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Histone acetylation gets complicated

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Cell Cycle News & Views

A new role for cytoplasmic p53: Binding and destroying double-stranded RNA

Comment on: Grinberg S, et al. Cell Cycle 2010; 9:2442–55.

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The tumor suppressor p53 is mutated or inactivated by epigenetic mechanisms in more than 50% of all human cancers. In resting conditions, baseline levels of p53 reportedly contribute to the maintenance of homeostasis by favoring anti-oxidant mechanisms and by regulating energy metabolism.[1] In response to various types of stress (e.g., DNA damage, oncogene deregulation), p53 is subjected to multiple post-translational modifications that inhibit its degradation by the proteasome system, resulting in p53 accumulation at both nuclear and extranuclear sites.[2] In the nucleus, p53 controls a large number of transcriptional programs, which can lead to cell cycle blockage, activation of autophagy or induction of apoptotic cell death.[2] Cytoplasmic p53 has been shown to exert lethal effects by interacting with members of the BCL-2 protein family at mitochondria,[3] and inhibits autophagy.[4] The subcellular shuttling of p53 (from the nucleus to the cytoplasm and from the cytoplasm to mitochondria, and vice versa) is regulated by additional post-translational modifications including MDM2-mediated (poly)monoubiquitination and HAUSP-mediated deubiquitination.[2] Thus, p53 is at the hub of a complex and highly dynamic system that regulates several aspects of the cell metabolism.

In *Cell Cycle*, Grinberg et al. show that recombinant wild type (WT) p53 as well as cytoplasmic extracts from two distinct p53-proficient human cancer cell lines (i.e., HCT 116 colon carcinoma and LCC2 human breast cancer cells) can catalyze the removal of nucleotides from the 3′ end of both double-stranded and single-stranded RNA (dsRNA and ssRNA, respectively).[5] This enzymatic activity could not be detected in lysates from *p53*$^{-/-}$ HCT 116 cells or from p53-null H1299 non-small cell lung cancer (NSCLC) cells, and could be prevented by pre-incubation of cytoplasmic fractions with a p53-neutralizing antibody.[5] Moreover, in extracts from *p53*$^{+/+}$ (but not in *p53*$^{-/-}$) HCT 116 cells, the degradation of dsRNA increased when cells were subjected to p53-activating stimuli (e.g., γ irradiation) before lysis. Finally, the reintroduction of WT p53 (but not of the catalytic dead p53^{R175H} mutant) in p53-deficient HCT 116 cells resulted in the restoration of a cytoplasmic dsRNAse activity.[5] Altogether, the results provided by Grinberg et al. demonstrate that p53 possesses intrinsic 3′→5′ exonuclease activity that can actively degrade dsRNA substrates in cellula.

dsRNA is a molecular species with multiple biological functions. Baseline levels of endogenous dsRNA participate in physiological processes including gene expression, heterochromatin remodeling, RNA editing, and translation control by natural antisense RNA and microRNA.[6] Moreover, high levels of dsRNA may be generated from perturbations of the RNA metabolism by genotoxic agents, upon aberrant transcription from damaged DNA or as a result of viral infection, and have been suggested to operate as an intracellular danger signal.[7] One of the best characterized responses to dsRNA accumulation is coordinated by the interferon-induced protein kinase RNA-activated (PKR), which rapidly shuts off protein translation by phosphorylating the α subunit of the eukaryotic translation initiation factor 2 (eIF2α). PKR-mediated eIF2α phosphorylation limits viral spreading by blocking translation and participates (at least in some instances) in the induction of autophagy, which also operates as an antiviral mechanism.[7] As cytoplasmic p53 inhibits autophagy,[4,8] the results presented in *Cell Cycle* by Grinberg and co-workers[5] open the fascinating possibility that at least part of the autophagy-inhibitory potential of p53 might derive from an indirect effect on dsRNA-mediated activation of PKR (**Fig. 1**). In this context, however, it should be noted that while both WT and mutant (p53^{R175H}) p53 suppress autophagy when they are localized in the cytoplasm,[9] only the former exerts bona fide dsRNAse activity.[5] Still, as both WT p53 and p53^{R175H} bind dsRNA with the same efficiency,[5] it cannot be excluded that p53 (at least in part) inhibits autophagy by limiting the cytoplasmic availability of dsRNA, thereby hindering the dsRNA-mediated activation of PKR. Further experiments are urgently awaited to elucidate these incognita and to evaluate p53's main function, the maintenance of cellular homeostasis, in view of its newly discovered ability to bind and degrade dsRNA.

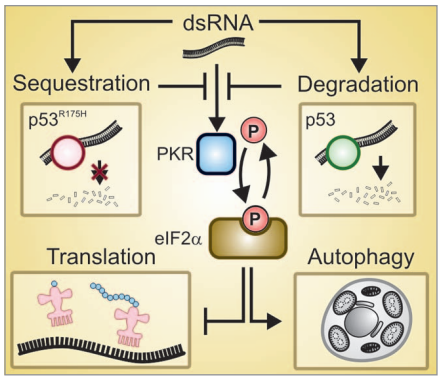

Figure 1. Cytoplasmic functions of p53 in relationship to its capacity to bind and degrade dsRNA. At least in some instances, phosphorylation of the α subunit of the eukaryotic translation initiation factor 2 (eIF2α), which can be catalyzed by protein kinase RNA-activated (PKR), is required for full-blown autophagy. Wild-type p53 efficiently binds to and degrades dsRNA (an activator of PKR), which may explain (at least part of) the autophagy-inhibitory potential of cytoplasmic p53. dsRNAse-deficient p53 mutants (e.g., p53R175H) maintain their dsRNA-binding as well as their autophagy-inhibitory activity, implying that p53-mediated dsRNA sequestration might suffice to limit PKR activation and hence to inhibit autophagy.

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References

1. Vousden KH, et al. Nat Rev Cancer 2009; 9:691-700.
2. Galluzzi L, et al. Antioxid Redox Signal 2010.
3. Morselli E, et al. Cell Res 2008; 18:708-10.
4. Maiuri MC, et al. Curr Opin Cell Biol 2010; 22:181-5.
5. Grinberg S, et al. Cell Cycle 2010; 2442-55.
6. Wang Q, et al. Microbiol Mol Biol Rev 2004; 68:432-52.
7. Garcia MA, et al. Biochimie 2007; 89:799-811.
8. Galluzzi L, et al. Cell Cycle 2010; 9:250-5.
9. Morselli E, et al. Cell Cycle 2008; 7:3056-61.

Schizosaccharomyces pombe gene deletion library nearing completion: New perspectives for cell cycle research

Comment on: Spirek M, et al. Cell Cycle 2010; 9:2399–402.

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Cell cycle studies in the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* have contributed enormously to our understanding of the eukaryotic cell cycle. The cell cycles of these organisms are currently the best understood of all eukaryotes. The genomic-postgenomic era has brought with it a basic change in experimentation, enabling researchers to look more comprehensively at the regulation and molecular mechanisms operating in the eukaryotic cell cycle. One of the "early" breakthrough achievements in the *Schizosaccharomyces pombe* post-genomic cell cycle research was obtained by genome-wide transcription profiling of synchronised cultures, which revealed that over 400 genes are periodically transcribed.[1] These results gave a new impetus to *Schizosaccharomyces pombe* cell cycle research by highlighting the importance of certain genes and key regulators, which were then subjected to detailed molecular analysis. Further progress of similar significance can be expected from screening of gene deletion libraries ("deletomes") consisting of deletion mutants each of which possessing a different ORF/gene deletion.

In the budding yeast *Saccharomyces cerevisiae* such collections of mutants have already been successfully used in the investigation of the cell. In a recent study, a quantitative flow cytometry assay was used to screen a large set of mutants with single deletions in genes coding for known or putative transcription factors.[2] Twenty percent of the nonessential transcription factors turned out to play some role in cell cycle. However, mutants often have complex phenotypes because many genes are indirectly involved in multiple processes. One approach for discovering indirect functions and interactions with other genes (or proteins) is to combine the mutant allele of interest with arrayed gene deletion libraries (Synthetic Genetic Array, SGA),[3] and ask which combination of alleles leads to lethality or a new, synthetic phenotype. The SGA approach can be refined by combination with specific screening methods. A very recent report on its combination with a high-content screening (HCS) system identified 182 mutations that influenced spindle dynamics, 90 of which had defects apparent only in the double mutant backgrounds.[4] Interestingly, the screen identified numerous genes whose links to spindle regulation is difficult to understand mechanistically from their annotated functions (e.g., metabolism and ribosome biogenesis). These findings confirmed the complexity of the regulation of spindle formation and demonstrated the power of the deletome-based approach in cell cycle research.

The *Schizosaccharomyces pombe* cell cycle research has profited less from gene deletion libraries because the systematic deletion of all ORFs of the genome lagged behind the *Saccharomyces cerevisiae* deletion project. A genome-wide haploid and diploid deletion library was only constructed and made available recently by the Bioneer corporation and the Korea Research Institute of Biotechnology and Bioscience (http://pombe.bioneer.co.kr/). This library contains 4,836 diploid heterozygous deletion strains covering 98.4% of the organism's open reading frames.[5] However, the PCR-based technology[6] applied failed to delete certain ORFs. In *Cell Cycle*, Spirek et al.[5] report on the application of a different strategy which is suitable for deletion of genes which require large regions of homology. This strategy enabled them to create 29 novel deletion strains which were missing in the Korean library. This extension of the collection increased the coverage of the genome to 99%. The nearly complete library will surely be a valuable resource for functional genomics and cell cycle research in *Schizosaccharomyces pombe*. The application of the incomplete library has already led to the identification of novel genes involved in the DNA damage response, among them 23 ORFs which had previously been classified as "sequence orphans" or as "conserved hypothetical" genes.[7] When considering the significance of a *Schizosaccharomyces pombe* deletion library, one has to bear in mind that *Schizosaccharomyces pombe* is not just another yeast. Any experiment to be performed with this library will be much more important than a simple repetition of an experiment done with *S. cerevisiae* because these two species are almost as distantly related to each other as to animals[8] and their cell cycles are very different.[9]

References

1. Rustici G, et al. Nat Genet 2004; 36:809-17.
2. White MA, et al. Genetics 2009; 181:435-46.
3. Tong AHY, et al. Science 2001; 294:2364-8.
4. Vizeacoumar FJ, et al. J Cell Biol 2010; 188:69-81.
5. Spirek M, et al. Cell Cycle 2010; 9:2399-402.
6. Bahler J, et al. Yeast 1998; 14:943-51.
7. Deshpande GP, et al. DNA Repair 2009; 8:672-9.
8. Sipiczki M. Genome Biol 2000; 1:1011.
9. Bahler J. Ann Rev Genet 2005; 39:69-94.

A role for Cdk4 in angiogenesis

Comment on: Abedin ZR, et al. Cell Cycle 2010; 9:2456–63.

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The mammalian cell cycle is directed in part by cyclins and their associated serine-threonine kinases, termed cyclin-dependent kinases, or Cdks. Cdk4, in conjunction with the D-type cyclins (cyclin D1, D2 and D3), mediates progression through the G_1 phase when the cell prepares to initiate DNA synthesis. Activation of Cdk4/cyclin D complexes contributes to hyperphosphorylation of the retinoblastoma (Rb) and subsequent release of the E2F transcription factor. Once the cell has made the G_1/S transition, Cdk4 activity is negatively regulated by INK4 inhibitors that act by binding directly to Cdk4 and preventing D-type cyclin activity.[1]

Alterations in cell cycle progression can have far-reaching and sometimes-detrimental effects, including deregulated proliferation of tumor cells. Amplification and/or overexpression of the Cdk4 locus has been detected in various tumor types, including sarcomas, gliomas, breast tumors, lymphomas and melanomas and this overexpression correlates with increased Cdk4 activity.[1] A missense mutation in the Cdk4 gene (R24C) that confers resistance to INK4 inhibitors has also been observed in a small percentage of familial melanomas.[2,3] Mice that are homozygous for this mutation develop malignancies of varying types such as insulinomas, tumors of the breast, liver, pituitary, Leydig cells, skin (carcinogen-induced) and gut and therefore serve as an excellent model to study the role that Cdk4 plays in tumorigenesis.[4,5]

Tumor progression in colorectal cancer is measured by several parameters, including vascularity, and the extent to which these tumors are vascularized can sometimes predict the likelihood of recurrence following therapy. Angiogenesis, or the formation of new blood vessels from those that already exist, is critical for tumor progression as it provides the tumor with the means by which it can acquire the oxygen and nutrients that are required for growth.[6] Although the factors that regulate this process are often secreted by the tumor itself, their expression and the events that control angiogeneic-associated proliferation are linked to the cell cycle.

In their recent work, Abedin et al. report that Cdk4 regulates colorectal tumor angiogenesis.[7] Min mutant mice harbor a mutation in the *adenomatous polyposis coli* (Apc) gene that is most commonly mutated in human colon cancers. Depending on the genetic background, heterozygous mice develop intestinal adenomas by 3–6 months of age and when crossed with $Cdk4^{R24C}$ mice ($Cdk4^{R24C/R24C}$:Apc^{+Min}), develop intestinal and colon tumors that are highly vascularized with red blood cells in the lumen of the blood vessels.[7] Although it is not surprising that this phenotype mirrors that obtained in colorectal tumors isolated from Min-heterozygous/Ink4a null-mutant ($Ink4a^{-/-}$:Apc^{+Min}) mice,[8,9] this study, for the first time, provides evidence that supports the direct involvement of Cdk4 in this process.

One important question to ask from a mechanistic perspective is: How does Cdk4 regulate angiogenesis? A logical answer and one that is supported by the data is that constitutive activation of Cdk4 results increased levels of E2F activity. $Cdk4^{R24C/R24C}$:Apc^{+Min} tumors express high levels of vascular endothelial growth factor-B (VEGF-B) and fibroblast growth factor-2 (Fgf-2), both of which are E2F-target genes. They also express elevated levels of cyclin A, which is also a target of E2F.[7] Although a role for cyclin A in angiogenesis has not been established, it is possible that this increase in expression may mediate some of the events that are associated with the formation of new blood vessels. However, the role of cellular proliferation in Cdk4-mediated angiogenesis is still not well defined. It is of interest to note that $Cdk4^{R24C/R24C}$:Apc^{+Min} mice have neither a higher tumor incidence nor an increase in tumor volume when compared to mice that are wild-type at the Cdk4 locus. This is again similar to what is observed in $Ink4a^{-/-}$:Apc^{+Min} mice where there was also no significant increase in tumor cell proliferation.[9] While genetic targeting of Cdk4 in mice has revealed that the gene is not essential for cell cycle progression, it is required for the proliferation of certain cell types such as pancreatic β-cells.[10] Considering that Cdk4 is required for Neu- and Ras-induced breast tumorigenesis,[11,12] future studies aimed at determining whether loss of Cdk4 has any effect on colorectal tumor development and angiogenesis will certainly be of significance, especially if one is to consider the use of Cdk4-inhibitors in cancer therapy.

References

1. Malumbres M, et al. Nat Rev Cancer 2009; 9:153-66.
2. Wolfel T, et al. Science 1995; 269:1281-4.
3. Zuo L, et al. Nat Genet 1996; 12:97-9.
4. Rane SG, et al. Mol Cell Biol 2002; 22:644-56.
5. Sotillo R, et al. EMBO J 2001; 20:6637-47.
6. Takebayashi Y, et al. Cancer 1996; 78:226-31.
7. Abedin ZR, et al. Cell Cycle 2010; 9:2456-63.
8. Gibson SL, et al. Cancer Res 2003; 63:742-46.
9. Gibson SL, et al. Cancer Biol Ther 2005; 4:1389-94.
10. Rane SG, et al. Nat Genet 1999; 22:44-52.
11. Reddy HK, et al. Cancer Res 2005; 65:10174-8.
12. Reddy HK, et al. Genes & Cancer 2010; 1:69-80.

FANCs regulate firing of DNA replication origins

Comment on: Song IY, et al. Cell Cycle 2010; 9:2375–88.

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Firing of DNA replication origins on the eukaryotic chromosomes is both spatially and temporally regulated and this regulation may be closely associated with control of gene expression as well as separation of chromosome domains in the nucleus.[1] Eukaryotic DNA replication proceeds in two steps; first assembly of pre-replicative complex (pre-RC) at presumptive origins in early G_1 followed by "activation" of selected pre-RC ("firing") through association of other replication factors including Cdc45 to generate active replication forks. The firing step requires actions of Cdk and Cdc7 which phosphorylate pre-RC components. However, the precise mechanism of maturation of pre-RC into an active replication fork structure has been elusive.

In *Cell Cycle*, Song and colleagues in the laboratory of Cyrus Vaziri at the University of North Carolina reported the unexpected participation of additional proteins in this complex process.[2] These workers evaluated the roles of FANCD2, the effector of the Fanconi Anemia (FA) DNA repair pathway in cell cycle progression of cultured human cells. They observed that DNA replication is significantly compromised in cells depleted of FANCD2. The requirement of FANCD2 for efficient DNA replication was observed not only in cancer cell lines but also in untransformed primary cells. In cells released from quiescence, both onset of the S phase and S phase progression were delayed after depletion of FANCD2. Detailed analyses of the distinct stages of DNA replication using velocity sedimentation of nascent DNA and DNA fiber studies showed that DNA chain elongation is not affected by FANCD2 depletion, but that the number of initiation events is reduced in FANCD2-deficient cells. Moreover, FANCD2 associates in a complex with Cdc45 specifically in the chromatin fraction and chromatin loading of the initiation factor Cdc45 is downregulated in FANCD2-depleted cells, further consistent with a role for FANCD2 in origin firing. Other FA proteins including FANCA, FANCG and FANCI involved in FANCD2 monoubiquitination are also required for efficient DNA replication, suggesting that the monoubiquitination plays a critical role in FANCD2-mediated promotion of DNA replication.

Previous studies with replication-competent Xenopus egg extracts have also indicated a role for FANCD2 in DNA replication.[3] In those studies, the FA pathway was proposed to mediate processing and repair of replication-associated DNA damage.[3] Similar to replicating Xenopus egg extracts, association of FANCD2 with chromatin in human cells is dependent on DNA replication. However, because a DNA damage response was not detected in FANCD2-depleted S-phase cells,[2] the FA pathway may not play a major role in repair of replication-associated DNA damage in human cells. Instead, in human cells FANCD2 may be required specifically for the activation of pre-RC through loading of Cdc45. The newly-described requirement of FANCD2 for initiation of DNA replication may explain the poor growth phenotype of FA patient cell lines.

The findings in this report raise new questions regarding the relationship between the FA pathway and the DNA replication machinery. How and when does FANCD2 associate with chromatin? What exactly is the role of FANCD2 in activation of pre-RC? Does FANCD2 directly interact with Cdc45? What is the role of FANCD2 monoubiquitination in this process? Following DNA damage or during S phase, the FA core complex becomes activated and monoubiquitinates FANCD2. This monoubiquitination has been suggested to target FANCD2 to chromatin where it interacts with other DNA repair proteins.[4] However, the mechanism for chromatin association of FANCD2-Ub remains unclear. One major hypothesis is that a ubiquitin-interacting motif (UIM)-containing protein, which localizes in chromatin, serves as a receptor for FANCD2-Ub.[4] Thus, factors in the pre-RC or the initiation complex could be candidates for the putative FANCD2-Ub receptor. FANCD2-Ub is not detected on chromatin until the G_1/S transition, suggesting that an S phase initiation signal may be required for this chromatin binding. Cdc7 kinase is required for firing at pre-RC and it would be interesting to examine whether chromatin binding of FANCD2 depends on Cdc7.

During serum starvation (G_0/G_1 phase), the level of FANCD2 is significantly reduced, and following re-stimulation with serum (entry into cell cycle), FANCD2 expression increases and is maintained during S phase. Interestingly, FANCD2 protein levels decrease in GINS-depleted cells.[2] Is FANCD2 stabilized upon initiation of DNA replication? The ubiquitinated FANCD2 (chromatin-bound FANCD2) may be more stable than the unmodified form.

The FA pathway is activated in an ATR-dependent manner following replication fork stalling.[5] Direct phosphorylation (SQ/TQ) of FANCD2 (and FANCI) by ATR or ATM has been well studied and appears to promote FANCD2 monoubiquitination in response to DNA damage.[4] It is possible that, even during the course of unperturbed cell cycle progression, the FA pathway is weakly activated in an ATR-dependent fashion during initiation of replication, thereby facilitating FANCD2 loading onto the pre-RC and promoting chromatin binding of Cdc45 or stabilization of the initiation complex. Once associated with the activated pre-RC, FANCD2 itself may also be stabilized. Elucidation of mechanisms by which FANC proteins participate in DNA replication certainly deserves further experiments not only to understand the molecular basis for the pathology of FA, but also to uncover additional layers of regulation that lead to timely and efficient initiation of DNA replication in vertebrates.

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References

1. Masai H, et al. Ann Rev Biochem 2010; 79:89-130.
2. Song JY, et al. Cell Cycle 2010; 9:2375-88.
3. Sobeck, et al. Mol Cell Biol 2006; 26:425-37.
4. Moldovan GL, et al. Annu Rev Genet 2009; 43:223-49.
5. Andreassen PR, et al. Genes Dev 2004; 18:1958-63.

Breaking into the brachyury world: ΔNp63 joins in

Comment on: Cho MS, et al. Cell Cycle 2010; 9:2434–41.

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The crucial role of the transcription factor Trp63 in regulating various facets of epithelial development and differentiation is epitomized by the striking phenotype of the p63 knockout mice.[1-2] These animals have a denuded skin bereft of a mature stratified epidermis and aplasia of multiple ectodermal appendages such as mammary glands, hair and teeth. This phenotype strongly suggests that developmental processes that rely heavily on proper epithelial-mesenchymal interactions are particularly affected in the absence of p63. Consistent with this view, p63-null animals also suffer from severely arrested limb development due to the physical and functional impairment of the Apical Ectodermal Ridge (AER). Deciphering the underlying molecular mechanism by which p63 transcriptionally regulates these developmental processes has proven to be quite challenging, in part, due to the existence of a growing number of p63 isoforms. Broadly, p63 isoforms can be grouped into two major classes: TAp63 isoforms that contain an N-terminal transactivation domain and ΔNp63 isoforms, which lack this domain. Furthermore, alternative splicing generates various C-terminal variants representing long (p63α) or short (p63β and γ) isoforms, each of which display distinctive biochemical and functional properties.

Since the established p63 mouse knockout models lack both TA and ΔNp63, it has been difficult to ascertain the relative contribution of these isoforms to the observed phenotype. However, recent studies have strongly suggested that ΔNp63 and not TAp63 isoforms are predominantly expressed in most epithelial cells, and hence likely to be functionally more relevant—a view that is strengthened by the paucity of any developmental phenotype in the TAp63 specific knockout animals.[3-5] In addition, contrary to previous assumption that ΔNp63 might function primarily as a dominant negative, the current consensus is that ΔNp63 is a potent transcriptional regulator on its own right and can regulate distinct sets of target genes and dictate a stratified epithelial identity.[3,5] Hence, there is an emerging need for identifying specific molecular targets of ΔNp63, particularly other developmentally regulated transcription factors, which act together with ΔNp63 in mediating some of the biological functions of p63. The paper by Elsa Flores and colleagues in *Cell Cycle* addresses this issue by demonstrating that the archetypal T-box family member brachyury is a direct ΔNp63 target.[6]

The initial evidence hinting that brachyury might be specifically regulated by p63 and not its family members, p53 and p73 came from global microarray analysis of mouse embryonic fibroblasts (MEFs) deficient for each p53 family member.[7] Given that brachyury and p63 share overlapping expression profiles during mouse embryonic development, in particular in the AER, the authors reasoned that brachyury could be downstream of the p63 pathway. Indeed, expression of brachyury was considerably attenuated in p63 null animals lacking both ΔNp63 and TAp63 as opposed to animals missing TAp63 alone. Armed with this piece of data, the authors then performed chromatin immunoprecipation experiments to show that ΔNp63 bound to a specific p63-Response Element (p63-RE) present in the proximal promoter region of the brachyury (T) gene. Interestingly, p53 and p73 did not occupy this site in vivo further supporting the notion that despite similarities in DNA-recognition elements, binding of p53 family members to their target elements is governed by additional factors. To further verify the importance of this p63-RE, the authors performed reporter assays in MEFs deficient in both p53 and p63. As expected, the segment of the brachyury promoter containing the p63-RE was strongly transactivated by ΔNp63 and not TAp63 and this response was mitigated when the p63-RE was mutated. Interestingly, the ability to activate the brachyury promoter was restricted to only the ΔNp63α and ΔNp63β isoforms, while the ΔNp63γ protein did not show any activity. This result is in sharp contrast to prior studies where the shortest ΔNp63γ isoform have been shown to possess similar if not higher transcriptional activity when compared to ΔNp63α and ΔNp63β. Possible explanations for this difference include cell type context, the varying nature of the p63-RE being studied and the usual caveats associated with transient transfection experiments with artificial reporter constructs.

Another aspect of the ΔNp63-brachyury connection tackled in this paper deals with the putative oncogenic roles of these two factors. Indeed high levels of both ΔNp63 and brachyury have been detected in several human cancers. In agreement with this, the authors found that in osteosarcoma (Os) cell lines, the transcripts for ΔNp63 and brachyury showed concordant expression with high levels of ΔNp63 accompanied by high levels of brachyury. Importantly knockdown of ΔNp63 in the Os cell lines with high levels of ΔNp63 resulted in dramatic reduction in brachyury mRNA and conversely, overexpression of ΔNp63α and ΔNp63β in ΔNp63-low cell lines drove the endogenous levels of brachyury higher. Finally, the authors show that the diminished levels of ΔNp63 and consequently brachyury in the Os cells treated with siRNA against ΔNp63 resulted in decreased cell proliferation, migration and invasion behavior. On one hand, this result fits well with a recent report showing that brachyury inhibition in human lung carcinoma cells results in loss of mesenchymal markers and reduced migration and invasion of human lung carcinoma cells.[8] On the other hand, the decreased migration and invasion behavior of Os cells upon loss of ΔNp63 is quite surprising given that in most epithelial cells, knockdown of ΔNp63 is accompanied by a shift towards mesenchymal morphology and an increase in motility.[9] This discrepancy most likely reflects the disparate nature of the origin of the cell types (carcinoma vs sarcoma for e.g) chosen for these experiments.

The findings by Cho et al. establishes a new candidate in the ΔNp63-regulated transcriptional landscape and like most studies also raises several interesting questions, which are likely to spur new avenues of future research. It is important to note that brachyury is highly expressed in chordoid tumors and a recent study has established that brachyury gene duplication confers major susceptibility to familial chordoma, a rare bone cancer believed to originate from notochordal remnants.[10] One tantalizing prospect is that high levels of ΔNp63 might affect tumors with chordoid

features or those of mesenchymal origin by influencing brachyury levels. The ΔNp63-brachyury nexus hence may be relevant for a spectrum of human cancers that express high levels of these transcription factors and offer new targets and strategies for therapy.

References

1. Mills AA, et al. Nature 1999; 398:708-13.
2. Yang A, et al. Nature 1999; 398:714-8.
3. Romano RA, et al. PLoS ONE 2009; 4:e5623.
4. Su X, et al. T Cell Stem Cell 2009; 5:64-75.
5. Crum CP, et al. Annu Rev Pathol; 5:349-71.
6. Cho MS, et al. Cell Cycle 2010; 9:2434-41
7. Lin YL, et al. PLoS Genet 2009; 5:e1000680.
8. Fernando RI, et al. J Clin Invest; 120:533-44.
9. Barbieri CE, et al. Cancer Res 2006; 66:7589-97.
10. Yang XR, et al. Nat Genet 2009; 41:1176-8.

Kip3 clusters kinetochores

Comment on: Wargacki M, et al. Cell Cycle 2010; 9:2581–8.

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During mitosis chromosome segregation relies on the action of microtubules within the mitotic spindle. Each chromosome is attached to spindle microtubules via its sister kinetochores, the proteinacious complexes found at opposite sides of its centromere.[1] The subset of microtubules that becomes attached to the kinetochores is referred to as kinetochore microtubules (kMTs). While each kinetochore attaches to ~25 microtubules in higher eukaryotes,[2] the budding yeast kinetochore binds only a single microtubule.[3] Chromosome movement is coupled to kMT polymerization/depolymerization dynamics. During metaphase, as sister kinetochores establish bipolar attachments with dynamic kMTs, chromosomes are congressed and aligned at the center of the spindle.[1] In budding yeast, congression clusters the sister kinetochores in two regions on either half of the spindle.[4] The mechanism(s) regulating the dynamics of individual kMTs to attain metaphase chromosome congression remains largely unclear.

Cells utilize motor proteins to facilitate and coordinate mitotic events. Kinesin-8 is a conserved subclass of Kinesin microtubule motors that regulates microtubule dynamics in diverse organisms. When Kinesin-8 is knocked-down in higher eukaryotic cells chromosomes are unable to achieve metaphase congression resulting in failure to enter anaphase and mitotic arrest.[5, 6] The budding yeast Kinesin-8, Kip3, functions both as a conventional plus-end directed motor and a plus-end specific microtubule depolymerase.[7, 8] Yeast cells lacking Kip3 do not arrest in mitosis, suggesting that Kip3 is not essential for establishing bipolar sister kinetochore attachments and/or alignment prior to anaphase onset. However, recent work by Wargacki and colleagues[9] provides evidence that Kip3 function is important to achieve proper kinetochore clustering during metaphase.

Imaging fluorescently-tagged kinetochores in live yeast cells, Wargacki et al.[9] observed variability in kinetochore positioning along the spindle axis in the absence of Kip3. One possibility is that Kip3 uses its length-dependent microtubule depolymerization activity[8] to ensure that the kMTs of sister kinetochores are of equal length. This would in turn cluster sister kinetochores equidistant from either spindle pole. Paradoxically the authors report a decrease in kMT length in the absence of Kip3, and it remains unresolved how the loss of this depolymerase results in shorter kMTs.

Movement and congression of chromosomes requires coordination between the dynamic states of sister kMTs. Interestingly, Wargacki and colleagues[9] find that the shorter kMTs are accompanied by increased inter-kinetochore distance, which may reflect perturbed tension at kinetochore-microtubule attachments. Kip3 may therefore play a role in coordinating sister kinetochore directional movement by correlating kMT dynamics with the amount of tension and/or compressive forces experienced at either kinetochore. Presumably such coordination in higher eukaryotes must be extended to all ~25 microtubules at each kinetochore rather than the single kMT found in budding yeast. Perhaps the complexity in synchronizing large numbers of kMTs is one reason why higher eukaryotes display more adverse metaphase defects than do budding yeast following perturbation of Kinesin-8. However, the molecular mechanism(s) through which Kip3 controls the dynamics of individual kMTs remains to be determined.

In previous work, Tytell and Sorger[10] observed an increase in lagging chromatids during anaphase and that a subset of chromatids experienced prolonged interruptions during anaphase poleward movement in cells lacking Kip3. Based on these and other findings Tytell and Sorger[10] concluded that Kip3 facilitates the synchronous anaphase movement of chromatids toward the spindle poles, a process intimately connected to kMT depolymerization. In light of the current study, Wargacki and colleagues[9] propose that defects in kinetochore clustering and the resulting disparate starting positions upon anaphase onset may be the major cause of the lagging anaphase chromatids. While the failure to congress could explain in large part the accumulation of lagging chromatids, it does not directly account for discontinuous poleward movement unless unclustered chromatids also experience subsequent problems with anaphase movements. As it stands, it would appear that Kip3 distinctly functions in metaphase to cluster chromatids and in anaphase to facilitate poleward chromatid movement. Determining the overlap between Kip3 function during metaphase and anaphase may be particularly tractable in budding yeast because the loss of Kip3 does not prevent progression into anaphase.

Overall, these findings highlight the conserved role of Kinesin-8 in chromosome congression across different systems and raise interesting questions about the mechanisms underlying the role of Kip3 in regulating kinetochore clustering.

References

1. Walczak CE, et al. Int Rev Cytol 2008; 265:111-58.
2. McDonald KL, et al. J Cell Biol 1992; 118:369-83.
3. Winey M, et al. J Cell Biol 1995; 129:1601-15.
4. Goshima G, et al. Cell 2000; 100:619-33.
5. Goshima G, et al. J Cell Biol 2003; 162:1003-16.
6. Mayr MI, et al. Curr Biol 2007; 17:488-98.
7. Gupta ML, Jr, et al. Nat Cell Biol 2006; 8:913-23.
8. Varga V, et al. Nat Cell Biol 2006; 8:957-62.
9. Wargacki, et al. Cell Cycle 2010; 9:2581-8
10. Tytell JD, et al. J Cell Biol 2006; 172:861-74.

Kip3-ing kinetochores clustered

Comment on: Wargacki M, et al. Cell Cycle 2010; 9:2581–8.

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Metaphase is the brief and highly conspicuous period during cell division where chromosomes become positioned at the spindle equator through the process of "congression". It is generally believed that congression promotes accurate chromosome segregation during anaphase, and this logic has driven research over several decades to identify the underlying mechanisms. Recent studies have demonstrated that Kinesin-8 microtubule motors are important for congression in organisms ranging from fission yeast to humans,[1-3] and that they probably act by modulating the dynamics of kinetochore-microtubule plus ends where they concentrate.[2,4-6] It has been unclear, however, if this role is conserved in the budding yeast Saccharomyces cerevisiae. Previous work by Tytell and Sorger showed that kinetochores lag during mitosis in yeast cells lacking the Kinesin-8 Kip3, and they proposed that Kip3 synchronizes poleward kinetochore movements during anaphase.[7] Gardner et al. have suggested that Kip3 is not involved in kinetochore positioning during pre-anaphase mitosis, and that the primary function of Kip3 is to govern spindle length by regulating the dynamics of interpolar microtubules.[8]

In *Cell Cycle*, Wargacki and colleagues demonstrate that Kip3 is required for chromosome congression like other Kinesin-8 motors.9 In budding yeast, metaphase sister kinetochores appear bi-lobed, forming clusters on either side of the spindle equator. Using quantitative fluorescence microscopy and live cell imaging, the authors find that kinetochores attach spindle microtubules normally in kip3Δ cells but are declustered (Fig. 1), indicating that kinetochores are scattered along the spindle axis in the mutant. This notion is confirmed through an analysis of single centromere (CEN3) positioning. Wargacki et al. show that KIP3 deletion causes CEN3 mis-positioning and, consistent with previous work,[7] that centromeric chromatin between sister kinetochores is hyperstretched, an effect probably caused by abnormally high microtubule-induced pulling forces on kinetochores. A final important observation in the Wargacki et al. study is that spindle length is normal in kip3Δ cells, suggesting that Kip3-dependent effects on kinetochore positioning are not indirect consequences of interpolar microtubule length regulation by the motor. Discrepancies between previous reports[8,10] are probably due to technical differences in how spindle lengths were measured; Wargacki et al. used spindle pole body markers instead of fluorescent tubulin to identify spindle poles.

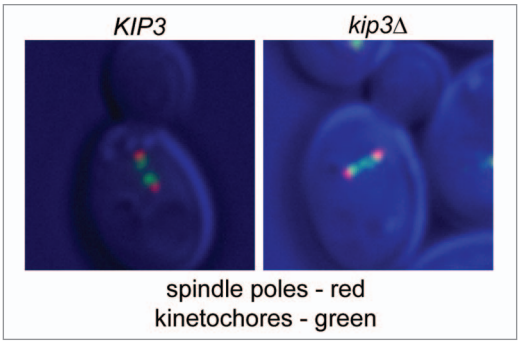

Figure 1. Kinetochores fail to congress in kip3Δ cells.

The work by Wargacki et al. is important because it establishes that Kip3, like the other Kinesin-8 motors analyzed to-date, performs a critical function during chromosome congression. It also supports the idea that proper kinetochore positioning during metaphase contributes to the accuracy of sister chromatid segregation during anaphase. As Wargacki et al. point out, sister chromatids will be scattered along the anaphase spindle if they initiate poleward movements from heterogeneous positions, reasoning which might account for the lagging chromosomes previously observed by Tytell and Sorger.[7] The next challenge is to define how Kip3 influences kinetochore positioning, and how this activity is integrated with other major chromosome positioning factors such as Kip1 and Cin8.[7,8] In vitro, Kip3 is a plus end-directed microtubule depolymerase that is capable of destabilizing filaments assembled from the GTP-mimic GMPCPP.[5,6] It is unclear, however, how loss of Kip3 would lead to increased pulling forces on kinetochores, a phenotype that suggests that Kip3 antagonizes microtubule shortening. At kinetochore-microtubule plus ends, Kip3 might act via other mechanisms to control kinetochore movements, as recently suggested for its human orthologue Kif18A.4 Future work is needed to reveal the precise mechanism(s) by which Kinesin-8 proteins govern kinetochore movements and their overall positioning during mitosis.

References

1. Savoian MS, et al. J Cell Sci 2004; 117: 3669-77.
2. Stumpff J, et al. Dev Cell 2008;14: 252-62.
3. West RR, et al. J Cell Sci 2002; 115: 931-40.
4. Du Y, et al. Curr Biol 2010; 20: 374-80.
5. Gupta ML, et al. Nat Cell Biol 2006; 8: 913-23.
6. Varga V, et al. Nat Cell Biol 2006; 8: 957-62.
7. Tytell JD, et al. J Cell Biol 2006; 172: 861-74.
8. Gardner MK, et al. Cell 2008; 135: 894-906.
9. Wargacki MM, et al. Cell Cycle 2010; 9;2581-8.
10. Straight AF, et al. J Cell Biol 1998; 143: 687-94.

Novel insights into the cytoplasmic functions of p53

Comment on: Grinberg S, et al. Cell Cycle 2010; 9:2442–55

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The tumor suppressor protein p53 plays a pivotal role in preventing malignant development. Approximately 50% of all human tumors harbor an inactivating mutation, many others show a compromised function by elevation of its inhibitors, reduction of its activators, or inactivation of downstream targets.[1] Most studies so far focused on the transcriptional activities of p53 which allow the "guardian of the genome" to govern life and death of cells by regulating cell cycle arrest, senescence, apoptosis, changes in metabolism, and others either via transcriptional activation or repression. The ability of p53 to bind directly to specific DNA sequences and transactivate nearby genes is the capacity that distinguishes wild-type p53 from virtually all cancer associated mutant forms.[2] Despite its transcriptional properties p53 was shown to play a role in DNA replication and homologous recombination.[1]

An emerging area of research reveals that p53 possesses also functions outside of the nucleus. A cytoplasmic pool of p53 can activate apoptosis by directly targeting mitochondria for cell death induction.[3] Another cytoplasmic property of basal p53 levels is its ability to inhibit autophagy.[4] Inhibition of autophagy under inappropriate conditions can be oncogenic. In line with that, autophagy has been reported to contribute to apoptosis and the clearance of damaged proteins and organelles. Therefore, it is not surprising that stress-activated wild-type p53 can switch to the induction of autophagy by stimulating the transcription of positive autophagy regulators.[5] In contrast, several cancer-associated p53 mutants that lost their transactivating activity retained their ability to inhibit autophagy.[6]

The control of centrosome replication displays another cytoplasmic effect of p53.[1] Interestingly, cytoplasmic p53 was shown to control the translation of certain mRNA transcripts and possess 3′→ 5′ exonuclease activity of DNA and ssRNA.[7]

Grinberg et al. in *Cell Cycle* now adds a new facet to the growing complexity of p53. The authors could show that p53 exerts also 3′→ 5′ exonuclease activity towards dsRNA. dsRNA plays a role in various important biological processes like gene activation, heterochromatin remodelling, RNA-editing, IFN responses and translation control mediated by natural antisense RNA and microRNA.[8] p53 was shown to play a role in the host defence against viral infections by its ability to directly activate the transcription of dsRNA-activated protein kinase (PKR) leading to translational inhibition and cell apoptosis.[9] Grinberg et al. now showed evidences that p53 might also directly provide antiviral function by its dsRNA exonuclease activity. PKR is activated by low concentrations of dsRNA longer than 30bp, but inhibited by high amounts. Therefore, the dsRNA exonuclease activity of p53 could also alter the levels or length of dsRNA and therefore the response of PKR versus translational inhibition and apoptosis.

Even more interesting is the potential impact of p53 on the RNAi and microRNA machinery which might open a plethora of new p53 functions.

Notably, the gain-of function mutant p53-R175H did not exhibit exonuclease activity, so the question is if and how this nuclease cleavage activity contributes to the tumor suppressor functions of p53. It will be interesting to test also other mutants, taking loss-of-function and gain-of-function, DNA-contact and conformational mutants into the analysis. The authors showed that irradiation increased the stability and therefore the amount of nuclear but also cytoplasmic p53 with a subsequent increase of cytoplasmic dsRNA 3′→ 5′ exonuclease activity. It might be interesting to test if sub-apoptotic and apoptotic stimuli or different posttranslational modifications of p53 provoke differences in its dsRNA 3′→ 5′exonuclease activity.

The ssRNA 3′→ 5′exonuclease activity of p53 showed a kind of sequence specificity, being permissive to RNA containing AU-rich elements which is a destabilizing sequence often found in proto-oncogene and cytokine mRNAs characterized by a short half live.[7]

It will be challenging to investigate further whether the dsRNA exonuclease activity of p53 has some kind of sequence specificity or preference or if it rather displays a general mechanism for viral defence and PKR regulation.

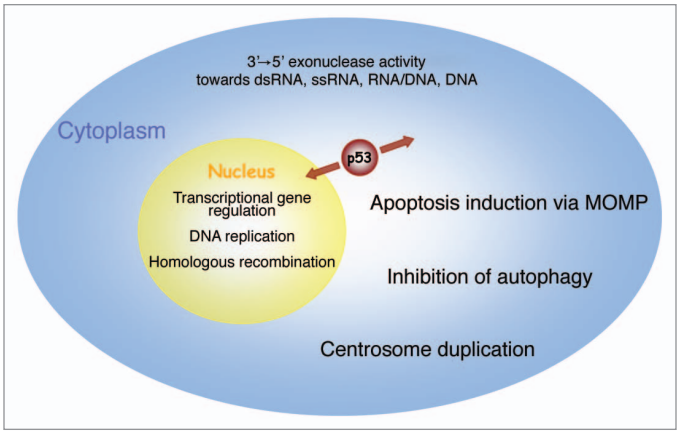

Figure 1. Overview of p53 activities in the nucleus and in the cytoplasm; MOMP (mitochondrial outer membrane permeabilization).

References

1. Green DR, et al. Nature 2009; 458: 1127-30.
2. Levine AJ, et al. Nat Rev Cancer 2009; 9: 749-58.
3. Vaseva AV, et al. Biochim Biophys Acta 2009; 1787: 414-20.
4. Vousden KH, et al. Cell 2009; 137: 413-31.
5. Vousden, KH, et al. Nat Rev Cancer 2009; 9: 691-700.
6. Morselli E, et al. Cell Cycle 2008; 7: 3056-61.
7. Bakhanashvili M, et al. J Mol Med 2008; 86: 75-88.
8. Grinberg S. Cell Cycle 2010; 9: 2442-55.
9. Yoon CH, et al. Proc Natl Acad Sci USA 2009; 106: 7852-7.

Integration of transcriptional signals at the tumor cell invasive front

Comment on: Fuxe J, et al. Cell Cycle 2010; 9:2363–74

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Cancer is one of the world wide leading causes of death, of which the mortality rate is in large part due to metastasis. However, the molecular mechanisms that determine the spread of tumor cells to distal organs are not well understood. The process that leads to metastasis is multifaceted and involves a series of steps that include: invasion into adjacent tissue, intravasation, transport through the circulatory system, extravasation and growth in a distal organ.[1] Despite the clinical importance, the genetic and biochemical determinants remain marginally defined. An important process that contributes to migration and invasion of tumor cells is thought to be epithelial to mesenchymal transition (EMT), when the polarity of epithelial tumor cells is disrupted and cells take on a mesenchymal spindle-shape morphology.[2]

EMT in cancer cells; From skepticism to acceptance

EMT is a critical process during development that determines the body plan and is characterized by a loss of epithelial cell-cell junction proteins, such as E-cadherin and zona occludens (ZO-1) and at the same time acquire the expression of fibroblasts markers, such as vimentin and fibroblast-specific protein-1 (FSP-1). Upon EMT cells become highly motile and invasive, allowing survival in an anchorage-independent environment and providing them with stem cell-like properties (Figure 1).[2] Whereas EMT in specific developmental processes is broadly accepted, its functional role in cancer biology was initially met with skepticism, mainly due to the absence of convincing evidence in clinical samples.[3] The epithelial to mesenchymal transition is difficult to capture in fixed tumor biopsies of cancer patients, partially due to its transient nature as well as that tumor cells after EMT are difficult to be distinguished from stromal cells or other tumor associated fibroblasts. However, EMT in cancer has recently gained in acceptance and has been observed in patient tumor samples, in particular at the invasive front of solid tumors.[4]

Interplay TGF-β and cancer stem cell pathways

In *Cell Cycle,* Fuxe et al. review the current knowledge of signaling pathways that are fundamental for EMT of cancer cells.[5] A mayor inducer of the dedifferentiating EMT process is the transforming growth factor (TGF)-β signaling pathway.[6] The signal is transduced by members of the Smad family that are the transcriptional effectors of TGF-β. Since the intrinsic DNA binding activity of Smads is weak, they need to cooperate with other partner transcription factors to regulate gene transcriptional responses.[7] Fuxe et al. elegantly discuss the emerging concept that TGF-β signaling and cancer stem cell pathways, such as Ras, Wnt, Hedgehog and Notch are intimately linked for the induction of EMT at tumor invasive niches. Transcriptional Smad containing complexes, termed EMT promoting Smad complexes (EPSC) by Fuxe et al., have a key role in the integration of external and internal cues that control EMT.[5] An important external trigger for EMT that needs further dissection in future studies is the inflammatory tumor microenvironment. What is not discussed in the review by Fuxe et al. is the important role of non-Smad signaling pathways downstream of TGF-β receptors in controlling epithelial plasticity by inducing a loss of cell polarity.[8]

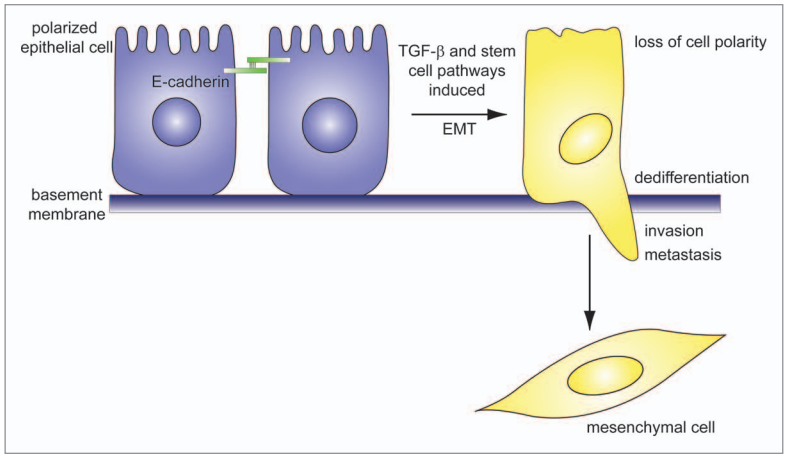

Figure 1. During the epithelial to mesenchymal transition (EMT) cells loose polarity and adopt properties required for motility and invasiveness. The EMT process (depicted by arrows) is stimulated by a combination of TGF-β and stem cell pathways. Characteristic is the dedifferentiation and loss of E-cadherin at the cell membrane.

EPSC as biomarkers and opportunities for therapeutic intervention

Fuxe et al. review the many EMT associated factors like Snail and AP1 family members that have been shown to participate in EPSC.[5] A challenge for future studies is to determine which particular EPSC are formed in a spatio-temporal manner and which specific steps of EMT are regulated by distinct EPSC. Importantly, these results require verification in animal models and biopsies of cancer patients. Ultimately, EPSC may associate with disease progression and poor prognosis and may be used as biomarkers to stratify cancer patients. In addition, EPSC may be a potential entry point for the development of therapies for cancer patients; domain mapping of Smad-protein interactions in EPSC can be used for development of small molecular compounds that disrupt functional EPSC. Current strategies to inhibit TGF-β-induced invasion and metastasis target TGF-β ligand or TGF-β receptor are prone to have side effects; TGF-β is a highly pleiotropic cytokine and has a dual role in cancer and other patho-physiological processes.[9] Specific interference of EPSC holds promise to block tumor cell invasion and metastasis, while leaving other TGF-β signaling arms largely intact.

References:

1. Nguyen DX, et al. Nat Rev Cancer 2009; 9:274-84.
2. Kalluri R, et al. J Clin Invest 2009; 119:1420-8.
3. Tarin D, et al. Cancer Res 2005; 65:5996-6000; discussion 6000-1.
4. Vincent T, et al. Nat Cell Biol 2009; 11:943-50.
5. Fuxe J, et al. Cell Cycle 2010; 9: 2363-74
6. Miettinen PJ, et al. J Cell Biol 1994; 127:2021-36.
7. Itoh S, et al. Eur J Biochem 2000; 267:6954-67.
8. Ozdamar B, et al. Science 2005; 307:1603-9.
9. Akhurst RJ, et al. Trends Cell Biol 2001; 11:S44-51.

Discovery that polyploid cells can undergo mitosis

Comment on: Leysi-Derilou Y, et al. Cell Cycle 2010; 9:2589–99.

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Leysi-Derilou et al. in the laboratory of Nicolas Pineault show for the first time that polyploid megakaryocytes (MKs) are capable of undergoing mitosis. MKs are hematopoietic cells that give rise to platelets. MK maturation is marked by a progressive increase in ploidy (up to 128N) due to repeated DNA replication without cell division, a process termed endomitosis, resulting in a large multilobulated, polyploid nucleus.[1] Although the impact of polyploidization during MK maturation is not fully understood, it is generally considered that polyploidization is essential for efficient platelet production by MKs. Genes related to platelet production are upregulated in highly polyploid MKs.[2]

Prior time-lapse microscopy studies characterized endomitosis from the 2N to 4N stage as an aborted mitosis with failure at a late stage of cytokinesis.[3-5] During anaphase, endomitotic cells form normal central spindles, and the cleavage furrow nearly completes ingression, but the cells do not undergo abscission, leading to regression of the cleavage furrow to produce polyploid cells. The reasons for this aborted cytokinesies have begun to be elucidated. Polyploid endomitotic MKs form intact midzone structures with normal localization of essential components of mitosis such as Survivin, Aurora B, INCENP, PRC1 (protein regulating cytokinesis 1), MKLP1 and 2 (mitotic kinesin-like protein), MgcRacGAP and microtubules.[4,6] However, in contrast to normal cytokinesis, the contractile ring of MK undergoing endomitosis lacks non muscle myosin IIA and has decreased levels of RhoA and actin.[4] RhoA signaling is required for establishing the actomyosin ring at the cleavage furrow, and generating the contractile force for completion of cytokinesis. Thus, decreased Rho/Rock signaling at the contractile ring may cause failure of late cytokinesis leading to endomitosis.

To gain greater insight into MK differentiation and endomitosis, Leysi-Derilou, et al., used live cell imaging to track the formation and fate of polyploid human MKs derived *in vitro* for 5—10 days from cord blood (CB) and bone marrow (BM) CD34+ cells.[7] They established methods to determine the ploidy level of MKs by combining cell history, cell size and Hoechst nuclear staining. These approaches enabled them to observe low-incidence events as they followed the fate of over 2000 polyploid MKs. Their data confirm that endomitosis is a failure of mitosis at cytokinesis; all visualized endomitotic events showed cleavage furrow formation and subsequent regression. More importantly, they report that polyploid MKs occasionally do complete cytokinesis and divide into separate daughter cells, although this happens significantly more often in MKs from CB than from BM (16% vs. 1.6% of 8N divisions result in complete cytokinesis in CB vs. BM, respectively). Division of 8N polyploid cells could be symmetric (most) or asymmetric (rare), the latter giving rise to 2 MKs (2N and 6N), or even to 3 MKs (4N, 2N and 2N). Although the 2N daughter cells usually die soon after cytokinesis, the higher ploidy daughter cells are viable and maintain the ability to undergo subsequent endomitotic events. The cell death in resulting 2N cells may be due to an abnormal distrubution of the homologous chromosomes, which are not split evenly between the daughter cells. In previous time-lapse studies, only an endomitotic fate was observed for polyploid MKs. This discrepancy may have occurred because previous studies recorded polyploid MKs during a 6-24 hour period, and tracked fewer than 50 polyploid MKs undergoing endomitosis.[3-5] Also, these studies used BM-derived MKs, which undergo cytokinesis more rarely than CB. Our lab has also observed polyploid cell division when the human erythroleukemia (HEL) cell line stably expressing H2B-GFP undergoes TPA induced megakaryocytic differentiation as recorded by fluorescence time-lapse microscopy. This novel finding is reminiscent of the recent discovery that platelets themselves are capable of division (analogous to cytokinesis) to form multiple functional platelets after they are released into the blood stream.[8]

Whether polyploid divisions of MKs are an in vitro culture artifact or have physiological relevance for megakaryocytopoiesis in vivo remains to be investigated. One step to clarify this would be to determine whether the small 6N peak observed occasionally in fresh BM samples from humans and mice by conventional flow cytometry methods is due to individual 6N cells or caused by doublets of 2N and 4N cells. This can be achieved using Imagestream flow cytometry, which allows imaging and analysis of DNA content on thousands of individual cells.

The data present suggest that primary polyploid MKs divide and thereby contribute to the expansion of polyploid MKs. Explorations into the etiology of the differences in polyploid divisions between CB and BM derived MKs may lead to a better understanding of endomitosis and megakaryocytopoiesis.

References

1. Battinelli E, et al. Curr Opin Hematol 2007; 14:419-26
2. Raslova H, et al. Blood 2007; 109:3225-34.
3. Geddis AE, et al. Cell Cycle 2007; 6:455-60.
4. Lordier L, et al. Blood 2008; 112:3164-74.
5. Papadantonakis N, et al. Cell Cycle 2008; 7:2352-6.
6. Geddis AE, et al. Cell Cycle 2006; 5:538-45.
7. Leysi-Derilou, et al. Cell Cycle. 2010; 9:2589-99
8. Schwertz H, et al. Blood. 2010;115:3801-3809.

Histone acetylation gets complicated

Comment on: Gatta R, et al. Cell Cycle 2010; 9:2149–59

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The highly dynamic structure of chromatin in eukaryotic cells allows compaction of DNA, regulation of gene expression and cell cycle progression at the same time. The combination of several post translational modifications (PTMs) of histones, as the "histone code" model suggests, specifies different chromatin states affecting all DNA related processes. Among the different modifications, up to present, acetylation seemed to be the easiest to interpret since it correlates with opened chromatin structure.[1] Even though multiple acetylation sites, deposited by several histone acetyl transferases [HATs; also called Lysine(K) acetyltransferases, KATs [2]], have been identified both on the N-terminal tails and in the core domains of histones, these modifications were always associated with active transcription in interphase cells.[3,4] In S phase the epigenetic state of the cell is challenged as the chromatin undergoes a wave of disruption and subsequent restoration during replication. Finally in M phase the chromatin reaches the highest level of compaction in the form of mitotic chromosomes.[5] Several DNA regions need to be transcribed during each cell cycle step, which might counteract the condensation required for the given cell cycle phase.[6] The exact processes ensuring active chromatin state in these conditions remain unknown. Acetylation is an unstable mark removed by histone deacetylases (HDACs), thus the controlled action of the opposing activities of HATs and HDACs is essential for proper regulation of both transcription and cell cycle.[5,7]

The article published by R. Gatta and R. Mantovani in *Cell Cycle* challenges our view on histone acetylation and shows that it can anti-correlate with gene expression.[8] Using a modified ChIP protocol, allowing single nucleosome resolution, the authors provide evidence that several acetylation marks (H3K9, H3K18, and H2BK120) are present at the transcription start site and/or at the transcribed region of cell cycle regulated genes, such as PCNA and CyclinB2, during the repressed state. Their results highlight that a switch of histone marks including actetylations, methylations and ubiquitination take place at cell cycle promoters when the corresponding genes turn on or off. A genome wide ChIP-Seq analysis of histone PTMs in synchronized cell populations would enlarge our knowledge on how the cell phase-dependent transcriptome is established. Cell cycling, in this respect, has the unique feature that genes are constantly turned on and off as the cell progresses. This is in contrast with differentiation, where, starting from a pluripotent stage, lineage specific genes get silenced or turn on for the rest of the cells' fate. Switches similar to cell cycle can be envisaged in stress response, where the responsive genes have to turn on to overcome the stress situation and then get silenced once the cell returns to non-stressed conditions. Although histone PTMs have been mapped following gene expression changes during hematopoetic differentiation,[9] until now not much is known about the role of acetylation in such a system. At the same time, global histone acetylation marks clearly shift during differentiation, as it has been shown for histone H4 in human embryonic stem cells.[10]

Another intriguing question raised by the authors is the possibility of cell cycle-dependent action of HATs on promoters. They provide evidence that GCN5/KAT2A, the founding member of a family of HAT enzymes,[11] is present on the PCNA promoter exclusively when the gene is expressed.[8] On the other hand, GCN5/KAT2A is recruited to the chromatin around the promoter of CyclinB2 during its "off" state, suggesting a role in repression.[8] Consequently, the same HAT seems to be able to activate a set of genes and repress others. Clearly, the picture is more complicated than it seems, as in vertebrate cells GNC5/KAT2A and PCAF/KAT2B, two highly identical HATs, coexist.[11] Moreover, both enzymes are components of at least two multisubunit complexes; SAGA and ATAC.[12] Many questions remain opened: (1) what is the role of the different acetylation marks in combination with other histone PTMs at a genome wide level; (2) is the effect of acetylation locus specific; (3) which HATs deposit this mark, are they strictly specific or do their activities overlap; (4) which HDACs remove the acetyl group when it is no longer required; (5) as these enzymes do not exert their function alone in the cell, into what complex they are incorporated to impact a genomic locus at a given stage. The observations reported by the Mantovani group provide a first step to answer these questions.[8] Future experiments will shed light on the exact role of HATs and the marks they deposit on the genome during transcriptional changes in the context of cell cycle.

References

1. Wang Z, et al. Nat Genet 2008; 40:897-903.
2. Allis CD, et al. Cell 2007; 131:633-6.
3. Kouzarides T. Cell 2007; 128:693-705.
4. Koch CM, et al. Genome Res 2007; 17:691-707.
5. Probst AV, et al. Nat Rev Mol Cell Biol 2009; 10:192-206.
6. McManus KJ, et al. Biochem Cell Biol 2006; 84:640-57.
7. Wang Z, et al. Cell 2009; 138:1019-31.
8. Gatta R, et al. Cell Cycle 2010; 9:2149-59.
9. Cui K, et al. Cell Stem Cell 2009; 4:80-93.
10. Phanstiel D, et al. Proc Natl Acad Sci U S A 2008; 105:4093-8.
11. Nagy Z, et al. Oncogene 2007; 26:5341-57.
12. Nagy Z, et al. Cell Mol Life Sci 2010; 67:611-28.