155. Akira, S. & Takeda, K. Toll-like receptor signalling. *Nature Rev. Immunol.* **4**, 499–511 (2004).
156. Tsenova, L., Bergtold, A., Freedman, V. H., Young, R. A. & Kaplan, G. Tumour necrosis factor α is a determinant of pathogenesis and disease progression in mycobacterial infection in the central nervous system. *Proc. Natl Acad. Sci. USA* **96**, 5657–5662 (1999).
157. Schluter, D. *et al.* Both lymphotoxin-α and TNF are crucial for control of *Toxoplasma gondii* in the central nervous system. *J. Immunol.* **170**, 6172–6182 (2003).
158. Pasparakis, M. *et al.* Peyer's patch organogenesis is intact yet formation of B lymphocyte follicles is defective in peripheral lymphoid organs of mice deficient for tumor necrosis factor and its 55-kDa receptor. *Proc. Natl Acad. Sci. USA* **94**, 6319–6323 (1997).
159. Kuprash, D. V. *et al.* Novel tumor necrosis factor-knockout mice that lack Peyer's patches. *Eur. J. Immunol.* **35**, 1592–1600 (2005).
160. Grivennikov, S. I. *et al.* Distinct and nonredundant *in vivo* functions of TNF produced by T cells and macrophages/neutrophils: protective and deleterious effects. *Immunity* **22**, 93–104 (2005).
161. Beutler, B. *et al.* Identity of tumour necrosis factor and the macrophage-secreted factor cachetin. *Nature* **316**, 552–554 (1985).
162. Brennan, F. M., Jackson, A., Chantry, D., Maini, R. & Feldmann, M. Inhibitory effect of TNF-alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. *Lancet* **2**, 244–247 (1989).
163. Williams, R. O., Feldmann, M. & Maini, R. N. Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc. Natl Acad. Sci. USA* **89**, 9784–9788 (1992).
164. van Deventer, S. J. Anti-TNF antibody treatment of Crohn's disease. *Ann. Rheum. Dis.* **58**, 14–20 (1999).
165. Mease, P. J. *et al.* Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. *Lancet* **356**, 385–390 (2000).
166. Chaudhari, U. *et al.* Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. *Lancet* **357**, 1842–1847 (2001).
167. Berry, M. A. *et al.* Evidence of a role of tumor necrosis factor α in refractory asthma. *N. Engl. J. Med.* **354**, 697–708 (2006).
168. Ashkenazi, A. Targeting death and decoy receptors of the tumor necrosis factor superfamily. *Nature Rev. Cancer* **2**, 420–430 (2002).
169. Gray, P. W., Barret, K., Chantry, D., Turner, M. & Feldmann, M. Cloning of human tumor necrosis factor (TNF) receptor cDNA and expression of recombinant soluble TNF-binding protein. *Proc. Natl Acad. Sci. USA* **87**, 7380–7384 (1990).
170. Heller, R. A. *et al.* Amplified expression of tumor necrosis factor receptor in cells transfected with Epstein–Barr virus shuttle vector cDNA libraries. *J. Biol. Chem.* **265**, 5708–5717 (1990).
171. Coussens, L. M. & Werb, Z. Inflammation and cancer. *Nature* **420**, 860–867 (2002).

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Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
MYC | *TP53* | *TSC1* | *VHL*
National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary/
actinomycin D | bevacizumab | doxorubicin | etanercept | infliximab | melphalan | mitomycin C
UniProtKB: http://www.uniprot.org
AID | BRAF | CCL2 | CXCL12 | CD13 | IKKβ | IL-2 | IL-6 | lymphotoxin | MIF | MYD88 | p65 | SMAC | TERT | TLR2 | TLR4 | TLR6 | TNF | TNFR1 | TNFR2 | TRAIL | TSC1 | versican | VHL

FURTHER INFORMATION
Frances Balkwill's homepage: http://www.cancer.qmul.ac.uk/research/cancer_inflammation/index.html
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OPINION

The ups and downs of p53: understanding protein dynamics in single cells

Eric Batchelor, Alexander Loewer and Galit Lahav

Abstract | Cells living in a complex environment must constantly detect, process and appropriately respond to changing signals. Therefore, all cellular information processing is dynamic in nature. As a consequence, understanding the process of signal transduction often requires detailed quantitative analysis of dynamic behaviours. Here, we focus on the oscillatory dynamics of the tumour suppressor protein p53 as a model for studying protein dynamics in single cells to better understand its regulation and function.

How are signals received by a cell translated into decisions such as growth, death and movement? In the past several decades there has been a great deal of success in identifying the proteins and genes that are activated or repressed in response to specific inputs and in assembling them into signal transduction pathways. However, even though we now have maps of many signalling pathways, new questions have arisen owing to the complexity of the pathways they represent. How can we move beyond describing the structure of biological networks to developing a detailed, quantitative understanding of their function and behaviour? One promising approach is to investigate the dynamics of key proteins within the network (FIG. 1). In this context, dynamics is defined as the change of any variable that can be quantitatively measured over time, such as protein concentration, activity, modification state or localization. These data are complementary to the information originally used to describe the network, and have great potential to provide new insight into the relationship between network structure and function. For example, if the activity of a signalling molecule is measured at only a single point in time, the signal could be interpreted as binary: being either on or off. If, however, the signalling activity is quantitatively measured with high temporal resolution over a long period it could show a large number of distinct behaviours. Detailed analysis of dynamic behaviours in diverse systems and under various conditions has the potential to provide new levels of understanding of how cells detect inputs and translate them into outputs.

The analysis of cellular dynamics often requires measurements in single cells, as measurements of averaged dynamics in a population of cells can be misleading. For example, in response to certain doses of antibiotics, some cells live but others die[1]. These different outcomes might reflect differences in the initial state of the cell (such as its cell cycle state, basal level of network components or local environment), which in turn lead to differences in the quantitative behaviour of the information processing network. By visualizing the dynamic behaviour and identifying how it varies among cells (or cell types), we might be able to explain varying behaviours both within cell populations and in different cell types.

Single cell analyses of signalling systems have already revealed important information about the role of dynamics in regulating various cellular responses. For example, in mammalian cells the transcription factor nuclear factor-κB (NF-κB) shows pulses of nuclear localization on stimulation[2,3]. Single-cell analysis of luciferase expression from a synthetic NF-κB-responsive promoter suggested that the pulses are involved in maintaining target gene expression[3,4]. In *Saccharomyces cerevisiae*, the mitogen-activated protein kinase Fus3 shows oscillations in activity in response to mating pheromone[5]. The Fus3 oscillations correlate with oscillations in mating gene expression and the formation of new mating projections, as determined by fluorescence microscopy and flow cytometry using cells expressing fluorescent fusion proteins[5].

In this Perspective, we focus on the p53 network as a model for studying the dynamics of a signal transduction pathway in single

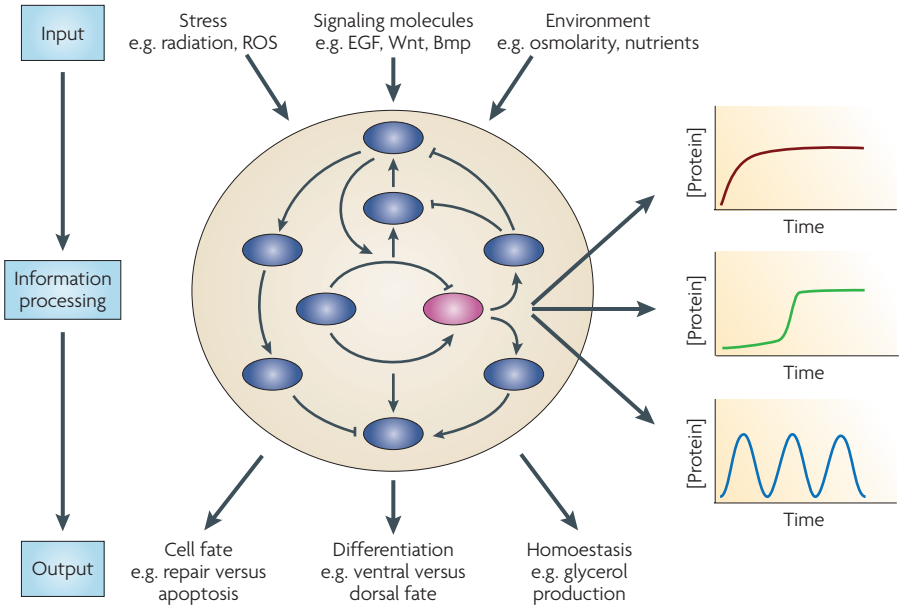

Figure 1 | **Dynamics in signal transduction pathways.** A complex protein network senses information about the intracellular and extracellular environment (input), processes the information, and triggers a response (output). Currently, the information processing step is usually represented as a static drawing of binary (inhibitory or activating) arrows connecting different components of the network. An important aspect that is missing from such diagrams is the dynamic behaviour of key members of the network. For example, the dark pink protein might show different dynamic behaviours in response to different inputs, in different cell types, or even between genetically identical cells. These dynamics can provide new insights about the specific interactions that are functional in each condition and the role of these interactions in triggering the right outcome. EGF, epidermal growth factor; ROS, reactive oxygen species.

cells to better understand its structure and function. This is an area of p53 research that is still in its early stages of development, and therefore the data covered in this article should be viewed in this context. We will discuss the proper characterization of p53 dynamics in single cells, especially as it relates to the structure of the network that shapes this dynamic response. We will also describe possible functions of p53 dynamics, in terms of both the fate of individual cells and the survival of the entire organism.

p53 dynamics in single cells

Owing to its important role in maintaining genomic integrity[6–8], the p53 network has been studied extensively over the past three decades (FIG. 2). p53 is upregulated in response to many forms of cellular stress, including various types of DNA damage[9]. The presence of stress is detected and transmitted to p53 by the action of signal transducers that post-translationally modify p53 and affect its stability and activity. Following activation, p53 regulates the transcription of hundreds of genes[7,10]. These genes code for proteins with a wide range of functions, including inhibition of cell cycle progression, activation of apoptosis, and regulation of p53 itself directly or through the upstream signal transducers[7,10] (FIG. 2).

Previous studies revealed that p53 undergoes a complex dynamic response to DNA damage. Work by Lev Bar-Or *et al.*[11] indicated that, in response to double strand breaks (DSBs) caused by γ-irradiation, p53 levels increased dramatically then decreased in a series of damped oscillations, in which the amplitude of the oscillations decreases in time (FIG. 3). Single live-cell analyses using fluorescently tagged p53 and higher temporal resolution revealed that these population studies masked the true behaviour of the network. Instead of damped oscillations, individual cells show series of undamped p53 pulses with fixed amplitude and duration, independent of the amount of γ-irradiation[12,13] (FIG. 3). The initial characterization of the pulses as damped oscillations was a result of averaging across a population of cells. The apparently lower amplitude of p53 in later pulses, as observed in western blots, is a results of several factors, including a reduction in the number of cells pulsing at later times[12] and loss of synchronization between individual cells[13,14].

Oscillations of p53 activity have also been observed *in vivo* using a mouse model. Hamstra *et al.*[15] constructed a transgenic mouse line in which firefly luciferase was expressed from a p53-responsive promoter. On irradiation, oscillations of luciferase were observed in a p53-dependent manner in the intestinal tissue. The timing of the oscillations was consistent with those observed in cultured cancer cells[11,12], indicating that oscillatory dynamics in the p53 network is not limited to cultured human cancer cells. Interestingly, the response *in vivo* was tissue

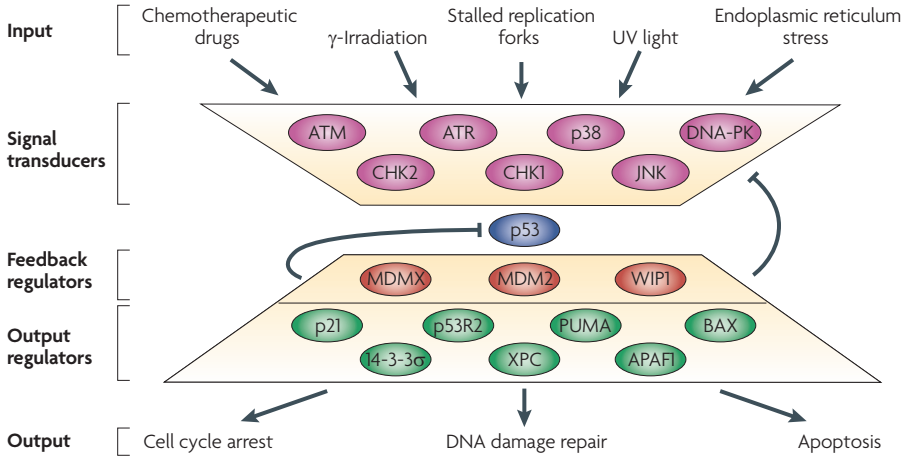

Figure 2 | **The p53 signalling network.** Stress signals (inputs) are detected and transduced to p53 through several kinases (signal transducers). On activation, p53 upregulates the transcription of numerous genes. Some p53 targets act as feedback regulators, altering the activity of the kinases or the stability of p53. Other p53 targets are output regulators that trigger specific cellular outcomes, including cell cycle arrest and apoptosis. APAF1, apoptotic protease-activating factor; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3 related; CHK, checkpoint kinase; DNA-PK, DNA-dependent protein kinase; JNK, JUN N-terminal kinase; p53R2, p53-inducible ribonucleotide reductase small subunit 2-like protein; UV, ultraviolet.

specific, suggesting that cell type-specific differences in the p53 network might give rise to differences in p53 dynamic responses to the same stimulus.

Understanding p53 dynamics

The frequency of p53 pulses in response to γ-irradiation appears to be tightly controlled. Do these pulses truly constitute oscillations or are they actually a series of independent pulses? Is this distinction biologically relevant in a sense that it would provide mechanistic insight into the function of the network, or is it merely a matter of semantics? We believe that it is crucial to distinguish between oscillations and repeated independent pulses, as the functions of the two behaviours are generally different, and they are used to 'solve' two different problems. Oscillators tend to be autonomous, and are often important for setting a well-regulated timescale or sequence to biological events. By contrast, pulse generators respond to stimulation with a single burst of activity, which can be repeatedly triggered. In addition, the network structures and kinetic parameters that are required for regulating these behaviours are different. For example, oscillators can arise from a single negative feedback loop, whereas pulse generators often exhibit excitability as a result of a fast and strong positive feedback that is crucial in setting an activation threshold[16,17].

The p53 system shares several features with oscillating systems and specifically with a class of dynamic systems called limit cycle oscillators[16–18]. The components of limit cycle oscillators change in concentration or location in a regular, repetitive pattern. In general, the changes are resistant to small fluctuations from a basal temporal trajectory. In mathematical terms, this trajectory is referred to as a stable limit cycle[16–18] (FIG. 4a). When operating in the stable limit cycle, oscillators tend to have characteristic frequencies and amplitudes. Specific examples of biological limit cycle oscillators include the regulation of sustained cyclic AMP oscillations in the amoeba *Dictyostelium discoideum*[19,20], regulation of circadian rhythms such as the PER–TIM system in *Drosophila melanogaster*[21] or the KaiABC system in cyanobacteria[22,23], and the eukaryotic mitotic clock[24,25].

An important (but not sufficient) component of oscillators is a negative feedback[26]. We have recently shown[14] that p53 dynamics in response to DNA double strand breaks (DSBs) are shaped by a combination of two negative feedbacks, including the negative feedback between p53 and MDM2, and the negative feedback between p53, ataxia telangiectasia mutated (ATM) and checkpoint kinase 2 (CHK2) mediated by the phosphatase WIP1 (also known as PPM1D)[27–30] (FIG. 3). Careful analysis of the dynamics of these feedbacks revealed that the upstream regulators ATM and CHK2 show oscillations that are both required for and shaped by p53 dynamics[14].

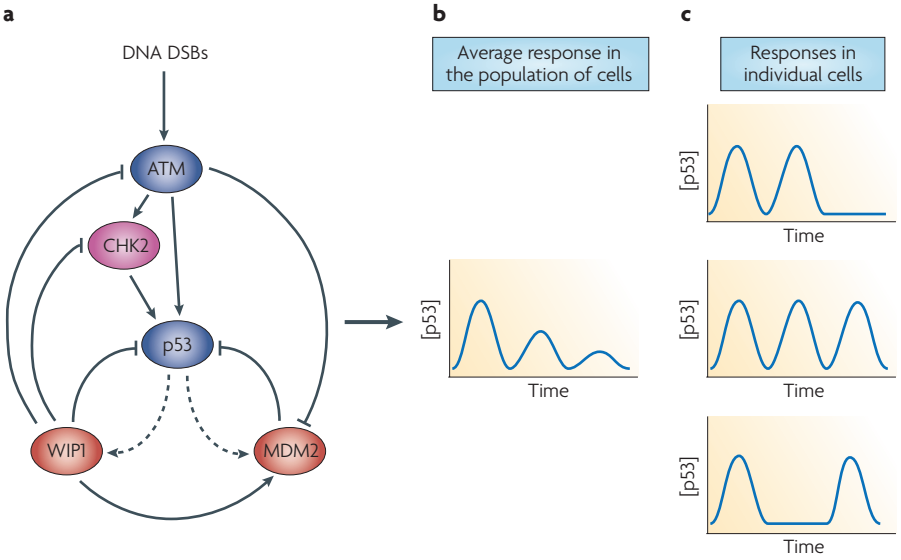

Figure 3 | **The response of p53 to DNA double strand breaks (DSBs). a** | In response to DSBs ataxia telangiectasia mutated (ATM) kinase is activated[64], and activates checkpoint kinase 2 (CHK2)[65,66]. Both of these kinases upregulate p53 by disrupting its interaction with one of its target genes, the E3 ubiquitin ligase MDM2 (REFS 52,53,67–69). p53 also upregulates the transcription of the phosphatase WIP1 which negatively feeds back on the entire circuit by dephosphorylating ATM, CHK2, p53 and MDM2. Solid lines represent protein–protein interactions, dashed lines represent transcriptional activation. **b** | Measurements averaged over populations of cells show damped oscillations of p53. **c** | Single cell measurements show a series of undamped pulses with different cells showing different numbers of pulses.

Computational work on p53 dynamics has suggested that additional negative feedbacks on p53, such as the feedback mediated by ARF[31], may also play a part in regulating the dynamic response of p53 to DNA damage. Additional work is required to determine whether this and other known negative feedbacks on p53 (REF. 32) are required for p53 oscillations.

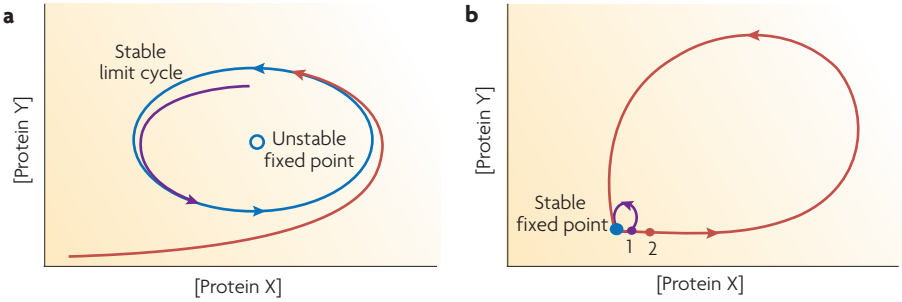

Figure 4 | **Phase plane trajectories of oscillators and pulse generators. a** | The concentration of proteins composing an oscillator follow a cyclic path in phase space called a limit cycle (blue). The limit cycle is stable: fluctuations of protein concentrations away from the limit cycle are suppressed. If the concentrations of the proteins in a system are initially different from values on the limit cycle, the system will relax away from unstable fixed points and will gradually approach the limit cycle. Two sample trajectories with different initial protein concentrations are shown in purple and red. **b** | The concentration of proteins in a pulse generator will remain at a stable fixed point as long as the system is unperturbed. If the system is perturbed slightly, as indicated in point 1, it relaxes back to the stable point with relatively small changes in the protein concentrations (purple trajectory). However, if the system receives a large enough perturbation, such as to point 2, the concentrations of the proteins in the system change greatly, making a large excursion in phase space before relaxing back to the stable fixed point (red trajectory).

The p53 system also shares features with excitable pulse generator systems. In these systems, the basal state is an off state and it is referred to as a stable steady state. Minor fluctuations from the stable steady state can immediately return back to the stable point (FIG. 4b). However, fluctuations from the steady state that are of sufficient size are amplified, usually through a strong positive feedback. The system is then excited and undergoes a large change in component concentrations, relative to the initial perturbation, before it returns to the stable steady state (FIG. 4b). Examples of pulse generator systems include protein networks regulating action potentials in neurons[33–36] and the switch from the vegetal growth state to the competent state in *Bacillus subtilis*[37]. Recently, we showed that transient activation of ATM and CHK2 results in a full pulse of p53 (REF. 14) away from its basal state, suggesting that there may be an excitable mechanism controlling p53 pulses. The fact that a simple negative feedback loop can generate sustained oscillations but not excitability suggests the existence of a positive feedback in the network controlling p53 levels. Several positive feedbacks have been identified in the p53 network[32], but additional studies are required to determine whether these or as yet unidentified positive feedback are important in shaping the p53 response to DSBs.

So, does the p53 system behave as a limit cycle oscillator or as a pulse generator? Is it possible that the p53 system switches between both kinds of dynamic behaviours? In general, biological systems can generate different dynamic behaviours, switching between qualitatively distinct regimes depending on the concentrations of network components and values of kinetic parameters for a given condition. For example, in the absence of growth factors, mammalian cells are in a stable, non-oscillatory steady state in which the concentrations of cell cycle factors remain fixed. In the presence of growth factors, the cell cycle regulators are forced out of the stable steady state into a limit cycle and continue to cycle as long as growth factors are present[38,39]. Theoretical analysis of the developmental decision in *B. subtilis* showed that multiple regimes of qualitatively distinct behaviour exist depending on key parameters in the network of interactions governing the system[37]. These regimes include both oscillatory and excitable pulse-generating regions[37]. Another example is the behaviour of giant squid axons. Experimental and mathematical analysis showed that stimulation away from a stable steady state above a threshold voltage leads to nerve signal propagation in an excitable manner[33,35]. However, applying an additional biasing current to the nerve leads to the formation of an unstable state surrounded by a stable limit cycle, converting the system from a pulse generator to an oscillator[34]. A similar case may hold for the p53 network: depending on cellular conditions, such as the amount of DNA damage or initial concentrations of regulatory proteins at the time of damage, the network may shift between pulse-generating and oscillatory behaviour. One could even speculate that in the absence of severe DNA damage p53 levels are low and excitable. Any brief damage elicits one pulse of p53. Sustained external damage might destabilize the steady state of the system and lead to a series of oscillations until damage is repaired.

On the basis of our present understanding of the structure and function of the p53 network, we currently favour the hypothesis that the network acts as a pulse generator in response to DSBs. It seems reasonable that, like a pulse generator, p53 is maintained in an off state until a stimulus is present. When a stimulus such as DNA damage is present, p53 shows a pulse only if the stimulus is large enough to push p53 over an activation threshold. After the completion of the pulse, if the stimulus is still present, and of sufficient magnitude, a subsequent pulse of p53 occurs. In this scenario, repeated activation would not require the formation of a stable limit cycle, but could arise owing to repeated perturbation from a stable steady state (FIG. 4). We therefore choose the word pulses for describing p53 dynamics throughout the second half of this article while keeping in mind that additional research is required to determine the proper classification and characterization of the dynamic behaviour of p53.

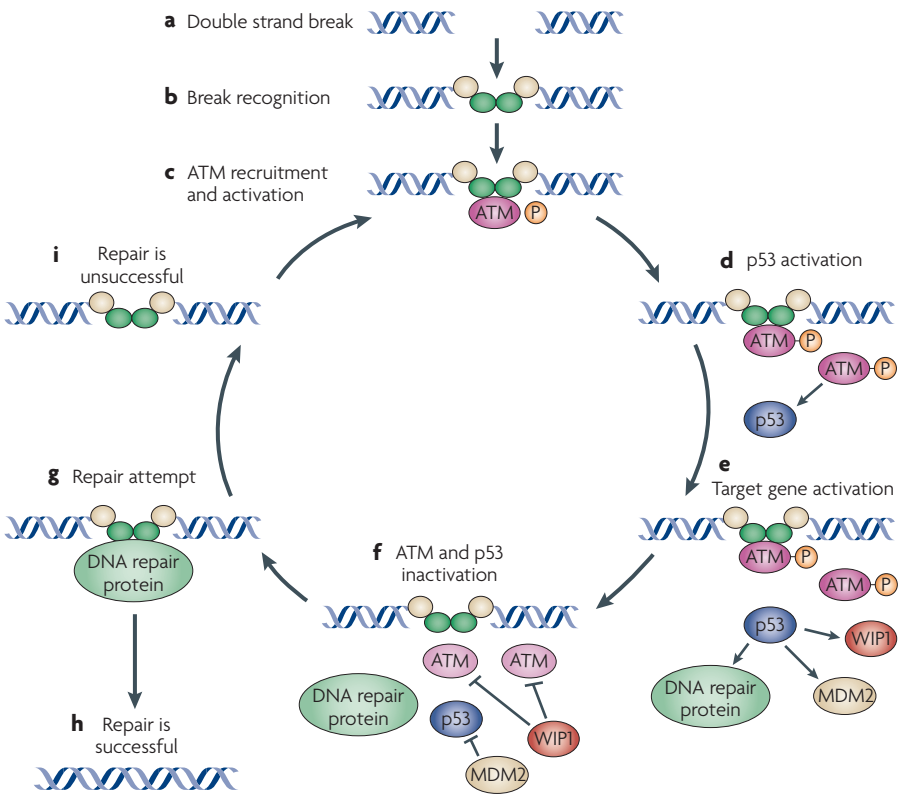

Figure 5 | **Model for the potential role of ataxia telangiectasia mutated (ATM) pulses in the DNA repair process.** When a DNA double strand break (DSB) occurs (**a**), several proteins form a complex at the break site (**b**). ATM is recruited and activated by break recognition proteins (**c**). Once activated, ATM stabilizes p53 and triggers its accumulation (**d**). p53 then transcriptionally upregulates the expression of several genes, including DNA repair genes and the inhibitor of ATM, WIP1 (**e**). Inhibition of ATM by WIP1 might lead to dissociation of ATM from the break (**f**), potentially allowing DNA repair proteins access to the DSB (**g**). If the DSB is repaired, ATM would not be recruited again and the signalling to p53 would be halted (**h**). If the DSB is not repaired (or if new breaks are detected), a new round of ATM recruitment would begin, resulting in a subsequent pulse of ATM and p53 (**i**).

Possible functions of the pulses

Even before we develop a complete understanding of the exact mechanism controlling p53 dynamics, we can begin to address the

role of its dynamics in responding to DNA damage. One possible explanation is that ATM pulses[14] are the pulses that are important for proper repair of DNA DSBs, and the p53 pulses are only an epiphenomenon of this behaviour (FIG. 5). In this scenario, when a DSB occurs, break recognition proteins including the MRE11–RAD50-NBS1 (also known as nibrin) complex (MRN complex)[40,41] localize to the site of damage. ATM is recruited to sites of DSBs through interactions with the MRN complex, which is believed to be required for kinase activation[42]. Following activation of ATM, a pulse of p53 occurs[14], leading to the transcription of several target genes including DNA repair proteins and negative feedback regulators. The negative regulator WIP1 has an important role in inhibiting ATM[27], resulting in a pulse of ATM activity[14]. We hypothesize that the inhibition of ATM may lead to dissociation of ATM from damage sites, thereby allowing repair proteins access to the damage. If a repair attempt is unsuccessful, a new round of ATM and p53 activation is initiated. In support of this idea, mutually exclusive binding of ATM and the repair cofactor XRCC4 to a site-specific DSB has recently been reported[41]. By contrast, many other proteins that associate with DSBs, including MDC1 (mediator of DNA damage checkpoint 1) and TP53BP1 (p53 binding protein 1), appear to remain stably associated with damage sites for prolonged periods[43]. This suggests that, if there is shuttling of proteins on and off damage sites, it may be specific to a subset of the proteins involved in the DSB response. Further studies, together with the development and refinement of technology for measuring kinase activity in single cells, are required to quantify the binding of ATM to the breaks over time and to determine how this affects the repair rate and the dynamics of many additional ATM substrates[44].

On the other hand, it is possible that ATM pulses serve a purely regulatory function for driving p53 pulses in response to DSBs. The question then becomes what is the function of p53 pulses in regard to its activity as a transcription factor? One of the simplest explanations is that the p53 pulses keep p53 below a threshold concentration to prevent premature activation of certain target genes, for example pro-apoptotic genes. Although this is certainly plausible, it is unlikely to be the entire reason for the pulsatile behaviour of p53, as simpler regulatory mechanisms, such as maintaining a constant low level of p53, could be used with similar results.

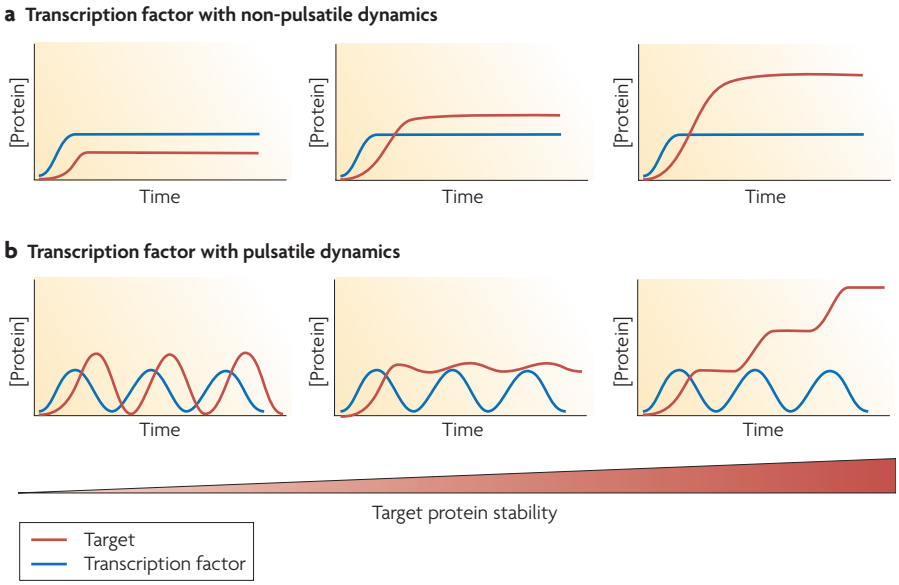

Figure 6 | **Pulsing transcription factors can give rise to multiple dynamic patterns of their target genes. a** | If, following activation, a transcription factor is increased to a new concentration that remains constant in time, the level of its target genes will also remain constant in time, with a steady-state level that depends on the stability of the target protein. **b** | If a transcription factor undergoes pulses upon activation, the dynamics of its target genes can take on a wider range of qualitatively distinct behaviours. For example, targets can show pulsatile, steady or step-like expression patterns depending on the stability of the target protein.

Are there any cellular benefits that could arise from p53 pulses? One possibility is that p53 pulses may coordinate regulation of the target genes of p53. Recent work in *S. cerevisiae* showed that the transcription factor Crz1 undergoes bursts of nuclear localization in response to Ca^{2+} (REF. 45). Using single-cell time lapse microscopy, it was shown that the frequency but not the amplitude of Crz1 pulses increased with greater concentrations of Ca^{2+}. This behaviour led to expression from various Crz1 target promoters in fixed ratios across a wide range of Ca^{2+} concentrations. Although the absolute level of activity of any given promoter depended on the concentration of Ca^{2+}, the ratio of the activity of one promoter to that of another promoter was independent of the Ca^{2+} concentration. Such fixed ratios would not occur if the amplitude of Crz1 bursts changed with Ca^{2+} concentration. These results suggest that frequency-modulated pulses might coordinate the expression levels of multiple target genes without the need to specifically tune the activity of individual promoters. The fact that the amplitude of p53 pulses is independent of stimulus strength (γ-irradiation dose) suggests that the ratio of p53 target genes may be fixed in a manner similar to the targets of Crz1. It might be beneficial for cells to coordinate upregulation of large sets of cell cycle arrest or DNA damage repair genes using such a regulatory mechanism. However, as opposed to the Crz1 system, the frequency of p53 pulses appears to be tightly regulated and fixed[13]. Frequency modulation of p53 pulses, if it indeed exists and plays a part in the function of the protein, has yet to be observed.

Recently, the varying functions of p53 targets pointed us to an alternative hypothesis for the function of its pulses. We suspect that the pulsatile dynamics of p53 increases the range of possible target gene dynamics compared with constant p53 levels. p53 regulates hundreds of genes[46], which are involved in diverse functions such as cell cycle regulation and apoptosis. It is likely that cells would benefit from having distinct temporal expression patterns for genes in different programmes. Constant p53 levels do not allow this wide range of behaviours, as target genes with similar promoter activity but different protein degradation rates result in qualitatively similar temporal expression patterns (FIG. 6a). Pulses of p53, however, allow a broader range of temporal patterns depending on the stability of the mRNA and protein of the target gene (FIG. 6b). One simple example of an expression pattern that can result from a pulsing transcription factor is a pulsatile pattern. This pattern arises when the degradation rates of the mRNA and

protein of the target gene are faster than the frequency of the transcription factor pulses. We observed such a pattern for the p53-regulated cell cycle regulator p21 (REF. 14). On the other hand, if the mRNA and protein half-life of the target gene are comparable to the frequency of the transcription factor pulse, the expression pattern remains constant following induction. If, however, the mRNA is rapidly degraded but the resulting protein is relatively stable, the target gene will show a step-like expression pattern. Further studies of the dynamics of different p53 target genes in response to DSBs are required to determine whether p53 pulses do translate into various dynamic patterns of its target genes.

Additionally, it has recently been suggested that p53 pulses may be a fast and effective method to regulate post-translational modifications of p53 to effect an orderly temporal pattern of cellular responses[47,48]. Currently, over 30 sites of post-translational modification in p53 are known, including numerous sites of phosphorylation, acetylation and ubiquitylation[49–51]. Although some of the sites (such as serine 15, serine 18, and carboxy-terminal lysines) are important for regulating p53 stability[52–54], other sites (such as lysine 373 and lysine 382) alter the gene-regulatory functions of the protein[55]. It has been speculated that specific patterns of post-translational modification on p53 might act as a molecular bar code, indicating specific cellular response programmes such as DNA repair or apoptosis[56,57]. If this is true, orderly progression from one stage to the next might require eliminating a large number of current modifications and establishing a new bar code. Instead of reversing a large number of modifications on the current set of p53 molecules, it might be more efficient to degrade the existing p53 and synthesize new p53 with a different bar code.

Connecting p53 pulses and cancer

As is often noted, p53 is estimated to be mutated in half of all cancers, and mutations in the p53 network are estimated to occur in nearly all cancers. An interesting possibility is that some mutations in the p53 network could affect the regulation of p53 dynamics, which might then contribute to the transformation of cells and the development of cancer. Recent work has suggested a correlation between a particular polymorphism in the p53 network, changes in p53 dynamics and predisposition to cancer. A single nucleotide polymorphism (SNP309G) in the *MDM2* promoter increases expression of MDM2 (REF. 58). Hu *et al.*[59] showed that cultured cells containing the wild-type SNP309T/T or heterozygous SNP309T/G sequences showed pulsatile p53 responses to DSBs caused by γ-irradiation. By contrast, cells carrying the homozygous SNP309G/G sequences responded to γ-irradiation by increasing the concentration of p53 and maintaining it at high levels without pulses[59]. Evidence has linked the SNP309G/G allele to earlier onset of several cancers, including breast carcinoma and colon carcinoma, in certain groups of patients[60]. However, contradictory data have also been presented that failed to find a statistical significance between SNP309G and tumour onset in other groups[61–63]. Clearly, more work is required to determine whether there is a connection between SNP309G, the alteration of p53 dynamics and tumour progression. However, understanding the role of p53 dynamics in healthy cells, and developing new ways to manipulate p53 dynamics in transformed cells may one day prove invaluable for cancer treatment.

Concluding remarks

Our ability to monitor and interpret dynamic changes is an important step for understanding a wide range of signalling events in both healthy cells and in the context of disease. As was clearly the case for the p53 network, proper identification of network dynamics depends strongly on our ability to monitor cellular events at the single-cell level. It is likely that we will identify additional systems exhibiting pulsatile or oscillatory dynamics as more analyses are performed at the single-cell level with the appropriate temporal resolution. Combining our current knowledge of the interactions that exist within various networks with new information regarding the dynamics of network components is likely to prove valuable in attempts to manipulate and control signalling systems for therapeutic purposes.

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1. Cohen, A. A. *et al.* Dynamic proteomics of individual cancer cells in response to a drug. *Science* **322**, 1511–1516 (2008).
2. Hoffmann, A., Levchenko, A., Scott, M. L. & Baltimore, D. The IκB–NF-κB signaling module: temporal control and selective gene activation. *Science* **298**, 1241–1245 (2002).
3. Nelson, D. E. *et al.* Oscillations in NF-κB signaling control the dynamics of gene expression. *Science* **306**, 704–708 (2004).
4. Nelson, D. E., See, V., Nelson, G. & White, M. R. Oscillations in transcription factor dynamics: a new way to control gene expression. *Biochem. Soc. Trans.* **32**, 1090–1092 (2004).
5. Hilioti, Z. *et al.* Oscillatory phosphorylation of yeast Fus3 MAP kinase controls periodic gene expression and morphogenesis. *Curr. Biol.* **18**, 1700–1706 (2008).
6. Lane, D. P. Cancer. p53, guardian of the genome. *Nature* **358**, 15–16 (1992).
7. Vogelstein, B., Lane, D. & Levine, A. J. Surfing the p53 network. *Nature* **408**, 307–310 (2000).
8. Levine, A. J. p53, the cellular gatekeeper for growth and division. *Cell* **88**, 323–331 (1997).
9. Horn, H. F. & Vousden, K. H. Coping with stress: multiple ways to activate p53. *Oncogene* **26**, 1306–1316 (2007).
10. Riley, T., Sontag, E., Chen, P. & Levine, A. Transcriptional control of human p53-regulated genes. *Nature Rev. Mol. Cell Biol.* **9**, 402–412 (2008).
11. Lev Bar-Or, R. *et al.* Generation of oscillations by the p53–Mdm2 feedback loop: a theoretical and experimental study. *Proc. Natl Acad. Sci. USA* **97**, 11250–11255 (2000).
12. Lahav, G. *et al.* Dynamics of the p53–Mdm2 feedback loop in individual cells. *Nature Genet.* **36**, 147–150 (2004).
13. Geva-Zatorsky, N. *et al.* Oscillations and variability in the p53 system. *Mol. Syst. Biol.* **2**, 2006 0033 (2006).
14. Batchelor, E., Mock, C. S., Bhan, I., Loewer, A. & Lahav, G. Recurrent initiation: a mechanism for triggering p53 pulses in response to DNA damage. *Mol. Cell* **30**, 277–289 (2008).
15. Hamstra, D. A. *et al.* Real-time evaluation of p53 oscillatory behavior *in vivo* using bioluminescent imaging. *Cancer Res.* **66**, 7482–7489 (2006).
16. Murray, J. D. *Mathematical Biology* (eds Antman, S. S., Marsden, J. E., Sirovich, L. & Wiggins, S.) 175–256 (Springer, New York, 2002).
17. Edelstein-Keshet, L. *Mathematical Models in Biology* 311–380 (Society for Industrial and Applied Mathematics, Philadelphia, 2005).
18. Strogatz, S. H. *Nonlinear Dynamics and Chaos* 196–297 (Perseus Books Publishing, LLC, Cambridge, 1994).
19. Halloy, J., Lauzeral, J. & Goldbeter, A. Modeling oscillations and waves of cAMP in *Dictyostelium discoideum* cells. *Biophys. Chem.* **72**, 9–19 (1998).
20. Tyson, J. J. & Murray, J. D. Cyclic AMP waves during aggregation of *Dictyostelium* amoebae. *Development* **106**, 421–426 (1989).
21. Leloup, J. C., Gonze, D. & Goldbeter, A. Limit cycle models for circadian rhythms based on transcriptional regulation in *Drosophila* and *Neurospora*. *J. Biol. Rhythms* **14**, 433–448 (1999).
22. Nakajima, M. *et al.* Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation *in vitro*. *Science* **308**, 414–415 (2005).
23. Johnson, C. H., Mori, T. & Xu, Y. A cyanobacterial circadian clockwork. *Curr. Biol.* **18**, R816–R825 (2008).
24. Pomerening, J. R., Sontag, E. D. & Ferrell, J. E. Jr. Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2. *Nature Cell Biol.* **5**, 346–351 (2003).
25. Tyson, J. J., Csikasz-Nagy, A. & Novak, B. The dynamics of cell cycle regulation. *Bioessays* **24**, 1095–1109 (2002).
26. Novak, B. & Tyson, J. J. Design principles of biochemical oscillators. *Nature Rev. Mol. Cell Biol.* **9**, 981–991 (2008).
27. Shreeram, S. *et al.* Wip1 phosphatase modulates ATM-dependent signaling pathways. *Mol. Cell* **23**, 757–764 (2006).
28. Fujimoto, H. *et al.* Regulation of the antioncogenic Chk2 kinase by the oncogenic Wip1 phosphatase. *Cell Death Differ.* 13 1170–1180 (2006).
29. Lu, X., Nguyen, T. A. & Donehower, L. A. Reversal of the ATM/ATR-mediated DNA damage response by the oncogenic phosphatase PPM1D. *Cell Cycle* **4**, 1060–1064 (2005).
30. Lu, X., Nannenga, B. & Donehower, L. A. PPM1D dephosphorylates Chk1 and p53 and abrogates cell cycle checkpoints. *Genes Dev.* **19**, 1162–1174 (2005).
31. Proctor, C. J. & Gray, D. A. Explaining oscillations and variability in the p53–MDM2 system. *BMC Syst. Biol.* **2**, 75 (2008).
32. Harris, S. L. & Levine, A. J. The p53 pathway: positive and negative feedback loops. *Oncogene* **24**, 2899–2908 (2005).

33. Hodgkin, A. L. & Huxley, A. F. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544 (1952).
34. Fitzhugh, R. Impulses and physiological states in theoretical models of nerve membrane. *Biophys. J.* **1**, 445–466 (1961).
35. Fitzhugh, R. Thresholds and plateaus in the Hodgkin–Huxley nerve equations. *J. Gen. Physiol.* **43**, 867–896 (1960).
36. Nagumo, J., Arimoto, S. & Yoshizawa, S. An active pulse transmission line simulating nerve axon. *Proc. IRE.* **50**, 2061–2070 (1962).
37. Suel, G. M., Garcia-Ojalvo, J., Liberman, L. M. & Elowitz, M. B. An excitable gene regulatory circuit induces transient cellular differentiation. *Nature* **440**, 545–550 (2006).
38. Murray, A. W. & Kirschner, M. W. Dominoes and clocks: the union of two views of the cell cycle. *Science* **246**, 614–621 (1989).
39. Gonze, D. & Goldbeter, A. A model for a network of phosphorylation–dephosphorylation cycles displaying the dynamics of dominoes and clocks. *J. Theor. Biol.* **210**, 167–186 (2001).
40. Mirzoeva, O. K. & Petrini, J. H. DNA damage-dependent nuclear dynamics of the Mre11 complex. *Mol. Cell. Biol.* **21**, 281–288 (2001).
41. Berkovich, E., Monnat, R. J. Jr & Kastan, M. B. Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair. *Nature Cell Biol.* **9**, 683–690 (2007).
42. Lee, J. H. & Paull, T. T. ATM activation by DNA double-strand breaks through the Mre11–Rad50–Nbs1 complex. *Science* **308**, 551–554 (2005).
43. Bekker-Jensen, S., Lukas, C., Melander, F., Bartek, J. & Lukas, J. Dynamic assembly and sustained retention of 53BP1 at the sites of DNA damage are controlled by Mdc1/NFBD1. *J. Cell Biol.* **170**, 201–211 (2005).
44. Matsuoka, S. *et al.* ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* **316**, 1160–1166 (2007).
45. Cai, L., Dalal, C. K. & Elowitz, M. B. Frequency-modulated nuclear localization bursts coordinate gene regulation. *Nature* **455**, 485–490 (2008).
46. Wei, C. L. *et al.* A global map of p53 transcription-factor binding sites in the human genome. *Cell* **124**, 207–219 (2006).
47. Lahav, G. The strength of indecisiveness: oscillatory behavior for better cell fate determination. *Sci. STKE* **2004**, pe55 (2004).
48. Zhang, T., Brazhnik, P. & Tyson, J. J. Exploring mechanisms of the DNA-damage response: p53 pulses and their possible relevance to apoptosis. *Cell Cycle* **6**, 85–94 (2007).
49. Kruse, J. P. & Gu, W. SnapShot: p53 posttranslational modifications. *Cell* **133**, 930–930.e1 (2008).
50. Brooks, C. L. & Gu, W. Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation. *Curr. Opin. Cell Biol.* **15**, 164–171 (2003).
51. Bode, A. M. & Dong, Z. Post-translational modification of p53 in tumorigenesis. *Nature Rev. Cancer* **4**, 793–805 (2004).
52. Banin, S. *et al.* Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science* **281**, 1674–1677 (1998).
53. Canman, C. E. *et al.* Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science* **281**, 1677–1679 (1998).
54. Toledo, F. & Wahl, G. M. Regulating the p53 pathway: *in vitro* hypotheses, *in vivo* veritas. *Nature Rev. Cancer* **6**, 909–923 (2006).
55. Li, A. G. *et al.* An acetylation switch in p53 mediates holo-TFIID recruitment. *Mol. Cell* **28**, 408–421 (2007).
56. Sims, R. J. 3rd & Reinberg, D. Is there a code embedded in proteins that is based on post-translational modifications? *Nature Rev. Mol. Cell Biol.* **9**, 815–820 (2008).
57. Murray-Zmijewski, F., Slee, E. A. & Lu, X. A complex barcode underlies the heterogeneous response of p53 to stress. *Nature Rev. Mol. Cell Biol.* **9**, 702–712 (2008).
58. Bond, G. L. *et al.* A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* **119**, 591–602 (2004).
59. Hu, W. *et al.* A single nucleotide polymorphism in the MDM2 gene disrupts the oscillation of p53 and MDM2 levels in cells. *Cancer Res.* **67**, 2757–2765 (2007).
60. Bond, G. L. *et al.* MDM2 SNP309 accelerates tumor formation in a gender-specific and hormone-dependent manner. *Cancer Res.* **66**, 5104–5110 (2006).
61. Petenkaya, A. *et al.* Lack of association between the MDM2-SNP309 polymorphism and breast cancer risk. *Anticancer Res.* **26**, 4975–4977 (2006).
62. Campbell, I. G., Eccles, D. M. & Choong, D. Y. No association of the MDM2 SNP309 polymorphism with risk of breast or ovarian cancer. *Cancer Lett.* **240**, 195–197 (2006).
63. Krekac, D. *et al.* MDM2SNP309 does not associate with elevated MDM2 protein expression or breast cancer risk. *Oncology* **74**, 84–87 (2008).
64. Bakkenist, C. J. & Kastan, M. B. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* **421**, 499–506 (2003).
65. Ahn, J. Y., Schwarz, J. K., Piwnica-Worms, H. & Canman, C. E. Threonine 68 phosphorylation by ataxia telangiectasia mutated is required for efficient activation of Chk2 in response to ionizing radiation. *Cancer Res.* **60**, 5934–5936 (2000).
66. Matsuoka, S. *et al.* Ataxia telangiectasia-mutated phosphorylates Chk2 *in vivo* and *in vitro*. *Proc. Natl Acad. Sci. USA* **97**, 10389–10394 (2000).
67. Chehab, N. H., Malikzay, A., Stavridi, E. S. & Halazonetis, T. D. Phosphorylation of Ser-20 mediates stabilization of human p53 in response to DNA damage. *Proc. Natl Acad. Sci. USA* **96**, 13777–13782 (1999).
68. Khosravi, R. *et al.* Rapid ATM-dependent phosphorylation of MDM2 precedes p53 accumulation in response to DNA damage. *Proc. Natl Acad. Sci. USA* **96**, 14973–14977 (1999).
69. Wu, X., Bayle, J. H., Olson, D. & Levine, A. J. The p53-mdm-2 autoregulatory feedback loop. *Genes Dev.* **7**, 1126–1132 (1993).

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DATABASES

UniProtKB: http://www.uniprot.org

ARF | ATM | ATR | CHK2 | MDM2 | MRE11 | NBS1 | p21 | p53 | PPM1D | RAD50 | TP53BP1 | XRCC4

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