ANALYSIS

Transcriptional control of human p53-regulated genes

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Abstract | The p53 protein regulates the transcription of many different genes in response to a wide variety of stress signals. Following DNA damage, p53 regulates key processes, including DNA repair, cell-cycle arrest, senescence and apoptosis, in order to suppress cancer. This Analysis article provides an overview of the current knowledge of p53-regulated genes in these pathways and others, and the mechanisms of their regulation. In addition, we present the most comprehensive list so far of human p53-regulated genes and their experimentally validated, functional binding sites that confer p53 regulation.

Response element
A short sequence of DNA in or near a gene that can bind one or more transcription factors that can regulate the transcriptional activity of that gene.

Extracellular matrix
The complex, multi-molecular material that surrounds cells. It comprises a scaffold on which tissues are organized, provides cellular microenvironments and regulates various cellular functions.

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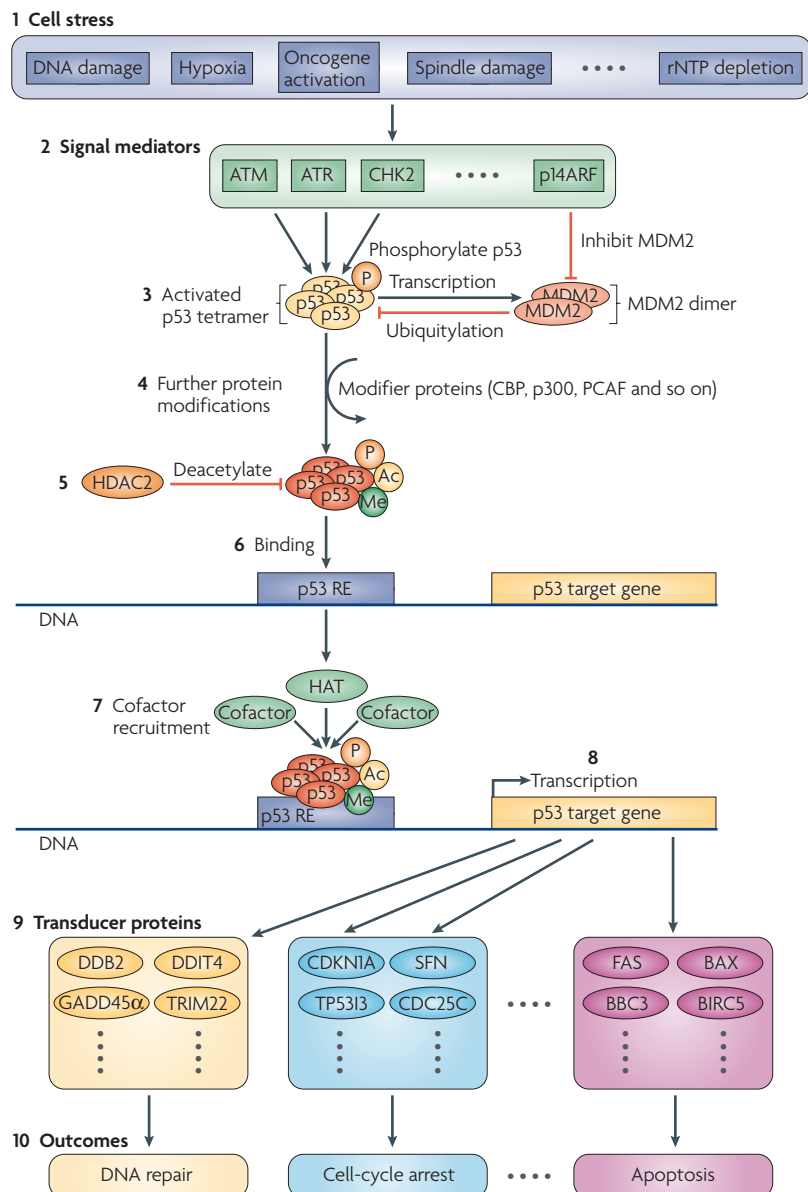Correspondence to T.R.
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The p53 pathway responds to various cellular stress signals (the input) by activating p53 as a transcription factor (increasing its levels and protein modifications) and transcribing a programme of genes (the output) to accomplish a number of functions (FIG. 1). Together, these functions prevent errors in the duplication process of a cell that is under stress, and as such the p53 pathway increases the fidelity of cell division and prevents cancers from arising. The goals of this Analysis article are: first, to bring together as one source a list of p53-regulated genes and the criteria that permit this classification; second, to analyse the p53 response elements (REs) in DNA that bind the p53 protein and promote transcriptional control; third, to organize and explore the functions of the p53-regulated genes; and, finally, to review useful algorithms that can detect p53-regulated genes by their associated REs in DNA from various sources.

The purpose of this exercise is to amalgamate a large body of literature that has mostly been assembled one gene and one publication at a time. This has not permitted an appreciation of the cooperative and broad nature of the functions of many p53-regulated genes in altering the cell and the extracellular matrix, or the role of the p53 response in communicating with various organ systems of the body. There is good evidence that the nature of the stress signal and the cell type can both affect the modulation of the transcriptional pattern of p53-responsive genes that respond with a transcriptional programme[1,2]. Because we have imperfect information about cell and tissue types and the nature of the stress signal for every gene discussed here, we can provide only a broad overview of the transcriptional programme regulated by the p53 protein. Where detailed information is available about cell and tissue type, and the stress response, it will be discussed.

Criteria for p53-responsive genes

Four sets of experimental criteria have been used to identify a p53-regulated gene. The first is the presence of a p53 RE in the DNA close to or in the gene. The second is a demonstration that the gene is either upregulated or downregulated at the RNA and protein levels by the activated wild-type p53 protein (but not by the mutant protein). The third line of evidence is to clone the p53 RE from that gene, place it near a test gene, such as luciferase, and demonstrate that the p53 protein can regulate the test gene. The fourth approach is to use chromatin immunoprecipitation with a p53-specific antibody to demonstrate the presence of the p53 protein on the RE site in the DNA. In some cases, a gel-shift assay is also used to demonstrate that the p53 protein binds *in vitro* to the p53 RE sequence from that gene.

These criteria may be modified depending on the cell or tissue specificity of some p53-regulated genes or on the nature of the stress signal that the p53 pathway responds to. In this article we have included a list of p53-responsive genes that have met a minimum of three out of four of these criteria. On the basis of these criteria, Supplementary information S1 (table) and S2 (table) contain 129 genes and 160 p53 REs from both the human and viral genomes (several of the genes contain more than one p53 RE). Supplementary information S1 (table) provides the gene name, the full description of this name, its accession number, a description of the p53 RE and, if it has one, its spacer. Supplementary information S2 (table) provides the gene name, the location of the p53 RE, whether this RE functions as a transcriptional activator or a repressor, the distance from the transcriptional start site (TSS) of the p53 RE, the proposed functions of the gene product, and a reference to the publication that describes these properties of the p53-regulated gene. TABLE 1 lists

Figure 1 | Mechanisms of p53 activation and regulation of downstream targets.
Step 1: Cells undergo stress, which can eventually lead to cancer. Step 2: Signal mediator proteins activate p53 by phosphorylating certain residues or inhibiting ubiquitylation by MDM2 (double minute-2). Step 3: Both processes increase the half-life of p53 by inhibiting ubiquitylation. The increased half-life, from minutes to hours, quickly leads to higher levels of p53. Step 4: Further p53 modifications by acetyltransferases (CBP, p300, PCAF) and methyltransferases (SET9) can further stabilize the p53 protein and increase site-specific DNA binding. Step 5: The deacetylase HDAC2 can inhibit p53 binding to DNA by deacetylating the protein. Step 6: The p53 tetramer binds to a p53 response element (RE) to regulate transcription of a nearby gene. Step 7: p53 also recruits cofactors such as histone acetyltransferases (HATs) and TATA-binding protein-associated factors (TAFs). Step 8: In this example, p53 mediates transactivation of its target gene, but p53 can also mediate transcriptional repression. Step 9: The p53 protein transactivates many genes, the protein products of which are involved in various pathways. Step 10: The most important pathways involved in tumour suppression that are activated by p53 lead to DNA repair, cell-cycle arrest, senescence and apoptosis. ATM, ataxia telangiectasia mutated; BAX, BCL2-associated X protein; BBC3, BCL2-binding component-3; BIRC5, survivin; CDKN1A, cyclin-dependent kinase inhibitor-1A; CHK2, checkpoint kinase-2; DDB2, damage-specific DNA-binding protein-2; DDIT4, DNA-damage-inducible transcript-4; FAS, TNF receptor subfamily, member 6; GADD45α, growth arrest and DNA-damage inducible α; p14ARF; SFN, stratifin; TP53I3, tumour protein p53-inducible protein-3; TRIM22, tripartite motif containing-22.

the 15 p53 cluster sites that are present in Supplementary information S1 (table) and S2 (table) and the number of half-sites found in each (p53 REs with more than two half-sites are referred to as cluster sites). We should note that the p53-target list found in Supplementary information S1 (table) and S2 (table) is probably not exhaustive, and is likely to grow as additional experimental evidence is acquired (see below).

The p53 consensus motif

Two different groups first identified a p53 consensus sequence in the DNA to which the p53 protein bound with high affinity and specificity[3,4]. The sequence was degenerate and was composed of 5′-RRRCWWGYYY-3′, where R is a purine, Y a pyrimidine, W is either A or T (adenine or thymine), G is guanine and C is cytosine[3,4]. The p53-binding site in the genomes of many organisms is composed of a half-site RRRCWWGYYY followed by a spacer, usually composed of 0–21 base pairs, which is then followed by a second half-site RRRCWWGYYY sequence (FIG. 2a). By labelling each quarter-site RRRCW as ⟶ and WGYYY as ⟵, the first discovered p53 consensus sequence can be graphically represented as ⟶⟵ spacer ⟶⟵. This configuration of the four quarter-sites is often referred to at the head-to-head (HH) orientation. The two other possible orientations of the quarter-sites are tail-to-tail (TT, ⟵⟶ spacer ⟵⟶) and head-to-tail (HT, ⟶⟶ spacer ⟶⟶). (TH is not used because the complementary strand would contain an HT-orientated site.)

In almost all natural p53-binding sites, the two half-sites share the same quarter-site orientations. Experiments have shown that the tetramer p53 protein can bind all three (HH, TT and HT) quarter-site orientations with equally high affinity[4]. However, only a few of the experimentally validated p53-binding sites in this analysis do not have the head-to-head (HH) orientation. Owing to allowed insertions and deletions relative to the consensus sequence, half-sites can vary in size between 8 and 12 base pairs, although most have 10. As mentioned above, some p53 REs have more than two half-sites, and as such are referred to as cluster sites. Various experiments have shown that the level of binding affinity and subsequent transactivation increases linearly with the number of adjacent half-sites[5–7]. Finally, some genes contain multiple p53-binding sites in different locations within the gene and promoter region, and each p53 RE can contribute to the p53 response. For example, a ⟶ ⟶ ⟶ ⟵⟶ cluster site is present in the promoter of CDKN1A (cyclin-dependent kinase inhibitor-1A, also known as p21) ~900 base pairs 3′ to a canonical ⟶⟵ spacer ⟶⟵ site, and both of these sites contribute to the induction of CDKN1A transcription after a p53 stress response[8–10].

Functions of p53-regulated genes

The mechanisms of p53-pathway activation and the cellular outcomes produced by p53-activated genes are presented in FIG. 1. Many proteins are involved in the p53 pathway in order to respond to stress signals and to produce the proper response.

Table 1 | **Cluster sites regulated by p53**

Gene name(s)	Short description	Number of half-sites
BTG2 (TIS21)	BTG family protein-2	3
CDKN1A (p21)	Cyclin-dependent kinase inhibitor-1A	2.5
DDB2	Damage-specific DNA-binding protein-2	4
GML	GPI-anchored molecule-like protein	3
HRAS (c-Ha-Ras)	Harvey rat sarcoma viral oncogene homologue	8
IGFBP3	Insulin-like growth factor binding protein-3	11
MDM2	Transformed 3T3-cell double minute 2	4
PCNA	Proliferating cell nuclear antigen	5
SH2D1A (SAP)	SH2 domain protein-1A, Duncan disease SH2 protein	4
TP53I3 (PIG3)	Tumour protein p53-inducible protein-3	7.5
TP73 (p73)	Tumour protein p73	3
TRPM2	Transient receptor potential cation channel M2	3
TYRP1 (TRP1)	Tyrosinase-related protein-1	6
VDR	Vitamin D (1,25-dihydroxyvitamin D3) receptor	3
HBV	Hepatitis B virus	3

The table lists genes that contain cluster-site response elements (REs) that have been shown experimentally to confer transcriptional regulation by p53. A cluster-site RE is defined as any RE that contains three or more half sites, each separated by no more than 15 base pairs.

Ubiquitin ligase
An enzyme that couples the small protein ubiquitin to Lys residues on a target protein, marking that protein for destruction by the 26S proteasome.

Polyubiquitylation
A process whereby a ubiquitin ligase protein attaches multiple ubiquitin molecules, one after the other, to a single Lys residue and thereby marks the protein for degradation by the 26S proteasome.

Senescence
An almost irreversible stage of permanent G1 cell-cycle arrest that is linked to morphological changes (flattening of the cells), metabolic changes and changes in gene expression (for example, β-galactosidase).

Autophagy
A pathway for the recycling of cellular contents, through which materials inside the cell are packaged into vesicles and are then targeted to the vacuole or lysosome for bulk turnover.

Endosome
A vesicle formed by invagination of the plasma membrane.

Stress signals decide the transcriptional programme. The p53 pathway responds to a wide variety of stress signals. These include several types of DNA damage: telomere shortening, hypoxia, mitotic spindle damage, heat or cold shock, unfolded proteins, improper ribosomal biogenesis, nutritional deprivation in a transformed cell, and even the activation of some oncogenes by mutation[11,12] (FIG. 1). These stress signals are detected by various proteins, the activities of which mediate the information about cellular damage (through protein modifications) to the p53 protein or to its negative regulator, MDM2 — a ubiquitin ligase that both blocks p53 transcriptional activity directly (sterically) and mediates the degradation of the p53 protein[13].

In many cells, the half-life of the p53 protein varies between 6 and 20 minutes. After a stress signal, MDM2 polyubiquitylates itself, which results in the degradation of MDM2 and an increase in the half-life of p53 from minutes to hours. Other mediators of the stress response act through protein modifications of p53. These rapid mechanisms of p53 modification and the greatly increased half-life of p53 do not depend on the slower mechanisms of transcription (of a damaged DNA template) or RNA transport. Thus, the response to stress is rapid, and it has been proposed (but not proven) that the nature of the stress signal determines the type of protein modification and, therefore, the transcriptional programme of the p53 protein.

This is one way to integrate cellular stress signals at a single cellular protein, whereby the activated p53 then binds to the p53 REs in the DNA and promotes a transcriptional programme that responds to that particular stress signal. There have been a number of experiments that suggest that, in addition to a transcriptional response to cellular damage, the p53 protein can act directly to trigger a response such as apoptosis[14]. Although this is an active area of research, detailed mechanisms describing how p53 acts on or in the mitochondria to promote apoptosis are still lacking.

Outcomes of transcriptional activation. There are three main outcomes after the activation of p53: apoptosis, senescence or cell-cycle arrest. The first two are terminal for the cell, whereas cell-cycle arrest permits repair processes to act and damage to be reversed, so that the cell survives. The choice between these three outcomes in a stressed cell depends on a number of other variables, which indicates that the p53 pathway is sensing the activities of other signal-transduction pathways. For example, glucose starvation of normal cells results in the AMP kinase-mediated phosphorylation by p53 on Ser15 but no further activation of p53-mediated transcription. By contrast, glucose starvation of a transformed cell results in p53-mediated apoptosis[2]. In some cell types in which p53 activation typically results in apoptosis, this can be reversed or reduced by treatment with interleukin-6 (REFS 15,16). The introduction of an activated *RAS* oncogene into a normal cell results in p53-mediated senescence[17]. As part of this senescent state, p53-mediated transcripts produce cytokines that attract inflammatory cells, which, in turn, eliminate the RAS-transformed cell from an organ[17]. So it is clear that elements of the p53 pathway are regulated by inputs from other signal-transduction pathways, resulting in different programmes of transcription by p53.

Although these three functional responses (apoptosis, senescence and cell-cycle arrest) are well appreciated, there are a number of other cellular processes that are altered by gene products regulated by the p53 protein. These include both positive and negative feedback loops in the p53 pathway[18], regulation of other signal-transduction pathways and autophagy[2,19], alterations in the extracellular matrix, alterations in the cytoskeleton of cells, activation of the endosome compartment of cells with increased exosomal and endosomal activity[20], and the regulation of protein translation[21–24], heat-shock proteins[25,26] and DNA-repair processes[7,27–29].

The above processes all occur within or around a cell at the molecular and cellular levels, but there are also physiological and systemic consequences of a p53 stress response. Exosomes produced by p53 activation of the endosomal compartment in an apoptotic cell after a p53 response to stress combine with dendritic cells in the body and can enhance the immunization process for antigens in the stressed cell[20]. Various p53-regulated genes that are expressed and act in the central nervous system can alter communication between neurons and, in some situations, result in neurodegeneration[30]. Regulation by p53 of the leukaemia inhibitory factor (*LIF*) gene in the uterus can directly regulate the efficiency of embryo implantation in mice[31]. Therefore, the p53-mediated transcriptional process can have systemic consequences in a host and communicate a stress signal throughout the body. These types of function are listed in Supplementary information S2 (table).
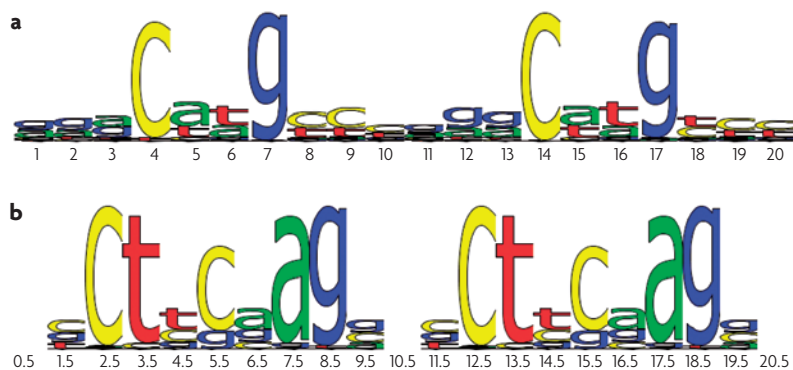
Figure 2 | **The p53-PHMM binding site motif. a** | The match-state sequence logo for the palindromic p53 motif: $1\ 2\ 3\ 4\ 5\ 6\ 7\ 8\ 9\ 10\ \tilde{10}\ \tilde{9}\ \tilde{8}\ \tilde{7}\ \tilde{6}\ \tilde{5}\ \tilde{4}\ \tilde{3}\ \tilde{2}\ \tilde{1}$ (which correspond to nucleotide positions 1–20 in the figure). Model position $\tilde{a}$ has the complement nucleotide-emission distribution of a. The height of each letter is made proportional to its frequency at each position, and the letters are sorted in descending frequency order. The height of the entire stack at each position is then adjusted to signify the information content (in bits) of that position[103]. The match-state nucleotide positions 4, 7, 14 and 17 (model positions 4, 7, $\tilde{7}$ and $\tilde{4}$, respectively) are the most conserved and are the main points of contact with the p53 protein. **b** | The insert-state sequence logo for the combined-palindromic p53 motif: $1\ 2\ 3\ 4\ 5\ \tilde{5}\ \tilde{4}\ \tilde{3}\ \tilde{2}\ \tilde{1}\ 1\ 2\ 3\ 4\ 5\ \tilde{5}\ \tilde{4}\ \tilde{3}\ \tilde{2}\ \tilde{1}$. The inserted nucleotides occur in-between the match states; thus, insert position 1.5 is in-between position 1 and 2 in the match-state positions shown in part **a**. The specificity motif of the insert-state emissions is different from that of the match-state emissions. For example, there is a bias for T's to be inserted around the well-conserved C's (at nucleotide positions 4 and 14) and A's around the well-conserved G's (at nucleotide positions 7 and 17). Although insertion events in the motif are rare, the allowed nucleotide insertions at certain positions can be very specific. The match-state sequence logo, insert-state sequence logo and transition probabilities (not shown) make up the p53 profile hidden Markov model (PHMM).

Modes of p53 regulation

The p53 protein can either activate or repress the transcription of a gene. The main mode of transcriptional activation is through direct, sequence-specific DNA binding. However, a number of the genes listed in Supplementary information S2 (table) are transcriptionally repressed by p53. p53 uses both direct and indirect methods to repress gene transcription.

Activation through direct binding and recruitment. Almost all p53-activated genes have at least one putative DNA-binding site that moderately matches the consensus p53 response element. Through protein–protein interactions, p53 can bind to and then recruit general transcription proteins (TATA-binding protein-associated factors (TAFs)) to the promoter-enhancer region of p53-regulated genes to induce transcription[32,33]. Recent experiments have shown that p53 can also recruit the histone acetyltransferases (HATs) CBP, p300 and PCAF to the promoter-enhancer region of genes (through high-affinity protein–protein binding)[34,35]. These HATs acetylate Lys residues of histones in chromatin, increasing transcriptional activity.

Repression through direct and indirect means. In some genes, the binding of p53 to its RE results in direct repression of that gene. It is not clear what distinguishes an RE sequence from being a transcriptional-activator site versus a transcriptional-repressor site. At present, three generally accepted methods of direct p53-mediated

Exosome
A membrane vesicle that is secreted into the extracellular milieu as a consequence of multivesicular-body fusion with the plasma membrane.

repression are known: first, binding-site overlap (steric interference); second, p53 squelching of transcriptional activators; and third, p53-mediated recruitment of histone deacetylases (HDACs).

The p53-mediated repression by steric interference involves sequence-specific DNA binding by p53 that overlaps the binding site of another (more powerful) transactivating protein. Examples of genes repressed by the method of p53 steric interference include *AFP* (α-fetoprotein), *BCL2* (B-cell lymphoma-2) and *HBV* (hepatitis B virus). In these examples, the corresponding activators that are occluded by DNA-bound p53 are FOXA1 (forkhead box A1), POU4F1 (POU domain class 4 transcription factor-1) and both RFX1 (regulatory factor X1) and ABL1 (Abelson tyrosine kinase), respectively[36–38]. An entire family of cell-cycle regulatory genes now seem to share the same squelching mechanism, whereby p53 binds to and suppresses bound and unbound activators of the CCAAT box, namely heterotrimeric NF-Y (nuclear transcription factor-Y) and CEBP (CCAAT/enhancer binding protein). Examples of genes that share this mechanism are *cyclin A2*, *CDC25C*, *CDC2*, the heat-shock protein *HSP70* gene, the kinase-encoding *CHK2* and *CDK1* genes, fibronectin-1 (*FN1*), *BRCA1* (breast cancer-1, early onset) and *PTGS2* (prostaglandin-endoperoxide synthase-2, also known as *COX2*)[26,39–48].

The p53 squelching (inactivation) of other DNA-bound and DNA-unbound activators occurs through p53-mediated protein–protein interactions. Examples of p53 squelching of other transactivating genes are *cyclin B1*, *TERT* (telomerase reverse transcriptase), *IGF1R* (insulin-like growth factor receptor-1), *ALB* (albumin) and *MMP1* (matrix metallopeptidase-1). The corresponding DNA-bound proteins that are inactivated by direct p53 binding are transcription factors Sp1, Sp1, Sp1, CEBPβ and AP1 (activator protein-1), respectively[49–53]. Owing to the observation that p53 binds the transcription machinery proteins TBP (TATA-box-binding protein), TAF6 (TBP-associated factor-6, also known as TAFII70), TAF9 (also known as TAFII31) and others *in vitro*, it was initially believed that p53 repression was achieved through p53 binding and suppression of these TATA-box-bound basal factors *in vivo*[32,33,54,55]. Experimental evidence suggests that the preferred *in vivo* method of p53-mediated squelching is achieved by binding and inhibiting the transactivators of the CCAAT box[26,44,56]. However, it remains unclear whether or not these squelching mechanisms of repression are used *in vivo* under normal physiological conditions.

The p53-mediated recruitment of HDACs occurs through p53 binding to the repressor protein SIN3A, which, in turn, binds the histone deacetylase HDAC1 (REF. 57). After p53-mediated recruitment to the promoter-enhancer region of a gene, HDAC1 deacetylates Lys residues of histones in chromatin, thereby repressing gene transcription[57,58]. Examples of genes repressed through this p53-mediated mechanism include *MAP4* (microtubule-associated protein-4), *STMN1* (stathmin-1) and the heat-shock protein *HSP90AB1* gene (REFS 25,57).
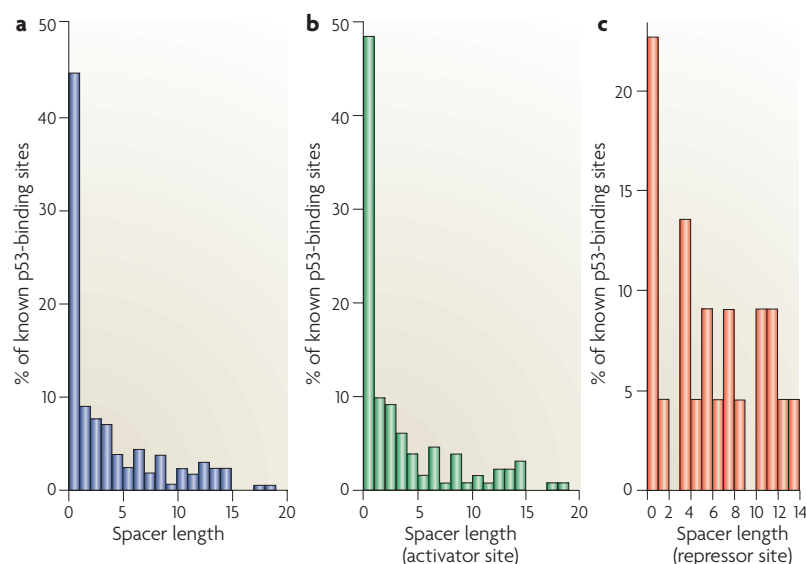
Figure 3 | **Histograms of spacer lengths by regulation types. a** | The histogram of all 160 spacer lengths of known, functional p53-binding sites reveals the following: first, almost 50% of the p53-binding sites have no spacer sequence (spacer length = 0 base pairs), and second, the distribution is relatively uniform for spacer lengths between 4 and 14 base pairs. This distribution does not match experimental results, which suggest a bimodal distribution with peaks at 0 and 10 base pairs, and which place the two half-sites on the same face of the DNA double helix[84,88]. **b,c** | Activation sites have a different distribution of spacer lengths compared with repressor sites. Most importantly, repressor sites do not show a great preference for 0-base-pair spacers.

There are two generally accepted modes of indirect p53-mediated repression. The first comes about by p53-mediated activation of *CDKN1A*, which, in turn, inhibits the cyclin D–CDK4 complex through direct binding. The consequence of this inhibition of cyclin D–CDK4 is the absence of hyperphosphorylation of the retinoblastoma (RB) protein from the G1 stage of the cell cycle[59]. Unphosphorylated RB represses the function of the E2F family of transcription factors through direct binding (forming an E2F–DP1–RB complex), thereby inhibiting the many downstream targets of E2F (including cyclin E, cyclin A, DNA polymerase and thymidine kinase) and halting the cell cycle in G1 phase. It seems that many genes are fully or partially repressed through p53-mediated induction of *CDKN1A* and ensuing repression of E2F by RB dephosphorylation[59]. Supplementary information S2 (table) shows only those genes that are directly repressed by p53 (and thus have an experimentally validated p53 RE). In the second mode of indirect p53-mediated repression, p53 binds to another transcription factor and, together, they repress a gene without a p53-specific RE.

Less established modes of p53 regulation. Investigators have also put forth other, sometimes controversial, models for mechanisms of p53-mediated repression and activation. One model proposes that the switch between p53-mediated activation and repression is determined by the length of the spacer[60]. The hypothesis is that p53 proteins bound to a 3-base-pair spacer-binding site are ineffective in recruiting the necessary additional activation proteins while simultaneously occluding them from

adjacent or overlapping REs. Investigators were able to convert direct p53 repression of *BIRC5* (survivin) into direct p53 activation by deleting the 3-base-pair spacer present in the p53 RE[60]. In our analysis, we show that experimentally validated repressor sites do have longer spacers (FIG. 3). However, many activator sites also have spacers of three or more base pairs.

Another model proposes that the existence of an adjacent response element (designated 'EP'), which binds the proteins RFX1 and ABL1, is sufficient to transform an activating p53 RE into a repressing RE[38]. Interestingly, Ori *et al.* succeeded in transforming the direct p53 repression found in the enhancer of *HBV* into direct p53 activation by mutating the adjacent EP response element. They also succeeded in transforming the direct p53 activation of MDM2 into direct p53 repression by inserting an EP response element adjacent to the p53 RE.

Yet another model proposes that the orientation of the quarter-sites within the p53-binding element determines activation versus repression. Johnson *et al.* propose that head-to-head (HH) p53-binding sites produce p53 activation, whereas head-to-tail (HT) sites produce p53 repression[61]. Interestingly, they succeeded in converting the p53-repressed ABC transporter gene, *ABCB1*, into p53-activated *ABCB1* by replacing the HT p53 RE in the promoter with an HH p53 RE. No experiments were performed with tail-to-tail (TT) p53-binding sites. However, it should be noted that all other experimentally validated repressing p53 REs in this analysis have an HH configuration, and that the HT cluster site in the 5′ untranslated region (UTR) of TP53I3 (tumour protein p53-inducible protein-3, also known as *PIG3*) confers p53 transactivation, rather than repression.

In the case of *HSP90AB1*, investigators discovered a biphasic p53 regulatory system in which the cofactor p300 mediated p53 activation, and the cofactors SIN3A and HDAC1 mediated p53 repression[25]. Another important cofactor for p53 regulation in some genes, including *CAV1* (caveolin-1), is E2F (REF. 62). Combining these observations leads to the following conclusions: first, properties of the p53 RE and adjacent cofactor REs confer the potential for direct p53 activation, repression, or both; and second, the induction of the right combination of p53 and cofactor proteins is required to regulate any potentially functional target site, when either activating or repressing.

Factors that affect p53 regulation

Experiments have shown that many factors can affect the mode and degree to which p53 regulates different target genes. These factors include cofactors, spacer lengths, quarter-site orientation, nucleosomes and post-translational modifications of p53.

The role of post-translational modifications of p53. An area of controversy is the role of post-translational modifications of p53 in determining the mode and efficacy of p53 transcriptional regulation. Experiments have shown that post-translational modifications of p53, such as phosphorylation, methylation and acetylation, alter the

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Protein methylation
A type of post-translational modification, mediated by enzymes, whereby a hydrogen atom is replaced with a methyl group, typically on an Arg or Lys amino-acid residue in the protein sequence.

Protein acetylation
A type of post-translational modification, mediated by enzymes, whereby a hydrogen atom is replaced with an acetyl group.

stability and DNA-binding affinities of p53 (REFS 63–66). Investigators have shown that p53 needs post-translational modifications in the C-terminal domain to bind to naked DNA *in vitro*, but requires no modifications in the presence of chromatin to bind to p53 REs[9,67]. This also fits with experiments that showed that the deacetylated C-terminal domain inhibits binding to p53-binding sites in linear DNA and promotes binding to sites in nonlinear, circularized DNA[68]. The p53-binding sites in circularized DNA segments mimic *in vivo* conditions, whereby DNA is wrapped around histones. These experiments suggest that the C-terminal domain of the p53 protein confers DNA structure specificity (whereas the DNA-binding domain confers sequence specificity). In direct contradiction to these results, other researchers have shown that some of the experimentally validated p53-binding sites do not require any phosphorylation or acetylation of the p53 protein in order to confer high-affinity binding *in vitro* in the absence of chromatin[69].

Nevertheless, there are cases in which these post-translational modifications seem to have a major role. Investigators found that the induction of *p53AIP1* (p53-regulated apoptosis-inducing protein-1) depends on the phosphorylation of the Ser46 residue of p53 (REF. 70). Investigators also found that phosphorylation of the Ser15 and Ser392 residues conferred p53 activation of the adenomatosis polyposis coli (*APC*) gene, whereas non-phosphorylated p53 served as a repressor of *APC*[71].

The strongest evidence to support the idea that post-translational modifications of p53 are relevant to the p53 regulatory mechanism is the fact that HDAC inhibitors have been shown to simultaneously increase levels of acetylated p53 and induce apoptosis and senescence in cancerous and normal cells[58,72]. HDAC inhibitors are currently in clinical trials as cancer chemotherapeutics and initial results are promising[72]. Although post-translational modifications of p53 are certainly important, the ability to properly quantify which ones are relevant, under which conditions, has been elusive. Further experimentation is needed to shed light on this complex mechanism of regulation in the p53 pathway.

The flexible CATG effect. It has been shown experimentally that in the head-to-head (HH) orientation, p53 greatly prefers the repeated RRRCATGYYY motif[73,74]. On the basis of X-ray crystallography studies of the p53 DNA-binding core domain bound to a p53-RE DNA sequence, the most important bases for interactions with the p53 protein are the central RCWWGY, which come into close contact with the amino acids from the p53 core domain[75]. In conjunction with this, the most conserved positions after aligning all experimentally validated, functional p53-binding sites are the central CWWG nucleotides within each half-site, especially the C and G (FIG. 2). Therefore, changes in the nucleotides in these central positions should affect binding affinity the most. Indeed, binding-affinity measurements of 20 p53-binding sites revealed that 50% of the high-affinity sites contained the CATG sequence at the centre of both half-sites[69]. Investigators also found that replacing the central CATG with CTAG in both half-sites reduced transactivation 20-fold[76].

It is known that the CATG sequence element is unusually flexible and exhibits extreme bending and kinking in many DNA–protein complexes[77,78]. Therefore, it is widely assumed that the flexibility of the p53 RE also affects binding affinities. p53–DNA-binding affinity experiments have shown that p53 exhibits higher binding affinity for sites in cell-cycle control target genes than for sites in apoptosis target genes, and that these differences coincide with the prevalence of the highly flexible CATG in both groups[69].

p53 RE sites that are not functional. Investigators have repeatedly found that p53-mediated regulation of minimal promoters can be profoundly different from that of their respective full-length promoters. Examples include experiments that showed that p53 would no longer bind in the natural promoter[79], and experiments that showed that the p53 RE was no longer functional in the natural promoter, even though the presence of bound-p53 on the RE was confirmed[80,81]. These results indicate that the presence of cofactor sites and the p53-RE occlusion by nucleosomes or other proteins have an important role in p53 regulation. Examples of genes that contain p53-binding sites that have been shown not to be functional *in vivo* include: the intron 5 cluster site in the apoptosis-inducing factor *AIFM2*, the -328 site in *TP53I3*, and the promoter cluster site in human *BAX* (BCL2-associated X protein) [79–81]. In addition, experiments have shown that an adjacent Sp1 RE is necessary to confer p53-mediated activation of *BBC3* (BCL2-binding component-3, also known as *PUMA*) and *BAX*[82,83]. Clearly, binding of p53 to DNA is not sufficient for transcription.

The effects of distance and DNA looping. It is well known that the distance between a *cis*-element binding site and the transcription start site (TSS) can greatly affect the degree of regulation of a gene. In the case of p53, researchers showed that inserting an additional 200-base-pair segment between a p53 RE and the TATA box eliminated a 45-fold p53-mediated induction[84]. It is also known that eukaryotic cells contain transcription factor (TF)-binding proteins that bind together — 'sticky' TF proteins — and can mediate DNA looping. This process can bring distal TF-bound binding sites close to the TATA box, and can confer regulation. In the case of p53, investigators using electron microscopy techniques showed that p53 tetramers stack in register (on top of each other) when bound to a p53 RE, and thereby link distant p53-binding sites through DNA looping[85]. They also showed that, alone, distant p53-binding sites are poor inducers of transcription, but that in the presence of a site proximal to the TSS, induction by the distal site is increased 25-fold[85]. p53-tetramer stacking translocates distally bound p53 protein to the promoter and increases the concentration of local p53 near the TSS.

In the absence of a proximal p53 RE, other 'sticky' proteins may serve as a surrogate, provided that their REs are present close to the TSS and the distal p53 RE. An example of a proven sticky protein that mediates

DNA looping is the p53 cofactor Sp1. An example of Sp1-mediated DNA looping may be found in *MDM2*, where a functional single-nucleotide polymorphism (SNP), SNP309 T/G, within a cluster of Sp1-binding sites affects the level of regulation of nearby oestrogen and p53 REs, and has been associated with an early onset of breast cancer in pre-menopausal women[86]. In contradiction to this model, other investigators have hypothesized that distal sites may reduce transcription by attracting p53 proteins away from the TSS[87]. For example, in Polo-like kinase-2 (*PLK2*) the distant site is a repressor, whereas nearer sites are activators[87]. Further investigation will be necessary to determine exactly how and when distant p53 REs regulate gene expression.

The effects of spacers. Experiments have shown that the spacers that separate the half-sites can greatly affect the binding affinity for the p53 protein. For example, Tan *et al.* showed that mutating the spacer of a p53 RE from a GG to a T increased binding affinity 6.6-fold[7]. Two series of experiments that used minimal promoter assays found a bimodal induction distribution, whereby the two induction peaks occurred with spacer-lengths of 0 and 10 base pairs[84,88]. The authors hypothesized that optimum binding occurred with the half-sites aligned along the same face of the double helix (stereospecific alignment), either with the half-sites adjacent or separated by a helical turn (10 base pairs). Other researchers showed that, under certain experimental conditions, specific spacers with spacer lengths of size 4, 13 and 14 considerably decreased the RE's binding affinity for p53 as compared with having no spacer at all; however, a spacer length of 10 was not tested[89].

Unfortunately, only one spacer, as opposed to all possible spacers, of a certain length was tested for binding affinity in these experiments. Interestingly, our database of 160 functional p53-binding sites does not show a bimodal distribution of spacer lengths. It is possible that spacer lengths may affect binding affinity and regulatory function differently, in that high binding affinity does not necessarily confer regulatory function. Although it is obvious that different spacers affect the function of p53 REs differently, the ability to quantify these effects has been elusive.

Rescue by p63 and p73. Yet another proposed p53-mediated activation mechanism is the rescue of weak p53-binding sites by the p53 homologues p63 and p73. Investigators have found that in mouse fibroblasts both p63 and p73 are required for p53-dependent transactivation of *Noxa* and *Bax*[90].

To our knowledge, no experiments have been performed that elucidate how these seemingly disparate determinants of p53 regulation (spacer lengths, quarter-site orientation, cofactors, nucleosomes and post-translational modifications of p53) might relate to each other in determining functional p53 repression and/or activation. It is obvious that our understanding of the mechanism(s) that determine p53 repression versus activation is not complete, and requires further study.

LINE element
A long interspersed sequence that contains a promoter region, untranslated region and one or more open reading frames, and is generated by retrotransposition.

Experimental approaches and considerations

There are special considerations that need to be taken into account when attempting to experimentally validate putative p53-binding sites. Wei and his colleagues have used chromatin immunoprecipitation with p53-specific antibodies to collect all of the tight binding sites for p53 in the genome of a cancer cell line[91]. They then sequenced the DNA fragments selected for by p53 binding and identified the genes associated with p53. They went on to validate, using other criteria, that a subset of these genes did indeed have a p53 RE that was regulated by p53. This has been a useful approach for identifying candidates, but it is clear that binding to an RE is not necessarily equivalent to regulating a gene. In addition, this approach requires tight binding and longer residence times of p53 at a site that could just store p53 proteins on the DNA for rapid use (not diffusion-limited) at a regulated gene. This method could also be used to identify p53-like sites on retroviruses and LINE elements (repetitive elements in the genome), both of which are observed by p53 RE algorithms (see below)[92].

In addition to this approach, others have used RNA microarrays to explore the increases and decreases in the steady-state levels of RNAs in cells after the induction of p53 or exposure to a stress signal[1]. This too has been useful for identifying new p53-regulated genes, but they need to be shown to be directly regulated by p53, and not the consequence of a secondary event (such as the induction of a transcription factor by p53 that then acts on other genes). Moreover, any stress signal used to induce p53 (such as exposure to ultraviolet (UV) radiation) may well induce the transcription of a gene by a pathway not involving p53. For example, the *GADD45a* (growth arrest and DNA-damage-inducible-α) gene is induced by p53 (following exposure to UV radiation, as verified by chromatin immunoprecipitation) but is also induced by UV radiation in a p53-null or mutant cell (by another mechanism). For these reasons, an inducible *p53* gene or a temperature-sensitive *p53* gene in a cell are often better used to increase p53 levels and activity than a DNA-damaging agent. However, the protein products of an inducible *p53* gene in an unstressed cell might not have the same protein modifications (acetylation, for example) that are observed *in vivo* in a stress response. Those modifications (which include acetylation, phosphorylation, methylation, ubiquitylation and sumoylation) could well lead to the choice of a transcriptional programme resulting from that particular stress signal (UV radiation versus ionizing radiation, for example)[1]. Finally, the choice of cell lines used to follow p53-regulated genes in these experiments ignores the fact that there are cell-type and tissue-type specificities in the p53 response.

Computational methods of identification

Several algorithms have been devised to detect p53 REs in the DNA of all organisms and identify possible p53-responsive genes[92–98]. These computational algorithms have been used extensively to help the experimental process of finding functional p53-binding sites, transcriptional gene targets of p53 and functional SNPs in the p53 pathway. Although these algorithms have been
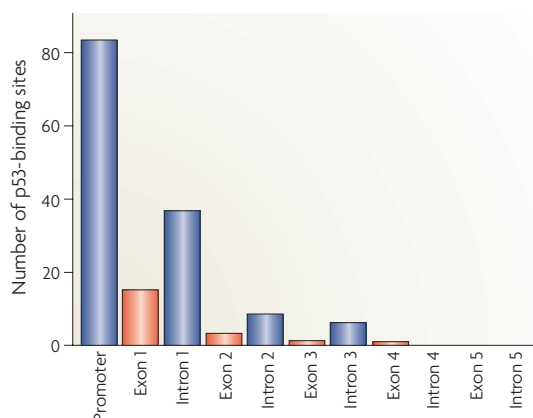
Figure 4 | **Histogram of p53-binding sites by gene region.** The histogram of 160 functional p53-binding sites (by gene region) reveals several things. First, there are slightly more p53 response elements (REs) upstream of the transcriptional start site (TSS) than downstream (83 of the 160 sites are completely within the promoter region, 3 straddle the TSS and 74 are downstream of the TSS). Second, there are significantly more p53 REs in non-coding regions (blue) than in coding regions (red). Third, there is an exponential decay of p53 REs as the distance from the TSS increases. Thirteen of the 15 exon-1 REs (87%) are in the 5′-untranslated region. Note that some p53 REs straddle both coding and non-coding regions and are counted twice.

extremely useful, they also have serious drawbacks. All the algorithms attempt to approximate the relative binding affinity of a putative p53-binding site by training a probabilistic model from a data set of experimentally validated, functional p53 REs (known as a 'training set'). Therefore, the strength and predictive power of any such model is completely dependent on the sampling size and quality of the training set.

In addition, experiments have repeatedly shown that relative binding affinity is not the only relevant factor when it comes to response elements (see above). Other important variables that affect the degree of function of a p53 RE include adjacent cofactor-binding sites, spacer length, distance from the TSS and nucleosome positioning. For these reasons, all the algorithms that approximate relative binding affinity alone have very high false-positive rates (for most TF-binding sites)[97]. In order to seriously boost predictive power, future algorithms will need to include at least some of the additional variables mentioned above.

The common position-specific score matrix. By far the most common computational method for predicting p53 REs (and other REs) is the position-specific score matrix (PSSM, or weight matrix), which attempts to estimate the binding affinity of a putative site[95]. Besides the drawbacks mentioned above, PSSMs have other serious limitations in their attempts to approximate relative binding affinity. The PSSM model contains the probabilities of each nucleotide at each position in the motif (or the logarithms of the probabilities) and is therefore static in length. So, PSSMs cannot model possible nucleotide insertions into, or possible deletions from,

the consensus motif, because any nucleotide insertion or deletion throws off the PSSM reading frame. This is clearly a problem because the p53 RE is very degenerate and ~30% of the 160 functional p53-binding sites in Supplementary information S2 (table) have at least one nucleotide insertion or deletion relative to the consensus. Any PSSM approach would therefore mis-score at least 30% of the binding sites in the data set. Examples of genes that contain these degenerate sites are: *BAI1* (brain-specific angiogenesis inhibitor-1), *CAV1*, *EEF1A1* (eukaryotic translation elongation factor-1 α1), *HSP90AB1*, *PCBP4* (poly(rC)-binding protein-4), *SH2D1A* (SH2 domain protein-1A), *TYRP1* (tyrosinase-related protein-1) and *LIF*.

The more powerful profile hidden Markov models. Profile hidden Markov models (PHMMs) provide a coherent theory for probabilistic profiling of degenerate binding sites that are subject to random insertions and deletions in the presence of natural selection[99,100]. The theory of selection postulates that critical nucleotides are conserved over evolutionary time, whereas noncritical nucleotides and tolerated insertions into a DNA-binding site are not conserved. It is exactly these conserved, critical nucleotides that serve as the blueprint for the match-state emissions (that is, the distributions of emitted nucleotides at each position in the p53 motif) of a PHMM of a binding site. The additional hidden insertion and deletion states give the model the ability to train for observed probabilistic insertions and deletions at different positions in the motif. In short, the training set of observed insertions and deletions serves to fine-tune the model to be properly sensitive to allowed deviations from the most prevalent consensus motif[101]. This inherent trained flexibility is the main strength of PHMMs. Their main drawback is that they require larger training sets to train extra parameters dependent on rare-occurrence insertions and deletions.
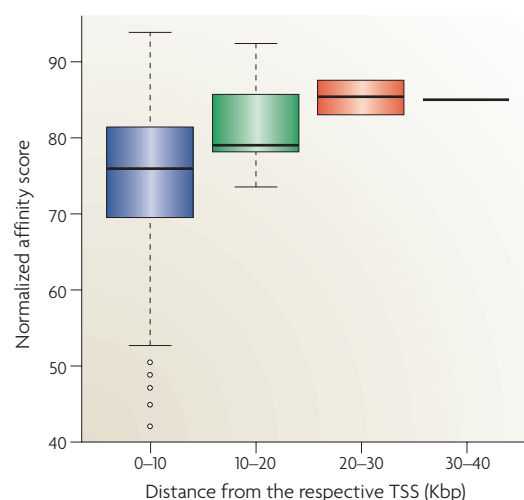

Figure 5 | **Box plots of normalized affinity scores by 10-kilobase-pair block distances from the transcriptional start site.** All low-affinity sites are within the first 10-kilobase pair (Kbp) block from the transcriptional start site (TSS). Median scores of the 10-Kbp blocks rise as a function of distance.

Profile hidden Markov model
(PHMM). A model that is designed to capture the statistical characteristics of biological sequence data (DNA, RNA or protein). All PHMMs typically have three hidden states (match, insertion and deletion) per nucleotide or amino-acid position.
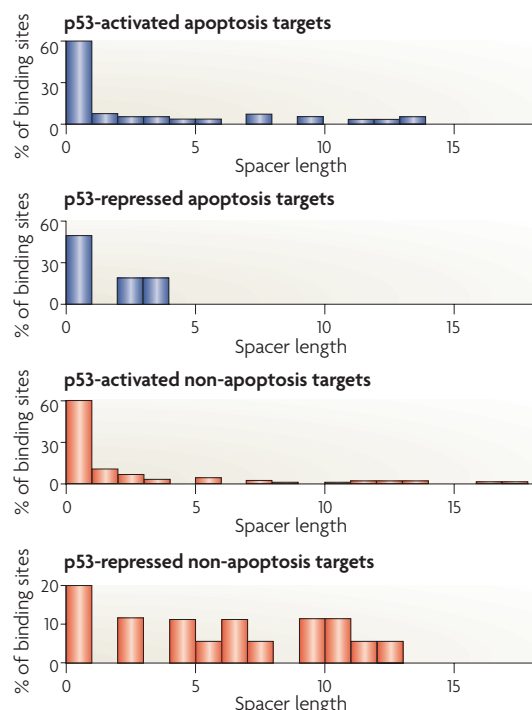
Figure 6 | **Histogram of spacer lengths by regulation type and gene-target function.** Non-apoptosis target sites (red) have a higher frequency of repressor sites compared with apoptosis target sites (blue). In addition, non-apoptosis target repressor sites have no preference for 0-base-pair-length spacers (bottom histogram). Thus, p53-repressor binding sites have significantly longer spacers on average.

PHMMs are hidden Markov models (HMMs) in which each position in the motif has three possible hidden states: match, insert and delete[99]. Each match and insert state has potentially different nucleotide-emission distributions. The delete state has no emissions and is therefore completely silent. PHMMs assume that each nucleotide position contributes independently to the overall binding free energy of the binding site[95,102]. The parameters that must be trained are the probabilities for the transitions to and from the match, insert and delete states (for each position), and the match- and insert-state emissions (for each position). The p53HMM algorithm, which can be used online, is a PHMM that has been trained on the data set of 160 functional p53 REs, and can be used to score putative p53-binding sites. In addition, the p53HMM algorithm has been optimized to increase predictive power by leveraging the redundancy of information found in the repeated, palindromic p53-binding motif.

Faithfulness within the p53 RE. Analysis of the 37 p53-binding sites identified by el Deiry *et al.*[3] and Funk *et al.*[4] showed that the left half-site seemed to be more faithful (that is, highly conserved) than the right half-site, which is graphically expressed as ⟶⟞⟵ spacer ⟶⟞⟵. It also seemed that the right quarter-sites (⟵) of the motif were more faithful than the left quarter-sites (⟶) within each half-site, graphically expressed as ⟶⟞⟵ spacer ⟶⟞⟵.

However, these differences were not statistically significant[92]. Our findings with the current data set of 160 p53-binding sites show no significant differences in the faithfulness between the quarter-sites. Additional evidence that the half-sites share the same binding properties is given by the fact that the best computational predictor in this analysis assumes and leverages that the two half-sites share the same binding preferences.

Analysis and conclusions

This comprehensive analysis of the p53 REs, the genes they regulate and the properties they confer can add new information to our understanding of the p53 pathway and p53-mediated transcriptional control. First, the p53 RE in a gene (FIG. 4) is most commonly located in the 5′ promoter-enhancer region of the gene (~50%) or in intron 1 (~25%). More rarely it is located in introns 2 or 3 of a gene. Surprisingly, some functional p53 REs are in exon 1 or even exon 2. When this occurs, however, the p53 RE is predominately in the 5′ UTR or the intron–exon boundary. Furthermore, because ~50% of the experimentally validated p53 sites are downstream of the TSS, intronic and 5′-UTR regions are equally important to promoter-enhancer regions in conferring p53 regulation. The p53 RE is commonly located close to additional transcription factor RE sites.

Second, the distance of the p53 RE from the TSS helps to determine the threshold for accepting any putative p53 RE on the basis of the normalized affinity score of the p53 protein for the known p53 REs (FIG. 5). Functional, low-affinity p53 RE sites in the DNA only exist around the TSS. Therefore, computational methods can use a dynamic affinity threshold to reduce false-positives during p53-site searches.

Third, ~50% of the p53 RE sites have no spacer between the half-sites, and the distribution of spacer lengths is relatively uniform for spacer lengths between 4 and 15 base pairs (FIG. 3a). This distribution contradicts *in vitro* experiments that predict functional p53 RE sites on the basis of the half-sites being located on the same face of the DNA helix. Interestingly, the distribution of spacer lengths in the p53 RE is different for genes that are transcriptionally activated by p53 and those that are repressed by p53 (FIG. 3b,c). The spacer lengths in the p53 REs of repressed genes do not show a great preference for zero length or small spacers. This difference between spacer length and gene activation or repression is especially clear for those genes not involved in apoptosis (FIG. 6). p53-regulated non-apoptosis genes that are repressed by p53 have no preference for zero-length spacers.

The list of known p53-regulated genes collected in one place gives us a new feeling for the breadth of functions regulated in response to stress signals. After a p53-mediated response to stress, there are changes in the intracellular compartments, cytoskeleton, endosomal and exosomal functions, heat-shock induction and cellular-repair processes. There are also changes in the extracellular matrix, increased secretion of exosomes and proteins that affect angiogenesis, growth-factor functions and the immune response. The p53 response sets up a series of positive and negative feedback loops that regulate p53-mediated

functions as well as other signal-transduction pathways. In addition to these local effects of a p53 response, systemic signals are p53 regulated. Both exosomes and cytokines engage the immune response of the body. p53-mediated responses in the brain alter signal transmission in the central nervous system. Moreover, angiogenic signals and interactions with growth factors and their receptors can all have wider systemic impact. Clearly, the list of genes involved in a stress response mediated by p53 has a broad effect on the host as well as the host cell.

1. Zhao, R. *et al.* Analysis of p53-regulated gene expression patterns using oligonucleotide arrays. *Genes Dev.* **14**, 981–993 (2000).
2. Feng, Z. *et al.* The regulation of AMPK β1, TSC2, and PTEN expression by p53: stress, cell and tissue specificity, and the role of these gene products in modulating the IGF-1–AKT–mTOR pathways. *Cancer Res.* **67**, 3043–3053 (2007).
3. Funk, W. D., Pak, D. T., Karas, R. H., Wright, W. E. & Shay, J. W. A transcriptionally active DNA-binding site for human p53 protein complexes. *Mol. Cell. Biol.* **12**, 2866–2871 (1992).
4. el Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W. & Vogelstein, B. Definition of a consensus binding site for p53. *Nature Genet.* **1**, 45–49 (1992).
   This paper presents the head-to-head p53-binding motif and the analysis that led to it.
5. Kern, S. E. *et al.* Oncogenic forms of p53 inhibit p53-regulated gene expression. *Science* **256**, 827–830 (1992).
6. Bourdon, J. C. *et al.* Further characterisation of the p53 responsive element — identification of new candidate genes for *trans*-activation by p53. *Oncogene* **14**, 85–94 (1997).
   This paper combines experimental and computational methods to explore new p53 targets. It also discusses the importance of clusters of p53 half-sites in the regulatory expression of target genes.
7. Tan, T. & Chu, G. p53 binds and activates the xeroderma pigmentosum *DDB2* gene in humans but not mice. *Mol. Cell. Biol.* **22**, 3247–3254 (2002).
8. el Deiry, W. S. *et al.* Topological control of p21^WAF1/CIP1 expression in normal and neoplastic tissues. *Cancer Res.* **55**, 2910–2919 (1995).
9. Espinosa, J. M. & Emerson, B. M. Transcriptional regulation by p53 through intrinsic DNA/chromatin binding and site-directed cofactor recruitment. *Mol. Cell* **8**, 57–69 (2001).
10. Saramäki, A., Banwell, C. M., Campbell, M. J. & Carlberg, C. Regulation of the human *p21*^(waf1/cip1) gene promoter via multiple binding sites for p53 and the vitamin D3 receptor. *Nucleic Acids Res.* **34**, 543–554 (2006).
11. Vogelstein, B., Lane, D. & Levine, A. J. Surfing the p53 network. *Nature* **408**, 307–310 (2000).
    This review presents the p53 regulatory network, response mechanisms to cell stress and the pathways that suppress cancer occurrence and growth.
12. Levine, A. J., Hu, W. & Feng, Z. The p53 pathway: what questions remain to be explored? *Cell Death Differ.* **13**, 1027–1036 (2006).
13. Levine, A. J. p53, the cellular gatekeeper for growth and division. *Cell* **88**, 323–331 (1997).
    This review presents the p53 regulatory network and how it functions to reduce the occurrence and how it suppresses the growth of cancer.
14. Manfredi, J. J. p53 and apoptosis: it's not just in the nucleus anymore. *Mol. Cell* **11**, 552–554 (2003).
15. Rollwagen, F. M., Yu, Z. Y., Li, Y. Y. & Pacheco, N. D. IL-6 rescues enterocytes from hemorrhage induced apoptosis *in vivo* and *in vitro* by a *bcl-2* mediated mechanism. *Clin. Immunol. Immunopathol.* **89**, 205–213 (1998).
16. Yonish-Rouach, E. *et al.* Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature* **352**, 345–347 (1991).
17. Yang, G. *et al.* The chemokine growth-regulated oncogene 1 (Gro-1) links RAS signaling to the senescence of stromal fibroblasts and ovarian tumorigenesis. *Proc. Natl Acad. Sci. USA* **103**, 16472–16477 (2006).
18. Harris, S. L. & Levine, A. J. The p53 pathway: positive and negative feedback loops. *Oncogene* **24**, 2899–2908 (2005).
    This paper presents how feedback loops are a common motif in the regulatory pathways involving p53.

19. Feng, Z., Zhang, H., Levine, A. J. & Jin, S. The coordinate regulation of the p53 and mTOR pathways in cells. *Proc. Natl Acad. Sci. USA* **102**, 8204–8209 (2005).
20. Yu, X., Harris, S. L. & Levine, A. J. The regulation of exosome secretion: a novel function of the p53 protein. *Cancer Res.* **66**, 4795–4801 (2006).
21. Clemens, M. J. Targets and mechanisms for the regulation of translation in malignant transformation. *Oncogene* **23**, 3180–3188 (2004).
22. Elela, S. A. & Nazar, R. N. The ribosomal 5.8S RNA as a target site for p53 protein in cell differentiation and oncogenesis. *Cancer Lett.* **117**, 23–28 (1997).
23. Prats, A.-C. & Prats, H. Translational control of gene expression: role of IRESs and consequences for cell transformation and angiogenesis. *Prog. Nucleic Acid Res. Mol. Biol.* **72**, 367–413 (2002).
24. Yin, X., Fontoura, B. M. A., Morimoto, T. & Carroll, R. B. Cytoplasmic complex of p53 and eEF2. *J. Cell Physiol.* **196**, 474–482 (2003).
25. Zhang, Y. *et al.* Repression of *hsp90β* gene by p53 in UV irradiation-induced apoptosis of Jurkat cells. *J. Biol. Chem.* **279**, 42545–42551 (2004).
26. Agoff, S. N., Hou, J., Linzer, D. I. & Wu, B. Regulation of the human hsp70 promoter by p53. *Science* **259**, 84–87 (1993).
27. Morris, G. F., Bischoff, J. R. & Mathews, M. B. Transcriptional activation of the human proliferating-cell nuclear antigen promoter by p53. *Proc. Natl Acad. Sci. USA* **93**, 895–899 (1996).
28. Tanaka, H. *et al.* A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. *Nature* **404**, 42–49 (2000).
29. Smith, M. L. *et al.* Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. *Science* **266**, 1376–1380 (1994).
30. Feng, Z. *et al.* p53 tumor suppressor protein regulates the levels of huntingtin gene expression. *Oncogene* **25**, 1–7 (2006).
31. Hu, W., Feng, Z., Teresky, A. & Levine, A. J. p53 regulates maternal reproduction through LIF. *Nature* **450**, 721–724 (2007).
32. Farmer, G., Colgan, J., Nakatani, Y., Manley, J. L. & Prives, C. Functional interaction between p53, the TATA-binding protein (TBP), and TBP-associated factors *in vivo*. *Mol. Cell. Biol.* **16**, 4295–4304 (1996).
33. Thut, C. J., Chen, J. L., Klemm, R. & Tjian, R. p53 transcriptional activation mediated by coactivators TAFII40 and TAFII60. *Science* **267**, 100–104 (1995).
34. Gu, W., Shi, X. L. & Roeder, R. G. Synergistic activation of transcription by CBP and p53. *Nature* **387**, 819–823 (1997).
35. Gu, W. & Roeder, R. G. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* **90**, 595–606 (1997).
36. Lee, K. C., Crowe, A. J. & Barton, M. C. p53-mediated repression of alpha-fetoprotein gene expression by specific DNA binding. *Mol. Cell. Biol.* **19**, 1279–1288 (1999).
37. Budhram-Mahadeo, V. *et al.* p53 suppresses the activation of the Bcl-2 promoter by the Brn-3a POU family transcription factor. *J. Biol. Chem.* **274**, 15237–15244 (1999).
38. Ori, A. *et al.* p53 binds and represses the HBV enhancer: an adjacent enhancer element can reverse the transcription effect of p53. *EMBO J.* **17**, 544–553 (1998).
39. Agostino, S. D. *et al.* Gain of function of mutant p53: the mutant p53/NF-Y protein complex reveals an aberrant transcriptional mechanism of cell cycle regulation. *Cancer Cell* **10**, 191–202 (2006).
40. Basile, V., Mantovani, R. & Imbriano, C. DNA damage promotes histone deacetylase 4 nuclear localization and repression of G2/M promoters, via p53 C-terminal lysines. *J. Biol. Chem.* **281**, 2347–2357 (2006).
41. Ceribelli, M., Alcalay, M., Viganò, M. A. & Mantovani, R. Repression of new p53 targets revealed by ChIP on chip experiments. *Cell Cycle* **5**, 1102–1110 (2006).

This paper presents experiments that show direct p53-mediated repression through p53-mediated inactivation (squelching) of NF-Y.
42. Chae, H. D., Yun, J. & Shi, D. Y. Transcription repression of a CCAAT-binding transcription factor CBF/HSP70 by p53. *Exp. Mol. Med.* **37**, 488–491 (2005).
43. Imbriano, C. *et al.* Direct p53 transcriptional repression: *in vivo* analysis of CCAAT-containing G2/M promoters. *Mol. Cell. Biol.* **25**, 3737–3751 (2005).
44. Yun, J. *et al.* p53 negatively regulates *cdc2* transcription via the CCAAT-binding NF-Y transcription factor. *J. Biol. Chem.* **274**, 29677–29682 (1999).
45. Jung, M. S. *et al.* p53 and its homologues, p63 and p73, induce a replicative senescence through inactivation of NF-Y transcription factor. *Oncogene* **20**, 5818–5825 (2001).
46. Matsui, T. *et al.* Negative regulation of Chk2 expression by p53 is dependent on the CCAAT-binding transcription factor NF-Y. *J. Biol. Chem.* **279**, 25093–25100 (2004).
47. Iotsova, V. & Stehelin, D. Down-regulation of fibronectin gene expression by the p53 tumor suppressor protein. *Cell Growth Differ.* **7**, 629–634 (1996).
48. Subbaramaiah, K. *et al.* Inhibition of cyclooxygenase-2 gene expression by p53. *J. Biol. Chem.* **274**, 10911–10915 (1999).
49. Innocente, S. A. & Lee, J. M. p53 is a NF-Y- and p21-independent, Sp1-dependent repressor of cyclin B1 transcription. *FEBS Lett.* **579**, 1001–1007 (2005).
50. Kanaya, T. *et al.* Adenoviral expression of p53 represses telomerase activity through down-regulation of human telomerase reverse transcriptase transcription. *Clin. Cancer Res.* **6**, 1239–1247 (2000).
51. Ohlsson, C., Kley, N., Werner, H. & LeRoith, D. p53 regulates insulin-like growth factor-I (IGF-I) receptor expression and IGF-I-induced tyrosine phosphorylation in an osteosarcoma cell line: interaction between p53 and Sp1. *Endocrinology* **139**, 1101–1107 (1998).
52. Kubicka, S. *et al.* p53 represses CAAT enhancer-binding protein (C/EBP)-dependent transcription of the albumin gene. A molecular mechanism involved in viral liver infection with implications for hepatocarcinogenesis. *J. Biol. Chem.* **274**, 32137–32144 (1999).
53. Sun, Y., Zeng, X.-R., Wenger, L., Firestein, G. S. & Cheung, H. S. P53 down-regulates matrix metalloproteinase-1 by targeting the communications between AP-1 and the basal transcription complex. *J. Cell. Biochem.* **92**, 258–269 (2004).
54. Seto, E. *et al.* Wild-type p53 binds to the TATA-binding protein and represses transcription. *Proc. Natl Acad. Sci. USA* **89**, 12028–12032 (1992).
55. Truant, R., Xiao, H., Ingles, C. J. & Greenblatt, J. Direct interaction between the transcriptional activation domain of human p53 and the TATA box-binding protein. *J. Biol. Chem.* **268**, 2284–2287 (1993).
56. Farmer, G., Friedlander, P., Colgan, J., Manley, J. L. & Prives, C. Transcriptional repression by p53 involves molecular interactions distinct from those with the TATA box binding protein. *Nucleic Acids Res.* **24**, 4281–4288 (1996).
57. Murphy, M. *et al.* Transcriptional repression by wild-type p53 utilizes histone deacetylases, mediated by interaction with mSin3a. *Genes Dev.* **13**, 2490–2501 (1999).
58. Harms, K. L. & Chen, X. Histone deacetylase 2 modulates p53 transcriptional activities through regulation of p53-DNA binding activity. *Cancer Res.* **67**, 3145–3152 (2007).
    This paper shows that HDAC2 represses p53-mediated regulation of genes by reducing the acetylation of Lys residues in the p53 protein.
59. Löhr, K., Möritz, C., Contente, A. & Dobbelstein, M. *p21/CDKN1A* mediates negative regulation of transcription by p53. *J. Biol. Chem.* **278**, 32507–32516 (2003).
    This paper presents evidence that many genes are indirectly repressed by p53 through p53-mediated induction of *CDKN1A* (*p21*).

60. Hoffman, W. H., Biade, S., Zilfou, J. T., Chen, J. & Murphy, M. Transcriptional repression of the anti-apoptotic survivin gene by wild type p53. *J. Biol. Chem.* **277**, 3247–3257 (2002).

61. Johnson, R. A., Ince, T. A. & Scotto, K. W. Transcriptional repression by p53 through direct binding to a novel DNA element. *J. Biol. Chem.* **276**, 27716–27720 (2001).

62. Bist, A., Fielding, C. J. & Fielding, P. E. p53 regulates caveolin gene transcription, cell cholesterol, and growth by a novel mechanism. *Biochemistry* **39**, 1966–1972 (2000).

63. Vousden, K. H. Activation of the p53 tumor suppressor protein. *Biochim. Biophys. Acta* **1602**, 47–59 (2002).

64. Luo, J. *et al.* Acetylation of p53 augments its site-specific DNA binding both *in vitro* and *in vivo*. *Proc. Natl Acad. Sci. USA* **101**, 2259–2264 (2004).

65. Gu, W., Luo, J., Brooks, C. L., Nikolaev, A. Y. & Li, M. Dynamics of the p53 acetylation pathway. *Novartis Found. Symp.* **259**, 197–205 (2004).

66. Chuikov, S. *et al.* Regulation of p53 activity through lysine methylation. *Nature* **432**, 353–360 (2004).

67. Barlev, N. A. *et al.* **Acetylation of p53 activates** transcription through recruitment of coactivators/histone acetyltransferases. *Mol. Cell* **8**, 1243–1254 (2001).

68. McKinney, K. & Prives, C. Efficient specific DNA binding by p53 requires both its central and C-terminal domains as revealed by studies with high-mobility group 1 protein. *Mol. Cell. Biol.* **22**, 6797–6808 (2002).

69. Weinberg, R. L., Veprintsev, D. B., Bycroft, M. & Fersht, A. R. Comparative binding of p53 to its promoter and DNA recognition elements. *J. Mol. Biol.* **348**, 589–596 (2005).

70. Oda, K. *et al.* p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. *Cell* **102**, 849–862 (2000).

71. Jaiswal, A. S. & Narayan, S. p53-dependent transcriptional regulation of the APC promoter in colon cancer cells treated with DNA alkylating agents. *J. Biol. Chem.* **276**, 18193–18199 (2001).

72. Minucci, S. & Pelicci, P. G. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nature Rev. Cancer* **6**, 38–51 (2006).

73. Halazonetis, T. D., Davis, L. J. & Kandil, A. N. Wild-type p53 adopts a 'mutant'-like conformation when bound to DNA. *EMBO J.* **12**, 1021–1028 (1993).

74. Osada, M. *et al.* Differential recognition of response elements determines target gene specificity for p53 and p63. *Mol. Cell. Biol.* **25**, 6077–6089 (2005).

75. Cho, Y., Gorina, S., Jeffrey, P. D. & Pavletich, N. P. Crystal structure of a p53 tumor suppressor–DNA complex: understanding tumorigenic mutations. *Science* **265**, 346–355 (1994).

76. Inga, A., Storici, F., Darden, T. A. & Resnick, M. A. Differential transactivation by the p53 transcription factor is highly dependent on p53 level and promoter target sequence. *Mol. Cell. Biol.* **22**, 8612–8625 (2002).

77. Balagurumoorthy, P., Lindsay, S. M. & Harrington, R. E. Atomic force microscopy reveals kinks in the p53 response element DNA. *Biophys. Chem.* **101–102**, 611–623 (2002).

78. Olson, W. K., Gorin, A. A., Lu, X. J., Hock, L. M. & Zhurkin, V. B. DNA sequence-dependent deformability deduced from protein–DNA crystal complexes. *Proc. Natl Acad. Sci. USA* **95**, 11163–11168 (1998).

79. Wu, M. *et al.* AMID is a p53-inducible gene downregulated in tumors. *Oncogene* **23**, 6815–6819 (2004).

80. Contente, A., Dittmer, A., Koch, M. C., Roth, J. & Dobbelstein, M. A polymorphic microsatellite that mediates induction of PIG3 by p53. *Nature Genet.* **30**, 315–320 (2002).

81. Thornborrow, E. C., Patel, S., Mastropietro, A. E., Schwartzfarb, E. M. & Manfredi, J. J. A conserved intronic response element mediates direct p53-dependent transcriptional activation of both the human and murine *bax* genes. *Oncogene* **21**, 990–999 (2002).

82. Koutsodontis, G., Vasilaki, E., Chou, W.-C., Papakosta, P. & Kardassis, D. Physical and functional interactions between members of the tumour suppressor p53 and the Sp families of transcription factors: importance for the regulation of genes involved in cell-cycle arrest and apoptosis. *Biochem. J.* **389**, 443–455 (2005).
This paper presents experiments that confirm that cofactor-binding sites adjacent to p53-binding sites can be crucial in conferring p53-mediated regulation.

83. Thornborrow, E. C. & Manfredi, J. J. The tumor suppressor protein p53 requires a cofactor to activate transcriptionally the human BAX promoter. *J. Biol. Chem.* **276**, 15598–15608 (2001).

84. Cook, J. L. Distance constraints and stereospecific alignment requirements characteristic of p53 DNA-binding consensus sequence homologies. *Oncogene* **11**, 723–733 (1995).

85. Stenger, J. E. *et al.* p53 oligomerization and DNA looping are linked with transcriptional activation. *EMBO J.* **13**, 6011–6020 (1994).

86. 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).

87. Burns, T. F., Fei, P., Scata, K. A., Dicker, D. T. & El-Deiry, W. S. Silencing of the novel p53 target gene *Snk/Plk2* leads to mitotic catastrophe in paclitaxel (taxol)-exposed cells. *Mol. Cell. Biol.* **23**, 5556–5571 (2003).

88. Wang, Y., Schwedes, J. F., Parks, D., Mann, K. & Tegtmeyer, P. Interaction of p53 with its consensus DNA-binding site. *Mol. Cell. Biol.* **15**, 2157–2165 (1995).

89. Tokino, T. *et al.* p53 tagged sites from human genomic DNA. *Hum. Mol. Genet.* **3**, 1537–1542 (1994).

90. Flores, E. R. *et al.* p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. *Nature* **416**, 560–564 (2002).

91. Wei, C.-L. *et al.* A global map of p53 transcription-factor binding sites in the human genome. *Cell* **124**, 207–219 (2006).

92. Hoh, J. *et al.* The p53MH algorithm and its application in detecting p53-responsive genes. *Proc. Natl Acad. Sci. USA* **99**, 8467–8472 (2002).

93. Barash, Y., Elidan, G., Friedman, N. & Kaplan, T. in *RECOMB '03: Proceedings of the Seventh Annual International Conference on Research in Computational Molecular Biology* 28–37 (ACM Press, New York, USA, 2003).

94. Djordjevic, M., Sengupta, A. M. & Shraiman, B. I. A biophysical approach to transcription factor binding site discovery. *Genome Res.* **13**, 2381–2390 (2003).

95. Stormo, G. & Fields, D. Specificity, free energy and information content in protein–DNA interactions. *Trends Biochem. Sci.* **23**, 109–113 (1998)..
This paper presents the theory and applicability of position-specific score matrices.

96. Tompa, M. *et al.* Assessing computational tools for the discovery of transcription factor binding sites. *Nature Biotechnol.* **23**, 137–144 (2005).

97. Wasserman, W. W. & Sandelin, A. Applied bioinformatics for the identification of regulatory elements. *Nature Rev. Genet.* **5**, 276–287 (2004).

98. Yeo, G. & Burge, C. B. Maximum entropy modeling of short sequence motifs with applications to RNA splicing signals. *J. Comput. Biol.* **11**, 377–394 (2004).

99. Krogh, A., Brown, M., Mian, I. S., Sjölander, K. & Haussler, D. Hidden Markov models in computational biology. Applications to protein modeling. *J. Mol. Biol.* **235**, 1501–1531 (1994).

100. Eddy, S. R. Profile hidden Markov models. *Bioinformatics* **14**, 755–763 (1998).

101. Durbin, R., Eddy, S., Krogh, A. & Mitchison, G. *Biological Sequence Analysis* 1st Edn (Cambridge Univ. Press, 1998).
This book provides an excellent introduction to computational methods used to analyse biological sequence data (DNA, RNA and proteins).

102. Stormo, G. D. DNA binding sites: representation and discovery. *Bioinformatics* **16**, 16–23 (2000).

103. Schneider, T. D. & Stephens, R. M. Sequence logos: a new way to display consensus sequences. *Nucleic Acids Res.* **18**, 6097–6100 (1990).