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Characterization of PrP Binding Proteins

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Characterization of PrP binding proteins

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SUMMARY

Prions cause spongiform degeneration in various mammalian species. The scrapie prion protein (PrP^{Sc}) is part of the infectious particle and may mediate infection and spreading of the disease in the brain. It was therefore of interest to purify and analyse PrP ligands (Plis). Plis were identified on ligand blots using either intact PrP or peptides corresponding to the central portion of PrP. Here, characterization of a 110 and a 125 kDa Pli is reported. Both Plis were found in total membrane fractions and could be extracted with carbonate indicating that they are not integral membrane proteins. On sucrose gradients both PrP ligands sedimented with high density particles.

1. INTRODUCTION

The infectious particle causing scrapie in sheep, bovine spongiform encephalopathy in cattle or Creutzfeldt-Jakob disease in humans contains the disease-specific form of the prion protein (PrP^{Sc}). The function of the normal, cellular form of PrP (PrP^{C}) is unknown while the prion-specific isoform PrP^{Sc} is correlated with pathological changes in the infected animal. A number of observations suggest that other cellular proteins may interact with PrP^{Sc} during the infection and replication.

1. Different strains of hamster adapted prions replicate in different regions of the brain (Hecker *et al.* 1992). When prions were injected intraocularly the pathological lesions were first found in the contralateral geniculate nucleus and the superior colliculus followed by the visual cortex. These findings suggest anterograde transport of prions along neuroanatomical pathways (Scott & Fraser 1989; Kimberlin & Walker 1986).
2. PrP^{Sc} was found to stimulate astrocyte proliferation suggesting a role as a cell type-specific growth factor (DeArmond *et al.* 1992). Gliosis is very pronounced in prion infected animals and appears to be correlated to the concentration of PrP^{Sc} (Jendroska *et al.* 1991).
3. An amyloid-forming peptide induced apoptosis in cultures of hippocampal neurons (Forloni *et al.* 1993). These effects as well as the cell surface localization of PrP^{C} suggested that there might be receptors or binding proteins for PrP involved.

Using the ligand blot technique two major PrP ligands of 110 and 45 kDa (denominated Pli 110 and Pli 45, respectively) have been identified (Oesch *et al.* 1990). Purification of Pli 45 subsequently showed that it was identical to glial fibrillary acidic protein (GFAP). Pli 110 was found in different tissues and was equally abundant in normal and infected animals (Oesch *et al.* 1990). In this article Pli 110 is further characterized as a periferal membrane protein.

2. RESULTS

(*a*) *PrP ligands can be extracted at alkaline pH or by detergents*

Hamster brain membranes were prepared by differential centrifugation (Linstedt & Kelly 1991). Briefly, normal hamster brain homogenates were centrifuged at 2000 *g*. The postnuclear supernatant was centrifuged at 100 000 *g* and the resulting pellet was osmotically shocked to release cytoplasmic proteins. This membrane fraction contained two PrP binding proteins of 110 and 125 kDa (Pli 110 and Pli 125, respectively; figure 1) as determined by ligand blotting (Oesch *et al.* 1990). Immobilized proteins were probed with a peptide (denominated P5) corresponding to the central portion of the PrP polypeptide (amino acids 140–174 of hamster PrP; Oesch & Prusiner 1992). The membrane fraction was extracted with either sodium carbonate (pH 11.5) or 1% Triton X-100, 1 M NaCl, 20 mM TrisHCl pH 7.4. After centrifugation at 100 000 *g*, pellets and supernatants were analysed for PrP ligands (figure 1, top panel) or the integral membrane protein synaptophysin (figure 1, bottom panel). PrP ligands were extracted with both, carbonate and detergents while synaptophysin was only extracted by detergents. These results suggest that PrP ligands are associated with membranes but they are not integral membrane proteins.

(*b*) *Sedimentation of PrP ligands on sucrose gradients*

Membranes from normal hamster brains were separated on a discontinuous sucrose gradient as

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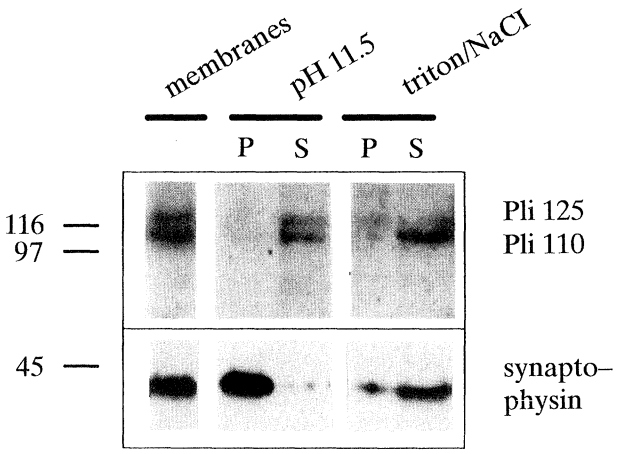

Figure 1. Total membranes were either treated with carbonate (pH 11.5) or 1% Triton X-100, 1 M NaCl followed by high speed centrifugation. Supernatants (S) and pellets (P) were electrophoresed on 10% SDS polyacrylamide gels and transferred to nitrocellulose. PrP ligands were revealed by binding of ^{125}I-labelled peptide P5 (10 nM) followed by autoradiography (18 h). The same blot was incubated subsequently in anti-synaptophysin antibodies, anti-mouse IgG coupled to alkaline phosphatase and color substrate (NBT/BCIP) to reveal alkaline phosphatase activity. Molecular mass standards are indicated at the left.

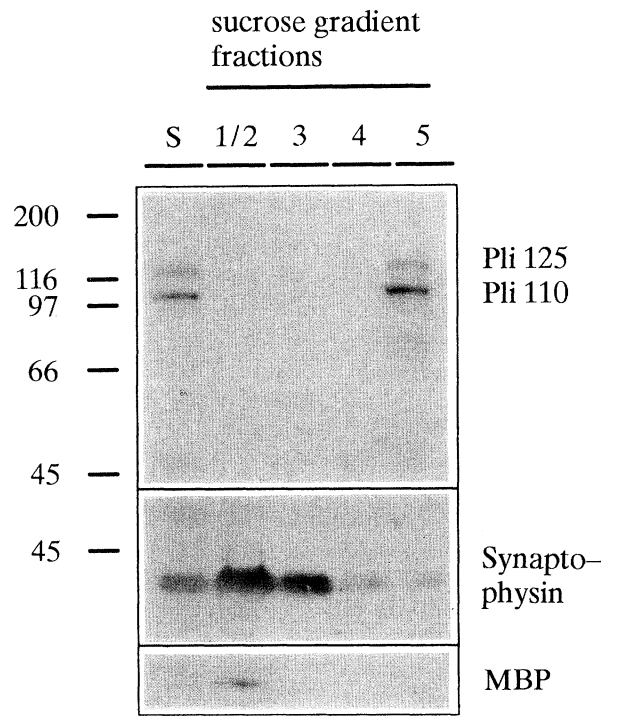

Figure 2. Postnuclear supernatant (S) was loaded onto a discontinuous sucrose gradient as described by Coleman *et al.* (1982). Fractions 1–5 were collected and aliquots were processed for detection of PrP ligands and synaptophysin as described in figure 1. Myelin basic protein (MBP) was detected with monoclonal anti-MBP antibodies followed by the same detection procedure as for synaptophysin. Sucrose gradient fractions 1 and 2 were not separated. Molecular mass markers are indicated at the left. The molecular mass of MBP is ~18 kDa.

described (Colman *et al.* 1982). Fractions corresponding to cytosol (1), myelin (2), plasma membranes (3), mitochondrial membranes (4) or polysomes (5) were collected, concentrated by centrifugation and analysed for PrP ligands. Figure 2 shows the distribution of PrP binding proteins on the sucrose gradient. Pli 110 and 125 were detected in the starting material and at the bottom of the sucrose gradient (fraction 5). The sucrose gradient fractions were also analysed for myelin basic protein (MBP) and synaptophysin by Western blotting (figure 2, bottom panels). MBP was concentrated in the top fraction. Synaptophysin, a constituent of synaptic vesicles, was distributed in fractions 1–3. Fraction 4 contains predominantly mitochondrial membranes (Hehman & Hauswirth 1992). Fraction 5 is enriched in free polysomes and other complexes with high density (Colman *et al.* 1982). Thus, PrP ligands are not associated with plasma membranes, vesicles or mitochondrial membranes but rather with either polysomes or other complexes of high density.

3. DISCUSSION

The function of PrP in the normal or the infected animal is still enigmatic. Mice lacking a functional PrP gene do not appear to have any obvious defect (Büeler *et al.* 1992; Brenner *et al.* 1992). Attempts were therefore made to characterize proteins which interact with the prion protein. Here we show that Pli 110 and a previously unrecognized PrP binding protein, Pli 125, purify with membranes but can be extracted at alkaline pH suggesting that they do not represent integral membrane proteins. Density gradient centrifugation showed that both, Pli 110 and Pli 125, sediment with particles of high density. The composition of this fraction are currently under investigation. Binding of PrP or peptide P5 to unfixed frozen sections of hamster brain reveals binding to regions of neuronal cell bodies (B. Oesch, F. Coufal, S. B. Prusiner and S. J. DeArmond, manuscript in preparation). These binding sites as well as the PrP ligands identified above may therefore represent intracellular binding sites.

The relevance of this finding is unclear. There are precedents for secretory proteins to be internalized and retrogradely transported in neurons. Nerve growth factor or brain-derived neurotrophic factor (BDNF) have intracellular binding sites (Rakowicz-Szulczynska *et al.* 1986; Wetmore *et al.* 1991). BDNF was also localized to cytoplasmic granules of cholinergic neurons of the nucleus basalis of Meinert. These neurons do not produce BDNF mRNA suggesting that BDNF is transported retrogradely from the target area. Hippocampal neurons also contain BDNF in the nucleus (Wetmore *et al.* 1991). There are several potential nuclear translocation signals in BDNF but it remains to be determined how BDNF is translocated to the nucleus in neurons. Similarly, interleukin 1 and fibroblast growth factors are internalized via a cell surface receptor and subsequently translocated into the nucleus (Heguy *et al.* 1992; Woodward *et al.* 1992).

In the future, it will be important to analyse

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whether PrP can be transported retrogradely and therefore may act as a neurotrophic signaling molecule. The N-terminus of mature PrP 33–35 is rich in basic amino acids and proline (KKRPKPGG) reminiscent of a nuclear localization sequence. In this context it is interesting to note that PrP was also localized in the nucleus as well as the cytoplasm of scrapie infected neuroblastoma cells (Pfeifer *et al.* 1993; Taraboulos *et al.* 1990; McKinley *et al.* 1991).

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REFERENCES

Brenner, H.R., Herczeg, A. & Oesch, B. 1992 Normal development of nerve-muscle synapses in mice lacking the prion protein gene. *Proc. R. Soc. Lond.* B **250**, 151–155.

Büeler, H., Fischer, M., Lang, Y. *et al.* 1992 Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature, Lond.* **356**, 577–582.

Colman, D.R., Kreibich, G., Frey, A.B. & Sabatini, D.D. 1982 Synthesis and incorporation of myelin polypeptides into CNS myelin. *J. Cell Biol.* **95**, 598–608.

DeArmond, S.J., Kristensson, K. & Bowler, R.P. 1992 PrPSc causes nerve cell death and stimulates astrocyte proliferation: a paradox. *Prog. Brain Res.* **94**, 437–446.

Forloni, G., Angeretti, N., Chiesa, R. *et al.* 1993 Neurotoxicity of a prion protein fragment. *Nature, Lond.* **362**, 543–546.

Hecker, R., Taraboulos, A., Scott, M. *et al.* 1992 Replication of distinct scrapie prion isolates is region specific in brains of transgenic mice and hamsters. *Genes Dev.* **6**, 1213–1228.

Heguy, A., Baldari, C.T., Macchia, G., Telford, J.L. & Melli, M. 1992 Amino acids conserved in interleukin-1 receptors (IL-1Rs) and the Drosophila toll protein are essential for IL-1R signal transduction. *J. biol. Chem.* **267**, 2605–2609.

Hehman, G.L. & Hauswirth, W.W. 1992 DNA helicase from mammalian mitochondria. *Proc. natn. Acad. Sci. U.S.A.* **89**, 8562–8566.

Jendroska, K., Heinzel, F.P., Torchia, M. *et al.* 1991 Proteinase-resistant prion protein accumulation in Syrian hamster brain correlates with regional pathology and scrapie infectivity. *Neurology* **41**, 1482–1490.

Kimberlin, R.H. & Walker, C.A. 1986 Pathogenesis of scrapie (strain 263K) in hamsters infected intracerebrally, intraperitoneally or intraocularly. *J. gen. Virol.* **67**, 255–263.

Linstedt, A.D. & Kelly, R.B. 1991 Synaptophysin is sorted from endocytic markers in neuroendocrine PC12 cells but not transfected fibroblasts. *Neuron* **7**, 309–317.

McKinley, M.P., Taraboulos, A., Kenaga *et al.* 1991 Ultrastructural localization of scrapie prion proteins in cytoplasmic vesicles of infected cultured cells. *Lab. Invest.* **65**, 622–630.

Oesch, B., Teplow, D.B., Stahl, N., Serban, D., Hood, L.E. & Prusiner, S.B. 1990 Identification of cellular proteins binding to the scrapie prion protein. *Biochemistry* **29**, 5848–5855.

Oesch, B. & Prusiner, S.B. 1992 Interaction of the prion protein with cellular proteins. In *Prion diseases of humans and animals* (ed. S. B. Prusiner, J. Collinge, J. Powell & B. Anderton), pp. 398–406. New York: Ellis Horwood.

Pfeifer, K., Bachmann, M., Schroder, H.C., Forrest, J. & Muller, W.E. 1993 Kinetics of expression of prion protein in uninfected and scrapie-infected N2a mouse neuroblastoma cells. *Cell Biochem. Funct.* **11**, 1–11.

Rakowicz-Szulczynska, E.M., Rodeck, U., Herlyn, M. & Koprowski, H. 1986 Chromatin binding of epidermal growth factor, nerve growth factor, and platelet-derived growth factor in cells bearing the appropriate surface receptors. *Proc. natn. Acad. Sci. U.S.A.* **83**, 3728–3732.

Scott, J.R. & Fraser, H. 1989 Transport and targeting of scrapie infectivity and pathology in the optic nerve projections following intraocular infection. *Prog. Clin. Biol. Res.* **317**, 645–652.

Taraboulos, A., Serban, D. & Prusiner, S.B. 1990 Scrapie prion proteins accumulate in the cytoplasm of persistently infected cultured cells. *J. Cell Biol.* **110**, 2117–2132.

Wetmore, C., Cao, Y.H., Pettersson, R.F. & Olson, L. 1991 Brain-derived neurotrophic factor: subcellular compartmentalization and interneuronal transfer as visualized with anti-peptide antibodies. *Proc. natn. Acad. Sci. U.S.A.* **88**, 9843–9847.

Woodward, W.R., Nishi, R., Meshul, C.K., Williams, T.E., Coulombe, M. & Eckenstein, F.P. 1992 Nuclear and cytoplasmic localization of basic fibroblast growth factor in astrocytes and CA2 hippocampal neurons. *J. Neurosci.* **12**, 142–152.

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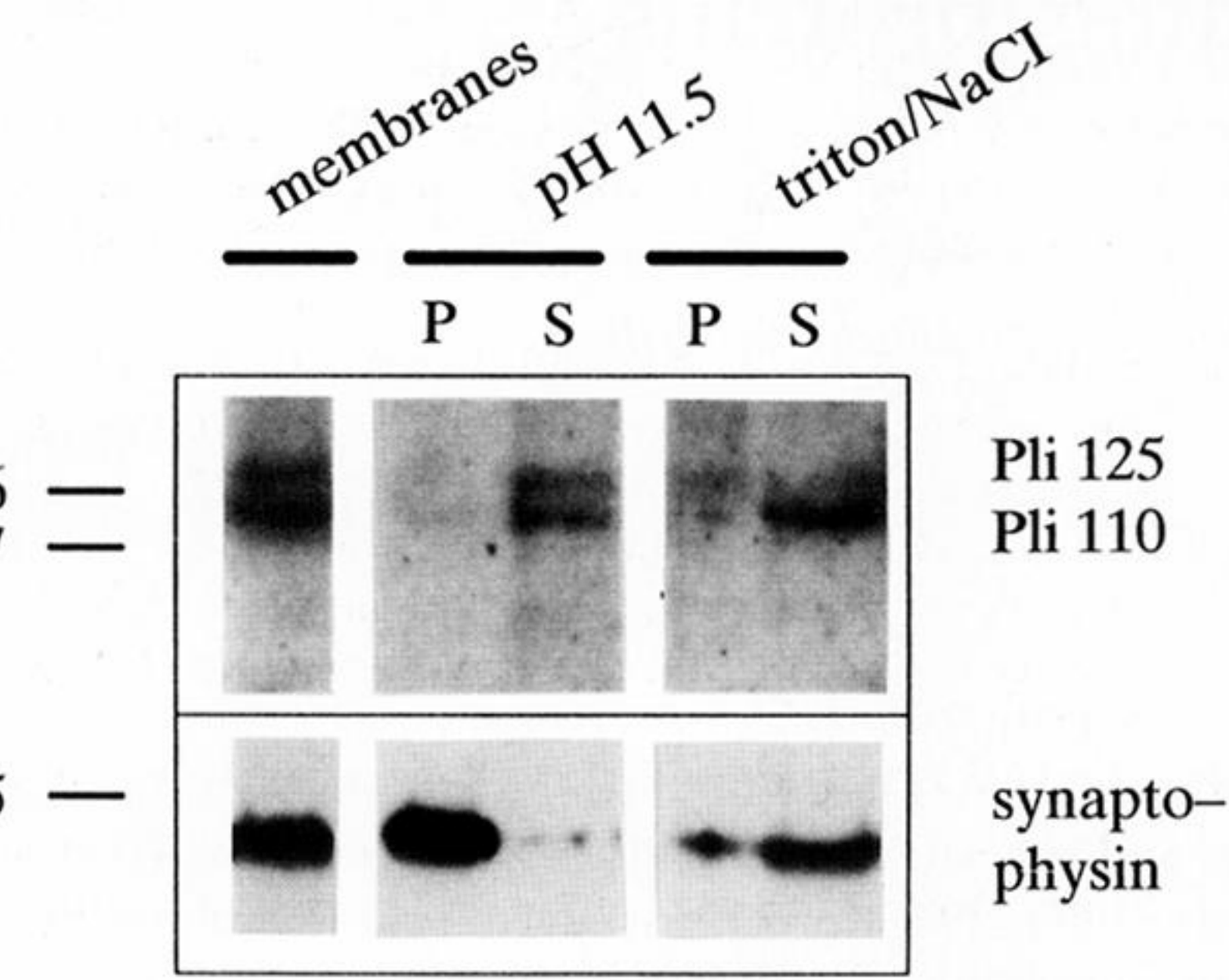

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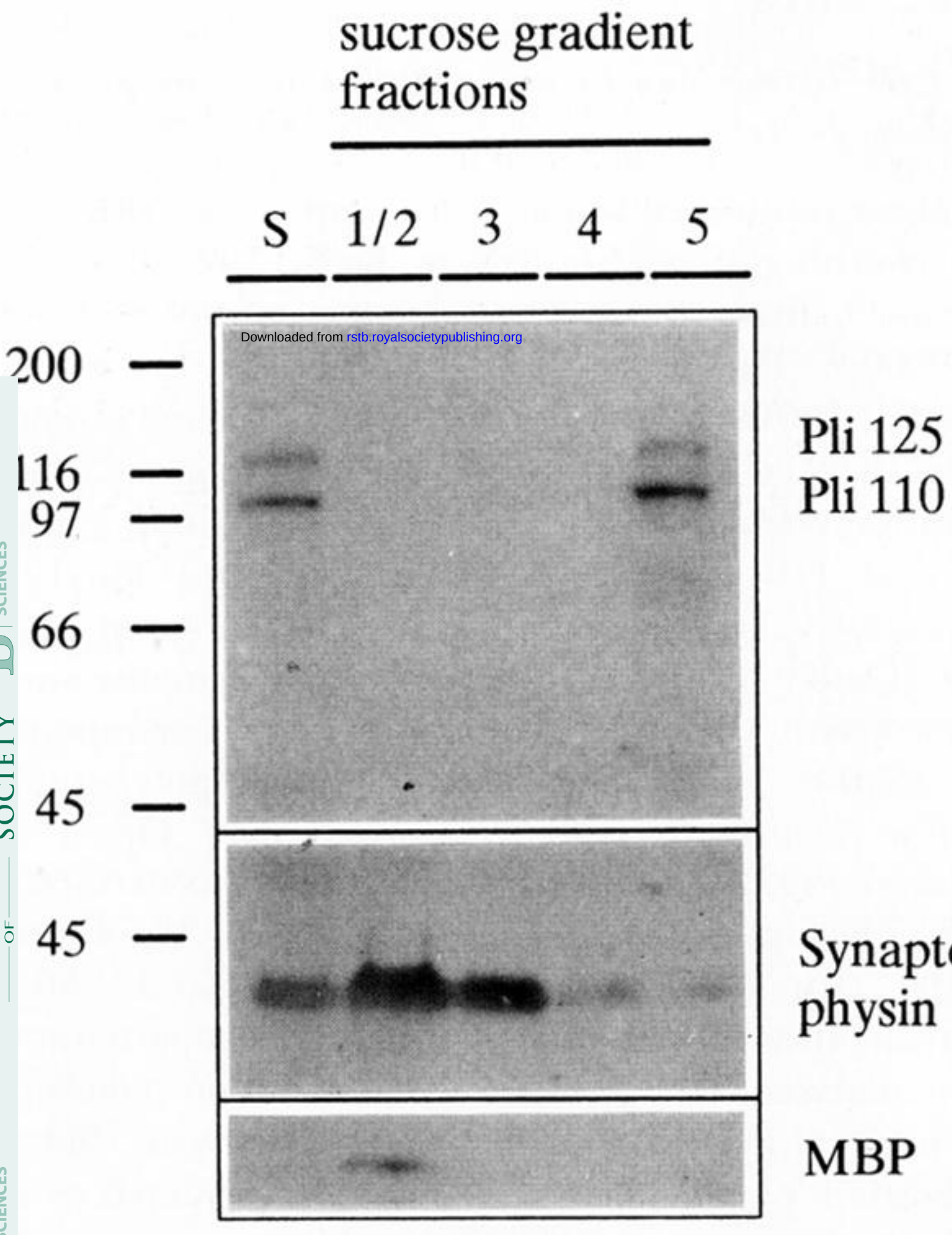

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