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Hypothesis

Identification of putative internalization signals in prion proteins

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Prion proteins were found to contain regions in their cytoplasmic domains that have significant structural homologies to molecular trafficking signals for internalization of membrane proteins. These regions may facilitate the endocytosis of prion proteins, which appears to be a first step in their conversion to the abnormal, amyloidogenic form.

Prion protein; Endocytosis; Signal peptide

1. INTRODUCTION

Prions are proteinaceous infectious particles (PrP) that are associated with certain inherited and transmissible neurodegenerative diseases in humans (kuru, Creutzfeldt–Jakob disease, Gerstmann–Straussler syndrome) and animals (scrapie in sheep and bovine spongiform encephalopathy) (for review, see [1]). The pathognomonic or 'scrapie' form, PrP^{Sc}, is derived from the cellular protein, PrP^{C}, that is normally present as an integral membrane protein on a variety of neuronal and non-neuronal cells [2], but whose function is not known. An emerging consensus is that the conversion of PrP^{C} to PrP^{Sc} involves conformational changes in PrP, possibly from a helical to an insoluble β-pleated sheet structure [3], and occurs in an endocytic compartment after internalization of PrP^{C} from the plasma membrane [4]. An understanding of the mechanism(s) by which PrP^{C} is endocytosed is therefore important in deciphering the process of PrP^{C}-to-PrP^{Sc} conversion.

PrP^{C} is unusual in that it is postulated to be anchored in the membrane by two transmembrane regions as well as by a glycosylphosphatidylinositol (GPI) anchor [5]. One mechanism for the endocytosis of GPI-anchored proteins is through caveolae, and this has been suggested for PrP^{C} [4]. Another mechanism, by analogy with several transmembrane proteins, may be through clathrin-coated pits, assisted by specific 'internalization signals' resident in the cytoplasmic domain of PrP^{C} (reviewed in [6,7]). Such signals consist of short segments (4–10 amino acid residues) of the cytoplasmic domains of some Type I and Type II transmembrane proteins [6,7], and are thought to be binding sites for adaptins which are found in clathrin-coated pits [8]. Several classes of internalization signals have been described, and the essential feature in many of these is a tight turn [6] or loop [9] containing an aromatic residue, usually tyrosine. Neighboring amino acid residues and the 'distance' of the signal from the membrane interface may also be important, and some proteins may require more than one internalization signal for optimal endocytosis [10,11]. Examples of consensus sequences that function as internalization signals are, using the single-letter code for amino acids and with X as any amino acid: YXRF, FXNPXY, and a tyrosine residue flanked by polar or basic amino acids at certain preferred positions relative to tyrosine [6,7,9].

2. IDENTIFICATION OF HOMOLOGIES TO INTERNALIZATION SIGNALS

An analysis of the PrP primary structure previously identified a well conserved 'aromatic palindrome' region (PrP:147–163 **RYYRENMYRYPNQVYYR**) containing six of the 14 tyrosine residues in murine PrP [12]. Based on the topography of PrP [5], this region is located mainly in the cytoplasmic (PrP:137–155) domain. Although the function of this region is not known, it was suggested that it may act as a binding site for other proteins [12]. The presence of conserved tyrosine residues in this region, as well as in the adjacent region of the cytoplasmic domain, prompted an analysis of structural homologies to internalization signals.

Secondary structure analysis using the methods of Chou and Fasman [13] and of Garnier et al. [14] predicted a probability for turn structures at PrP:142 and 146, and in the region PrP:151–160. Based on the β-turn structure exhibited by the model pentapeptide, YPNDV

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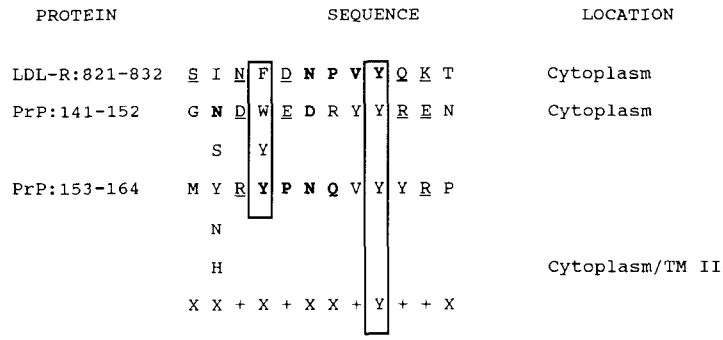

Fig. 1 The region of the LDL-R cytoplasmic domain, LDL-R:821–832 containing the six-residue internalization signal having the consensus sequence FXNPXY [17]. PrP:141–152 and 153–164 containing six-residue segments that are similar to the LDL-R signal, begin and end with aromatic residues (boxed) and have intervening β-turn structures (indicated by bold letters) that were either predicted or found by 2D NMR. Residues at positions that coincide with the motif XX+X+XX+Y++X (where + is a basic or polar residue) [9] are underlined. Amino acid residues occurring at variable positions in mammalian PrP's [20] are indicated below those positions.

[15], it had been speculated that the segment PrP:156–160 (YPNQV) would also have a β-turn structure [12]. In fact, recent data [16] acquired using 2D nuclear magnetic resonance to study the conformation of a synthetic cognate peptide, PrP:145–165, revealed the presence of a β-turn involving the region PrP:157–160 (YPNQ). This region is conserved in all mammalian PrP's, and is located at the proposed boundary between the cytoplasmic and putative second transmembrane (TM II) domains (PrP:156–159).

An analysis of the sequences of the regions predicted or found to have β-turn structure showed that the regions PrP:141–152 (WEDRYY) and PrP:155–160 (YPNQVY) are similar to the six residue internalization signal (FDNPVY) in the low-density lipoprotein receptor (LDL-R). The consensus sequence reported for this internalization signal, as mentioned earlier, is FXNPXY [17]; and the tetrapeptide, NPVY, contained within the LDL-R sequence was shown by 2D NMR to adopt a β-turn structure in solution [18]. An amino-terminal aromatic residue and a carboxy-terminal aromatic or large hydrophobic residue, and a turn in the intervening region, have been suggested [17] to provide sufficient structural information for this signal. A somewhat different motif for internalization signals has also been suggested [9] based on an analysis of the cytoplasmic domains of several integral membrane proteins known to be internalized, including the LDL-R. This motif requires the presence of basic or polar amino acid residues (+) at certain preferred positions relative to a tyrosine residue: XX+X+XX+Y++X. The PrP hexapeptide segments and adjacent residues also share similarities with this motif. These homologies are shown in Fig. 1.

3. DISCUSSION

Based on these structural homologies, it is proposed that the regions, PrP:141–152 and PrP:153–164, contain internalization signals for endocytosis of PrP^C, although the exact boundaries are not defined. Of importance is that these regions are conserved in all mammalian PrP's (except for positions indicated in Fig. 1), and this would be expected to result in the preservation of these signals among PrP's from various species. Whether only one or both of these signals are required for optimal endocytosis is not known.

An internalization signal in the region PrP:153–164 requires special consideration, since it is located partly in the putative TM II domain, which has been proposed to be an amphipathic helix [5]. This would suggest that it may not be accessible for binding to adaptins. Speculatively, exposure of this region in the cytoplasm could arise through conformational changes that might occur upon binding to a ligand or association with other integral membrane proteins. The determination of the function of PrP^C and a more detailed understanding of its structure in the membrane would help to clarify this issue.

It should be acknowledged that these may not be the only, or even primary, internalization signals in PrP, nor do they necessarily exclude other mechanisms of endocytosis that are independent of such signals. For example, internalization may occur through caveolae [4], which is consistent with the mechanism of potocytosis (endocytosis) of GPI-anchored proteins [19]. However, the unusual membrane topography proposed for PrP^C [5] could indicate that it is endocytosed by more than one mechanism. This hypothesis advances the testable prediction that insertion of these PrP segments into the cytoplasmic domain of an internalization deficient model membrane protein should increase its rate of endocytosis [9,17]. Amino acid residues critical for internalization could then be assessed by site-directed or cassette mutagenesis of the transplanted signals [9,17]. Relevant to the conversion of PrP^C to PrP^{Sc} and the pathogenesis of prion disease, alterations in the local PrP^C structure, such as elimination of the turn structure,

should decrease the internalization rate of PrP^C, possibly diminishing its conversion to PrP^{Sc}.

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