GTP-bound, catalytically inactive TG2 requires high (>1 mM) Ca^{2+} ion concentrations for activation, although one high-affinity site ($K_d = 0.1$ – 15 μM) has been identified by isothermal titration calorimetry^{9,12} and visualized in the GTP-bound crystal structure.⁶

Because TG2 activity requires relatively high concentrations of calcium and is inhibited by GTP/GDP, intracellular TG2 is catalytically silent under normal physiological conditions. However, these allosteric regulatory features do not explain why extracellular TG2 is also predominantly inactive in most tissues and organs.¹³ This feature is influenced by the redox sensitivity of the protein. Early studies showed that TG2 is susceptible to reversible inactivation by the formation of an intramolecular disulfide bond between cysteine residues other than the active site Cys277.^{14,15} More recently, the switch between the (active) reduced state and the (inactive) oxidized state of TG2 has been characterized.^{16,17} This redox switch centers on a triad of cysteine residues, the vicinal Cys370/Cys371 and Cys230, which have an unusually high redox potential of -190 mV.¹⁶ Through mutation analysis and alkylation studies, Cys230 was identified as the key redox sensor. Under oxidizing conditions, the inter-strand disulfide-bond Cys230-Cys370 forms first and facilitates formation of the more stable Cys370-Cys371 disulfide bond that inactivates TG2.¹⁷ Interestingly, the oxidation of TG2 is dependent on the presence of calcium ions, and oxidized TG2 appears to adopt an open conformation.^{7,17}

In summary, Ca^{2+} , guanine nucleotides, and the redox potential collaborate to maintain mammalian

Figure 2. The three states of TG2: Reduced, guanine nucleotide bound TG2 is closed and inactive. Upon dissociation of GTP and binding of Ca^{2+} , the enzyme opens to the active conformation, which can then be oxidized to the open, inactive, disulfide-bonded form, a process that is reversed by thioredoxin (adapted from Refs. 16 and 17).

TG2 is in at least three distinct states, depending on local conditions (Fig. 2). The two most abundant states—the GTP/GDP bound form and the Ca^{2+} bound oxidized form—are inactive, whereas catalytic activity is only expressed in the transient Ca^{2+} -bound, reduced form. Under homeostatic conditions, the cytosol has a high concentration of GTP, a low redox potential, and extremely low ($<1 \mu\text{M}$) levels of free Ca^{2+} . Consequently, the intracellular pool of TG2 is in a reduced, catalytically inactive state. An influx of calcium ions can then activate the enzyme, as demonstrated in apoptotic states.^{18,19} Similar arguments are also applicable to the mitochondrial and nuclear pools of TG2, because free Ca^{2+} concentration is comparably low in those compartments under ordinary conditions. In contrast, the extracellular matrix in most organs (e.g., intestine, liver) has a considerably lower concentration of GTP or GDP, a relative abundance of Ca^{2+} ions, and an oxidizing environment. Consequently, a vast fraction of the extracellular TG2 reservoir is also inactive.¹³

Both thermodynamic and kinetic factors are responsible for activating extracellular TG2. The high redox potential of the vicinal disulfide bond poises TG2 favorably for reduction relative to other disulfide bonded extracellular proteins, as the local environment becomes hypoxic. Indeed, this may be the mechanism for hypoxia induced, TG2 catalyzed serotonylation of fibronectin in rodent models of pulmonary hypertension.²⁰ In addition, and perhaps more importantly, selective recognition of TG2 by the protein cofactor thioredoxin 1 leads to highly efficient activation of oxidized TG2.¹⁶ The precise mechanisms for thioredoxin secretion into the extracellular matrix remain to be established, although at least one pro-inflammatory signal has been identified. Specifically, cultured monocytic and epithelial cells that are responsive to interferon- γ secrete thioredoxin in response to elevated levels of this pro-inflammatory cytokine.¹⁶

Analogous to the recognition of oxidized TG2 by thioredoxin, other protein–protein interactions might also regulate TG2 activity in the extracellular matrix. Indeed, TG2 is known to interact with a number of extracellular proteins such as fibronectin, integrin, and calreticulin, as reviewed elsewhere.²¹ Although binding to fibronectin does not alter the

enzymatic activity of TG2,²² one or more of the other protein partners could do so. Similarly, in the intracellular environment, GTP-bound TG2 has been shown to physically associate with other proteins. For example, in the presence of epinephrine, it associates with the $\alpha 1$ -adrenergic receptor and induces phospholipase C activity.²³ Given the inverse relationship between guanine nucleotide binding and transglutaminase activity, this G-protein like activity of TG2 may also be regulated by protein–protein interactions. Last but not least, noncovalent interactions between extracellular TG2 and cell surface carbohydrates can also modulate TG2 activity. For example, heparan sulfate has a binding site on the surface of the closed form of TG2.^{24,25}

In addition to the above allosteric mechanisms for regulating TG2, nature may have also evolved covalent modification strategies for regulating the catalytic activity of this mammalian enzyme. For example, protein kinase A catalyzes phosphorylation of Ser216 of human TG2, which in turn leads to activation of nuclear factor- κB , although the mechanistic basis of this phenomenon remains to be elucidated.²⁶ Recent evidence also suggests that a small amount of a constitutively active proteolytic derivative of TG2 may be present in the cytosol, although the biochemical origins and physiological role of this fragment is unknown.²⁷

Regulation by synthetic allosteric factors

To date, there have been several reports of small molecules that inhibit TG2 activity by binding to its active site.^{28–32} In contrast, very few synthetic allosteric regulators of TG2 activity have been identified, all of which inhibit catalytic activity.

Even though the nucleotide binding site of TG2 would be the most obvious target for allosteric inhibition, few small molecules are known to target this site, most of which are very close analogs of the natural ligands (and are, therefore, neither cell permeable nor bioavailable). This is consistent with the observation that the nucleotide binding site of TG2 is markedly different from similar sites in ATP-dependent kinases, for which a plethora of inhibitors exist that mimic nucleobase interactions.³³ The sugar phosphate analog, glucosamine-6-phosphate, is a weak (millimolar) ligand for the GTP site; it

Figure 3. Structures of LDN-27219-analog (A)³⁵ and CK-IV-38 (B).³⁶

appears to induce a similar conformational change as GTP itself.³⁴

Thienopyrimidinone compounds such as the LDN-27219 analog 8 [Fig. 3(A)] are also thought to be allosteric inhibitors of TG2 activity based in their noncompetitive inhibition characteristics.^{35,37} The binding site of these inhibitors has not yet been verified, and some controversy exists whether it is the GTP binding pocket itself, or a site that is coupled to the GTP binding site.^{35,38} The acylidene oxindoles such as CK-IV-38 [Fig. 3(B)] comprise another class of allosteric inhibitors based on their noncompetitive inhibition characteristics. Their binding site also remains to be determined.³⁶

Mutations and transcriptional variants

Four shorter variants of TG2 have been discovered, all resulting from alternative splicing of the transcript. One variant, transglutaminase homologue (TGH, also TGase-short) lacks the C-terminal 139 amino acids and its terminal residues 539–548 are altered from the full-length transcript.^{39,40} A second variant, transglutaminase homolog 2 (TGH2) lacks the C-terminal 338 amino acids and has an altered sequence in its terminal residues 287–349.⁴⁰ Two other variants (tTGv1 and tTGv2) result from alternative splicing in exons 12 and 13. They share the first 620 amino acids with full-length TG2, but have divergent 54 or 25 amino acid C-terminal sequences.⁴¹

Because TGH lacks key residues lining the nucleotide-binding pocket, it is unresponsive to GTP, as are the tTGv1 and tTGv2 variants. In each case, the protein retains some Ca-dependent transglutaminase activity.⁴¹ Upregulation of TGH has been detected under apoptotic conditions and in Alzheimer's disease patients and is believed to have a differential biological role compared with full-length TG2.⁴² In all likelihood, these variants have distinct calcium dependence, which could explain how TG2 is activated without the need for high Ca^{2+} concentration inside the cell. Although TGH2 has not yet been characterized, its sequence is significantly modified immediately following the active site

Cys277, a region of the protein that includes the catalytic triad of His335 and Asp358. As such, TGH2 is likely catalytically incompetent.

A number of missense mutations in the gene encoding human TG2 have been detected and implicated in diseases such as early onset Type 2 diabetes (Fig. 4).^{43,44} Three of these functionally annotated mutations map to the vicinity of the putative Ca^{2+} binding site S3 and have been shown to modestly reduce the transamidation activity of the enzyme, underscoring the role of Ca^{2+} in allosteric regulation of TG2 function.^{45,46} Given the observation that TG2-null mice are developmentally and reproductively normal,^{47,48} perhaps it is not surprising that mutations in the corresponding human gene have subtle, if any, phenotypes.

Transglutaminase 1 (TG1)

Structure

TG1 (also known as keratinocyte transglutaminase) is primarily expressed in epithelial tissues and is activated upon terminal differentiation of keratinocytes (Fig. 5). In contrast to TG2 and TG3, the crystal structure of mammalian TG1 has not yet been solved, although a β -barrel domain of TG1 has recently been crystallized (PDB accession code 2XZZ).⁵²

The basic architecture of the modeled TG1 enzyme is similar to that of other transglutaminases,⁵³ where an N-terminal β -sandwich (Ser94 to Phe246) is followed by the catalytic domain (Asn247-Arg572) and two β -barrels (Gly573-Arg688 and Thr689-Ala816). The vicinal Cys residues that undergo disulfide bond formation in TG2 are also conserved in TG1, although redox-dependent activity of TG1 has not been reported. A unique feature of TG1 is the presence of a 10 kDa N-terminal region that serves as a membrane anchor after post-translational modification via myristoylation.⁵⁴

Regulation

TG1 is synthesized as a 92 kDa protein, and further modified into a 106 kDa form. Modifications include

Figure 4. Structure of TG2 in the open (PDB 2Q3Z) conformation, colored by domain (blue: N-terminal domain, green: catalytic domain, yellow: first C-terminal β -barrel, red: second C-terminal β -barrel). The thiol of the active site Cys277 is visualized with a yellow sphere. Three positions whose mutation has been implicated with human disease (M330R, I331N, N333S, all in early-onset Type 2 diabetes) are depicted on the protein structure as brown spheres.^{43,44} These mutations map to the vicinity of the putative calcium-binding site S3.⁴⁵ The vicinal disulfide bond between Cys370 and Cys371 is depicted in the inset.

constitutive *N*-myristoylation at a lysine followed by regulated myristoylation or palmitoylation at two Cys residues, all which lie in the N-terminal region.^{54–56} The membrane-bound protein is catalytically inactive, although the membrane-dissociated form appears to have low activity.⁵⁷

During keratinocyte differentiation, TG1 is activated by two proteolytic cleavages adjacent to Gly93 and Gly573, which coincide with the C-terminal boundaries of the membrane anchoring domain and the catalytic domain, respectively.^{53,58} The resulting 67 kDa fragment includes the catalytic domain. The protease(s) responsible for activation of enzyme function remain to be identified.

As with TG2, Ca^{2+} concentration is a key determinant of TG1 activity.⁵⁹ Based on sequence conservation with TG3, at least three calcium-binding sites are predicted. Occupancy of one of these binding

sites is expected to result in the movement of a loop, thereby opening access to the active site. Because Mg^{2+} can inhibit enzyme activity, occupancy of the same site with Mg^{2+} is thought to relax the loop back to its original configuration.⁵³ Guanine nucleotides not appear to affect TG1 activity.⁵³

Recently, tazarotene-induced gene 3 (TIG3), a 164 amino acid protein whose expression can be induced by the synthetic retinoid tazarotene, has been shown to activate TG1. TIG3 appears to bind to TG1 and activate its catalytic function even in the absence of calcium ions.^{60,61} Further analysis of this phenomenon could yield important clues for the design of small molecule transglutaminase activators.

Mutations

In contrast to the other transglutaminases, more than 70 missense mutations in the TG1 gene

Figure 5. Model of the TG1 protein (generated using the Swiss-Model Automated Comparative Protein Modeling Server,⁴⁹ using the Factor XIII crystal structure 1EX0 as template, as described by Herman and coworkers⁵⁰). Amino acids 105–787 were modeled, the N-terminal membrane anchoring domain is thus missing. The domains of the conserved TG architecture are colored as for TG2 (blue: N-terminal domain, green: catalytic domain, yellow: first C-terminal β -barrel, red: second C-terminal β -barrel). The thiol of the active site Cys377 is visualized as a yellow sphere. Mutations with a disease annotation in the UniProt database⁵¹ are mapped as spheres on the structure, color coded by amino acid type (neutral residues: brown, acidic residues: red, basic residues: blue).

have been implicated in human diseases. Notable examples of inheritable disorders due to TG1 mutation include lamellar ichthyosis, autosomal recessive congenital ichthyosis, and nonbullous congenital erythrodermal ichthyosis.⁵⁰ Mutations are present on all domains of the protein, but strongly concentrate in the catalytic domain. Although most mutations await biochemical characterization to establish whether their effects are due to destabilization of the protein or altering regulatory sites, they effectively lead to reduced TG1 cross-linking activity during keratinocyte maturation.⁶²

Transglutaminase 3 (TG3)

Structure

Similar to TG1, TG3 (also known as epidermal transglutaminase) is expressed in keratinocytes and is involved in their terminal differentiation (Fig. 6). A 77 kDa protein, its architecture is similar to TG2, and consists of an N-terminal β -sandwich domain, followed by the catalytic domain and two β -barrel domains. The crystal structures of the zymogen form of TG2 as well as various cofactor-bound forms of the mature protein have been solved.^{63–65} The vicinal Cys residues that undergo redox-mediated disulfide bond formation in TG2 are absent in TG3.

Regulation

Similar to TG1, TG3 is expressed as an inactive zymogen that must undergo proteolytic processing. The

Figure 6. Crystal structure of TG3 in the proteolyzed, calcium-bound state (PDB 1L9N). The domains of the conserved TG architecture are colored as for TG2 (blue: N-terminal domain, green: catalytic domain, yellow: first C-terminal β -barrel, red: second C-terminal β -barrel) and the active site thiol of Cys272 is depicted as a yellow sphere. None of the single-nucleotide polymorphisms leading to an amino acid change of the TG3 protein have thus far been implicated in a human disease.

77 kDa pro-TG3 is cleaved into a 50 kDa N-terminal fragment containing the active site and a 27 kDa C-terminal fragment, which remains associated with the mature enzyme.^{66,67} The cleavage site (between Ala466 and Ala467) is located on a long loop connecting the catalytic domain and the first β -barrel.⁶⁵ Although the protease responsible for activating pro-TG3 has not yet been definitively identified, there is growing evidence that cathepsin L catalyzes this reaction during keratinocyte differentiation.⁶⁸

As is the case with other transglutaminases, TG3 requires Ca^{2+} for its catalytic activity. The mechanistic basis for this allosteric regulation is well understood. The zymogen binds a single Ca^{2+} ion extremely tightly.⁶³ Upon processing to the mature form, two additional ion binding sites with an average dissociation constant of 4 μM become available.^{63,64} In particular, coordination of a calcium ion by Asp324 induces the movement of a loop region, enabling substrate access to the active site.⁶⁴ Cooperativity has been observed between the two weak calcium binding sites. Replacement of either ion by Mg^{2+} leads to tighter metal coordination, thereby maintaining the loop in its inactive configuration.^{64,65} Thus, the relative concentrations of Ca^{2+} and Mg^{2+} in a keratinocyte may be important for regulation of TG3 enzyme activity.⁶⁵

As with TG2, guanine nucleotides also inhibit TG3 activity in an entirely analogous manner.^{65,69} The thienopyrimidinone LDN-27219 analog 8 [Fig. 3(A)] also cross reacts with TG3 with a potency that is comparable with its TG2 affinity.^{35,38}

Mutations and transcriptional variants

Two splicing isoforms of TG3 have been identified. The longer splice variant, resulting from a deletion of exons 9 and 10, lacks the amino acids lining the nucleotide binding pocket as well as the second Ca^{2+} binding site. Consequently, the mutant lacks GTPase activity and is not regulated by guanine nucleotides, yet it retains 25% transglutaminase activity compared with the full-length protein. The second splice variant is a result of deletion of exons 6 and 7, but due to a frameshift, translation terminates early in exon 8. The resulting protein is inactive. It is unclear whether these splicing isoforms are a result of a regulated process and whether they possess any physiological function distinct from the full-length enzyme.⁷⁰

A few missense mutations have also been identified, although none has yet been implicated in a human disease or has been otherwise functionally annotated.

Factor XIIIa (FXIIIa)

Structure

Factor XIII is a tetrameric enzyme complex involved in the blood clotting cascade. It exists as an A_2B_2

heterotetramer of which the A subunit (FXIIIa) is a transglutaminase zymogen and the B subunit (FXIIIb) is an inhibitory glycoprotein with no enzymatic function.⁴ Although the full complex has not yet been crystallized, the structure of the A_2 homodimer has been solved by x-ray crystallography.^{4,71–74} The architecture of this 83 kDa protein resembles that of the other transglutaminases with an N-terminal β -sandwich (residues 38–184) preceding the catalytic domain (residues 185–515) that harbors the active site Cys314 and two C-terminal β -barrels (residues 516–628 and 629–731).^{4,75}

Regulation

Like other transglutaminases, the catalytic activity of FXIIIa is tightly regulated via an intricate activation scheme involving Ca^{2+} , proteolysis, and substrate interactions.⁴ In plasma, the A_2 homodimer harbors an N-terminal peptide, which undergoes thrombin-catalyzed cleavage between Arg37 and Gly38 in order to activate enzymatic activity. Initially, the N-terminal peptide remains associated with the heterotetramer, but proteolytic cleavage weakens the protein–protein interactions between the A_2 and B_2 subunits.^{76,77} After cleavage and upon occupancy of high-affinity Ca^{2+} binding sites on the A subunit, the inhibitory B subunits dissociate, leaving the A_2 dimer to undergo a calcium-dependent conformational change to the catalytically active A_2^* species.^{77,78} It has been found that this dimer attains full activity upon cleavage of only one of the two activation peptides.⁷⁸

Macroscopically, FXIIIa binds one Ca^{2+} ion per A subunit with an apparent dissociation constant of $\sim 0.1 \text{ mM}$ and up to 7 additional Ca^{2+} ions upon increasing the external Ca^{2+} concentration to 2.5 mM.⁷⁹ There is evidence for regulatory low-affinity sites that can render the enzyme active at extremely high-calcium concentrations ($>100 \text{ mM}$) without the need for proteolysis.⁷⁶ Although the physiological relevance of this effect is unclear, intracellular FXIIIa lacking the inhibitory B subunits can be activated upon elevation of intracellular calcium concentrations in physiologically relevant ranges, independent of proteolysis.^{80–82}

Interestingly, when FXIIIa polymerizes fibrin in the blood clotting cascade, the resulting polymer accelerates activation of the zymogen by protein–protein interactions with the B subunits, so as to sensitize the complex toward more effective proteolysis.^{4,83}

Factor XIIIa does not possess a binding site for guanine nucleotides, and its activity is thus not regulated by GTP.^{84–86} Among known TG2 inhibitors that have an allosteric mode of action, the LDN-27219 analog 8 [Fig. 3(A)] cross reacts with FXIII, albeit with low potency, which would suggest that the unidentified binding site of this inhibitor on

Figure 7. Crystal structure of Factor XIII (PDB 1GGU). The domains of the conserved TG architecture are colored as for TG2 (blue: N-terminal domain, green: catalytic domain, yellow: first C-terminal β -barrel, red: second C-terminal β -barrel). The portion of the activation peptide that is resolved in the crystal structure is colored orange. The active site thiol of Cys314 is indicated as a yellow sphere. On this protein backbone are depicted those residues as spheres whose mutation has been implicated in Factor XIII deficiency conditions (the mutated residues were retrieved from the FXIII mutation database at <http://www.f13-database.de>⁸⁶). The types of the affected amino acids are color coded as for TG1 (neutral residues: brown, acidic residues: red, basic residues: blue).

transglutaminase enzymes is distinct from the nucleotide binding pocket.^{35,38}

Mutations

Similar to TG1, loss-of-function mutations in FXIIIa tend to give rise to a disease phenotype that is due to the diminished stability of fibrin clots. At least 37 missense mutations with a phenotype have been reported to date, most of which are predicted to destabilize the protein.^{86,87} The affected residues are mapped onto the structure in Figure 7.

Summary

In summary, the catalytic activity of transglutaminases is tightly controlled and nature has evolved an intricate scheme of allosteric ligands and modifications to induce or inhibit these enzymes. Some of these cues are shared by members of the family but others are specific to a subset of isoforms. Transglutaminases generally require calcium ions for their crosslinking function. For some members of the family, including TG2 and TG3, but not TG1 and FXIIIa, a nucleotide binding pocket is antagonistic to calcium and, when occupied, inhibits the enzyme. For TG3, magnesium can occupy a calcium-binding site and inhibit the enzyme. Overall, the relative local concentrations of these cofactors will likely determine the activity state of the enzyme. The local redox potential represents a means to keep extracellular TG2 inactive by oxidation of a pair of surface sulfides, which can transiently be reduced by the cellular reducing agent thioredoxin 1 to gain TG2 crosslinking function. While the cysteine redox sensor is conserved among some isoforms, including TG1, it is unknown whether it presents a physiological regulatory role for this intracellular protein.

Additionally, specific proteolytic events unmask the catalytic activity of TG1, TG3, and FXIII that are expressed as zymogens and might play a role in attaining intracellular TG2 activity by abrogating its GTP-mediated inhibition. Allosteric protein–protein with the B subunit interactions play a role in retaining plasma FXIIIa in its inactive A_2B_2 form, whereas the interaction of TIG3 with TG1 appears to induce its catalytic activity, albeit by an unknown mechanism. It is also not yet known whether interactions of TG2 with the extracellular matrix (except fibronectin) influence its activity state.

Although there has been considerable progress in understanding the regulatory cues for transglutaminase activity in recent years, for example, establishing the redox state as for TG2, a number of regulatory features await discovery or characterization, such as the induction of intracellular TG2 activity. Similarly, analysis of protein–protein interactions, such as the activation of TG1 by TIG3 could allow for the design of small-molecule TG activators.

References

1. Lorand L, Graham RM (2003) Transglutaminases: crosslinking enzymes with pleiotropic functions. *Nat Rev Mol Biol* 4:140–156.
2. Iismaa SE, Mearns BM, Lorand L, Graham RM (2009) Transglutaminases and disease: lessons from genetically engineered mouse models and inherited disorders. *Physiol Rev* 89:991–1023.
3. Mehta K, Eckert R, editors (2005). *Transglutaminases family of enzymes with diverse functions*. Basel: Karger, pp1–247.
4. Muszbek L, Bereczky Z, Bagoly Z, Komáromi I, Katona É (2011) Factor XIII: a coagulation factor with multiple

- plasmatic and cellular functions. *Physiol Rev* 91: 931–972.
5. Nurminskaya MV, Belkin AM (2012) Cellular functions of tissue transglutaminase. *Int Rev Cell Mol Biol* 294: 1–97.
 6. Liu S, Cerione RA, Clardy J (2002) Structural basis for the guanine nucleotide-binding activity of tissue transglutaminase and its regulation of transamidation activity. *Proc Natl Acad Sci USA* 99:2743–2747.
 7. Pinkas DM, Strop P, Brunger AT, Khosla C (2007) Transglutaminase 2 undergoes a large conformational change upon activation. *PLoS Biol* 5:2788–2796.
 8. Bergamini CM (1988) GTP modulates calcium binding and cation-induced conformational changes in erythrocyte transglutaminase. *FEBS Lett* 239:255–258.
 9. Király R, Csoos E, Kurtán T, Antus S, Szigeti K, Simon-Vecsei Z, Korponay-Szabó IR, Keresztessy Z, Fésüs L (2009) Functional significance of five noncanonical Ca^{2+} -binding sites of human transglutaminase 2 characterized by site-directed mutagenesis. *FEBS J* 276:7083–7096.
 10. Bergamini CM, Signorini M, Poltronieri L (1987) Inhibition of erythrocyte transglutaminase by GTP. *Biochim Biophys Acta* 916:149–151.
 11. Begg GE, Holman SR, Stokes PH, Matthews JM, Graham RM, Iismaa SE (2006) Mutation of a critical arginine in the GTP-binding site of transglutaminase 2 disinhibits intracellular cross-linking activity. *J Biol Chem* 281:12603–12609.
 12. Bergamini CM, Dondi A, Lanzara V, Squerzanti M, Cervellati C, Montin K, Mischiati C, Tasco G, Collighan R, Griffin M, Casadio R (2010) Thermodynamics of binding of regulatory ligands to tissue transglutaminase. *Amino Acids* 39:297–304.
 13. Siegel M, Strnad P, Watts RE, Choi K, Jabri B, Omary MB, Khosla C (2008) Extracellular transglutaminase 2 is catalytically inactive, but is transiently activated upon tissue injury. *PLoS ONE* 3:e1861.
 14. Folk J, Cole P (1966) Mechanism of action of guinea pig liver transglutaminase. *J Biol Chem* 241: 5518–5525.
 15. Boothe R, Folk J (1969) A reversible, calcium-dependent, copper-catalyzed inactivation of guinea pig liver transglutaminase. *J Biol Chem* 244:399–405.
 16. Jin X, Stamnaes J, Klöck C, Diraimondo TR, Sollid LM, Khosla C (2011) Activation of extracellular transglutaminase 2 by thioredoxin. *J Biol Chem* 286: 37866–37873.
 17. Stamnaes J, Pinkas DM, Fleckenstein B, Khosla C, Sollid LM (2010) Redox regulation of transglutaminase 2 activity. *J Biol Chem* 285:25402–25409.
 18. Fesus L, Piacentini M (2002) Transglutaminase 2: an enigmatic enzyme with diverse functions. *Trends Biochem Sci* 27:534–539.
 19. Orrenius S, Zhivotovsky B, Nicotera P (2003) Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol* 4:552–565.
 20. Wei L, Warburton RR, Preston IR, Roberts KE, Comhair SAA, Erzurum SC, Hill NS, Fanburg BL (2012) Serotonylated fibronectin is elevated in pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 302: L1273–L1279.
 21. Zemskov E, Janiak A, Hang J, Waghay A (2006) The role of tissue transglutaminase in cell-matrix interactions. *Front Biosci* 11:1057–1076.
 22. Turner P, Lorand L (1989) Complexation of fibronectin with tissue transglutaminase. *Biochemistry* 28: 628–635.
 23. Nakaoka H, Perez DM, Baek KJ, Das T, Husain A, Misono K, Im MJ, Graham RM (1994) Gh: a GTP-binding protein with transglutaminase activity and receptor signaling function. *Science* 264:1593–1596.
 24. Lortat-Jacob H, Burhan I, Scarpellini A, Thomas A, Imberty A, Vivès RR, Johnson T, Gutierrez A, Verderio EA (2012) Transglutaminase-2 interaction with heparin: identification of a heparin binding site that regulates cell adhesion to fibronectin-transglutaminase-2 matrix. *J Biol Chem* 287:18005–18017.
 25. Wang Z, Collighan RJ, Pytel K, Rathbone DL, Li X, Griffin M (2012) Characterization of heparin-binding site of tissue transglutaminase: its importance in cell surface targeting, matrix deposition, and cell signaling. *J Biol Chem* 287:13063–13083.
 26. Wang Y, Ande SR, Mishra S (2012) Phosphorylation of transglutaminase 2 (TG2) at serine-216 plays a role in TG2 mediated activation of nuclear factor-kappa B and in the downregulation of PTEN. *BMC Cancer* 12:277.
 27. Fraij BM (2011) Activation of tissue transglutaminase by removal of carboxyl-terminal peptides. *J Cell Biochem* 112:3469–3481.
 28. Watts RE, Siegel M, Khosla C (2006) Structure-activity relationship analysis of the selective inhibition of transglutaminase 2 by dihydroisoxazoles. *J Med Chem* 49:7493–7501.
 29. Marrano C, de Macêdo P, Keillor JW (2001) Evaluation of novel dipeptide-bound alpha, beta-unsaturated amides and epoxides as irreversible inhibitors of guinea pig liver transglutaminase. *Bioorg Med Chem* 9:1923–1928.
 30. Hausch F, Halttunen T, Mäki M, Khosla C (2003) Design, synthesis, and evaluation of gluten peptide analogs as selective inhibitors of human tissue transglutaminase. *Chem Biol* 10:225–231.
 31. Siegel M, Khosla C (2007) Transglutaminase 2 inhibitors and their therapeutic role in disease states. *Pharmacol Ther* 115:232–245.
 32. Pardin C, Pelletier JN, Lubell WD, Keillor JW (2008) Cinnamoyl inhibitors of tissue transglutaminase. *J Org Chem* 73:5766–5775.
 33. Datta S, Antonyak MA, Cerione RA (2007) GTP-binding-defective forms of tissue transglutaminase trigger cell death. *Biochemistry* 46:14819–14829.
 34. Jeong K-C, Ahn K-O, Lee BI, Lee C-H, Kim S-Y (2010) The mechanism of transglutaminase 2 inhibition with glucosamine: implications of a possible anti-inflammatory effect through transglutaminase inhibition. *J Cancer Res Clin Oncol* 136:143–150.
 35. Case A, Stein RL (2007) Kinetic analysis of the interaction of tissue transglutaminase with a nonpeptidic slow-binding inhibitor. *Biochemistry* 46:1106–1115.
 36. Klöck C, Jin X, Choi K, Khosla C, Madrid PB, Spencer A, Raimundo BC, Boardman P, Lanza G, Griffin JH (2011) Acylideneoxindoles: a new class of reversible inhibitors of human transglutaminase 2. *Bioorg Med Chem Lett* 21:2692–2696.
 37. Duval E, Case A, Stein RL, Cuny GD (2005) Structure-activity relationship study of novel tissue transglutaminase inhibitors. *Bioorg Med Chem Lett* 15:1885–1889.
 38. Schaertl S, Prime M, Wityak J, Dominguez C, Munoz-Sanjuan I, Pacifici RE, Courtney S, Scheel A, Macdonald D (2010) A profiling platform for the characterization of transglutaminase 2 (TG2) inhibitors. *J Biomol Screen* 15:478–487.
 39. Fraij BM, Birckbichler PJ, Patterson MK, Lee KN, Gonzales RA (1992) A retinoic acid-inducible mRNA from human erythroleukemia cells encodes a novel tissue transglutaminase homologue. *J Biol Chem* 267: 22616–22623.

40. Fraij BM, Gonzales RA (1996) A third human tissue transglutaminase homologue as a result of alternative gene transcripts. *Biochim Biophys Acta* 1306:63–74.
41. Lai T-S, Liu Y, Li W, Greenberg CS (2007) Identification of two GTP-independent alternatively spliced forms of tissue transglutaminase in human leukocytes, vascular smooth muscle, and endothelial cells. *FASEB J* 21:4131–4143.
42. Antonyak MA, Jansen JM, Miller AM, Ly TK, Endo M, Cerione RA (2006) Two isoforms of tissue transglutaminase mediate opposing cellular fates. *Proc Natl Acad Sci USA* 103:18609–18614.
43. Bernassola F, Federici M, Corazzari M, Terrinoni A, Hribal ML, De Laurenzi V, Ranalli M, Massa O, Sesti G, McLean WHI, Citro G, Barbetti F, Melino G (2002) Role of transglutaminase 2 in glucose tolerance: knockout mice studies and a putative mutation in a MODY patient. *FASEB J* 16:1371–1378.
44. Porzio O, Massa O, Cunsolo V, Colombo C, Malaponti M, Bertuzzi F, Hansen T, Johansen A, Pedersen O, Meschi F, Terrinoni A, Melino G, Federici M, Decarlo N, Menicagli M, Campani D, Marchetti P, Ferdaoussi M, Froguel P, Federici G, Vaxillaire M, Barbetti F (2007) Missense mutations in the TGM2 gene encoding transglutaminase 2 are found in patients with early-onset type 2 diabetes. Mutation in brief no. 982. *Online. Hum Mutat* 28:1150.
45. Király R, Demény M, Fésüs L (2011) Protein transamidation by transglutaminase 2 in cells: a disputed Ca^{2+} -dependent action of a multifunctional protein. *FEBS J* 278:4717–4739.
46. Salter NW, Ande SR, Nguyen HK, Nyomba BLG, Mishra S (2012) Functional characterization of naturally occurring transglutaminase 2 mutants implicated in early-onset type 2 diabetes. *J Mol Endocrinol* 48: 203–216.
47. Nanda N, Iismaa SE, Owens WA, Husain A, Mackay F, Graham RM (2001) Targeted inactivation of Gh/tissue transglutaminase II. *J Biol Chem* 276:20673–20678.
48. De Laurenzi V, Melino G (2001) Gene disruption of tissue transglutaminase. *Mol Cell Biol* 21:148–155.
49. Arnold K, Bordoli L, Kopp J, Schwede T (2006) The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* 22:195–201.
50. Herman ML, Farasat S, Steinbach PJ, Wei M-H, Toure O, Fleckman P, Blake P, Bale SJ, Toro JR (2009) Transglutaminase-1 gene mutations in autosomal recessive congenital ichthyosis: summary of mutations (including 23 novel) and modeling of TGase-1. *Hum Mutat* 30: 537–547.
51. UniProt Consortium (2012) Reorganizing the protein space at the Universal Protein Resource (UniProt). *Nucleic Acids Res.* 40:D71–D75.
52. Vollmar M, Krysztofinska E, Krojer T, Yue WW, Cooper C, Kavanagh K, Allerston C, Chaikuad A, Von Delft F, Arrowsmith CH, Weigelt J, Edwards A, Bountra C, Oppermann U. (in press) Crystal structure of the human transglutaminase 1 beta-barrel domain (PDB File 2XZZ).
53. Boeshans KM, Mueser TC, Ahvazi B (2007) A three-dimensional model of the human transglutaminase 1: insights into the understanding of lamellar ichthyosis. *J Mol Model* 13:233–246.
54. Steinert PM, Kim SY, Chung SI, Marekov LN (1996) The transglutaminase 1 enzyme is variably acylated by myristate and palmitate during differentiation in epidermal keratinocytes. *J Biol Chem* 271:26242–26250.
55. Chakravarty R, Rice RH (1989) Acylation of keratinocyte transglutaminase by palmitic and myristic acids in the membrane Anchorage region. *J Biol Chem* 264: 625–629.
56. Phillips MA, Qin Q, Mehrpouyan M, Rice RH (1993) Keratinocyte transglutaminase membrane anchorage: analysis of site-directed mutants. *Biochemistry* 32: 11057–11063.
57. Steinert PM, Chung SI, Kim SY (1996) Inactive zymogen and highly active proteolytically processed membrane-bound forms of the transglutaminase 1 enzyme in human epidermal keratinocytes. *Biochem Biophys Res Commun* 221:101–106.
58. Kim SY, Chung SI, Steinert PM (1995) Highly active soluble processed forms of the transglutaminase 1 enzyme in epidermal keratinocytes. *J Biol Chem* 270: 18026–18035.
59. Rice RH, Green H (1979) Presence in human epidermal cells of a soluble protein precursor of the cross-linked envelope: activation of the cross-linking by calcium ions. *Cell* 18:681–694.
60. Sturniolo MT, Chandraratna RAS, Eckert RL (2005) A novel transglutaminase activator forms a complex with type 1 transglutaminase. *Oncogene* 24:2963–2972.
61. Eckert RL, Sturniolo MT, Jans R, Kraft CA, Jiang H, Rorke EA (2009) TIG3: a regulator of type I transglutaminase activity in epidermis. *Amino Acids* 36:739–476.
62. Farasat S, Wei M-H, Herman M, Liewehr DJ, Steinberg SM, Bale SJ, Fleckman P, Toro JR (2009) Novel transglutaminase-1 mutations and genotype-phenotype investigations of 104 patients with autosomal recessive congenital ichthyosis in the USA. *J Med Genet* 46: 103–111.
63. Ahvazi B, Kim HC, Kee S-H, Nemes Z, Steinert PM (2002) Three-dimensional structure of the human transglutaminase 3 enzyme: binding of calcium ions changes structure for activation. *EMBO J* 21: 2055–2067.
64. Ahvazi B, Boeshans KM, Idler W, Baxa U, Steinert PM (2003) Roles of calcium ions in the activation and activity of the transglutaminase 3 enzyme. *J Biol Chem* 278:23834–23841.
65. Ahvazi B, Boeshans KM, Rastinejad F (2004) The emerging structural understanding of transglutaminase 3. *J Struct Biol* 147:200–207.
66. Kim IG, Gorman JJ, Park SC, Chung SI, Steinert PM (1993) The deduced sequence of the novel protransglutaminase E (TGase3) of human and mouse. *J Biol Chem* 268:12682–12690.
67. Kim H, Lewis M, Gorman J, Park SC, Girard J, Folk J, Chung S (1990) Protransglutaminase E from guinea pig skin. Isolation and partial characterization. *J Biol Chem* 265:21971–21978.
68. Cheng T, Hitomi K, van Vlijmen-Willems IMJJ, de Jongh GJ, Yamamoto K, Nishi K, Watts C, Reinheckel T, Schalkwijk J, Zeeuwen PLJM (2006) Cystatin M/E is a high affinity inhibitor of cathepsin V and cathepsin L by a reactive site that is distinct from the legumain-binding site. A novel clue for the role of cystatin M/E in epidermal cornification. *J Biol Chem* 281: 15893–15899.
69. Hitomi K, Kanehiro S, Ikura K, Maki M (1999) Characterization of recombinant mouse epidermal-type transglutaminase (TGase 3): regulation of its activity by proteolysis and guanine nucleotides. *J Biochem* 125: 1048–1054.
70. Zocchi L, Terrinoni A, Candi E, Ahvazi B, Bagetta G, Corasaniti MT, Lena AM, Melino G (2007)

- Identification of transglutaminase 3 splicing isoforms. *J Invest Dermatol* 127:1791–1794.
71. Fox BA, Yee VC, Pedersen LC, Le I, Bishop PD, Stenkamp RE, Teller DC (1999) Identification of the calcium binding site and a novel ytterbium site in blood coagulation factor XIII by X-ray crystallography. *J Biol Chem* 274:4917–4923.
 72. Weiss MS, Metzner HJ, Hilgenfeld R (1998) Two non-proline cis peptide bonds may be important for factor XIII function. *FEBS Lett* 423:291–296.
 73. Yee VC, Pedersen LC, Bishop PD, Stenkamp RE, Teller DC (1995) Structural evidence that the activation peptide is not released upon thrombin cleavage of factor XIII. *Thromb Res* 78:389–397.
 74. Yee VC, Pedersen LC, Le Trong I, Bishop PD, Stenkamp RE, Teller DC (1994) Three-dimensional structure of a transglutaminase: human blood coagulation factor XIII. *Proc Natl Acad Sci USA* 91:7296–7300.
 75. Muszbek L, Yee VC, Hevessy Z (1999) Blood coagulation factor XIII: structure and function. *Thromb Res* 94:271–305.
 76. Lorand L, Jeong JM, Radek JT, Wilson J (1993) Human plasma factor XIII: subunit interactions and activation of zymogen. *Meth Enzymol* 222:22–35.
 77. Radek JT, Jeong JM, Wilson J, Lorand L (1993) Association of the A subunits of recombinant placental factor XIII with the native carrier B subunits from human plasma. *Biochemistry* 32:3527–3534.
 78. Hornyak TJ, Shafer JA (1991) Role of calcium ion in the generation of factor XIII activity. *Biochemistry* 30: 6175–6182.
 79. Lewis BA, Freyssinet JM, Holbrook JJ (1978) An equilibrium study of metal ion binding to human plasma coagulation factor XIII. *Biochem J* 169:397–402.
 80. Muszbek L, Haramura G, Polgár J (1995) Transformation of cellular factor XIII into an active zymogen transglutaminase in thrombin-stimulated platelets. *Thromb Haemost* 73:702–705.
 81. Muszbek L, Polgár J, Boda Z (1993) Platelet factor XIII becomes active without the release of activation peptide during platelet activation. *Thromb Haemost* 69: 282–285.
 82. Abdalla S, Lother H, Langer A, El Faramawy Y, Quitterer U (2004) Factor XIIIa transglutaminase cross-links AT1 receptor dimers of monocytes at the onset of atherosclerosis. *Cell* 119:343–354.
 83. Lewis SD, Janus TJ, Lorand L, Shafer JA (1985) Regulation of formation of factor XIIIa by its fibrin substrates. *Biochemistry* 24:6772–6777.
 84. Achyuthan KE, Greenberg CS (1987) Identification of a guanosine triphosphate-binding site on guinea pig liver transglutaminase. Role of GTP and calcium ions in modulating activity. *J Biol Chem* 262:1901–1906.
 85. Iismaa SE, Wu MJ, Nanda N, Church WB, Graham RM (2000) GTP binding and signaling by Gh/transglutaminase II involves distinct residues in a unique GTP-binding pocket. *J Biol Chem* 275: 18259–18265.
 86. Ivaskevicius V, Seitz R, Kohler HP, Schroeder V, Muszbek L, Ariens RAS, Seifried E, Oldenburg J (2007) International registry on factor XIII deficiency: a basis formed mostly on European data. *Thromb Haemos* 97: 914–921.
 87. Biswas A, Ivaskevicius V, Seitz R, Thomas A, Oldenburg J (2011) An update of the mutation profile of factor 13 A and B genes. *Blood Rev* 25:193–204.