gene mutations that cause thalassemia (*14*). Analysis of seven RFLPs linked to the *G6PD* gene led to the identification of several haplotypes and a possible genealogy for the most common G6PD variants (*15–17*). It has been estimated that the A– variant, based on the fact that it has a selective advantage, could have arisen between 5000 and 10,000 years ago (*17*). Building on this work, Tishkoff *et al.* (*3*) have analyzed RFLPs associated with the normal forms of *G6PD* (B and A) and with the A– and Med G6PD deficiency variants in 591 individuals from 14 different populations worldwide. In addition to four of the seven known RFLPs, these investigators analyzed three new microsatellites (DNA containing variable numbers of tandem repeats) located in a noncoding region within 19 kilobases of the *G6PD* gene.

With mathematical modeling of the RFLP and microsatellite analysis, the authors were able to estimate when the A– and Med variants arose. They propose that the A– variant is more ancient, originating 6357 years ago (very close to the previous estimate) (*17*), whereas the Med variant is more recent, originating 3330 years ago. This is somewhat surprising as the A– variant is limited to Africa, whereas the Med variant ranges from Portugal to Indonesia, hinting that Med should be the more ancient variant (see the figure). However, there is considerable overlap in the range of the estimates: 3840 to 11,760 years for A– and 1600 to 6640 years for Med. We eagerly await analyses of other G6PD variants, such as Seattle and Union, which have broader geographical distributions than either A– or Med, suggesting that they may be even more ancient (see the figure).

It is intriguing that estimates for the origin of the A– and Med G6PD variants are consistent with the introduction of agriculture in the Middle East and Africa about 10,000 years ago, which provided conditions conducive to the spread of malaria. Volkman *et al.* (*18*), reporting on page 482 of this issue, have narrowed down previous calculations which estimate that ancestral *P. falciparum* arose between 5000 and 50,000 years ago (*19*). By analyzing sequence variations in the noncoding regions of some *P. falciparum* genes, they calculate that the ancestral strain of *P. falciparum* emerged 3200 to 7700 years ago. This estimate overlaps nicely with the Tishkoff *et al.* estimate for the age of the A– and Med G6PD variants. These molecular analyses provide further evidence for a close connection between malaria and G6PD deficiency, and yield a glimpse into the complexity of the co-evolution of a parasite and its host.

The Tishkoff *et al.* findings, however, do not answer two crucial questions: How does G6PD deficiency protect against *P. falciparum*, and is it only heterozygotes that are protected? (There has been much debate about whether hemizygotes, males with only one variant copy of the X chromosome–linked *G6PD* gene, are also protected.) Considering that malaria remains a devastating public health problem, these questions are not merely of academic interest. Malaria has been credited with contributing to the fall of the Roman Empire—it would be a vindication of human cultural evolution, if we could learn from natural selection how to defeat this terrible disease.

References

1. J. B. S. Haldane, *Ric. Sci.* **19** (Suppl. 1), 68 (1949).
2. L. Luzzatto, in *Genes and Resistance to Disease*, V. Boulyjenkov, K. Berg, Y. Christen, Eds. (Springer-Verlag, Berlin, 2000), pp. 105–119.
3. S. A. Tishkoff *et al.*, *Science* **293**, 455 (2001).
4. R. Notaro *et al.*, *Faseb J.* **14**, 485 (2000).
5. P. P. Pandolfi *et al.*, *EMBO J.* **14**, 5209 (1995).
6. L. Longo *et al.*, *Blood* **90** (Suppl.), 408a (1997).
7. U. Bienzle, O. Ayeni, A. O. Lucas, L. Luzzatto, *Lancet* **i**, 107 (1972).
8. C. Ruwende *et al.*, *Nature* **376**, 246 (1995).
9. E. A. Usanga, L. Luzzatto, *Nature* **313**, 793 (1985).
10. M. Cappadoro *et al.*, *Blood* **92**, 2527 (1998).
11. L. Luzzatto, A. Mehta, T. Vulliamy, in *The Metabolic and Molecular Bases of Inherited Disease*, C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Valle, Eds. (McGraw-Hill, New York, 2001), p. 4517.
12. Y. W. Kan, A. M. Dozy, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5631 (1978).
13. M. Ceppellini, *G. Batteriol. Virol. Immunol.* **69**, 39 (1976).
14. S. H. Orkin *et al.*, *Nature* **296**, 627 (1982).
15. T. J. Vulliamy *et al.*, *Hum. Mutat.* **8**, 311 (1996).
16. B. Kurdi-Haidar *et al.*, *Am. J. Hum. Genet.* **47**, 1013 (1990).
17. A. C. Kay *et al.*, *Am. J. Hum. Genet.* **50**, 394 (1992).
18. S. K. Volkman *et al.*, *Science* **293**, 482 (2001).
19. S. M. Rich, F. J. Ayala, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6994 (2000).

PERSPECTIVES: NEUROSCIENCE

The Meaning of a Mini

Patrik Verstreken and Hugo J. Bellen

Nerve cells communicate with one another by releasing neurotransmitter molecules at specialized junctions between neurons called synapses. In response to electrical impulses (action potentials) transmitted along the nerve axon, synaptic vesicles fuse with the neuron's presynaptic membrane, releasing their neurotransmitter contents into the synaptic cleft (see the figure). The neurotransmitter then diffuses across the synaptic cleft, activating receptors in the postsynaptic membrane. Usually, synaptic vesicles fuse with the presynaptic membrane in response to action potentials, but spontaneous fusion resulting in the release of a single vesicle's contents has been recorded in the absence of action potentials (*1*). When a single synaptic vesicle spontaneously fuses with the presynaptic membrane, neurotransmitter diffuses across the synaptic cleft and activates postsynaptic receptors, eliciting a miniature endplate potential or mini. Spontaneous fusion usurps some (but not all) of the molecular machinery required for action potential–dependent fusion. Indeed, genetic manipulation or toxin treatment that blocks neurotransmitter release evoked by action potentials also blocks spontaneous fusion (*2*, *3*).

Spontaneous fusion events and the minis they generate were reported more than 50 years ago by Fatt and Katz (*1*). Yet it is still not clear whether spontaneous fusion is important in neuronal communication or merely represents leakage from an otherwise efficient synaptic machinery. Saitoe *et al.* (*4*) have tackled this problem by examining the organization of developing neuromuscular junctions (NMJs)—specialized synapses between neurons and muscle cells—in a variety of fly mutants that have defects in different steps of neurotransmitter release. They report on page 514 of this issue that spontaneous neurotransmitter release is crucial for organizing sites of neuronal communication in the NMJ during *Drosophila* development.

In the NMJ of the developing fly, alignment of the presynaptic active zone and the postsynaptic density (which contains a high concentration of glutamate receptors) is essential for fast and reliable transmission of action potentials. Clustering of glutamate receptors in the postsynaptic density takes place in several stages. Initially, glutamate receptors are spread diffusely throughout the muscle membrane but are somewhat enriched in the vicinity of muscle nuclei (*5*, *6*). They then start to cluster at sites where the neuron and muscle cell will eventually make contact (*6*, *7*). Later, these broad receptor clusters are reduced (refined) still further when the innervating neuron makes contact with the muscle cell. Neuronal activity is required for the final clustering of receptors in the postsynaptic density, but action potentials do not appear to be involved. Tetrodotoxin (TTX), a drug that inhibits action potentials (but not minis) by inactivating sodium channels, does not prevent glutamate receptor clustering (*8*). However, postsynaptic gluta-

The authors are in the Program in Developmental Biology, Howard Hughes Medical Institute, Department of Molecular and Human Genetics, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA.

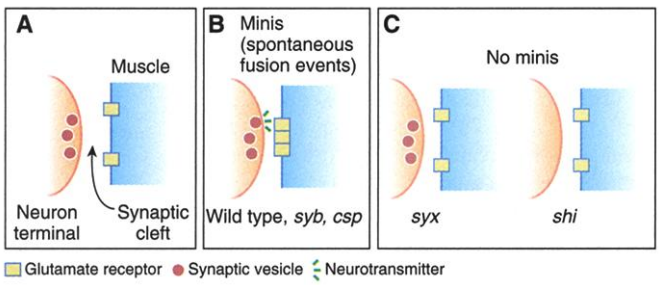
mate receptor clustering is prevented when sodium channel activity is blocked in a temperature-sensitive fly mutant (*6, 8*). Intriguingly, at the restrictive temperature (34°C), minis were mostly absent in this temperature-sensitive fly mutant. Unfortunately, wild-type NMJ activity is also severely impaired at the restrictive temperature, making these experiments difficult to interpret. Thus, neural activity seems to be required for clustering of postsynaptic glutamate receptors in the developing *Drosophila* NMJ, but the nature of this neural activity is still unclear.

Saitoe *et al.* (*4*) now demonstrate that spontaneous fusion, but not fusion evoked by action potentials, is required for glutamate receptor organization in the NMJ postsynaptic membrane. They observed that *Drosophila* mutants with defective synaptic vesicle proteins such as Syntaxin or Dynamin had neither evoked nor spontaneous fusions and did not show refinement of glutamate receptor clustering. In contrast, clustering was essentially normal in flies with defective Synaptobrevin or Cysteine String Protein—mutants that exhibit spontaneous fusion but not action potential–dependent fusion. In these mutants, activation of glutamate receptors did not seem to be important for clustering, because iontrophic glutamate receptor blockers did not interfere with this process. Thus, spontaneous fusion is likely to be important for refining postsynaptic receptor clustering in the *Drosophila* NMJ. It is possible that spontaneous fusion induces the secretion of unknown factors that are required for glutamate receptor clustering.

How does the clustering of postsynaptic glutamate receptors in the developing fly NMJ compare to the clustering of acetylcholine receptors in the NMJ of the developing mouse embryo? Acetylcholine receptor clustering in the developing mouse NMJ depends on the secreted protein agrin and its postsynaptic receptor MuSK (*9, 10*). Interestingly, postsynaptic acetylcholine receptors localize and cluster normally in the developing mouse NMJ in the absence of both evoked and spontaneous fusion events (*11*). Therefore, spontaneous fusion does not seem to be required for clustering of mouse NMJ acetylcholine receptors.

Glutamate receptors are the most common receptors in the vertebrate brain, but how they cluster in the postsynaptic membrane is still not well understood. Recently, a protein called Narp (neural activity–regulated pentraxin) secreted by cultured mouse presynaptic neurons, perhaps through spontaneous fusion, was found to induce the clustering of glutamate (AMPA) receptors (*12*). Interestingly, proteins similar to Narp exist in *Drosophila* and may evoke similar postsynaptic effects in fly NMJs.

Spontaneous fusion events may operate not only during glutamate receptor clustering in the developing fly NMJ, but also at other synapses, indicating their importance in many different aspects of neuronal communication.



What a mini can do. **(A)** During development of NMJs in the fruit fly, glutamate receptors become localized to broad presumptive fields of nerve contact in the absence of neural activity. **(B)** Spontaneous fusion of a single synaptic vesicle with the presynaptic membrane generates a mini in the postsynaptic membrane and provokes clustering of postsynaptic glutamate receptors. In *synaptobrevin* (*syb*) and *cysteine string protein* (*csp*) fly mutants, there is still glutamate receptor clustering because only vesicle fusion evoked by action potentials and not spontaneous fusion is abolished. **(C)** In the *syntaxin* (*syx*) and *dynamin* (*shi*) fly mutants, which have neither evoked nor spontaneous fusion, glutamate receptors do not cluster but stay dispersed in the NMJ postsynaptic (muscle) membrane. In the *syx* mutant there is an absolute block in neurotransmitter release, whereas in the *shi* mutant there is depletion of synaptic vesicles.

References and Notes

1. P. Fatt, B. Katz, *J. Physiol.* **117**, 109 (1952).
2. C. J. O'Kane, G. Schiavo, S. T. Sweeney, in *Neurotransmitter Release*, H. J. Bellen, Ed., vol. 23 of *Frontiers in Molecular Biology* (Oxford Univ. Press, Oxford, 1999), pp. 208–236.
3. G. Pennetta, M. Wu, H. J. Bellen, in *Neurotransmitter Release*, H. J. Bellen, Ed., vol. 23 of *Frontiers in Molecular Biology* (Oxford Univ. Press, Oxford, 1999), pp. 304–351.
4. M. Saitoe, T. L. Schwarz, J. A. Umbach, C. B. Gundersen, Y. Kidokoro, *Science* **293**, 514 (2001).
5. K. Broadie *et al.*, *Neuron* **15**, 663 (1995).
6. M. Saitoe, S. Tanaka, K. Takata, Y. Kidokoro, *Dev. Biol.* **184**, 48 (1997).
7. K. Broadie, M. Bate, *Nature* **361**, 350 (1993).
8. _____, *Neuron* **11**, 607 (1993).
9. M. Gautam *et al.*, *Cell* **85**, 525 (1996).
10. T. M. DeChiara *et al.*, *Cell* **85**, 501 (1996).
11. M. Verhage *et al.*, *Science* **287**, 864 (2000).
12. R. J. O'Brien *et al.*, *Neuron* **23**, 309 (1999).
13. We thank M. Crair, R. Gereau IV, G. Bhave, O. Kjaerulff, and especially T. Lloyd for comments.

PERSPECTIVES: ELECTRON TRANSFER

Sometimes You Can Go Home Again

Christopher Bardeen

From simple nucleophilic substitution reactions in organic chemistry to photosynthesis, electron transfer is a basic element of chemical reactions in liquids (*1*). The theoretical framework for understanding electron transfer rates in systems near equilibrium was developed by Marcus and verified experimentally by many workers. The advent of ultrafast lasers has provided physical chemists with a tool for studying how these systems evolve under nonequilibrium conditions. Such studies have revealed molecular details of how electrons move in dense media. On page 462 of this issue, Martini *et al.* go one step further, providing evidence that femtosecond pulses may be used not only to observe electron transfer dynamics but to control them as well (*2*).

The system we are concerned with here is an electron embedded in a molecular liquid. An electron is arguably the simplest possible reactant in a condensed phase environment because it lacks the intramolecular vibrational modes of a molecular solute. A dissolved electron can be prepared by a variety of methods in a variety of solvents, but its essential characteristics remain the same.

At equilibrium, the electron is nestled in a solvent cavity, kept in place by the solvent dipoles. Absorption of a photon excites the electron into a delocalized state, whose wave function may then relocalize to different sites in the solvent that are spatially separated from the original low-energy site. As time goes on, many electrons will lose energy and find their way back to their original cavities, a process known as recombination. But some will escape into the solvent, never to return.

Both types of events are examples of electron transfer from one solvent site to another, and both can be followed by observing the transient absorption spectra of the electron because its wave function, which depends on its spatial extent and environment, also determines its spectral behavior. By using femtosecond pulses, one can ob-

The author is in the Department of Chemistry, University of Illinois, Urbana, IL 61801, USA. E-mail: bardeen@scs.uiuc.edu