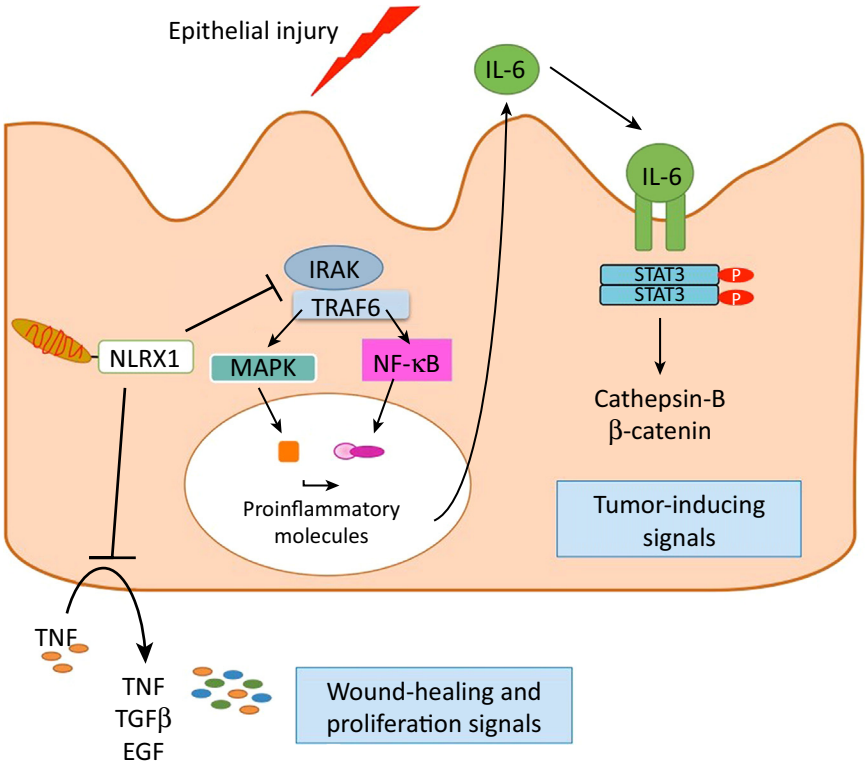

Figure 1. NLRX1 as a Cell-Intrinsic Tumor Suppressor in Intestinal Epithelial Cells (IEC). NLRX1 acts as a cell-intrinsic tumor suppressor in IEC by inhibiting several key tumor-promoting pathways. Following intestinal injury, NLRX1 inhibits epithelial proliferation by decreasing responsiveness to TNF and limiting the expression of wound-healing molecules. In addition, NLRX1 inhibits the activation of the NF-κB and MAPK signaling pathways, which leads to reduced activation of the IL-6/STAT3 axis and downstream tumor-promoting molecules. Abbreviations: EGF, epithelial growth factor; IRAK, IL-1 receptor-associated kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; NLRX1, NOD-like receptor X1; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TNF, tumor necrosis factor; TRAF, TNF receptor associated factor.

several cohorts of patients with CRC revealed significantly decreased expression of NLRX1 in CRC samples compared with healthy controls [4,5]. Although, the basis of NLRX1 downregulation in CRC remains to be determined, this observation lends further support to the concept of personalized medicine; for example, by targeting the IL-6/STAT3 axis in CRC with reduced NLRX1 expression. Such agents are already approved for the treatment of human disease and have shown promise for the treatment of chronic intestinal inflammation [10].

These results reveal another facet to the regulatory functions of NLR members in nonhematopoietic cells, such as IEC. Indeed, recent evidence suggests IEC-intrinsic roles of many NLRs for protection against intestinal tumorigenesis, including NLRP1b, NLRP6, NLRP12, and NLRC4/NAIPs [2,3]. Several mechanisms have been proposed to contribute to these protective effects, including activation of inflammasomes, regulation of secretory pathways and microbiota composition, extrusion of IEC from the epithelium, and inhibition of inflammatory responses. The current studies add NLRX1 to this group and highlight its role in the intrinsic regulation of IEC proliferative responses during wound healing and tissue repair. Further studies of how distinct NLR signaling circuits are integrated within IEC should continue to identify new avenues for the treatment of human intestinal disease and cancer.

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Spotlight

Immune Response Signaling: Combinatorial and Dynamic Control

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Macrophages mount complex responses to pathogens. Although several key signaling pathways have been identified, it remains unclear how they work together to provide specificity. In a recent paper, Gottschalk *et al.* report that

differential dose–response behaviors of the NFκB and MAPK pathways allow dose-specific gene expression programs.

How immune activation is controlled continues to be an important research question; a well-functioning immune system, or its dysregulation, is a key determinant of human health and disease. Immediate recognition of pathogen-derived substances or antigens by innate and adaptive immune receptor molecules has been studied in atomic detail to provide some clarity on the basis of molecular specificity and the distinction between self and nonself. However, our understanding of the specificity of the signaling pathways that are activated by these receptor–ligand interactions lags far behind.

Progress has been made in identifying the molecular components of prominent signaling pathways such as those controlling the activities of the transcription factor NFκB, interferon regulatory factors (IRFs), and kinases of the c-Jun N-terminal kinase (JNK) and MAPK/ERK families. Thus, recent studies have been able to focus on how the signaling characteristics of each pathway are generated. Experimental approaches that allow single-cell resolution, temporal sequence, and true quantitation are revealing new, emergent properties of pathways that in turn determine their biological function. However, these studies have largely been focused on the functioning of single signaling pathways.

The biological response involves the coordinated functioning of several pathways and combinations of pathways are known to be activated by the ligands of pathogen-sensing receptors or immune receptors. Also, immune response genes whose expression is upregulated by them are thought to integrate combinations of transcription factors (Figure 1).

The hypothesis of a combinatorial signaling code posits that regulatory modules encode information about the environment in combinations of intracellular signals (such as kinase activities) and effector-associated regulatory modules (such as gene regulatory modules) decode combinations of intracellular signals to provide a response (Figure 1). However, diagrams of immune response networks show that two prominent pathways, NFκB and MAPK, emanate from virtually all immune activation receptors, whether they are pathogen, cytokine, or antigen sensors [1].

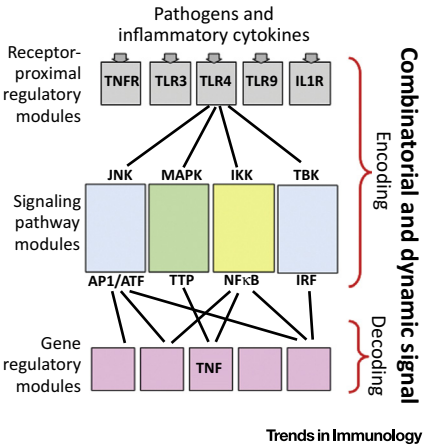

Figure 1. Encoding and Decoding of Inflammatory Signals. Schematic of the immune response signaling network, emphasizing a modular structure. In this view, receptors that sense the presence of pathogen-derived substances or inflammatory cytokines engage receptor-proximal regulatory modules that trigger the activation of several signaling pathways. These pathways regulate the activities of transcription factors or other regulators that combine in gene regulatory modules to control immune response gene expression. For example, endotoxin binding to Toll-like receptor 4 (TLR4) is known to trigger the activation at least four kinases; two downstream effectors are known to control tumor necrosis factor (TNF) protein expression [5]. Gottschalk *et al.* show that the endotoxin dose–response behaviors of MAPK and NFκB are quite distinct, supporting the view that cells encode the presence of pathogens in both combinatorial and dynamic intracellular signaling events, allowing gene regulatory modules to decode these to produce gene expression that is both ligand and dose appropriate. TNFR, TNF receptor; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; IKK, IκB kinase; TBK, TANK-binding kinase; AP-1, activator protein 1; ATF, activating transcription factor; TTP, tristetraprolin; IRF, interferon regulatory factor.

A recently published paper by Gottschalk *et al.* shows that these two prominent signaling pathways of NFκB and MAPK have differential dose–response behaviors; thus, the control of dose–response behavior allows cells to express different sets of genes at different doses of the same ligand [2]. Specifically, the authors found that tumor necrosis factor (TNF) protein production in individual cells as assayed by flow cytometry was highly thresholded, a phenomenon also described as ultrasensitivity, despite the fact that NFκB-responsive transcriptomes could be observed even at subthreshold concentrations. As TNF production is dependent not only on NFκB-driven transcription but also on MAPK-driven mRNA splicing, mRNA half-life stabilization, and protein processing, the authors hypothesized that the MAPK signaling pathway may have a higher threshold than the NFκB pathway. Probing IκBα degradation and MAPK/p38 or ERK phosphorylation showed differential dose responses, with the dose response of TNF production resembling that of Erk or p38 phosphorylation more than that of IκBα degradation. Interestingly, thresholded dose–response behavior in the expression of other genes was generally correlated with MAPK dependence, and while gene expression of macrophages obtained from human donors may differ in some aspects, the thresholded MAPK dose response was generally conserved.

The present paper emphasizes the importance of understanding the molecular mechanisms that function together in producing even simple emergent systems properties such as dose–response behavior. Within the Toll-like receptor (TLR) pathway, recent studies have begun to describe how signaling behavior may be 'encoded' and 'decoded'. As multiple molecular mechanisms function together and must be considered quantitatively, mathematical modeling is a hallmark of such mechanistic studies. Although this study does not provide extensive time-course information, dose–response behaviors are the result of kinetic molecular mechanisms that also produce dynamic

behavior. For example, one recent study explored the mechanisms by which TLR 'signal encoding' is thresholded and identified the formation of an oligomeric signalosome, the Myddosome, as a source of this ultrasensitivity [3]; a previous study showed substantial ultrasensitivity in the MAPK cascade itself [4] that is not present in the NFκB signaling module.

By contrast, 'signal decoding' may be mediated by both nuclear and cytoplasmic mechanisms. Specifically, in the case of TNF, mRNA processing, half-life, and translation and protein processing and secretion were shown to be controlled by MAPK [5]. Consistent with the present study, the key role of MAPK in TNF production renders NFκB activity a poor predictor of TNF production at the single-cell level [6]. However, these studies do not shed light on the differential dose–response behaviors of the MAPK and NFκB pathways and further quantitative molecular mechanistic studies particularly in the MAPK pathways are required to produce well-founded mathematical-model research tools. When such experimental and modeling frameworks are established, future studies may investigate whether the dose–response behavior might be stimulus specific such that different ligands might produce different gene expression responses, particularly at non-saturating concentrations.

The present paper challenges future studies to integrate the combinatorial and dynamic signaling codes [7] to derive a mechanistic understanding of how combinations of dynamic signaling events are encoded by signaling pathways and decoded by receptors. A first framework for TNF [5] provides one example (although lacking single cell-resolution), revealing that TNF's autocrine functions are largely restricted to pathogen ligands that do not engage the TIR-domain-containing adapter inducing interferon-β (TRIF) signaling pathway. It will be interesting and important to discover how combinatorial engagement of distinct signaling pathways, each with specific dynamic regulation [8], provides specificity. Such analyses, at the single-cell level, also allow the application of information theoretic techniques to characterize the performance of signaling networks. Considering the NFκB pathway alone, the information-carrying capacity in response to TLR4 appears to be more limited than anticipated due to substantial cell-to-cell heterogeneity [9,10]. Initial analysis of MAPK signaling at a single time point in response to TNF indicated little improvement [9]. However, within the framework of both combinatorial and dynamical coding, the information-carrying capacity of pathogen-responsive signaling may be much greater. Thus, with the advent of live-cell experimental probes that allow measurement of the activities of multiple pathways simultaneously, future work may address the mutual information benefit of a combinatorial code within a noisy cellular environment.

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