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p53 in the DNA-Damage-Repair Process

Ashley B. Williams[1,2] and Björn Schumacher[1,2]

[1]Medical Faculty, Institute for Genome Stability in Ageing and Disease, University of Cologne, 50931 Cologne, Germany

[2]Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), Center for Molecular Medicine Cologne (CMMC) and Systems Biology of Ageing Cologne, University of Cologne, 50931 Cologne, Germany

Correspondence: bjoern.schumacher@uni-koeln.de

The cells in the human body are continuously challenged by a variety of genotoxic attacks. Erroneous repair of the DNA can lead to mutations and chromosomal aberrations that can alter the functions of tumor suppressor genes or oncogenes, thus causing cancer development. As a central tumor suppressor, p53 guards the genome by orchestrating a variety of DNA-damage-response (DDR) mechanisms. Already early in metazoan evolution, p53 started controlling the apoptotic demise of genomically compromised cells. p53 plays a prominent role as a facilitator of DNA repair by halting the cell cycle to allow time for the repair machineries to restore genome stability. In addition, p53 took on diverse roles to also directly impact the activity of various DNA-repair systems. It thus appears as if p53 is multitasking in providing protection from cancer development by maintaining genome stability.

The loss of p53 is a major driver of cancer development mainly because, in the absence of this "guardian of the genome," cells are no longer adequately protected from mutations and genomic aberrations. Already the most ancestral forms of p53 have acquired functions in responding to DNA damage. Intriguingly, the evolutionary occurrence of p53 homologs appears to be associated with multicellularity. With the advent of metazoans, genome maintenance became a specialized task with distinct requirements in germ cells and somatic tissues. The function of p53 in the nematode *Caenorhabditis elegans* particularly well exemplifies the distinct requirements for genome maintenance in distinct tissues of metazoans and is, therefore, discussed as an instructive instance of ancestral p53 function.

In the nematode, DNA-damage checkpoints respond to DNA damage specifically in the germ cells (Gartner et al. 2000). When ionizing radiation (IR) induces DNA double-strand breaks (DSBs), mitotically dividing germ cells arrest through conserved DNA-damage checkpoint activity. The cell-cycle arrest is confined to the germ stem cell compartment that is maintained in the distal zone of the germline. Once the damage is repaired, the germ stem cells resume proliferation. Only cells during the late stages of meiotic pachytene undergo apoptosis in the presence of DSBs. Although the IR-induced cell-cycle arrest is unaffected by

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the presence or absence of the *C. elegans* p53 homolog CEP-1, it is required for DNA-damage-induced apoptosis (Derry et al. 2001; Schumacher et al. 2001). The proapoptotic action of CEP-1/p53 is strictly confined to meiotic pachytene cells. Before meiotic cells reach that stage, the translational repressor GLD-1 prevents *cep-1/p53* mRNA from being translated (Schumacher et al. 2005a). Only when GLD-1 is switched off in late pachytene does CEP-1/p53 protein become available. In case DSBs are present, CEP-1/p53 transcriptionally induces the BH3-only domain proteins EGL-1 and CED-13 (Hofmann et al. 2002; Schumacher et al. 2005b). Intriguingly, physiological DSBs are induced to ignite meiotic recombination by the endonuclease SPO-11. The orderly recombination events must be completed within the pachytene stage. Only failure in resolving recombination intermediates will lead to persistent DSBs in late pachytene cells. CEP-1/p53 is thus available to cull such cells that might otherwise result in genomically compromised oocytes. As oocytes are the cell type that major resources are deposited into by the mother, CEP-1/p53 safeguards the investments by eliminating genomically compromised cells before any major investments are made; thus, CEP-1/p53 fulfills an important function in ensuring the inheritance of stable genomes.

Although the ancestral p53 specifically surveys the presence of DSBs in meiotic cells, it responds to other types of DNA lesions also in mitotic cells. In contrast to IR, UV induces cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs), both of which lead to distortions in the DNA double helix and pose obstacles for both replication and transcription. CEP-1/p53 not only mediates apoptosis but is also required for arresting the cell cycle on UV-induced lesions (Derry et al. 2007). Halting the cell cycle is a prerequisite for repairing DNA lesions. Although on DSB formation only the two proapoptotic BH3-only proteins are transcriptionally induced through CEP-1/p53, transcriptome experiments following UV irradiation defined a range of genes that are induced or repressed dependent on CEP-1/p53 (Derry et al. 2007). The growth-arrest-specific 1 (Gas1) homolog *phg-1* was defined as a CEP-1/p53 target gene required for halting cell proliferation.

Also in mammals, p53 is stabilized and activated by DNA-damage checkpoint signaling following a range of genotoxic insults (reviewed in Shiloh et al. 2013). Although the proapoptotic function of p53 is highly conserved throughout evolution by transcriptional induction of BH3-only domain proteins that execute the programmed cell death, the target genes through which p53 halts the cell cycle have diversified. Indeed, p53 contributes to genome maintenance to a large part by allowing time for the DNA-repair machineries to remove the lesions before cell proliferation resumes. When DNA damage is present before the entry into S phase, p53 halts the cell cycle at the G_1 phase in part by transcriptionally inducing the cyclin-dependent kinase inhibitor *cdkn1a,* also known as p21 (el-Deiry et al. 1993). In nematodes, the p21 homolog *cki-1* is dispensable for the DNA-damage response as it specifically halts the cell cycle to allow differentiation of cells during unperturbed development (Buck et al. 2009).

During the time the cell cycle is halted, the highly specialized DNA-repair machineries pursue the damage removal. In addition to the well-defined role of p53 in regulating the cell cycle under the influence of DNA damage, p53 has also been directly implicated in the regulation of and participation in various DNA-repair pathways.

MULTITASKING FOR GENOME MAINTENANCE: P53 IN DNA REPAIR

Cells can revert the large variety of DNA lesions that are induced by endogenous and exogenous genotoxic attacks through a variety of sophisticated DNA-repair machineries, many of which somehow involve p53. Nucleotide excision repair (NER) removes a variety of helix-distorting lesions such as typically induced by UV irradiation, whereas base excision repair (BER) targets oxidative base modifications. Mismatch repair (MMR) scans for nucleotides that have been erroneously inserted during replication. DNA DSBs that are typically induced by IR

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are resolved either by nonhomologous end joining (NHEJ) or by homologous recombination (HR), whereas RECQ helicases assume various roles in genome maintenance during recombination repair and replication.

Functions of p53 in Nucleotide Excision Repair

It has been known for more than 20 years that p53 has important roles in the repair of UV-induced DNA damage, both via *trans*-activation and *trans*-repression activities (transcriptional regulation) and via activities not directly associated with gene regulation. Hinting at a role for p53 in DNA repair, studies showed that p53 has both sequence-dependent and sequence-independent DNA-binding activities and that it may be involved in recognizing structures associated with DNA damage (Lee et al. 1995; Liu and Kulesz-Martin 2001). Perhaps the earliest connection between p53 and NER came from the work of Smith and colleagues when they showed that human cell lines with disrupted p53 function (either via a dominant negative mutation or expression of the human papillomavirus E6 oncoprotein) showed significant losses of fitness and survival after UV irradiation (Smith et al. 1995). Importantly, this study presented both in vivo and in vitro functions for p53. First, the investigators showed the in vivo importance of p53 using host-cell-reactivation experiments, which correlated with cell survival, as shown by clonogenic assays. They further showed that extracts from the same p53-defective cells were defective for tolerating UV-induced DNA damage. Although this study did not clarify whether the function of p53 in UV resistance was direct or via activities of p53-associated factors, it did lay an important foundation for the further characterization of the functions of p53 in NER (for a detailed review of NER, see Marteijn et al. 2014).

Several studies, which followed quite quickly, assigned the requirement for p53 in the survival of UV-induced DNA damage more specifically to the NER pathway. Two important studies from Phil Hanawalt's group revealed that p53 was important for the regulation of global genome NER (GG-NER), but largely dispensable for transcription-coupled NER (TC-NER). In the first study, Ford and Hanawalt examined the functions of p53 in p53 heterozygous and homozygous Li–Fraumeni syndrome fibroblasts (Ford and Hanawalt 1995). Specifically, they showed that homozygous p53 mutant fibroblasts, which had spontaneously lost the wild-type p53 allele during culturing of p53 heterozygous lines (Yin et al. 1992), were defective for the removal of CPDs from overall genomic DNA compared with their heterozygous progenitors, suggesting a loss of GG-NER. In contrast, the homozygous cells remained proficient for TC-NER, the preferential removal of DNA damage from actively transcribed strands. They followed up and confirmed these findings by directly examining lesion repair using antibodies specific for CPDs and 6-4PPs (Ford and Hanawalt 1997). In a 1996 study, Mirzayans and colleagues (Mirzayans et al. 1996) independently confirmed a function for p53 in UV resistance in different cell lines and using different techniques from those of Ford and Hanawalt (1995). Their combined results showed that p53 was involved in repair mediated by DNA polymerases δ and ε; however, in contrast to the results from the Hanawalt group, p53 seemed to be important for both GG-NER and TC-NER. Perhaps an even more direct connection between p53 and NER was provided by Wang and colleagues (Wang et al. 1995) when they showed that p53 can bind to several components of the NER pathway including XPC, XBP, and CSB.

The literature cited above shows a division on the question of whether p53 is involved only in GG-NER or whether it also participates in TC-NER. Consistent results have generally confirmed that p53 is important in the GG-NER pathway, so the controversy mostly surrounds its involvement in TC-NER. Clouding the issue even further, three later studies from Altaf Wani's group provided additional evidence that p53 is primarily involved in GG-NER, contradicting the results of Mirzayans et al. (1996) and agreeing with the earlier results from the Hanawalt group (Wani et al. 2000; 2002; Zhu et al. 2000). Can these contradictions be reconciled?

One possible explanation is that the discrepancy may stem from the type of UV source used for the irradiation (Sengupta and Harris 2005). This explanation is based on the observation that loss of p53 reduces TC-NER after exposure to broad-spectrum UV (290–324 nm), but not after irradiation with narrow-band UV (254 nm), despite a universal requirement for efficient GG-NER (Mathonnet et al. 2003). Interestingly, because 254 nm UV is almost nonexistent in the spectrum of light reaching Earth from the Sun, p53 is likely part of the response to natural damage in both the transcribed and nontranscribed DNA strands.

These early studies clearly placed p53 in the NER pathway; however, they provided limited insight into whether the requirement for p53 in NER was via its *trans*-activation activity, or whether it directly acts in DNA-associated transactions during repair—in fact, p53 seems to act via both mechanisms. The involvement of p53 as a transcriptional regulatory in NER seems to be limited, as its only known relevant regulatory targets are the genes encoding the DDB2 protein (*p48*) and the XPC protein (*XPC*) (Hwang et al. 1999; Adimoolam and Ford 2002; Hastak et al. 2012). DDB2 associates with its binding partner DDB1 to form the UV-DDB heterodimer, which in turn binds to 6-4PPs and CPDs to help recruit XPC during the early steps in NER (reviewed in Sugasawa 2010). After UV-induced DNA damage, activated p53 induces the expression of *p48* and *XPC*, thus increasing the cell's capacity to locate and target DNA damage for repair. At least two observations support the *trans* requirement for p53 in regulating NER. First, endogenous expression of DDB2 in p53-deficient cells improves the efficiency of GG-NER (Fitch et al. 2003b). Second, XPC and DDB2 are recruited to sites of DNA damage, whereas p53 is not (Fitch et al. 2003a).

p53 may be most commonly associated with gene regulation at the transcriptional level, typically functioning as a transcriptional activator; thus, its function in regulating NER components transcriptionally is not surprising. In contrast, that p53 also appears to have functions in NER (and other DNA-repair pathways discussed below) independent of its transcriptional role were perhaps more unexpected. Two transcription-independent functions for p53 in NER have been reported: (1) modulation of the helicase activities of XPB and XPD (Wang et al. 1995; Léveillard et al. 1996), and (2) modulation of chromatin accessibility (Rubbi and Milner 2003). The GG- and TC-NER subpathways converge on the common repair pathway with the local relaxation of the DNA by the multimeric protein TFIIH via the helicase activity of its XPB and XPD subunits. At least in vitro, XPB and XPD can interact with p53 leading to a decrease in their helicase activity. Because TFIIH plays a central role in NER, it is likely that these changes could influence its function, although the details remain to be worked out. The chromatin relaxation function for p53 is also spatially and temporally associated with TFIIH during NER. Following the recognition of a lesion by TC-NER branch, the chromatin is relaxed throughout the genome. Rubbi and colleagues hypothesized that this global relaxation leads to enhanced global lesion detection. This activity seems to be via p53-dependent recruitment of the p300 histone acetylase to damage sites where it acetylates the histone H3 subunit. In this way, p53 may generally increase lesion detection across the entire genome making an additional contribution to the maintenance of genome stability.

Finally, a function for p53 in the regulation of NER during chronic exposure to the potent genotoxin aflatoxin B_1 (AFB_1) has recently been described. In p53-proficient mice, dietary exposure to AFB_1 led to an increase in NER activity as measured by an in vitro assay (Mulder et al. 2014). This up-regulation of NER was eliminated in p53 haploinsufficient mice and was independent of the levels of the NER proteins XPA and XPB. The mechanism for this effect is yet to be elucidated.

Functions of p53 in Base Excision Repair

The reported roles of p53 in the BER pathway reveal even more the diverse repertoire of functions for p53. As in NER, p53 has both transcription-dependent and transcription-independent functions in BER. Apurinic and

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apyrimidinic (AP) endonucleases are key players in BER that function downstream from the DNA glycosylases (such as OGG1) during the removal of damaged bases and the subsequent repair of the resulting AP sites. Interestingly, studies have shown several different connections between AP endonucleases and p53, the earliest of which were described in studies that showed an interaction between the bifunctional AP endonuclease APE1/Ref-1 and p53 (Jayaraman et al. 1997; Gaiddon et al. 1999). Jayaraman et al. made the initial observation that p53 was regulated by APE1/Ref-1 via redox-dependent and -independent pathways. Subsequently, Gaiddon et al. showed that Ref-1 enhances p53 DNA binding in vitro and that the effects of this regulation can be mirrored in vivo. Through these interactions, APE1/Ref-1 modulated the *trans*-activation and proapoptotic functions of p53. A 2005 study revealed that the function of APE1/Ref-1 was to promote the tetramerization of p53 (Hanson et al. 2005). Interestingly, only in 2014 was it discovered that APE1/Ref-1 can also modulate the DNA-binding activity of mutant p53 through a redox-dependent mechanism (Cun et al. 2014). A further connection between p53 and APE1/Ref-1 was reported by Seo and colleagues when they showed that selenomethione (SeMet) can activate p53 in a Ref-1-dependent manner (Seo et al. 2002b). These findings established a connection between the dietary nutrient selenium and the regulation of DNA repair. In fact, selenomethionine showed some protective effect against IR (Jeong et al. 2009). The effects of selenomethionine on p53 and BER have been extensively studied because selenomethionine can be used to stimulate p53 and its genome protective functions independent of genotoxic effects, thus supporting its use as a chemotherapeutic agent (for example, see Jung et al. 2013). Finally, in addition to its transcription-independent interaction with APE1, p53 also seems to directly repress transcription of the *APE1* gene (Zaky et al. 2008). This role for p53 is particularly interesting because it may seem contradictory—why would cells repress a DNA-repair gene when facing DNA damage? In fact, such a function could contribute to the tumor suppressor function of p53 as p53-dependent down-regulation of DNA-repair activity could skew the DNA-damage response in the direction of apoptosis to clear highly damaged genomes.

The studies above show that BER can influence the activity of p53, but the question remains whether or not p53 can also regulate BER? The first bona fide example of a function of p53 in BER came from Varda Rotter's group when they showed that cell extracts overproducing p53 had increased BER activity in vitro (Offer et al. 1999). Follow-up work indicated that this connection between p53 and BER was independent of the transcriptional activity of p53 because the p53 transactivation-deficient protein (p53-22-23) was actually more effective in controlling BER than wild-type p53 (Offer et al. 2001a). The investigators concluded that the transcription-independent function of p53 could represent a more acute response to DNA damage. This conclusion was supported by work in which they examined how the amount of DNA damage influences this pathway (Offer et al. 2002). The data in this study revealed a dose-dependent component of this regulation: on low doses of DNA-damaging agents (γ-irradiation or cisplatin), there was an immediate increase in BER activity; in contrast, higher doses led to a reduction in BER and p53-dependent apoptosis. Consistent with this biological function, they showed that p53 can enhance BER during G_0-G_1 stages of the cell cycle while reducing BER and inducing apoptosis during G_2-M; thus, p53 seems to act as a modulator of BER activity throughout the cell cycle (Offer et al. 2001b).

That p53 can functionally influence BER was further shown by its ability to regulate the 3-methyladenine (3-MeAde) DNA glycosylase. Zurer et al. (another study from the Rotter group) showed that nitric oxide treatment increases the activity of 3-MeAde (as expected because 3-MeAde is the first enzyme in the BER pathway) (Zurer et al. 2004). Wild-type p53 suppressed this increase by repressing the glycosylase mRNA, thus operating as a transcriptional regulator. Interestingly, the activity of the AP endonuclease protein was not altered under these conditions. In the absence of p53, elevated

glycosylase activity could lead to an increased number of AP sites and, without a concomitant increase the AP endonuclease activity to repair them, this imbalance could then lead to genome instability because of weakened DNA repair. Together, these observations suggest that this regulatory effect of p53 on a BER component could limit the mutagenic effects of some genotoxins.

Incidentally, some work has showed that p53 can also regulate the expression of two additional BER genes at the transcriptional level: *OGG1* (the gene for 8-oxoguanine glycosylase) (Chatterjee et al. 2006) and *MUTYH*, which encodes an adenine DNA glycosylase (Oka et al. 2014). The interaction between p53 and *MUTYH* is particularly interesting as research on MUTY has intensified in recent years because of its role in hereditary colorectal cancer (for a review, see de Oliveira et al. 2014).

More recent relationships between carcinogen exposure and p53 have also been reported. Hamann et al. showed that, after cadmium exposure, p53 exerts effects on BER by regulating OGG1 and APE1 (Hamann et al. 2012); although p53 directly regulated the expression of *APE1*, it appeared to affect OGG1 only indirectly. A function for p53 during chronic exposure to low doses of AFB_1 has also been reported (Mulder et al. 2015). Mulder et al. showed that BER activity was decreased in p53-proficient mouse livers, but was unchanged in p53-heterozygous knockouts. Notably, this effect is the opposite of what the same group reported for regulation of NER by p53 during AFB_1 exposure (above) (Mulder et al. 2014).

The above results show that p53 can certainly control various aspects of BER, but can the protein be placed directly at repair sites? Direct protein–protein and protein–DNA interactions between p53 and several BER components seem to suggest so. Wild-type p53 was shown to enhance the activity of OGG1 both in vitro and in vivo (Achanta and Huang 2004). When cells were exposed to the same levels of reactive oxygen species (ROS), wild-type p53 cells showed more rapid removal of the resulting lesions (8-oxoG) from the DNA compared with p53-defective cells. This enhancement occurred when p53 was bound to DNA in association with OGG1 and AP endonuclease, and it was hypothesized that p53 may stimulate the combined activities of OGG1 and AP endonuclease.

DNA polymerase β (DNA pol β) is the main DNA polymerase involved in short patch BER and acts downstream from the glycosylase and AP endonuclease to insert the new complementary base. Zhou et al. again showed that p53 can stimulate BER both in vitro and in vivo and that this activity correlates with the amount of p53. The novel aspect of this study was that they also showed that this effect depended on the ability of p53 to directly interact with DNA pol β because amino-terminal mutant forms of p53 that do not interact with DNA pol β fail to stimulate BER (Zhou et al. 2001) (subsequent work from the same group further corroborated a function for p53 in DNA replication [Zhou and Prives 2003]). One suggestion for the basis for this effect is that the interaction between DNA pol β and p53 might affect the stability of the polymerase because DNA pol β levels are drastically decreased in p53-deficient cells (Seo et al. 2002a). In this case, a direct interaction between p53 and a BER component could serve a critical function in ensuring proper BER function.

Functions of p53 in Mismatch Repair

Of the trifecta of classical DNA-repair pathways, p53 probably has the least known interactions with DNA MMR (Fig. 1), although that is not to say that the interactions are unimportant. The relationship between p53 and MMR seems to be centered on the MMR core component MSH2; however, it is important to note that MSH2 also functions to some extent in NER, HR, and BER, so p53-dependent effects on MSH2 may also influence other DNA-repair pathways.

Connections between p53 and MMR have been made in various systems and several examples demonstrate a role for MMR proteins in influencing p53-related processes. For example, Cranston et al. showed that p53 and MMR function synergistically in mice, as $Msh2^{-/-}$ $p53^{-/-}$ females arrested as embryos and, although the males survived, they quickly developed tumors relative to the single-mutant ani-

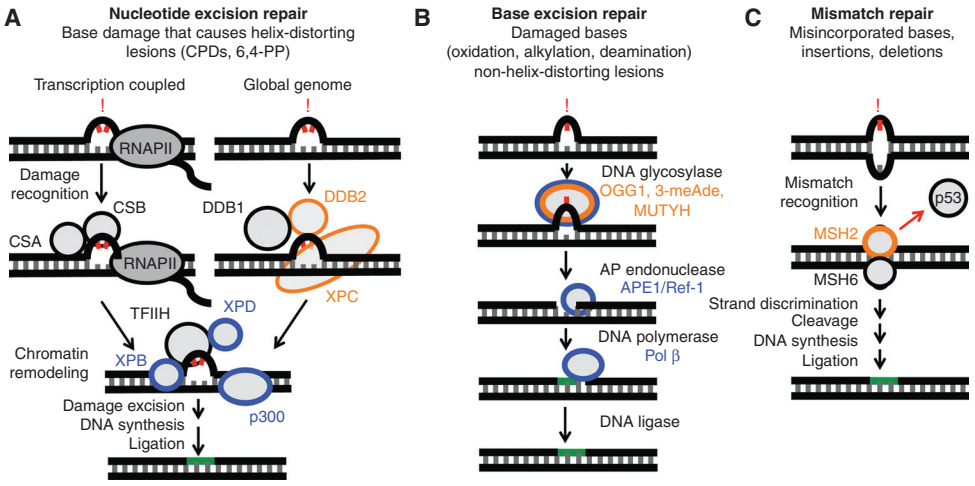

Figure 1. Examples of p53 interactions with DNA-repair pathways. (*A–C*) Simplified representations of canonical DNA-repair pathways: nucleotide excision repair (NER), base excision repair (BER), and mismatch repair (MMR). Factors with transcription-related interactions are highlighted in orange and factors with nontranscriptional interactions are highlighted in blue. Note that OGG1 has both transcription-dependent and -independent interactions with p53 during base excision repair. Red arrows indicate regulatory effects on p53.

mals (Cranston et al. 1997). Around the same time, Lin et al. showed that, in an MMR-defective colon carcinoma cell line, MMR and p53 can work together to modulate the cell's sensitivity to the genotoxic effects of cisplatin (Lin et al. 2001).

p53 has also been linked to the DNA-damage-response signaling pathway as the MMR proteins hMutL and PMS1/2 (components of the MMR pathway) are stabilized by the DNA-damage-response checkpoint protein ATM after DNA damage, leading to an increase in p53 activation (Luo et al. 2004). Interestingly, the MSH2–MSH6 complex can, at least in vitro, enhance the binding of p53 to DNA substrates with topological distortions, and this activity depends on the phosphorylation state of p53 (Subramanian and Griffith 2002, 2005). In one study, p53 and MSH2 were shown to colocalize to early recombination intermediates, suggesting that p53 is linked to recombination by a non-MMR function of MSH2 (Zink et al. 2002). More recently, p53 signaling was shown to be suppressed in MSH2-deficient cells (Pabla et al. 2011), and MUTS3 was found to be a powerful effector of p53-dependent tumorigenesis (van Oers et al. 2014).

The transcriptional function of p53 may also regulate MMR. p53, along with the transcription factor c-Jun, bind to motifs in the promoter region of *hMSH2* (Scherer et al. 1996, 2000). The biological effect of this binding is an up-regulation of *hMSH2* after UV exposure.

Finally, clinically relevant connections between MMR and p53 are abundant in the literature; however, in most cases, little mechanistic information exists to explain the observations. Nevertheless, for just a few interesting examples, see Martinez et al. (2009), Schröer et al. (2009), Haghighi et al. (2014), and Shen et al. (2014).

Functions of p53 in DNA Double-Strand Break Repair and Recombination

DNA DSBs are repaired by two pathways, depending on the stage of the cell cycle: NHEJ is active throughout the cell cycle, but especially important in G_1 (Deriano and Roth 2013) and HR, which is most active during late S and G_2 (Jasin and Rothstein 2013). Although HR-dependent repair is largely error free, NHEJ can be inaccurate, leading to genomic instability, although opinions are shifting in both of these

areas (Bétermier et al. 2014; Guirouilh-Barbat et al. 2014). p53 has diverse roles in each of these pathways and each pathway will be discussed individually here.

p53 in Nonhomologous End Joining

Under some conditions, p53 functions seem to intersect with NHEJ, although the biological outcome seems to be via effects on apoptosis. For example, DSBs are maintained in mice defective for XRCC4 and DNA ligase IV (thus lacking NHEJ), leading to p53-dependent apoptosis and embryonic lethality (Gao et al. 1998, 2000; Frank et al. 2000). In this background, loss of p53 can rescue the embryonic lethality (although probably at the expense of genome stability).

Mice deficient for p53 and NHEJ were reported to develop a number of pathologies including progenitor B-cell lymphomas (Guidos et al. 1996; Nacht et al. 1996; Vanasse et al. 1999; Frank et al. 2000; Gao et al. 2000; Difilippantonio et al. 2002; Gladdy et al. 2003). Subsequently, work from Fred Alt's group showed that the Artemis endonuclease could, in cooperation with p53, suppress the development of progenitor B-cell lymphomas (Rooney et al. 2004). Artemis also has an additional DNA-repair-independent function in p53 regulation. In U2-OS cells depleted for Artemis, p53 accumulates leading to cell-cycle arrest and apoptosis (Zhang et al. 2009); thus, at least one component of NHEJ can also exert a regulatory effect on p53.

Several other connections have been made between p53 and the repair of DSBs apparently by NHEJ, although these connections may be more circumstantial (Fig. 2). For example, two studies with I-SceI systems that facilitate an exogenous endonuclease to produce DSBs at specific sites have suggested a connection between p53 and NHEJ. Through the use of I-SceI-recognition sites, functions for p53 outside of DSB repair can be largely excluded, as DSBs can be specifically induced without the use of other genotoxic agents, which in many cases leads to

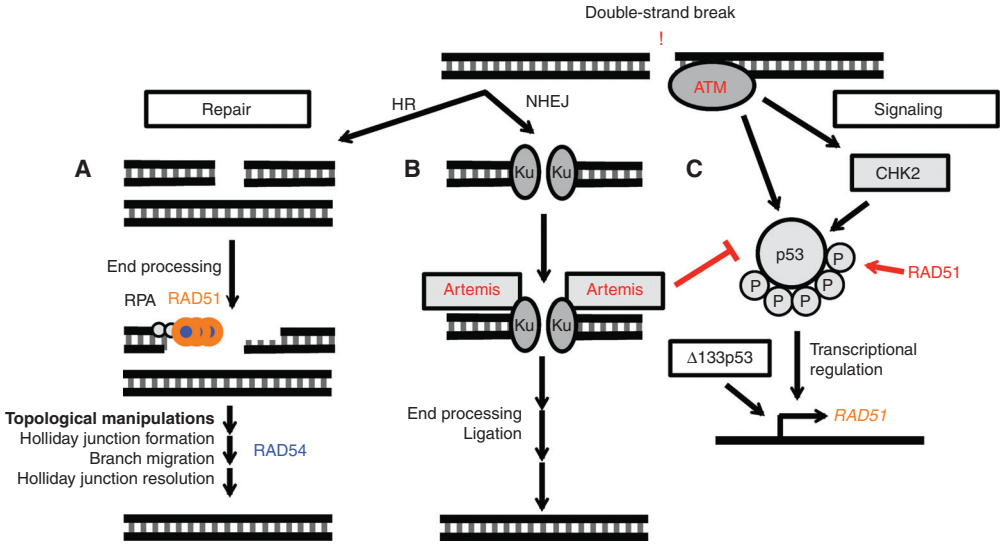

Figure 2. Examples of p53 interactions with double-strand break repair pathways (*left*) and p53 checkpoint signaling (*right*). (*A*,*B*) Simplified representations of double-strand break repair via homologous recombination (HR) and nonhomologous end joining (NHEJ). (*C*) Simplified representation of p53 signaling at double-strand breaks. Factors with transcription-related interactions are highlighted in orange and factors with nontranscriptional interactions are highlighted in blue. Note that RAD51 has both transcription-dependent and -independent interactions with p53 during base excision repair. Red labels and red arrows indicate regulatory effects on p53.

multiple types of the DNA damage. In the first study, wild-type p53 inhibited NHEJ that used microhomologies near the cut site (Akyüz et al. 2002). In the second study, pharmacological inhibition of p53 by pifithrin-α had little effect on overall end joining; however, high-fidelity end joining was diminished (Lin et al. 2003). This finding suggests another genome-protective function for p53: to minimize genome instability caused by low-fidelity NHEJ.

Of the two pathways for DSB repair, the roles of p53 in NHEJ remain the most nebulous. What is clear is that p53 has several genetic interactions with components of the NHEJ pathway that are manifested by downstream effects on cellular survival and cell-cycle control or effects on DNA repair; to date, the molecular mechanisms of these interactions remain poorly or entirely not understood.

p53 in Homologous Recombination

The roles of p53 in HR are clearer than those in NHEJ and, in fact, p53 has been shown to have both *trans*-activation-dependent and -independent functions in regulating HR, independent of its functions in cell-cycle checkpoint control (this separation of functions was first reported by Willers et al. 2000). To date, most of the roles of p53 in HR are independent of its transcriptional activity (see below); however, several studies have revealed what may turn out to be important *trans*-activation functions for p53 in the regulation of HR. After many experiments examining the role of p53 in the repair of I-SceI DSBs (including work with transactivation-defective mutants) Rieckmann et al. present data showing that p53 regulates HR-dependent repair of induced DSBs via its *trans*-activation function; however, they maintain that its role in HR repair of replication-associated DSBs is independent of its *trans*-activation function (Rieckmann et al. 2013). Further support that p53 can regulate DSB repair transcriptionally comes from several studies that showed direct interactions between p53 and the Rad51 promoter, with corresponding changes in *RAD51* expression (Hasselbach et al. 2005; Arias-Lopez et al. 2006; Fong et al. 2011). Most recently, Hine et al. confirmed that p53 can directly regulate *RAD51,* although its contribution was small compared with other transcription factors (Hine et al. 2014). Finally, the p53 isoform Δ133p53, which is expressed from an internal promoter in the p53 gene and is a regulatory target of full-length p53 itself, has also been shown to up-regulate several DNA-repair genes, including *RAD51* (Gong et al. 2015).

In addition to regulating the expression of Rad51, p53 also appears to modulate HR via direct interactions with the RAD51 and RAD54 proteins. The frequency of spontaneous and damage-induced HR between repetitive DNA sequences increases on p53 inhibition (Saintigny et al. 1999). A subsequent study several years later confirmed that this effect was because of a p53-RAD51 interaction by overexpressing a mutant RAD51 that was unable to interact with p53 (Linke et al. 2003). This overexpression resulted in a twofold to threefold increase in HR, similar to the earlier phenotype from p53 inhibition. This same study also reported an interaction between p53 and RAD54 via the carboxy-terminal domain of p53. The investigators conclude that p53 likely inhibits illegitimate recombination by inhibitory interactions with RAD51 and RAD54, suggesting yet another mechanism by which p53 could suppress genome instability. Although these studies clearly demonstrate that p53 can modulate HR, it has also been shown that interaction between RAD51 and p53 can stimulate the 5′ to 3′ exonuclease activity of p53 during the production of strand transfer intermediates; thus, RAD51 and p53 seem to affect each other bidirectionally.

Other studies have revealed functions for p53 in regulating HR that did not explicitly involve interaction with RAD51. Three studies from Lisa Wiesmüller's group are especially notable. First, they showed that even when the transactivation domain of p53 was fully inactivated, p53 could still regulate HR (interestingly, this finding is in stark contrast to the findings of Rieckmann et al. discussed above) (Boehden et al. 2003). Next, they showed that p53 can both repress and stimulate HR depending on the substrate sequence and that such effects

may be influenced by interactions with DNA topoisomerase I (Boehden et al. 2004). In a later study, they showed that p53 stimulates HR in the ribosomal gene cluster repeat, which they speculate represents a function for p53 in regulating the integrity of rDNA sequences (Boehden et al. 2005). Taken together, the investigators argue that p53 promotes genome stability through lesion-specific interactions with topoisomerase I, global repression of mutant genetic strand exchange, and by promoting directed recombination events to maintain rDNA.

p53 also seems to play a role in regulating replication-related recombination that is tightly linked to the ATM/ATR checkpoint kinases. When replication inhibitors or DNA cross-linking agents are used to induce replication stress leading to stretches of single-strand DNA, p53 suppresses HR (Romanova et al. 2004). Interestingly, this activity requires phosphorylation of p53 and a direct interaction with the ssDNA binding protein RPA. This phenotype was further clarified by Sirbu et al. when they showed that this antirecombinogenic activity requires that phosphorylation of p53 by ATR (Sirbu et al. 2011). Through this mechanism, p53 can modulate HR frequency associated with the S phase replication stress checkpoint, without altering the cell's capacity to use HR for repair of exogenously generated DSBs. A later study showed that the checkpoint components ATM and ATR, along with the DNA-dependent protein kinase (DNA-PK), can modulate the physical interaction between p53 and RPA (Serrano et al. 2013). On DNA damage, p53 is phosphorylated by DNA-PK and RPA is phosphorylated by both ATM at ATR at two sites. Only together can these phosphorylation events disrupt the p53-RPA interaction, liberating both proteins to carry out their DNA-damage-associated functions. This pathway also reveals some cross talk between p53's regulation of HR and NHEJ as DNA-PK is also a central component of the NHEJ pathway.

p53 and RecQ Helicases

The human genome encodes five DNA helicases belonging to the RecQ helicase family (Croteau et al. 2014). Each of the helicases has important responsibilities in the maintenance of genome stability via specific recombination functions. Deficiencies in three of these helicases cause well-characterized, although still not entirely understood, heritable human diseases: BLM (Bloom syndrome), WRN (Werner syndrome), and RECQL4 (Rothmund–Thompson syndrome, and others) (Monnat 2010). The diseases cause various phenotypes, especially developmental and immunological defects, genome instability, and increased cancer risk. WRN additionally results in premature aging. Interestingly, interactions between these three disease-related helicases and p53 have been identified.

BLM causes a predisposition to many different types of cancers, including epithelial tumors (breast, colon, lung), blood cell cancers (leukemias, lymphomas), connective tissue tumors (sarcomas), and several embryonic tumors. The relationship between BLM and p53 was established when it was shown that changes in p53 levels after DNA damage differed depending on *BLM* status (Collister et al. 1998). Garkavtsev et al. later showed that p53 and BLM directly interact and that they act together to control transcription and cellular growth (Garkavtsev et al. 2001). BLM has also been shown to influence HR at stalled replication forks by controlling the localization of p53 (Sengupta et al. 2003) and to regulate Holliday junction processing (Yang et al. 2002). In addition, BLM can affect apoptosis (Wang et al. 2001) and interacts with p53 in many clinically important contexts (for example, see Wirtenberger et al. 2006; Babbe et al. 2009; Kaneko et al. 2011).

As noted above, an important clinical feature of WRN is premature aging and, like BLM, WRN deficiency also results in an elevated, although more limited, cancer risk. Several studies have identified important connections between WRN activity and p53. Like BLM, WRN is also involved in p53-dependent apoptosis (Spillare et al. 1999). WRN and p53 interact physically and this interaction may affect the *trans*-activation functions of p53 (Blander et al. 1999). WRN-deficient cells do not induce the *p53* gene after DNA damage and this func-

tion may at least partially explain the cancer predisposition in WRN patients (Blander et al. 2000). Some insight into the molecular underpinning of the premature aging in WRN patients came from a mouse model that showed that *WRN* deficiency can accelerate aging in the absence of p53 (Lombard et al. 2000). Later work showed that p53 can also modulate the biochemical activities of WRN (Brosh et al. 2001) and that it can regulate WRN's Holliday junction–processing activity (Yang et al. 2002).

Interactions between RECQL4 and p53 have been more recently reported. RECQL4 has a DNA-damage-independent function related to p53, as it is important for the trafficking of p53 to mitochondria in normal, unstressed human cells (De et al. 2012). Interestingly, p53 itself represses the expression of *RECQL4* (Sengupta et al. 2005), perhaps serving to control its own cellular localization, although this interpretation is only speculative. The relationship between p53 and RECQL4 is an active area of research and new results are likely to emerge (for some recent findings, see Gupta et al. 2014 and Lu et al. 2015).

CONCLUDING REMARKS

With the central importance of p53 in controlling genome instability–driven cancer development, it might not be surprising that p53 controls DNA-damage checkpoints and impacts the activity of various DNA-repair systems. Given the many ways through which p53 guards the genome, it remains a major challenge to mechanistically dissect the distinct functions of p53. Studies in various model systems will continuously enrich the understanding of the physiological roles of p53 also in the context of genome evolution. Cytological and biochemical studies in the context of living organisms are likely to reveal yet additional functions of p53 in responding to genome instability.

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p53 in the DNA-Damage-Repair Process

Ashley B. Williams and Björn Schumacher

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