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## Review Article

# Progress and challenges in understanding the regulation and function of p53 dynamics

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The dynamics of p53 expression provide a mechanism to increase differentiation between cellular stresses and specificity in appropriate responses. Here, we review recent advances in our understanding of the molecular mechanisms regulating p53 dynamics and the functions of the dynamics in the regulation of p53-dependent cell stress responses. We also compare dynamic encoding in the p53 system with that found in other important cell signaling systems, many of which can interact with the p53 network. Finally, we highlight some of the current challenges in understanding dynamic cell signaling within a larger cellular network context.

## Introduction

With improvements in techniques for the imaging and tracking of single living cells over long time-scales, studies over the past two decades have found that the dynamics of cellular signaling pathways, i.e. the temporal patterns of expression of active signaling molecules, can serve an important regulatory function in cellular response systems. Examples of dynamic regulation have been found in a variety of organisms, including *Escherichia coli*, yeast, and human cells [1–13]. Dynamic regulation serves a particularly important function for cell signaling hubs, signaling molecules that are activated in response to several different stimuli and orchestrate the response of multiple downstream pathways. Since signaling hubs respond to many different stimuli and activate distinct, and sometimes conflicting, downstream pathways, the encoding of additional information through temporal dynamics provides a mechanism by which signaling pathways can increase specificity and functionality.

In human cells, an important signaling hub that uses dynamic regulation is the transcription factor p53 [7,14–19]. p53 co-ordinates the response to several cellular stresses [14,15]. Upon stress-mediated stabilization, p53 controls the expression of many target genes that affect a diverse array of downstream responses important for alleviating stresses and regulating cell fates [20–22]. Long-term time-lapse microscopy, imaging cells on the timescale of days, revealed that the dynamics of the nuclear concentration of p53, the temporal pattern of p53's accumulation and degradation in the nucleus, depend on the stress a cell is experiencing [23] (Figure 1). Stress-specific p53 dynamics are important for the temporal regulation of the different target gene activation programs in response to stresses, as altering p53 dynamics can lead to a different cell fate outcome in response to a given stress [24,25].

In addition to the insights gained from time-lapse microscopy, advances in understanding how p53 dynamics encode and shape the cellular response to stress have benefitted from an iterative process combining quantitative experimental techniques with predictive computational modeling [21,24–28]. Recently, such approaches have also enabled a deeper understanding of the dynamic processes shaping the regulation and function of several other cell signaling hubs, including NF-κB [6,11,29–31] and ERK [1,32,33]. In many cases, detailed understanding of dynamic cell signaling is limited to pathways studied in isolation, owing to our ability to image only a few distinct signaling molecules simultaneously. A current challenge in the field is to integrate these data and models of isolated pathways

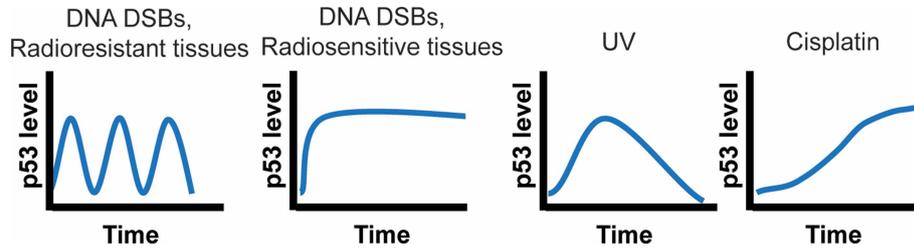*These authors contributed equally to this work.

**Figure 1. Distinct modes of p53 dynamics.**
p53 undergoes qualitatively distinct dynamics in response to different stress stimuli and in different tissues.

to expand our understanding of dynamic regulation at the level of individual cells and provide a more holistic view of cellular signaling dynamics through the parallel, and often overlapping, networks activated in response to many stimuli.

In this review, we will highlight advances in our understanding of p53 dynamics, both in terms of how it is regulated and what functions it provides. We will discuss how dynamic p53 regulation is altered due to cellular and stress contexts. We will also compare p53's dynamic regulation to that of other cell signaling hubs. Finally, we will highlight recent work in which analysis of p53 dynamics has been integrated with other pathways activated in response to specific cellular stresses.

## The regulation and functions of p53 dynamics

p53 is activated by numerous cellular stresses, including DNA double-strand breaks (DSBs), exposed single-stranded DNA, oxidative stress, oncogene activation, and ribosomal protein stress [34]. p53 has been well-characterized as the 'guardian of the genome' [14], and early work on p53 dynamics focused on the response to DNA damage. Through high temporal resolution profiling of populations of cells by Western blot analysis, Lev Bar-Or et al. [18] showed that the p53 response to DNA DSBs in MCF-7 breast carcinoma cells showed a damped oscillatory response, with sequential peaks of p53 levels decreasing over time following the damage. Subsequent single-cell analysis by Lahav et al. [7] showed that oscillations of p53 were undamped within any given cell, with the concentration of p53 rising to approximately the same levels for any pulse of the oscillation (Figure 1). The damped oscillations originally identified by Western blotting were attributed to desynchronization across individual cells in the population. Analysis of the single-cell response showed that the average amplitude, duration, and frequency of p53 pulses are fixed regardless of the dose of the DNA-DSB inducing agent. The length of time over which the oscillations occur can be highly variable, ranging from single pulses to oscillations that persist for days [19] or even weeks [35] in some cells. While most studies rely on the induction of DNA DSBs through the addition of an extrinsic DNA damaging agent such as ionizing radiation or the radiomimetic drug neocarzinostatin [36], even the small number of DNA DSBs generated during normal cell proliferation can trigger a single p53 pulse of stereotypical shape [37].

Following the initial identification of dynamic regulation of p53 in response to DNA DSBs, the response to several other p53-activating conditions was measured (Figure 1). Interestingly, p53 dynamics were found to be stress specific, with different dynamic patterns occurring in response to different stimuli. For example, in response to UV-C-induced DNA damage, p53 levels increased not in oscillations but instead in a single pulse [23]. In contrast with the fixed amplitude and duration pulses in response to different numbers of DNA DSBs, the single pulse of p53 in response to UV-C increased in both amplitude and duration proportional to the UV-C dose. The key regulatory feedback loops governing the distinct responses to DNA DSBs and UV-C-induced DNA damage were characterized by combining experimental quantification of p53 dynamics [7,18,23], development of differential-equation based computational models [19,26,30,38–43], and further experimental validation of computational predictions [26,44]. The switch between the distinct dynamic modes of p53 was shown to be dependent on differential alterations to p53 protein stability generated by the strength of negative feedback from the phosphatase WIP1 and the E3 ubiquitin ligase MDM2 through interactions with the upstream kinases ATM and ATR [23,26,44].

More recently, additional dynamic modes of p53 have been identified (Figure 1). Chen et al. [45] showed that, in contrast with transient exposure to DNA damaging agents such as ionizing or UV-C radiation, continuous exposure of cells to DNA damaging chemotherapeutics can generate a monotonic dynamic response,

in which p53 levels continuously rise without oscillations. Furthermore, longer-term imaging studies of the response to DNA DSBs have found that subpopulations of cells can switch dynamic modes from oscillations to a more monotonic response ∼24 h post-damage [46]. The switch in dynamics is dependent on levels of the caspase-2-piddosome.

Variations in the signaling networks governing p53 expression can generate cell-type and tissue-specific alterations to p53 dynamics. Stewart-Ornstein et al. [47] analyzed p53 dynamics in response to DNA DSBs in a panel of established cell lines and found that the dynamics ranged from relatively consistent oscillations to a single large pulse. Using a combined modeling and experimental approach, they showed that the different types of dynamics depended on two main factors: the efficiency of the DNA repair process and the strength of the activity of the damage sensing kinase ATM. Similarly, Yang et al. [48] showed that cell line differences in p53 dynamical responses to chemotherapeutic compounds could be generated by variability in ATM-mediated degradation of the negative feedback regulator MDM2. Tissue-specific p53 dynamics were observed in a mouse model by Hamstra et al. [49]. Following whole-body irradiation of mice, p53 oscillations were found to be strong in certain tissues, including the gut. More recently, it was shown that radioresistant mouse tissues have a transient p53 dynamic response *in vivo*, in contrast with a prolonged p53 response in radiosensitive tissues [50]. These results suggest that distinct modes of p53 dynamics may affect the degree of radiosensitivity or radioresistance for different tissues and tumor cells.

Several studies have identified functional impacts of p53 dynamics on the regulation of different stress response pathways. p53 controls the expression of over a hundred target genes, which affect a range of activities including cell cycle arrest, DNA repair, apoptosis, senescence, and metabolism [20,22]. Following a p53-activating stress, these pathways must be temporally regulated to provide an effective means of combating stresses — for example, DNA repair and cell cycle arrest mechanisms should be activated before senescence or apoptosis programs. Recent work has shown that p53 oscillations provide a mechanism to generate distinct temporal responses in target genes as a function of the mRNA decay rate of specific targets [21,28]. Target genes with short-lived mRNAs (relative to the p53 oscillation period) have an oscillatory temporal expression pattern that tracks p53 dynamics, enabling a threshold on the expression level of the short-lived targets. In contrast, target genes with long-lived mRNAs continue to increase in transcript level with each oscillatory pulse of p53, enabling a continuous accumulation of transcript throughout the damage response. Similarly, the protein expression levels of p53 targets are determined by the protein decay rates [27]. More recently, Harton et al. [25] used a microfluidic approach to control the delivery of a small molecule inhibitor of MDM2 to drive a range of p53 expression dynamics. Focusing on the activation of the two highly p53-responsive promoters of *MDM2* and *CDKN1A*, they showed that p53 dynamics can confer several different cell signaling features. The amplitude of p53 oscillations was important for establishing threshold in the activation levels of the promoters. The duration of individual p53 pulses was found to control a refractory period, with longer duration p53 pulses delaying the reactivation of subsequent p53 pulses. Finally, the *MDM2* and *CDKN1A* promoters each showed distinct frequency response profiles, with increased promoter responsiveness for distinct ranges of p53 oscillation frequencies. In terms of the *CDKN1A* promoter, these effects were shown to not only impact promoter activation but also affect cell cycle arrest fidelity. In a separate study, three distinct p53 target promoter archetypes were identified in terms of their temporal responsiveness to p53 oscillations [51]. The rates of activation of these archetypes were found to be dependent on the acetylation state of p53.

In addition to the effects on cell cycle arrest [25], p53 dynamics have been shown to functionally control several other cellular stress responses. By using the small molecule inhibitor of MDM2 Nutlin-3 following induction of DNA DSBs, Purvis et al. [24] sustained p53 levels, thereby eliminating the normal oscillatory response. In the condition of sustained p53, cells prematurely up-regulated the senescence program, providing the first direct evidence that p53 dynamics can control a cell fate program. Chen et al. [45] showed that distinct p53 dynamics patterns correlate with cell death in tumor cells responding to chemotherapeutic treatments. In colon carcinoma cells, Paek et al. [52] showed that the rate of accumulation of p53, rather than a threshold concentration, determined whether cells underwent apoptosis in response to cisplatin treatment. Chen et al. [53] found that knockdown of the p53 regulator MDMX activates a biphasic p53 dynamical response consisting of an initial high amplitude, long duration single pulse of p53 followed by low amplitude, short-duration oscillations of p53. Subsequent induction of DNA damage during the first phase favors apoptosis; in contrast, DNA damage during the oscillatory phase of the p53 response abrogates apoptosis and results in higher cell survival to the damaging agent.

# Dynamic encoding in other cell signaling pathways

Dynamic encoding has been well established in many physiological contexts, such as action potentials in neuronal firing or hormonal secretion. At the level of intracellular signaling, several other important cell signaling hubs in addition to p53 have recently been found to use dynamic encoding to differentiate between stimuli and perform downstream signaling functions. Here, we highlight two examples of the growing list of cell signaling networks found to use dynamic encoding.

A particularly well-characterized example is the transcription factor NF-κB. Like p53, it is also a stress response regulator activated in response to a variety of stimuli, with different dynamic expression patterns depending on the activating stress. For example, in response to TNFα NF-κB shuttles from the cytoplasm to the nucleus in damped oscillations [6,11], whereas in response to the DNA damage-inducing topoisomerase inhibitor etoposide NF-κB undergo undamped oscillations [11]. Exposure of macrophages to lipopolysaccharide (LPS) generates a single prolonged pulse of NF-κB [30]. Thus, in response to different stimuli NF-κB dynamics qualitatively change much like p53 dynamics. The similarity in the dynamic regulation results from a similarity in the network architecture of the feedback loops regulating p53 and NF-κB; namely, they both have time-delayed negative feedback mediators that act directly on the network hub (MDM2 for p53, IκB for NF-κB) as well as additional negative feedback from the hub to the upstream signaling kinase (WIP1 acting on ATM for the p53 network, A20 acting on IKK for the NF-κB network) [6,11,19,26,31,44]. The dynamics of NF-κB expression have been found to serve important functions for the temporal ordering and overall dynamics expression patterns for the large number of NF-κB target genes [6,30].

The EGF-stimulated ERK/MAPK pathway is an important regulator of cellular proliferation and differentiation. Stimulus-dependent dynamic encoding in the ERK pathway has been well-characterized in the response of PC-12 cells to different growth factors [54,55]. In response to EGF, there is a transient single pulse of ERK resulting in cell proliferation. In contrast, in response to NGF there is a sustained ERK response leading to cell differentiation. More recent analysis of individual cells has revealed that the situation is even more complex, with subsets of cells exhibiting either dynamic behavior in response to EGF or NGF, but with different percentages of cells in the proliferating or differentiating subpopulations based on the stimulus [32]. Studies in MCF10A primary-like cells showed that ERK exhibits asynchronous, frequency-modulated pulses of activity in steady-state conditions in the presence of EGF [1]. Alteration of EGFR activity changed the frequency of ERK pulses, while inhibiting MEK changed the amplitude [1]. Functionally, the pulse frequency of ERK activity was correlated with the rate of transition into S-phase, showing that in primary-like cells ERK signaling dynamics are likely an important regulatory mechanism for controlling cell proliferation rates. Advances in live-cell imaging have enabled a wealth of new data indicating both complex temporal and spatial regulation of ERK dynamics within cells, between cells, and within *in vivo* contexts [56].

In addition to p53, NF-κB, and ERK, differential dynamics in response to different stimuli or stimulation conditions have been identified for a growing list of cell signaling molecules, including the calcium-responsive NFAT1 and NFAT4 [57,58] and several yeast transcription factors including Crz1 and Msn2 [2–5]. As long-term live-cell imaging continues to be widely applied, it is likely that the list of dynamic signaling networks will continue to grow.

# Dynamic cross-talk between p53 and other signaling networks

As discussed, the temporal dynamics of p53 expression play a key role in regulating the expression dynamics of p53 target genes [21,27,28] and cell fate determination [24,25,35,45,46,52,53]. While the core regulatory network governing p53 dynamics is well-characterized [7,23,26,44], several additional cell signaling hubs can be induced in parallel to p53 in response to specific stresses. For example, in response to genotoxic stress NF-κB [59,60], JNK [61], p38 [62], and FOXO1 [63,64] are all activated in addition to p53. A key question that remains unanswered is understanding how these networks dynamically integrate with p53 to control gene expression and cell fate in response to cellular stresses. To date, the number of studies examining the cross-talk of these networks are limited; however, recent studies are beginning to provide insights into this important question.

One mechanism for dynamic integration of signaling pathways is direct cross-talk to p53 to refine p53 expression dynamics. Putative examples of this potential mechanism include regulation of p53 expression via the NF-κB network in response to DNA damage (Figure 2). Two interactions that link p53 and NF-κB are (1) the WIP1 phosphatase, a negative feedback mediator of p53 [26,44,65], which is also a known
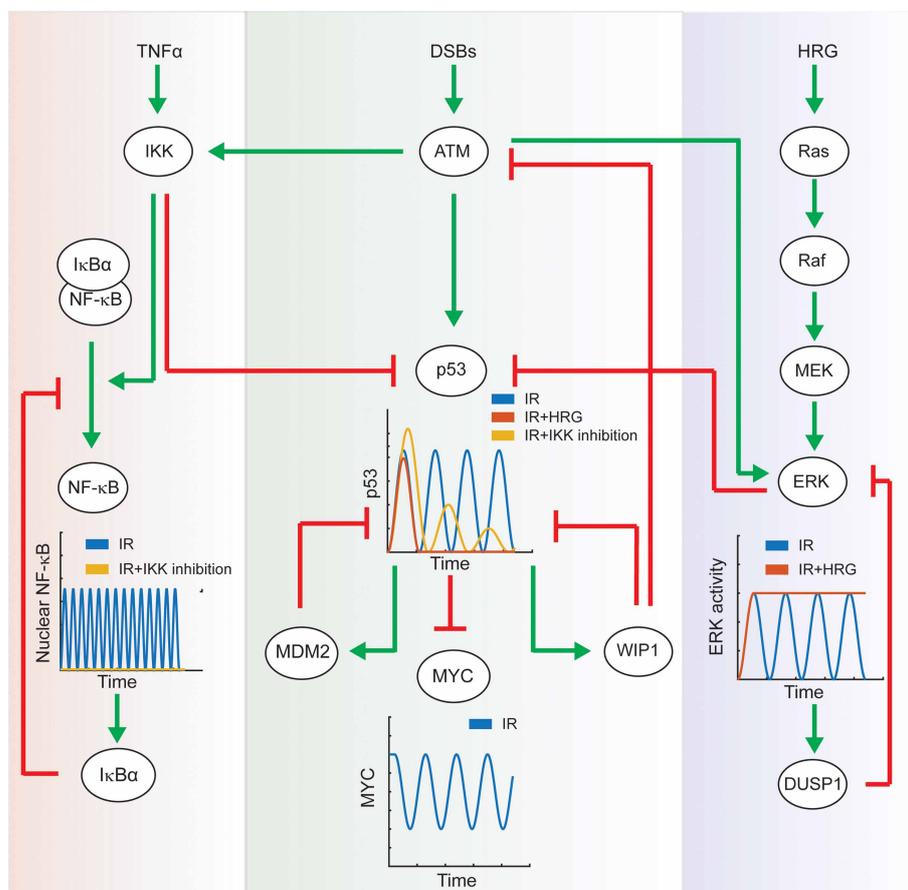
**Figure 2. The integration of multiple dynamic signaling networks.**

The NF-κB (left panel), p53 (middle panel), and ERK (right panel) pathways use dynamic encoding in response to stimulation.
Ongoing efforts are being applied to determine how the large network composed of these pathways and their interactions
shape cell signaling dynamics.

transcriptional target of NF-κB [66], and (2) the NF-κB activator IKK2 kinase can also regulate p53 through
phosphorylation [67]. Indeed, the latter mechanism was recently shown to affect p53 dynamics. Using pharma-
cological inhibition of IKK2, Konrath et al. [68] showed that loss of NF-κB activation significantly delayed the
peak timing of p53 expression and increased the inter-peak interval between sequential p53 pulses. These varia-
tions resulted in changes in the expression of known p53 target genes including *CDKN1A*, *PML*, *GADD45A*,
*BBC3*, and *PMAIP1*. Mathematical modeling predicted these downstream effects were through two potential
mechanisms both of which regulated p53 expression by modulating the degradation of p53. These findings
provide direct evidence that integration of additional genotoxic stress-responsive pathways act to refine p53
dynamics. Future studies examining the dynamics of both p53 and NF-κB activity within the same individual
cells are likely to provide additional insights into these mechanisms and their importance in cell fate
regulation.

In addition to the integration of genotoxic stress-responsive pathways, cells must also reconcile potential pro-
growth signals that coincide with DNA damage to determine the appropriate response. As discussed, the fre-
quency of ERK pulses controls the likelihood of cellular proliferation [1]. To determine how cells interpret the
coincidence of pro-growth signals and DNA damage, the Esposito lab used a combination of a FRET-based
ERK-specific biosensor, a fluorescently tagged p53, and population-level analysis of protein expression by
western blot to measure ERK activity and p53 expression dynamics [69]. When MCF-7 cells encountered both
proliferative and DNA damage signals, the integration of the two networks dictated the cell fate outcome.
Sustained p53 activation in the absence of ERK activity leads to cell cycle withdrawal. In contrast, the presence
of sustained ERK activity leads to diminished p53 activation and cell cycle re-entry. The precise mechanisms

dictating the balance of arrest and proliferation remain unclear, though it is hypothesized that ERK-mediated regulation of MDM2 dictates p53 expression. These studies highlight how the integration of multiple pathways with distinct temporal dynamics can encode additional information to specify cell fate outcomes (Figure 2).

Similar to ERK, the transcription factor MYC is a known regulator of cellular proliferation [70] and global regulator of transcription [71,72]. Interestingly, MYC expression was shown to exhibit oscillatory reductions in expression following p53 pulses in response to DNA damage [73]. These oscillations in MYC expression were due to the binding of p53 to *MYC*-distal regulatory elements, demonstrating dynamic cross-talk between p53 and MYC expression. Using an inducible system to regulate MYC expression, Porter et al. [73] found that repression of MYC is critical for selective induction of DNA damage responsive genes and removing the repression of MYC led to significant increases in cell death.

These studies highlight how the integration of additional networks with unique temporal dynamics may enable cells to respond specifically to a wide range of stimuli. By fine-tuning p53 with additional regulatory inputs, such as the NF-κB network, gene expression patterns can be tightly regulated. Integrating growth responsive signals through ERK or MYC can potentially improve the specificity of cell fate determination in response to genotoxic stress. While the number of studies exploring the integration of multiple signaling networks in conjunction with p53 are limited, the continued development of fluorescent biosensors should provide new insight into how multiple networks co-ordinate to regulate the genotoxic stress response.

## Future directions

With improvements in fluorescent tagging and time-lapse microscopy, there has been a wealth of new biology revealed for many important cell signaling systems. The similarities in how signaling dynamics can be used to enhance information processing at the subcellular level are striking, and it points to potential evolutionary selection of dynamic encoding as an effective method for biological signaling pathways to respond to multiple stimuli and orchestrate multiple downstream responses.

While our understanding of dynamic signaling has increased tremendously in recent years, several challenges remain that will need to be addressed. These studies often rely on live-cell imaging using fluorescent protein variants, and one limitation is the availability of spectrally distinct fluorophores that can be measured. In addition to improvements in distinct fluorescent protein variants, alternative solutions using current technologies are actively being pursued. For example, recent work has shown that signaling events can be constrained to distinct subcellular locations to spatially resolve separate interactions using the same color fluorophore [74]. Such improvements to increase the number of signaling events that can be simultaneously imaged and tracked will aid tremendously in efforts to understand how cell signaling pathways are dynamically integrated. Additionally, advances in microscopy such as super-resolution microscopy and light-sheet fluorescence microscopy are providing methods to increase spatial resolution and reduce phototoxicity [75]. Finally, efforts to improve the length of time and spatial penetrance of intravital microscopy will help in developing a better understanding of dynamic signaling within physiological contexts.

### Perspective
- Dynamic p53 expression regulates transcriptional programs and cell fate in response to cellular stress.
- Integration of p53 dynamics with dynamic signaling from parallel pathways provides increased specificity in response to multiple stimuli.
- Continued improvements in imaging technologies and data analysis are necessary to understand the integration of complex signaling networks.

### Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

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## Author Contributions

Z.Y., R.L.H., and E.B. wrote and edited the manuscript.

## Acknowledgements

## Abbreviations

DSBs, DNA double-strand breaks; LPS, lipopolysaccharide.

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