

# NSF – fusion and beyond

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In 1988, Block and collaborators in James Rothman's group at Stanford University purified a factor that restored intra-Golgi transport after inactivation of Golgi membranes by *N*-ethylmaleimide. They baptized this protein NSF for *N*-ethylmaleimide-sensitive factor<sup>1</sup> or *N*-ethylmaleimide-sensitive fusion protein<sup>2</sup>. The first models of NSF action were that it would act in concert with 'soluble NSF attachment proteins' (SNAPs) on transmembrane SNAP receptors (SNAREs) to form a '20S fusion complex', bridging the membranes destined to fuse<sup>3</sup>. This complex would be dissociated by ATP hydrolysis by NSF, and the hydrolysis energy would also drive membrane fusion<sup>4</sup>. Later, it became clear that, at least in some systems, NSF could act long before the actual fusion takes place<sup>5,6</sup>, raising the question of whether NSF is really a fusion protein in the sense of being directly involved in the merging of membranes. Instead, it was suggested that NSF and SNAP act as molecular chaperones that change the conformation of SNAREs at the expense of ATP<sup>7</sup>. This process would dissociate SNAREs that are tightly bound to each other on the same membrane<sup>8</sup> so that they can then bind to different SNAREs on the partner membrane and promote fusion<sup>9</sup>. Recent ultrastructural<sup>10</sup> and biochemical<sup>8,11,12</sup> evidence clearly supports this revised interpretation.

One of the main models used for investigations into the role of NSF in intracellular trafficking is the fusion of synaptic vesicles with the presynaptic membrane during neurotransmission. Hence, it is particularly surprising that three recent studies using this well-characterized system assigned a novel role to NSF in neurotransmission that is probably completely independent of SNAREs. Instead, Nishimune and colleagues<sup>13</sup>, Osten and coworkers<sup>14</sup> and Song *et al.*<sup>15</sup> suggest that NSF is an important constituent of the postsynaptic density and that it regulates the function of the AMPA glutamate receptor located there.

## The AMPA receptor

The AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate) glutamate receptor<sup>16</sup> resides in the postsynaptic membrane, where it acts in fast-excitatory synaptic transmission of the central nervous system. The receptor is part of the postsynaptic density, which is a specialization of the cytoskeleton and positioned at the cytoplasmic face of the postsynaptic membrane<sup>17</sup>. Each AMPA receptor consists of multiple subunits (GluR1 through GluR4), which can produce differently composed receptors possessing a variety of properties. All receptors that contain GluR2 are impermeable to  $\text{Ca}^{2+}$ . All of the AMPA receptor subunits have an extracellular N-terminus, followed by three membrane-spanning domains and an intracellular C-terminus of ~50 amino acid residues. This terminus is a major target for phosphorylation reactions that can modulate channel properties<sup>18</sup> and is also the site of interaction with synaptic proteins involved in the regulation of glutamate receptor function<sup>19</sup>. The three research teams used this presumed regulatory hot-spot as a 'bait' in the yeast two-hybrid-system to

*NSF (N-ethylmaleimide-sensitive fusion protein) was the first protein to be isolated as a crucial factor in intracellular membrane-fusion events, such as the fusion of synaptic vesicles with the presynaptic membrane during neurotransmission.*

*Although the activation of membrane SNARE proteins for subsequent fusion is clearly a primary role of NSF, recent studies have provided surprising evidence that NSF also interacts with glutamate receptors at the postsynaptic membrane in a way that does not seem to involve SNAREs. These results suggest that NSF might act as a molecular chaperone not only on SNAREs but also on other proteins.*

search for interacting factors from rat brains. All three groups identified, among a few other proteins, NSF as a strongly interacting factor. The positive signal in the two-hybrid system was validated by various techniques, including immunoprecipitations with antibodies to NSF<sup>14,15</sup> or GluR2<sup>14</sup> or binding of NSF to the GluR2 C-terminus on affinity columns<sup>13</sup>. There is no obvious amino acid sequence homology between this C-terminus and other NSF-binding proteins, suggesting that the binding between GluR2 and NSF is different from that between NSF and, for example, SNAP.

## Does NSF cheat on SNAREs?

Other lines of evidence support the idea that NSF resides in the postsynaptic density and plays an important role there. First, NSF is enriched within isolated postsynaptic density material<sup>20</sup>, particularly after transient cerebral ischemia (Ref. 21, confirmed in Ref. 15). Second, long-term potentiation (LTP) is inhibited by *N*-ethylmaleimide, which also inhibits NSF<sup>1</sup>, and addition of recombinant SNAP increases excitatory postsynaptic currents (EPSCs)<sup>22</sup>. Third, NSF and GluR2 colocalize in dendrites<sup>13,14</sup>. So, does this mean that NSF functions postsynaptically in concert with its classic partners, SNAPs and SNAREs? Not necessarily!

In fact, Osten and coworkers<sup>14</sup> isolated a GluR2–NSF– $\alpha$ / $\beta$ -SNAP complex from a hippocampal detergent extract, and this complex could only be maintained stably when a nonhydrolysable ATP analogue was included; magnesium-ATP readily disassembled the complex. This behaviour is reminiscent of that of the 20S complex of

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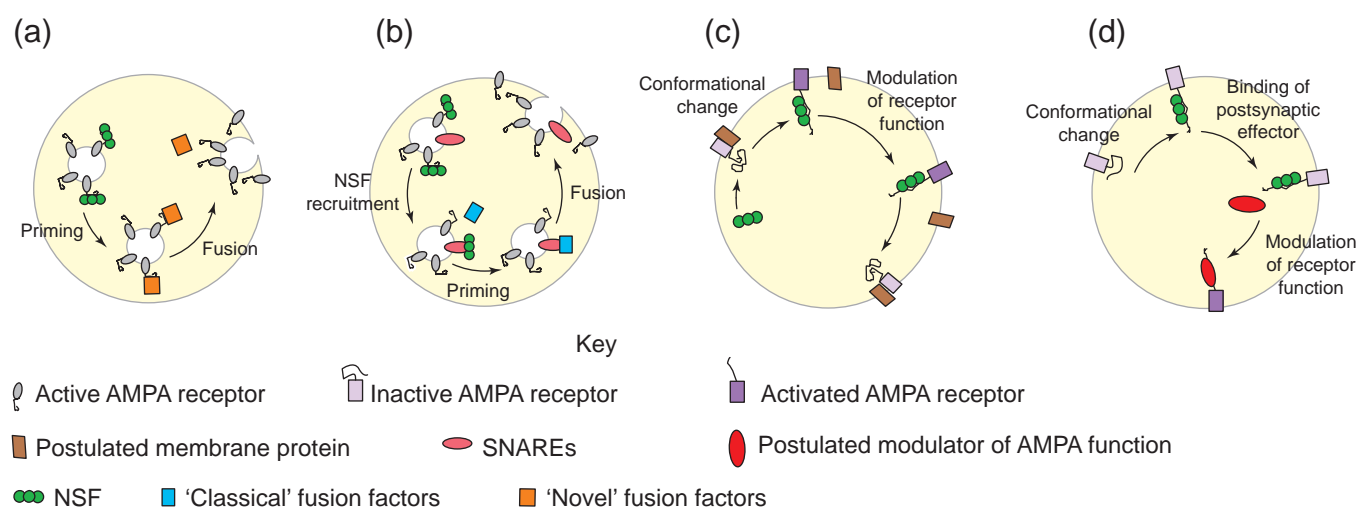


FIGURE 1

Various ways in which postsynaptic NSF–GluR2 interactions could activate synaptic transmission. (a) 'Receptor-insertion model': the NSF-requiring membrane-fusion reaction shuttles the AMPA receptor between a vesicular, intracellular pool and the pool in the postsynaptic plasma membrane. Synaptic transmission would be increased owing to increased availability of AMPA receptors. (b) 'NSF-supply model': GluR2 C-termini on the surface of AMPA-containing intracellular vesicles could act as a store for NSF, keeping the NSF concentration high in proximity of membrane SNAREs and related proteins and hence stimulate vesicle fusion with the plasma membrane. (c) 'AMPA-chaperone model 1': NSF and SNAP could dissociate the AMPA receptor from a hypothetical protein (X) of the postsynaptic density to which the receptor would normally be tightly bound. This would release and activate the receptor on the cell surface. (d) 'AMPA-chaperone model 2': AMPA channel function could be directly altered through a chaperone-like activity of NSF on the receptor, possibly followed by binding of a cytosolic protein(s) that activates the receptor on the cell surface directly or by mediating phosphorylation of the GluR2 C-terminus. Abbreviations: AMPA,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate; GluR2, subunit of AMPA glutamate receptor; NSF, N-ethylmaleimide-sensitive factor/fusion protein.

SNAREs–SNAPs–NSF<sup>3,23</sup>. However, neither of the major nerve terminal SNAREs, synaptobrevin and syntaxin, was coprecipitated with GluR2. When the researchers used 293T cells (lacking AMPA receptors) in immunoprecipitation experiments, neither NSF nor SNAP was precipitated with antibodies against GluR2. Their data<sup>14</sup> suggest that NSF alone, or in concert with a SNAP, acts on GluR2 in a mass ratio of about one NSF hexamer per AMPA receptor molecule and that NSF is crucial for the activity of AMPA receptors containing GluR2.

In addition to the solid biochemical data, there are also some functional data that support a role of NSF in a system analogous to, but different from, the SNARE complexes. Nishimune and colleagues<sup>13</sup> and Song *et al.*<sup>15</sup> used whole-cell patch-clamp recordings to determine AMPA receptor-mediated EPSCs in CA1 pyramidal rat neurons. Infusion into CA1 neurons of a high concentration of decapeptides, which block the interaction between NSF and the GluR2 subunit *in vitro*, caused a rapid reduction in EPSC amplitude. A decapeptide with a single amino acid exchange was noninhibitory. These authors also found that infusion with a monoclonal antibody that recognizes rat brain NSF caused a reduction in EPSC amplitude, whereas a monoclonal antibody that does not recognize the rat brain isoform of NSF had no effect<sup>13</sup>. This result was in agreement with the data previously obtained by Lledo *et al.*<sup>22</sup>.

Thus, NSF protein is present postsynaptically and interacts molecularly with GluR2<sup>13–15</sup>. It also interacts weakly or very weakly with GluR3<sup>14</sup> and GluR4c<sup>15</sup>. None of these subunits is absolutely required for AMPA receptor function<sup>15</sup> – so their interaction with NSF might therefore reflect a specific

mode of regulation for those AMPA receptors that contain subunits that do interact with NSF. Recombinant  $\alpha$ -SNAP added postsynaptically induces an increase in synaptic transmission, and agents that inhibit NSF reduce LTP in hippocampal slices<sup>22</sup>. In summary, the major roles of NSF activity in the brain might be modulation of synaptic transmission and mediation of specific forms of synaptic plasticity.

#### So, why is NSF on the postsynaptic side?

The above studies suggest that NSF and SNAPs can build '20S-complex-like structures' with non-SNARE receptors and that these structures are readily disassembled by ATP hydrolysis on NSF. It therefore seems likely that the AMPA receptor and the SNARE disassembly complex have similar modes of action. This would imply that the SNAP–NSF interaction would act on GluR2 in a chaperone-like priming reaction<sup>8,9,11,24</sup>, and this priming would be required continuously for receptor function. Experimental data suggest that SNAP and NSF could in this system not be functionally replaced by other chaperones, such as HSP60, HSP70, HSP90 or p97 ATPase, as none of these chaperones coprecipitates with GluR2 from hippocampal extracts, whereas NSF does<sup>13,14</sup>.

In principle, four scenarios seem possible:

- 'Receptor-insertion model' (Fig. 1a): the NSF-requiring reaction shuttles the receptor between a cytoplasmic AMPA vesicle pool and the pool on the postsynaptic membrane. NSF would act in the preparation of AMPA-containing vesicles in the postsynaptic cells for fusion with the postsynaptic membrane – but in a way that is independent of

SNAREs. In fact, cytochemical studies in cultured neurons have shown that AMPA receptor subunits are distributed diffusely in immature neurons; then, as the neurons mature and produce synapses, the receptor subunits become clustered in the spines and shafts of dendrites, hinting that there are targeting and concentration mechanisms for AMPA receptors during synaptogenesis<sup>25,26</sup>.

- 'NSF-supply model' (Fig. 1b): GluR2 C-termini on the surface of AMPA-containing intracellular vesicles could act as a store for NSF and keep the NSF concentration high in proximity to the membrane. When needed, NSF could be transferred from GluR2 C-termini to another protein, such as a SNARE, ultimately leading to fusion of the vesicles with the postsynaptic membrane. This interpretation would fit very well with the observation that yeast NSF and SNAP are tightly bound to vacuole membranes in the absence of SNAREs that function in vacuole fusion<sup>8</sup>, and NSF is also abundant in the complete absence of SNAP<sup>5</sup>. In this scenario, NSF would not have any direct effect on the function of GluR2.
- 'AMPA-chaperone model 1' (Fig. 1c): NSF and SNAP could dissociate the AMPA receptor from other postsynaptic density proteins to which the receptor would otherwise be bound. Either this would make the receptor available for new rounds of activity (a reaction analogous to 'priming' of SNAREs<sup>8</sup>) or the separated receptor could only then be properly sorted.
- 'AMPA-chaperone model 2' (Fig. 1d): AMPA activity could be altered directly through a chaperone-like action of NSF on the receptor. This activity could either have a direct effect on the receptor function or would allow a third factor, for example a protein kinase, to bind to the AMPA C-terminus.

The latter two possibilities, in particular, would be exciting novel roles for NSF. The receptor-insertion and the NSF-supply models would be more in line with the classical role for NSF, although there is no obvious SNARE that could participate in this event. However, there has not yet been an exhaustive search for alternative SNAREs. Clearly, more work is needed to determine which of the models is correct. This could include, for example, immuno-electron microscopy to investigate whether AMPA receptors and NSF colocalize on intracellular vesicles and/or whether they can be found together on the plasma membrane.

A close structural and probably functional relative of NSF, p97<sup>27</sup>, and its yeast counterpart, Cdc48p<sup>28</sup>, are also prime candidates for being chaperones that

might have functions beyond their direct role in membrane fusion<sup>29,30</sup>. They could act either alone or in concert with homotrimeric p47, which might be a functional correlate of the SNAPs<sup>31</sup>. An additional function of p97 would help to explain its abundance in various types of cells<sup>27</sup>, but so far the existence of such roles is completely speculative. However, there is no question that NSF and its fellow members of the AAA protein family of ATPases<sup>32</sup> (such as p97) still hold some surprises.

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