

Mice sans synaptotagmin

Erwin Neher and Reinhold Penner

In the past two years we have come a long way in the biochemical characterization of synaptic proteins. It has emerged that some 6–8 of them interact to form the so-called fusion complex (see refs 1–3 for review); and that homologues of some of the proteins are located at different places in the cell interior, wherever intracellular membranes are believed to fuse with one

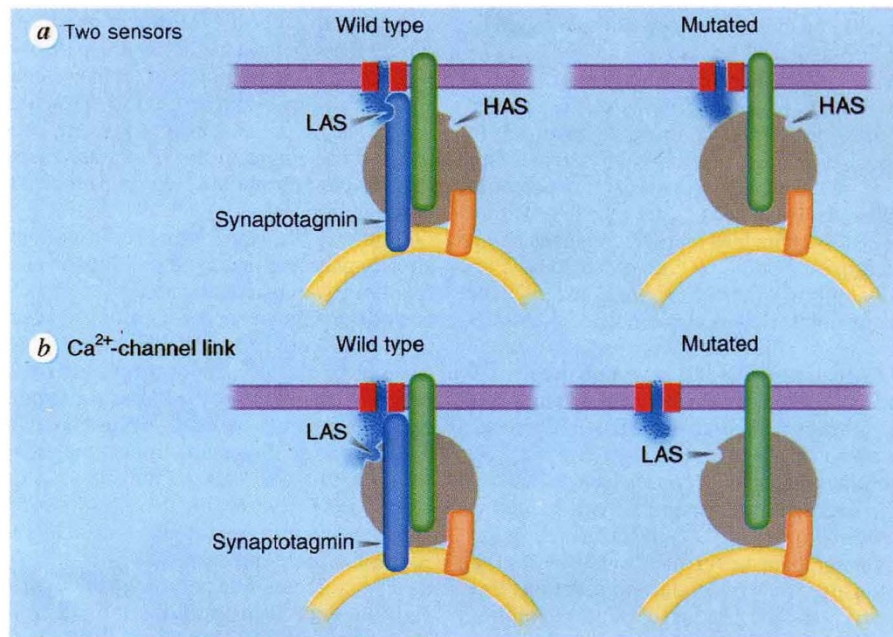
arrested at the 'active zone' (the site of neurotransmitter release) until an action potential arrives and increases the concentration of Ca^{2+} . It was therefore argued that synaptic vesicles possess a 'fusion clamp' which prevents fusion otherwise. Synaptotagmin, by the mere fact of its presence at sites of regulated fusion, was held up as a candidate for carrying out the

tagmin gene are phenotypically indistinguishable from heterozygotes or wild-type mice. Transgenic animals breathe normally, and respond to tactile stimulation; the retina and brain structure show no morphological abnormalities. If one believes that synaptotagmin I is central to neurotransmitter release, and that some functional interaction is required for establishment of a correct body plan, these findings are quite unexpected.

Nevertheless, Geppert *et al.* find that synaptic transmission in cultured hippocampal neurons is severely perturbed, but in a more subtle way than would be expected if there were just a single Ca^{2+} -sensor. They recorded from pairs of hippocampal neurons forming contacts in cell culture, stimulating the 'presynaptic' cells and then analysing responses in voltage-clamped 'postsynaptic' cells. With wild-type cells, large-amplitude, postsynaptic currents were observed, which were well synchronized with presynaptic action potentials. A slower 'asynchronous component', lasting some 100 milliseconds, contributed only little to the overall signal. In cells from mutant mice, on the other hand, the fast synchronous component was almost completely absent. The asynchronous phase, however, was unaltered or slightly increased.

Geppert *et al.* interpret the data in the light of a study by Goda and Stevens⁹, which describes two such components of neurotransmitter release: a fast component mediated by a highly efficient, low-affinity Ca^{2+} receptor; and a slower component mediated by a less efficient, high-affinity receptor. In this scheme, synaptotagmin would be the low-affinity sensor, which efficiently elicits secretion during the short period of the action potential when concentrations of Ca^{2+} at the release site reach very high levels (see left-hand part of *a* in the figure). Mutant mice lacking synaptotagmin (right-hand part of *a*) would be stimulated only through the inefficient (but high-affinity) receptor; they therefore show only a small, slow response, while residual Ca^{2+} , after having spread out diffusorally, decays back to basal levels.

This proposal explains the data very well, and provides a detailed picture of the events preceding fusion. But other interpretations are possible. One is offered by the so-called Ca^{2+} -voltage hypothesis¹⁰, which holds that transmitter release is regulated both by a Ca^{2+} -sensor and by a voltage sensor. It seems that the simple postulate that synaptotagmin is the voltage sensor would, by and large, explain the results (we are aware that its molecular properties appear to be incompatible with this assumption, but it might be coupled to a voltage sensor). A study on synaptotagmin-deficient PC12 cells can be interpreted in terms of the Ca^{2+} -voltage hypothesis, however, with the postulate



Models for the role of synaptotagmin in eliciting vesicle-membrane fusion. Each of the four diagrams shows a vesicle attached to the plasma membrane by a fusion complex. *a*, The two-sensor model⁹. Two Ca^{2+} -sensors, a low-affinity one (LAS) on synaptotagmin and a high-affinity one (HAS), respectively provide for fast synchronous and asynchronous release. The LAS normally senses short intensive pulses of Ca^{2+} when Ca^{2+} -channels open, but not in the absence of synaptotagmin (right). The HAS is not necessarily a single binding site, but may represent several Ca^{2+} -dependent steps preceding fusion. *b*, A speculative, alternative model, with only one low-affinity Ca^{2+} -sensor. Here, synaptotagmin's function is to link a Ca^{2+} -channel to the fusion machinery. In its absence (right), channels and release sites are randomly distributed, so that the Ca^{2+} -sensor no longer experiences short intensive pulses of Ca^{2+} .

another. Synaptic proteins are conserved between man and yeast, so there is no doubt that the fusion complex is one of the very basic molecular machines. It not only mediates synaptic transmission (by letting synaptic vesicles fuse with the plasma membrane) but also enables membranes to flow from their site of synthesis to the cell surface through various cycles of vesicle formation and fusion.

It seems, then, that we know which players take part in this game. But what does each of them do? A paper by Geppert *et al.*⁴, published in *Cell* last week, provides some clues to help start assigning specific functions to specific proteins.

Fusion of transmitter-containing vesicles during synaptic transmission is precisely regulated by calcium, in contrast to 'constitutive exocytosis' which goes on continuously. Synaptic vesicles seem to be

clamping function. When it was found that each molecule has two so-called C2-domains (which in protein kinase C bind calcium), the obvious conclusion was that synaptotagmin is the Ca^{2+} -sensor of regulated secretion — or, more precisely, a Ca^{2+} -controlled fusion clamp⁵. Geppert *et al.* have now looked at hippocampal neurons from transgenic mice lacking functional synaptotagmin I, one of the two isoforms of the molecule. They conclude that synaptotagmin I is one of the main Ca^{2+} -sensors for transmitter release.

Mutational studies on *Drosophila*^{6,7} and *Caenorhabditis elegans*⁸ provided the initial indications that synaptotagmin is essential for neurotransmission, and that lack of the molecule leads to severe dysfunction in secretion. But Geppert *et al.* find that, immediately after birth, mice homozygous for the knocked out synap-

WHITE DWARFS

Plugging the generation gap

Harry L. Shipman

that synaptotagmin is the Ca^{2+} -sensor rather than the voltage sensor (W. Huttner, personal communication).

Another (admittedly speculative), explanation is depicted in *b* in the figure. Here, synaptotagmin links the fusion particle and Ca^{2+} -channels. Although synaptogenesis and vesicle docking appear to be normal in Geppert and co-workers' mutant mice, it still may be that, unlike the situation in wild-type mice, Ca^{2+} -channels are not recruited to the molecular fusion complex. The lack of a well synchronized response might then be a simple consequence of the fact that the Ca^{2+} -sensor (which in these circumstances can be a single-type, low-affinity sensor) is not exposed to the extremely high local concentrations of Ca^{2+} which are thought to occur in microdomains at the mouths of open channels^{11,12}. The small component of asynchronous release could then be the response of the same sensor to more generalized but smaller increases in Ca^{2+} , which come about through the overlap and diffusional spread of microdomains.

From its molecular properties, synaptotagmin looks like a Ca^{2+} -sensor, but it may also be important for structural organization. The synaptotagmins bind to neurexins which are involved in many aspects of synapse organization¹³. Also, synaptotagmin I interacts with the clathrin-assembly protein complex AP2 (ref. 14), meaning that it may take part in endocytosis following exocytosis. Last — but not least — synaptotagmin interacts with N-type Ca^{2+} -channels *in vitro*¹⁵.

It will be interesting indeed to find out which of the properties of this multifaceted molecule is responsible for its involvement in exocytosis, and what exactly its Ca^{2+} -sensing ability is used for. Geppert *et al.* have provided some helpful pointers to that end. □

Erwin Neher and Reinhold Penner are in the Abteilung Membranbiophysik, Max-Planck-Institut für Biophysikalische Chemie, Am Fassberg, D-37077 Göttingen, Germany.

RESULTS published this month by Martin Barstow and colleagues¹ provide a clue to a long-standing mystery in stellar evolution: the genesis and life cycles of white dwarf stars. The trouble has been that hydrogen-rich white dwarfs are rather cool (in stellar terms), whereas their supposed progenitors, the hydrogen-rich 'planetary nebulae' stars, are extremely hot. Barstow *et al.* may have found the star that plugs the gap. The object known as RE1738+665, identified by the Wide Field Camera on the Rosat spacecraft, is the hottest hydrogen-rich white dwarf yet detected.

Most stars end their lives as white-dwarf stars, tiny balls no larger than the Earth. So, if you like, you can interpret the study of white dwarfs as a part of the study of stellar old age, or stellar geriatrics. White dwarfs are interesting in their own right, as in many respects they are much more varied than are their ancestors on the main sequence of stellar evolution. Some of the highest stellar temperatures, strongest magnetic fields and most unusual photospheric compositions are found in the bottom of the Hertzsprung–Russell diagram of luminosity against temperature, the place where these little stars are found. The life cycles of white dwarfs is, or should be, simple. Because nuclear reactions (for the most part, hydrogen burning) have ceased in their cores, they cool into oblivion.

When white-dwarf spectroscopy began in earnest in the 1950s, it became clear that, broadly speaking, white dwarfs are divided into two kinds. One group has photospheres that show hydrogen lines and little else in their spectra; these are called DAs because their spectra roughly resemble those of the main-sequence A stars. The second group's photospheres show little more than helium lines, and are classified into different spectral categories depending on whether the helium lines are He II (DO), He I (DB) or non-existent (DC, DQ or DZ)². There are many additional categories because some white-dwarf photospheres are polluted by species such as carbon, calcium, magnesium and iron. The circumstance requires some additional explanation because the surface gravities of white dwarfs are so large that the lightest constituent in their outer envelopes should float to the top, and everything else should sink out of sight.

A number of us have been trying to make sense of this complex picture for years, but even the broad question of why white dwarfs are divided into DAs and non-DAs has eluded us. Most of us thought that this distinction pre-dated the white-dwarf stage of a star's life cycle and

that white dwarfs cooled through two channels: the DAs and the non-DAs. But evidence for this picture was slim. In the late 1980s, Gilles Fontaine, François Wesemael and Jim Liebert, three leaders in the field, stunned the rest of us by proposing a single-channel scheme for white-dwarf chemical evolution. This model posited that there was really only one kind of white dwarf, which changed its face as it cooled (see for example refs 3,4). Hydrogen-rich DAs turned into DBs, which then turned back into DAs, changing their costumes like an old-time vaudeville actor. The thin hydrogen skin disappeared when it was mixed with the helium interior; then these helium-surfaced stars changed their faces when hydrogen floated to the top. This proposal explained a number of puzzling features of white-dwarf temperature distributions. For instance, at that time no DA white dwarfs were known with temperatures exceeding about 65,000 K, whereas there were plenty of non-DAs that had such extreme temperatures.

This proposal stirred up a commotion because it required that the hydrogen layer in all white dwarfs was a thin skin on the top of a mainly helium star, somewhere between 10^{-7} and 10^{-14} solar masses depending on exactly what you wanted the single-channel scheme to explain. But our basic understanding of the life cycles of red giants, the stars that turn into white dwarfs, indicated that the hydrogen-layer masses were much higher, about 10^{-4} solar masses⁵. How could a star manage to get rid of almost all, but not absolutely all, of its hydrogen? At least one investigator found the idea so nonsensical that he stopped working on white-dwarf stars for a while. But the single-channel model apparently explained a lot of things, including the extreme ultraviolet photometry of white dwarfs, the 'DB gap' or complete absence of DB stars with temperatures between 30,000 K and 45,000 K, and the pulsational properties of many white dwarfs. It attracted considerable support.

Enter Barstow and colleagues. The Wide Field Camera on board Rosat discovered 384 objects, and measured their extreme ultraviolet (EUV) count rates in two bands, 60–140 Å and 114–200 Å. Some of these sources were known from other catalogues, but some, including RE1738, were not. Spectra of the blue and red dots which sat within the Rosat error boxes were then obtained with ground-based optical telescopes. The nature of RE1738 prompted Barstow's team to obtain an ultraviolet spectrum from the International Ultraviolet Explorer (IUE).

- Söllner, T. & Rothman, J. E. *Trends Neurosci.* **17**, 344–348 (1994).
- Jahn, R. & Südhof, T. C. *A. Rev. Neurosci.* **17**, 219–246 (1994).
- Rothman, J. E. *Nature* **372**, 55–63 (1994).
- Geppert, M. *et al. Cell* **79**, 717–727 (1994).
- Popov, S. V. & Poo, M. M. *Cell* **73**, 1247–1249 (1993).
- Littleton, J. T., Stern, M., Schulze, K., Perin, M. & Bellen, H. J. *Cell* **74**, 1125–1134 (1993).
- DiAntonio, A. & Schwarz, T. L. *Neuron* **12**, 909–920 (1994).
- Nonet, M. L. *et al. Cell* **73**, 1291–1305 (1993).
- Goda, Y. & Stevens, C. F. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Parnas, H., Dufel, J. & Parnas, I. *Pflügers Arch.* **406**, 121–130 (1986).
- Llinas, R., Steinberg, I. Z. & Walton, K. *Biophys. J.* **33**, 323–351 (1981).
- Heidelberger, R., Heinemann, C., Neher, E. & Matthews, G. *Nature* **371**, 513–515 (1994).
- Hata, Y. *et al. Neuron* **10**, 307–315 (1993).
- Maycox, P. R., Link, E., Reetz, A., Morris, S. A. & Jahn, R. *J. Cell Biol.* **118**, 1379–1388 (1992).
- Bennett, M. K., Caiados, N. & Scheller, R. H. *Science* **257**, 255–259 (1992).