Neurobiology

Postsynaptic densities clarified?

from Gerry Shaw

THERE seems to be a curious rule concerning our understanding of elements of the neuronal cytoskeleton. The components which were discovered first have proved to be the most difficult to characterize biochemically and comprehend functionally. Thus neurofilaments and postsynaptic densities (PSDs) were visualized both at the light and electron microscope levels well before microtubules and microfilaments, yet the major subunits of microtubules and microfilaments were already characterized at a time when our knowledge of the biochemical make-up of neurofilaments and PSDs was confused. For neurofilaments, the mists began to clear about nine years ago as a result of data derived from studies of axonal transport[1]. But PSDs have remained shrouded in mist. In the light of a few recent papers on the subject it is worth asking when that is likely to change.

In the central nervous system, the PSD complex is visualized by electron microscopy of ultra-thin sections as a clump of very electron-dense material apparently adherent to the postsynaptic membrane. Fine filaments can often be seen to extend from this dense clump. The size of the complex is variable, as is the shape, and it has been suggested that such morphological differences are correlated with neurotransmitter type[2]. Much work has been directed towards identifying the protein constituents of PSDs. Presumably the protein that makes the fine filaments is a major component of total PSD protein. One would also expect to find some sort of protein or glycoprotein involved in gluing the complex to the postsynaptic membrane. Finally, if the complex acts as an anchoring structure, it is possible that a variety of different synapse-specific enzymes and receptors might be bound to it. These constituents would add biochemical complexity to the PSD complex. A further complication is that different proteins may be associated with different types of PSD. Accordingly, it is perhaps not surprising that the PSD has been so intractable.

An obvious way to find out which proteins make up PSDs is to examine biochemically a fraction of brain material which, by electron microscope criteria, is composed predominantly of PSDs. Using this approach, actin, fodrin, tubulin, calmodulin, synapsin I and a 50,000-molecular-weight (50K) protein (called the 'major postsynaptic density protein') have all been postulated as components of the PSD. Another approach has been to react sections of brain tissue with antibodies specific for various known proteins and to see, by light and electron microscopy, whether PSDs are stained. Actin, tubulin, an intermediate filament protein, synapsin 1, calmodulin and microtubule-associated protein 2 have been implicated as part of the PSD by this approach. In addition, a group of glycoproteins found in synaptic junction preparations and originally identified by their ability to bind lectins, could be PSD constituents (see, for example, ref. 3).

If all these results are correct, the PSD must be an extremely complex structure. So could some of them be due to co-purification of proteins found outside the PSD complex or to spurious immunoreactivities? Some data included in two recent papers, suggest that could sometimes be the case.

Thus Matus *et al.* found neurofilament proteins and glial fibrillary acidic protein (GFA), the components of the intermediate filaments of neurones and astrocytes, in the purified PSD fraction routinely used for biochemical experiments[4] and went on to show that purified radiolabelled GFA, added to brain material at the start of the standard PSD preparation, also ended up in the final PSD fraction[4], clearly indicating that its presence there was artefactual. Therefore the presence of a protein in the standard PSD preparation does not necessarily indicate that this protein is also an *in vivo* component. And a carefully controlled immunoelectron microscopical study of synapsin 1 showed that claims it was a true PSD constituent were wrong and probably founded on non-specific binding of the antibody to PSDs[5]. So immunological approaches may also be problematical; the PSD seems to be a 'sticky' structure presenting special problems to the biochemist and the immunologist.

More recently, Landis and Reese[6] examined the structure of dendritic spines in the mouse cerebellum using John Heuser's technique of rapid freezing, deep etching and rotary shadowing. They found that the PSD was composed of a mass of fine filaments with a diameter of about 4 nm, much smaller than that of actin-containing microfilaments clearly visualized in other regions of the dendritic spine. The diameter also makes it unlikely that the PSD filaments are composed of microtubule or intermediate filament proteins, at least in their usual conformations. What, then, are these filaments made of?

It would have been neat if the answer had been the 50K protein of PSD. However, the protein is apparently absent from cerebellar PSDs and, in an important paper, Kennedy *et al.* recently demonstrated that the 50K protein is indistinguishable from a component of a calmodulin-dependent protein kinase of brain tissue[7] and is part of an abundant 650,000-molecular-weight holoenzyme[7], data since confirmed by Kelly *et al.*[8] Moreover, Kennedy's group suggests that the complex is composed of nine α (50K) subunits and three β (60K) subunits, both of which can be detected in PSD preparations[9]. Gel filtration studies suggest that the diameter of the holoenzyme should be about 20 nm, and it is interesting that particles of about this size have been detected in electron microscopical studies of PSD preparations[10]. Since Kennedy *et al.* have a monoclonal antibody against the α subunit they should be able to check whether the protein is a true component of PSDs *in vivo*. In any case, it seems inconceivable that the PSD filaments are composed of subunits of the enzyme.

Bearing these results in mind, what do we really know about the components of PSDs? The answer seems to be that we can still only guess at the make-up of the PSD filaments, the dense material and the membrane attachment, if these are indeed distinct elements. We should, at least, soon know whether the major PSD protein is really a component of the PSD complex *in vivo* and, if so, in what form it appears in the complex. For the future, comparison of PSDs with some of the better understood cell-to-cell and cell-to-substrate contacts might also be illuminating. We might find proteins in PSDs related to proteins of desmosomes or adhesion plaques. Perhaps we must look for the protein components of the PSD filaments in the many minor bands found on polyacrylamide gels of PSD preparations (see ref. 11 for example).

It is even possible that the filament component may have been completely missed due to its lack of solubility in gel sample buffer, as was the case with the protein or proteins of the paired helical filaments found in the brain of patients suffering from senile dementia of the Alzheimer's type[12]. It has been supposed that such paired helical filaments are composed of modified neurofilament proteins, though recent data do not confirm this view, and indeed leave the identity of the subunits of these filaments an open question[12]. Since the paired helical filaments are found in neurones which typically have thousands of PSDs, could it be that they are the result of overproduction of a PSD filament protein? At present there are not enough data to muzzle speculation. □

1. Hoffman, P. N. & Lasek, R. J. *J. Cell Biol.* **66**, 351 (1975).
2. Gray, E. G. *J. Anat.* **93**, 420 (1959).
3. Mena, E. E. & Cotman, C. W. *Science* **216**, 422 (1982).
4. Matus, A. *et al. J. Cell Biol.* **87**, 346 (1980).
5. De Camilli, P. *et al. J. Cell Biol.* **96**, 1355 (1983).
6. Landis, D. & Reese, T. *J. Cell. Biol.* **97**, 1169 (1983).
7. Kennedy, M.B. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 7357 (1983).
8. Kelly, P.T., McGuinness, T.L. & Greengard, P. *Proc. natn. Acad. Sci. U.S.A.* **81**, 948 (1984).
9. Bennet, M.K. *et al. J. biol. Chem.* **258**, 12735 (1983).
10. Blomberg, F. *et al. J. Cell Biol.* **74**, 204 (1977).
11. Matus, A. *et al. J. Cell Biol.* **98**, 237 (1984).
12. Ihara, Y. *et al. Nature* **304**, 727 (1983).

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