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Sense and Response in the Cell

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ABSTRACT

Almost a century ago, Paul Ehlrich proposed a concept of the 'receptors' as sensing components of cellular signaling pathways for the mediation of downstream response in the context of toxin action. Since then, we have come a long way in describing the signal transduction through dynamic sense & response in various domains of cell physiology studies, beyond the static 'receptor-ligand' mechanism. A multi-nodal characteristic of such signaling modules generates an enriched version of temporal signals responsible for a fine-tuned cellular response based on the identity and severity of stimulus. Here, we review diverse nodes of dynamic signal transduction mechanism for an efficient cellular response.

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INTRODUCTION

A precise determination of identity and strength of the stimulus is a pre-requisite for the signal transduction in the cell (Lahav, 2004; Batchelor, Loewer and Lahav, 2009; Batchelor *et al.*, 2011; Purvis *et al.*, 2012). This is crucial as downstream responses have to be graded according to the stress in order to induce flexible cellular outcomes. The classical understanding of cellular signal transmission comes from the receptor-ligand mechanism. Although the specificity of ligands for receptors facilitates activation of their respective signaling pathways by the precise identification of stimulus, its static nature to trigger the response at a fixed time does not necessarily manifest into the gradation of response (Lahav, 2004; Purvis and Lahav, 2013). Signal transduction through the static mechanisms could be assumed to follow 'all or none' principle where receptor-ligand complex association or dissociation at a given time either generates or terminates the downstream transmission. For instance, G-protein coupled receptors (GPCRs) activate cyclic adenosine monophosphates (cAMPs) upon ligand binding to control membrane channel functions. The extracellular signal-regulated kinase (ERK) regulates proliferation and differentiation of rat neuronal precursor PC-12 cells in response to epidermal growth factor (EGF) and nerve growth factor (NGF) respectively. A major limitation to 'all or none' formulation is the generation of fixed rather than a tuned response under the varying stimulus. Deciphering precise signaling in cells is tough due to the simultaneous involvement of multiple regulatory networks (Alon, 2007; Brandman and Meyer, 2008; Ferrell, 2013; Kholodenko, 2006). Recent advancement in single cell imaging, optogenetic and computational tools have provided a paradigm shift in our understanding of signal transduction at single cell level (Welch et al.,2011; Albeck et al.,2008; Bakstad et al., 2012, Purvis et al., 2012, Choi et al., 2012; Gaglia *et al.*, 2013; Gaglia and Lahav, 2014). Temporal studies have led to the discovery of the dynamic signal transduction mechanism for efficient cell fate decision in response to a variable stimulus. In contrast to receptor-ligand, the dynamic mode is more enriched in information and flexible to carry out calibrations in the cellular responses.

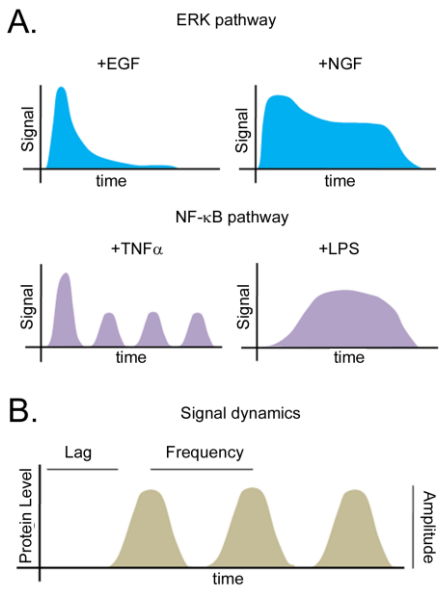

Figure 1. Dynamic signal transduction mechanism in the cell. A. The representations show distinct temporal activation of ERK, NF-κB in response to different stimulus. **B.** Temporal signals are characterized in terms of amplitude, frequency or duration.

Signal transduction in dynamic mode

Time-dependent measurements showed that EGF and NGF ligands trigger distinct dynamics of ERK activation. EGF induces transient whereas NGF triggers sustained activation of ERK (Gotoh et al., 1990; Nguyen et al., 1993; Traverse et al., 1992; Marshall, 1995). Similarly, upstream inflammatory signals like tumor necrosis factor-α (TNF-α) generates an oscillatory pattern of NF-κB for the stimulation of inflammatory response (Hoffmann et al., 2002; Nelson et al., 2004; Sung et al., 2009; Tay et al., 2010). On the contrary, a single persistent wave of NF-κB is introduced by bacterial lipopolysaccharide (LPS) in order to induce immune response gene activation (Covert et al., 2005; Lee et al., 2009; Werner et al., 2005, Barken et al., 2005, Ashall et al., 2009). As a response towards DNA-damage, tumour suppressor p53 also elicits either repetitive or sustained pulse dynamics to dictate cell cycle arrest or senescence respectively depending upon the severity of lesion (Purvis et. al., 2012; Geva-Zatorsky et al., 2010; Lahav et al., 2004; Batchelor et al., 2011) **(Figure 1A)**. The dynamics of signaling species are characterized by frequency, amplitude and duration of signals in response to the stress (Lahav et al., 2004) **(Figure 1B)**. These properties are accurately determined at single cell stage as an asynchronous or heterogeneous response at population level masks the actual fluctuation in obtained signals through average out effect (Cohen et al., 2008; Lee et al., 2009; Lev Bar-Or et al., 2000).

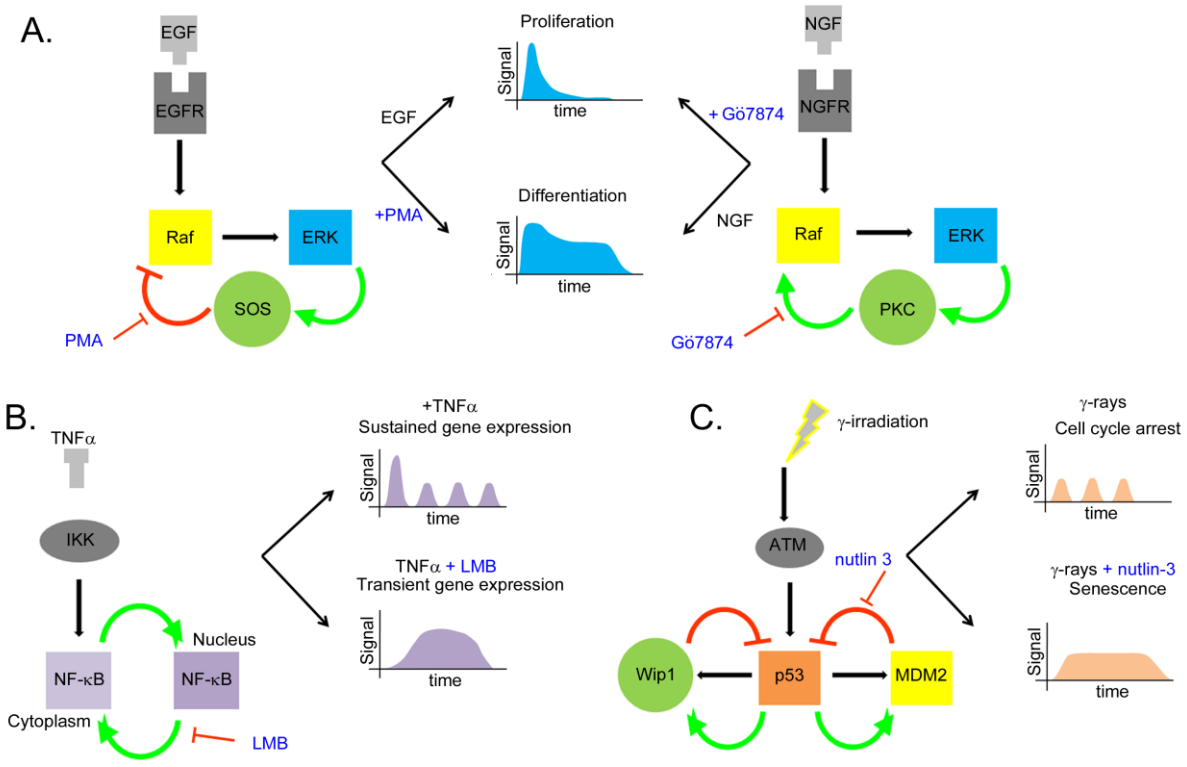

Figure 2. Signal dynamics decide cell fate. A. Decoupling of SOS with Raf through PMA removes negative regulation and results in the conversion of transient into sustained ERK activation. A transient signal stimulates cell proliferation naturally whereas the perturbation leads to cellular differentiation via sustained activation. **B-C.** Transformation of the dynamics of NF-κB by LMB or p53 through nutlin-3 also results in the modulation of cell fate.

At first glance, one may conclude that these responses are the consequence of receptor-ligand interaction, as proposed by the classical view. However, genetic or pharmacological interventions of key regulatory steps of these pathways revealed that the cellular response can be transformed through perturbation in signal dynamics. NF-κB shuttles in cytoplasm and nucleus that is essential for the generation of an oscillatory or sustained target gene expression (Hoffman et al., 2002). Inhibition of such translocation through pharmacological intervention by leptomycin B (LMB) sustains NF-κB in the nucleus and transforms sustained into transient activation of genes (Werner et al., 2005; Nelson et

al., 2004; Sung et al., 2009). Moreover, ERK naturally generates transient and sustained activation in response to EGFP and NGF respectively (Santos et al., 2007). However, intervention by protein kinase C (PKC activator) phorbol-12-myristate-13-acetate (PMA) transforms the transient into sustained ERK dynamics by EGF that ultimately provide the same outcome as NGF. Conversely, intervention by PKC inhibitor Gö7874 transformed the sustained into transient activation of ERK by NGF that mimics EGF pattern to promote proliferation (Grammer and Blenis, 1997). Next, modulation of the p53-MDM2 loop by nutlin-3 which decouples p53 and MDM2 and inhibit proteolysis of p53 further shows the transformation of oscillatory into a sustained pulse with the change in cell fate from arrest to senescence upon γ-irradiation (Purvis et al., 2012) **(Figure 2)**. These findings provide new insight into signal transduction mechanism. The precise determination of molecular dynamics, however, requires precise measurements at proper time intervals. An appropriate time-scale measurement is required due to the heterogeneity in the kinetics of biological events. Failure to do this may lead to wrong or incomplete inferences. This is clearly demonstrated by the ATM-p signals at high frequency in the initial duration of DNA-damage. ATM is modified within the initial 5 min of stress followed by its gradual loss (Jazayeri et al., 2006). When similar measurements were made at an hour interval for 10 hours, ATM-p showed a series of oscillations (Batchelor et al., 2008).

Unlike static mode, temporal quantities enrich the cellular system with the signaling dimensions to receive and process multiple information which facilitates the encoding and decoding based on the identity and strength of stimulus via (Purvis and Lahav, 2013). Recent studies show that Yeast transcription factor Msn2 undergoes a transient increase in the nucleus in response to glucose limitation. With the severity of stress, Msn2 leads to series of bursts with fixed amplitudes. However, upon oxidative stress, Msn2 elicits a different pattern with prolonged localization in the nucleus and increased amplitude (Hao and O'Shea, 2012). p53 also respond according to the strength and identity of the stimulus. p53 shows bursts of oscillatory cycles with fixed amplitude for the cell cycle arrest under increasing doses of γ-irradiation whereas it undergoes sustained activation with increasing amplitude under UV exposure for the induction of senescence or apoptosis (Purvis et. al.,2012, Lahav, 2004). Thus, dynamic signaling modules tune the biological response with the nature of the stimulus.

Signal Encoding

How does dynamic signaling mechanism 'sense' the nature of a defined stimulus in order to generate distinct temporal patterns of signal molecules for variable outcomes? Different shapes of the signaling dynamics are determined through the specific network structures of feedback regulation. Son of Sevenless (SOS) & ERK controls the negative feedback loop in order to trigger transient ERK activation. The sustained activation in response to NGF arises from receptor internalization (Sasagawa et al., 2005). Positive feedback also regulates between PKC & ERK (Santos et al., 2007). NF-κB elicits negative feedback regulation through its downstream gene IκB upon stimulation by TNFα. IκB kinase complex is activated by TNF-α which leads to the phosphorylation of IκB and subsequently, its proteasomal degradation. This triggers transcriptional activation of free NF-κB, including IκB which induces negative feedback for NF-κB itself. By contrast, A20 proteins dampen the sustained NF-κB activation (Basak et al., 2012; Werner et al., 2008). The sustained NF-κB dynamics is under the control of positive feedback from autocrine pathways in response to LPS. LPS activates Toll-like receptors 4 (TLR4) to synthesize TNFα in order to activate TNF receptor. p53 dynamics in response to different extent of DNA damage is also controlled through feedback regulation networks. Five critical feedback circuits such as p53-Wip1, p53–cyclin G, cyclin G–ATM, Wip1-ATM, and Mdm2-p53 are linked to cell survival, proliferation, arrest and apoptosis after DNA damage through p53 pulse; missing of any above transform the pulse into sustained response corresponding to cell death (Choi et al., 2012).

Decoding of signals from sense to response

How upstream dynamics of signaling molecules are translated into distinct cell fate? Although many studies have deciphered the inter-relation between temporal patterns and outcomes, only a few provide precise mechanism governing at a molecular level that induces different outcomes. One mechanism by which transient or sustained inputs from upstream species could display variable outcome depends upon the affinity of the downstream effectors. Low-affinity effector requires sustained input levels whereas high-affinity molecules can be activated by fluctuating levels in order to elicit a variable response. For instance, Transient or sustained pulse of calcium differentially activates JNK, NF-κB, and NFAT. JNF and NF-κB have low affinity for calcium and hence strong transient calcium bursts explicitly activate them. By contrast, NFAT has a high affinity for calcium and therefore, it is activated under low and sustained levels (Dolmetsch et al., 1997). Dynamics of yeast stress response factor Msn2 is decoded in a similar manner (Hao and O'Shea, 2012). Besides, a different mechanism has been proposed based on direct sensing of temporal changes in upstream regulators by specific network motifs in the responding network. An example includes ERK and TLR4 dependent pathways. In the ERK pathway, transient ERK activation triggers gene product c-Fos which undergoes rapid degradation (Murphy et al., 2002, 2004). However, sustained ERK continuously transcribe c-Fos which stabilizes in the nucleus upon phosphorylation (Nakakuki et al., 2010). These immediate gene products of the ERK pathway are linked to various cell fate (Amit et al., 2007; Murphy et al., 2004) **(Figure 3A)**. Unlike transient, sustained activation of TLR4 is necessary for the expression of interleukin 6 (IL6). LPS-stimulated TLR-4 generates two waves of transcription which determines C/EBPδ dependent response via NF-κB and ATF3 **(Figure 3B).** The key to the regulation of such an enriched version of the signal transduction mechanism lies in the negative feedback regulatory loop networks (Neves et al., 2008). The selective activation of a particular loop in these networks establishes a 'sense' whereas its subsequent feedback regulation determines the 'response' module of a signaling pathway. A major advantage provided by such a mechanism is the tendency to execute a flexible response towards the varying stress conditions. This is due to the ability to encode information through modulation in frequency, amplitude, and duration. This is necessary as the stress is distributed differentially across the cells owing to the heterogeneity in the microenvironment and a graded response is therefore required through the precise determination of strength and identity of the stimulus.

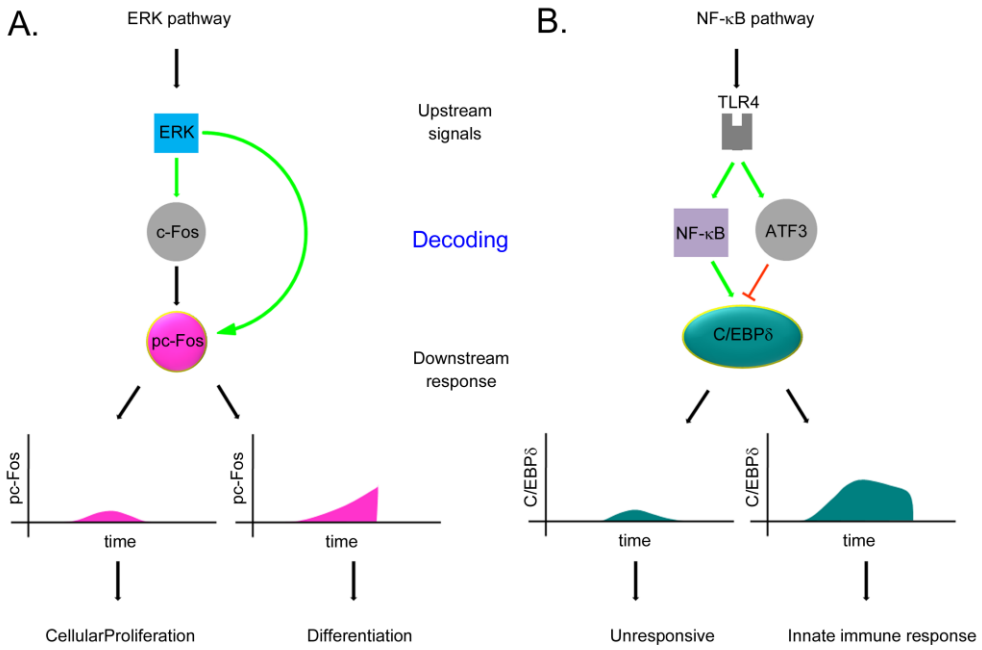

Figure 3. Decoding signal dynamics. A. ERK signals are translated into response via c-Fos. ERK activates c-Fos and leads to its phosphorylation (pc-Fos). Depending upon the decay rate of c-Fos which is usually slow, the downstream target products are selected in response through either transient or persistent pc-Fos level. **B.** Signal decoding in the NF-κB signaling pathway is performed through the differential activation of C/EBPδ via levels of NF-κB and ATF3.

Signal transduction via supra-molecular assemblies

Receptor signal transduction influences nearly every domain of the cell physiology. The classical view describes this process through successive activation of signaling molecules. Some well-studied examples include β-adrenergic receptor or EGFR. Ligand interaction induces conformation change that facilitates the recruitment of heterotrimeric G-proteins and phosphate exchange between guanosine diphosphate (GDP) and guanosine triphosphate (GTP). Such an exchange dismantles the subunits of heterotrimeric G- protein complex. α subunit detaches from β-γ complex and each entity triggers downstream amplification of secondary messenger cyclic adenosine monophosphate (cAMP) in order to control channel activities. Unlike GPCRs or RTKs, TNFR and Toll-like receptor/interleukin-1 superfamily do not possess enzymatic activity or coupled to any other intracellular enzymes. They require an adaptor protein to activate enzymatic reactions in order to stimulate NF-κB & MAP kinase for downstream outcomes**.** Recently, a new paradigm has been identified that comprises the higher order molecular assemblies or signalosome in intracellular spaces. Death domain (DD) fold complex structure shows helical symmetry which enables DD complex to generate filamentous structures (Hau, 2013). Signaling through spatial clustering of effectors in higher order structure such as amyloids and prions, DD signalosomes, Head-to-tail signalosomes, and multivalent signaling complexes provides an efficient way to concentrate activators for signaling (Hao and Fuxreiter, 2016). This also amplifies downstream signals by incorporating higher stoichiometry numbers of signaling enzymes into the signalosome (Hau, 2013). Besides, slow association kinetics of such assemblies may reduce the biological noise associated with transient fluctuations in conformation, stoichiometry or diffusion of participating molecules and allow the signaling to initiate upon sustained and strong stimulation.

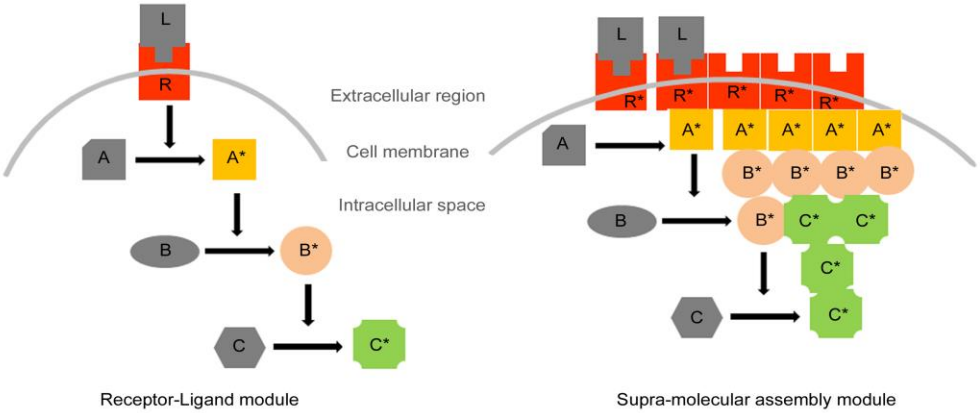

Figure 4. Dynamic signal modules in the cell. A general representation of receptor-ligand or supra-molecular assembly modules of cell signaling.

The supramolecular assembly may further facilitate the spatial compartmentalization which may delineate the cross-reactions in signals originated at signalosome. Signaling by higher order assemblies is distinct from receptor clustering **(Figure 4)**. Activated GPCRs and RTKs oligomerize at the cell surface however there is no clear evidence for intracellular signalosome. Extracellular ligand-bound domains oligomerize into dimers or trimers and cooperate with the assembly of intracellular higher order complexes to transmit the signals in innate immune pathways. For instance, the NF-κB pathway is triggered by TNFα through receptor-ligand binding in extracellular spaces which subsequently stimulates distinct dynamics of NF-κB inside the cell. This mode further incorporates either amyloid plaques of RIP1 or RIP3 or two-dimensional crystal lattice of TRAF6, as the assistance from supra-molecular signalosomes in order to tune NF-κB activation in the diverse stimulus (Hau, 2013 and Hao, and Fuxreiter, 2016). In response to DNA damage, different p53 dynamics is responsible for either cyclic or terminal fates. Such p53 signals are decoded via transcriptional activation of target gene products. p53 monomers exist in the dimer, tetramer, octamer or other higher order forms (Xu *et al.*, 2011; Gaglia *et al.*, 2013; Gaglia and Lahav, 2014; Vyas *et al.*, 2017). p53 tetramers are essential for transcriptional activation of p53 target genes which rapidly forms after DNA damage. Besides, p53 has a tendency to aggregate upon structural changes after genetic mutations(Xu *et al.*, 2011). This also suggests assistance from higher-order assemblies to p53 molecular dynamics based signaling. However, no clear evidence is present in support of p53 mediated signal transduction via higher-order machinery. Therefore, it is evident that extracellular ligand/receptor complexes and intracellular higher order signaling machines together may generate precise signal transduction across the cell membrane. These structures, therefore, provide unique mechanisms of signal transduction with reduced biological noise and temporal & spatial control of signaling.

CONCLUSION

Dynamic signal transduction in biological systems may integrate canonical receptor-ligand and non-canonical dynamic or supra-molecular assemblies for response generation. Besides, the strength of stimulus may also disturb synchronization of molecular dynamics with supra-molecular assemblies. However, a defined response via cell signaling point towards the co-ordination in molecular dynamics and supramolecular assemblies. Such integration may generate a robust sensory module to adapt to wide variations in stimulus due to its property to encode enriched information. Dynamic sensory modules, therefore, may exhibit 'multi-nodal' characteristics by integrating other nodes of signaling. The complexity thus arises; impose difficulties in disseminating signaling events precisely in biological systems. However, the innovative and careful investigation may reveal the secrets of integrations of nodes of different dynamic modules for the efficient manipulation of abrupt cellular states in the future.

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