PERSPECTIVES: NEUROSCIENCE

Driving the Growth Cone

Pico Caroni

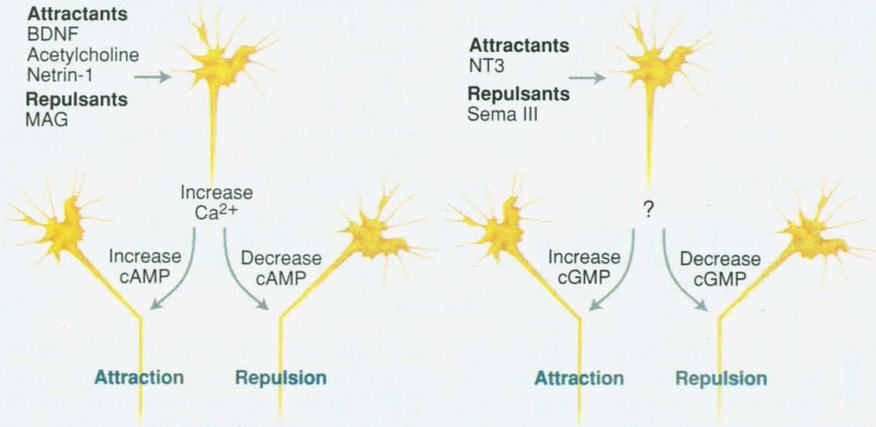
The nervous system is made of billions of neurons that are interconnected with astonishing precision. How is genetic information decoded to wire this most complex of computing machines? By the end of the last century, the founder of modern neuroscience, Ramón y Cajal, had already proposed that the tips of the growing neuronal processes, which he called growth cones, made the navigational decisions required to specifically wire neuronal networks. Cajal also proposed that growth cones sampled their environment, guided in part by diffusible attractive factors.

Since then, much progress has been made in identifying molecules that guide growth cones through specific ligand-receptor interactions. These guidance signals can be attractive or repulsive, diffusible or substrate bound. Specificity of guidance signals is achieved by their local distribution and by the selective expression of corresponding receptors by defined populations of neurons (*1*).

But how do growth cones make sense of the multiple signals that they encounter at any given time? How is information evaluated, integrated, and translated into a specific steering and elongation decision? Because axonal growth cones are small, difficult to isolate, and so diverse, our understanding of how signals are transduced at the growth cone has been slow in coming. In this issue on page 1515, Song *et al.* now report an important milestone for this question. They demonstrate that attractive and repulsive guidance mechanisms are mechanistically related and switched from one to the other by cyclic nucleotide levels within the growth cone (*2*).

Using an in vitro approach pioneered by Gundersen and Barrett (*3*), Poo's group produced gradients of diffusible agents such as membrane-permeable cylic adenosine 3′,5′-monophosphate (cAMP) analogs, the neurotransmitter acetylcholine, or the NGF relatives BDNF and NT3—all of which attract growth cones from embryonic frog spinal cord neurons (*4*). Poo and his co-workers went on to show that attraction by acetylcholine or BDNF, but not NT3, would switch to repulsion when cAMP concentrations in the growth cone were decreased or when cAMP-dependent protein kinase A was inhibited (*5*). Around the same time, Tessier-Lavigne and his colleagues provided the first evidence for a chemotactic system that would actually guide axons in a developing embryo (*6*). They showed that Netrin-1, produced by a group of cells at the base of the developing spinal cord (the floor plate), selectively attracts commissural axons to the floor plate, where they cross the midline and then turn in an anterior direction. Other populations of axons are repelled by Netrin-1 and navigate away from the midline, indicating that Netrin-1 can act as both an attractant or a repellant depending on the type of responding neurons (*7*). The two groups then teamed up to show that, as in the case of BDNF and acetylcholine, spinal cord neuron responses to Netrin-1 could be switched between attraction and repulsion by altering the cAMP system in the growth cone (*8*). For both kinds of turning, ligand-mediated receptor activation and calcium influx were required.

These experiments showed that for molecules that usually cause attraction, growth cone attraction and repulsion can be linked mechanistically. But what about the guidance molecules that predominantly produce repulsion? The new study by Song *et al.* (*2*) demonstrates that repulsion induced by soluble fragments of the myelin-associated protein MAG is mechanistically related to attraction by BDNF, acetylcholine, or Netrin-1. In all cases calcium influx is required and growth cone cAMP induces attraction, whereas cAMP inhibition leads to repulsion. Inhibition by MAG may be one of the factors that inhibits nerve regeneration in the adult central nervous system, but the significance of this mechanism is presently not clear. In contrast, genetic experiments have clearly established that inhibition by collapsin-1/semaphorin III/D (Sema III) is critical during the wiring of the nervous system. In vitro, repulsion by SemaIII was calcium-independent and could not be switched by cAMP. Instead, elevating growth cone cylic guanosine 3′,5′-monophosphate (cGMP) levels, the other key cyclic nucleotide second messenger, switched SemaIII guidance from repulsion to attraction. NT3-mediated attraction, which was not sensitive to calcium or cAMP, was also regulated by cGMP, with high levels again promoting attraction and low levels switching the response to repulsion. Thus, guidance factors that induce attraction or repulsion feed onto common signal transduction machinery in the growth cone, and growth cone cyclic nucleotide levels act as switching or gating mechanisms (see figure) to determine the ultimate growth direction toward or away from the signal (*2*). Generally, elevation of cyclic nucleotide levels promotes attraction, whereas their lowering results in repulsion.



Turning the growth cone's head. Guidance molecules function predominantly as attractants or repulsants. They can be grouped according to shared signal transduction machinery in the growth cone. In each case, elevating cyclic nucleotide second messenger levels (cAMP or cGMP) produces attraction, and inhibiting cyclic nucleotide signaling leads to repulsion.

Do the new findings tell us how growth cones may make sense of the complex array of guidance information that they encounter on their journey to their targets? One important concept that emerges is that the actual steering response will depend on the local levels of general second messengers in the growth cone. This suggests that coincident information can be integrated and filtered by the cell, thus greatly expanding the repertoire of possible steering behaviors in response to a limited number

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of guidance cues. Cyclic nucleotide levels are subject to sophisticated temporal and spatial regulation, introducing ample potential for precision, and crosstalk among signal transduction pathways. Converging signaling mechanisms also simplify customized solutions to the problem of assessing relative signal strength and thus interpreting the same local environment in neuron type– and neuron state–specific manners. In this context it is interesting to note that spinal cord neurons that behave very differently in their natural environment in the embryo exhibited similar responses in vitro. Probably, the complex in vivo environment provides unique spatial compositions of guidance cues, thus eliciting context-related responses.

These results raise a number of issues for further study. One is the molecular nature of the effector mechanisms that mediate attraction or repulsion. Attractive candidates are the small G proteins (heterotrimeric GTP-binding proteins) cdc42, Rac, and Rho, which regulate actin cytoskeleton morphogenesis and growth cone activity (*9*). RhoA can mediate growth cone collapse, is negatively regulated by cAMP, and may also mediate repulsion; cdc42 participates in chemotaxis and may also mediate attraction (*10*). A further issue is whether receptor complexes rather than single-receptor proteins may mediate attractive and repulsive responses (*11*). Finally, to what extent and how do the mechanisms described in these studies affect guidance in situ? The recent report that, after crossing the midline, commissural axons become insensitive to attraction by the floor plate is consistent with the possibility that attraction by Netrin-1 may be switched to repulsion in vivo (*12*). In a similar scenario, guidance by guidepost cells may involve initial attraction, followed by repulsion to promote further navigation of the growth cone toward the next guidance cues. Direct experimental testing of these possibilities is needed. It will be particularly important to determine how growth cones handle complex sets of guidance clues, and how parallel systems that use cAMP and cGMP interact. By recruiting other signaling components, contact-mediated guidance may add significant complexity to the attractively simple mechanisms uncovered by these in vitro studies.

It seems a safe bet that these discoveries will promote significant further progress. Similar switch mechanisms may operate in synapse formation, nerve sprouting, and synaptic plasticity in the adult (*13*). Clearly, however, the most exciting possibility raised by these findings is that the inhibitory signals that prevent nerve regeneration in the adult central nervous system could be attenuated by pharmacological interventions that raise cyclic nucleotide levels in injured axons, thus promoting their regeneration.

References and Notes

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PERSPECTIVES: NUCLEAR STRUCTURE

Duplicating a Tangled Genome

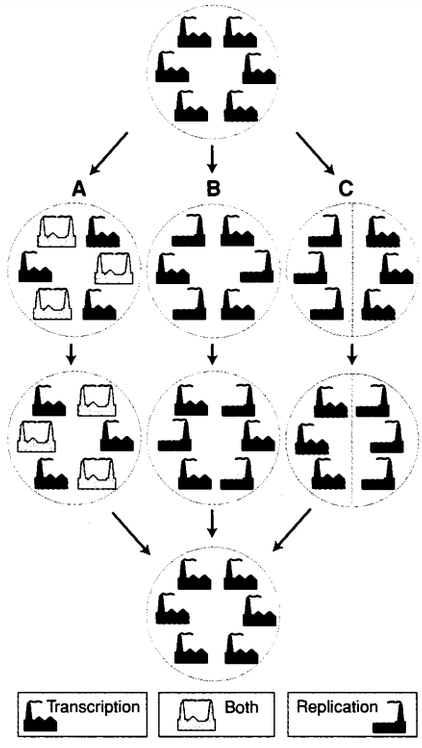
Peter Cook

The 46 DNA molecules that make up the diploid human genome are incredibly thin and very long, with a width of ~2 nanometers and a combined length of ~2 meters. All 46 molecules must be packed into a nucleus only ~10 micrometers wide, a packing problem analogous to folding a kite string that stretches from New York to Chicago into a sphere 10 meters across!

Enhanced online at www.sciencemag.org/cgi/content/full/281/5382/1466

Real-sized DNA strings are probably packed into real-sized nuclei by a combination of random bundling (like pasta in a bowl), coiling into higher order spirals, and looping by attachment to an underlying nuclear skeleton or "matrix" (*1*, *2*). Yet this tangle must still allow transcription of individual genes and the replication of all chromosomes. Somehow one complete new genome must be sorted out for inheritance by each daughter cell. One milestone in the elucidation of such structure-function relationships within these complex tangles was the isolation by Berezney and Coffey of the nuclear matrix in the 1970s (*3*). Now a report from Berezney's lab, on page 1502 of this issue, illuminates the order in the nuclear tangle during the process of DNA replication (*4*).

The new work builds on several observations. First, the cellular machines that replicate DNA (composed of DNA polymerase and associated proteins) do not act alone. Instead, tens (sometimes hundreds) are housed in enormous "factories" (with diameters of 0.1 to 1 μm); individual machines in each factory reel in loops of DNA as they replicate them (*5*). Second, transcription machines that copy DNA into RNA (RNA polymerases and associated proteins) are concentrated in analogous factories (*6*). Third, transcription happens continuously; it starts when a cell is born and goes on until it divides. In contrast, DNA is replicated only during the middle third of each cell cycle. Replication begins in many factories located in transcriptionally active regions and ends in a few large factories in less active regions (*5*, *7*). Fourth, structure-function relationships are remarkably stable. For example, clusters of



One or the other. Organizing replicating machines in nuclei. (A) Replicating machines are installed in functioning transcription factories. (B) Existing transcription factories are decommissioned and replaced by dedicated replication factories. (C) Zoning regulations ensure dedicated replication factories are grouped together.

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