The guardian’s choice: how p53 enables context-specific decision-making in individual cells

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p53 plays a central role in defending the genomic integrity of our cells. In response to genotoxic stress, this tumour suppressor orchestrates the expression of hundreds of target genes, which induce a variety of cellular outcomes ranging from damage repair to induction of apoptosis. In this review, we examine how the p53 response is regulated on several levels in individual cells to allow precise and context-specific fate decisions. We discuss that the p53 response is not only controlled by its canonical regulators but also controlled by interconnected signalling pathways that influence the dynamics of p53 accumulation upon damage and modulate its transcriptional activity at target gene promoters. Additionally, we consider how the p53 response is diversified through a variety of mechanisms at the promoter level and beyond to induce context-specific outcomes in individual cells. These layers of regulation allow p53 to react in a stimulus-specific manner and fine-tune its signalling according to the individual needs of a given cell, enabling it to take the right decision on survival or death.

Introduction

In every part of our life, we are challenged with making difficult decisions. No matter if we consider family issues, business strategies or politics, our approaches are often similar: when important decisions are about to be made, we collect all available information and consult with the people most affected to ensure that the outcome is as favourable as possible under the given circumstances. Cells in our body need to take decisions as well. They have to choose between proliferation and differentiation or sometimes even between survival and death. Wrong choices can have severe consequences: if damaged and genetically altered cells decide to proliferate instead of inducing apoptosis, cancer can arise. One of the proteins in charge of such vital decisions is the transcription factor (TF) p53.

However, even the guardian of our genome is not able to do the job on its own. To determine the appropriate cell fate, p53 relies not only on the source and degree of the stress, but it also combines this information with additional cell-intrinsic inputs and environmental cues. Here, we aim to elucidate how different layers of regulation allow the p53 response to integrate the various sources of information and enable individual cells to faithfully decide whether to live or to die (Fig. 1A).

P53 is activated by a variety of different stresses ranging from nutrient deprivation and hypoxia to damage of our genome [1]. For the purpose of this review, we focus on the well-characterized role of p53 in the response to genotoxic stress, specifically in form of double-strand breaks (DSB). As a TF, p53 controls...
Fig 1. Regulatory levels of the p53 response. (A) The p53 response to DNA damage is regulated and adjusted on several levels. As a first layer, a tightly regulated interplay of canonical regulators in combination with crosstalk to other signalling pathways allows a damage and context-specific response. The resulting p53 dynamics are decoded on the promoter level. In this step, several mechanisms allow the diversification of the single p53 input signal. Using stochastic gene expression, time-varying patterns of PTM as well as controlled oligomerization and interaction with cofactors, the expression of distinct target genes and different cellular outcomes can be induced. In the final step, the possible target expression dynamics are expanded and fine-tuned due to their specific mRNA and protein stabilities and depending on the cellular state. (B) Schematic overview of the p53 network in response to DSB. Upon genotoxic stress, the kinases ATR, DNA-PK and ATM mediate the stabilization and activation of p53 directly and via the checkpoint kinases CHK1 and CHK2. P53 can then induce the expression of its target genes including the negative regulators WIP1 and MDM2. These two proteins terminate the p53 response. (C) p53 dynamics in response to different stimuli. In response to DNA DSB, p53 shows a series of pulses with fixed amplitude and duration with the number of pulses increasing with the damage dose. After UV radiation, only a single pulse is formed whose amplitude and duration increases with the degree of damage. Upon cisplatin treatment, the p53 levels increase over time. (D) P53 dynamics control cell fate. In response to DNA DSB, p53 shows a series of pulses with fixed amplitude and duration. In contrast, certain chemotherapeutic drugs induce sustained high levels of p53. While pulsatile dynamics lead to cell cycle arrest and repair, high sustained levels are associated with terminal cell fates like apoptosis and senescence.
the expression of several hundreds of genes [2] and is thereby able to induce cell fates ranging from cell cycle arrest to senescence and apoptosis upon DNA damage [3]. Many tumours have mutations either of p53 itself or of members of its signalling pathway underlining its importance as a tumour suppressor [4]. To prevent inappropriate induction of senescence or cell death, the p53 level in the cell is tightly controlled through its activating pathways. These pathways are directly connected to the DNA damage response machinery and thereby allow a sensitive and precise response to genotoxic stress [5]. Under nonstressed conditions, p53 levels in the cell are kept low due to MDM2-mediated ubiquitination and subsequent proteasomal degradation of p53 [6]. When the cell senses genotoxic stress, three kinases of the PI3K like kinase family are activated: ATM, ATR and DNA-PKcs, which phosphorylate p53 on S15 and stabilize the TF [7–9] (Fig. 1B). Beside S15, several other residues of p53 are phosphorylated in the course of the DNA damage response. For instance, ATR and ATM activate the checkpoint kinases CHK1 and CHK2 which phosphorylate p53 on S20 and thereby contribute to preventing its degradation [10]. After accumulation in the nucleus, the TF promotes the expression of its target genes, mediating the cellular response to the damage. Among the target genes are negative regulators of p53 such as MDM2 and the phosphatase WIP1 (encoded by the gene PPM1D), which limit p53 accumulation [11,12]. In addition to stabilizing modifications, p53 is also phosphorylated on other residues that can modify its activity. For example, phosphorylation at S46 by the kinase HIPK2 is associated with the induction of genes involved in apoptosis [13,14] (for detailed review about phosphorylation of p53, see [15]).

This interplay of positive and negative regulators constitutes the core of the p53 network. However, it only represents one of several layers of regulation that enable cells to generate stimulus-specific responses, to diversify these responses and to fine-tune them according to their individual needs (Fig. 1A).

**P53’s dynamics: a key to understanding the encoding of individual stress levels**

A first layer of regulation is implemented at the level of p53 dynamics. As previously reviewed by Purvis and Lahav [16], the dynamics of proteins are defined as the change of their level, their localization or of other features over time. Several TFs show complex dynamical behaviours after activation. The resulting patterns range from transient and sustained responses to periodic activation. For instance, NF-κB, ERK and NFAT4 (NFATC3) exhibit repeated pulses of cytoplasmic to nuclear translocation upon defined stimuli [17–19]. TF dynamics contribute to encoding the kind and strength of stimuli and control subsequent cellular responses. Using single cell measurements and image analysis, the specific features of these dynamics can be quantified. Relevant features in the context of p53 include the amplitude and length (duration) as well as the timing of accumulation. Early studies of the temporal changes of p53 levels in individual breast carcinoma cells revealed that the tumour suppressor exhibits a series of discrete accumulation pulses with fixed amplitude and duration in response to DSB [20–23] (Fig. 1C). The features of these pulses are independent of the strength of the damage stimulus. Already low degrees of damage or transient input signals can trigger a full p53 pulse. For instance, damage occurring during proliferation induces spontaneous p53 pulses with the same characteristics as pulses that are formed in response to externally induced DSB [24]. This excitability of the p53 network allows it to react with high sensitivity to all levels of damage. Remarkably, no discrete threshold in the number of DSB was found for inducing a p53 pulse. Individual cells with a similar number of DSB exhibited different p53 dynamics depending on the state of a given cell [25]. These cell-specific activation thresholds occurred in genetically identical cell of the same type and could be explained by variability in WIP1 protein levels [26]. As WIP1 removes modifications of p53 and MDM2 that are required for p53 accumulation, cells with higher levels of WIP1 needed higher levels of active ATM and consequently more DSB to induce a p53 pulse.

While the features of p53 pulses do not generally reflect the severity of the stress, the number of pulses that are triggered in response to genotoxic stress increases with the damage dose, indicating that p53 dynamics encode the strength of the input. However, even isogenic cells in the same environment show variations in their p53 dynamics and sister cells soon lose their correlation most likely due to bursty gene transcription and the resulting stochastic changes in mRNA and protein abundance [22]. This heterogeneity in the response emphasizes the importance of measuring p53 dynamics in individual cells and raises the question how one molecular network can induce different dynamics in genetically identical cells.

This characteristic is even more striking when we consider different forms of DNA damage. In contrast to the pulsatile dynamics in response to DSB-inducing ionizing radiation, p53 shows different dynamics after UV radiation or treatment with chemotherapeutic drugs [27,28] (Fig. 1C). Treatment with the...
chemotherapeutic drug cisplatin, for example, induces a constant accumulation of p53. While the underlying mechanism for these altered dynamics is not well understood, the p53 response to UV radiation has been characterized in molecular detail. Here, only one single pulse is formed that increases in amplitude and duration with increasing dose. This can be explained by the observation that in contrast to DSB-inducing IR, UV radiation results in the exposure of single-stranded DNA as a repair intermediate, which leads to the activation of the kinase ATR. As the ATR- and ATM-driven p53 networks have different topologies of their negative feedbacks, also the resulting p53 dynamics differ [27]. But what is the purpose of this complex dynamical behaviour? It was shown by pharmacological and genetic perturbations that p53 dynamics determine gene expression programmes and define cell fates [29,30]. While a pulsatile response leads to cell cycle arrest, a sustained p53 response activates different sets of genes and induces senescence (Fig. 1D). Consistently, a further study indicated that low levels of the chemotherapeutic drug etoposide induced pulsatile dynamics and cell cycle arrest, while high doses led to a strong monotonic increase and apoptosis [31]. In this context, it was proposed that for the induction of apoptosis in response to cisplatin treatment, the p53 levels must exceed a time-dependent threshold [28].

While we mainly focus on context-specific decisions in individual cells of a given cell type here, it should be noted that studies comparing p53 dynamics in a variety of cell lines revealed diverging cell type-specific dynamics as well. For example, some cell lines showed dose-dependent changes in the features of the pulsatile p53 dynamics induced by IR, which could be linked to the state of the damage sensing network and the kinetics of DSB repair [32]. Nevertheless, the qualitative features of p53 dynamics are conserved not only across different cell lines but also across various species [33].

All things considered, p53 is robustly induced by various levels of damage, while its complex dynamics allow cell- and stimulus-specific responses. As ATM and WIP1 are central for regulating p53 dynamics, it is not surprising that these two proteins are major players when it comes to crosstalk with other signalling pathways. This integration of the context of the cell further shapes p53 dynamics and can lead to a different response to the same stimulus within the same cell type.

**Thinking outside the box: integration of external stimuli in the p53 response**

To enable appropriate decisions, p53 needs to integrate damage stimuli with inputs from other signalling sources. Cells are constantly challenged with varying internal and external signals, and these different contexts have to be considered for the cell’s final fate decision. The corresponding crosstalk between signalling pathways forms an additional layer of regulation, which can take place at multiple levels within the p53 network. For example, key players of the networks can interact directly with each other and change their corresponding activity. Alternatively, transcription of pathway regulators or target genes can be altered to rewire the network and modify signalling outcomes. For several pathways, such interactions with the p53 response were reported, including STAT, NF-κB and TGF-β signalling (Fig. 2).

TGF-β signalling regulates several cellular processes including proliferation and migration [34]. In brief, TGF-β binds to TGF-β-receptors, which induces the recruitment of SMAD2 and 3 (Fig. 2A). After complex formation with SMAD4, the proteins enter the nucleus and regulate the expression of their targets [35]. Several members of the p53 network were shown to interact with the TGF-β signalling pathway. For instance, the activity of ATM in response to ionizing radiation is modulated by TGF-β signalling [36]. In addition, recent studies have shown that the phosphatase WIP1 dephosphorylates SMAD4 at Thr277, a MAPK phosphorylation site that modulates the nuclear accumulation of SMAD4 [37]. Thereby, WIP1 oppresses TGF-β’s antiproliferative effects. Moreover, p53 itself plays an important role in TGF-β-induced growth arrest and physically associates with SMAD2/3 in a TGF-β-dependent manner [38]. Several targets of TGF-β signalling require joint control of p53 and SMAD proteins for activation. For instance, the PAI-1 (SERPIN1) promoter contains neighbouring p53- and SMAD-binding elements and p53 forms a complex with SMAD2/3 on the promoter [39]. Depletion of p53 impaired PAI-1 expression and growth arrest in response to TGF-β stimulation. Additionally, mutant p53 is suggested to repress TβRII expression and thereby impairs several stages of TGF-β signalling [40].

The p53 response was also shown to interact with the JAK/STAT pathway. Signal transducers and activators of transcription (STAT) proteins are a family of latent TFs involved in development, cell growth, proliferation and cell death [41]. Several ligands such as interferons, interleukins and growth factors activate the JAK/STAT pathway upon binding to their cell surface receptors and induce the phosphorylation of STAT proteins by JAK tyrosine kinases, which enables them to enter the nucleus (Fig. 2B). Several STATs were shown to modulate the p53 response. STAT3 binds directly to the p53 promoter and inhibits its...
expression [42], which leads to reduced induction of growth arrest upon UV radiation. In addition, STAT3 antagonizes p53-dependent repression at specific target promoters [43]. Beside STAT3, STAT1 is also a modulator of the p53 response. It interacts with p53 and fosters apoptosis induction in response to genotoxic stress by altering expression of p53 target genes like BAX, NOXA (PMAIP1) and FAS [44].

Another well-documented example for a pathway that is tightly connected to p53 is the NF-κB network. NF-κB is a dimeric TF whose targets are involved in the promotion of cell survival and immunity [45]. When bound to inhibitors of the IκB family, NF-κB is unable to enter the nucleus and is therefore inactive [46]. When extracellular ligands like TNF-α bind to membrane receptors, a complex called IKK is activated, which phosphorylates IκBα (NFKBIA) and induces its proteasomal degradation [46]. As a consequence, NF-κB is able to enter the nucleus (Fig. 2C). Several players of the p53 response were shown to interact with the NF-κB network. Firstly, the phosphatase WIP1 dephosphorylates S536 of the p65 (RELA) subunit of NF-κB, a modification that is essential for its transactivation abilities [47,48]. WIP1 is also a direct target of NF-κB [49], forming a negative feedback loop. Secondly, in response to DNA damage, ATM mediates the activation of the IKK complex and is therefore crucial for the activation of both the p53 and NF-κB pathways upon genotoxic stress [50,51]. Beside its role in the NF-κB network, the IKK subunit IKK2 (IKBKB) was also reported to phosphorylate p53 on S362 and S366 [52], which promotes its degradation via β-TrCP1 independent of MDM2. Mutation of these serine residues increases the stability of p53 as well as target gene expression. Furthermore, it could be shown that loss of IKK2 activity leads to increased p53 stability, promoter binding and target gene expression [53].

While many reports such as the examples given above highlight individual interactions mediating signalling crosstalk, we lack a more systematic understanding of the interplay between pathways. To address this issue, a recent study combined time-resolved data at single cell level with pharmacological perturbations and mathematical modelling to provide a framework for systematic identification of interactions points between signalling pathways [54]. In this case, NF-κB signalling was modulated using an IKK2 inhibitor and consequences on p53 dynamics upon DSB induction were monitored by time-lapse microscopy. Interestingly, several features of the p53 dynamics were changed including a delayed timing of the peak of p53 accumulation and significantly longer...
interpeak intervals. Notably, these changes resulted in differences in target gene expression profiles. Using two complementary computational approaches, it was shown that the modulated p53 dynamics could not be explained by single interactions between NF-κB and p53. Instead, crosstalk at multiple independent nodes was necessary. These nodes could be narrowed to processes affecting the activation and degradation of p53 as well as the degradation of MDM2. The framework for investigating crosstalk between signalling pathways introduced in this study can now be applied to help to systematically decipher the manifold interactions between p53 and other cellular networks.

**Promoter puzzles: decoding and diversifying the p53 response**

To enable cells to take appropriate fate decisions, cell-specific p53 responses need to be translated into context-specific expression patterns of its target genes. To this end, the transcriptional activity of p53 at the promoters of its targets is subject to further layers of regulation. Time-varying post-translational modifications (PTMs), regulated oligomerization and interaction with alternating cofactors allow to further diversify the p53 response.

The central process in the regulation of target gene expression is the conversion of free p53 accumulated in the nucleus to an active TF at the promoter. In essence, this allows the cell to decode the information contained in the dynamic p53 response. The cis-regulatory architecture, location and sequence of p53 response elements in target gene promoters and, as a consequence, the affinity of the TF to these binding sites varies widely. Not surprisingly, target gene mRNAs are expressed with different temporal patterns upon damage induction [55]. However, several studies demonstrated that despite varying expression patterns, the dynamics of p53 binding to its target promoters are similar across many loci including pro-apoptotic and pro-arrest genes [55–57]. This indicates that upon induction of DSB, a linear relationship exists between overall p53 levels in the nucleus and the amount of TF bound at the promoter for most genes. The correlation between p53 levels and chromatin binding seems to be retained when p53 dynamics are altered either pharmacologically using the MDM2 inhibitor Nutlin-3 or by using UV as an alternative damage stimulus [56,58]. However, TF binding has so far mainly been measured at the population level using methods such as ChIPseq. Due to the heterogeneity of p53 dynamics in individual cells within a population, measuring p53 recruitment to chromatin on the single cell level may reveal regulated recruitment to target gene promoters [59].

Remarkably, despite similar temporal changes in p53 binding to target gene promoters in response to DSB, even isogenic cells show a high degree of variability during transcription. One factor contributing to this heterogeneity is transcriptional bursting (Fig. 3). Due to stochastic activation and inactivation of the promoter, mRNAs are not continuously transcribed. Instead, gene expression switches between transcriptionally inactive (OFF) and active (ON) states, during which a variable number of transcripts are generated (Fig. 3A) [60]. Transcriptional bursting can not only lead to differences in the behaviour of individual cells within a population, diverse bursting patterns for different genes also allow a high variability in gene expression [61]. Studies on the single cell level employing single molecule fluorescence in situ hybridization (smFISH) showed that p53-mediated target gene expression is burst-like as well [56]. Upon p53 activation, the frequency of target gene promoter activation is increased, while the number of transcripts produced per burst remains unaltered. Furthermore, the transcriptional activity of p53 targets is gene-specific with changes between the first pulse and the second pulse. The target genes could therefore be grouped according to their pattern of promoter activity into ‘sustained’, ‘pulsatile’ and ‘transient’ responders, which suggests that direct regulation of stochastic bursting contributes to establishing distinct patterns of gene expression (Fig. 3B). Similarly, Hariton et al. [62] demonstrated that promoters can differentially decode p53 dynamics despite similar temporal patterns of chromatin recruitment by employing signal processing features such as activation thresholds, refractory periods or dynamic filtering. Moreover, time-resolved RNA sequencing similarly revealed multiple groups of p53 dependent expression patterns [55]. However, as the amount of RNA and protein is determined by the balance of production and degradation processes (Fig. 3A), the corresponding dynamics of total mRNAs are also shaped by their respective half-life, which was supported by additional molecular analyses [63,64]. To further investigate the p53-driven information transfer along the central dogma of molecular biology, a recent study established a fluorescent reporter system that allowed simultaneous tracking of p53 dynamics and the RNA and protein levels of its target p21 (CDKN1A). Overall, they observed a tight correlation between p53 protein level and p21 transcription. Interestingly, while the transcription magnitude
rapidly saturated, the probability for the induction of transcription was determined by p53 levels [65]. How could we explain the diversification of target gene promoter activity despite uniform p53 dynamics in the nucleus? One crucial factor may be PTMs of the TF that do not only stabilize it but also modulate its activity. The role of acetylation for promoter binding and activation of target gene transcription has been steadily studied over the past decade. P53 is acetylated on several lysine residues predominantly in the C-terminal domain including K320, K372 and K382 [9,66,67]. The C-terminal parts of p53 control site-specific DNA binding and are important for structural features of the DNA-binding domain [68]. Early studies indicated that acetylation of the C terminus enhances site-specific DNA binding [66,67,69,70] while others have shown that acetylation of p53 induces transcription by recruitment of co-activators or facilitating histone acetylation instead [71]. Loss of acetylation at one site can be compensated by other sites, while simultaneous loss of acetylation at eight lysine residues completely blocks p53 dependent activation of both growth arrest and apoptosis [72]. Consequently, acetylation is deemed indispensable for p53 activation. In this context, single cell studies have shown that p53 binds only transiently to chromatin and that its bound fraction and residence time increases after DNA damage-dependent acetylation on its C terminus [59]. However, does the acetylation state of p53 also contribute to diversification of target gene expression patterns and cell fate decision?

Knights et al. [73] proposed that p53 acetylation cassettes coordinate gene expression in response to DNA damage. Acetylation of K320 blocks the phosphorylation of important serine residues on the N terminus and restricts activation to high-affinity p53-binding sites that are involved in arrest. In contrast, K373 acetylation induces phosphorylations on the N terminus and triggers binding to low-affinity-binding sites such as those found in pro-apoptotic genes. Furthermore, recent studies provided evidence that the C-terminal acetylation state of p53 mediates gene-specific regulation of transcriptional activity. Specifically, the archetype of transient expression observed in the smFISH-based study of stochastic gene expression mentioned above is regulated via acetylation and methylation of K370 and K382 [56]. Interestingly, lysine residues in the DNA-binding domain were found to be acetylated after DNA damage. K120 is acetylated by MOZ (KAT6A), hMOF (KAT8) and TIP60 (KAT5) [74–76] upon exposure to different kinds of DNA damage. Modification of this residue was shown to be crucial for p53-mediated apoptosis and senescence rather than growth arrest. Importantly, acetylation of p53 does not only have direct effects on its DNA-binding capabilities, but influences target gene expression also via an indirect mechanism. On the promoter of distinct p53 targets, p53 forms a complex with MDM2 [77–79], which represses transcription. Acetylated p53 does not recruit MDM2 to these promoters and is therefore able to activate transcription [72]. Beside its role in the choice of the correct
cellular outcome, acetylation of p53 also serves as a fine-tuning mechanism for nonsevere intrinsic damage. During proliferation, p53 shows single accumulation pulses similar to pulses occurring after high degrees of externally induced damage. However, these pulses do not result in a full damage response due to the lack of an activating p53 PTM profile. Consequently, acetylation functions as a fine-tuning mechanism to filter p53 pulses induced by transient damage frequently occurring in proliferating cells [24].

In addition to PTMs and the dynamical behaviour of p53, other mechanisms have been proposed to influence the cell fate decision. Under basal conditions, most p53 molecules in the cell form dimers [80]. Upon DNA damage, two p53 dimers rapidly form tetramers that bind DNA [80,81]. The oligomerization process itself is crucial for the transcriptional activity of p53 and might contribute to the decision between survival and death. Mutations in the oligomerization domain strongly impair DNA binding and mutants that lack the ability to form oligomers are transcriptionally inactive [82–84]. In this context, the oligomerization status itself might regulate which set of genes is activated [85]. p53 mutants that only form dimers induced the transcription of genes involved in growth arrest, whereas pro-apoptotic genes were only triggered by tetrameric p53, which may be connected to the affinity of the corresponding p53-binding sites [86,87]. Similarly, mutations that impair cooperative binding to the DNA due to changes in the quaternary structure of the p53 tetramer also show altered cellular outcomes: While high DNA-binding cooperativity is crucial for p53 dependent apoptosis, it is dispensable for the induction of arrest [88–90].

Considering the important role of oligomerization, it is crucial to understand how the oligomerization process is regulated. Tetramerization of p53 is triggered rapidly after induction of DNA damage [80]; however, the process is independent of the rise in total p53 levels. PTM of residues located in the tetramerization domain was shown to alter p53’s oligomeric conformation. For instance, phosphorylation on S392 triggers tetramerization of p53 while this effect is reversed by additional phosphorylation of S315 [91,92]. Another way to regulate the formation of tetramers is the interaction with proteins modulating the oligomerization process. Proteins of the S100 family associate with the tetramerization domain of monomeric/dimeric p53 and abrogate the formation of tetramers [93]. Similarly, the multifunctional protein p32 (encoded by C1QBP) was shown to interfere with p53’s tetramerization [94]. This way, p32 inhibits DNA binding, occupancy at target genes and p53 dependent transcription.

Beside PTM and oligomerization, other factors contribute to diversifying similar promoter-binding dynamics to a specific target gene expression. For example, p53 interacts with other TFs as well as with several coregulators that modify the surrounding chromatin structure to specifically induce defined cell fate programmes (for detailed reviews, see [95,96]).

When considering these different layers of regulation, it is important to keep in mind that they do not act in isolation, but instead synergize to precisely control p53’s activity. For instance, PTMs alter the oligomerization status while the modification patterns themselves can be changed between p53 pulses. In turn, PTMs alter the stability of p53 and can thereby shape p53 dynamics. Interestingly, the oligomerization process seems to be independent of the accumulation of p53 after genotoxic stress and tetrameric p53 was even shown to be less stable than monomeric or dimeric forms [80,85]. However, certain PTMs, such as phosphorylation of S20 or acetylation on K320, depend on tetramerization of p53, indirectly linking the oligomerization status of p53 with the TF’s dynamics [66,97]. Together, these mechanisms allow diverse and context-specific target gene expression patterns despite having a single protein input.

The finishing touch: adapting the p53 response to the cell cycle phase

Even after the expression of p53 target genes is set, cells still fine-tune the response depending on their state. A well-studied example is the regulation of p21 depending on the cell cycle phase [98–100]. While the p53 response upon genotoxic stress is uniform in different cell cycle phases, p21 accumulation is heterogeneous and only observed during G1 and G2 phase. This could be explained by PCNA-CRL4<sub>cdt2</sub>-dependent degradation of p21 during S-phase, which leads to its delayed accumulation in G2. Blocking the PCNA-p21 interaction by mutation of p21’s PIP box domain causes a more homogeneous p21 response with p21 accumulating in all cell cycle phases. Also in unstressed cells, p21 accumulates in a p53-dependent manner during the mother’s G2 and daughter’s G1 phases due to DNA damage acquired in the course of proliferation [99,101]. High levels of p21 lead to a G1 arrest through CDK inhibition, whereas low levels of p21 do not alter G1 progression. In this case, p21 is degraded via the ubiquitin ligases CRL4<sub>cdt2</sub> and SCF<sub>skp2</sub> at the G1/S transition. All in all, p21 passes on information about DNA damage from mother to daughter cells and thereby alters the cell’s decision between proliferation and arrest [101].
Perspective

The examples given here can provide only a glimpse of the manifold layers of regulation in the p53 pathway. To get a more comprehensive view on the p53 response, we are challenged with developing systematic approaches relying on well-controlled, quantitative and time-resolved experiments in combination with theoretical approaches such as mathematical modelling. This will help our intuitive understanding and provide means to decipher the complex molecular networks mediating cellular decisions. Crosstalk with the NF-κB pathway is a good paradigm to show how p53 is interconnected with the complex web of signalling networks in the cell. While for several other networks, single interaction nodes have been determined, it is not clear yet how they affect the p53 response. For instance, it remains to be elucidated to what extent activation of JAK/STAT signalling or stimulation with TGF-β modulate p53 dynamics and the cellular outcome upon genotoxic stress. Integration of these and other extracellular signals would allow p53 to adjust its dynamic response to cellular stress depending on complex information from the immune system and neighbouring tissues.

Another challenge is to understand how p53 dynamics are decoded and to elucidate their function. A current hypothesis for the function of the p53 pulses is that they allow more diverse gene expression patterns. p53 target genes themselves serve as filters for p53 pulses, and their final expression pattern is determined by the half-life of mRNA and protein [63]. Furthermore, p53 dynamics can prevent too high accumulation of target proteins such as p21 to avoid terminal cell fates like senescence or apoptosis [65]. Yet, changes in p53’s transcriptional activity and its modification state between the first pulse and the second pulse could hint towards another function of p53 pulses [56]. Pulsatile behaviour might allow an effective change in PTM patterns which in turn modulates target gene transcription (Fig. 3C). Variations in the PTM patterns may indicate that the underlying interplay of modifying enzymes changes between the first and second pulses and might even point towards the involvement of additional regulators that are so far not fully appreciated. However, for what purpose could p53’s PTM be altered in the course of the response? One potential explanation is that the status of the genome continuously changes while the cell reacts to damage. When the first p53 pulse is triggered, the DNA repair machinery has just detected the newly formed DSB, and, as a consequence, most of the breaks remain unrepaired. However, when the second and subsequent pulses are induced, the cell already recognized and marked the lesions and restored many of them. Only a fraction of more complex and hard-to-repair breaks remain. Through distinct molecular barcodes, the p53 pathway might be able to differentiate between acute and sustained damage [102]: The resulting PTM can alter the oligomerization status of p53 as well as DNA binding and the interaction with its cofactors, which together allows the induction of appropriate response programmes.

As a central decision-maker in the cell, the guardian of our genome collects information from all available sources and is itself influenced by ‘crosstalking’ to other cellular regulators. The many different layers of regulation in the p53 system often provide challenges when we aim to investigate specific aspects of the signalling pathway. Even isogenic cells show a high degree of heterogeneity given their individual state and local environment. In different cell lines, the deviations of the p53 response are even stronger. Diverging dynamics as mentioned above are only the tip of the iceberg when comparing p53 signalling in different cell types. Virtually all layers of the p53 response can be affected in different cell lines. For instance, different sets of active interconnected signalling pathways as well as cell type-specific chromatin modifications can shift the final cell fate decision towards another outcome (for a recent review, see [103]). Taking this into account, it is not too surprising that there are often controversial reports regarding specific aspects of p53-mediated cell decisions. However, if we embrace the complexity and use systematic approaches to investigate it, we have the opportunity to use the well-studied p53 system as paradigm to fully understand the regulatory potential of cellular signalling and decision-making.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Both authors drafted, edited and revised the manuscripts.
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