flammatory factors, including lipoxins and resolvins (*8*), are generated at injury sites and dampen neutrophil-mediated inflammation. Additionally, the production of reactive oxygen species (ROS) by host cells is necessary for defense against pathogens but also provides a cue that limits inflammation fueled by neutrophils. Chronic granulomatous disease (CGD) patients deficient in ROS production have sustained sterile (pathogen-free) inflammation, and their neutrophils form larger neutrophil swarms in vitro (*9*). Macrophages, another white blood cell present in wounds, cloak damage signals and prevent neutrophil swarming (*10*). They can also promote the reverse migration of neutrophils from sites of tissue damage (*11*).

Kienle *et al.* have identified an elegant, neutrophil-intrinsic mechanism that limits swarm size: negative regulation of the receptors that recognize self-produced swarm signals (see the figure). Notably, only receptors for intermediate-target attractants, like LTB4, are affected. Receptors for end-target attractants like C5a (complement component 5a) are unaffected. As a result, neutrophils remain sensitive to exogenous signals that promote functions necessary for pathogen killing. This self-limiting mechanism occurs through the activity of a cytoplasmic GRK-family protein, GRK2 (G protein–coupled receptor kinase 2). GRK proteins are cytoplasmic enzymes that phosphorylate activated G protein–coupled receptors. This phosphorylation results in receptor desensitization and, in some cases, internalization. Internalized receptors can be degraded or returned to the cell surface, as is the case with the CXCL2 receptor but not the LTB4 receptor. In this way, a cell can dynamically alter its sensitivity to various ligands.

It is tempting to think that by increasing swarms, more neutrophils would reach the wound, and the magnitude of their combined defenses would easily overcome the threat. This is not always the case. A particularly surprising observation by Kienle *et al.* is that persistent swarming did not result in better control of infection in mice with neutrophils lacking GRK2. In these animals, both increased cell speed and larger neutrophil clusters were observed at the wound. Without an adequate pause in motility, these neutrophils could not mount a successful defense response. Thus, a cell-intrinsic mechanism ensures that neutrophils successfully transition from the recruitment phase to the defensive phase. The study also suggests that a bigger swarm is not necessarily better in clearing pathogens. This is similar to what is seen in patients with CGD, where increased neutrophil swarming is associated with impaired microbial killing, although these effects may not be related.

Not all cellular swarms are beneficial to the body. Excessive or inappropriate neutrophilic inflammation is associated with debilitating diseases, including CGD and other autoinflammatory disorders. Additionally, the collective migration of cancer cells can drive metastasis, and self-propagating swarming may promote this behavior. Given the variety of contexts in which swarms occur, insights into their termination are of great general interest as well. Insect swarms can be both beneficial (bees) and devastatingly costly to agriculture (locusts). For decades, engineers and computer scientists have worked to incorporate aspects of swarm intelligence into technological applications. Robot swarms show promise in a variety of contexts, including environmental remediation (*12*).

At first glance, the model of self-control suggested by Kienle *et al.* seems deceptively simple. Long-range alarm signals trigger self-propagating neutrophil swarms that converge at sources of infection or injury. However, these swarms are intrinsically transient if receptors become desensitized as ligand concentrations increase close to target sites. The selectivity of this response enables neutrophils to prioritize signals that induce effector functions essential for clearing the infection and limiting collateral tissue damage. It is unclear how neutrophils prioritize signals to arrive at and depart from damaged tissues simultaneously (*4*). A tissue factor—myeloid-derived growth factor—has recently been identified that promotes neutrophil reverse migration and limits neutrophil inflammation (*13*). The signals that orchestrate and integrate these types of complex behaviors in homeostasis and disease are still largely unknown and likely involve the combined influence of self-propagated and exogenous cues. ■

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SIGNALING

Preparing macrophages for the future

Temporal dynamics of a key immune transcription factor shape the epigenome and future cell responses

By **Nagarajan Nandagopal, Ashwini Jambhekar, Galit Lahav**

Cells live in complex environments and must respond appropriately to extracellular signals. Such responses often involve regulating the expression of hundreds of genes through transcription factors (TFs). Many TFs are activated by multiple signals and regulate the expression of distinct genes in response to each. How extracellular information is "encoded" in TF activity and subsequently "decoded" to orchestrate gene expression is a fundamental question in biology. Intriguingly, some TFs such as nuclear factor κB (NF-κB) and p53 encode signaling information in their temporal dynamics (*1*). Studies have shown that signaling dynamics can be used to control the induced expression levels (*2*), types (*3*), or ratios (*4*) of genes. On page 1349 of this issue, Cheng *et al.* (*5*) report a previously unknown role for TF dynamics: They show that NF-κB dynamics not only control how genes respond in the present but also reconfigure the cell to control gene expression in response to future stimulation.

In macrophages, which act as sentinel cells of the innate immune system, NF-κB dynamics was shown to encode signal identity. For example, activation by toxins released from invading bacteria leads to sustained NF-κB activity, whereas activation by inflammatory signals from other immune cells leads to oscillations in NF-κB activity (*6*, *7*). Rather than focusing directly on which genes' expression are induced by NF-κB, and by how much, Cheng *et al.* analyzed the NF-κB "epigenome," a set of factors that influences the potential for expression of genes to be induced upon TF activation.

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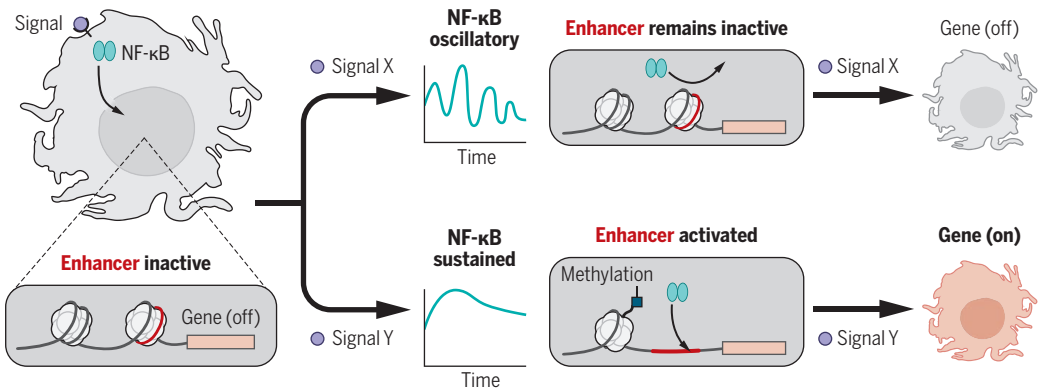
Signal-specific nuclear factor κB dynamics

In macrophages, the transcription factor nuclear factor κB (NF-κB) can be activated either with oscillatory or sustained temporal dynamics, depending on the signal. Under oscillatory dynamics, inactive enhancers remain nucleosome-bound. By contrast, sustained dynamics can remodel enhancers through nucleosome displacement and histone 3 Lys^4 (H3K4) methylation. After a second exposure to the same signal, enhancers that were activated by sustained NF-κB dynamics induce expression of their associated genes, whereas these genes remain inactive under oscillatory dynamics.



The status of "enhancers," DNA sequences bound by TFs to control gene expression, is a particularly important aspect of the epigenome. In some cases, enhancers are bound by nucleosomes, which obstruct TF binding. Such enhancers cannot activate genes until the nucleosomes are displaced. Other enhancers are readily available for TFs to bind and facilitate gene expression and are considered to be active. The set of genes whose expression can be induced and their expression levels depend on how these two classes of enhancers are distributed in the genome. This layer of regulation provides a means for different cell types to regulate different sets of genes by using the same TFs. It also enables a cell to change its response to the same signal and thus adapt to the environment.

Cheng *et al.* investigated the status of NF-κB–bound enhancers in macrophages derived from mice after stimulating the cells with signals previously shown to activate NF-κB with different dynamics. They found that signals that led to sustained NF-κB activity increased the number of active enhancers compared with signals that produced oscillatory activity. To directly test whether this difference was due to NF-κB dynamics, the authors blocked normal oscillations by disengaging a well-characterized negative-feedback loop in the NF-κB pathway, which did not alter other features of the response such as activation intensity. Removing NF-κB oscillations in this manner increased the number of active NF-κB–dependent enhancers. Thus, although both oscillatory and non-oscillatory NF-κB dynamics induce gene expression through already active enhancers, non-oscillatory activation also reconfigures the epigenome by activating additional enhancers.

What effect does this have on the cell? Because the epigenome defines the set of genes whose expression can be induced by NF-κB, one scenario is that by increasing the number of active enhancers, non-oscillatory NF-κB dynamics change the set of genes that could respond to future stimulation of the pathway. Indeed, analysis of gene expression after a second phase of sustained NF-κB activation showed induction of hundreds of genes not induced upon repeat stimulation of oscillatory NF-κB (see the figure). Thus, it appears that the functions of NF-κB as an inducer

"...NF-κB dynamics not only control how genes respond in the present but also reconfigure the cell to control gene expression in response to future stimulation."

of gene expression and as a modifier of the epigenome (likely in conjunction with other proteins) are separable according to its temporal dynamics. On the basis of modeling, the authors suggest that this separation is achieved through the many steps involved in unwrapping inactive enhancers from nucleosomes, which demands persistent nuclear NF-κB—that is, non-oscillatory dynamics.

Modifying the epigenome provides NF-κB with the ability to record its activation and affect future cellular responses. Other forms of this phenomenon, broadly called epigenetic transcriptional memory, have been described (*8*), including in macrophages (*9*). It will be interesting to compare enhancer activation by NF-κB to other mechanisms of transcriptional memory, in terms of stability, fidelity, and lifetime. At a mechanistic level, it remains to be determined how NF-κB activity duration translates to nucleosome displacement and how the proposed multistep reaction mechanism compares with classic kinetic proofreading (*10*) or circuit-based mechanisms for duration sensing (*11*). The responses of target genes to other TFs are sensitive to oscillation frequency and duration (*12*); the relevance of the proposed mechanism to these pathways will be worth investigating.

At the level of the cellular response, the functional consequences of organizing NF-κB target genes into multiple cohorts are currently unclear. Does it lead to macrophage adaptation to particular immune threats, as suggested previously (*9*, *13*)? Maybe this response can be "tuned" by changing the duration or intensity of the initial stimulus. The discovery by Cheng *et al.* thus opens up several new lines of inquiry that will help to better understand how NF-κB orchestrates specific responses to different stimuli. More generally, other oscillatory TFs have known roles in regulating the epigenome (*14*, *15*). It will be fascinating to see whether similar principles apply to decoding dynamics and controlling the activation of target genes in other systems. ■

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