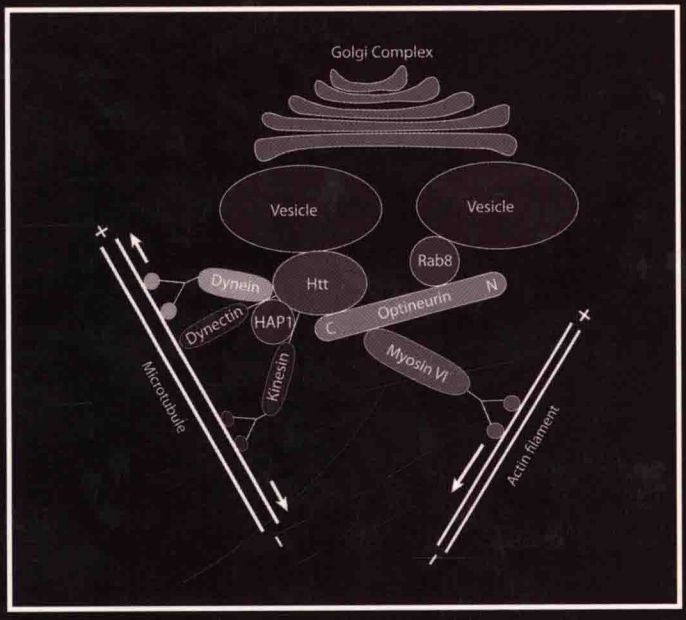
INTERNATIONAL REVIEW OF CELL AND MOLECULAR BIOLOGY

Edited by
Kwang W. Jeon



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Contents

CHAPTER ONE

Cellular Functions of Tissue Transglutaminase

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Abstract

Transglutaminase 2 (TG2 or tissue transglutaminase) is a highly complex multifunctional protein that acts as transglutaminase, GTPase/ATPase, protein disulfide isomerase, and protein kinase. Moreover, TG2 has many well-documented nonenzymatic functions that are based on its noncovalent interactions with multiple cellular proteins. A vast array of biochemical activities of TG2 accounts for its involvement in a variety of cellular processes, including adhesion, migration, growth, survival, apoptosis, differentiation, and extracellular matrix organization. In turn, the impact of TG2 on these processes implicates this protein in various physiological responses and pathological states, contributing to wound healing, inflammation, autoimmunity, neurodegeneration, vascular remodeling, tumor growth and metastasis, and tissue fibrosis. TG2 is ubiquitously expressed and is particularly abundant in endothelial cells, fibroblasts, osteoblasts, monocytes/macrophages, and smooth muscle cells. The protein is localized in multiple cellular compartments, including the nucleus, cytosol, mitochondria, endolysosomes, plasma membrane, and cell surface and extracellular matrix, where Ca^{2+}, nucleotides, nitric oxide, reactive oxygen species, membrane lipids, and distinct protein–protein interactions in the local microenvironment jointly regulate its activities. In this review, we discuss the complex biochemical activities and molecular interactions of TG2 in the context of diverse subcellular compartments and evaluate its wide ranging and cell type-specific biological functions and their regulation.

Key Words: Transglutaminase, Protein cross-linking, Transamidation, GTPase, Cell signaling, Stem cells, Therapeutic target.

Abbreviations

AKAP13	protein kinase A anchor protein 13
ANT1	adenine nucleotide translocator 1

CFTR	cystic fibrosis transmembrane conductance regulator
CREB cAMP	response element-binding protein
ECM	extracellular matrix
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EMT	epithelial mesenchymal transition
ERK	extracellular signal-regulated kinase
FAK	focal adhesion kinase
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
FXIIIA	Factor XIIIA
GPCR	G protein coupled receptor
HIF1	hypoxia inducible factor 1
HRE	hypoxic response element
IFN	interferon
IGF	insulin-like growth factor
IGFBP	insuline-like growth factor-binding protein
LAP	latency-associated peptide
LBTP	latent TGFβ-binding protein
LDLR	low density lipoprotein receptor
LPS	lipopolysaccharide
LRP	low density lipoprotein receptor-related protein
MEK	mitogen-activated protein kinase kinase
MFG-E8	milk fat globulin EGF factor 8
MMP	matrix metalloproteinase
MSC	mesenchymal stem cell
MTA1	metastatic tumor antigen 1
MT-MMP	membrane-type matrix metalloproteinase
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor
PDI	protein disulfide isomerase
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PPAR	peroxisome proliferator-activated receptor
Rb	retinoblastoma protein
ROCK	Rho kinase
ROS	reactive oxygen species
SUMO	small ubiquitin-like modifier
TG	transglutaminase
TG2	transglutaminase 2
TGF	transforming growth factor
TNF	tumor necrosis factor

TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
VLDLR	very low density lipoprotein receptor

1. Introduction

Transglutaminase 2 (TG2), also known as tissue transglutaminase (TG), is an 80-kDa protein that consists of four domains (Gentile et al., 1991; Liu et al., 2002). TG2 is the only ubiquitously expressed member of the TG family of enzymes that all catalyze Ca^{2+}-dependent protein deamidation, transamidation, and cross-linking (Iismaa et al., 2009; Lorand and Graham, 2003). Since the discovery of TG2 in 1957, a large number of its enzymatic substrates have been identified in intracellular compartments, including the cytosol, nucleus, and mitochondria, and extracellularly, on the cell surface and in the extracellular matrix (ECM) (Csosz et al., 2008; Facchiano and Facchiano, 2009). Availability of the protein's crystal structure (Han et al., 2010; Liu et al., 2002; Pinkas et al., 2007) facilitated our understanding how the transamidating activity of TG2 is regulated in cells by reversible conformational changes of the protein. These include Ca^{2+}-dependent activation, which shifts TG2 to the "open" (extended) conformation, thereby unmasking the enzyme's active center, and inhibition by GTP, GDP, and ATP, which constrains it in the "closed" (compact) conformation (Begg et al., 2006a,b; Casadio et al., 1999; Di Venere et al., 2000; Kiraly et al., 2011; Liu et al., 2002; Monsonego et al., 1998; Pinkas et al., 2007; Zhang et al., 1998). Although recent studies suggested that transamidating activity of TG2 inside and outside the cells is tightly controlled and might be suppressed *in vivo* in the absence of mechanical or chemical stresses (Siegel et al., 2008), it is likely that precise regulation of the enzyme's activity involves other important mechanisms, including the binding of Ca^{2+} ions to noncanonical sites (Kiraly et al., 2009), reversible reduction/oxidation via a formation of intramolecular disulfide bonds (Stamnaes et al., 2010), and NO-mediated nitrosylation (Lai et al., 2001). The fact that sphingophospholipids were shown to sensitize TG2 to Ca^{2+} regulation (Lai et al., 1997) suggests that other lipids that bind to TG2, such as cholesterol and phosphoinositides (Harsfalvi et al., 1987; Zemskov et al., 2011a), small molecules, or as-yet-unidentified TG2-interacting proteins, may also modulate its transamidating activity (Singh et al., 2001). Finally, generation of alternative spliced isoforms (Antonyak et al., 2006; Festoff et al., 2002; Fraij et al., 1992; Lai et al., 2007; Tee et al., 2010) and limited

proteolysis of the molecule (Fraij, 2011) was reported to influence the transamidating activity of TG2.

Besides its classical transamidating/protein cross-linking activity, TG2 possesses several other enzymatic functions (Iismaa et al., 2009, Lorand and Graham, 2003; Mehta et al., 2010; Park et al., 2010). Its GTPase activity allows intracellular TG2 to link transmembrane α_{1B}/α_{1D} adrenergic, thromboxane A2, and oxytocin receptors to cytoplasmic signaling targets such as phospholipase C (PLC)δ1, increasing inositol-1,4,5-trisphosphate levels upon stimulation of these receptors with appropriate agonists (Baek et al., 1993, 1996; Im and Graham, 1990; Im et al., 1990; Nakaoka et al., 1994; Park et al., 1998; Vezza et al., 1999). Biochemical studies revealed that the transamidating and GTPase activities of this protein are mutually exclusive: Ca^{2+}-bound TG2 has no GTPase activity, whereas GTP-bound TG2 does not exhibit TG activity (Feng et al., 1999a,b). The protein can also hydrolyze ATP (Iismaa et al., 1997), an activity which is believed to facilitate the promineralization capacity of TG2 in osteoblasts (Nakano et al., 2010).

Moreover, TG2 was found to display protein disulfide isomerase (PDI) activity *in vitro* (Hasegawa et al., 2003) and *in vivo* (Malorni et al., 2009; Mastroberardino et al., 2006). More recently, and even more surprisingly, TG2 was reported to phosphorylate insulin-like growth factor-binding protein-3 (IGFBP-3) on the cell surface, and p53 tumor suppressor protein, histones and retinoblastoma protein (Rb) in the nucleus, suggesting that it has an intrinsic serine/threonine protein kinase activity (Mishra and Murphy, 2004, 2006a,b; Mishra et al., 2006, 2007).

Finally, the vast array of TG2 functional activities in the cell is not limited to its enzymatic functions. TG2 was found engaged in the formation of noncovalent complexes with various cytoplasmic, cell surface, ECM, nuclear, and mitochondrial proteins (Iismaa et al., 2009; Lorand and Graham, 2003; Park et al., 2010). This emerging adapter/scaffolding function of TG2, which is independent of its enzymatic activities, appears to regulate cell adhesion, ECM remodeling, survival, growth, migration, and differentiation due to modulation of several signaling pathways (Belkin, 2011; Wang and Griffin, 2011).

An emerging theme in the field suggests that precise tuning of the numerous TG2 activities is defined by the microenvironment and localized protein–protein interactions within various cellular compartments (Park et al., 2010). Importantly, recent studies began to unravel the complex mechanisms of TG2 turnover, intracellular trafficking, and targeting to specific cellular compartments (Antonyak et al., 2011; Cho et al., 2011; Jeong et al., 2009; Luciani et al., 2009; Peng et al., 1999; Scarpellini et al., 2009; Zemskov et al., 2007, 2011a). In this review, we focus on the emerging mechanisms of spatial compartment-dependent regulation of TG2 activities in various cell types and their role in key cellular processes.

We abstain from in-depth discussion of various mechanistic aspects of transamidating and GTPase functions of TG2, as excellent reviews on these topics are published elsewhere (Bergamini, 2007; Facchiano and Facchiano, 2009; Kiraly et al., 2011; Mhaouty-Kodja, 2004, Siegel and Khosla, 2007). Likewise, we do not extensively discuss the involvement of TG2 in human disease states, as recent comprehensive reviews in this field either elaborate on the numerous pathophysiological aspects of TG2 function (Iismaa et al., 2009) or focus on its role in inflammation (Elli et al., 2009; Iismaa et al., 2009; Kim, 2006), wound healing and tissue fibrosis (Collighan and Griffin, 2009; Verderio et al., 2004), autoimmunity (Briani et al., 2008; Sollid, 2000), cardiovascular diseases (Bakker et al., 2008; Sane et al., 2007), cancer (Chhabra et al., 2009; Mehta et al., 2010), and neurodegeneration (Bailey et al., 2005; Jeitner et al., 2009; Malorni et al., 2008; Mastroberardino and Piacentini, 2010).

2. Enzymatic and Nonenzymatic Activities of TG2

2.1. TG2 as transglutaminase

TG2 was the first identified member of the TG family of Ca^{2+}-dependent enzymes that is now known to contain eight enzymatically active and one inactive member in humans (Facchiano and Facchiano, 2009; Lorand and Graham, 2003). It shares the same overall four-domain tertiary structure and several conserved secondary structure elements with other mammalian TGs (Grenard et al., 2001; Liu et al., 2002; Lorand and Graham, 2003; Nemes et al., 2005). Unlike closely related TG1, TG3, and Factor XIIIA (FXIIIA) TGs, TG2 does not require proteolysis for activation. In humans, it is encoded by a single *TGM2* gene located on chromosome 20q11–12. TG2 has a highly conserved catalytic triad of Cys277-His335-Asp358, which is shared by all other enzymatically active TGs as well as cysteine proteases that belong to the papain-like superfamily (Lorand and Graham, 2003). While these residues form the enzyme's active site within a substrate binding channel of the second (catalytic) domain, the adjacent Trp241 and Trp332 residues are involved in stabilization of the transition state (Iismaa et al., 2003; Liu et al., 2002). Like other TGs, TG2 catalyzes covalent cross-linking, transamidation, and deamidation of proteins (Fig. 1.1). More than one hundred of its enzymatic substrates have been identified in a variety of cellular compartments (Esposito and Caputo, 2005; Facchiano and Facchiano, 2009). Therefore, this enzymatic activity enables TG2 to generate an immense array of posttranslational modifications in target proteins.

Despite sharing the same enzymatic reaction of forming acyl-enzyme intermediates with other TGs, both donor- and acceptor-group specificity for TG2 distinguish it from homologous TGs such as FXIIIA (Gorman and

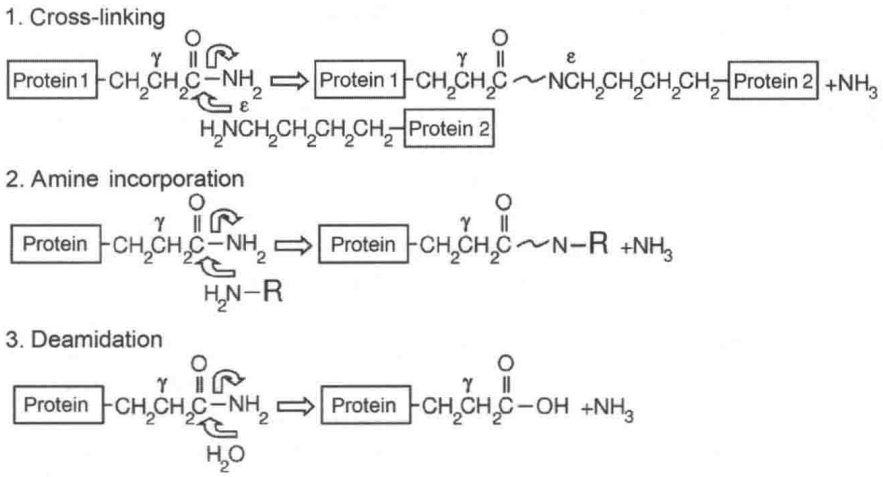

Figure 1.1 TG2 acting as transglutaminase catalyzes several types of posttranslational modifications of proteins. (1) *Protein cross-linking.* TG2-mediated transamidation reactions proceed via formation of a N^{ε}(γ-glutamyl)lysine isopeptide bond between the acceptor Gln residue of the protein 1 and deprotonated Lys donor residue of the protein 2. TG2 displays specificities toward both their Gln and Lys substrates. (2) *Protein aminylation.* TG2-mediated transamidation reactions occur via incorporation of an amine (H_2NR) into the Gln residue of the acceptor protein. Diamines and polyamines may act as a tether in a bis-glutaminyl adduct between two protein molecules. (3) *Deamidation of proteins.* TG2-mediated hydrolysis reactions in the absence of amine cosubstrates convert the Gln residues of the reactive protein into the Glu residues. Electron movements are shown by curved arrows. The *de novo* formed covalent bonds are shown by curved lines.

Folk, 1984, Hettasch et al., 1997; Khew et al., 2010), and TG1 and TG3 (Lorand and Graham, 2003). Although the distinction between reactive and nonreactive glutamines and lysines is dictated primarily by secondary and/or tertiary structural elements in the TG2 substrate proteins, the enzyme also displays preference at the level of primary sequence, mostly around reactive glutamine residues (Aeschlimann et al., 1992; Coussons et al., 1992). Using phage display combinatorial and bioinformatics approaches, the consensus sequences p-Q-X-(P,T,S)-I (Keresztessy et al., 2006) and Q-X-P-Φ-D-(P), Q-X-P-Φ, and Q-X-X-Φ-D-P (Sugimura et al., 2006) were defined as preferred for TG2-specific transamidation (where P and I stand for polar and aliphatic, and X and Φ stand for nonconserved and hydrophobic amino acids, respectively). Further developing these findings, a highly specific peptide for TG2-mediated transamidation, HQSYVDPWMLDH, was isolated from phage display libraries (Hitomi et al., 2009) and was subsequently shown to enable the detection of active TG2 *in situ* (Itoh et al., 2011). No such information is available with regard to consensus sequences containing the TG2-reactive lysines.