forces exerted on the skull during predation and feeding (*8*). The skulls of *Suchomimus*, *Baryonyx*, and their kin are long and narrow, and their teeth are subcircular in basal cross section, with either very fine serrations or none at all. The anterior end of a spinosaur snout is expanded into a pincerlike "terminal rosette," containing the largest teeth in the skull. As demonstrated by *Suchomimus* and other new discoveries, spinosaur skulls had a substantial secondary palate (formed by medial extensions of the maxillae).

The cranial adaptations in spinosaurs parallel those of crocodilians. Early crocodylomorphs had skulls similar to those of typical theropods and bladelike teeth with serrations running along the front and back margin (*9*). With the assumption of an aquatic habit, the snout of crocodilians became elongate, the bones of the palate joined to form a solid roof of the mouth with rearward-placed internal nostrils, and the teeth became conical (*10*). These changes have been considered adaptations toward a piscivorous (fish-eating) diet from one based primarily on the meat of terrestrial animals. A narrow snout may allow more rapid passage through the water, and teeth with a rounded cross section function better as piercers and graspers rather than as slicers and slashers (that is, as meat hooks rather than steak knives). The solid secondary palate of crocodilians allows them to absorb the torsional loads generated by struggling fish (and, in some species, by their habit of rolling in the water to disarticulate limbs from their prey) (*8*).

Might spinosaurs have been specialized fish eaters? This hypothesis has been suggested before by Charig and Milner (*5*) for *Baryonyx* on the basis of both the anatomical similarity with crocodilians and the presence of digestive acid-etched fish scales within the rib cage of the type specimen. Large fish are known from the faunas containing other spinosaurids, including the 3.5-m coelacanth *Mawsonia* in the mid-Cretaceous of northern Africa and Brazil (*11*), and the highly diverse fish community co-occurring with *Irritator* and *Angaturama* (*12*). Charig and Milner (*5*) indicate the presence of the acid-etched remains of a young specimen of the plant-eating ornithopod dinosaur *Iguanodon* in the body cavity of *Baryonyx*, so it almost certainly fed on the meat of terrestrial animals as well as fish.

The anatomical differences in the feeding apparatus of spinosaurs, regardless of the proportion of fish in their diet, may go some way in explaining the high diversity of extremely large theropods in North Africa in the mid-Cretaceous. *Spinosaurus* co-occurs with at least two other multiton theropods: the allosaur *Carcharodontosaurus* and *Deltadromeus*, a coelurosaur more closely related to advanced forms such as dromaeosaurid "raptors" and tyrannosaurs than to either allosaurs or spinosaurs (*13*), with possible evidence for an abelisaurid ceratosaur in the same region (*4*). Perhaps these different enormous carnivores were capable of coexisting by exploiting different parts of the potential food supply and in particular because spinosaurs had more immediate access to the freshwater part of the food web than theropods of more typical morphology.

Clearly the crocodile analogy does not extend beyond the skull of spinosaurs. The postcranial skeleton of *Suchomimus*, *Baryonyx*, and related forms lacks any particular specializations for aquatic life. Nevertheless, the remarkable skulls of spinosaurid dinosaurs represent an intriguing case of convergence between distantly related reptilian forms. Additionally, the skeletal specializations of *Suchomimus* helps to reinforce the idea that there is much more to theropod history than the beginnings of avian flight.

References

1. For instance, Q. Ji *et al.*, *Nature* **393**, 753 (1998).
2. P. C. Sereno *et al.*, *Science* **282**, 1298 (1998).
3. E. Stromer, *Abh. Kgl. Bayer. Akad. Wiss. Math. Phys. Kl.* **28**, 1 (1915); *Abh. Bayer. Akad. Wiss. Math. Naturwiss. Abt.* **22**, 1 (1934).
4. S. Bouaziz *et al.*, *Bull. Soc. Geol. France* **1988**, 335 (1988); E. Buffetaut, *Neues Jahrb. Geol. Palaeontol. Monatsh.* **1989**, 79 (1989); D. A. Russell, *Bull. Mus. Natl. Hist. Nat. Paris Ser. 4* **18**, 349 (1996); P. Taquet and D. A. Russell, *C. R. Acad. Sci. Paris* **327**, 347 (1998). Russell and Taquet and Russell refer their material to the species *Spinosaurus maroccanus* and *Cristatusaurus lapparenti*; Sereno *et al.* (*2*) provisionally consider the former of these species synonymous with *Spinosaurus aegyptiacus* and the latter as a dubious taxon indistinguishable at present from *Baryonyx*.
5. A. J. Charig and A. C. Milner, *Nature* **324**, 359 (1986); in *Dinosaur Systematics: Perspectives and Approaches*, K. Carpenter and P. J. Currie, Eds. (Cambridge Univ. Press, Cambridge, 1990), pp. 127–140; *Bull. Nat. Hist. Mus.* **53**, 11 (1997).
6. L. I. Viera and J. A. Torres, *Munibe Cienc. Nat.* **47**, 57 (1995).
7. D. M. Martill *et al.*, *J. Geol. Soc. London* **153**, 5 (1996); A. W. A. Kellner and D. A. Campos, *Neues Jahrb. Geol. Palaeontol. Abh.* **199**, 151 (1996); A. W. A. Kellner, *Mem. Queensland Mus.* **39**, 611 (1996).
8. A. B. Busbey, in *Functional Morphology in Vertebrate Paleontology*, J. J. Thomason, Ed. (Cambridge Univ. Press, Cambridge, 1995), pp. 173–192.
9. For example, A. D. Walker, *Philos. Trans. R. Soc. London B* **330**, 1 (1990).
10. A. P. Russell and X.-C. Wu, *Zoology* **100**, 164 (1998)
11. S. Wenz, *Mem. Soc. Geol. France* **139**, 187 (1980); *Ann. Paleontol. Vertebr.* **67**, 128 (1981); J. G. Maisey, *Am. Mus. Novit.* **2866**, 1 (1986).
12. J. G. Maisey, Ed., *Santana Fossils* (T. F. H. Publications, Neptune City, NJ, 1991).
13. P. Sereno *et al.*, *Science* **272**, 986 (1996).

PERSPECTIVES: NEUROSCIENCE

Gathering Glycine Receptors at Synapses

Stanley C. Froehner

Synapse formation in the central nervous system is exceptionally complex. A single neuron may receive input from thousands of synaptic connections on its cell body and dendrites—some inhibitory, some excitatory. To integrate these signals rapidly and specifically, the neuron anchors high concentrations of receptors at postsynaptic sites, matching the correct receptor with the neurotransmitter released from the presynaptic terminal. Receptor-associated proteins are thought to be involved in forming these postsynaptic specializations, possibly by linking the receptor to the postsynaptic cytoskeleton (*1*). This idea has been most thoroughly studied at the neuromuscular junction, where nicotinic receptors are associated with the clustering protein rapsyn (*2, 3*). Now, the laboratories of J. R. Sanes and H. Betz have tested this theory on neurons in the central nervous system. In a report on page 1321 of this issue, they show by targeted gene disruption that gephyrin, a protein associated with the glycine receptors, is required for the formation of inhibitory synapses in the spinal cord and brain (*4*). Their results also reveal an intriguing link between gephyrin and neurological diseases related to molybdenum deficiency.

Glycine receptors are members of the pentameric family of ligand-gated ion channels, which also includes nicotinic acetylcholine receptors, γ-aminobutyric acid (GABA) receptors (another type of inhibitory receptor), and, more distantly, NMDA- and AMPA-type glutamate receptors. In earlier experiments, Betz and colleagues found that a 93-kilodalton peripheral membrane protein, now called gephyrin, was localized at inhibitory synapses on motor neurons in a complex with the glycine receptor (*5*). A clue to gephyrin's function came from experiments in which depletion of gephyrin with antisense oligonucleotides

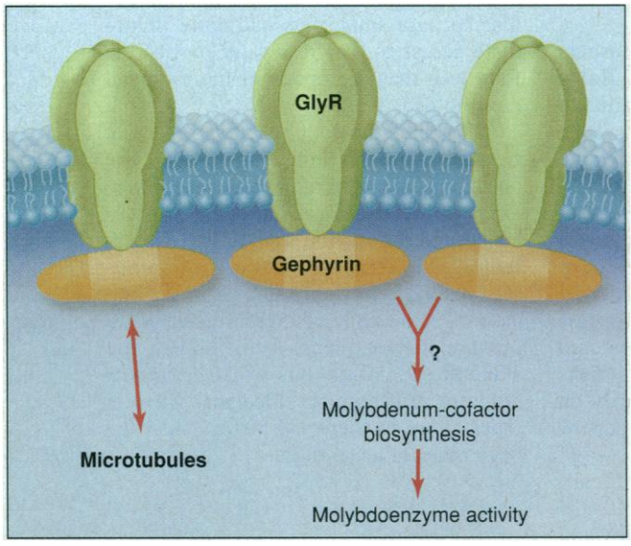
The author is in the Department of Cell and Molecular Physiology, University of North Carolina, Chapel Hill, NC 27599–7545, USA. E-mail: froehner@med.unc.edu

blocked the accumulation of glycine receptors at synapses on cultured neurons (*6*). Recently, a similar approach has been used to show that $GABA_A$ receptor clustering at synapses on cultured hippocampal neurons requires gephyrin (*7*). These findings set the stage for a genetic test of gephyrin's role in inhibitory synapse formation, as reported by Feng *et al.* (*4*).

Mutant mice lacking gephyrin are born without apparent developmental abnormalities but die within 1 day. Neonatal animals respond in an exaggerated way to a light touch on the skin, becoming rigid and hyperextended and having difficulty breathing. These impairments would be predicted to occur if inhibitory inputs to the motor neurons were compromised. This prediction is borne out at the molecular level. In the spinal cord, brainstem, and hypothalamus of these mice, where inhibitory synapses are prominent, neither gephyrin nor glycine receptor clusters were found in association with presynaptic terminals, even though the abundance of receptors was normal. The effect seems to be specific to glycinergic synapses, as synaptic clustering of glutamate receptors was unaffected. Thus, gephyrin is required for the synaptic clustering of glycine receptors.

Mice deficient in glycine receptor expression have similar abnormalities, but the consequences are not as severe. This raised the possibility that disruption of glycinergic transmission may not be the whole story in the gephyrin mutant. Gephyrin is related in primary structure to the products of a group of genes from bacteria, *Drosophila*, and plants that are involved in the biosynthesis of molybdopterin, a cofactor required for the activity of molybdenum-dependent enzymes (*8*). The activities of two such enzymes, sulfite oxidase and xanthine dehydrogenase, were virtually absent in the gephyrin mutants. Thus, in addition to its critical role in synaptic organization, gephyrin may also mediate metabolic aspects of neuronal function (see the figure). Molybdenum deficiency or mutations in certain molybdoenzymes cause neurological problems in humans that in many ways mimic the phenotype of the gephyrin-negative mouse, or can be accounted for by impairments at inhibitory synapses (*9*). Abnormalities in gephyrin expression or function and in molybdoenzymes could potentially be the underlying basis for maladies such as stiff baby syndrome or even neonatal seizures.

Are the phenotypic abnormalities in the gephyrin mutant mice due to compromised inhibitory synaptic transmission or to molybdenum cofactor deficiencies? A pharmacological test done by Feng *et al.* supports the former. Strychnine blocks glycine receptor function, presumably without altering molybdenum-requiring processes. The behavioral characteristics of neonatal mice injected with strychnine were very similar to those of the mice without gephyrin, consistent with the idea that the phenotype is primarily attributable to the failure of glycinergic synaptic activity. Genetic rescue experiments with constructs of gephyrin that lack the glycine receptor binding site but retain regions necessary for molybdenum cofactor biosynthesis may be informative in differentiating more precisely between synaptic and metabolic defects in these mutant mice.



Power in numbers. Glycine receptors are anchored at synapses by association with gephyrin. Gephyrin may link glycine receptors to the microtubule-based cytoskeleton. At the same time, gephyrin may also be required for the synthesis of a cofactor that regulates molybdenum-dependent enzymes.

In addition to gephyrin and rapsyn, two other receptor-associated proteins have received much attention: the PSD-95 protein family, associated with NMDA receptors (*10, 11*), and GRIP, which binds the carboxyl-terminal tail of AMPA receptors (*12*). Rapsyn, PSD-95, and GRIP are scaffolding proteins. All three have multiple sites for interactions with other proteins (eight tetratricopeptide repeats and a zinc finger in rapsyn; three PDZ domains, an SH3 domain, and an inactive guanylate kinase domain in PSD-95; and seven PDZ domains in GRIP) (*13, 14*). An emerging theme is that each of these proteins anchors receptors at synaptic sites, while simultaneously serving as a platform on which diverse but functionally interdependent components of the postsynaptic machinery can be assembled. These components include enzymes such as nitric oxide synthase, regulatory proteins involved in Ras signaling, cytoskeletal proteins, receptor tyrosine kinases, transmembrane proteins that link to the extracellular matrix, and potentially even other ion channels (*14*).

The idea that these receptor-associated proteins are central organizers of synaptic function is clearly gaining support. Gephyrin appears to fall into a slightly different category, however. Although gephyrin shares with other receptor-associated proteins the ability to bind to the cytoskeleton via its microtubule binding property (*5*), it has no obvious protein-protein interaction domains. Gephyrin may instead be a multifunctional enzyme, at least judging by its sequence homology with genes known to mediate molybdenum cofactor synthesis. Clearly, a biochemical test is needed. What role this cofactor plays at synapses remains a mystery, nor is it known how activity at inhibitory synapses modulates the production of this cofactor and the enzymes that it regulates.

Genetic tests of the importance of PSD-95 and GRIP in glutamate receptor clustering in vivo can be expected soon. Thus, clustering events proximal to the postsynaptic receptor are being defined at a rapid pace. But what about the control of receptor clustering by the innervating neurons? At the neuromuscular junction, the motor neuron controls clustering by secreting agrin, a protein that eventually becomes incorporated into the synaptic basal lamina (*15*). The agrin signaling pathway is not fully defined, but activation of a muscle-specific receptor tyrosine kinase (MuSK) (*16*), an increase in intracellular calcium (*17*), perhaps phosphorylation of the acetylcholine receptor (*18, 19*), and rapsyn (*3*) are all involved. Because no defects in central nervous system synapse formation have been found in mice lacking agrin, MuSK, or rapsyn, the signaling system used by neurons must be different. The one common feature linking neuromuscular and neuronal synaptic receptor clustering is the increase in intracellular calcium concentration. In spinal cord neurons, calcium flux may be regulated by the postsynaptic receptor itself. Blockade of glycine receptors or calcium channels prevents synaptic receptor clustering in cultured neurons (*20*). Because glycine receptors are excitatory in

embryonic neurons, this calcium-dependent process may be active only when synapses are being formed. Thus, while a more complicated signaling pathway is needed to cluster nicotinic receptors at neuromuscular junctions, local activity may be sufficient to induce the formation of glycine receptor clusters in neurons. Although this might explain how clustering of a single type of receptor can be controlled, the sorting of different receptor types to specific postsynaptic sites must require even more complex regulation.

References

1. M. Colledge and S. C. Froehner, *Curr. Opin. Neurobiol.* **8**, 357 (1998).
2. S. C. Froehner, C. W. Luetje, P. B. Scotland, J. Patrick, *Neuron* **5**, 403 (1990).
3. M. Gautum *et al.*, *Nature* **377**, 232 (1995).
4. G. Feng *et al.*, *Science* **282**, 1321 (1998).
5. J. Kirsch *et al.*, *J. Biol. Chem.* **266**, 22242 (1991).
6. J. Kirsch, I. Wolters, A. Triller, H. Betz, *Nature* **366**, 745 (1993).
7. C. Essrich *et al.*, *Nature Neurosci.* **1**, 563 (1998).
8. P. Prior *et al.*, *Neuron* **8**, 1161 (1992).
9. J. L. Johnson and S. K. Wadman, Eds., *The Metabolic Basis of Inherited Diseases* (McGraw-Hill, New York, 1995), pp. 2271–2283.
10. H.-C. Kornau, L. T. Schenker, M. B. Kennedy, P. H. Seeburg, *Science* **269**, 1737 (1995).
11. E. Kim, K. Cho, A. Rothschild, M. Sheng, *Neuron* **17**, 103 (1996).
12. H. Dong *et al.*, *Nature* **386**, 279 (1997).
13. M. K. Ramarao, J. B. Cohen, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 4007 (1998).
14. R. J. O'Brien, L. F. Lau, R. L. Huganir, *Curr. Opin. Neurobiol.* **8**, 364 (1998).
15. U. J. McMahan *et al.*, *Curr. Opin. Cell Biol.* **4**, 869 (1992).
16. D. J. Glass *et al.*, *Cell* **85**, 513 (1996).
17. L. J. Megeath and J. R. Fallon, *J. Neurosci.* **18**, 672 (1998).
18. M. Ferns, M. Deiner, Z. Hall, *J. Cell Biol.* **132**, 937 (1996).
19. B. G. Wallace, Q. Zhican, R. L. Huganir, *Neuron* **6**, 869 (1991).
20. J. Kirsch and H. Betz, *Nature* **392**, 717 (1998).

A Cellular Striptease Act

Zena Werb and Yibing Yan

The cell surface is a dynamic place. During its life history the cell alters the repertoire of proteins displayed on its surface many times. Membrane-anchored adhesion molecules, receptors, ligands, and enzymes are removed and replaced as the cell proceeds through development and as its activation state changes. How is this wholesale refurnishing of the cell membrane orchestrated? One key mechanism is proteolytic processing of the ectodomain (extracellular domain) of such membrane proteins. Cleavage or shedding of the ectodomains of plasma membrane proteins—widely observed in cells in culture—is blocked by inhibitors of metalloproteinases (*1, 2*). This result suggests that transmembrane and soluble metalloproteinases, such as matrix metalloproteinases (MMPs) and their relatives, are rate-limiting for cleavage and shedding. Other evidence also implicates serine proteinases in these processing events (*3, 4*).

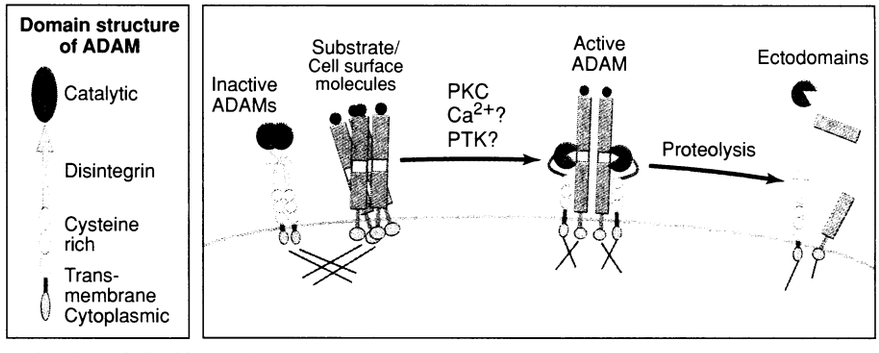
Enhanced online at www.sciencemag.org/cgi/content/full/282/5392/1279

The first such "sheddase" characterized was the tumor necrosis factor–α (TNF-α) converting enzyme (TACE) (*5*). The study by Peschon and colleagues (*6*) on page 1281of this issue now points to TACE's essential role in the shedding of ectodomains during mouse development. The surprise comes from the observation that mice lacking TACE do not show a phenotype indicative of a lack of TNF-α availability. Rather, they show the same phenotype as mice engineered to be without the epidermal growth factor (EGF) receptor—because TACE-mediated proteolysis makes available ligands for the EGF receptor, particularly transforming growth factor–α (TGF-α).

TACE turns out to be a membrane-anchored proteinase that is a member of the ADAM (a disintegrin and metalloproteinase) domain family of proteins that combines features of both cell surface adhesion molecules and proteinases (*8*). ADAMs all have a common domain organization, which endows these proteins with several potential functions—proteolysis, adhesion, signaling, and fusion (see figure below). The proteolytically competent ADAMs, such as TACE (ADAM17), are zinc-dependent metalloproteinases, closely related to the MMP family with which they share small molecule inhibitors and even one tissue inhibitor, TIMP-3 (*9, 10*). Several newly discovered MMPs appear to be hybrids of both MMP and ADAM domains (*11*), indicating that these two types of enzymes are part of one, larger family.

The ADAM proteinases are themselves targets of proteolytic events that ultimately strip off the catalytic domains (*5, 8*). This action could be a mechanism of functionally blunting the effects of the proteinases (see the figure on the next page). These soluble ADAMs may have proteolytic activity, as is the case for snake venom enzymes (*8*), but soluble TACE is much less active than membrane-bound enzyme (*5, 6*). The residual adhesive domains of ADAMs left after cleavage may have regulatory or adhesive functions. In support of this idea, a catalytic domain-deleted mutant of *Kuz* (ADAM10/SUP17), first identified as being required for cleavage of Notch during neural development in *Drosophila*, exerts a dominant negative effect (*8, 12*). During sperm maturation fertilin, a heterodimeric ADAM essential for sperm-egg interaction (*13*), also loses its catalytic domains by proteolytic processing. The remaining adhesive disintegrin domain is then competent to bind integrins.

How does TACE act? TACE is widely expressed in the animal. Mutation of the catalytic domain of TACE (*6*) reveals several distinct functions for this ADAM in development. Ligands for the EGF receptor, which is essential for epithelial development (*7*), are usually made and used locally (*14*). Although the growth factor precursors may have some biological activity (*15*), the new results imply that the membrane-anchored forms are essentially inactive precursors (*6*). TACE also cleaves ectodomains of other receptors and ligands, such as TNF-α, the p75 TNF receptor, and L-se-



Activation of sheddases. The ADAM proteases (as dimers) and substrates are anchored apart in the plane of the membrane. Upon activation (via protein kinases and other pathways) they are brought together and proteolysis takes place, leading to free ectodomains.

The authors are at the Department of Anatomy, University of California, San Francisco, CA 94143-0452, USA. E mail: zena@itsa.ucsf.edu; yan@cgl.ucsf.edu