sively for sample loading in capillary electrophoresis systems (*14*). Relatively few of the wide range of mechanical valves that exist today (*15*) have been integrated into chemical analysis systems, not least because of noncompatible fabrication procedures. Coupling of these valves or new valving strategies into more complex systems is sorely needed. Materials that expand in response to an external stimulus (such as an electric field) also hold considerable promise for impacting this field.

Progress on the construction of fully integrated chemical systems has lagged behind component development. In a fully integrated chemical analysis system, the input to the device is a gas or liquid sample plus reagents, and the output is an electronic signal indicating the presence and/or concentration of the tested compounds. Successes include the combination of liquid metering, reaction, analysis, and detection steps necessary for DNA identification in a single device (*16*).

But not all microfabricated chemical systems require full integration. Microfabricated DNA hybridization arrays have already had a tremendous impact on genetic analysis (*17*, *18*). In these systems, the power of the analysis lies not in the movement and control of multiple liquid samples but in the multiple simultaneous hybridization events on the chip. Gas-sensing systems such as the "electronic nose" for sensing low concentrations of gas-phase compounds have a similar advantage in being able to detect many different chemical species (*19*). Nonsensing applications include chemical production, for example, of harmful chemicals from harmless precursors on site, avoiding storage of toxic material (*20*).

For chemical integrated systems to be successful, the devices need to be cheap, durable, and compact. Packaging, often overlooked in research advances, can present a significant stumbling block to the ultimate success of the device. The applications for these systems will be numerous, but two areas are particularly promising. Airborne contaminants pose a threat at home, at work, and in the battlefield. A microfabricated device for their detection should, however, be able to handle gases and liquids on the same chip, because the species of interest (for example, a biological warfare agent), though found in a gaseous or suspended state, may need to be analyzed in solution.

The second area is genetic testing and diagnosis. The technology for identifying pathogens and inborn genetic disease susceptibilities on the basis of DNA sequence information exists, but these methods are not cost effective for widespread use. Miniaturized genetic testing would provide accurate diagnosis of infectious diseases and predisposition to future ailments. This type of analysis could also be applied to other fields, including food-borne pathogens, agriculture pest isolation, and forensic identification.

The potential impact of microfabricated integrated chemical systems is substantial. The current state of chemical analysis is analogous to that of computing technology in the mid-1970s. At that time, Cray had just introduced its first supercomputer, Cray I, with a peak performance of <0.2 gigaflops (*21*). Today, one of Apple's G4 personal computers can do 15 gigaflops (*22*). The bulk of chemical analysis and testing, aside from simple test kits such as pregnancy tests and glucose measurements, is still done in large centralized laboratories. The sequencing of DNA associated with the Human Genome Project is a good example: A handful of labs produced the bulk of the current draft sequence.

If future technology developments in chemical analysis parallel those in the computer industry—and there is every indication that they will—then the power to analyze a variety of chemical information will be disseminated to the individual. Given the wide range of potential chemical and biochemical tests, the microfabricated chemical analysis device will be as ubiquitous in the future as the computer chip is today.

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PERSPECTIVES: DEVELOPMENT

Putting the Brakes on Regeneration

Lisa McKerracher and Benjamin Ellezam

Neurons communicate with each other through specialized cytoplasmic processes called axons and dendrites. During development, axons and dendrites follow separate differentiation pathways. Nerve damage in the adult caused by injury or disease usually results from loss of the long axons. Current thinking is that axons of the optic nerve, brain, and spinal cord fail to regenerate after injury because of an inhospitable environment that contains growth-inhibitory proteins. Accordingly, strategies to stimulate nerve regeneration in the central nervous system (CNS) have focused on altering the environment. An important question, however, is whether changes acquired during development also limit the ability of adult neurons to regrow their axons. On page 1860 of this issue, Goldberg *et al.* (*1*) reveal that postnatal retinal ganglion cells (RGCs) in the rat cannot grow axons as fast as embryonic RGCs, even under the most favorable test environments. With this finding, these investigators provide evidence that the poor growth of adult CNS axons is not simply a consequence of the local environment, but is a property acquired during development. Their key observation is that contact between RGCs and amacrine cells—interneurons that form part of the retinal circuitry—provides the developmental switch that drives RGCs into a dendritic growth mode, and that this switch limits the ability of older neurons to grow axons (see the figure).

Cultured neurons from embryonic animals grow axons much more rapidly than do neurons from older animals. Goldberg *et al.* (*1*) set out to discover the reasons for these differences by measuring axonal growth rates. These investigators screened

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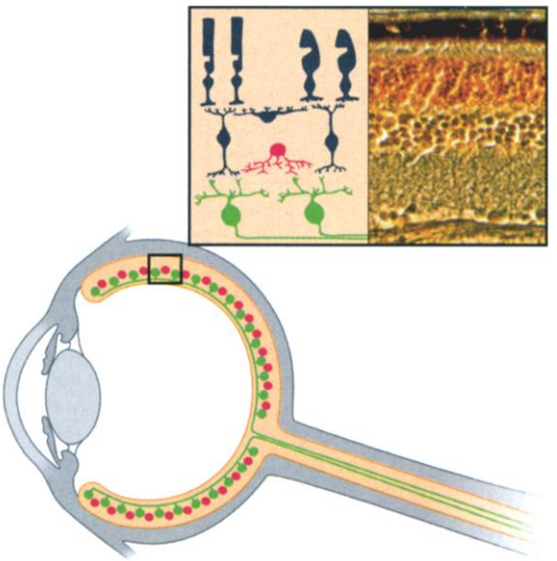
the typical list of molecular suspects for their ability to enhance axon growth of postnatal day 8 (P8) and embryonic day 20 (E20) RGCs. They tested neurotrophins, Schwann cells, growth-promoting substrates, and high cAMP levels—none of these treatments helped older neurons to accelerate their axon growth rates. They analyzed RGCs that overexpressed bcl-2 (an anti-apoptotic protein), RGCs transplanted into the developing brain, and RGCs that overexpressed the TrkB neurotrophin receptor. And still the young neurons grew their axons the fastest.

Taken together, these results support the idea that the difference in axon growth rates is intrinsic and not dependent on the environment. Goldberg *et al.* are careful to note that in the population of P8 RGCs a small number (about 1%) grew more quickly. Do these represent a subpopulation of neurons with a greater capacity for regeneration? Adult RGCs can regenerate axons after injury when a peripheral nerve is attached to the cut optic nerve stump, although there is considerable variability in success rates (*2*). It seems unlikely that there is a set number of regeneration-competent neurons in the CNS. In the spinal cord, different treatments give widely differing success rates. Fast growth rates may predict successful regeneration, although it is not known whether the intracellular mechanisms for speed of growth are related to growth capacity.

Recently, exciting progress has been made in elucidating new ways to stimulate regeneration of adult RGC axons in the optic nerve. Altering the inhibitory environment of the optic nerve (*3*) and blocking growth-inhibitory signaling pathways with antagonists of Rho (*4*) both promote regeneration of adult rat RGCs. Yet, the total regeneration distances are short compared with the distances obtained using the same strategies in spinal cord (*5*, *6*)—it is almost as though optic nerve axons just give up growing. Implantation of living peripheral nerve segments into the eye, which offers sustained treatment, does not promote growth much further in the optic nerve, only a few millimeters (*7*). Similarly, lens injury stimulates regeneration of RGCs (*8*), but again axons stop growing long before they reach their target. Could the inability of RGC axons to grow long distances be a developmentally acquired property?

Goldberg and colleagues found that dissociated, purified embryonic RGCs had a fast axonal growth rate, which did not slow down when dissociated cells were allowed to reach maturation in vitro. In contrast, RGCs dissociated at the postnatal stage or cultured in explants where they remained in contact with other retinal cells had dramatically reduced axonal growth rates. This change in growth rate between E20 and P8 neurons could be signaled by other retinal cells. Coculture experiments revealed that the culprits are amacrine cells that signal RGCs to switch to a dendritic growth mode. Interestingly, the decrease in axon growth rate occurs when RGCs switch from axon to dendrite growth as they begin to acquire polarity (*9*).



Neurons of the retina. RGCs (green) and amacrine cells (red) are part of the circuitry of the retina. Only RGCs project axons into the optic nerve. Contact between RGCs and amacrine cells during retinal maturation may induce RGCs to stop elongating their axons, and to switch to growth of dendrites instead. The box shows a detailed diagram of retinal circuitry **(left)** and a silver-stained section of adult rat retina **(right)**.

To further explore RGC differentiation, Goldberg *et al.* examined the types of projections elaborated by RGCs dissociated before or after maturation. The trend was surprising: E20 RGCs grew axons, P8 RGCs grew dendrites. Thus, contact between RGCs and amacrine cells may stimulate dendrite growth but impair the capacity of RGCs to grow axons. If this signal remains long after development is finished, it could suppress axonal regeneration in the adult. The new work demonstrates that the interaction between RGCs and amacrine cells is a new type of growth-inhibitory process, and that interneurons must be included as a variable in studies of CNS regeneration.

An important next step will be to determine if the intrinsic changes in RGCs that accompany retinal maturation can be reversed. Hope that the change in growth capacity is reversible comes from experiments on regenerating RGCs. A change to axon growth in cell culture can be induced by axotomy (cutting of the axon) close to the neuronal cell body, which elicits the formation of a new axon from an existing dendrite (*10*). In vivo, some RGCs respond similarly by growing axons from dendrites when a peripheral nerve graft is attached to the retina after optic nerve crush (*11*). Additional evidence that the change in growth capacity can be reversed comes from studies of conditioning lesions (accelerated axon growth in response to a second axon injury) of adult neurons. A second axon lesion closer to the cell body accelerates slow transport of cytoskeletal components into axons, resulting in faster axon growth rates (*12*). Slow axonal transport rates increase when RGCs regenerate in peripheral nerve grafts (*13*), suggesting that RGCs can return to an axon growth mode. Recently, conditioning lesions were found to promote robust regeneration in the CNS, which implies that increasing the rate of growth also improves the success of regeneration (*14*). The challenge now will be to discover the signals that switch neurons back to the axon-growth mode. The Goldberg *et al.* work suggests that a better understanding of the developmental switch from axonal to dendritic growth may hold the key to regeneration in the CNS. Meanwhile, the ability of amacrine cells to influence RGC axon growth underscores the importance of neighboring neurons for the repair of injured nerves. Regulation of axon growth by interneurons offers a new explanation for how axon regeneration in the CNS may be suppressed.

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