An isomerase completes the circuit for a redox switch

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The activity of human transglutaminase 2 (TG2), which forms protein cross-links between glutamine and lysine residues, is controlled by an allosteric disulfide bond. However, the mechanism by which this bond is formed, like many systems regulated by oxidative cysteine modifications, was not clear. A new study from Khosla and colleagues shows that TG2 is oxidatively inactivated by the protein disulfide isomerase ERp57, providing the first example of a defined and reversible protein-controlled redox switch and pointing to new strategies to inhibit undesirable TG2 activity in pathological states.

Cysteine residues in proteins are highly conserved, reflecting functional conservation. Due to the properties of its thiol group, Cys usually serves catalytic (redox and non-redox), structure-stabilizing, regulatory, and metal-binding functions. A unique feature of Cys residues is the capacity of forming protein disulfide bonds, which can be classified as structural, catalytic, or allosteric. Catalytic disulfides, present in numerous thiol-disulfide oxidoreductases, are transiently formed in enzyme active sites. In contrast, structural disulfides, common in extracellular proteins and ectodomains of membrane proteins, are a permanent feature of proteins and contribute to their structural stabilization. Allosteric disulfides regulate protein function when they undergo a redox change. In contrast to catalytic and structural protein disulfides, there are few examples of allosteric disulfides (1, 2); whether this paucity represents limited usage of this redox switch or the inherent challenges in capturing transient states that are highly dependent on local environment, or both, is not fully understood. Moreover, identifying the pathways by which these redox switches occur—whether induced by cellular metabolites or proteins—has been challenging. Until now, there was no example of a protein disulfide redox switch reversibly and allosterically modulated by two distinct proteins, comparable to the kinase/phosphatase or O-GlcNAc transferase/O-GlcNAcase pairs known for other post-translational modifications. Previous work from Khosla and colleagues identified the protein that activates TG2 by reducing (i.e. breaking) its allosteric disulfide bond in vitro (3) and in vivo (4). Their new study completes the circuit through the discovery of a protein that forms this disulfide bond in TG2 in the extracellular environment (5). This series of discoveries provides a refined framework for understanding the role of TG2 and its modulators and potentially points to new therapeutic approaches for conditions where TG2 is misregulated, such as celiac disease.

TG2 catalyzes the transamidation of glutamine to lysine residues, resulting in isopeptide bond formation in protein substrates. It is found in the intracellular and extracellular environments of many organs. Because of its cross-linking activity, TG2 stiffens the tissue extracellular matrix (ECM). Yet, TG2 is dormant in the ECM of most organs under normal physiological conditions and is only activated under specific conditions such as tissue injury or inflammatory stimuli (6). TG2 activity is regulated at a post-translational level and is responsive to nucleotides, calcium, and redox state. Binding to GTP or GDP inactivates the enzyme (by forming the closed conformer), whereas the absence of guanine nucleotides and the presence of Ca\(^{2+}\) activates the enzyme (forming the open conformer) (6, 7). The formation of a Cys\(^{370}\)–Cys\(^{371}\) vicinal allosteric disulfide bond results in an inactive open conformation (6, 7). Previous reports by Khosla and colleagues have shown that TG2 is specifically and efficiently activated by extracellular thioredoxin-1 (Trx) (3, 4), which reduces the allosteric disulfide bond (Fig. 1). Certain cues, such as proinflammatory signals, can increase Trx secretion, leading to TG2 activation. Yet, some pieces of the regulatory puzzle remained unsolved. Since TG2 is inactive in most tissues and secreted via a non-classical pathway, Khosla and colleagues reasoned that TG2 must be released into the ECM in its reduced form and subsequently inactivated via oxidation. But how is this oxidation occurring? The highlighted paper addresses this issue: The authors find that protein disulfide isomerase (PDI) ERp57 (also known as PDIA3), which is typically found in the ER but can also be secreted into the extracellular environment by a non-classical pathway, is responsible for TG2 inactivation in vitro and in vivo (Fig. 1). Oxidative inactivation might be required not only for the freshly secreted protein but also following reductive activation to switch off TG2.

To address the oxidative inactivation of TG2, the authors first determined the rate constants of reduced TG2 with low molecular weight oxidants (cystine, GSSG, and H\(_2\)O\(_2\)). These reactions were kinetically slow at physiological concentrations. Thus, they searched for specific proteins that can oxidize TG2. For this, they used human umbilical vein endothelial cells (HUVEC), which secrete PDI and its homologs (ERp5, ERp57, and ERp72); these proteins contain Trx domains, but their...
Figure 1. Redox control of transglutaminase 2 (TG2) through an allosteric disulfide bond is achieved by distinct proteins. Upon nonclassical secretion, reduced TG2 is inactivated through ERp57-mediated oxidation of an allosteric disulfide bond (Cys370–Cys372) (5). Specific stimuli (e.g. inflammatory signals) can activate TG2 through Trx-mediated reduction of this allosteric disulfide (4, 5). The redox potentials of the disulfides of Trx, TG2, and ERp57 are indicated. For simplicity, only one of the two CGHC redox motifs of ERp57 is shown, but both can oxidize TG2. Adapted with permission from research originally published in the Journal of Biological Chemistry. Yi et al. Endoplasmic reticulum–resident protein 57 (ERp57) oxidatively inactivates human transglutaminase 2. J. Biol. Chem. 2018; 293:2640–2649. (© the American Society for Biochemistry and Molecular Biology (5).)

These results also have implications for human health: Abnormally up-regulated TG2 activity is a hallmark of celiac disease, caused by an inappropriate immune response to dietary gluten that leads to intestinal dysfunction. In the absence of a lysine acceptor, water serves as TG2’s co-substrate, resulting in deamidation of glutamine residues. This activity has been implicated in the pathogenesis of celiac disease, since a gluten-deamidated peptide has higher affinity for certain MHC II (molecules responsible for presenting antigenic peptides to T cells) present in celiac patients and exacerbates duodenal inflammation (9). How TG2 is activated by injury or inflammation remained an unsolved question for years. Khosla’s group showed that interferon-γ–stimulated macrophages exposed to inflammatory stimuli mediate the extracellular release of Trx, which activates TG2 in the extracellular matrix of the small intestine (4). This generates an inflammatory amplification loop. An inflammatory Trx-TG2 axis may also increase protein cross-linking in other maladies in which TG2 has been implicated, such as renal fibrosis and neurodegenerative diseases. In summary, the highlighted research contributes to the understanding of the role of TG2 and its modulators in disease pathogenesis and points to new therapeutic approaches for diseases involving this enzyme.

References
1. Azimi, I., Wong, J. W., and Hogg, P. J. (2011) Control of mature protein function by allosteric disulfide bonds. Antioxid. Redox Signal. 14, 113–126 CrossRef Medline
2. Butera, D., Cook, K. M., Chiu, J., Wong, J. W., and Hogg, P. J. (2014) Control of blood proteins by functional disulfide bonds. Blood 123, 2000–2007 CrossRef Medline
3. Jin, X., Stammaes, J., Klock, C., DiRaimondo, T. R., Sollid, L. M., and Khosla, C. (2011) Activation of extracellular transglutaminase 2 by thioredoxin. J. Biol. Chem. 286, 37866–37873 CrossRef Medline
4. Plugis, N. M., Palanski, B. A., Weng, C. H., Albertelli, M., and Khosla, C. (2017) Thioredoxin-1 selectively activates transglutaminase 2 in the extracellular matrix of the small intestine: Implications for celiac disease. J. Biol. Chem. 292, 2000–2008 CrossRef Medline
5. Yi, M. C., Mellkonian, A. V., Ousey, I. A., and Khosla, C. (2018) Endoplasmic reticulum–resident protein 57 (ERp57) oxidatively inactivates human transglutaminase 2. J. Biol. Chem. 293, 2640–2649 CrossRef Medline
6. Eckert, R. L., Kaartinen, M. T., Nurminskaya, M., Belkin, A. M., Colak, G., Johnson, G. V., and Mehta, K. (2014) Transglutaminase regulation of cell function. Physiol. Rev. 94, 383–417 CrossRef Medline
7. Pinkas, D. M., Strop, P., Brunger, A. T., and Khosla, C. (2007) Transglutaminase 2 undergoes a large conformational change upon activation. PLoS Biol. 5, e327 CrossRef Medline
8. Iversen, R., Myrsling, S., Hnida, K., Jorgensen, T. J., and Sollid, L. M. (2014) Activity-regulating structural changes and autoantibody epitopes in transglutaminase 2 assessed by hydrogen/deuterium exchange. Proc. Natl. Acad. Sci. U.S.A. 111, 17146–17151 CrossRef Medline
9. Møllerberg, Ø., McAdam, S. N., Körner, R., Quaas, H., Kristiansen, C., Madsen, L., Fugger, L., Scott, H., Norén, O., Roepstorff, P., Lundin, K. E. A., Sjöström, H., and Sollid, L. M. (1998) Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. Nat. Med. 4, 713–717 CrossRef Medline