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Recent Progress Using Systems Biology to Better Understand Molecular Mechanisms of Immunity

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Abstract

The immune system is composed of multiple dynamic molecular and cellular networks, the complexity of which has been revealed by decades of exacting reductionist research. However, understanding of the immune system sufficient to anticipate its response to novel perturbations requires a more integrative view, or systems approach, to immunology. While methods for unbiased high-throughput data acquisition and computational integration of the resulting datasets are still relatively new, they have begun to substantially add to our understanding of immunological phenomena. Such approaches have expanded our view of interconnected signaling and transcriptional networks and have highlighted the function of non-linear processes such as spatial regulation and feedback loops. In addition, advances in single cell measurement technology have demonstrated the potential sources and functions of response heterogeneity in system behavior. The success of the studies reviewed here often depended upon integration of one or more systems biology approaches with more traditional methods. We hope these examples will inspire a broader range of immunologists to probe questions in a quantitative and integrated manner, to advance the common efforts to understand the immune “system”.

Keywords

global analysis; high-throughput; computational modeling; signaling networks; transcriptional networks; single-cell analysis; heterogeneity

1. Introduction

The enormous complexity of the vertebrate immune system, both with respect to its many components and the elaborate interconnections that control its operation, has created a tension in the field of immunology. On the one hand, the success of traditional experimental approaches in identifying cellular and molecular players and ascribing major functions to many of them, and the development of a rich set of ‘working models’ describing major intercellular and intracellular activities, have provided strong impetus to continue using the same tools and techniques in dissecting the system. On the other hand, some have come to appreciate that we are far from a ‘predictive’ understanding of immune function, and that the very complexity that traditional investigation has uncovered speaks to a need for additional methodologies to organize, expand, and fully utilize the mass of information in modern data sets, so that important but non-canonical aspects of immune operation can be discerned.

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The result is the development of two schools of thought about the future. One is wedded to the experimental methods and fine-grained studies that have done so well by the field for decades; the other seeks to attack the complexity problem using new computational and global-scale methods, that is, to take a systems biology approach to immunology. But we think this is a false dichotomy. The promise of “systems immunology” will not be realized until immunologists stop seeing systems biology as a distinct field of study, but rather as a complementary means of approaching immunological questions from another angle. Just as reductionist approaches fail to elucidate the complex workings of integrated systems, systems biology methods have limited value without coordination with traditional scientific methods. Systems biology approaches can range from using high-throughput techniques to measure large numbers of changes in the system, to using quantitative methods to assess the relationship between a few interconnected components. Importantly, it is not the methods themselves, such as proteomics and computational modeling, which should be considered systems biology, but the use of these tools to understand how integration of multiple non-linear processes result in emergent biological behaviors of a system.

Signals downstream of varying combinations of immune receptors are integrated to induce activation and differentiation of cells into successors with unique phenotypes and functions. In the context of complex intercellular interactions, quantitative differences in ligand stimulation, such as density and duration, have significant biological consequences, with stimulation of select receptor combinations yielding results not predicted by responses downstream of individual receptors in isolation. These processes are further complicated by the movement of cells and soluble ligands through the microenvironment and by dynamic changes in cell number as a result of cell proliferation and death during the response. Thus, the need for quantitative analysis and consideration of the interplay between network components is clear and the potential of systems biology is evident, leading to increased use of these high-throughput methods and computational approaches in immunological studies. However, is systems immunology living up to its potential?

What has the use of systems biology approaches taught us about the immune response that other immunological methods could/have not? We have previously reviewed a spectrum of systems biology methods of immunological interest and discussed insights potentially gained from these techniques (1). Here we focus on the current state of systems immunology, reviewing recent contributions of these approaches to our knowledge of signal transduction and gene regulation in particular. Examples provided in this review demonstrate that with proper experimental design, integrated and quantitative studies of immune receptor signaling highlight biological phenomena that would not be readily identified by purely reductionist approaches.

2. Signaling

Living organisms adapt their cellular processes in response to a variety of stimuli. For immune cells, receptor triggering allows sensing of pathogens and foreign antigens. Subsequent responses depend on transmission of these signals to the nucleus, resulting in changes in gene expression. This signal transduction relies on a relatively fast cascade of reversible covalent modifications (2) or via the reversible binding of allosteric regulators (3). Among the covalent post-translational modifications, phosphorylation is arguably the most studied (4). Thus, the combinatorial action of over 500 kinases and 100 phosphatases in the human genome determines the response of cells to particular stimuli (5). Understanding signaling cascades triggered by immune receptors is not only critical for our understanding the basic biology of signal transduction, but also has clear therapeutic implications. For example, defects in signaling components downstream of Toll like receptors (TLRs) have

been implicated in susceptibility to infection and multiple inflammatory disorders, resulting in increased efforts to target these pathways using novel drug therapies (6).

2.1 Stimulation induced phosphorylation

In the last few years, the development of several mass spectrometry instruments with shorter cycle times and higher resolving power (7, 8), as well as software tools allowing reliable identification and quantification of the data sets, have led to an explosion in the number of protein phosphorylation sites described (9–11). Phosphoproteomics can give quantitative information on thousands of proteins, including those for which phospho-antibodies are not currently available. The predominant use of existing reagents to canonical phosphoproteins limits investigation to the same small set of molecules in the majority of experimental studies. In contrast, phosphoproteomics allows for the unbiased analysis of signaling networks and has led to the identification of novel phosphosites and phosphoproteins downstream of various immune receptors including the T cell receptor (TCR), Toll like receptors (TLRs), cytokine receptors, and chemokine receptors (12–15). The biological relevance of mass spectrometry-identified phosphorylation sites is made clear when combined with complementary experimentation. For example, the use of pulldown and siRNA knockdown assays was used to reveal protein interactions and a functional role for a novel TCR signalosome component (12). THEMIS, whose TCR-dependent phosphorylation was detected by a phosphoproteomic approach, associates with LAT and positively modulates NFAT/AP-1 and ERK activity, and subsequently IL-2 production. As another example, in a proteomic study identifying TLR-induced phosphorylation of the B-cell adaptor for PI3-kinase in macrophages, use of kinase inhibitors and RNAi showed that this protein is phosphorylated by the tyrosine kinase Syk and is a negative regulator of IL-6 and IL-10 production (16).

Other high-throughput techniques are being increasingly used in combination with mass spectrometry to identify important components of signal transduction. In a recent screen for novel substrates for IKK γ , positional scanning peptide array technology was used to identify the preferred phosphorylation motif, followed by a bioinformatics approach based on phosphorylation sites verified by independent mass spectrometry (17). This approach highlighted TRAF4 as a novel substrate and subsequent experiments showed that phosphorylation by IKK γ is required for the function of TRAF4 as a negative regulator. In a study of lymphocyte antigen receptor signaling, both mass spectrometry analysis of CARMA1 binding partners and RNAi screens in two lymphoma cell lines identified casein kinase 1 α as a regulator of NF κ B (18). TCR induced association of this serine/threonine kinase with CARMA1 is important for subsequent NF κ B activation and proliferation, and is required for constitutive NF κ B activity in a subset of lymphomas, suggesting it as a potential therapeutic target. The identification of signaling node candidates using these techniques, and the validation of candidate relevance using functional studies, provide clear evidence that these approaches are useful for highlighting unexplored pathway components, and therefore expanding our understanding of immune system organization and operation.

While it is important that groups continue to characterize the functions of specific molecular players in a response, these global datasets have intrinsic value and the potential to enhance our understanding of immune receptor induced signal transduction when they are viewed as a whole. Using knowledge based software and publicly available databases (19), datasets can be organized based on pathway commonality, molecular and cellular function, protein localization, and shared motifs, allowing discovery of signaling modules whose activation was not previously attributed to the studied receptor. A recent study in T cells identified novel TCR-responsive phosphorylation sites in protein modules involved in patterning of surface proteins, endocytosis of the TCR, and polarization of microtubules (20), while a phosphoproteomic study in macrophages highlighted several unexpected hotspots for

phosphorylation downstream of LPS stimulation, including signaling modules associated with the DNA damage response and cytoskeletal changes (13). Based on these diverse outcomes, it is clear that a view of immune receptor-induced phosphorylation events as leading primarily to gene transcription is shortsighted. Such *in silico* analysis of phosphoprotein hits also has the potential to highlight drug candidates, as suggested by a recent phosphoproteomic study of CXCR4, a co-receptor for the human immunodeficiency virus-1(21, 22), which found a subset of genes from ligand-responsive phosphosites that had previously been associated with viral replication (15).

2.2 Signaling networks and pathway cross-talk

With well-executed network analysis, proteomic datasets can elucidate how phosphorylation and protein-protein interactions facilitate information transfer downstream of immune receptor triggering. For example, when analyzed in the context of a database of protein interaction information, experimentally observed TCR-responsive phosphorylation sites suggested a system-wide influence of serine-threonine phosphorylation on protein-protein interactions that exceeded the extent that would have been predicted from the current literature (20). Compared to randomly selected phosphoproteins, proteins with TCR-induced serine-threonine phosphorylation sites had higher connectivity, suggesting an efficient route for information transfer. In another TCR signaling study (12), thorough kinetic phosphoproteomic analysis of TCR signaling revealed distinct waves of phosphorylation events, potentially suggesting close physical proximity or similar functions for these proteins. These peaks of phosphorylation broadened at later time points as the signal propagated. By also assessing the phosphoproteome in mutant mice, this study identified a subset of phosphorylation events independent of the central LAT/SLT-76 scaffold proteins, which are thought to direct most TCR-induced signaling pathways (23). Thus, in the context of cells deficient in key network components, systems biology approaches can highlight the existence of alternative signaling mechanisms.

Elucidating network interactions is necessary for understanding how signals are integrated downstream of multiple receptors. A recent study of type I interferon (IFN) regulation downstream of several types of innate stimuli screened hits from mass spectrometry for a functional role using overexpression and RNAi assays (24). Using 58 bait proteins known or suspected to be involved in IFN production, 71 novel protein-protein interactions were identified, while functional analysis revealed 22 molecules that modulated IFN expression or antiviral activity. Together these techniques not only highlighted specific processes, such as the role of TBK1 ubiquitinylation in antiviral immunity, but also facilitated the construction of an innate immunity interaction network, whereby IFN is regulated downstream of various TLRs, highlighting several nodes of potential receptor cross-talk (24). While shared network components suggest that two receptors or pathways could influence one another, assessing signaling upon stimulation with multiple ligands is required to reveal mechanisms that are defined by the interaction of two responses. This was clearly demonstrated during a large-scale survey of pathway interactions which assessed 22 distinct ligands, alone and in pairwise combinations (25); this study revealed multiple novel interaction mechanisms responsible for non-additive responses, and suggested that a relatively small number of such mechanisms could facilitate context-dependent responses downstream of varying combinations of stimuli.

2.3 Spatial regulation of signaling

Large-scale datasets and databases of phosphorylation kinetics and protein-protein interactions facilitate the construction of complex signaling networks. However, these networks do not explain the impact of the concentration and spatial distribution of specific signaling intermediates on the behavior of the system. High-resolution microscopy has been

used to reveal dynamic changes in the organization of membrane proteins and signaling components in response to receptor triggering (26, 27). Due to recent advances, fluorescence cross correlation spectroscopy can be used to measure protein numbers, protein-protein interactions or diffusion rates and dissociation constants within a cell. This approach has been used to measure the diffusion constant of CD3zeta and LAT in live T cells and to demonstrate their co-movement in cells activated on bilayers (28). These types of quantitative measurements are essential for the construction of mechanistic models of T cell activation.

Construction of dynamic mathematical models using quantitative data is a valuable approach to test and refine hypotheses, and thus to elucidate mechanisms by which complex non-linear processes determine the response to immune receptor stimulation. Simulation approaches can describe a biochemical network in terms of concentrations, relying on the mass action law and diffusion (29–31), or as single particles or agents (29, 30, 32), modeling the interactions and trajectories of individual proteins. While spatially-resolved agent-based simulations are the closest match to the underlying biochemical reality, differential equation-based models have a richer theory for analyzing the system's behavior. It is important to note that the same model can in principle be used in both mass action and agent-based simulations, and both require the same set of experimentally measured inputs; reaction rates and diffusion constants. Rules-based models (30–32) allow for reduction of model complexity. For example, the association of a MAPK with a scaffold can be described by two rules specifying the on-rates for the interaction for the phosphorylated and unphosphorylated MAPK, independent of rates for complexes between the scaffold and its other binding partners. This simplifies the process of specifying variants that represent alternative hypotheses about a signaling mechanism.

The large number of parameters required as inputs is the major criticism brought forward against quantitative modeling approaches. While it is true in general that models with too many degrees of freedom are not informative, experimental constraints and direct measurements of parameters can constrain the model enough to distinguish between different hypotheses bearing on the connectivity of a signaling network. For example, spatially-resolved concentration ratios, relative amounts of phosphorylated intermediates, and intercellular gradients for multiple components of the MAPK cascade were sufficient to build a detailed model the MAPK activation in yeast (31). While a number of parameter remained undetermined, this approach was able to distinguish between alternative models of the signaling cascade. The same authors also used single-cell imaging data of biochemical polarization for a detailed study of chemogradient sensing (33). By comparing model predictions with experimental results, this study showed that the observed biphasic polarization response of to a chemoattractant gradient is not compatible with the accepted notion of a global regulation of the inhibitory signaling components, but instead requires a local regulation of the phosphatase PTEN.

3. Transcription

Stimulation of immune receptors and subsequent transcriptional activation results in diverse gene products, ranging from proteins that target an infection, such as complement factors and cytotoxic effector molecules, those which impact the migration and differentiation of additional cells, chemokines and cytokines, to products controlling proliferation and cell death. The range of mechanisms controlling gene expression extends well beyond activation of transcription upon binding of inducible transcription factors to specific promoters (34). Thus systems biology approaches are useful in elucidating the complex layers of gene regulation (35). Transcriptome-wide gene expression analysis using microarrays has become a well-established technique to identify candidates or look at broad gene expression patterns,

allowing investigators to visualize global differences in transcriptional programs downstream of a component of stimulus of interest. With new technology (e.g., RNA-Seq) and analytical methods, the field is moving toward a more complete view of transcriptional regulation. We highlight examples where integration of multiple systems biology approaches yielded insight into how genes are regulated downstream of immune receptor triggering.

3.1 Transcriptional networks

Identifying the full set of genes involved in a response remains an important goal even in well-studied signaling responses in immunity. As with signaling events, temporal dynamics of gene expression must also be taken into account. In an early example of a systems immunology approach, microarray-based transcriptome analysis was employed to measure cellular responses at multiple time points following LPS stimulation of macrophages (36). Sets of genes with similar expression kinetics were clustered, and these clusters were mined for transcription factors induced early in the response and computationally scanned for the presence of *cis*-regulatory elements. This analysis led to the identification of activating transcription factor 3 (ATF3) as a transcription factor that is both expressed early in the response to LPS, and which has putative binding site over-representation within a cluster of induced genes. Computational analysis was used to identify a gene expression network regulated by ATF3, which was partially verified experimentally; macrophages deficient in ATF3 demonstrated its negative regulatory role in LPS-induced signaling. Thus, integration of system-wide gene expression measurements and computational analysis led to novel insights into the innate transcriptional network.

Computational modeling is a useful tool for uncovering how transcription factor dynamics determine the quality of a response. For example, by simulating LPS-induced IL-6 production, modeling was used to elucidate an NF- κ B-ATF3-C/EBP β regulatory circuit; NF κ B induces expression of both IL-6 and C/EBP β , which positively regulates IL-6 in only coordination with NF κ B, while ATF3 attenuates transcription of both genes (37). This model predicted that only prolonged stimulation would result in robust *Il6* transcription and that this would be dependent on C/EBP β . These predictions were experimentally validated and together these approaches revealed a mechanism by which feed-forward motifs facilitate discrimination between transient and persistent stimuli. In the B cell lineage, computational modeling helped to elucidate how gene regulatory networks allow the intensity of B cell receptor (BCR) signaling to modulate cell fate (38). It had previously been demonstrated that IRF-4 was expressed in a graded manner in stimulated B cells and that higher concentrations of this transcription factor induced expression of Blimp-1 and plasma cell differentiation (39). Using a combination of mathematical modeling and experimental approaches, a subsequent study showed that the strength of BCR stimulation determined the levels and dynamics of IRF-4 expression, and that this expression was sufficient to predict B cell fate (38). Modeling approaches have also been used extensively to study the regulation of NF- κ B and have been instrumental in characterizing the molecular mechanisms shaping NF- κ B dynamics, including negative feedback loops and cross-talk between multiple NF- κ B activating pathways (Reviewed in (40)).

The integration of multiple systems methods allows for the inference of large-scale gene networks. One particularly extensive analysis of an immunological gene regulatory network was carried out using a combination of microarray and RNAi perturbation to dissect TLR responses (41). Based on the expression profiles of mouse dendritic cells following stimulation with several distinct TLR ligands, candidate regulators and response signature genes were selected using a computational approach. RNAi perturbation was subsequently carried out to see how these regulators affect the expression of the signature genes. This study revealed the global topology of an immune transcriptional network, with 24 hub

regulators that controlling more than 25% of the 118 signature genes and 76 specific regulators each impacting the expression of 4 to 25 genes. Further analysis revealed mechanisms by which inflammatory and antiviral programs regulate each other through the cross-inhibition of transcription factors, and suggested that the network architecture consists of largely coherent feed-forward circuits, which, as discussed above, may facilitate discrimination between persistent and transient stimulation (37). The approaches utilized in this study have recently been reviewed by the authors (35).

Systems methods have also yielded insights into the *in vivo* response to LPS in humans. Microarrays were performed on peripheral blood leukocytes isolated from human subjects at 2, 4, 6, 9, and 24 hours post injection of LPS (42). Pathway analysis was used to describe the response, revealing changes in interesting gene modules including those related to mitochondrial function, protein synthesis and degradation. Another group expanded upon this analysis and utilized this dataset to perform Network Component Analysis, a computational method for deriving regulatory networks from transcriptomic data (43, 44). This approach enabled assembly of a dynamic network of transcriptional regulation encompassing 10 transcription factors and 99 target genes. In yet another example, transcriptional control of macrophage activation was elucidated by combining microarray and motif scanning approaches (45). The rapid development of analytical methods is opening new avenues for integration of dynamic transcription measurements and computational tools to map transcriptional networks and identify novel regulatory interactions.

3.2 Indirect regulation of gene expression

The use of systems biology approaches has significantly advanced our understanding of how transcription is regulated beyond the direct activation and inhibition of transcription factors, and has been especially instrumental in explaining the lineage specific transcriptome (44–46). For example, these approaches recently revealed that lineage-determining transcription factors also play an important role in shaping the transcriptomic response to stimulation. In an effort to identify LPS-induced enhancers in macrophages, a genome-wide chromatin immunoprecipitation (ChIP-Seq) analysis of p300 binding sites was performed (46). The p300-marked enhancer sites after LPS stimulation were enriched for PU.1, an important transcription factor for macrophage lineage commitment. Further analysis showed that this PU.1 binding is pre-existing in unstimulated macrophages and is coincident with the active chromatin modification H3K4me1, suggesting that lineage commitment transcription factors maintain an open chromatin structure for specific sets of genes thus determine the lineage specific responses to perturbation. This concept is consistent with later work showing that TLR4 signaling-responsive genes show histone modifications associated with activation and the presence of RNA polymerase II, even under unstimulated conditions (47). In addition, a recent study using ChIP-Seq for genome wide identification of PU.1 binding sites in B-cells and macrophages revealed that there is very little overlap in the two cell types (48). The specificity is determined by the differences in co-factors; PU.1 cooperates with E2A in B cells, and with C/EBP in macrophages. This mechanistic understanding of lineage specific transcriptome determination wouldn't have been achieved without a genome-wide analysis.

MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression (49) and their importance has been demonstrated in multiple immune pathways (50, 51). A single miRNA may target multiple genes, and a single gene may be targeted by multiple miRNAs, creating a complex interaction network ideal for systems approaches (reviewed in (52)). By utilizing integrated miRNA, transcriptome, and proteome profiling with miRNA target prediction, a recent study examined the role of miRNA-mediated gene regulation in T cell activation (53). With these datasets, a large network of miRNAs was uncovered that regulates key aspects of T cell activation, including cell proliferation, survival, and ERK/MAPK signaling. For

example, knockdown of miR-221, which was predicted to have a target set enriched in proliferation related genes, resulted in increased BrdU incorporation upon stimulation. While the computational target prediction used may lead to a high number of false positives, experimental confirmation of small subset of the identified regulatory miRNAs suggests that the identified network of miRNAs involved in the activation response of T cells may indeed be extensive. Thus, in addition to yielding specific hypotheses to be explored further, this global view has highlighted an additional network of gene regulation in stimulated T cells.

4. Heterogeneity

Our current understanding of the molecular network within a cell is largely based on data derived from the average expression of molecules in thousands or millions of cells. While this technique has led to important insights into the systems biology of immune cells, recent technological advancements have allowed an increased appreciation of the degree of heterogeneity occurring within seemingly homogenous immune cell populations. Heterogeneity in gene expression may be due to several factors, such as the stochastic nature of molecular interactions, the burst-like nature of transcription, and deterministic properties such as environmental variation and the presence of distinct functional subtypes within a seemingly homogenous population (54–60). The reliance of systems-level approaches on mixed population data has been one of necessity: it has not been possible to simultaneously measure a large fraction of the network using single cell technologies. New advancements in the analysis of mRNA and protein are now allowing researchers to break the single cell barrier.

4.1 Response heterogeneity

Advances in microfluidic technology have allowed a large number of genes to be quantified in single cells by qPCR (61). This approach was recently applied to the assessment of vaccine-induced T cell responses, and this examination of gene expression at the single cell level revealed the presence of distinct subsets of responding CD8⁺ T cells (62). These subsets could not have been discriminated using population level gene expression measurements. These data also reveal the high degree of gene expression heterogeneity among responding T cells in both the central and effector memory compartments, highlighting the great deal of complexity of responses within these populations. The increased sensitivity afforded by considering responses at the single cell level may allow for an improved understanding of the correlates of protection, and thereby allow the development of more effective vaccines and ultimately a deeper understanding of how the immune system functions.

Single cell analysis has also revealed a high degree of T cell heterogeneity at the protein expression level. A microfluidic chip capable of quantifying release of twelve proteins from approximately 10⁴ single cells in parallel was applied to the analysis of polyfunctionality in cytotoxic T lymphocytes (63). The data revealed a large degree of variation in terms of co-expression of sets of the measured proteins. The co-expression combinations were non-random, as not all combinations that would be expected by chance were found, suggesting that the observed differences may represent high-dimensional polyfunctionality in these cells. A similar microfluidic approach was used to probe the relationship between cytokine secretion and cytolytic activity in HIV-specific T cells (64), revealing discordance between IFN γ secretion and killer function, with only a minority of reactive cells expressing both IFN γ and lytic ability. This is a key insight into CD8⁺ T cell biology that could have important implications for how vaccine effectiveness is measured.

Flow cytometry has long provided immunologists with the ability to assess protein expression at the single cell level, and recent advances in this field have increased its value

for systems biology approaches (65). The ability to measure multiple markers per cell is key to the assessment of molecular networks acting at the single cell level, and thus multiparameter flow experiments have enabled correlation analysis, using each of the thousands to millions of cells collected as individual data points (66). The application of “mass cytometry”, a melding of mass spectrometry and traditional flow cytometry, holds the promise to extend the number of parameters/cell from 20 up to 40–60 (67). CyTOF has been applied to the analysis of CD8⁺ T cells, and revealed that virus specific T cells are found in distinct niches within a continuum of cell phenotypes; these data describe the entire circulating CD8⁺ compartment at once, allowing specific subsets to be identified with the phenotypic “cloud”, and at the same time deepen our understanding of CD8⁺ T cell differentiation as a gradient of phenotypes and as a complex set of niches (68). The large scale of measurement by CyTOF, in terms of both the number of parameters and the number of cells measured, make this technique very powerful for such data-driven approaches to understanding immune compartment heterogeneity.

4.2 Sources of response heterogeneity

The use of live-cell microscopy has provided significant insight into variables influencing response heterogeneity. In a recent study using imaging to follow NF- κ B activation after TNF stimulation, responses at the individual cell level were observed to be digital, with higher doses of TNF yielding a higher percentage of responding cells in the population (69); therefore measurement of the mean level of activation for all cells in the population yields a typical logarithmic dose-response curve to TNF. A more recent analysis identified variation in cell size and shape, NF- κ B levels, and I κ B translation and degradation rates as key factors in determining this type of NF- κ B response heterogeneity (70). Other studies suggest that heterogeneity can result from stochastic processes. For example, a study of B cell fate determination used live-cell microscopy and mathematical modeling to provide evidence that isotype switch and plasmablast differentiation are guided by intracellular stochastic competition (71). Another recent report demonstrated that viral induced type I interferon (IFN) gene expression occurs as a stochastic digital switch due to cell intrinsic noise (72). Computational modeling and experimental validation showed that, through paracrine signal propagation, the overall system topology lead to a homogenous functional outcome, leading the authors to suggest that variability in single cell IFN responses may function to prolong the antiviral state of the population. While intercellular communication can facilitate coherent responses, it may also drive heterogeneity in the context of environmental variability. For example, the cell-cell heterogeneity pattern of SV40 infection was largely explained by the population context of the cell, such as its position relative to other cells and cell density (73).

Cell-to-cell heterogeneity in protein concentration can have a significant functional impact on responses downstream of receptor ligation. A recent study (74) assessed the influence of variable protein concentration on TRAIL induced apoptosis using experimentally observed distributions of protein expression levels and *in silico* experiments based on a previously published model of apoptosis (75). These experiments simulated the time between treatment with TRAIL and permeabilization of the mitochondrial outer membrane and found that the concentration of specific proteins had more impact than others on the dynamics of apoptosis and that the extent of the effect was dependent on the concentration of other proteins (74). Such was the case with Bcl-2, which impacted cell fate with even a small increase in levels, in a manner sensitive to the levels of interacting proteins; this was confirmed experimentally. These results demonstrate the interdependence of protein abundances and show how slight differences in protein levels can impact response heterogeneity.

In the case of T cell activation, concentrations of specific proteins determine the sensitivity of the TCR. A combination of computational modeling and experimental methods was used

to demonstrate that a competing ERK mediated positive-feedback loop and SHP-1 mediated negative-feedback loop tune the threshold for T cell activation (76). The mathematical model predicted that this threshold is highly sensitive the levels of SHP-1 expression. Indeed, subsequent studies from these authors showed the impact of signaling protein heterogeneity on response variability; levels of CD8 modulate antigen responsiveness in an analog manner, while levels of the phosphatase SHP-1 digitally regulate responses downstream of the TCR (77). Stochastic differences in the expression of these proteins correlated with diverse degrees of TCR induced ERK activation within a monoclonal population of naïve T cells, which shows that the range of protein expression detected by flow cytometry is relevant to individual cell performance. The frequency of T cell hypo- or hyper-responsiveness was limited by the co-regulation of CD8 and SHP-1, demonstrating how coordination of protein changes across a network may restrain response variation resulting from differential expression of a single pathway component.

Further understanding of the functional roles of cellular heterogeneity in the immune response should elucidate the inter- and intra-cellular networks underlying the biology of the system. Cell-cell heterogeneity may play a role in providing robustness of the population, as is readily observed in model systems such as yeast and bacteria, in which heterogeneity maintains a fraction of the population in a state that, while reducing fitness under current conditions, becomes favorable under certain future conditions, such as changing nutrient sources (78, 79). It is conceivable that this “bet-hedging” plays an important role in providing robustness to the functioning of the immune system, as a perfectly predictable system would be more easily exploited by rapidly evolving pathogens. A mathematical model of the NF- κ B negative feedback loop suggests that this network motif functions to produce greater cell-cell variability in NF- κ B nuclear translocation, which serves to stabilize overall NF- κ B activity at the population level (80). The studies discussed in this section suggest ways in which cell-cell variability may be functionally tuned and exploited by the immune system. Future advancements in single cell measurement will clarify our understanding of the importance of regulated cell-cell heterogeneity in determining subsequent responses.

5. Concluding remarks

As demonstrated in this review, systems biology approaches have made significant contributions to our understanding of immunology. Although we remain far from the goal of true systems-wide descriptions at all levels of a response linked to comprehensive, dynamic computational models capable of predicting immune behavior, the techniques and technologies of systems biology continue to advance at a rapid pace. We believe the recent successes highlighted here will increase the acceptance of these approaches among the wider immunology community, which will lead to even greater success and innovation. The challenge of understanding complex systems is not confined to the niche of “systems immunology”, but is the fundamental challenge faced by all immunologists. We hope the work reviewed here inspires others in the field to push forward toward our goals of understanding the immune “system”.

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References

1. Germain RN, Meier-Schellersheim M, Nita-Lazar A, Fraser ID. Systems biology in immunology: a computational modeling perspective. *Annu Rev Immunol.* 2011; 29:527–585. [PubMed: 21219182]
2. Deribe YL, Pawson T, Dikic I. Post-translational modifications in signal integration. *Nat Struct Mol Biol.* 2010; 17:666–672. [PubMed: 20495563]
3. Shen A. Allosteric regulation of protease activity by small molecules. *Mol Biosyst.* 2010; 6:1431–1443. [PubMed: 20539873]
4. Hunter T. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell.* 1995; 80:225–236. [PubMed: 7834742]
5. Cohen P. The role of protein phosphorylation in human health and disease. The Sir Hans Krebs Medal Lecture. *Eur J Biochem.* 2001; 268:5001–5010. [PubMed: 11589691]
6. Hennessy EJ, Parker AE, O'Neill LA. Targeting Toll-like receptors: emerging therapeutics? *Nat Rev Drug Discov.* 2010; 9:293–307. [PubMed: 20380038]
7. Michalski A, Damoc E, Hauschild JP, Lange O, Wiegand A, Makarov A, Nagaraj N, Cox J, Mann M, Horning S. Mass spectrometry-based proteomics using Q Exactive, a high-performance benchtop quadrupole Orbitrap mass spectrometer. *Mol Cell Proteomics.* 2011; 10:M111, 011015. [PubMed: 21642640]
8. Nagaraj N, D'Souza RC, Cox J, Olsen JV, Mann M. Feasibility of large-scale phosphoproteomics with higher energy collisional dissociation fragmentation. *J Proteome Res.* 2010; 9:6786–6794. [PubMed: 20873877]
9. Deutsch EW, Mendoza L, Shteynberg D, Farrah T, Lam H, Tasman N, Sun Z, Nilsson E, Pratt B, Prazen B, Eng JK, Martin DB, Nesvizhskii AI, Aebersold R. A guided tour of the Trans-Proteomic Pipeline. *Proteomics.* 2010; 10:1150–1159. [PubMed: 20101611]
10. Cox J, Mann M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol.* 2008; 26:1367–1372. [PubMed: 19029910]
11. Shteynberg D, Deutsch EW, Lam H, Eng JK, Sun Z, Tasman N, Mendoza L, Moritz RL, Aebersold R, Nesvizhskii AI. iProphet: multi-level integrative analysis of shotgun proteomic data improves peptide and protein identification rates and error estimates. *Mol Cell Proteomics.* 2011; 10:M111, 007690. [PubMed: 21876204]
12. Brockmeyer C, Paster W, Pepper D, Tan CP, Trudgian DC, McGowan S, Fu G, Gascoigne NR, Acuto O, Salek M. T cell receptor (TCR)-induced tyrosine phosphorylation dynamics identifies THEMIS as a new TCR signalosome component. *J Biol Chem.* 2011; 286:7535–7547. [PubMed: 21189249]
13. Weintz G, Olsen JV, Fruhauf K, Niedzielska M, Amit I, Jantsch J, Mages J, Frech C, Dolken L, Mann M, Lang R. The phosphoproteome of toll-like receptor-activated macrophages. *Mol Syst Biol.* 2010; 6:371. [PubMed: 20531401]
14. Osinalde N, Moss H, Arrizabalaga O, Omaetxebarria MJ, Blagoev B, Zubiaga AM, Fullaondo A, Arizmendi JM, Kratchmarova I. Interleukin-2 signaling pathway analysis by quantitative phosphoproteomics. *J Proteomics.* 2011; 75:177–191. [PubMed: 21722762]
15. Wojcechowskyj JA, Lee JY, Seeholzer SH, Doms RW. Quantitative phosphoproteomics of CXCL12 (SDF-1) signaling. *PLoS One.* 2011; 6:e24918. [PubMed: 21949786]
16. Matsumura T, Oyama M, Kozuka-Hata H, Ishikawa K, Inoue T, Muta T, Semba K, Inoue J. Identification of BCAP-(L) as a negative regulator of the TLR signaling-induced production of IL-6 and IL-10 in macrophages by tyrosine phosphoproteomics. *Biochem Biophys Res Commun.* 2010; 400:265–270. [PubMed: 20728433]
17. Marinis JM, Hutti JE, Homer CR, Cobb BA, Cantley LC, McDonald C, Abbott DW. IkappaB Kinase alpha Phosphorylation of TRAF4 Downregulates Innate Immune Signaling. *Mol Cell Biol.* 2012; 32:2479–2489. [PubMed: 22547678]
18. Bidere N, Ngo VN, Lee J, Collins C, Zheng L, Wan F, Davis RE, Lenz G, Anderson DE, Arnould D, Vazquez A, Sakai K, Zhang J, Meng Z, Veenstra TD, Staudt LM, Lenardo MJ. Casein kinase 1alpha governs antigen-receptor-induced NF-kappaB activation and human lymphoma cell survival. *Nature.* 2009; 458:92–96. [PubMed: 19118383]

19. Tiwari A, Sekhar AK. Workflow based framework for life science informatics. *Comput Biol Chem.* 2007; 31:305–319. [PubMed: 17931570]
20. Mayya V, Lundgren DH, Hwang SI, Rezaul K, Wu L, Eng JK, Rodionov V, Han DK. Quantitative phosphoproteomic analysis of T cell receptor signaling reveals system-wide modulation of protein-protein interactions. *Sci Signal.* 2009; 2:ra46. [PubMed: 19690332]
21. Gorry PR, Ancuta P. Coreceptors and HIV-1 pathogenesis. *Curr HIV/AIDS Rep.* 2011; 8:45–53. [PubMed: 21188555]
22. Wu Y, Yoder A. Chemokine coreceptor signaling in HIV-1 infection and pathogenesis. *PLoS Pathog.* 2009; 5:e1000520. [PubMed: 20041213]
23. Smith-Garvin JE, Koretzky GA, Jordan MS. T cell activation. *Annu Rev Immunol.* 2009; 27:591–619. [PubMed: 19132916]
24. Li S, Wang L, Berman M, Kong YY, Dorf ME. Mapping a dynamic innate immunity protein interaction network regulating type I interferon production. *Immunity.* 2011; 35:426–440. [PubMed: 21903422]
25. Natarajan M, Lin KM, Hsueh RC, Sternweis PC, Ranganathan R. A global analysis of cross-talk in a mammalian cellular signalling network. *Nat Cell Biol.* 2006; 8:571–580. [PubMed: 16699502]
26. Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. *Science.* 2010; 327:46–50. [PubMed: 20044567]
27. Dustin ML, Depoil D. New insights into the T cell synapse from single molecule techniques. *Nat Rev Immunol.* 2011; 11:672–684. [PubMed: 21904389]
28. Lillemeier BF, Mortelmaier MA, Forstner MB, Huppa JB, Groves JT, Davis MM. TCR and Lat are expressed on separate protein islands on T cell membranes and concatenate during activation. *Nat Immunol.* 2010; 11:90–96. [PubMed: 20010844]
29. Cowan AE, Moraru, Schaff JC, Slepchenko BM, Loew LM. Spatial modeling of cell signaling networks. *Methods Cell Biol.* 2012; 110:195–221. [PubMed: 22482950]
30. Smith AM, Xu W, Sun Y, Faeder JR, Marai GE. RuleBender: integrated modeling, simulation and visualization for rule-based intracellular biochemistry. *BMC Bioinformatics.* 2012; 13(Suppl 8):S3. [PubMed: 22607382]
31. Angermann BR, Klauschen F, Garcia AD, Prustel T, Zhang F, Germain RN, Meier-Schellersheim M. Computational modeling of cellular signaling processes embedded into dynamic spatial contexts. *Nat Methods.* 2012; 9:283–289. [PubMed: 22286385]
32. Sneddon MW, Faeder JR, Emonet T. Efficient modeling, simulation and coarse-graining of biological complexity with NFsim. *Nat Methods.* 2011; 8:177–183. [PubMed: 21186362]
33. Meier-Schellersheim M, Xu X, Angermann B, Kunkel EJ, Jin T, Germain RN. Key role of local regulation in chemosensing revealed by a new molecular interaction-based modeling method. *PLoS Comput Biol.* 2006; 2:e82. [PubMed: 16854213]
34. Smale ST. Selective transcription in response to an inflammatory stimulus. *Cell.* 2010; 140:833–844. [PubMed: 20303874]
35. Amit I, Regev A, Hacohen N. Strategies to discover regulatory circuits of the mammalian immune system. *Nat Rev Immunol.* 2011; 11:873–880. [PubMed: 22094988]
36. Gilchrist M, Thorsson V, Li B, Rust AG, Korb M, Roach JC, Kennedy K, Hai T, Bolouri H, Aderem A. Systems biology approaches identify ATF3 as a negative regulator of Toll-like receptor 4. *Nature.* 2006; 441:173–178. [PubMed: 16688168]
37. Litvak V, Ramsey SA, Rust AG, Zak DE, Kennedy KA, Lampano AE, Nykter M, Shmulevich I, Aderem A. Function of C/EBPdelta in a regulatory circuit that discriminates between transient and persistent TLR4-induced signals. *Nat Immunol.* 2009; 10:437–443. [PubMed: 19270711]
38. Sciammas R, Li Y, Warmflash A, Song Y, Dinner AR, Singh H. An incoherent regulatory network architecture that orchestrates B cell diversification in response to antigen signaling. *Mol Syst Biol.* 2011; 7:495. [PubMed: 21613984]
39. Sciammas R, Shaffer AL, Schatz JH, Zhao H, Staudt LM, Singh H. Graded expression of interferon regulatory factor-4 coordinates isotype switching with plasma cell differentiation. *Immunity.* 2006; 25:225–236. [PubMed: 16919487]
40. Cheong R, Hoffmann A, Levchenko A. Understanding NF-kappaB signaling via mathematical modeling. *Mol Syst Biol.* 2008; 4:192. [PubMed: 18463616]

41. Amit I, Garber M, Chevrier N, Leite AP, Donner Y, Eisenhaure T, Guttman M, Grenier JK, Li W, Zuk O, Schubert LA, Birditt B, Shay T, Goren A, Zhang X, Smith Z, Deering R, McDonald RC, Cabili M, Bernstein BE, Rinn JL, Meissner A, Root DE, Hacohen N, Regev A. Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. *Science*. 2009; 326:257–263. [PubMed: 19729616]
42. Calvano SE, Xiao W, Richards DR, Felciano RM, Baker HV, Cho RJ, Chen RO, Brownstein BH, Cobb JP, Tschoeke SK, Miller-Graziano C, Moldawer LL, Mindrinos MN, Davis RW, Tompkins RG, Lowry SF. A network-based analysis of systemic inflammation in humans. *Nature*. 2005; 437:1032–1037. [PubMed: 16136080]
43. Seok J, Xiao W, Moldawer LL, Davis RW, Covert MW. A dynamic network of transcription in LPS-treated human subjects. *BMC Syst Biol*. 2009; 3:78. [PubMed: 19638230]
44. Liao JC, Boscolo R, Yang YL, Tran LM, Sabatti C, Roychowdhury VP. Network component analysis: reconstruction of regulatory signals in biological systems. *Proc Natl Acad Sci U S A*. 2003; 100:15522–15527. [PubMed: 14673099]
45. Ramsey SA, Klemm SL, Zak DE, Kennedy KA, Thorsson V, Li B, Gilchrist M, Gold ES, Johnson CD, Litvak V, Navarro G, Roach JC, Rosenberger CM, Rust AG, Yudkovsky N, Aderem A, Shmulevich I. Uncovering a macrophage transcriptional program by integrating evidence from motif scanning and expression dynamics. *PLoS computational biology*. 2008; 4:e1000021. [PubMed: 18369420]
46. Ghisletti S, Barozzi I, Mietton F, Polletti S, De Santa F, Venturini E, Gregory L, Lonie L, Chew A, Wei CL, Ragoussis J, Natoli G. Identification and characterization of enhancers controlling the inflammatory gene expression program in macrophages. *Immunity*. 2010; 32:317–328. [PubMed: 20206554]
47. Escoubet-Lozach L, Benner C, Kaikkonen MU, Lozach J, Heinz S, Spann NJ, Crotti A, Stender J, Ghisletti S, Reichart D, Cheng CS, Luna R, Ludka C, Sasik R, Garcia-Bassets I, Hoffmann A, Subramaniam S, Hardiman G, Rosenfeld MG, Glass CK. Mechanisms establishing TLR4-responsive activation states of inflammatory response genes. *PLoS Genet*. 2011; 7:e1002401. [PubMed: 22174696]
48. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, Cheng JX, Murre C, Singh H, Glass CK. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell*. 2010; 38:576–589. [PubMed: 20513432]
49. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004; 116:281–297. [PubMed: 14744438]
50. Baltimore D, Boldin MP, O'Connell RM, Rao DS, Taganov KD. MicroRNAs: new regulators of immune cell development and function. *Nat Immunol*. 2008; 9:839–845. [PubMed: 18645592]
51. O'Neill LA, Sheedy FJ, McCoy CE. MicroRNAs: the fine-tuners of Toll-like receptor signalling. *Nat Rev Immunol*. 2011; 11:163–175. [PubMed: 21331081]
52. Watanabe Y, Kanai A. Systems Biology Reveals MicroRNA-Mediated Gene Regulation. *Front Genet*. 2011; 2:29. [PubMed: 22303325]
53. Grigoryev YA, Kurian SM, Hart T, Nakorchevsky AA, Chen C, Campbell D, Head SR, Yates JR 3rd, Salomon DR. MicroRNA regulation of molecular networks mapped by global microRNA, mRNA, and protein expression in activated T lymphocytes. *J Immunol*. 2011; 187:2233–2243. [PubMed: 21788445]
54. Suter DM, Molina N, Gatfield D, Schneider K, Schibler U, Naef F. Mammalian genes are transcribed with widely different bursting kinetics. *Science*. 2011; 332:472–474. [PubMed: 21415320]
55. Snijder B, Pelkmans L. Origins of regulated cell-to-cell variability. *Nat Rev Mol Cell Biol*. 2011; 12:119–125. [PubMed: 21224886]
56. Balazsi G, van Oudenaarden A, Collins JJ. Cellular decision making and biological noise: from microbes to mammals. *Cell*. 2011; 144:910–925. [PubMed: 21414483]
57. Raj A, van Oudenaarden A. Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell*. 2008; 135:216–226. [PubMed: 18957198]

58. Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S. Stochastic mRNA synthesis in mammalian cells. *PLoS Biol.* 2006; 4:e309. [PubMed: 17048983]
59. Chubb JR, Trcek T, Shenoy SM, Singer RH. Transcriptional pulsing of a developmental gene. *Curr Biol.* 2006; 16:1018–1025. [PubMed: 16713960]
60. Raser JM, O'Shea EK. Noise in gene expression: origins, consequences, and control. *Science.* 2005; 309:2010–2013. [PubMed: 16179466]
61. Kalisky T, Quake SR. Single-cell genomics. *Nat Methods.* 2011; 8:311–314. [PubMed: 21451520]
62. Flatz L, Roychoudhuri R, Honda M, Filali-Mouhim A, Goulet JP, Kettaf N, Lin M, Roederer M, Haddad EK, Sekaly RP, Nabel GJ. Single-cell gene-expression profiling reveals qualitatively distinct CD8 T cells elicited by different gene-based vaccines. *Proc Natl Acad Sci U S A.* 2011; 108:5724–5729. [PubMed: 21422297]
63. Ma C, Fan R, Ahmad H, Shi Q, Comin-Anduix B, Chodon T, Koya RC, Liu CC, Kwong GA, Radu CG, Ribas A, Heath JR. A clinical microchip for evaluation of single immune cells reveals high functional heterogeneity in phenotypically similar T cells. *Nat Med.* 2011; 17:738–743. [PubMed: 21602800]
64. Varadarajan N, Julg B, Yamanaka YJ, Chen H, Ogunniyi AO, McAndrew E, Porter LC, Piechocka-Trocha A, Hill BJ, Douek DC, Pereyra F, Walker BD, Love JC. A high-throughput single-cell analysis of human CD8(+) T cell functions reveals discordance for cytokine secretion and cytotoxicity. *J Clin Invest.* 2011; 121:4322–4331. [PubMed: 21965332]
65. Bendall SC, Nolan GP, Roederer M, Chattopadhyay PK. A deep profiler's guide to cytometry. *Trends Immunol.* 2012
66. Irish JM, Kotecha N, Nolan GP. Mapping normal and cancer cell signalling networks: towards single-cell proteomics. *Nat Rev Cancer.* 2006; 6:146–155. [PubMed: 16491074]
67. Ornatsky O, Bandura D, Baranov V, Nitz M, Winnik MA, Tanner S. Highly multiparametric analysis by mass cytometry. *J Immunol Methods.* 2010; 361:1–20. [PubMed: 20655312]
68. Newell EW, Sigal N, Bendall SC, Nolan GP, Davis MM. Cytometry by time-of-flight shows combinatorial cytokine expression and virus-specific cell niches within a continuum of CD8+ T cell phenotypes. *Immunity.* 2012; 36:142–152. [PubMed: 22265676]
69. Tay S, Hughey JJ, Lee TK, Lipniacki T, Quake SR, Covert MW. Single-cell NF-kappaB dynamics reveal digital activation and analogue information processing. *Nature.* 2010; 466:267–271. [PubMed: 20581820]
70. Kalita MK, Sargsyan K, Tian B, Paulucci-Holthauzen A, Najm HN, Debusschere BJ, Brasier AR. Sources of cell-to-cell variability in canonical nuclear factor-kappaB (NF-kappaB) signaling pathway inferred from single cell dynamic images. *J Biol Chem.* 2011; 286:37741–37757. [PubMed: 21868381]
71. Duffy KR, Wellard CJ, Markham JF, Zhou JH, Holmberg R, Hawkins ED, Hasbold J, Dowling MR, Hodgkin PD. Activation-induced B cell fates are selected by intracellular stochastic competition. *Science.* 2012; 335:338–341. [PubMed: 22223740]
72. Rand U, Rinas M, Schwert J, Nohren G, Linnes M, Kroger A, Flossdorf M, Kaly-Kullai K, Hauser H, Hofer T, Koster M. Multi-layered stochasticity and paracrine signal propagation shape the type-I interferon response. *Mol Syst Biol.* 2012; 8:584. [PubMed: 22617958]
73. Snijder B, Sacher R, Ramo P, Damm EM, Liberali P, Pelkmans L. Population context determines cell-to-cell variability in endocytosis and virus infection. *Nature.* 2009; 461:520–523. [PubMed: 19710653]
74. Gaudet S, Spencer SL, Chen WW, Sorger PK. Exploring the contextual sensitivity of factors that determine cell-to-cell variability in receptor-mediated apoptosis. *PLoS Comput Biol.* 2012; 8:e1002482. [PubMed: 22570596]
75. Albeck JG, Burke JM, Spencer SL, Lauffenburger DA, Sorger PK. Modeling a snap-action, variable-delay switch controlling extrinsic cell death. *PLoS Biol.* 2008; 6:2831–2852. [PubMed: 19053173]
76. Altan-Bonnet G, Germain RN. Modeling T cell antigen discrimination based on feedback control of digital ERK responses. *PLoS Biol.* 2005; 3:e356. [PubMed: 16231973]

77. Feinerman O, Veiga J, Dorfman JR, Germain RN, Altan-Bonnet G. Variability and robustness in T cell activation from regulated heterogeneity in protein levels. *Science*. 2008; 321:1081–1084. [PubMed: 18719282]
78. Acar M, Mettetal JT, van Oudenaarden A. Stochastic switching as a survival strategy in fluctuating environments. *Nat Genet*. 2008; 40:471–475. [PubMed: 18362885]
79. Eldar A, Elowitz MB. Functional roles for noise in genetic circuits. *Nature*. 2010; 467:167–173. [PubMed: 20829787]
80. Paszek P, Ryan S, Ashall L, Sillitoe K, Harper CV, Spiller DG, Rand DA, White MR. Population robustness arising from cellular heterogeneity. *Proc Natl Acad Sci U S A*. 2010; 107:11644–11649. [PubMed: 20534546]
81. Ghisletti S, Barozzi I, Mietton F, Polletti S, De Santa F, Venturini E, Gregory L, Lonie L, Chew A, Wei CL, Ragoussis J, Natoli G. Identification and characterization of enhancers controlling the inflammatory gene expression program in macrophages. *Immunity*. 2010; 32:317–328. [PubMed: 20206554]

Figure 1.

Contributions of systems biology approaches to our current understanding of immune activation. Global, quantitative and computational methods have been successfully used to elucidate complex non-linear processes involved in the regulation of responses downstream of immune receptor triggering. Some examples include (a) spatial regulation of signaling intermediates (33), (b) impact of component concentration variation (74), (c) signaling feedback loops (76), (d) pathway cross-talk (25), (e) transcriptional regulatory circuits (37), (f) network interference by miRNAs (53), and (g) global regulation of gene expression through chromatin modifications (81).