Synaptic transmission: **Two players team up for a new tune**

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An unexpected link has been found between two molecules that were thought to perform seemingly unrelated functions. The AMPA-subclass glutamate receptor GluR2 interacts with the membrane-fusion protein NSF in a manner that appears important for receptor-mediated intracellular signalling.

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Neurotransmitter receptors do not diffuse freely in the plasma membrane, but are clustered at specific subsynaptic sites. This clustering is mediated by proteins that bind to the intracellular tails of the receptor molecules, organizing them into microdomains within the postsynaptic plasma membrane. Some receptor clustering proteins have been well-characterized, such as rapsyn, in the case of the nicotinic acetylcholine receptor, and gephyrin, in the case of the glycine receptor. Screens for proteins that interact with members of the glutamate receptor family have identified PSD-95/SAP-90, which is involved in clustering of glutamate receptors of the NMDA class. More recently, similar molecular fishing expeditions have detected additional proteins, including GRIP, which selectively interacts with glutamate receptors of the AMPA class. These proteins both contain ‘PDZ’ domains, which mediate protein–protein interactions [1]. The latest fish, landed independently by three groups [2–4], is a major surprise; it is a well-known protein, but from another world of cell biology — the ATPase known as NEM-sensitive factor or NEM-sensitive fusion protein (NSF).

NSF is a conserved soluble protein that has a well-defined role in intracellular membrane transport [5]. A typical transport process in which NSF plays a part consists of the generation or ‘budding’ of a transport vesicle, the transport of the vesicle to its destination, and finally the binding or ‘docking’ and fusion of the transport vesicle with the target membrane. NSF is needed for vesicle docking and fusion. To perform its function, NSF needs additional proteins, which mediate its attachment to membranes; these cofactors are known as ‘soluble NSF attachment proteins’ (SNAPs) [5].

The first targets of NSF and SNAPs were identified in neurons, where they act in the exocytosis of synaptic vesicles [6]. These targets include the synaptic vesicle protein synaptobrevin, also known as VAMP, and the synaptic membrane proteins SNAP-25 and syntaxin. A number of relatives of these synaptic proteins have been identified in other cell types and species — including yeast — and they have been collectively termed ‘SNAP receptors’ (SNAREs). Genetic and biochemical studies have provided convincing evidence that SNAREs are crucial for intracellular membrane fusion. Complementary sets of SNAREs need to be present on the membranes destined to fuse, and most intracellular fusion events have their own unique set (although some SNAREs appear to operate in more than one transport process) [7].

SNAREs are small proteins, with molecular weights in the range 14,000–35,000 kDa. They contain single membrane-anchor domains, usually located at the carboxy-terminal end. Purified neuronal SNAREs are only partially structured; when complementary SNAREs are mixed, they rapidly form a stable and highly structured complex. The core of the complex consists of a long bundle of four different and intertwined α-helices, with all the membrane-anchor domains exposed on one side of this structure [8]. Complex assembly would therefore be expected to pull the two membranes very close together, and may initiate bilayer mixing [9].

NSF disassembles SNARE complexes [10]. *In vitro*, SNAPs bind first to the SNARE complex, followed by NSF. Binding of NSF requires ATP, the hydrolysis of which is required for disassembly. With non-hydrolyzable ATP analogs, NSF gets ‘stuck’ in the bound state, resulting in a large complex consisting of SNAREs, SNAPs and NSF [6]. When NSF is deficient, SNARE complexes cannot be disassembled and transport is blocked [11]. NSF’s primary role is thus to disentangle and re-energize the SNAREs after fusion, when they are all aligned in parallel in the same membrane [9]. Furthermore, complementary SNAREs in the same membrane have a tendency to form ‘non-constructive’ complexes, so that NSF is continuously required to maintain SNAREs in a reactive state [12].

NSF is a hexamer of identical subunits. Each subunit contains three distinct domains: two similar ATPase domains, D1 and D2, and an amino-terminal domain that is required for substrate binding. The carboxy-terminal ATPase domain, D2, does not participate in the catalytic cycle of the protein; rather, it holds the hexamer together [13–15]. The substrate-binding amino-terminal domains form the business end of NSF. When ATP bound to the D1 domain is hydrolysed, the protein undergoes a major

conformational change that appears largely to involve the amino-terminal domains [16].

The discovery that the NSF interacts with the AMPA receptor GluR2 [2–4] comes as a major surprise. Even the most sceptical biochemist, however, should be convinced by the evidence that the interaction is specific. The GluR2 side of the interaction is mediated by a discrete stretch of ten amino acids in the receptor's carboxy-terminal cytoplasmic tail (residues 844–853) [2–4]. Although this region of AMPA receptors is highly conserved, only weak or no interactions were found with GluR1, GluR3 and GluR4 (with the exception of a GluR4 splice variant [2]). Mutagenesis experiments indicated that residue asparagine 851 of GluR2 is crucial for binding to NSF.

In contrast, NSF needs to be intact for the interaction; none of its isolated domains showed any GluR2 binding activity [3]. Direct binding between GluR2 and NSF was observed in *in vitro* assays when either binding partner was added as recombinant protein [2–4]. Addition of a decapeptide corresponding to the interacting region of GluR2 abolished binding, whereas peptides corresponding to the equivalent sequences of non-binding glutamate receptors did not. Furthermore, GluR2 and NSF were found to coprecipitate from brain extracts.

There are similarities between the binding of NSF to GluR2 and its binding to SNARE complexes. Both complexes dissociate upon ATP hydrolysis and can be maintained in a stable form by non-hydrolyzable ATP analogs [4]. In contrast to the binding of NSF to SNARE complexes, its binding to GluR2 does not require SNAPs, although SNAPs were found to coprecipitate with GluR2 and GluR2–NSF complexes. Quantitative analysis of the GluR2/NSF immunoprecipitates revealed an approximately one-to-one stoichiometry between the oligomeric AMPA receptors and NSF [4].

What, if anything, is the physiological significance of the interaction between GluR2 and NSF? To address this question, Nishimune *et al.* [3] perfused CA1 pyramidal neurons in hippocampal slices with the decapeptide corresponding to the NSF-binding domain of GluR2. On electrical stimulation, they observed a significant reduction of the amplitude of excitatory postsynaptic currents which developed within 10–20 minutes after injection. Other parameters, such as the rise time of the excitatory postsynaptic potential, were unchanged. No such changes were observed when the corresponding peptide of GluR4 or a scrambled peptide were used.

Such a response can be interpreted as a reduction of the number of active receptors in the presence of the decapeptide that is known to inhibit the GluR2–NSF interaction. A similar reduction was observed when an anti-NSF antibody was injected. This antibody had previously been shown to inhibit NSF's action in membrane transport. The effects of microinjected peptides were also examined on evoked [3] and spontaneous [2] synaptic activity in cultured hippocampal neurons; in both cases, inhibition of excitatory postsynaptic currents was observed.

How can these results be explained? Two interpretations are possible, one conservative and one heretical. The conservative view would be that the depression of the AMPA receptor response is simply the result of an inhibition of intracellular membrane transport caused by inactivation of NSF. The run-down of active receptors might reflect interference with normal receptor turnover. The synapse might depend on constant, constitutive recycling of vesicles containing spare receptors, and inhibition of this recycling could explain the observations. This would be in line with the established function of NSF: the exocytosis that mediates receptor recycling would be mediated by as yet unknown SNAREs which would be the functionally relevant targets of NSF in these experiments. This model does not, however, address the interaction between GluR2 and NSF.

The second, and more heretical, possibility is that the reduction in AMPA receptor response is primarily a consequence of preventing the binding of NSF to GluR2. Such an explanation would integrate the *in vitro* interaction with the physiological findings, but it requires some imagination concerning the substrate specificity of NSF. Given the evolutionary conservation of SNARE-mediated membrane fusion, it is likely that ATP-driven disassembly of SNARE complexes is the original function of NSF. Sequence conservation between distantly related SNAREs is rather low, although the overall structure of SNARE complexes appears to be conserved [17]. Perhaps the carboxy-terminal tail of this GluR2, in a kind of 'evolutionary opportunism', has evolved a surface that resembles that of SNARE complexes, allowing NSF to bind.

The molecular consequences of NSF's action on GluR2 needs clarification. NSF belongs to the AAA protein superfamily — the acronym deriving from 'ATPases Associated with various cellular Activities' — which are characterized by a highly conserved ATP-binding module [18]. Although the activities and targets of these diverse proteins have no obvious common feature, it has been speculated that AAA proteins share the ability to change the conformation of their target proteins while expending energy — that they are a special group of molecular chaperones [18]. For instance, NSF might disassemble receptor oligomers, or receptor complexes with proteins such as GRIP or other PDZ proteins. It is not yet known whether NSF induces a conformational change in the receptor, as it does in its target SNARE complexes. Future work will hopefully establish for sure whether NSF really is a physiologically

important regulator of postsynaptic receptor activity, and, if so, precisely how it acts to fulfill this role.

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