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Cell Cycle News & Views

The p53-HAT connection: PCAF rules?

Comment on: Love IM, et al. Cell Cycle 2012; 11:2458–66;
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Multiple structural, biochemical and in vivo studies have solidified the role that histone acetyltransferases (HATs) play in regulation of the p53 pathway.[1] HATs are known to modulate p53 functions in many ways, from regulating its stability to promoting acetylation-dependent interactions with DNA as well as various co-factors and chromatin modifiers.[1] Of these, p300, CBP, PCAF and Tip60 are the most well-studied p53 co-factors that can regulate (sometimes selectively) a number of bona fide p53 targets involved in cell cycle, apoptosis, DNA repair, metabolism and other processes.[2] Of particular current interest is the time- and stress-dependent interplay between different acetyltransferases. Yet, though we now know the players, we still have only limited knowledge of their performances.

A paper by Love et al. in a previous issue of *Cell Cycle*, has contributed new insight into the function of one such HAT.[3] Using multiple p53-activating stress conditions in combination with siRNA-mediated knockdown of specific HATs in several cancer cell lines, the authors have demonstrated the dependence of p21-driven cell cycle arrest on the histone acetyl transferase activity of PCAF. They found that PCAF, but not p300 or CBP (two closely related and well characterized transcriptional co-activators) is absolutely required for maximal p21 expression in several settings. Intriguingly, their work indicates that PCAF is exclusively important for the activation of cell cycle arrest through p53- but not Rb-dependent pathways. A pictorial description of possible events leading to p21 activation is shown in **Figure 1**.

Key features of the paper by Love et al. are summarized as follows. First, PCAF-dependent effects on p21 transcription are apparently unrelated to its reported MDM2-directed E3 ligase activity, which otherwise would result in subsequent elevation of p53 levels.[4] Second, acetylation of a previously identified PCAF site within p53, Lys320,[5] is not necessary for p21 transactivation, although the acetyl-transferase activity of PCAF, per se, is indispensable. Accordingly, previously identified PCAF sites within the H3 core histone, Lys9 and Lys14, are markedly acetylated at the strong p53 "distal" binding site within the p21 promoter following stress-induced p53 activation. Since the same lysine residues are known to be acetylated by other HATs (e.g., GCN5, SRC-1 and GCN5, p300, Tip60 and SRC-1), stress- or co-factor-dependent specificity of those modifications would need to be investigated in future studies. Finally, somewhat unexpectedly, the authors did not observe any significant changes in the levels of PCAF at the distal p53 binding site within the p21 promoter before and after p53 activation. So, it is unclear what brings PCAF to the promoter of p21 gene. Detailed analysis of the nucleosome content at that region, as reported

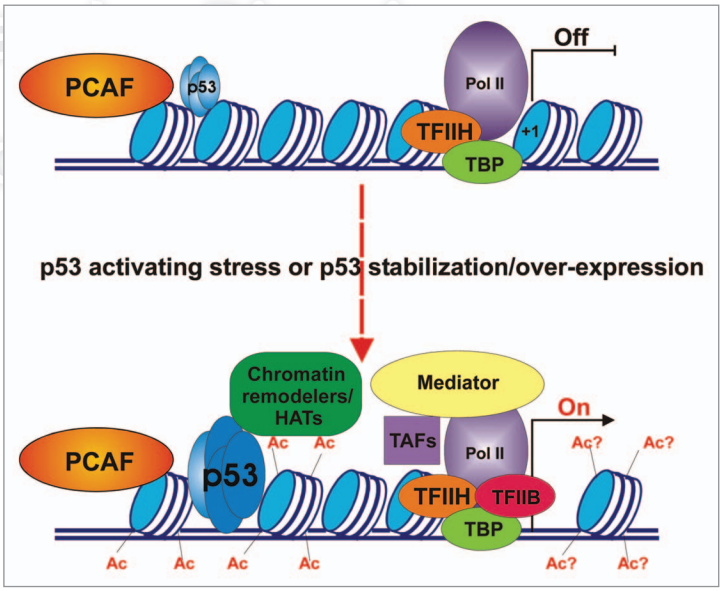

Figure 1. Involvement of PCAF in p53-dependent activation of the p21 promoter. Upper panel: chromatin landscape and factors present at the p21 promoter in the absence of p53-activating stress signals. The p21 promoter is enriched with nucleosomes (blue and white cylinders) that dwell in positions proximal to the transcription start site (+1 position) and at p53 binding sites. Only low levels of p53 (represented by four miniature light blue ovals) occupy its distal and proximal binding sites. PCAF (orange oval) is present but inactive at the distal region of the p21 promoter. Some components of the transcription pre-initiation complex (PIC), including DNA-dependent Polymerase II (Pol II), TATA-binding protein (TBP) and transcription factor IIH (TFIIH) are present at the proximal promoter region near the transcription start site (TSS, shown by black vertical line) of the p21 gene. Transcription of the p21 gene is either off or very low (OFF). Lower panel: chromatin landscape and factors present at p21 promoter following p53 activating stress signals. Once levels of p53 have increased and the protein is bound to its site(s) within the p21 promoter, the PCAF complex is activated and acetylates its targets within core histones both within the distal portion of the promoter (Ac) and possibly within the TSS (Ac?). This results in the recruitment of other chromatin remodelers/modifiers, stress-specific transcription association factors (TAFs) and Mediator complex, eventually leading to active PIC formation and promoter opening (On).

by Laptenko et al.,[6] could be informative in that regard.

Unlike the p300 and CBP HATs, human PCAF functions within a complex of more than 20 proteins.[7] The acetyltransferase activity of the PCAF complex toward nucleosomal substrates is known to be markedly higher than of the PCAF enzyme itself. Several subunits within the complex show 100% identity to TAFs (TATA-binding protein-associated factors), while others are highly homologous but not identical to them. One such subunit, TAFII31 (a part of the TFIID complex), has already been shown to stabilize and activate p53,[8] so this may provide some connection between p53-PCAF-dependent events at the distal p53 sites and the region of the promoter that is close to the start site. Finally, the largest subunit of the PCAF complex, TRAPP/PAF400, a member of the ATM super family and a component of the Tip60 HAT complex, may facilitate multiprotein assemblies (e.g., chromatin remodeling complexes) on targeted promoters .

A good scientific study, in its attempt to answer a few specific questions, inevitably generates more questions. Among those prompted by the report by Love et al. are: what brings PCAF to the p21 promoter in the absence of high levels of p53? What other subunits/activities, if any, of the PCAF complex are vital for p21 promoter activation? What PCAF-dependent changes in chromatin occur within the p21 transcription start site following stress? Future experiments will hopefully be able to address these and other questions.

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A critical role for TORC1 in cellular senescence

Comment on: Kolesnichenko M, et al. Cell Cycle 2012; 11:2391–401; PMID:22627671; and Pospelova TV, et al. Cell Cycle 2012; 11:2402–7; PMID:22672902

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Cellular senescence is a process initiated either when cells proliferate past their potential (replicative senescence) or by activation of an oncogenic stress (oncogene-induced senescence). Both of these events are characterized by the activation of a DNA damage response, which is initiated by eroded telomeres in the case of replicative senescence, and aberrant products of DNA replication in the case of oncogene-induced senescence.[1] Senescence plays a critical tumor-suppression role in vivo, and alterations in the senescence program are a hallmark of cancer cells. Bypass of senescence is critical for tumor progression and involves the p53 and pRB tumor-suppressor pathways.[2] Indeed, expression of DNA tumor virus oncoproteins that target p53 and pRB can bypass senescence in cultured cells,[3] and concomitant loss of pRB and p53 bypasses senescence in human diploid fibroblasts.[4] In addition to being an obligatory step for tumor progression, bypass of senescence creates a favorable environment in which additional tumor-promoting mutations can be acquired. For example, inactivation of p53 in the context of telomere erosion promotes rampant genomic instability mediated by cycles of aberrant DNA damage/DNA repair events.[5]

In a new study, Kolesnichenko et al. describe a critical role for the mTOR pathway in senescence induction.[6] This work demonstrates that inhibition of mTOR is sufficient to delay RAS-induced senescence as well as replicative senescence. Using a combination of inhibitory molecules, shRNA-mediated knockdown and expression of inhibitory proteins, the authors demonstrate that inhibition of the TORC1 complex is sufficient to delay senescence induction. These findings are further corroborated by the independent work of Pospelova and colleagues showing that rapamycin treatment delays senescence induction in murine fibroblasts.[7] These intriguing findings raise the question of why mTOR inhibition inhibits senescence induction. The work of Kolesnchenko and colleagues provides two clues to explain this phenotype. First, mTOR inhibition results in the activation of the pro-survival factor AKT, a factor that could explain how cells can proliferate in the face of an ongoing senescence-inducing signal. In addition, the authors find reduced levels of p53 and its target gene p21 upon mTOR inhibition. These findings are particularly significant considering the critical role for both p53 activation and p21 induction in senescence induction.

In conclusion, the finding that inhibition of the TORC1 complex has a profound effect on the onset of senescence might explain why rapamycin treatment had limited success in the treatment of cancer.[8] On the other hand, rapamycin slows aging and thus delays cancer in mice.[9]

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Timeless tunes: Replicating happy endings

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DNA replication is at the heart of the inheritance of genetic material. A single replication fork can progress through hundreds of kilobases of DNA, melting parental double-stranded DNA and leaving newly synthesized strands in its wake. A beautiful illustration showing how the replication machinery accomplishes this complex task is one of the triumphs of molecular biology. However, it is known that DNA replication is not always as processive as the textbooks suggest. Specifically, the rate of fork progression varies depending on the regions being replicated, and the replication fork even stalls in some circumstances, during replication of heterochromatin or damaged DNA, for example. A stalled replication fork has two fates. It may restart DNA replication, or it may collapse after prolonged stalling. A collapsed replication fork is particularly dangerous for the genome, because the DNA intermediate left by the collapsed fork may form a double-stranded break, a highly mutagenic lesion that can undergo illegitimate recombination. To circumvent replication fork collapse, cells are equipped with specialized proteins that stabilize the stalled replication fork. Timeless and Tipin are highly conserved in eukaryotes, from yeast to humans, and form a complex to protect stalled replication forks.

In a paper published in *Cell Cycle*, Noguchi and his group investigated how Timeless plays a role in telomere replication in human cells.[1] Telomeres consist of tandem arrays of short repetitive DNA (TTAGGG/CCCTAA in mammals) at the ends of chromosomes and numerous associated proteins. Telomeres are essential for the stable maintenance of genomic DNA, because they protect the DNA termini from undergoing accidental recombination and exonuclease attack. Dysfunctional telomeres lead to genetic instability that eventually results in senescence and cancer development. Because of the heterochromatic nature of telomeres, it has been recognized that telomere DNA is one of the genomic regions that impede replication fork progression. Indeed, in vitro DNA replication experiments using SV40 DNA, and cell extracts demonstrated that telomere DNA is replicated less efficiently and incurs more fork stalling than non-telomeric DNA.[2] Moreover, overexpression of telomere-DNA binding protein TRF1 in HeLa cells led to an accumulation of replicating telomeres, consistent with a slower replication rate of telomeres under those circumstance. Furthermore, experiments using TRF1-deleted murine cells showed that TRF1 is essential for efficient telomere DNA replication.[3] Collectively, these results confirm that the telomere is a difficult-to-replicate region.

There is an apparent contradiction between two earlier studies, however, with TRF1 described as an anti-replication protein in one report[2] and a pro-replication protein in the other.[3] One potential explanation for the inconsistency might be that TRF1 requires other protein(s) to perform its pro-replication function, and the second factor was missing in the TRF1-overexpression experiments. Noguchi and his colleagues investigated this possibility by testing whether Timeless is required for proficient telomere DNA replication.[1] They found that Timeless-knockdown cells displayed telomere length shortening and an increased frequency of dysfunctional telomeres. In vitro replication assays of SV40 DNA revealed that Timeless-depleted extracts supported non-telomere replication proficiently, while telomere replication was inefficient. They then demonstrated that addition of recombinant TRF1 to the replication system slowed telomere replication. Importantly, Timeless depletion and TRF1 addition did not produce additive effects on telomere replication, suggesting that Timeless and TRF1 function in the same pathway. These results suggest a model as described in **Figure 1**. A replication fork frequently stalls at telomeres because of the molecularly crowded nature of telomeric chromatin. Timeless presumably encounters TRF1 at telomeres and protects the stalled fork from undergoing collapse. In the absence of Timeless, the stalled forks easily collapse, leading to an abrupt shortening of telomeres. Several questions remain to be

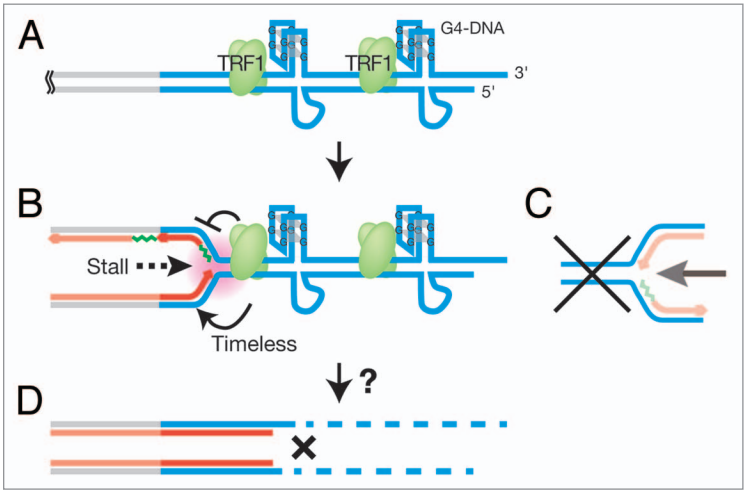

Figure 1. Hard life at telomeres. (A) Mammalian telomeres consist of repetitive DNA that potentially forms higher-ordered structures [G-quartet(G4)-DNA] and numerous proteins, including telomere DNA-binding protein TRF1. (B) Replication fork is frequently stalled at telomeres. Overexpressed TRF1 slows down fork progression at the telomere, while endogenous TRF1 together with Timeless protein facilitates it. Timeless protects the stalled replication fork from collapse. (C) Telomeres are unique in that the most distal replication fork is not coupled with another fork progressing inversely. (D) Prolonged fork stalling may lead to the formation of a DNA double-strand break. Because of the lack of another fork compensating the telomere replication (C), the break immediately results in the abrupt single-step shortening of telomere DNAs.

answered. Given that Timeless moves along the genomic DNA as a component of the replication machinery,[4] it will be particularly interesting to see how Timeless (or the replication machinery) interacts with telomeric chromatin. In such studies, a dynamic transaction between the regional chromatin at telomeres and the replication machinery may be revealed.

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A new mode of cell cycle stimulation: Cyclin E and CDK2-mediated cytoplasmic retention of repressive E2F complexes

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FGF, fibroblast growth factor, normally exerts a positive effect on the proliferation of most cell types. Chondrocytes, the cellular constituent of cartilage, can be quite different: in certain settings, they exit the cell cycle and initiate differentiation when exposed to FGF.[1] The molecular mechanisms behind this uncommon response to growth factor stimulation are still incompletely understood. The FGF-stimulated chondrocyte cell cycle arrest is highly dependent on the function of two key cell cycle regulators, the retinoblastoma protein sister proteins p107 and p130.[2] These two pocket proteins function by binding to "repressor E2F" transcription factors, bringing them to the cell nucleus, and assisting in the downregulation of the expression of genes important for cell cycle progression.[3] The crucial role played by p107 and p130 in this setting is manifested in mice knockout for both genes: they have higher proliferation of chondrocytes and display cartilage and skeletal developmental defects.[4]

Mitogenic stimulation of cells triggers the accumulation of cyclin D, which associates with CDK4 or CDK6 and initiates the phosphorylation, and inactivation, of pocket proteins. Subsequently, the cyclin E gene is de-repressed, and cyclin E can associate to CDK2 to continue the wave of regulatory phosphorylation events. In contrast, the cell cycle arrest of chondrocytes and most other cell types is accompanied by the dephosphorylation of pocket proteins, as it is in this hypophosphorylated state that they can bind to E2F proteins and repress their activity. Kolupaeva et al. have previously shown that following FGF exposure of chondrocytes, dephosphorylation of p107 by the phosphatase PP2A accompanies cell cycle exit.[5] They now report on the effect of cyclin E and CDK2 overexpression on p107/p130 phosphorylation and on proliferation.[6] The idea was that if the role of cyclin E and CDK2 is to participate in the phosphorylation of p107/p130, the overexpression of cyclin E-CDK2 should counteract the effects of FGF treatment and lead to two consequences: maintaining p107/p130 in a phosphorylated state and preventing cell cycle exit. The result was surprising, however: the cyclin E-CDK2-expressing cells did continue to cycle, but they did so while p107/p130 were in a hypophosphorylated state.

Repressive E2Fs (E2F4 and E2F5) lack a nuclear localization signal, and instead rely on association to hypophosphorylated pocket proteins to reach the nucleus and repress their target genes.[7] Kolupaeva et al. reveal that when cyclin E-CDK2 are ectopically expressed, the repressive E2F4/p130 complex fails to make its way to the nucleus; this prevents the repression of E2F target genes and impairs FGF-stimulated cell cycle exit. Interestingly, Kolupaeva et al. also show that while in the cytoplasm, the E2F-pocket protein complexes are associated to cyclin E-CDK2. These results highlight a novel means to promote proliferation for cyclin E-CDK2 that might be independent of pocket protein phosphorylation. This is interesting in the context of previous findings reported by the authors that cyclin D-CDK4 ectopic expression not only prevents FGF-mediated cell cycle exit, but also leads to p107 phosphorylation. Because, cyclin E expression and associated CDK2 activity are higher following cyclin D-CDK4 overexpression, it will be interesting to determine whether the cytoplasmic association between cyclin E-CDK2 and pocket proteins also occurs in the cyclin D-CDK4-overexpression setting, and if a catalytically inactive CDK2 would still lead to higher proliferation when cyclin E is overexpressed.

As the authors pointed out, cyclin E overexpression is detected in several types of cancers, and it would be interesting to determine the contribution of the cytoplasmic retention of E2F-p107/p130 by cyclin E-CDK2 to tumorigenesis. Moreover, if this function of cyclin E-CDK2 is independent of its kinase activity, this would represent a novel therapeutic target to block, in conjunction with conventional CDK inhibitors. This will await more detailed studies on the precise mode of interaction of cyclin E-CDK2 with the E2F-pocket protein complexes and on the exact composition of cytoplasmic pocket protein complexes.

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"Double hit" makes the difference

Comment on: Menendez JA, et al. Cell Cycle 2012; 11:2782–92; PMID:22809961; http://dx.doi.org/10.4161/cc.20948

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Metformin (N', N'-dimethylbiguanide) is an anti-diabetic drug prescribed to more than 100 million patients in the world. In addition to its efficacy for the treatment of diabetes, several recent studies have shown that it has anti-tumoral properties.[1] We and others have shown that metformin targets cancer cell metabolism by inhibiting mitochondrial complex 1 activity.[2,3] This energetic stress leads to a decrease of intracellular ATP concentration, and cancer cells will increase their rate of glycolysis.[2] This compensatory response is not sufficient to restore ATP levels, but is adequate to maintain viable cells in most of the cancer cells. Indeed, metformin blocks cell growth but can also induce apoptosis in some cancer cell models.[4] The increase of glycolysis induced by metformin is somehow inconsistent with the observed inhibition of proliferation, since cancer cells use preferentially glycolysis to grow faster. This switch to glycolysis, also known as the "Warburg effect," is linked to oncogenic transformation[5] and is accompanied by the hyperactivation of the mTOR pathway. In cancer cells, the increase of glycolysis induced by metformin is associated with a strong inhibition of the mTOR pathway via the AMPK. This new metabolic order established by metformin may explain the paradoxical effect of metformin. In view of the above scenario, Menendez et al. decided to test the synthetic lethality of metformin and combined metformin treatment with glucose starvation. They showed that the treatment of breast cancer cells with metformin alone does not induce apoptosis but arrests cells in G_0/G_1. Glucose starvation by itself induces few apoptosis, but the combination of metformin with the absence of glucose induces massive apoptosis. This is not altogether surprising, since the dual action of metformin and glucose starvation block the two main ways of production of ATP (i.e., mitochondrial respiration and glycolysis) (**Fig. 1**). This is an interesting observation, which could be valuable for future anticancer therapy; however, glucose starvation is not therapeutically feasible. Thus, the use 2-deoxyglucose (2-DG), an inhibitor of glycolysis, could be useful. We and others found that the combination of 2-DG and metformin inhibits prostate cancer cell proliferation and breast tumor growth in xenograft models.[2,6] Although it induces a slight apoptotic response in vitro, 2-DG alone is not efficient in vivo to alter tumor growth[6] but improves the curative action of radiotherapy;[7] similarly, it reinforces metformin action. Another interesting issue raised by Menendez et al. is the use of such dual therapy to target cancer stem cells. Metformin has been shown to selectively kill cancer stem cells and the chemotherapy-resistant subpopulation of cancer stem cells.[8,9] Cancer stem cells greatly depend on aerobic glycolysis to sustain their stemness and immortality. The synthetic lethality induced by metformin and glucose starvation may help to improve chemotherapy action and avoid cancer relapse. In conclusion, targeting cancer cell metabolism with a "dual hit therapy" opens new avenues for the future treatment of cancer.

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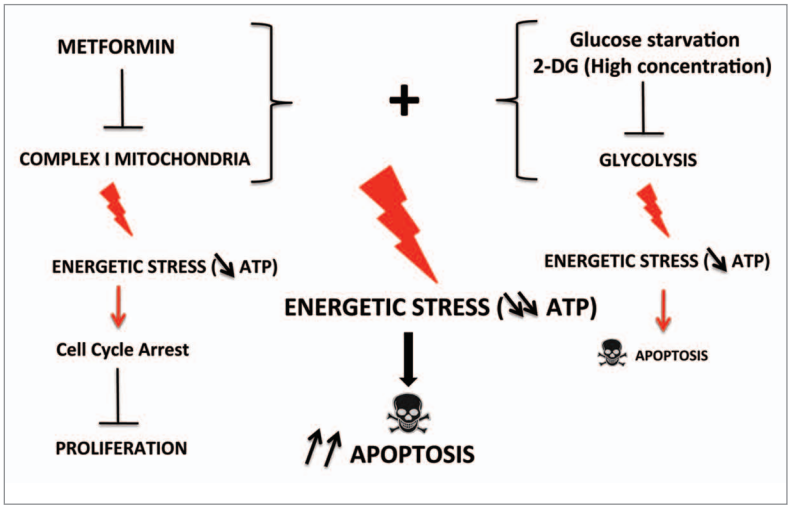

Figure 1. The combination of metformin and glucose starvation induces a strong energetic stress. Metformin inhibits the mitochondrial complex 1 and glucose starvation, or 2-DG inhibits ATP production from glycolysis. The combination of the two energetic stresses induces a massive energetic stress and leads to a strong apoptotic response.