hot off the press

Get your fingers out of p53's way!

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The p53 tumour suppressor acts as a major barrier against cancer [1,2]. Inactivation of p53's tumour suppressive functions appears to be almost a must for a tumour to develop; such inactivation can occur directly through mutations in the *TP53* gene encoding human p53, as is the case in about half of all human cancers, or through alterations in other components of the intricate p53 pathway.

The tumour suppressor activities of p53 rely on its ability to modulate a variety of biological processes in stressed as well as in unperturbed cells. Most notable in that regard is the capacity of p53 to enforce growth arrest or apoptosis in response to varying degrees and types of stress, as well as its role in ensuring genome stability [1,2]. In this issue of *EMBO reports*, Yuan and colleagues characterize a new mechanism of repression of a p53 proapoptotic target in normal cells that is relieved upon DNA damage [3].

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p53 is a transcription factor that can transcriptionally activate numerous genes while repressing many others. Transcriptional activation by p53 typically involves the binding of p53 to cognate DNA sequences known as p53 response elements (p53REs). While some p53REs are located within target gene promoters, others can be found within introns or sometimes even at a considerable distance from the target gene. The mechanisms underlying transcriptional repression by p53 appear to be more diverse and often less direct. Together, the combined impact of p53 on the transcriptome of a given cell

might dictate which biological outcome is favoured. For example, robust activation by p53 of the *CDKN1A* gene, encoding the cyclin-dependent kinase inhibitor p21/Waf1, is likely to drive growth arrest. Conversely, preferential transactivation of proapoptotic p53 target genes such as *Puma*, *Noxa*, *Bax*, *CD95* and others will often initiate apoptosis. It is thus not surprising that great attention has been given over the years to the mechanisms that regulate selective target gene activation by p53, particularly those that affect the choice between growth inhibitory genes and proapoptotic genes. These studies have revealed that much of the selectivity is driven by an exquisite set of protein–protein interactions, as well as by a broad palette of p53 post-translational modifications (PTMs), most notably phosphorylation, acetylation and ubiquitylation [2,4]. Given the centrality of p53 as a key tumour suppressor, it is not surprising that cancer cells acquire substantial alterations in the pattern of p53 PTMs [4].

Unlike growth arrest, cell death is a one-way road. Premature triggering of the apoptotic programme is therefore highly inadvisable. To avoid such undesirable 'accidents', the cell uses sophisticated mechanisms for selective inhibition of proapoptotic p53 target genes, to assure that they stay 'locked' unless an apoptotic programme needs to be triggered. Some of those mechanisms are 'class-specific', as is the case for deacetylation that prevents p53 from efficiently activating a substantial subset of proapoptotic genes [4]. Other mechanisms, however, are tailored intricately to restrict the expression

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of individual genes. An elegant example of such gene-specific safeguard mechanisms was provided by Gomes and Espinosa, who found that the proapoptotic p53 target gene *Puma* is constitutively repressed through the action of the insulator protein CTCF in a complex with cohesin [5]. Remarkably, in the absence of a proper signal to trigger p53-mediated apoptosis, this mechanism results in production of an abortive transcript rather than of mRNA encoding functional PUMA protein.

Yuan and colleagues now describe another interesting mechanism for gene-specific repression of a p53 proapoptotic target in non-stressed cells [3]. In earlier work, these researchers identified Apak (ATM and p53-associated KZNF protein, also known as ZNF420) as a negative regulator of p53, which effectively inhibits p53-mediated apoptosis [6]. Apak belongs to the family of KRAB-type zinc finger proteins (KZNFs), typically involved in transcriptional repression. Tian and co-workers showed that Apak binds directly to p53, and concomitantly—through its KRAB domain—also recruits the co-repressor KAP1 (KRAB box-associated protein 1; Fig 1, left). KAP-1, in turn, recruits the histone deacetylase HDAC1, which quenches p53 acetylation and thereby hampers the ability of p53 to transactivate proapoptotic target genes. That study positioned Apak as a 'class-specific' p53 modulator. In their present study [3], the same authors now report that Apak also doubles as a gene-specific repressor, selectively targeting the proapoptotic p53 target gene *p53AIP1*. Yuan and colleagues identified a particular DNA sequence—TCTTN(2–30)TTGT—as the binding motif favoured by Apak. Remarkably, *p53AIP1* harbours such a sequence within its first intron, and the authors confirmed the binding of Apak to this region. What makes this observation of particular interest is the fact that the Apak

Fig 1 | Proposed model for regulation of p53-dependent apoptosis by Apak. Left: in unstressed cells Apak interacts with p53 and inhibits p53 activity by two mechanisms. (i) Apak binds to KAP1, which recruits HDAC1. HDAC1 hampers p53 acetylation, thereby selectively preventing p53-mediated transcriptional activation of proapoptotic target genes. (ii) Apak binds directly to the p53RE located within intron 1 of the proapoptotic *p53AIP1* gene, thus competitively hindering the binding of p53 to that p53RE and preventing *p53AIP1* transactivation. This ensures that p53-driven apoptosis is not triggered prematurely. Right: in response to DNA damage, phosphorylation of Apak disrupts the Apak–p53 and Apak–DNA interactions, allowing p53 to bind the p53RE within intron 1 of the *p53AIP1* gene and induce its expression. Apak eventually translocates to the nucleolus. Furthermore, p53 undergoes phosphorylation (P) and acetylation (Ac); acetylated p53 binds preferentially to proapoptotic genes such as *CD95* and *Noxa*, elevating their expression. Together with the augmented transactivation of *p53AIP1*, this leads to apoptosis.

binding motif overlaps with the p53RE located within this region, which is responsible for the ability of p53 to transactivate *p53AIP1*. Indeed, Yuan and colleagues went on to show that Apak could compete with p53, preventing it from binding to its cognate p53RE and thereby strongly repressing *p53AIP1* transcription (Fig 1, left). Thus, Apak can dampen p53-mediated apoptosis by at least two complementary molecular mechanisms, class-specific as well as gene-specific.

This ‘off’ state predominates in non-stressed cells. By contrast, when cells are exposed to genotoxic stress, Apak and its partner KAP1 are phosphorylated through an ATM-dependent mechanism [6,7]. Consequently, Apak dissociates from p53 and KAP1 is prevented from driving HDAC1-mediated p53 deacetylation; the resultant acetylated p53 is now free to bind to and transactivate a variety of proapoptotic genes including *CD95* and *Noxa* (Fig 1, right). Moreover, Apak phosphorylation

also results in its dissociation from the *p53AIP1* intron 1, enabling p53 to bind to its p53RE and trigger expression of *p53AIP1* mRNA (Fig 1, right). Together, reversal of the two complementary repressive activities of Apak enables the orchestration of a robust proapoptotic transcription programme, eventually leading to cell death. At later stages of the process, Apak is sequestered to the nucleolus [3], further ensuring that it stays out of p53’s way as long as the genotoxic signal persists.

It is unlikely that this is the only instance in which direct competition is used to repress a p53 target gene by preventing p53’s access to a p53RE. Given the large number of zinc finger proteins and their diverse DNA sequence preferences, it will not be at all surprising if additional KZNFs or other zinc finger proteins are found to compete with p53 over p53REs within additional proapoptotic target genes, making the Apak case part of a grander plot. Similarly, other proteins might use

DNA-binding competition to selectively block the activation of growth arrest genes by p53. In fact, such an example already exists. Just like Apak, the ZBTB2 protein—a member of another subfamily of DNA-binding zinc finger proteins—also binds to p53 directly as well as competing with its specific binding to DNA, except that in this case the molecular arena encompasses the p53RE of the *CDKN1A* gene, encoding p21 [8]. Thus, the nature of the particular competitors and their regulation by different signals might dictate which genes are eventually bound and activated by p53, thereby contributing another page to the unfolding story of how selective transcription by p53 regulates cell fate decisions.

While the findings of Yuan and colleagues highlight an interesting new regulatory mechanism, many questions remain. Perhaps most importantly, it has to be established whether ‘fixation’ of Apak binding to *p53AIP1*, leading to a constitutive inability of p53 to transactivate this gene even under conditions of excessive stress, contributes to illegitimate survival of cancer cells and plays a role in human cancer. Similarly, one wonders whether translocation of Apak to the nucleolus, where many p53-related transactions take place [9], might not only serve to sequester Apak away from the real action but might actually contribute positively to p53 activation in response to genotoxic stress. These, along with many other obvious questions, will hopefully be answered by further investigation of this intriguing protein.

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