

glume, which can secondarily alter the fate of the SMs when de-repressed.

None of these models adequately address the fact that the *bd1* SM has different fates in the tassel and ear. It is unlikely that *bd1b* partially compensates for the loss of BD1 in the tassel, as proposed for the *zag1/zmm2* duplication in maize (14), because we have been unable to detect *bd1b* transcript in any tissues (15). It is possible that a different tassel-specific factor may function redundantly with *bd1*. Given the intense selective pressure on the maize ear, it is not surprising that the ear and tassel are genetically distinct.

The expression pattern and mutant phenotype of *bd1* show similarities to the *FIM-BRIATA/UFO* genes of *Antirrhinum* and *Arabidopsis*, respectively (16, 17). Both genes are expressed in a ring at the base of the floral meristem adjacent to the sepals, and the *Antirrhinum* mutant shows a partial loss of lateral determinacy within the meristem. In the case of *UFO*, the basal floral meristems may be replaced with cofilence branches (18). In *Arabidopsis*, the *UFO* and *LEAFY* genes have been proposed to be coregulators of floral meristem identity (19). Therefore, BD1 may interact with other SM identity factors to impose determinate meristem fates. As in wild type, the maize *LEAFY* ortholog is expressed in the SPMs and SMs of *bd1* mutants (5). However, the genetic interaction between *bd1* and *leafy* is unknown and awaits identification of *leafy* mutants in maize.

To date, *bd1* is the only maize mutant that specifically displays altered SM identity. Several maize mutants that affect SM determinacy have been described, such as *Tassel-seed6* (20) and *indeterminate spikelet1* (21). Both these mutants display SMs that initiate more than two florets per spikelet, and interestingly, both show normal patterns of *bd1* expression in the SM (fig. S1). The latter result indicates that SM identity is acquired before SM determinacy. Recently, it has been shown that SM identity and determinacy are interdependent, as two genes that control SM determinacy, *indeterminate spikelet1* and *indeterminate floral apex1*, also show SM identity defects as a double mutant (22).

The grass spikelet is conventionally interpreted as a strongly contracted branch system—literally, a little spike (23). If this interpretation is correct, then genes should exist that, when mutated, cause the spikelet to revert to a branchlike structure. We have identified a gene that regulates spikelet versus branch meristem fates within the inflorescence of maize, and whose sequence and expression are conserved in other grasses such as rice and sorghum. Our data suggest that the expression of *bd1* is fundamental to grass spikelet formation and may have played a role in the origin of this evolutionary novelty.

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## Supporting Online Material

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Materials and Methods  
Fig. S1

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## The I $\kappa$ B–NF- $\kappa$ B Signaling Module: Temporal Control and Selective Gene Activation

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Nuclear localization of the transcriptional activator NF- $\kappa$ B (nuclear factor  $\kappa$ B) is controlled in mammalian cells by three isoforms of NF- $\kappa$ B inhibitor protein: I $\kappa$ B $\alpha$ , - $\beta$ , and - $\epsilon$ . Based on simplifying reductions of the I $\kappa$ B–NF- $\kappa$ B signaling module in knockout cell lines, we present a computational model that describes the temporal control of NF- $\kappa$ B activation by the coordinated degradation and synthesis of I $\kappa$ B proteins. The model demonstrates that I $\kappa$ B $\alpha$  is responsible for strong negative feedback that allows for a fast turn-off of the NF- $\kappa$ B response, whereas I $\kappa$ B $\beta$  and - $\epsilon$  function to reduce the system's oscillatory potential and stabilize NF- $\kappa$ B responses during longer stimulations. Bimodal signal-processing characteristics with respect to stimulus duration are revealed by the model and are shown to generate specificity in gene expression.

The transcription factor NF- $\kappa$ B regulates numerous genes that play important roles in inter- and intracellular signaling, cellular stress responses, cell growth, survival, and apoptosis (1–3). As such, the specificity and temporal control of gene expression are of crucial physiological interest. Furthermore, the realization of the potential of NF- $\kappa$ B as a drug target for chronic inflammatory diseases

or within chemotherapy regimens (4, 5) is dependent on understanding the specificity mechanisms that govern NF- $\kappa$ B-responsive gene expression.

Five related mammalian gene products participate in NF- $\kappa$ B functions (RelA/p65, cRel, RelB, p50, p52), but the predominant species in many cell types is a p65:p50 heterodimer. Its activity is largely controlled by three I $\kappa$ B isoforms (I $\kappa$ B $\alpha$ , - $\beta$ , and - $\epsilon$ ) that bind to NF- $\kappa$ B, preventing its association with DNA and causing its localization to the cytoplasm. Signals from various stimuli are transduced to the I $\kappa$ B kinase (IKK) complex, which phosphorylates each I $\kappa$ B isoform, leading to its ubiquitination and proteolysis (6). I $\kappa$ B degradation allows NF- $\kappa$ B to translocate to the nucleus and bind DNA (Fig. 1A). The specific role of each I $\kappa$ B protein in regulating NF- $\kappa$ B is not understood. Mice

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with an engineered deletion of one *IκB* gene show notable molecular compensation by the remaining *IκB* family members (7, 3) and mild phenotypes (8, 9). The *IκBα*<sup>-/-</sup> mouse, however, is perinatal lethal with multi-organ inflammation presumably caused by up-regulation of many NF-κB-responsive genes (10, 11). This phenotype is largely rescued by placing the *IκBβ* coding region under transcriptional control of *IκBα* (12). *IκBα* synthesis is controlled by a highly NF-κB-responsive promoter generating autoregulation of NF-κB signaling (13).

The interactions of IKK, *IκB* isoforms, and NF-κB can be thought of as a negative feedback-containing signal-transduction module (Fig. 1A) that receives signals from pathways emanating from cell-surface receptors (input) and transmits signals to nuclear promoter-bound protein complexes regulating gene expression (output). In a minimal system with negative feedback (Fig. 1B), cross-regulation between the two signaling components determines the strength of the negative feedback (constants  $\beta$  and  $\gamma$ ) and thus the propensity for oscillations, whereas self-regulation (constants  $\alpha$  and  $\delta$ ) determines the degree of damping. Depending on the values of these constants, the response to persistent stimulation may thus vary from continual oscillations, to damped oscillations, to a virtually monotonic rise to a plateau level (Fig. 1B). We examined the dynamics of *IκB*-NF-κB signaling experimentally by measuring nuclear NF-κB (NF-κBn) activity with the electrophoretic mobility shift assay (EMSA) (14). Two phases of NF-κB activation were revealed in response to the stimulation of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in various human and mouse cell lines (Fig. 1C). Although the degree and precise timing of postinduction attenuation are variable (15, 16), the output resembles strongly damped oscillations but is distinct from that predicted for a minimal two-component system (Fig. 1B). We conclude that the coordinated degradation, synthesis, and localization of all three *IκB* isoforms is required to generate the characteristic NF-κB activation profile.

To address their differential functions, we constructed a computational model based on ordinary differential equations. The model behavior depends on the specific values of various control parameters, including those describing (i) the synthesis of each *IκB* isoform (transcription, mRNA stability, and translation), (ii) the stability of free and NF-κB-bound *IκB* proteins, (iii) the formation of binary and tertiary IKK-*IκB*-NF-κB complexes, (iv) the enzymatic rate constants of IKK-containing complexes, and (v) the transport rates affecting localization of each of the components (*IκBα*, - $\beta$ , and - $\epsilon$ ; NF-κB; and derived complexes). Many of the 30 independent model parameters have been previously

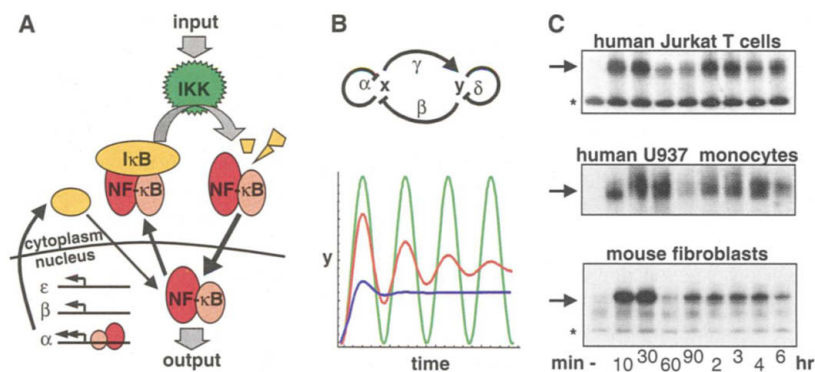
determined biochemically, and the values of others may be constrained by published data (14). We used reverse genetics to create three *IκB*-NF-κB signaling modules of reduced complexity and to provide further constraints for effective model parameter fitting.

We engineered mice deleted for *IκBβ* and - $\epsilon$  with the use of standard homologous recombination technology of embryonic stem cells (14). These mice, as well as existing *IκBα* gene-deleted mice (10), were intercrossed appropriately to yield embryonic fibroblasts in which the nuclear localization of NF-κB was controlled by a single *IκB* isoform (14). TNF $\alpha$  stimulation of fibroblasts that contained only the *IκBα* isoform resulted in a highly oscillatory NF-κB response, with four equally spaced peaks over the course of the 6-hour experiment (Fig. 2A, top). In contrast, in cells harboring only *IκBβ* (Fig. 2A, center) or - $\epsilon$  (Fig. 2A, bottom), NF-κBn increased monotonically to a plateau at 1 hour with no notable subsequent repression.

The simplified computational models of  $\beta^{-/-}\epsilon^{-/-}$ ,  $\alpha^{-/-}\beta^{-/-}$ , and  $\alpha^{-/-}\epsilon^{-/-}$  cells each contain 17 partially overlapping parameters that control the output, 9 of which have been biochemically determined previously. In addition, five transport parameters are constrained by ratios derived from steady state nuclear and cytoplasmic localization (17, 18). Their absolute values, as well as *IκB* synthesis parameters, were determined by semiquantitative fitting of the model outputs to the experimental data.

Combining the three models of genetically reduced signaling modules results in a computational model of the *IκB*-NF-κB signaling module in wild-type cells. Varying their relative contributions revealed discrete functional roles for the mammalian *IκB* proteins in NF-κB regulation (Fig. 2D). *IκBα* mediates rapid NF-κB activation and strong negative feedback regulation, resulting in an oscillatory NF-κB activation profile. *IκBβ* and - $\epsilon$  respond more slowly to IKK activation and act to dampen the long-term oscillations of the NF-κB response. The interplay between these isoforms can result in remarkably rapid responses to the onset or cessation of stimulation and can allow a relatively stable NF-κB response during long-term stimulation (Fig. 2D, middle). In the absence of strong damping mechanisms, high negative feedback efficiency can lead to long-term oscillations in the output (Fig. 2D, top), as observed, for example, in p53-mdm2 cross-regulation (19). In wild-type NF-κB signaling, pronounced oscillations are absent and may be detrimental, as *IκBβ*<sup>-/-</sup>*IκBε*<sup>-/-</sup> females have a dramatically shortened fertility span (3). Our results also suggest that varying relative synthesis levels of *IκBα*, - $\beta$ , and - $\epsilon$  may constitute a mechanism for altering the responsiveness of the NF-κB signal transduction pathway, a mechanism that cells or cell lineages may use in response to environmental or developmental cues.

To determine the individual contributions of



**Fig. 1.** Negative feedback and the *IκB*-NF-κB signaling module. (A) The *IκB*-NF-κB signaling module. NF-κB is held inactive in the cytoplasm by three *IκB* isoforms. Cell stimulation activates the IKK complex, leading to phosphorylation and degradation of *IκB* proteins. Free NF-κB translocates to the nucleus, activating genes, including *IκBα*. *IκBβ* and - $\epsilon$  are synthesized at a steady rate, allowing for complex temporal control of NF-κB activation involving a negative feedback. (B) A two-component system ( $x$  and  $y$ ) with a negative feedback exhibits dynamic behavior that depends on the relative efficiency of the feedback regulation ( $\beta$  and  $\gamma$ ) regulating oscillation persistence versus self-regulation ( $\alpha$  and  $\delta$ ) causing oscillation damping. This relationship can be described mathematically as  $dx/dt = S - \alpha x - \beta y$  and  $dy/dt = \gamma x - \delta y$ , where  $S$  represents the stimulus. The output,  $y$ , ranges from persistent oscillations (green line, high feedback efficiency and no damping,  $\alpha = \delta = 0$ ), to damped oscillations (red line, intermediate feedback efficiency and intermediate damping), to gradual rising to a plateau level (blue line, low feedback efficiency and high damping). In simulations corresponding to the red line,  $\alpha$  and  $\delta$  are 30% of the respective values used for the generation of the blue line. (C) EMSA for NF-κBn in TNF $\alpha$ -stimulated human T cells, human monocytes, and mouse fibroblasts. Nuclear extracts were prepared at the indicated times after the beginning of persistent stimulation with TNF $\alpha$  (10 ng/ml). Equal amounts of nuclear protein were reacted with a radioactively labeled double-stranded oligonucleotide containing a consensus  $\kappa B$  site sequence (14). Arrows indicate specific nuclear NF-κB binding activity; asterisks indicate nonspecific DNA binding complexes.

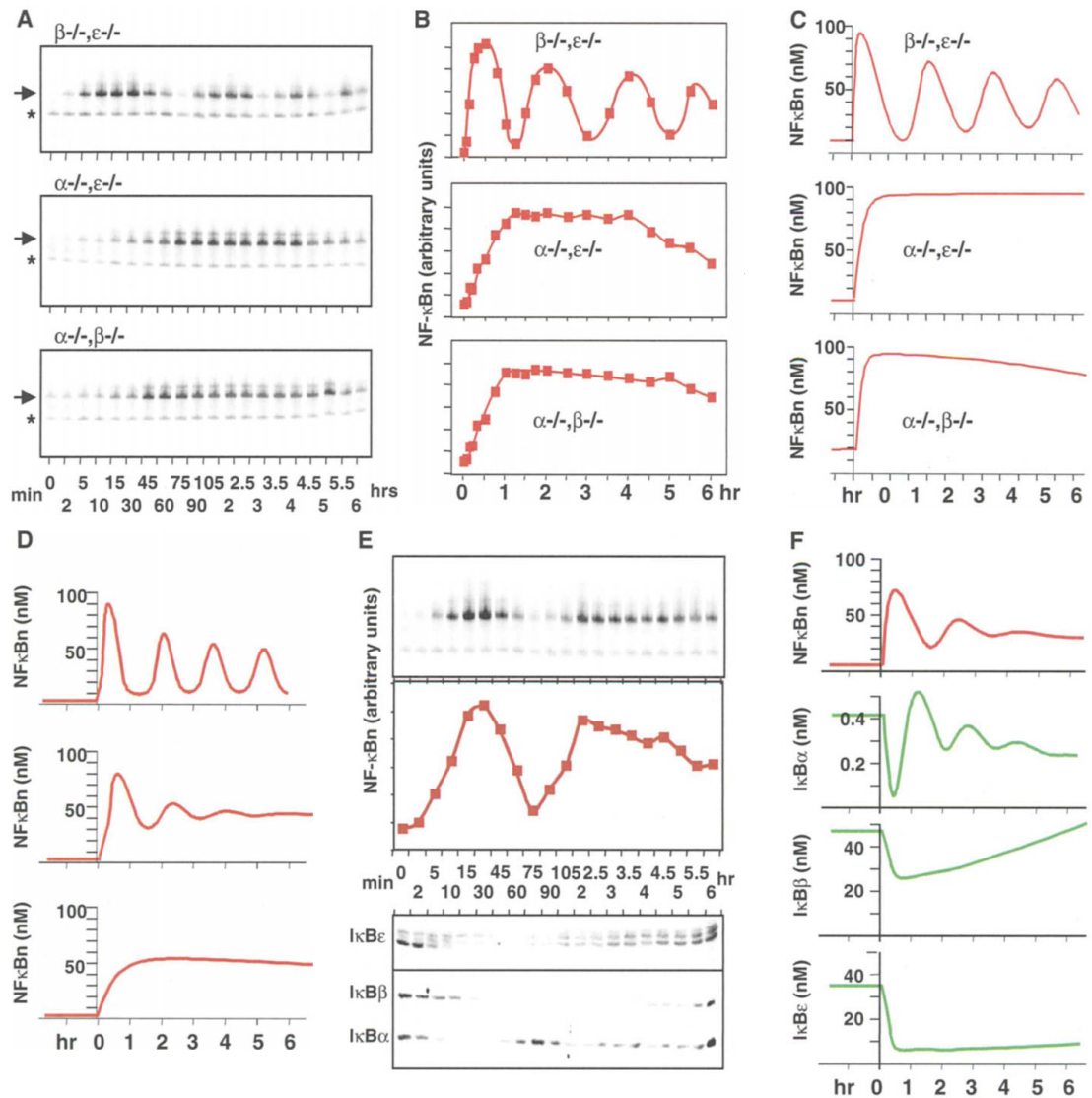
the single-I $\kappa$ B isoform models in wild-type fibroblasts, we analyzed nuclear and cytoplasmic extracts during TNF- $\alpha$  stimulation by EMSA for NF- $\kappa$ Bn (Fig. 2E, top) and by Western blot for I $\kappa$ B proteins (Fig. 2E, lower panels). NF- $\kappa$ Bn first appeared within 5 minutes, as I $\kappa$ B $\alpha$  levels rapidly decreased, and was at its maximum level at 30 min, when cytoplasmic I $\kappa$ B $\beta$  and - $\epsilon$  disappeared. After 60 min,  $\kappa$ B-binding activity was reduced concomitantly with a transient increase in I $\kappa$ B $\alpha$  protein levels. After 2 hours, NF- $\kappa$ Bn returned to about maximum levels and then decreased to about half of maximum levels as I $\kappa$ B $\alpha$  levels stabilized and I $\kappa$ B $\beta$  and - $\epsilon$  proteins reappeared. Model-fitting allowed the determination of I $\kappa$ B $\beta$  and - $\epsilon$  mRNA synthesis parameters that resulted in an optimal fit of the reconstituted model (Fig. 2F) with experimental wild-type responses. These pa-

rameters were about sevenfold lower than those determined for the respective single-I $\kappa$ B models, suggesting previously unrecognized cross-regulation in the expression of I $\kappa$ B genes. The outputs of the resulting "wild-type" model describe NF- $\kappa$ Bn (Fig. 2F, top) and cellular I $\kappa$ B isoform levels (Fig. 2F, lower panels) in good qualitative and quantitative agreement with experimental data, thus justifying its use as a predictive tool in experimentation.

We then investigated how transient stimuli control NF- $\kappa$ B activation. Simulated short pulses led to transient NF- $\kappa$ B activation responses, whose duration was insensitive to the duration of the stimulus within the first hour (Fig. 3A). Measuring NF- $\kappa$ Bn in transiently stimulated cells confirmed this prediction: TNF- $\alpha$  pulses of 5, 15, 30, and 60 min led to DNA binding activity profiles of sim-

ilar duration, equivalent to the first peak of persistently stimulated NF- $\kappa$ B activity (Fig. 3B). To explore the signal-processing characteristics of the I $\kappa$ B-NF- $\kappa$ B signaling module, we considered the availability of NF- $\kappa$ Bn for binding to an arbitrary  $\kappa$ B-responsive promoter. We plotted the duration of NF- $\kappa$ Bn availability above an arbitrarily chosen threshold concentration presumed to allow for efficient binding of the  $\kappa$ B element of some promoters (Fig. 3C) as a function of the duration of TNF- $\alpha$  stimulation. For long stimulations, NF- $\kappa$ Bn lasts as long as the stimulus. For stimulations of less than 1 hour, the duration of the response is largely invariant. Hence, the I $\kappa$ B-NF- $\kappa$ B signaling module has bimodal signal processing characteristics: One mode of signal processing ensures that even short stimulations result in substan-

**Fig. 2.** A computational model based on genetically reduced systems. (A) Analysis of NF- $\kappa$ Bn by EMSAs of nuclear extracts prepared at indicated times after stimulation with TNF- $\alpha$  (10 ng/ml) of fibroblasts of the indicated genotype. Arrows indicate specific nuclear NF- $\kappa$ B binding activity; asterisks indicate nonspecific DNA binding complexes. (B) The NF- $\kappa$ B-specific mobility shift in cells of the indicated genotype was quantitated by phosphorimager and normalized and graphed against a linear time scale. (C) Computational modeling of each genetically simplified signaling module results in characteristic kinetics of the NF- $\kappa$ Bn response. Model-fitting allows previously undetermined biochemical parameters to be estimated. (D) Models of the simplified signaling modules are combined, with increasing I $\kappa$ B $\beta$  and - $\epsilon$  transcription rates, while keeping the I $\kappa$ B $\alpha$  transcription rate constant. Model behaviors are shown that result as the constitutive mRNA synthesis parameters for I $\kappa$ B $\beta$  and I $\kappa$ B $\epsilon$  are increased fivefold (top to middle) and then sevenfold (middle to bottom). The bottom panel represents the NF- $\kappa$ Bn output predicted by a model with mRNA synthesis parameters identical to those employed in the single I $\kappa$ B isoform models shown in Fig. 2C. (E) Biochemical analysis of NF- $\kappa$ B and I $\kappa$ B isoforms in wild-type fibroblasts. NF- $\kappa$ Bn (top) assayed by EMSA at the indicated times after persistent stimulation with TNF- $\alpha$ . The specific NF- $\kappa$ B-specific mobility shift was quantitated by phosphorimager and normalized and graphed at the indicated nonlinear time scale. Western blots of corresponding cytoplasmic fractions are probed with anti-bodies specific to I $\kappa$ B $\alpha$  and - $\beta$  (bottom) and I $\kappa$ B $\epsilon$  (above). (F) Verifica-



tion of the computational model for wild-type cells. I $\kappa$ B $\alpha$  and - $\beta$  mRNA synthesis parameters were determined by qualitative model fitting to yield the graphed outputs in response to persistent stimulation of NF- $\kappa$ Bn (top) and total cellular concentrations of I $\kappa$ B $\alpha$ , - $\beta$ , and - $\epsilon$  (lower panels as indicated).



tial NF- $\kappa$ B responses; the other mode, operative at stimulations longer than 1 hour, generates responses proportional in duration to the stimulus.

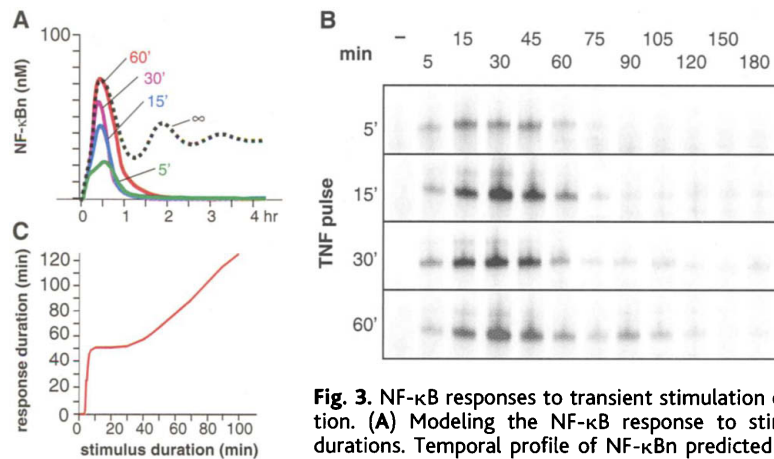
One implication of the above-described temporal regulatory switch is that some NF- $\kappa$ B-responsive genes might be efficiently activated by a stimulation pulse as short as 15 min (Fig. 4A, blue stippled line). However, there may be other genes that require longer (>1 hour) exposure to NF- $\kappa$ B. We tested this hypothesis by examining the behavior of two NF- $\kappa$ B-regulated genes that display different transcriptional activation profiles (3). One gene encodes the chemokine IP-10, which displays detectable mRNA levels within 30 min of TNF- $\alpha$  stimulation (Fig. 4B) (14). A 15-min pulse of TNF- $\alpha$  was sufficient to activate IP-10 transcription, although, as might be expected, persistent stimulation yielded greater amounts of IP-10 mRNA at later time points. In contrast, the chemokine gene RANTES required at least 2 hours of stimulation for detectable expression and was not induced by a shorter, transient stimulation (Fig. 4B). The model predicts that in cells lacking I $\kappa$ B $\alpha$ , NF- $\kappa$ B will have a longer nuclear lifetime after a short TNF- $\alpha$  pulse (Fig. 4A, red lines) than in wild-type cells. Indeed, transient stimulation led to an extended peak of  $\kappa$ B-binding activity, similar to responses resulting from longer stimulations (compare upper panels in Fig. 4, B and C). In turn, RANTES gene transcription was induced not only with persistent stimulation but also with transient stimulations as short as 15 min in I $\kappa$ B $\alpha$ <sup>-/-</sup> cells (Fig. 4C).

Thus, the bimodal temporal signal-processing characteristics of the I $\kappa$ B-NF- $\kappa$ B module result in not only quantitative but also qualitative regulation of gene expression defining two classes of genes: those that require persistent NF- $\kappa$ B activation and those that do not. The latter genes (e.g., IP-10) undergo a standard activation program irrespective of the precise duration of a transient TNF- $\alpha$  stimulus. I $\kappa$ B-NF- $\kappa$ B signal processing ensures that even very short stimulations can produce easily detectable transcriptional activation of such genes. Conversely, the former genes (e.g., RANTES) may or may not require expression of NF- $\kappa$ B-induced transcription factors with which NF- $\kappa$ B must synergize for gene induction. RANTES induction is protein synthesis-independent (3), and although the mechanism for the apparent delay in RANTES induction is unknown, it is noteworthy that histone H4 acetylation in macrophages was shown to be lipopolysaccharide-inducible on the RANTES promoter while it was constitutive on many other chemokine promoters (20). If H4 acetylation, a marker for chromatin accessibility, precedes NF- $\kappa$ B binding to the endogenous promoter, it may be induced by a NF- $\kappa$ B-independent

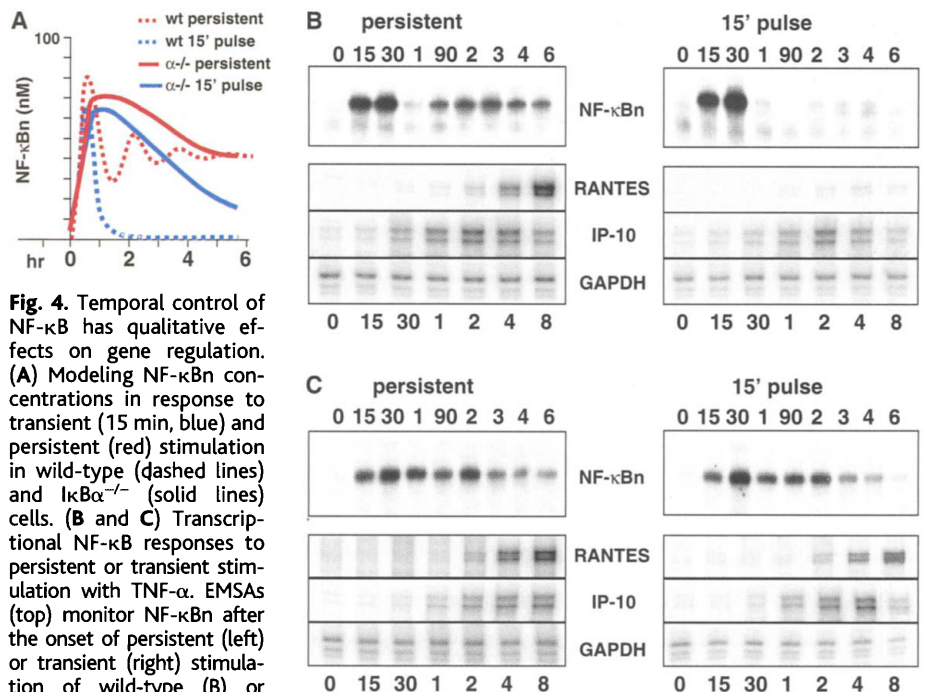
pathway. Our results imply that specificity in gene expression can be achieved by using two signal transduction pathways in temporally distinct ways. Gene induction only occurs when the two pathways are temporally coordinated.

Our analysis has revealed the I $\kappa$ B-NF- $\kappa$ B signaling module as a biological system that regulates cellular behavior through the control of system dynamics. The generation of genetically reduced systems enabled the com-

putational analysis of complex dynamic behavior. Exploration of the computational model, in turn, provided insights into the physiologically relevant differential functions of heretofore seemingly redundant system components. Their distinct but coordinated regulation in synthesis and degradation allows for a transcriptional response system with signal-processing characteristics that exhibit both rapid signal responsiveness and stable long-term responses.



**Fig. 3.** NF- $\kappa$ B responses to transient stimulation of varying duration. (A) Modeling the NF- $\kappa$ B response to stimuli of various durations. Temporal profile of NF- $\kappa$ Bn predicted by the computational model of the pathway in the wild-type cells in response to stimulations of the same intensity but varying durations as indicated. (B) Experimental NF- $\kappa$ Bn data for transient TNF stimulation regimes. Each panel shows the results from EMSAs with nuclear extracts after the onset of a transient stimulation with TNF- $\alpha$  for 5, 15, 30, and 60 min. (C) Graph of the duration of above-threshold (20 nM) NF- $\kappa$ Bn as a function of the duration of the transient stimulus as predicted by the computational model.



**Fig. 4.** Temporal control of NF- $\kappa$ B has qualitative effects on gene regulation. (A) Modeling NF- $\kappa$ B concentrations in response to transient (15 min, blue) and persistent (red) stimulation in wild-type (dashed lines) and I $\kappa$ B $\alpha$ <sup>-/-</sup> (solid lines) cells. (B and C) Transcriptional NF- $\kappa$ B responses to persistent or transient stimulation with TNF- $\alpha$ . EMSAs (top) monitor NF- $\kappa$ Bn after the onset of persistent (left) or transient (right) stimulation of wild-type (B) or I $\kappa$ B $\alpha$ <sup>-/-</sup> (C) fibroblasts with TNF- $\alpha$ . Ribonuclease protection assays (bottom) monitor the transcript levels of chemokine genes RANTES and IP-10 as well as the housekeeping gene glyceraldehyde phosphate dehydrogenase (GAPDH) at indicated times (in min and hours) after the onset of persistent (left) or transient (right) stimulation of wild-type (B) or I $\kappa$ B $\alpha$ <sup>-/-</sup> (C) fibroblasts with TNF- $\alpha$ .

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## Supporting Online Material

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Materials and Methods  
SOM Text

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# The Neurotrophin Receptor p75<sup>NTR</sup> as a Positive Modulator of Myelination

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Schwann cells in developing and regenerating peripheral nerves express elevated levels of the neurotrophin receptor p75<sup>NTR</sup>. Neurotrophins are key mediators of peripheral nervous system myelination. Our results show that myelin formation is inhibited in the absence of functional p75<sup>NTR</sup> and enhanced by blocking TrkC activity. Moreover, the enhancement of myelin formation by endogenous brain-derived neurotrophic factor is mediated by the p75<sup>NTR</sup> receptor, whereas TrkC receptors are responsible for neurotrophin-3 inhibition. Thus p75<sup>NTR</sup> and TrkC receptors have opposite effects on myelination.

The neurotrophin receptor p75<sup>NTR</sup> (1) is now known to have more diverse functions than that of being a helper for the Trk receptors. We show that the brain-derived neurotrophic factor (BDNF) acts through p75<sup>NTR</sup> to enhance myelin formation. The neurotrophins, a

family of growth factors including nerve growth factor (NGF), BDNF, neurotrophin-3 (NT3), and neurotrophin-4/5 (NT4/5), exert their biological actions mostly in neuronal cells by regulating survival, differentiation, and cell death (2). All known neurotrophins bind the receptor p75<sup>NTR</sup>, but others of the Trk family of tyrosine kinase receptors are more selective about which neurotrophin they will bind. NGF binds to TrkA, BDNF and NT4/5 bind to TrkB, and NT3 binds to TrkC. Alternative splicing of the *trkB* and *trkC* genes results in full-length receptor isoforms

(TrkB-FL and TrkC-TK<sup>+</sup>) that contain an intact tyrosine kinase domain and the truncated isoforms (TrkB-T1 and -T2 and TrkC-TK<sup>-</sup>) that lack the kinase domain (3, 4).

The myelin sheath is a specialized membrane component in the nervous system that maximizes the conduction efficiency and velocity of neuronal action potentials. The myelination program involves a number of signals between the neuronal and myelin-forming cells that include, in the peripheral nervous system (PNS), neuregulins (5), adenosine triphosphate (6), steroid hormones (7), Desert hedgehog (8), and the neurotrophins BDNF and NT3 (9). Removal of BDNF inhibited myelination, whereas removal of NT3 enhanced myelination in vitro and in vivo (9).

To identify the neurotrophin receptors responsible, we determined which receptor mRNAs were present during myelination both in sciatic nerve and in Schwann cell/dorsal root ganglia neuron (SC/DRG) cocultures by non-quantitative reverse transcription-polymerase chain reaction (RT-PCR). The mRNAs for p75<sup>NTR</sup> and TrkC-TK<sup>+</sup> were present in both actively myelinating sciatic nerve and cocultures (Fig. 1A). TrkB-T1 mRNA was also detected in sciatic nerve and in cocultures, whereas only a minute amount of TrkB-FL was observed. Myelination in the sciatic nerve, determined by the expression of the major myelin

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**Fig. 1.** p75<sup>NTR</sup>, TrkB, and TrkC are present during development in sciatic nerve and SC/DRG cocultures. (A) Expression of neurotrophin receptors, the myelin protein PMP22, and the ribosomal protein L19 was analyzed by RT-PCR from purified rat DRG, SC, pre-myelinating SC/DRG cocultures before induction of myelination (SC/DRG 0 days), actively myelinating cocultures after 4 days of induction (SC/DRG 4 days), newborn mouse sciatic nerve, and adult mouse brain. (B) Protein levels of the myelin protein P<sub>0</sub> and the neurotrophin receptors p75<sup>NTR</sup>, TrkC-TK<sup>+</sup>, and TrkB-T1 were analyzed by Western blot in SC/DRG cocultures and in rat sciatic nerve at the times indicated. The results are presented as the mean ± SD.

