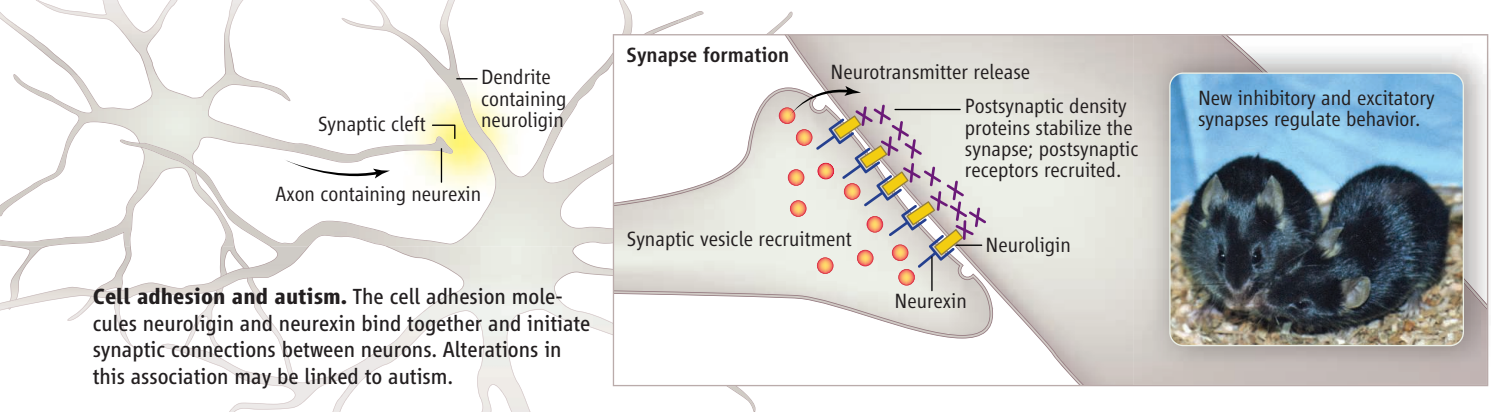
MEDICINE

Testing Hypotheses About Autism

A challenging, but potentially fruitful approach to evaluate proposed candidate genes for autism is to study analogous behaviors in mouse models.

Jacqueline N. Crawley

The Beatles' insight, that something in the way you move attracts me like no other, aptly describes how neurons connect with each other. Specific forms of cell adhesion molecules expressed in neuronal processes—a neurexin in axons and a neuroligin in dendrites—attract and bind in mid-synaptic space with exquisite selectivity (*1*, *2*). The resulting synaptic connections regulate excitatory and inhibitory transmission of information in neural circuits (see the figure). On page 71 of this issue, Tabuchi *et al.* (*3*) report that mice with a mutation in neuroligin-3 display increased activity of inhibitory synapses in the brain. This specific mutation was identified previously in the genomes of two brothers with autism (*4*), raising interest in the role of neurexin-neuroligin complexes in autism spectrum disorders.



Cell adhesion and autism. The cell adhesion molecules neuroligin and neurexin bind together and initiate synaptic connections between neurons. Alterations in this association may be linked to autism.

The search is on for genes that underlie autism, a neurodevelopmental disorder of unknown cause that is diagnosed by abnormal social interactions, impaired communication, and repetitive behavior (*5*). Concordance for autism spectrum disorders reaches >90% for identical twins, compared to <10% for fraternal twins and siblings (*6, 7*), indicating a strong genetic component. Like the mutation studied by Tabuchi *et al.*—R451C, in which arginine at position 451 in neuroligin-3 is substituted with cysteine—many genetic polymorphisms have been associated with autism, but each was found in only a few individuals, and seldom replicated across studies (*6, 7*). No ubiquitous single-gene mutation linked to the disorder has yet been detected. Because multiple genes, each accounting for a small percentage of the variance, typify many neuropsychiatric disorders (*8*), focus is now shifting to alternate genetic strategies to reveal the genetic basis for autism (*7*). The true significance of so many unreplicated candidate genes for autism remains a fascinating conundrum. A logical working hypothesis is that many factors can disrupt developing brain pathways necessary for sociability, interactive communication, and flexible executive function (*9*).

Targeted mutations in mice offer a rational strategy to systematically test hypotheses about each candidate gene for autism. If the mutation results in a phenotype analogous to the symptoms of autism, then that gene may play a critical role. Because no biochemical or neuroanatomical markers for autism are known, the mouse phenotype is defined by behavioral criteria relevant to the three diagnostic symptoms of autism.

Why is it hard to develop good mouse models of autism, and why are model animals essential? The challenge is to design mouse behavioral tasks with sufficient analogies to the three diagnostic symptoms. Fortunately, mice are a social species, with high levels of social interaction. Behavioral neuroscientists are generating useful assays for autism-like social and communication deficits, and for motor stereotypies, repetitive behaviors, and perseverative habits (*10–19*). A caveat is the apparent circular logic in modeling symptoms (face validity) without knowing causes (construct validity). In fact, perfecting animal models as mechanistic hypotheses emerge, while investigating proposed genetic, biochemical, and environmental factors in model organisms, is a common iterative process at early stages of understanding a human disease. Altered neuronal connectivity and larger brain volume at early ages in autism (*20*) suggest exploring genes that control programmed cell death and/or dendritic pruning in rodent models. Poor attentional disengagement at very young ages, detected in the Baby Siblings project (*21*), may implicate inhibitory neurophysiological mechanisms similar to those described by the Sudhof team (*3*). Ultimately, robust mouse models provide translational tools for developing effective treatments.

Neuroligin-3 R451C mice displayed deficits in some, but not all, of the social tasks assessed by the authors, and not on the conventional parameters. These mice also showed faster acquisition and reversal in some components of a spatial learning and memory task, a finding counterintuitive to the resistance to change in routine that is a hallmark of autism. The authors are in good company. Mice with mutations in various candidate genes for autism, and proposed inbred strain models, display behavioral abnormalities relevant to only one or two diagnostic symptoms of autism, and some show confounding physical dysfunctions (*11–17*). Currently, only one strain, BTBR, appears to model all three symptoms without confounding abnormalities (*18, 19*).

Faster learning of the Morris water maze by R451C mice is remarkable. The R451C mutation increased the frequency of inhibitory synaptic events in mouse brain structures without changing the total number of inhibitory synapses. It is intriguing to speculate how more inhibitory neurotransmission during early development could improve cognitive performance. As the authors state, cases

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of savant abilities are associated with autism, but exceedingly rarely, and not in the two brothers with the R451C polymorphism (*4*). Cognitive researchers may want to explore the appealing notion that alterations in neurexin-neuroligin complexes shift the balance of excitatory and inhibitory synapses to enhance learning and memory.

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BIOPHYSICS

Going with the Flow

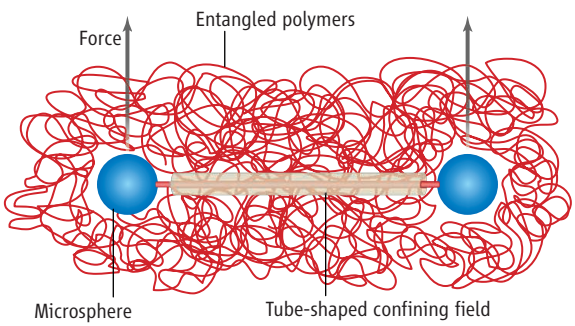
A growing synergy between rheology and biophysics is yielding insights into the flow properties of polymers and biomolecules.

Ronald G. Larson

The field of rheology—the study of the deformation and flow of polymers, colloids, or emulsions—long had to content itself with macroscopic experiments, because the microstructures that produce the rheological response were beyond the reach of experimental tools. But now, new tools borrowed from biophysics allow these microscopic substructures to be probed directly, as illustrated by a recent use of optical tweezers to study the dynamics of entangled polymers (*1*). Another recent study exemplifies borrowing in the reverse direction: the use of a microrheological tool to study the actin filament network of a cell (*2*). These examples are just a small sampling of a growing synergy between rheology and biophysics that is yielding deeper understanding of the dynamics of biopolymers such as DNA and actin filaments and of conventional synthetic polymers such as polyethylene.

Since the mid-1990s, long double-stranded DNA molecules have been widely used to study the flow properties of polymers (*3*, *4*). Stained by intercalating dyes, double-stranded DNA molecules were visualized as they deformed, tumbled, stretched, and relaxed as a result of flow, yielding a thorough basis for understanding the flow behavior of dilute polymers (*5*–*8*).

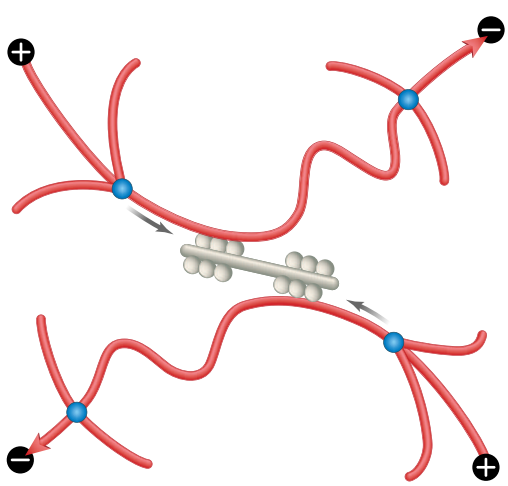
However, dilute polymer solutions, in which the polymer molecules do not overlap, are rare in practical applications. Far more important are the higher-concentration regimes in which polymer molecules overlap and entangle with each other, as occurs, for example, when molten polyethylene is blown into plastic film or when silk solutions are spun by spiders into a thread.



An insider view of entanglement. In (*9*), a probe DNA molecule was held stretched by two optically trapped microspheres in a solution of other DNA molecules. The confining tube potential (orange region) was explored by displacing the spheres perpendicular to the probe chain and measuring the resulting forces.

Since the seminal work of de Gennes (*9*) and Doi and Edwards (*10*), theories for the rheology of densely entangled polymers have relied on the tube model. In this model, each polymer molecule is confined by entanglements with its neighboring molecules to a tubelike region that roughly follows the contorted, random-walk contour of the polymer molecule. Polymer motion is preferentially directed along this tube rather than perpendicular to it, with dramatic consequences for polymer dynamics and rheology. The tube model has remained largely phenomenological, with the properties of the confining tube determined only indirectly through observation of polymer motion and rheological properties. However, this may now be changing.

In a recent study, Robertson and Smith (*1*) attached small beads, which served as handles for optical traps, to both ends of a single long probe DNA molecule, which they mixed with a densely entangling solution of other, similar, DNA molecules (see the first figure). By stretching the probe molecule to nearly full extension using the traps and allowing surrounding molecules to relax, they created a straightened version of the tube. When both optical traps were displaced by the same



Motor-driven fluctuations. In (*12*), actin filaments (red) were pulled by ATP-powered myosin motors (gray) in the direction of the arrows. The resulting tensile forces are marked with (+); contractile forces are marked with (–). The cross-links (blue) link the mobile actin chains into a soft-solid network.

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CREDITS: (TOP) ADAPTED FROM (*1*); (BOTTOM) ADAPTED FROM (*2*)

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