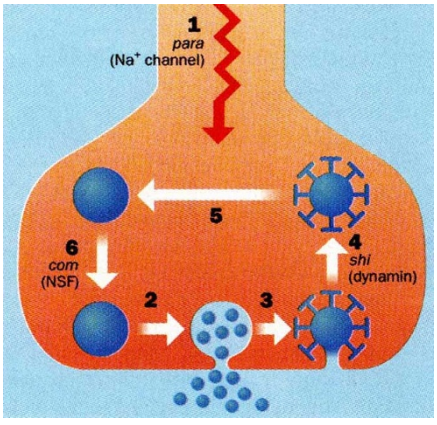
wasps successful in removing a nymph. In contrast, the success rate of predatory wasps can be as high as 90% in the absence of tending females[6]. Offspring–parent signalling appears to play a central role in defence in these subsocial insects. As in eusocial taxa, communication among group members permits an adaptive response to a rapidly changing feature of the environment.

Reginald B. Cocroft
Section of Neurobiology and Behavior,
Mudd Hall,
Cornell University,
Ithaca, New York 14853, USA

Classic clues to NSF function

SIR — Most intracellular membrane fusion events require the action of the *N*-ethylmaleimide-sensitive fusion protein (NSF)[1]. The role of NSF in vesicular transport remains highly controversial, and various models have proposed that NSF acts at a post-docking stage close to membrane fusion[2], at a post-docking but pre-fusion stage[3], or at a pre-docking stage[4,5]. A recent study of *in vitro* vacuolar fusion in yeast[6] supports the latter hypothesis, as does re-examination of a 1976 report on the temperature-sensitive *Drosophila* mutant *comatose*[7], which is now known to be an NSF mutant[8].

Like many other *in vitro* membrane fusion assays[1], homotypic fusion of yeast vacuolar vesicles requires NSF (Sec18p in yeast). Using an elegant approach, Mayer *et al.*[6] have succeeded in kinetically defining the stage of action of NSF in the complex process of priming, docking and fusion of vesicles that comprises the vacuolar fusion assay. They found that the action of Sec18p is complete even before docking can occur, implying that NSF acts to prime vesicles for subsequent docking and/or fusion.



Neurotransmission and synaptic vesicle recycling in *Drosophila* synapses. Stages depicted: 1, action potential propogation; 2, synaptic vesicle exocytosis and neurotransmitter release; 3, coated pit formation; 4, endocytic vesicle formation; 5, vesicle uncoating and reloading with neurotransmitter; 6, priming of vesicle for docking and/or fusion. The putative stages at which paralytic mutants are blocked in this cycle are illustrated.

Although the literature is replete with data on NSF function in constitutive membrane traffic[1] such as the vacuolar fusion assay, the only functional evidence for a role of NSF in regulated membrane traffic, as reported in *Nature*[8], is that the temperature-sensitive paralysis exhibited by *comatose* mutants of *Drosophila* are due to point mutations in the *dNSF-1* gene. The original, classic work on *comatose* by Siddiqi and Benzer[7], when reinterpreted 20 years later, provides important information on the molecular function of NSF in neurotransmission *in vivo*.

Siddiqi and Benzer[7] observed the kinetics of onset and recovery from temperature-induced paralysis in three *Drosophila* mutants: *para* (*paralysed*), *shi* (*shibire*) and *com* (*comatose*), all of which result from a presynaptic block of neurotransmission. *Para* mutants became paralysed within seconds of a temperature shift and recovered almost instantaneously, whereas *com* mutants required a minute to become fully paralysed and 30 minutes to recover; *shi* mutants were intermediate, displaying complete paralysis at 30 seconds and recovering after 20 minutes. Although phenomenological at the time, these data have profound functional significance as it is now known that the *para* gene codes for a voltage-dependent sodium channel[9], the *shi* gene for dynamin[10] and the *com* gene for NSF[8].

Neurotransmission begins with an action potential and results in the release by exocytosis of neurotransmitter, which then signals to the postsynaptic cell (stages 1 and 2 in the figure). Nevertheless, this complex process can take place in less than 200 microseconds. However, the depletion of synaptic vesicles by exocytosis has to be balanced by replenishment of new synaptic vesicles via endocytosis in a process estimated to require many seconds[11] (stages 3–6 in the figure). The kinetics of temperature-induced paralysis fit this model, as *para* flies exhibiting defects in voltage-dependent Na^+ channels (required to generate an action potential) recover from paralysis almost instantaneously, as would be predicted if an essential switch for neurotransmission was suddenly turned on. Indeed, such fast kinetics are exhibited not only by different allelic mutants of *para*, but also by *nap* (*no action potential*) and *tip-E* (*temperature-induced paralysis-E*) *Drosophila* mutants, which have different Na^+ channel defects[12]. In contrast, *shi* flies exhibiting mutations in dynamin (required for endocytic vesicle formation) take 20 minutes to recover fully from paralysis, as would be predicted if an essential switch for synaptic vesicle replenishment was suddenly turned on (the synaptic vesicle pool in *shi* mutants is replenished after 15 minutes[13]).

As the time taken for *com* flies to recover from paralysis is even longer than that required for *shi* flies and as the known allelic mutants of *com* display similar kinetics of paralysis, this suggests that the action of NSF is slower than the process of synaptic vesicle recycling. The new work on vacuolar fusion[6] suggests that this action is the priming of vesicles for cell membrane docking and fusion. Synaptic vesicle recycling is thought to involve two processes, endocytosis and vesicle re-priming, both requiring many seconds[11]. The slow kinetics of *shi* and *com* mutants[7] support a model in which dynamin acts in endocytosis and NSF in re-priming recycled synaptic vesicles (see figure). Such a slow, priming action of NSF is consistent with the hypothesis that NSF acts as a molecular chaperone to fold *Botulinum* neurotoxin substrates into a conformation competent for vesicle docking and/or fusion[4]. In addition to the *Drosophila* mutants discussed above, there are many temperature-sensitive paralytic mutants with differing kinetics whose mutant genes are unknown[12]. On identification of these genes, a rich source of data from previous decades should lead to insights on their *in vivo* molecular function in synaptic vesicle dynamics based on the kinetics of their paralytic phenotypes.

Alan Morgan
The Physiological Laboratory,
University of Liverpool,
PO Box 147, Liverpool L69 3BX, UK
e-mail: *amorgan@liverpool.ac.uk*

1. Rothman, J. E. *Nature* **372**, 55–63 (1994).
2. Sollner, T., Bennett, M. K., Whiteheart, S. W., Scheller, R. H. & Rothman, J. E. *Cell* **75**, 409–418 (1993).
3. O'Connor, V. O., Augustine, G. J. & Betz, H. *Cell* **76**, 785–787 (1994).
4. Morgan, A. & Burgoyne, R. D. *Trends Cell Biol.* **5**, 335–339 (1995).
5. Rexach, M. F. & Schekman, R. W. *J. Cell Biol.* **114**, 219–229 (1991)
6. Mayer, A., Wickner, W. & Haas, A. *Cell* **85**, 83–94 (1996).
7. Siddiqi, O. & Benzer, S. *Proc. Natl Acad. Sci. USA* **73**, 3253–3257 (1976).
8. Pallanck, L., Ordway, R. W. & Ganetzky, B. *Nature* **376**, 25 (1995).
9. Loughrey, K., Kreber, R. & Ganetzky, B. *Cell* **58**, 1143–1154 (1989).
10. Van der Bliek, A. M. & Meyerowitz, E. M. *Nature* **351**, 411–414 (1991).
11. Betz, W. J. & Wu, L.-G. *Curr. Biol.* **5**, 1098–1101 (1995).
12. Ganetzky, B. & Wu, C.-F. *Annu. Rev. Genet.* **20**, 13–44 (1986).
13. Van de Goor, J., Ramaswami, M. & Kelly, R. *Proc. Natl Acad. Sci. USA* **92**, 5739–5743 (1995).

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. Priority will be given to letters of fewer than 500 words and five references.