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Breakthroughs and Views

Hypothesis: a glycoprotein-degradation complex formed by protein–protein interaction involves cytoplasmic peptide:*N*-glycanase

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Abstract

A cytoplasmic peptide:*N*-glycanase has been implicated in the proteasomal degradation of newly synthesized misfolded glycoproteins that are exported from the endoplasmic reticulum to the cytosol. Recently, the gene encoding this enzyme (Png1p) was identified in yeast and shown to bind to the 26S proteasome through its interaction with a component of the DNA repair system, Rad23p. Moreover, a mouse homologue of Png1p (mPng1p), which has an extended N-terminal domain, was found to bind not only to the Rad23 protein, but also to various proteins related to the ubiquitin/proteasome pathway. An extended N-terminus of mPng1p, which is not found in yeast, contains a potential site of protein–protein interaction called the PUB/PUG domain. The PUB/PUG domain is predicted to be helix-rich and is found in various proteins that may be involved in the ubiquitin/proteasome-related pathway. This review will discuss the consequence of the deglycosylation reaction by peptide:*N*-glycanase in cellular processes. In addition, the potential importance of the PUB/PUG domain for the formation of a putative "glycoprotein-degradation complex" will be discussed.

Keywords: PUB/PUG domain; UBA domain; UBX domain; Peptide:*N*-glycanase

Secretion of proteins is an essential biological process in virtually all types of cells. In eukaryotes, proteins that go through the secretory path are transported into the endoplasmic reticulum (ER) through a protein channel (translocon) during or immediately following translation. Subsequently, these proteins acquire the correctly folded state and, in some cases, subunit structure in the ER with the aid of various lumenal chaperones such as BiP or protein disulfide isomerase, and eventually exit the ER by vesicular transport to their respective destinations. However, if these proteins are unable to guide folding to the correct state, the cell eliminates them. The system utilized to maintain the quality of proteins is generally called the "quality control system" [1].

The quality control machinery for secretory proteins has the capacity to differentiate unfolded or misfolded proteins from correctly folded proteins in the ER. Misfolded proteins are retained in the ER and eventually degraded by the mechanism called "ER-associated degradation" [2]. It is now clear that this degradation process does not occur in the ER. Subsequent to the retro-translocation of the defective proteins or glycoproteins from the ER to the cytosol, degradation process occurs in the cytosol and involves the proteasome. Further characterization of this quality control system may be medically important, since human genetic diseases such as autosomal dominant neurohypophyseal diabetes insipidus, α1-antitrypsin (A1Pi Z type) or polyglutamine diseases [spinobulbar muscular atrophy (SBMA)] are probably caused by the toxic effects of the misfolded protein or aggregates, which are believed to accumulate due to the deficiency in the level of degradation machinery in the cytosol [3,4].

The last several years have seen an explosion of information about proteins that interact with the

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proteasome and may play important regulatory roles in cellular processes as diverse as the cell cycle, DNA excession repair, and destruction of misfolded protein and glycoproteins. One of the molecules that interact individually with the proteasome in the cytosol is peptide:*N*-glycanase (PNGase). This enzyme is the subject of this review.

Cytoplasmic PNGase: its connection with proteasomal degradation

PNGase is a deglycosylating enzyme that has been suggested to be linked to proteasome-dependent degradation for misfolded glycoproteins that are translocated from the ER to the cytosol [5–8]. Recently the gene encoding this enzyme (*PNG1*) was identified in yeast, and orthologues of the gene product (Png1p) were found widely distributed in higher eukaryotes [9]. The Png1p orthologues found in higher eukaryotes turned out to have extended sequences at both the amino- and the carboxy-termini of the common central core ([9]; see Fig. 1), implying that this enzyme may have acquired these extensions during evolution of these organisms.

Quite recently, Rad23 protein was identified as a PNGase-binding protein in yeast [10]. Evidence has shown that a fraction of the Rad23 protein (Rad23p) binds to the 26S proteasome through the Rad23p N-terminus ubiquitin-like (UbL) domain [11]. In fact, the physical interaction of Png1p with the 26S proteasome occurs in a Rad23p-dependent manner in yeast [10]. The formation of a Rad23p–Png1p complex was found to be distinct from the well-established Rad23p–Rad4p complex required for DNA repair [10]; both complexes were found to involve binding to the C-terminal domain of Rad23p [10,12]. In addition, recent evidence showed that the UBA domain of Rad23p actually binds to ubiquitin/multiubiquitin [13–17]. These observations suggest that Rad23 may serve an "escort" function to connect the 26S proteasome with other proteins and thereby control the stability and/or the functions of these proteins.

Deglycosylation vs. proteolysis: which comes first?

Given the fact that PNGase can be physically associated with the 26S proteasome, it is not unreasonable to envisage that prior to the precessive proteolysis by the proteasome, the deglycosylation may occur as in the case of deubiquitinaton [18,19]. One pitfall of this assumption, however, is the fact that neither the biochemically purified mammalian cytoplasmic PNGase nor the purified yeast Png1p acts on intact glycoprotein substrates in vitro [9,20], in spite of the abundant evidence that deglycosylation can occur on glycoproteins in vivo [8]. Perhaps there is a yet unknown component(s) that facilitates the deglycosylation of glycoproteins by cytoplasmic PNGases. Now that the physical interaction of Png1p and the 26S proteasome is evident, the 19S proteasome complex is one of the obvious candidates that aid the unfolding of glycoprotein substrates [21–23]. Alternatively it is also possible that functional PNGase is located in proximity to the "dislocon," the site where glycoproteins are dislocated from the ER lumen into the cytosol, so that PNGase may be able to act on proteins which are readily unfolded while they pass through the protein channel. A final possibility is that PNGase or complex containing this enzyme can recognize and bind to the carbohydrate chain of unfolded proteins in the cytosol, but that it only deglycosylates the glycopeptides formed by proteasomal proteolysis of the glycoproteins. In this case, deglycosylation should come after the proteolysis (Fig. 2). So far the order of the proteolysis and the deglycosylation processes in the cytosol have not yet been determined in any system.

Deglycosylation of misfolded glycoproteins: a double-edged sword?

While the notion that bulky *N*-glycan chains on misfolded glycoproteins might impair the efficient action by the proteasome sounds reasonable, it would be important to note that, in some cases, PNGase-action can in fact be disadvantageous to cells. It has been known that when cells are overloaded with misfolded proteins they are accumulated as inclusion bodies (aggresomes [24]) in the cytosol. Similar proteinaceous aggregates can be seen in various neuropathies [25]. In the case of the cystic fibrosis transmembrane regulator (CFTR) [24] or the prion protein (PrP) [26], "non-glycosylated" species were notably enriched when these proteins were accumulated in the cytosol. Although it remains to be determined if these are the genuine "unglycosylated" species which were never glycosylated in the ER or they had been glycosylated but then deglycosylated in the cytosol by the action of PNGase, it would be safe to say that aglyco-form of misfolded proteins, compared with their glycosylated counterparts, will be subjected to the exposure of hydrophobic amino acid residues to the aqueous environment of the cytosol and subsequent aggregation. Thus, if the "unglycosylated species" are formed by the action of PNGase, the action of this enzyme could promote the accumulation of aggregates of misfolded proteins. Since it is proposed that the Scrapie-form of the prion protein (PrP^{Sc}) may arise de novo due to the compromised action of the proteasome in the sporadic and the familial forms of the prion diseases [26], one

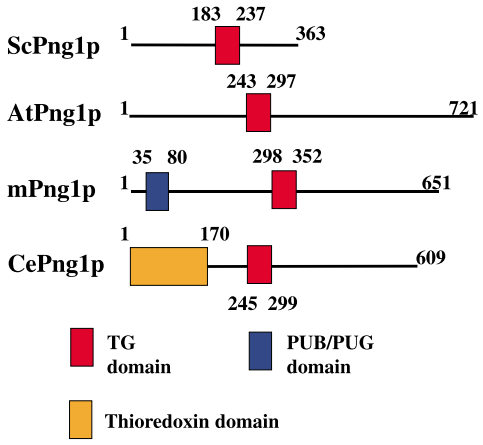

Fig. 1. Schematic representation of Png1p homologues: TG, transglutaminase-like domain [42,43], which includes catalytic triad for Png1p [8,44]; ScPng1p, *Saccharomyces cerevisiae* Png1p; mPng1p, mouse Png1p; and AtPng1p, *Arabidopsis thaliana* Png1p.

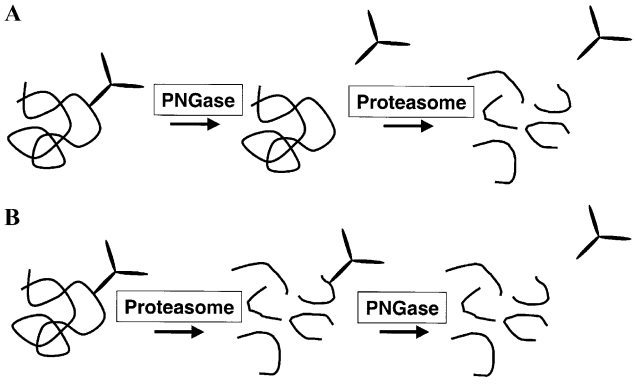

Fig. 2. The alternative route for misfolded glycoprotein degradation PNGase action on misfolded glycoproteins in the cytosols is either prior to (A) or after (B) degradation by the proteasome.

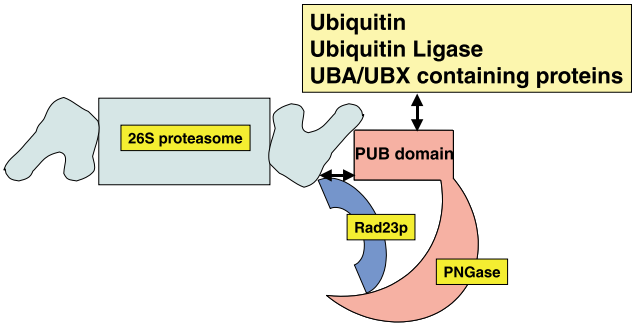

Fig. 3. Schematic representation of "glycoprotein degradation complex." The addition of an N-terminal extension of mouse Png1p including the PUB domain may be an evolutionary change which allows mouse Png1p to form "a glycoprotein degradation complex."

can speculate that inhibition of PNGase action could be a potential therapeutic target for control of this devastating disease.

PNGase action on misfolded proteins, nevertheless, could also serve a useful function. For instance, there is an evidence to suggest that cytoplasmic deglycosylation by PNGase is involved in the formation of a melanoma-specific antigenic peptide derived from tyrosinase, a key enzyme for melanogenesis [27]. Tyrosinase is known to be subjected to deglycosylation by PNGase during proteasomal degradation [28,29]. When the amino acid sequence of antigenic peptide was analyzed, it was found that a single amino acid conversion occurs on the peptide compared with the deduced amino acid sequence; that is, Asn in the recognition sequence (Asn–X–Ser/Thr) is changed to Asp [27]. The antigenic (Asp)-peptide sensitizes the tyrosinase-specific cytotoxic T lymphocytes (CTL) at a 100-fold lower concentration than the equivalent Asn-peptide [27]. This result can be explained if the action by PNGase during proteasomal degradation of tyrosinase in myeloma cell produces a change in sequence (Asp-peptide) from that which is encoded by the genome. Accidentally or not, this change would be advantageous for CTL-mediated recognition of the cancer cells as abnormal.

PUB/PUG domain: a potential protein–protein interaction domain

Recently the PUB/PUG domain was identified as a novel sequence motif within the N-terminal region of Png1p from vertebrates and insects [30,31]. This domain (amino acid residues 35–80 in mouse) is highly homologous across the species (mouse vs. human, 100% identical; mouse vs. Fugu, 82% identical, 10% similar; and mouse vs. fruit fly, 36% identical, 17% similar). Based on a secondary structure prediction, the PUB/PUG domain is predicted to have 4α-helices based on secondary structure prediction. Our preliminary experimental evidence shows that the PUB/PUG domain is important in the binding of the mPng1p to a wide variety of ubiquitin/proteasome pathway-related proteins [8,32]. Those proteins include the mouse homologue of Rad23p (mHR23B), a 19S proteasome subunit S4 (Rpt2p), ubiquitin, a hypothetical protein, which has 1 UBA and 1 UBX domain (Y33K), and autocrine motility factor receptor, which has a RING finger motif [33] and a CUE domain [34], both of which are implicated in the ubiquitination reaction. Interestingly, all of the interactions except that of Rad23p orthologue utilize the N-terminal domain of mPng1p [8,32]. These facts may reflect an evolutionary change whereby addition of an N-terminal extension to Png1p, including the PUB/PUG domain, allows mPng1p to control various regulatory protein–protein interactions for proteasome-mediated glycoprotein degradation.

Glycoprotein degradation complex: a specialized platform for glycoproteins?

The functional importance of the PUB/PUG domain in proteins is not yet proven. It is noteworthy, however, that some of the proteins that have PUB/PUG domains were found to have either a UBA or a UBX domain, a sequence motif present in multiple enzyme classes in the ubiquitin-related pathway [35]. This, together with our recent observations [32], implies that the acquisition of the PUB/PUG domain by animal Png1p may have resulted in its ability to directly bind to the 26S proteasome.

Recent studies suggest that both UBA and, most likely, UBX domains function as protein–protein interaction domains [13–17,36–40]. We hypothesize that the PUB/PUG domain, as proposed for UBA or UBX domain, may also serve as a protein–protein interaction domain. One can readily envision that, together with UBA or possibly UBX domains, the PUB/PUG domain can generate a number of protein–protein interaction networks in many cellular processes including the ubiquitin–proteasome pathway. In this case, the acquisition of PUB/PUG domain may result in the formation of a "glycoprotein-degradation complex" in order to facilitate a series of reactions such as retro-translocation, ubiquitination, deglycosylation, deubiquitination, and degradation of proteins at one cellular site (Fig. 3). Demonstration of the occurrence as well as the characterization of these complexes would be the next logical step to validate this hypothesis. In this connection, the recent finding of the lectin–ubiquitin ligase, Fbx2, involved in ER-associated degradation is very interesting [41]. Since it is likely that Fbx2 represents a glycoprotein-specific ubiquitin ligase, it is tempting to speculate that this dual-functional protein might also be a member of the "scavenger factory" that retrieves the building blocks (sugars and amino acids) of defective glycoproteins.

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References

[1] A. Helenius, Philos. Trans. R. Soc. Lond. B: Biol. Sci. 356 (2001) 147–150.
[2] A.A. McCracken, J.L. Brodsky, J. Cell Biol. 132 (1996) 291–298.
[3] J. Rutishauser, M. Spiess, Swiss Med. Wkly 132 (2002) 211–222.
[4] C.K. Bailey, I.F. Andriola, H.H. Kampinga, D.E. Merry, Hum. Mol. Genet. 11 (2002) 515–523.
[5] E.J. Wiertz, T.R. Jones, L. Sun, M. Bogyo, H.J. Geuze, H.L. Ploegh, Cell 84 (1996) 769–779.
[6] T. Suzuki, K. Kitajima, Y. Emori, Y. Inoue, S. Inoue, Proc. Nat. Acad. Sci. USA 94 (1997) 6244–6249.
[7] T. Suzuki, Q. Yan, W.J. Lennarz, J. Biol. Chem. 273 (1998) 10083–10086.
[8] T. Suzuki, H. Park, W.J. Lennarz, FASEB J. 16 (2002) 635–641.
[9] T. Suzuki, H. Park, N.M. Hollingworth, R. Sternglanz, W.J. Lennarz, J. Cell Biol. 149 (2000) 1039–1052.
[10] T. Suzuki, H. Park, M.A. Kwofie, W.J. Lennarz, J. Biol. Chem. 276 (2001) 21601–21607.
[11] C. Schauber, L. Chen, P. Tongaonkar, I. Vega, D. Lambertson, W. Potts, K. Madura, Nature 391 (1998) 715–718.
[12] J.F. Watkins, P. Sung, L. Prakash, S. Prakash, Mol. Cell. Biol. 13 (1993) 7757–7765.
[13] B.L. Bertolaet, D.J. Clarke, M. Wolff, M.H. Watson, M. Henze, G. Divita, S.I. Reed, Nat. Struct. Biol. 8 (2001) 417–422.
[14] T.G. Ortolan, P. Tongaonkar, D. Lambertson, L. Chen, C. Schauber, K. Madura, Nat. Cell Biol. 2 (2000) 601–608.
[15] L. Chen, U. Shinde, T.G. Ortolan, K. Maura, EMBO Rep. 2 (2001) 933–938.
[16] C.R. Wilkinson, M. Seeger, R. Hartmann-Petersen, M. Stone, M. Wallace, Wallace, C. Semple, C. Gordon, Nat. Cell Biol. 3 (2001) 939–943.
[17] H. Rao, A. Sastry, J. Biol. Chem. 277 (2002) 11691–11695.
[18] T. Yao, R.E. Cohen, Nature 419 (2002) 403–407.
[19] R. Verma, L. Aravind, R. Oania, W.H. McDonald, J.R. Yates III, E.V. Koonin, R.J. Deshaies, Science 298 (2002) 611–615.
[20] T. Suzuki, A. Seko, K. Kitajima, Y. Inoue, S. Inoue, J. Biol. Chem. 269 (1994) 17611–17618.
[21] B.C. Braun, M. Glickman, M. Glickman, R. Kraft, B. Dahlmann, P.M. Kloetzel, D. Finley, M. Schmidt, Nat. Cell Biol. 1 (1999) 221–226.
[22] E. Strickland, K. Hakala, P.J. Thomas, G.N. Demartino, J. Biol. Chem. 275 (2000) 5565–5572.
[23] N. Benaroudj, A.L. Goldberg, Nat. Cell Biol. 2 (2000) 833–889.
[24] J.A. Johnston, C.L. Ward, R.R. Kopito, J. Cell Biol. 143 (1998) 1883–1898.
[25] J. Hardy, K. Gwinn-Hardy, Science 282 (1998) 1075–1079.
[26] J. Ma, S. Lindquist, Proc. Natl. Acad. Sci. USA 98 (2001) 14955–14960.
[27] J.C. Skipper, R.C. Hendrickson, P.H. Gulden, V. Brichard, A. Van Pel, Y. Chen, J. Shabanowitz, T. Wolfel, C.L. Slingluff Jr., T. Boon, D.F. Hunt, V.H. Engelhard, J. Exp. Med. 183 (1996) 527–534.
[28] R. Halaban, E. Chen, Y. Zhang, G. Moellmann, D. Hanlon, M. Michalak, V. Setaluri, D.N. Hebert, Proc. Natl. Acad. Sci. USA. 94 (1997) 6210–6215.
[29] C.A. Mosse, L. Meadows, C.J. Luckey, D.J. Kittlesen, E.L. Huczko, C.L. Slingluff, J. Shabanowitz, D.F. Hunt, V.H. Engelhard, J. Exp. Med. 187 (1998) 37–48.
[30] T. Suzuki, H. Park, E.A. Till, W.J. Lennarz, Biochem. Biophys. Res. Commun. 287 (2001) 1083–1087.
[31] T. Doerks, R.R. Copley, J. Schultz, C.R. Ponting, P. Bork, Genome Res. 12 (2002) 47–56.
[32] H. Park, T. Suzuki, W.J. Lennarz, Proc. Natl. Acad. Sci. USA 98 (2001) 11163–11168.
[33] C.A. Joazeiro, A.M. Weissman, Cell 102 (2000) 549–552.
[34] C.P. Ponting, Biochem. J. 351 (2000) 527–535.
[35] K. Hofmann, P. Bucher, Trends Biochem. Sci. 21 (1996) 172–173.

[36] T. Dieckmann, E.S. Withers-Ward, M.A. Jarosinski, C.F. Liu, I.S. Chen, J. Feigon, Nat. Struct. Biol. 5 (1998) 1042–1047.
[37] D.J. Clarke, G. Mondesert, M. Segal, B.L. Bertolaet, S. Jensen, M. Wolff, M. Henze, S.I. Reed, Mol. Cell. Biol. 21 (2001) 1997–2007.
[38] B.L. Bertolaet, D.J. Clarke, M. Wolff, M.H. Watson, M. Henze, G. Divita, S.I. Reed, J. Mol. Biol. 313 (2001) 955–963.
[39] A. Buchberger, M.J. Howard, M. Proctor, M. Bycroft, J. Mol. Biol. 307 (2001) 17–24.
[40] A. Buchberger, Trends Cell Biol. 12 (2002) 216–221.
[41] Y. Yoshida, T. Chiba, F. Tokunaga, H. Kawasaki, K. Iwai, T. Suzuki, Y. Ito, K. Matsuoka, M. Yoshida, K. Tanaka, T. Tai, Nature 418 (2002) 438–442.
[42] K.S. Makarova, L. Aravind, E.V. Koonin, Protein Sci. 8 (1999) 1714–1719.
[43] V. Anantharaman, E.V. Koonin, L. Aravind, Hum. Mol. Genet. 10 (2001) 1627–1630.
[44] S. Katiyar, T. Suzuki, B.J. Balgobin, W.J. Lennarz, J. Biol. Chem. 277 (2002) 12953–12959.
[45] K. Madura, Cell Cycle 1 (2002) 235–244.