phatidylinositol (GPI)–linked brevican. Interestingly, immature glial cells elaborate larger extracellular matrices than do mature glial cells (*9*). The extracellular matrix in the developing brain is more permissive for growth, but it is not known whether the injected immature astrocytes (*8*) altered the extracellular matrix to a growth-permissive form in the adult cat. If they did, it would help to explain how injecting immature astrocytes restored plasticity.

Future experiments are likely to explore the nature of the perineuronal net and examine its properties in more detail. A number of useful enzymes already exist to do this, including that used by Pizzorusso *et al.* For example, whereas chondroitinase ABC removes CS side chains of CSPGs and degrades hyaluronan, the enzyme streptomyces hyaluronidase could be used to specifically degrade hyaluronan alone. This would only affect the macromolecular aggregation of lecticans in the perineuronal net, leaving the CS side-chain interactions via tenascin intact. Unfortunately, all of the chondroitinases that degrade CS also degrade hyaluronan (*10*). But some specificity could be achieved with keratanases, which degrade keratan sulfate glycosaminoglycans on phosphacan and aggrecan in the perineuronal net without affecting hyaluronan. Experimenting with different enzyme combinations will enable exploration of the properties of the perineuronal net that permit and restrict plasticity.

From a therapeutic viewpoint, it will be important to elucidate whether chondroitinase treatment facilitates invasion of the cerebral cortex by glial tumor cells, a possible detrimental side effect of restoring plasticity. It has been shown that catabolism of a brevican CSPG (called BEHAB) in the brain facilitates glial tumor invasion of cortical tissues (*11*). The time course over which chondroitinase treatment affects the perineuronal net also will be a critical factor in attempts to increase plasticity. It takes at least 8 weeks for the perineuronal net to reestablish itself after a single chondroitinase injection (*12*), which might be a useful time period during which to restore plasticity. Clearly, the road to a useful therapy for enhancing neuronal plasticity in the brain is long, but Pizzorusso and colleagues are among those who have taken the first crucial step.

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PERSPECTIVES: SIGNAL TRANSDUCTION

Decoding NF-κB Signaling

Alice Y. Ting and Drew Endy

Pressing a piano key causes a hammer to hit a string. Until the resulting oscillation of the string dampens of its own accord, an audible tone is produced for as long as the key is depressed. A pianist can modify this tone by depressing foot pedals that indirectly control how hard the string is hit and whether or not string vibration is sustained after the key is released. Similarly, signal transduction pathways in living systems are activated by information in the environment; by encoding and transmitting this information, they produce "music" that is interpreted by cells and results in direct changes to cellular behavior. On page 1241 of this issue, Hoffmann *et al.* (*1*) use a combination of experiments and computer modeling to make sense of a particular signal transduction pathway, and they illustrate how the pathway can be manipulated to switch on different target genes.

Hoffmann *et al.* chose to study the NF-κB/IκB signaling pathway. NF-κB (nuclear factor κB) is a transcription factor that helps to govern the expression of genes involved in both the innate and adaptive immune response (*2, 3*). The ability of NF-κB to regulate gene expression is controlled by chemical modifications such as the addition of phosphate groups, and by interactions with other proteins notably members of the IκB family (inhibitors of NF-κB). The binding of NF-κB to IκB helps to localize NF-κB in the cytoplasm. Upon activation of the NF-κB signaling pathway by tumor necrosis factor (TNF), IκB kinases target IκBs for degradation. This allows NF-κB to accumulate in the nucleus, where it binds to DNA, resulting in the expression of target genes. One of the genes activated by NF-κB is that encoding IκBα. Newly synthesized IκBα binds to NF-κB and attenuates the pathway response to TNF, thereby creating a negative feedback loop within the NF-κB/IκB signaling pathway (see the figure). The new work explores two unanswered questions. First, why are there different isoforms of IκB (for example, IκBα, IκBβ, and IκBε)? Second, how does NF-κB control the expression of different genes, or classes of genes, each of which might be required at different times depending on the needs of the cell?

Hoffmann and colleagues start with experimental observations that show how NF-κB activity varies dynamically in response to sustained pathway activation [e.g., (*1, 4*)]. Given knowledge about how IκBα is regulated, they use an abstract linear model of a simple system containing negative feedback to explore how such a system might produce time-dependent oscillations. For a broad range of model parameters, "self-regulation" of model variables results in dampened oscillatory behavior. Hoffmann *et al.* posit that the NF-κB/IκB signaling pathway could produce dampened oscillations of NF-κB activity through the combined action of different IκB isoforms. To examine the individual contributions of each of the IκBs, Hoffmann *et al.* created and characterized knockout cell lines (or "genetically reduced" systems) that each contained only one of the three IκB isoforms. Their surprising observation was that IκBα cells (lacking both β and ε isoforms) behaved quite differently from cells containing only IκBβ or IκBε. The IκBα cells displayed prominent undampened oscillations in nuclear NF-κB upon pathway activation by TNF, whereas IκBβ and IκBε cells produced a monotonic increase in nuclear NF-κB (see the figure).

To better understand the observed differences in the regulation of NF-κB, Hoffmann *et al.* constructed a quantitative model for both the wild-type and knockout cells. According to the model, TNF treatment of IκBα cells causes IκBα degradation, which results in NF-κB translocation to the nucleus (first peak, see the figure, panel A). NF-κB replenishes the level of IκBα, promoting a re-inhibition of NF-κB (first dip, see the figure, panel A). Continued cycles of IκBα degradation and synthesis give rise to oscillations in nuclear NF-κB activity. In the wild-type model

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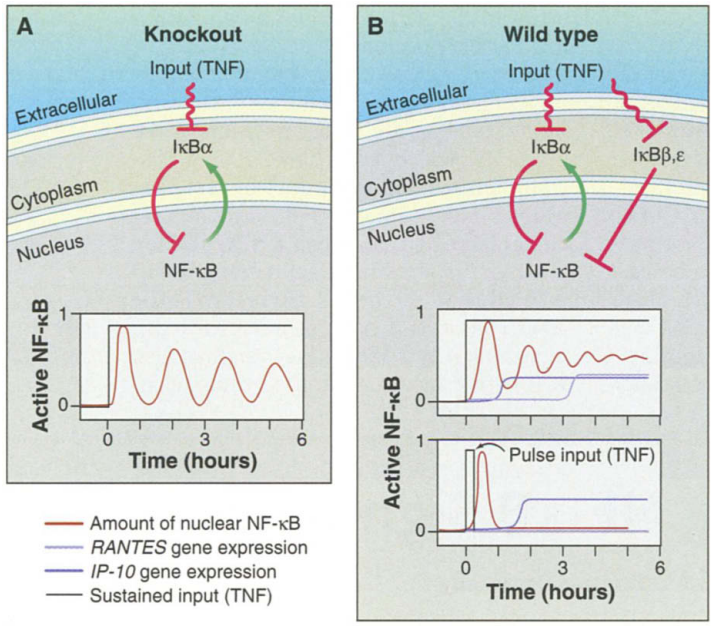
(see the figure, panel B), TNF stimulation triggers degradation of all three IκB isoforms. NF-κB activity rises as a result and, as before, stimulates expression of IκBα; however, IκBβ and IκBε remain at low levels because, unlike IκBα, their expression is not linked to NF-κB (in other words, the impact of IκBβ and IκBε on NF-κB activity is unidirectional; they inhibit NF-κB, but NF-κB does not regulate their production). Thus, the steady oscillations observed in the IκBα-deficient cells are dampened in the wild-type cells by the actions of IκBβ and IκBε. Returning to the piano metaphor, stimulation with TNF represents depressing a key and results in oscillations of the corresponding string (the NF-κB/IκB pathway). In wild-type cells, the different IκB isoforms act in concert to naturally dampen the oscillations, whereas removal of IκBβ and IκBε is analogous to pressing a pedal to raise the dampers, which sustains the tone.

A limitation of computational modeling is that, in the absence of complete information about cell parts and interconnections, it is easy to omit critical parameters that might influence the state of a cell or signaling pathway. This is illustrated in the Hoffmann *et al.* work. The authors assigned values for several model parameters using experimental data from the three knockout cell lines and then applied these same values to derive a model for the full wild-type pathway. When they used this model to predict the behavior of wild-type cells, the outcome was very different from what was actually measured, even though many of the parameters were empirically obtained. Such discrepancies could be due to compensatory changes in expression and signaling state from one cell line to the next, or to additional pathway components and regulatory mechanisms beyond the current model.

Finally, Hoffmann *et al.* applied their models to explore the question of differential gene regulation by NF-κB (see the figure, panel B). Some genes, such as *IP-10*, are early genes that are activated quickly by NF-κB; others such as *RANTES* are late genes that are activated only after prolonged exposure to NF-κB. The authors hypothesized that IκBα is necessary for this differential control because it helps to rapidly attenuate NF-κB activity in response to a short pulse of TNF. To test this hypothesis, Hoffmann *et al.* treated cells lacking IκBα with both pulsed and sustained TNF inputs. Because feedback inhibition of NF-κB is absent in these knockout cells, either input results in sustained NF-κB activation and expression of both early and late genes (*IP-10* and *RANTES*, respectively). This experiment demonstrates how feedback inhibition can influence encoding of a time-dependent signal and produce precise temporal regulation of transcription factor activity.



Toning down NF-κB oscillations. (**A**) The NF-κB signaling pathway in mouse knockout cells lacking two IκB isoforms (IκBβ and IκBε). Activation of the NF-κB signaling pathway by TNF reduces IκBα-mediated inhibition of NF-κB; NF-κB in turn increases production of IκBα. Because the cells lack IκBβ and IκBε, the system produces undampened NF-κB oscillations in response to sustained TNF input. (**B**) The feedback loop in wild-type cells containing three IκB isoforms: IκBα, β, and ε. Activation of the pathway by TNF again reduces IκBα-mediated inhibition of NF-κB; NF-κB still promotes production of IκBα. However, IκBβ and ε are independent of NF-κB feedback and act to dampen the response of the NF-κB signaling pathway to TNF input. The system now produces dampened oscillations in response to TNF input, but a quick attenuation of NF-κB activity in response to a short TNF pulse. Such pathway control allows for the differential regulation of the expression of early and late genes, such as *IP-10* and *RANTES*, respectively.

Our understanding of biological signaling will benefit from advances in three general areas. The first area is the development of methods for manipulating and quantifying pathway inputs. For example, in situ characterization and external manipulation (*5*) of environmental information would help to determine how a graded increase in TNF input could reduce a feedback-induced dip in NF-κB activity, how a periodic input signal could amplify or suppress NF-κB oscillations, and when such input and behavior would be physiologically relevant. The second area is the construction of theoretical and computational frameworks to describe the encoding, transmission, and decoding of information in molecular systems. For example, exciting work by Samoilov *et al.* describes how very simple chemical reaction networks might respond to (that is, decode) periodic single- and multifrequency signals (*6*). The third area is the creation of new methods for making better measurements of a cell's state. Hoffmann *et al.* make sole use of gel shift assays to measure NF-κB levels; this method is destructive and averages signals from a heterogeneous population of cells. It is exciting to imagine the in vivo data that could be obtained with noninvasive single-cell fluorescence techniques. For example, the apparent slight dampening observed in IκBα cells might be a population effect due to a loss of synchronization among individual cells. In addition, the effect of cell lysis on activity and localization of NF-κB is unclear. Chemistry, molecular biology, and protein engineering will continue to contribute to signaling research, generating new methods for measuring not only the expression of biomolecules and their localization but also currently hidden information such as the production of small molecule messengers, enzyme activation, and protein interactions (*7*). By thoughtfully combining experimental manipulations, measurements, and theory, we should be able to "lift the lid" on the signal transduction instruments encountered in the living world, helping to reveal both their detailed inner workings and general principles of design.

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