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LETTER TO THE EDITOR

Calnexin leads glycoproteins into the fold

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Calnexin is an unglycosylated, type I membrane protein of the endoplasmic reticulum (ER) that consists of a 461 residue calreticulin-like lumenal domain, a single transmembrane segment, and a cytosolic domain of 90 residues. It interacts transiently with a diverse array of newly translocated membrane and secretory proteins and in prolonged fashion to misfolded or incompletely folded/assembled proteins. In many cases, dissociation of calnexin can be correlated with some aspect of polypeptide folding or subunit assembly (reviewed in [1, 2]). This behaviour suggests a molecular chaperone function and recent studies have confirmed that calnexin functions as a chaperone in facilitating the formation of the fully disulfide-bonded form of vesicular stomatitis virus G glycoprotein [3]. Furthermore, it promotes proper folding of the heavy chain of class I histocompatibility molecules and subsequent assembly of the heavy chain with β_2-microglobulin (A. Vassilakos and D. B. Williams, unpublished data). Calnexin has also been shown to be a component of the ER quality control system that prevents incompletely folded or misfolded proteins from progressing along the secretory pathway [4, 5].

One of calnexin's most interesting characteristics is its marked preference for N-linked glycoproteins. This is evidenced by the fact that virtually all proteins isolated as complexes with calnexin are capable of binding specifically to concanavalin A [6]. Also, treatment of cells with tunicamycin [6] or with inhibitors of the oligosaccharide processing enzymes, glucosidases I and II [7], prevents interaction of calnexin with most newly synthesized proteins. Inhibition of α-mannosidase activity, however, does not affect calnexin binding. These findings suggest that the removal of glucose residues within the ER is somehow important for calnexin interactions. One possible explanation for these results is that calnexin possesses lectin-like properties with specificity for early oligosaccharide processing intermediates [2]. To test this possibility, we prepared a soluble form of calnexin that consists only of its ER luminal domain and incubated the purified protein with a mixture of radiolabelled $Glc_{0\text{-}3}Man_9GlcNAc_2$ oligosaccharides. Soluble calnexin bound specifically to a single species in the mixture, the $Glc_1Man_9GlcNAc_2$ oligosaccharide. Limited substrate specificity studies suggested that, in addition to a requirement for the single glucose residue, at least one of the terminal mannoses is required for optimal binding [8]. Oligosaccharides were not altered following incubation with calnexin in any of these experiments, suggesting that calnexin is a lectin rather than an enzyme that modifies oligosaccharide structure such as a glycosidase. However, examination of the primary sequence of calnexin revealed no similarity to the carbohydrate-recognition domains of the galectins, the C-type lectins, or mannose-6-phosphate receptors.

Although the lectin properties of calnexin provide an explanation for its glycoprotein preference, it is not immediately apparent how they relate to its function as a molecular chaperone. Molecular chaperones generally bind to the polypeptide backbone of a folding protein, thereby preventing aggregation and maintaining the polypeptide in a conformation competent for folding. Indeed, calnexin has been shown to bind to unglycosylated segments of proteins [5, 9]. We attempted to assess the relative contributions of a glycoprotein's oligosaccharide versus polypeptide moieties during interaction with calnexin. Purified complexes of calnexin with either soluble or membrane glycoproteins were prepared and the accessibility of oligosaccharides in the complexes was probed by digestion with endoglycosidase H. Remarkably, all oligosaccharides were accessible to this probe and could be removed without any detectable dissociation of the complexes [8]. This suggests that once a stable complex between calnexin and a glycoprotein is formed, the lectin-oligosaccharide interaction contributes little to the overall association.

In an effort to accommodate all of these observations, we propose a two-step model for the interaction of calnexin with newly synthesized glycoproteins [8]. Following the attachment of the $Glc_3Man_9GlcNAc_2$ oligosaccharide to a nascent polypeptide chain and the removal of the outermost two glucoses by glucosidases I and II, initial binding to calnexin occurs through the monoglucosylated oligosaccharide. This serves to bring the two proteins in proximity whereupon the unfolded glycoprotein associates with calnexin through segments of its polypeptide chain. The features of an unfolded glycoprotein recognized by calnexin in this second step are unknown but, by analogy to other chaper-

ones, they may consist of exposed hydrophobic segments or patches. Subsequently, the glycoprotein folds in association with calnexin (or during cycles of calnexin binding and release) until sites for calnexin binding are buried in the folded molecule. The ER enzyme UDP-glucose: glycoprotein glucosyltransferase may also participate in calnexin-mediated glycoprotein folding. This enzyme adds back a single glucose to oligosaccharides following their complete deglucosylation by glucosidases I and II [10]. It may offer a glycoprotein additional opportunities to bind (or re-bind) to calnexin in those instances where all three glucoses have been removed before folding is complete.

Why does calnexin utilize oligosaccharide binding for its initial interaction with unfolded glycoproteins? Calnexin is unique among molecular chaperones in that it is an intrinsic membrane protein. Such a disposition within the ER membrane may limit access of its polypeptide binding site to newly translocated polypeptides. The $Glc_1Man_9GlcNAc_2$ oligosaccharide constitutes a large, accessible binding site for calnexin that is present on nascent polypeptide chains at an early stage in the folding process [11]. The presence of calnexin and possibly other carbohydrate-binding chaperones in diverse species from yeasts to humans may also help to explain why the process of N-glycosylation via a large, pre-assembled oligosaccharide has been preserved since the emergence of early eukaryotes.

References

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