Life or death

p53-induced apoptosis requires DNA binding cooperativity

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The tumor suppressor p53 provides exquisite protection from cancer by balancing cell survival and death in response to stress. Sustained stress or irreparable damage trigger p53's killer functions to permanently eliminate genetically-altered cells as a potential source of cancer. To prevent the unnecessary loss of cells that could cause premature aging as a result of stem cell attrition, the killer functions of p53 are tightly regulated and balanced against protector functions that promote damage repair and support survival in response to low stress or mild damage. In molecular terms these p53-based cell fate decisions involve protein interactions with cofactors and modifying enzymes, which modulate the activation of distinct sets of p53 target genes. In addition, we demonstrate that part of this regulation occurs at the level of DNA binding. We show that the killer function of p53 requires the four DNA binding domains within the p53 tetramer to interact with one another. These intermolecular interactions enable cooperative binding of p53 to less perfect response elements in the genome, which are present in many target genes essential for apoptosis. Modulating p53 interactions within the tetramer could therefore present a novel promising strategy to fine-tune p53-based cell fate decisions.

Throughout lifetime the cells of our body are continuously exposed to a large variety of environmental and intrinsic hazards that cause damage to the genome. In case these genetic or epigenetic aberrations are replicated and passed on during cell division danger exists that proliferation and survival promoting mutations accumulate so that sooner or later malignant progeny arises posing a threat to the organism as a whole. Early eradication of aspiring cancer cells through activation of an apoptotic cell death program is therefore an efficient means to protect the organism from a full-blown tumor disease. However, considering that moderate damage resulting from mild stress is often reparable, the decision to kill a stressed cell needs to be well-thought-out. Unreflected killing of valuable cells could eventually result in a depletion of stem cell pools and premature aging as a consequence. Every single cell is therefore continuously confronted with the choice: repair and live or die. Too much death poses the risk of aging, too little death the risk of cancer. Balancing these risks for the benefit of the organism is a central task of the tumor suppressor protein p53. Summoned under conditions of stress, p53 functions like a hub in a highly-connected intracellular signaling network to integrate a plethora of inputs from the inside and outside of the cell to trigger a well-balanced cell fate decision.[1]

The Choice of Targets

How p53 executes this cell fate decision is therefore a question of considerable biomedical interest (**Fig. 1**). Since it is known that p53 functions as a sequence-specific DNA binding transcription factor, tremendous efforts have been made in the last decade to identify the p53-regulated targets in the genome that execute the appropriate cell fate responses.[2] The induction
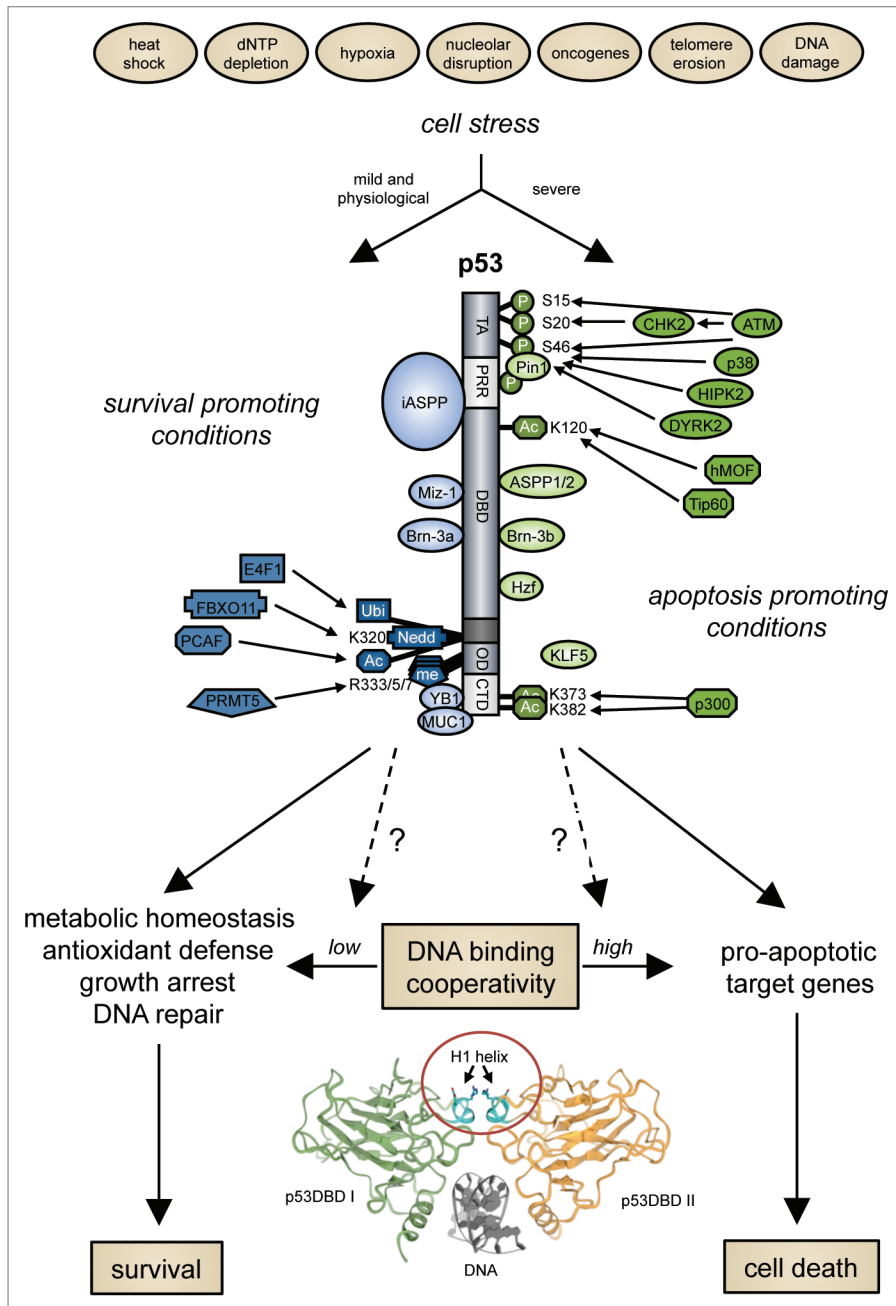
Figure 1. DNA binding cooperativity—a new variable in the p53-based cell fate decision. Post-translational modifications of p53: P, phosphorylation; Ac, acetylation; me, methylation; Ubi, ubiquitylation; Nedd, neddylation.

activation in acute myeloid leukemia cells bearing an activated Ras oncogene.[10,11]

Only when cells have encountered sustained and irreparable damage that is incompatible with further survival, p53 shifts to the most extreme and irrevocable antiproliferative response—apoptotic cell death.[12,13] In line with the importance of this activity numerous studies have identified many different proapoptotic p53 target genes including *BAX*, *FAS*, *TP53I3* (*PIG3*), *TNFRSF10B* (*KILLER/DR5*), *LRDD* (*PIDD*), *P53AIP1*, *APAF1*, *PERP*, *PMAIP1* (*NOXA*) and *BBC3* (*PUMA*)—to name just the most commonly cited. Of note, accumulating evidence shows that p53-induced apoptosis does not only require activation of these proapoptotic target genes but also involves transcription-independent functions of p53 in the cytoplasm.[14-16]

Not enough, a recent review lists a total of 129 transcriptional targets of p53 with experimentally validated binding sites and global approaches using chromatin-immunoprecipitation in conjunction with microarrays (ChIP-chip) or massively parallel sequencing reveal increasingly more sites within the genome that are bound by p53.[2,17-20] The majority of these genomic sequences contain a common consensus motif to which p53 binds with high affinity and specificity. This motif is composed of two decameric half-sites RRR CWW GYY Y, where R is a purine, Y a pyrimidine and W is either adenine (A) or thymine (T), separated by a spacer, usually composed of 0–21 base pairs.[2,21,22] Considering that most of the p53-regulated genes contain response elements that more or less concur with the consensus motif, it remains a mystery how p53 can distinguish between the various genomic binding sites with their associated target genes and selectively activate a subset of them to drive cell fate into the desired direction.[2,12,13,23-25]

The Role of Cofactor Recruitment

One way to target p53 to the promoters of specific target genes is through interaction with partner proteins. Considering the vast amount of p53 binding proteins described so far we will focus on a small

of a transient cell cycle arrest that allows for damage repair depends critically on the genes *p21* (*CDKN1A*), *14-3-3σ* (*SFN*) and *GADD45A*, with the first being crucial for cell cycle arrest in the G_1 phase and the latter two for arrest in G_2.[3] In the case of prolonged damage p53-mediated transactivation of the sestrins (*SESN1* and *SESN2*) causes inhibition of mTOR signaling and helps to maintain the arrest

reversible, while activation of mTOR under these conditions triggers a shift to cell cycle exit termed senescence.[4-8] Another way for p53 to permanently stop cell proliferation without compromising cell viability is induction of differentiation.[9] For example, differentiation follows experimental reactivation of p53 in a murine model of Ras-dependent liver cancer or genotoxic stress induced-p53

fraction with a clear role in redirecting p53 towards a specific cellular outcome.

For example, the proteins of the ASPP family have turned out to be potent regulators of p53's apoptotic function.[26] The apoptosis promoting members, ASPP1 and ASPP2, specifically stimulate p53 binding to the promoters of the proapoptotic target genes *BAX* and *PIG3* but not to the promoters of *p21* or *MDM2*.[27] On the other hand, the inhibitory ASPP family member, iASPP, competes with the other ASPP proteins and blocks p53-mediated apoptosis.[28] Interestingly, iASPP discriminates between two common polymorphic variants of p53 that differ at codon 72.[29] iASPP preferentially binds the proline 72 (P72) variant and inhibits its activity, providing an intriguing explanation for why the arginine 72 (R72) variant is a more potent inducer of apoptosis than the P72 variant.

Another family of proteins that regulates p53 is the Brn3 family of POU domain transcription factors that interact with the p53 DNA binding domain (DBD). While Brn3a stimulates p53-dependent transcription of *p21* and inhibits its ability to activate the *BAX* and *NOXA* promoters, Brn3b functions in the opposite manner by assisting p53 to activate *BAX* but not *p21* expression.[30-32]

The zinc-finger protein Hzf is a target gene of p53 and by interacting with the p53 DBD regulates its target selectivity.[33,34] Hzf promotes p53 binding to the *p21* and *14-3-3σ* promoters early after DNA damage. Inactivation of Hzf—experimentally or by degradation in response to sustained DNA damage—prevents p53 binding to these promoters and allows relocalization to the response elements in the proapoptotic target genes *BAX*, *PUMA*, *NOXA* and *PERP*.[35] A notable exception to the regulation of target selectivity is *MDM2* which appears to be unaffected by Hzf.

Similarly, Miz1 also interacts with the DNA binding domain of p53 to prevent the activation of the proapoptotic targets *BAX* and *PUMA*.[36] Together with Miz1 being a potent transactivator of *p21* expression this results in promotion of cell survival. c-Myc via interaction with Miz1 suppresses *p21* induction by p53 and thus switches the p53-response from cytostatic to apoptotic.[36,37]

The Role of Post-Translational Modifications

Discriminatory effects on target selectivity can also be exerted by interacting proteins that modulate p53's DNA binding properties via covalent post-translational modifications including phosphorylation, acetylation, methylation, ubiquitylation, neddylation, sumoylation and even addition of N-acetyl glucosamine. Here we will highlight those modifications that most prominently influence p53's promoter selectivity.

Among the phosphorylation sites, serine 46 (S46) has clear discriminatory function for p53 as a transcriptional activator. p53 is phosphorylated at this residue by homeodomain interacting protein kinase 2 (HIPK2), dual-specificity tyrosine-phosphorylation-regulated kinase 2 (DYRK2), AMPK, protein kinase C delta or p38 mitogen activated protein kinase in response to severe cellular damage.[38-44] S46-phosphorylated p53 is recognized by the peptidyl-prolyl cis/trans isomerase Pin1 leading to dissociation of the apoptosis-inhibiting protein iASPP from p53 and induction of apoptosis via, for example, transactivation of *p53AIP1*, a proapoptotic factor that promotes the release of mitochondrial cytochrome *c* during apoptosis.[45,46]

While numerous studies have implicated acetylation of lysine residues in the C-terminus of p53 as being important for p53's transcriptional activity in general, acetylation of lysine 120 (K120) in the DNA binding domain by the MYST family histone acetyl transferases hMOF and Tip60 specifically results in increased binding to proapoptotic targets like *BAX* and *PUMA* while the nonapoptotic targets *p21* and *MDM2* remain unaffected.[47,48] On the other hand, acetylation of lysine 320 (K320) by the transcriptional coactivator p300/CBP-associated factor (PCAF) predisposes p53 to activate *p21* and decreases its ability to induce proapoptotic target genes. Cells ectopically expressing a mutant p53 where K320 is mutated to glutamine (K320Q) to mimic acetylation, display reduced apoptosis after some forms of DNA damage.[49] Vice versa K317R (corresponding to human K320R) knockin mice, where K317 acetylation is missing,

consistently display increased apoptosis and higher expression of relevant target genes in several cell types.[50] However, K320 is not only a target for acetylation but it is also ubiquitylated by the zinc-finger protein E4F1.[51] This modification facilitates p53-dependent activation of *p21* and *Cyclin G1* expression without affecting the expression of the proapoptotic gene *NOXA*, overall resulting in reduced p53-mediated cell death in response to UV.

p53-mediated cell cycle arrest is also favored following methylation of at least two arginine residues (R333 and R335) by the arginine methyltransferase PRMT5.[52,53] Consistently, depletion of PRMT5 by siRNA in cancer cell lines leads to increased apoptosis following p53 activation.

The Role of DNA Binding Cooperativity

Together these data highlight the complexity of how p53 binding proteins modulate—in a covalent or non-covalent manner—the DNA binding properties of p53 to influence the cell fate decision in favor of survival or death. Despite this substantial body of knowledge, very little is known about the molecular details. Even a structurally simple modification such as the acetylation of K120 does not directly explain why p53's specificity for certain promoter sequences changes and p53 is redirected to proapoptotic target genes. The recent progress in solving the 3D structures of p53 in contact to DNA, however, promises that it will be possible to gain a clearer view of how p53's sequence specificity is regulated by either modifications or through association with interaction partners.

One striking result of the recent structural studies was that the p53 molecules within the tetramer, which assembles as a dimer of dimers on two cognate half sites in the DNA, do not only interact through their oligomerization domains but also tightly and specifically via their DBDs. Nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography and computational studies indicate that the oppositely charged glutamate (E180) and arginine (R181) residues in the short helix
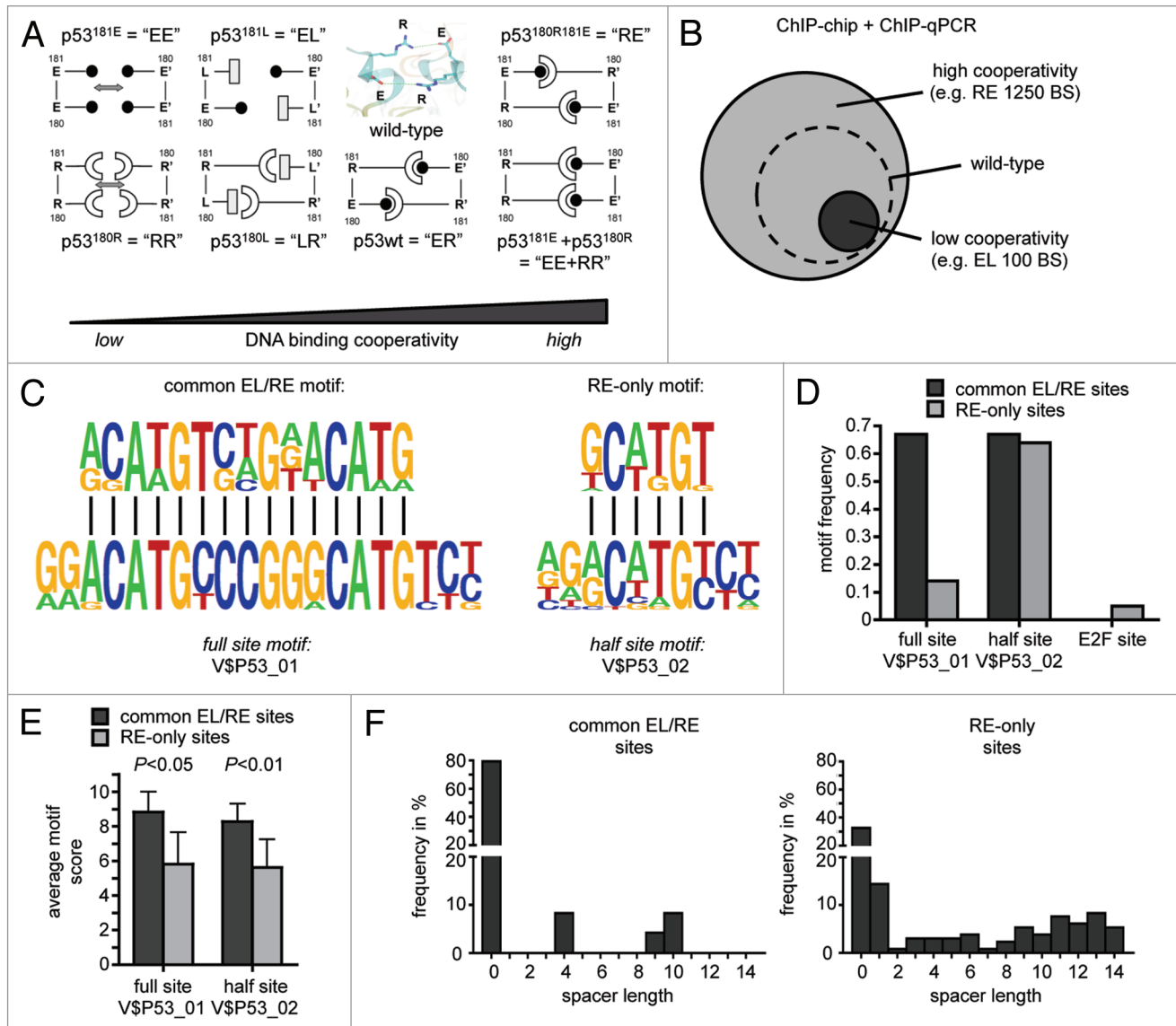
Figure 2. Role of cooperativity for DNA binding of p53 in the human genome. (A) Schematic representation of the dimerization patterns of wild-type p53 and the H1 helix mutants used in this study. The small insert shows the 3D structure of the double salt bridge in the wild-type molecule. To disrupt the intradimer interface we introduced modest charge-neutralizing (E180→L "LR" and R181→L "EL") and more severe charge-inverting (E180→R "RR" and R181→E "EE") mutations into the H1 helix of the full-length p53 molecule. The short names denote the amino acid sequence at positions 180 and 181 in the mutant proteins, e.g., "ER" for E180, R181 in the wild-type. To assure that functional defects are truly due to defective core domain interactions and are not caused by structural misfolding of the core domain or disturbed interaction with other cellular proteins, we also introduced the two most severe mutations E180R and R181E together into a single p53 molecule (double mutant E180R, R181E "RE") and used the two complementing mutants "EE" and "RR" in functional rescue studies. (B) p53 DNA binding cooperativity determines the number of binding sites in the genome. The number of binding sites was estimated by bioinformatic analysis combining ChIP-chip results with experimental validation rates determined by ChIP-qPCR.[9] (C) De novo motif discovery in validated common EL/RE and RE-only binding sequences. Twenty-meric and decameric consensus motifs are shown for comparison. (D and E) Frequency and average motif scores of the TRANSFAC motifs V$P53_01 (full site), V$P53_02 (half-site) and V$E2F_01 (E2F site as a control) in validated common EL/RE and RE-only binding sequences. Results are presented as the mean ± SD. (F) Distribution of spacer lengths in validated common EL/RE and RE-only binding sequences as determined by the spacer-tolerant p53MH algorithm.

H1 of the DBDs engage in intermolecular interactions to form a so-called double salt bridge as part of the DBD dimer interface.[54-58] This dimer interface was further confirmed when Fersht and colleagues succeeded in obtaining a structure of full-length p53 bound to DNA by using a combination of small angle X-ray scattering, NMR and electron microscopy.[59] In vitro studies with recombinant p53 DBDs carrying targeted mutations in the critical residues highlighted that the dimer interface is crucial for a long-known property of p53, called DNA binding cooperativity, which simply means that four interacting p53 subunits cooperate to bind DNA better than four non-interacting subunits.[54]

To better understand the relevance of this dimer interface and the resulting DNA binding cooperativity for the biology of p53, we analyzed the consequences

of expressing dimer interface mutants of full-length p53 in cells.[9] For this, we generated a panel of p53 expression constructs with mutations in the H1 helix residues E180 and R181 that reduced or increased interactions between neighboring p53 subunits (**Fig. 2A**). This mutant panel covers the whole cooperativity range from barely detectable to super-physiological DNA binding cooperativity.

The expression of these mutants in p53-null cell lines resulted in distinct biological outcomes. Low cooperativity mutants induced *p21* and *MDM2* expression leading to a selective cell cycle arrest while high cooperativity mutants activated *BAX*, *NOXA* and other proapoptotic target genes causing cell death. Likewise, when p53 function in p53^{-/-} HCT116 cells was restored with the panel of cooperativity mutants at physiological expression levels the extent of apoptosis induced by genotoxic stress correlated directly with DNA binding cooperativity, indicating that p53's killing function strongly depends on its ability to bind DNA in a cooperative manner.

DNA Binding Cooperativity Enables Binding to Imperfect Binding Elements

One hypothesis was that the binding of p53 to apoptotic target genes requires higher levels of cooperativity than binding to survival genes. To test this we compared the genomic binding profiles of a low (EL) and high (RE) cooperativity mutant by chromatin immunoprecipitation coupled to the unbiased detection of binding sites (BS) with genome-wide promoter tiling microarrays (ChIP-chip). Bioinformatic analysis combining ChIP-chip results with experimental validation rates determined by ChIP-qPCR revealed approximately 1,250 BS for the high cooperativity mutant RE in the promoter regions of the human genome (**Fig. 2B**). Interestingly, the low cooperativity mutant EL showed only approximately 100 BS, which represent a subset of the RE BS. This led us to the conclusion that the DNA binding cooperativity serves to increase the number of BS in the genome.

To understand the differences between BS that are strongly dependent on

cooperativity (RE-only BS) and those, which are bound independently of cooperativity (common BS of EL and RE), we performed motif analysis on experimentally validated "common EL/RE" and "RE-only" BS. De novo motif discovery as well as screening the bound sequences for p53 binding motifs of the TRANSFAC database revealed that common EL/RE but not RE-only BS were strongly enriched for the 20-meric p53 full-site motif (V\$P53_01) (**Fig. 2C and D**). In contrast, the decameric p53 half-site motif (V\$P53_02) was identified with equal frequency in both sets of BS. Nevertheless, in both cases, the average motif score as a measure of similarity to the consensus was significantly lower among the validated RE-only sites (**Fig. 2E**), suggesting that RE tolerates mismatches to the consensus binding site better than EL. Another explanation for the absence of 20-meric full sites in RE-only sequences—despite the presence of decameric half-sites—are spacer elements that separate two half-sites. Applying a spacer-tolerant algorithm, we indeed identified spacer-containing full sites much more frequently in RE-only than in common EL/RE sequences (**Fig. 2F**). Together, these results indicate that the sequence requirements for recruitment of RE are less stringent than for EL and that DNA binding cooperativity increases the number of binding sites in the genome by enabling binding to imperfect, i.e., mismatch- and spacer-containing, response elements.

A Role for Cooperativity in Binding and Activating Imperfect Binding Sites

To experimentally confirm that the extent of DNA binding cooperativity determines binding to imperfect response elements, we performed electrophoretic mobility shift assays. It has been previously shown that even subtle changes in the core CWWG sequence of a RRR CWWG YYY half-site can dramatically reduce DNA binding affinity, which is known to be maximal for CATG.[2] Mutation of the invariable C or G nucleotides typically results in a complete loss of binding activity, whereas changing of the central AT to AA, TT or TA reduces binding only.[2] Consistently, binding of wild-type p53

and even more pronounced of low cooperativity mutants (RR, LR and EL) was reduced when the core CATG sequence was mutated to CAAG, CTTG or CTAG (**Fig. 3A**). In contrast, the high cooperativity mutants RE and EE + RR bound these non-CATG sequences even better than wild-type p53. Similarly, spacer elements in between the two half-sites completely abolished the binding of low cooperativity mutants whereas high cooperativity mutants were still bound (**Fig. 3B**). H1 helix interactions therefore strongly influence the sequence specificity of the p53 tetramer in the way that high cooperativity renders p53 tolerant to deviations from the consensus sequence.

To investigate whether cooperativity also affects transactivation of target genes in a way predicted by the DNA binding experiments, we analyzed luciferase reporter plasmids containing the consensus-like 5' p53 binding site of the *p21* promoter in comparison to derivative constructs containing central CTAG sequences and/or variable spacers (**Fig. 4A**). Activation of these reporters by our panel of cooperativity mutants was measured following transfection into p53-null H1299 cells. The parental promoter construct—with central CATG sequence and without any spacer—yielded high levels of reporter activity and was preferentially activated by low cooperativity mutants (**Fig. 4B**). Mutation of the central CATG to CTAG in both half-sites as well as the insertion of a 5 or 14 bp spacer reduced the maximal activity of the reporter (**Fig. 4C**). However, this decrease primarily affected the transactivation by low cooperativity mutants so that the difference between low and high cooperativity mutants became less apparent (**Fig. 4D**). In fact, insertion of a 14 bp spacer rendered the promoter with a CATG core independent of cooperativity so that all p53 H1 helix mutants induced equal reporter activity levels (**Fig. 4B**). By combining a central CTAG sequence with a spacer insertion we even obtained reporters that were preferentially induced by high cooperativity mutants (**Fig. 4B and D**). Together these experiments illustrate that the level of DNA binding cooperativity determines which promoter sequences are activated by p53.
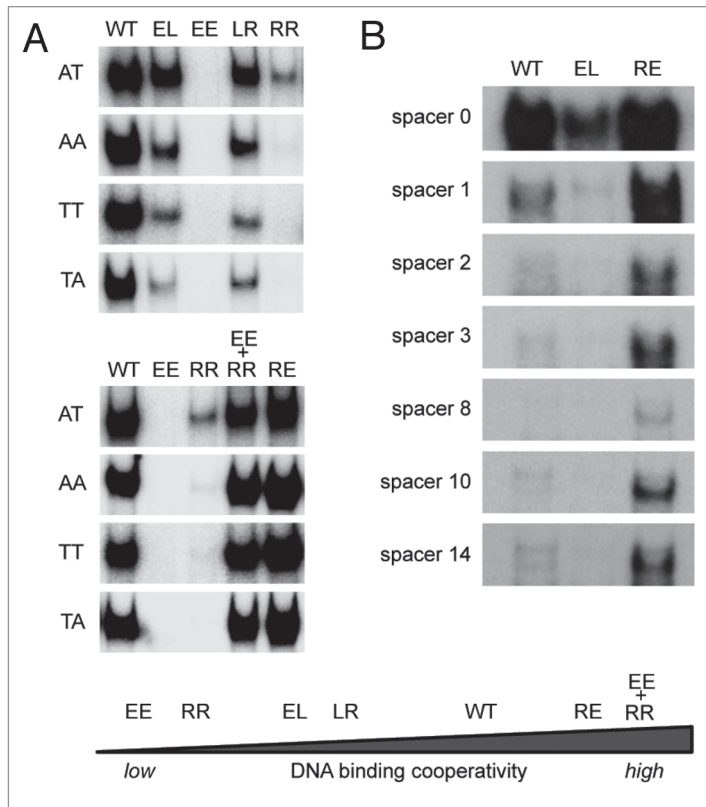
Figure 3. Impact of DNA binding cooperativity on sequence selectivity of p53. (A) Shown are electrophoretic mobility shift assays (EMSA) for DNA binding of in vitro translated wild-type p53 and the indicated H1 helix mutants to dsDNA oligonucleotides (5'-GGG AGC TTA GGC WWG TCT AGG CWW GTC TA-3') with WW denoting AT, AA, TT or TA sequences in the center of each half site. EMSAs were performed as previously described.[9,72] Compared to H1 helix mutants with reduced DNA binding cooperativity (EE, RR, LR, EL), mutants with increased DNA binding cooperativity (RE and EE+RR) revealed an increased ability to bind the lower affinity non-CATG sequences. (B) Same as in (A) using dsDNA oligonucleotides containing the 5' p53 binding site in the *p21* promoter (5'-TCT GGC CGT CAG GAA CATG TCC (N)$_{1-14}$ CAA CATG TTG AAG CTC TGG CAT A-3') with increasing central spacer sequences (N)$_{1-14}$. The high cooperativity mutant (RE) showed an increased ability to bind the spacer-containing motifs, while the low cooperativity mutant (EL) was largely unable to bind these spacer-containing elements.

The Consensus Sequence Binding-Transactivation Paradox

Curiously, high cooperativity mutants often bound perfect consensus-like response elements at least equally well if not even stronger than low cooperativity mutants, but failed to efficiently transactivate reporter constructs made up of these binding sites. Although not fully understood at present, we can envision two possible mechanisms. First, because high cooperativity mutants bind to many more sites in the genome than low cooperativity mutants, essential cofactors that might be present in limiting amounts could be sequestered, so that the local availability of these factors on a given promoter might be insufficient to support high expression levels of the target gene. This idea is experimentally supported by our data showing that coexpression of a high cooperativity mutant also limits transactivation in a heterologous reporter system, which depends on the transactivation domain but not the DNA binding domain of p53.[9] Second, it still remains unclear how p53 and other transcription factors (TF) efficiently drive a promoter to maturation.[60-62] Many different chromatin-modifying enzymes and chromatin remodellers have been identified as essential players involved in this transactivation process.[63,64] In one scenario, TFs stably associate with a binding site in the promoter and serve as a docking site for the various cofactors that one after the other are recruited to the promoter for its activation. In a contrasting model, various different preformed TF-cofactor complexes exist in the nucleoplasm and the TF functions as a shuttling factor to transport these factors to the target gene promoters.[65-67] In the latter model, stable association of p53 with the promoter DNA (as in the case of high cooperativity mutants on consensus binding sites) could compromise the hypothetical shuttling function and be detrimental to the transactivation process. Therefore, an efficient shuttling of highly cooperative p53 would only be possible on imperfect, low-affinity binding sites. No matter which model applies, excessively high levels of cooperativity prevent efficient transactivation of genes with perfect p53 binding elements so that high cooperativity contributes to shifting the expression profile to target genes with imperfect binding sites.

Imperfect binding sites are enriched in proapoptotic target genes. Importantly, there is evidence that low-affinity and spacer-containing sequences are more common in proapoptotic than in non-apoptotic genes, which could explain that the apoptotic potential of p53 correlates with the level of DNA binding cooperativity. It has been known for a while that the cellular level of p53 can dictate the response of the cell such that lower levels of p53 result in arrest whereas higher levels result in apoptosis.[68] It has therefore been hypothesized that only high levels of p53 protein, for example following stabilization in response to massive DNA damage, allow for sufficient binding to proapoptotic target genes, which in many cases contain p53 binding elements that only poorly resemble the consensus binding sequence and which—compared to response elements in cell cycle arrest targets—show very little evolutionary conservation.[69] To investigate whether the imperfect response elements in proapoptotic target genes resemble the binding sequences that we found to be preferentially bound by high cooperativity mutants, we analyzed 60 p53 binding sites found in 39 experimentally validated bona fide p53 target genes (**Sup. Table 1**).[70] The p53 response elements in non-apoptotic genes were indeed significantly enriched for the half-site RRR CATG YYY, whereas central CAAG,
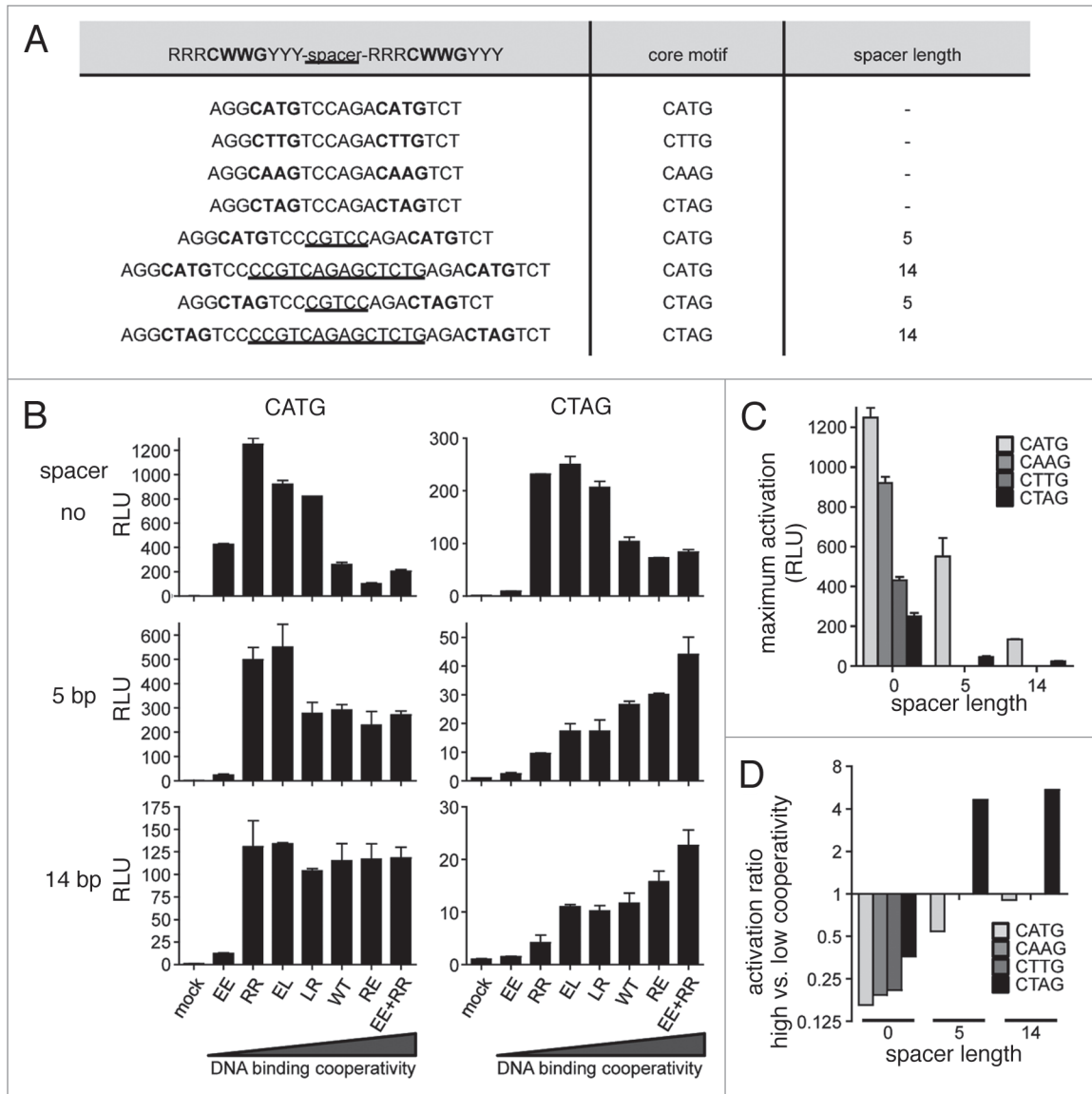
Figure 4. Impact of DNA binding cooperativity on sequence selectivity of transactivation. (A) Shown are p53 binding sequences that differ from the consensus sequence with respect to the CATG in the core of a half-site (bold) and with respect to spacer length (underlined). (B) Luciferase reporter assays. Single copies of the sequences in (A) were cloned into pGL4.23[luc2/minP] and tested for transactivation by the indicated p53 cooperativity mutants. Firefly luciferase activity was measured 48 hours following co-transfection of 100 ng reporter plasmid and 5 ng p53 expression plasmid (pCMVneo-BamHI) into p53-null H1299 cells. The p53 mutants are shown in the order of increasing DNA binding cooperativity. Mean ± SD. (C) Shown is the maximum p53-induced reporter activation for the different promoter sequences. (D) Shown is the ratio of the reporter activities induced by "EE + RR" (high cooperativity) and "RR" (low cooperativity) for the different promoter sequences.

CTTG or CTAG sequences as well as spacers between the two half-sites were significantly more common in the proapoptotic genes (**Fig. 5**). Our study therefore provides the first direct experimental evidence that the activation of the apoptosis program indeed requires p53 binding to imperfect binding sites, which are overrepresented in the promoters of many known proapoptotic target genes, and that this depends on the cooperative nature of DNA binding by the p53 tetramer.

Open Questions

Considering the relevance of DNA binding cooperativity for binding and activation of proapoptotic target genes, it can be hypothesized that known p53 binding proteins or post-translational modifications that affect p53-based cell fate decisions act via modulating this cooperativity. For example, chromatin-associated factors only present on proapoptotic promoters could be envisioned to attach to p53 and stimulate DBD interactions to allow a more stable binding to the imperfect binding sequences in these promoters. So far, direct evidence for this is missing and will be difficult to obtain, because the cooperativity status of p53, i.e., the interaction strength of neighboring p53 subunits in a p53 tetramer, cannot be easily measured in living cells. However, there is some indirect evidence that at least a few of the known apoptosis-promoting factors might function via modulation of cooperativity.[9]
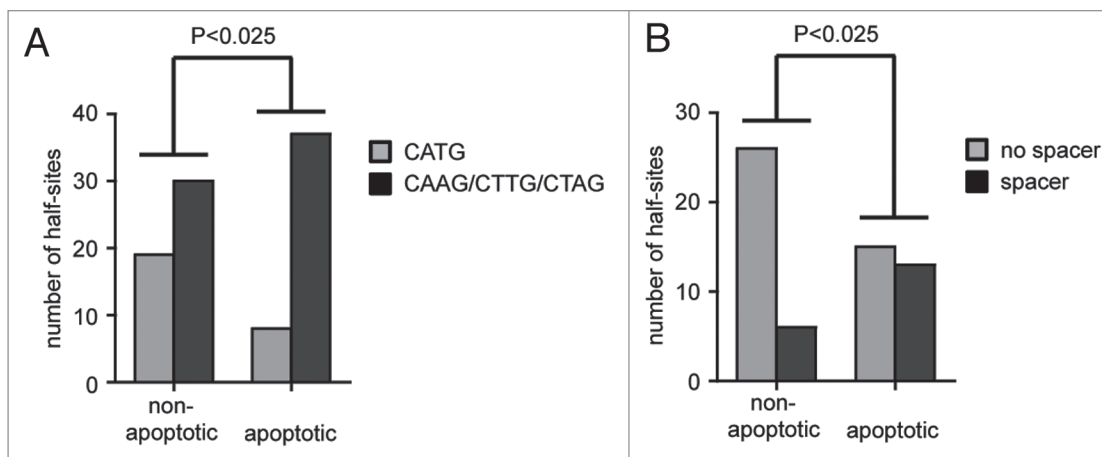
Figure 5. Proapoptotic target genes in a list of experimentally validated p53 target genes (**Sup. Table 1**) are enriched for p53 response elements with spacers and non-CATG core sequences. Statistical significance was calculated by Pearson's Chi-square test. (A) Number of half-sites with a central CATG versus CAAG, CTTG or CTAG sequence in non-versus proapoptotic target genes. (B) Presence of spacer containing response elements in non-versus proapoptotic target genes.

First of all, known apoptosis-promoting conditions such as ectopic expression of ASPP2 or the apoptosis-enhancing mutation of serine 46 to phenylalanine appear to be less effective when cooperativity is impaired. Second, ASPP2 was able to increase apoptosis induced by low cooperativity mutants but could not further increase the apoptotic function of the engineered high cooperativity p53 "EE+RR", suggesting that ASPP2 binding to the p53 DBD—possibly in a hit-and-run mechanism—enhances cooperativity to enable p53 to bind to proapoptotic target genes. While it is clear that cooperativity is essential for high-level induction of apoptosis by p53, it remains to be elucidated, whether an increase in cooperativity mediates the proapoptotic activity of p53-stimulating cofactors or modifying enzymes.

Importantly, mutations that reduce cooperativity by interfering with H1 helix interactions are found in human tumors. Apart from many somatic mutations affecting residues E180 and R181, even families with Li Fraumeni-like syndrome carrying germline mutations in the H1 helix have been described. Interestingly, the R181H mutation (EH) has been identified very early in a family with familial breast carcinoma, but was excluded as a cancer-promoting mutation in part because the mutant protein retained the ability to suppress proliferation of p53-null Saos-2 cells in culture.[71] Nevertheless, similar germline mutations (R181C and R181L) have been found in other families. We confirmed that these p53 mutant proteins were indeed able to induce cell arrest, but showed strongly impaired apoptotic activity.[9] This implies that H1 helix mutations cause a loss of DNA binding cooperativity resulting in an increased cancer risk. However, formal confirmation, which could be provided by the analysis of cooperativity mutant mice, remains to be obtained. In summary, cooperativity appears to be essential for both p53's apoptotic activity and its tumor suppressor function.

Note

Supplementary materials can be found at: www.landesbioscience.com/supplement/SchlerethCC9-20-Sup.pdf

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