artificial) contains no innate knowledge at all, we have to make crucial assumptions about the structure of the learning device, its rate and style of learning, and the kinds of input that it "prefers" to receive. The emergence of language in the hominid line must have involved a certain amount of tinkering with the primate brain, leading ultimately to a brain that was capable of learning language.

References

1. J. R. Saffran, R. N. Aslin, E. L. Newport, *Science* **274**, 1926 (1996).
2. L. Fenson *et al.*, *Monogr. Soc. Res. Child Dev.* **59** (1994).
3. N. Chomsky, *Rules and Representations* (Columbia Univ. Press, New York, 1980).
4. S. Crain, *Behav. Brain Sci.* **14**, 597 (1991).
5. E. Gold, *Inf. Control* **16**, 447 (1967).
6. D. Lightfoot, *The Language Lottery: Towards a Biology of Grammars* (MIT Press, Cambridge, MA, 1980); F. Newmeyer, *Linguistic Theory in America* (Academic Press, New York, 1980).
7. M. Piatelli-Palmarini, *Cognition* **31**, 1 (1989).
8. J. Elman *et al.*, *Rethinking Innateness: A Connectionist Perspective on Development* (MIT Press and Bradford Book, Cambridge, MA, 1996).
9. J. Elman and D. Ziupser, *J. Acoust. Soc. Am.* **83**, 1615 (1988).
10. J. Elman, *Cognition* **48**, 71 (1993).
11. K. Lund and C. Burgess, *Behav. Res. Methods Instrum. Comput.* **28**, 203 (1996).
12. P. Jusczyk, A. Friederici, J. Wessels, V. Svenkerud, A. Jusczyk, *J. Mem. Lang.* **32**, 402 (1993); J. Mehler, G. Lambertz, P. Jusczyk, C. Amiel-Tison, *C. R. Acad. Sci. Paris* **303**, 637 (1986).

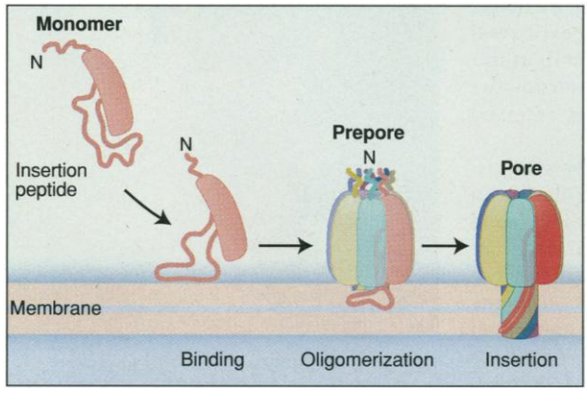
BIOCHEMISTRY

Crossing the Hydrophobic Barrier: Insertion of Membrane Proteins

Donald M. Engelman

Lipid bilayers are thin, flexible self-sealing boundaries that are used by cells to create regions of different composition and electrochemical potential. To accomplish transmembrane functions, proteins inserted within and across the hydrophobic barrier must cope with hydrophilic interactions with the solutions inside and outside a cell or compartment and hydrophobic interactions with the membrane. Usually, proteins are assisted in their insertion by proteinaceous machinery. But can they insert spontaneously? The structure of α hemolysin, reported by Song *et al.* in this issue of *Science*, reveals how a protein, in this case a toxin produced by a pathogenic bacterium, can penetrate a lipid bilayer (*1*)—by the spontaneous formation of an oligomeric β barrel (see figure).

It is much easier to understand the stability of observed transmembrane structures than to fathom the process by which they are positioned within a membrane. The membrane proteins whose high-resolution structures have been solved contain either bundles of α helices or β barrels in the regions presumed to span the lipid bilayer (*2–5*). One can rationalize each of these structures by recognizing that the main-chain hydrogen bonds need to be satisfied in an environment that lacks hydrogen bond donors or acceptors, and that the hydrophobic effect will stabilize the association of a transmembrane structure with the hydrophobic region of a lipid bilayer if the amino acid side chains contacting this region are predominantly apolar (*6*). Detailed knowledge of the final, folded state of a protein, however, does not inform us directly about the process of insertion, particularly if the protein in question is inserted into the membrane in a process catalyzed by cellular machinery. On the other hand, proteins whose functions require them to be stable in an aqueous environment and also capable of inserting themselves into membranes provide an opportunity to examine, biochemically and structurally, the determinants of an insertion event.



Binding, oligomerization, and transmembrane insertion of α hemolysin. The hemolysin is soluble as a monomer, binds through interactions of loops to the surface of a lipid bilayer, oligomerizes to form a heptamer stabilized by amino-terminal "latches," and inserts two strands of a β barrel from each subunit to form a 14-stranded β barrel across the bilayer. The structure that is known definitively is the final pore structure. There is less direct evidence to support the details of other steps. The molecular features of the bilayer at different stages are as yet completely undefined.

Spontaneous transmembrane insertions of both α helices and β barrels are found in the world of toxins, where the capacity to insert is packaged in a soluble molecule. Colicin A has a membrane-insertion domain that sequesters a hydrophobic helical hairpin whose insertion into the bilayer is postulated to be the primary step in colicin action (*7*). The α-hemolysin toxin studied by Song *et al.* is now shown to act by inserting β-barrel structures into bilayers, as previously surmised for aerolysin (*1*, *8*). Other cases of spontaneous insertion have been documented, including the insertion of porins from denatured states in solution into lipid bilayers (*9*). Despite the fact that proteinaceous machinery is used for the insertion of many membrane proteins, a number of cases exist in which the insertion event does not require the participation of structures other than the inserting polypeptide and the lipid bilayer. How might this occur? Which intermediate states might one imagine?

The answer to the issue of intermediates will undoubtedly vary for specific cases, but three themes emerge in the examples we have thus far. The first is the role of oligomerization in the process. Both the α hemolysin and proaerolysin change oligomeric state in the process of insertion. α Hemolysin binds to the membrane as a monomer, subsequently forms a heptamer, and then inserts. This sequence is based on studies of mutants that block steps in the process (*10*, *11*). Thus, one wonders whether the energy of oligomerization may drive the process, producing an intermediate state that relaxes to the transmembrane form. The structure shows that a large surface area is buried in the oligomerization event; hence, a large amount of energy could be available. This is undoubtedly one of the directions that will be explored by Song *et al.*

A second theme is the exposure of regions of the protein that are kept sequestered in the soluble form of the molecule. The colicin A structure shows a hydrophobic helical hairpin surrounded by another structure, sequestering it from the aqueous environment (*7*). A body of work indicates that confor-

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mational rearrangements are also part of the insertion pathway of α hemolysin (*1*), and the structure supports this view. Binding of the soluble protein to the membrane may result in the exposure of previously buried hydrophobic regions that could promote interaction with and insertion across the bilayer.

The role of the bilayer in this process is clearly important, but difficult to address experimentally. Occupancy of bilayer surface area by intermediates making hydrophobic contact with the lipid bilayer is likely to produce distortions of the bilayer structure; 10 amino acids in a β strand at the bilayer surface will occupy more space than the surface area of a typical lipid. Because the area per lipid molecule in a bilayer tends to be conserved (*12*), spreading the lipid headgroups at the surface of a lipid bilayer may cause the bilayer to thin locally, distorting the bilayer profile from its equilibrium position. Such effects, multiplied by protein oligomeric association, for example, could destabilize the bilayer and promote a relaxation to a state in which both the transmembrane form of a protein and of the bilayer have lower energy.

Understanding the energetics and possible intermediates of these spontaneous insertion processes can lead to important developments in our concepts of how membrane proteins may have arisen during evolution, how some membrane proteins are placed in position biosynthetically, and how membrane protein folding may proceed. Practical results may also ensue: study of transmembrane phenomena may lead to better drug delivery.

References and Notes

1. L. Song *et al.*, *Science* **274**, 1859 (1996).
2. R. Henderson and N. Unwin, *Nature* **257**, 28 (1975).
3. J. Deisenhofer, O. Epp, K. Miki, R. Huber, H. Michel, *ibid.* **318**, 618 (1985).
4. M. S. Weiss *et al.*, *Science* **254**, 1627 (1991).
5. S. W. Cowan *et al.*, *Nature* **358**, 727 (1992).
6. D. M. Engelman and T. A. Steitz, *Cell* **23**, 411 (1981).
7. M. W. Parker, F. Pattus, A. D. Tucker, D. Tsernoglou, *Nature* **337**, 93 (1989).
8. M. W. Parker *et al.*, *ibid.* **367**, 292 (1994).
9. T. Surrey and F. Jähnig, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7457 (1992).
10. S. Bhakdi *et al.*, *Arch. Microbiol.* **165**, 73 (1996).
11. B. Walker, O. Braha, S. Cheley, H. Bayley, *Chem. Biol.* **2**, 99 (1995).
12. B. A. Lewis and D. M. Engelman, *J. Mol. Biol.* **166**, 211 (1983).

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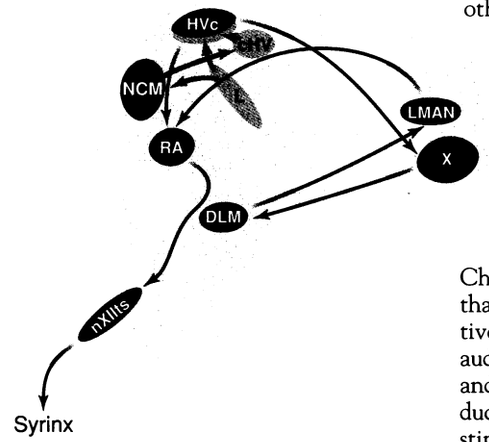
PSYCHOLOGY

Plasticity of a Different Feather?

Allison J. Doupe

Neuroscientists are keenly interested in understanding the neural mechanisms underlying learning and memory. On page 1909 of this issue, Chew *et al.* (*1*) describe an intriguing form of experience-dependent neural plasticity in songbirds, with some astonishing new twists: when a bird repeatedly hears a song, the response of certain neurons to this song progressively decreases and then recovers only at one of six fixed, "quantal" times. The duration of the decrease in neural response depends on the nature of the initial auditory experience and on the occurrence of episodes of protein synthesis at approximately the same fixed intervals. This phenomenon is quite surprising, which makes it all the more important to find out whether it exists in other brain systems and in other animal species. Even more critical is whether the decreased neural response and its quantized recovery underlie behavioral learning and forgetting. Songbirds are a particularly useful system for tackling this behavioral question, because they learn and remember for many months the songs of their own species (conspecific songs), both during juvenile life in order to sing them and as adults to identify other individual birds.

The neuronal plasticity that Chew and colleagues observe occurs in the caudomedial neostriatum (NCM), a high-level auditory area in the brain of songbirds. NCM is not part of the "song system" known to be required for song learning and production (see figure), but is one indirect source of the song system's auditory inputs. Earlier work on gene expression from the Nottebohm laboratory showed that the immediate-early gene *ZENK* was induced in this area in both male and female songbirds when they were presented with songs (*2*). The auditory induction of *ZENK* expression in NCM was especially striking because, within the song system itself, the gene was resolutely resistant to activation by song playback, despite the presence of well-described song-responsive auditory neurons in those areas. Moreover, *ZENK* was much more strongly induced by songs of conspecific birds than by songs of other species (heterospecifics). Thus, induction of *ZENK* correlated with the biological relevance of the auditory stimuli. In addition, repeated presentation of a song would result in this song being ineffective at inducing *ZENK* for at least 24 hours, although a novel song could still trigger a full *ZENK* induction (*3*).



The songbird brain. In this schematic cross section, the traditionally defined song system is shown in black; these discrete brain areas form a network of neurons required for song learning and production. NCM is one of a number of high-level auditory areas (shown in red) that indirectly project to the song system.

Using neurophysiological recordings, Chew and colleagues then demonstrated that NCM neurons have strong and relatively unselective responses to a variety of auditory stimuli, including both conspecific and heterospecific songs. As with *ZENK* induction, repeated presentations of the same stimulus led to a gradual and long-lasting decrease of multi-unit and single-unit neuronal responses; similar results have been reported by Stripling and co-workers (*4*). Chew *et al.* termed this decrease in neural responsiveness "habituation," a term borrowed from behavioral studies of response decrements (*5*), and showed that habituation was specific for each song presented, even for very similar stimuli such as forward and reversed versions of the same song.

The neuronal habituation in NCM may be most analogous to the loss of neural responsiveness to repeated visual stimuli described in primate inferotemporal (IT) cortex (*6*), and, like that adaptation, raises interesting questions about the possible mechanisms. For instance, does the habituation of the multi-unit response to multiple

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