Cell Cycle News & Views

RB in breast cancer: Differential effects in estrogen receptor-positive and estrogen receptor-negative disease

Comment on: Ertel A, et al. Cell Cycle 2010; 9:4153–63.

Elizabeth A. Musgrove and Robert L. Sutherland*; Cancer Research Program, Garvan Institite of Medical Research; Sydney, NSW Australia; *Email: r.sutherland@garvan.org.au; DOI: 10.4161/cc.9.23.13889

The RB pathway is one of the best-studied and most frequently altered pathways leading to loss of proliferative control in cancer. It can be disrupted by a variety of means including, but not limited to, epigenetic silencing, allelic loss or mutation of *RB1*. In some cancers including breast cancer, amplification of the *CCND1* gene or overexpression of cyclin D1 is commonly observed.[1] In others, the *CDK4* gene is amplified or mutated within the p16[INK4A]-binding domain. Finally, many cancers lack p16[INK4] expression due to deletion or promoter hypermethylation of *CDKN2A*. By increasing cyclin D1-CDK4 kinase activity, these genetic events all restrict the ability of underphosphorylated RB to inhibit cell cycle progression.

Genetic alterations at the *RB1* locus, or lack of RB expression, occur in up to a third of breast cancers.[2] Breast cancer can be divided into a number of biologically distinct phenotypes, and RB loss is more common in some of these subtypes, i.e. basal breast cancers, which are typically estrogen receptor (ER)-negative, poorly-differentiated and aggressive and luminal B cancers, which are a poor-prognosis subgroup accounting for approximately a third of ER-positive breast cancers.[3] Interestingly, *RB* deletion in mouse mammary progenitor cells leads to the formation of tumors with basal or luminal B characteristics,[4] suggesting that *RB* loss may not simply be a marker of these subtypes.

Confounding issues in examining the association between RB loss and breast cancer therapeutic responsiveness and outcome include the poor correlation between *RB1* LOH and low or absent RB protein expression[5] as well as technical difficulties in measuring RB expression by immunohistochemistry. In addition, cyclin D1 overexpression or p16[INK4A] methylation, both of which are common in breast cancer[1,6], are usually mutually exclusive with RB loss but are expected to have similar biological consequences. In a previous issue of *Cell Cycle*, Ertel et al.[7] used a "RB-loss signature" composed of genes that are upregulated by RB deletion or repressed by RB activation to identify breast cancers where the RB pathway is deregulated. In ER-positive cancers the RB-loss signature was correlated with cyclin D1 overexpression and low RB1 levels but not with p16[INK4A] levels, whereas it was correlated with p16[INK4A]/*CDKN2A* expression but not cyclin D1 or RB expression in ER-negative cancer. Although aberrant expression of these genes is to some degree a marker of RB pathway deregulation, the correlation is not strong, and none are useful biomarkers of pathway activity in all breast cancer subtypes.

Although in both ER-positive and ER-negative breast cancer, RB pathway deregulation was associated with increased proliferation, it was correlated with poorer outcome in ER-positive disease but with better outcome in ER-negative disease.[7] In ER-positive breast cancer this relationship was apparent both in patients treated with surgery alone and in patients receiving adjuvant antiestrogen (tamoxifen) therapy, indicating that ER-positive breast cancers with RB pathway deregulation are inherently more aggressive. They are also more likely to metastasise, possibly because of the decreased expression of cell-cell communication genes that Ertel et al. find to be associated with the RB-loss signature.[7] These observations are consistent with the widely-held view that more proliferative cancers have a poorer outcome, and the association between markers of increased proliferation and poor response to endocrine therapy.[8]

In ER-negative cases, the association of RB pathway deregulation with better outcome appears to be due to improved response to some types of chemotherapy.[7,9] This is likely to result, at least in part, from an impaired cellular DNA damage/genotoxic stress response in the absence of functional RB.[2] In ER-positive cancers, RB pathway deregulation may be a marker of increased sensitivity to small molecule inhibitors of CDK4.[7] These important findings provide impetus for further studies aimed at developing a clinically useful panel of biomarkers that can identify cancers with RB pathway deregulation and sensitivity to new therapies following independent validation in other patient cohorts. The importance of Ertel et al.'s work lies not only in that it points towards a potential role for measuring RB pathway aberrations in order to inform treatment decisions in breast cancer. It also illustrates the power of using gene signatures based on biological function to probe determinants of prognosis and response to therapy, as has been suggested in the context of endocrine therapy.[8]

References
1. Buckley MF, et al. Oncogene 1993; 8:2127-33.
2. Bosco EE, et al. Cell Cycle 2007; 6:667-71.
3. Herschkowitz JI, et al. Breast Cancer Res 2008; 10:R75.
4. Jiang Z, et al. J Clin Invest 2010; 120:3296-309.
5. Borg A, et al. Cancer Res 1992; 52:2991-4.
6. Hui R, et al. Clin Cancer Res 2000; 6:2777-87.
7. Ertel A, et al. Cell Cycle 2010; 9:4153-63.
8. Musgrove EA, et al. Nat Rev Cancer 2009; 9:631-43.
9. Derenzini M, et al. Clin Cancer Res 2008; 14:2199-209.

miR-31 in cancer: Location matters

Comment on: Aprelikova O, et al. Cell Cycle 2010; 9:4387–98.

Christina H. Stuelten* and David S. Salomon; National Institutes of Health; National Cancer Institute; Bethesda, MD USA
*Email: chrisstu@mail.nih.gov; DOI: 10.4161/cc.9.23.13928

In epitheial tumors, a significant part of the tumor mass is comprised of non-parenchymal cells such as immune cells, cells of the microvasculature and fibroblasts, often referred to as tumor stroma or tumor microenvironment. The tumor stroma not only supplies scaffolding and nutrition to parenchymal tumor cells, but stromal and parenchymal cells also partake in an extensive crosstalk that can alter tumor biology. Fibroblasts are ubiquitous stromal cells that secrete extracellular matrix, such providing mechanical support to parenchymal cells. In tumors, cancer associated fibroblasts (CAFs) overproduce extracellular matrix thus contributing to the rigidity of tumors. Furthermore, CAFs secrete cytokines and growth factors into the tumor microenvironment thus promoting tumor growth and/or metastases.[1,2]

MicroRNAs (miRNAs) are small, non-coding, single-stranded RNAs that negatively regulate gene expression on a post-transcriptional level. Individual miRNAs can target multiple genes and such orchestrate complex biological phenotypes like cell migration or proliferation. On the other side, alterations of miRNA expression patterns might cause significant disturbances of cell and tissue homeostasis. Indeed, recently altered miRNA expression has been associated with cardiovascular diseases, diabetes and metabolic diseases, neurodenerative diseases and cancer.[3]

In their present study, Aprelikova et al.[4] set out to test if posttranscriptional regulation of gene expression in endometrial CAFs as compared to endometrial normal fibroblasts (NFs) isolated from the same patient contributes to tumor progression. Having ascertained differential effects of CAFs and matching NFs on endometrial tumor cells in a xenograft model, Aprelikova et al.[4] determined which miRNAs and mRNAs were differentially expressed in CAFs and NFs and found miR-31 was the most downregulatated miRNA in CAFs, while one of its target genes, SATB2, was upregulated in CAFs. Release of miR-31 relayed suppression of SATB2 expression in fibroblasts increased migration of tumor cells towards fibroblasts in vitro while there was no effect on tumor cell proliferation. This indicates that expression levels of miR-31/SATB1 in stromal cells might influence the metastatic capacity of endometrial tumors.

Altered expression levels of miR-31 and SATB2 have been shown for several solid tumors. Interestingly, miR-31 expression is downregulated in carcinoma of the breast, prostate, ovary and stomach, but upregulated colorectal, liver and head-and-neck tumors.[5] Likewise, increased expression of SATB2 as shown to correlate with higher tumor grade in breast cancer and reduced expression of SATB2 to correlate with higher tumor stage and metastasis in colon cancer.[6,7]

These results together imply that metastasis is affected by stromal as well as parenchymal expression of miR-31 and its target gene SATB2. However, the downstream effects of altered mi-R31/SATB2 expression must be quite different for stromal cells, parenchymal breast cancer cells derived from breast cancer and parenchymal colon cancer cells, respectively. In parenchymal breast cancer cells the effect of miR-31 on metastases can be phenocopied by concurrent downregulation of the miR-31 target genes integrin α_5, radixin and rhoA,[8] an in colon cancer cells miR-31attentuates expression of TIAM1 which is involved in regulation of cell motility;[9] This implies that miR-31 affects the metastatic capacity of parenchymal cells
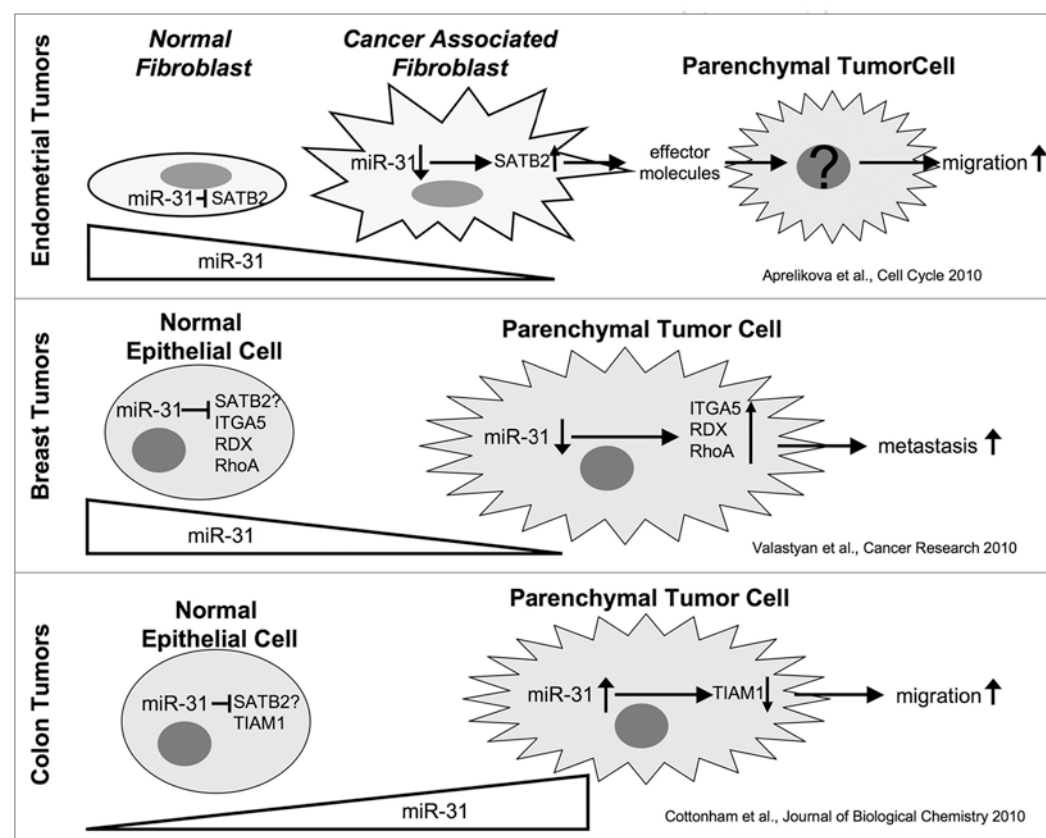
Figure 1. Effects of miR-31 on tumor cell migration and metastasis are cell type and tissue dependent.

by directly altering their biological properties. In contrast, Aprelikova et al.[4] demonstrated that altered miR-31/SATB2 levels in fibroblasts alter migration of tumor cells that were separated from the fibroblasts by the membrane of a transwell chamber, that is without direct contact between fibroblasts and tumor cells. The effect of altered fibroblast miR-31/SATB2 levels on tumor cells

therefore has to be relayed by soluble factors that are released from the fibroblasts into the surrounding microenvironment and subsequently alter behavior of neighboring tumor cells. Thus the data of Aprelikova et al.[4] adds a new perspective to the tissue- and cell specific role of miR-31/SATB2 tumor-stromal interactions, tumor cell migration and metastasis.

References
1. Proia DA, et al. Cell Cycle 2005; 4:1022-5.
2. Bhowmick NA, et al. Science 2004; 303:848-51.
3. Kanwar JR, et al. Front Biosci (Schol Ed) 2:1113-26.
4. Aprelikova O, et al. Cell Cycle 2010; 9:4387-98.
5. Valastyan S, et al. Cell Cycle; 9:2124-9.
6. Patani N, et al. Cancer Cell Int 2009; 9:18.
7. Wang S, et al. J Pathol 2009; 219:114-22.
8. Valastyan S, et al. Cancer Res; 70:5147-54.
9. Cottonham CL, et al. J Biol Chem 2010; 285:35293-302.

Nucleolar structure: It's all in a tangle

Comment on: Mayan M, et al. Cell Cycle 2010; 9:4328–37.

Justin M. O'Sullivan; Institute of Natural Sciences; Massey University; Auckland, New Zealand; Email: j.m.osullivan@massey.ac.nz; DOI: 10.4161/cc.9.23.13929

The nucleolus is the most obvious and best-studied structure within the nucleus. It has central roles in cellular metabolism, aging and the cell cycle. However, the repetitive nature of the DNA skeleton (rDNA) that forms the nucleolar core makes it recalcitrant to detailed structural resolution. Recent methodological advances have provided tantalizing insights into the structure of this most enigmatic organelle.

In yeast, the rDNA locus is made up of ~200 repeats each consisting of the 35S rDNA (i.e., the 18S, 5.8S, 28S and intervening sequences) and 5S rDNA genes and two intergenic sequences (i.e., IGS1, IGS2). Inter- and intra-rDNA repeat interactions have been proposed as part of a structure that increases local concentrations of the cis- and trans-acting factors, DNA and protein, that are required for efficient rDNA transcription and replication.[1,2] While theoretically attractive, these conformations have not been empirically confirmed.

In a previous issue, Mayan and Aragón[3] presented suggestive observations relating to the structure of the yeast rDNA array. They identified an interaction that involves sequences either side of the 5S rDNA (IGS1-IGS2) and, while it is weak, seems to connect yeast 35S rDNA promoter and terminator regions. This interaction is similar to previously identified interactions between the 35S rDNA promoter and terminator regions in rats[4] and mice.[5] Perhaps more importantly, there is also clear evidence for connections between IGS1 and the 25S rDNA and between regions located within the 18S and 25S rDNA genes.

The IGS1-25SrDNA connection is consistent with an interaction we have previously documented in the yeast rDNA.[6] Together these results appear to substantiate earlier assertions that the terminal regions (promoters and terminators) of yeast rDNA repeats are connected.[1,2] However, interpreting the results of proximity-based ligation assays of repetitive regions (e.g., the nucleolus) is particularly problematic because interactions that form within a repeat cannot be differentiated from those that form between different repeats (**Fig. 1**). Hence, it remains an open question as to whether these are intra-[1] or inter-repeat[2] interactions.

Mayan and Aragón make the intriguing observation that reducing the level of Reb1p (and to a lesser degree the Net1p) causes a reduction in the frequency of the IGS1-IGS2 interaction, and also of the interaction bridging the 18S and 25S genes.[3] Thus it appears that there is an inter-relationship between these two interactions. The link may be mediated by an interaction that occurs between the 25S rDNA and the enhancer/IGS1 sequence, which also requires enhancer and replication fork binding proteins (i.e., Fob1p and Sir2p).[6]

Taken together with previous observations,[1,2,6] the results of Mayan and Aragón offer a suggestive glimpse of the spatial organization of the *S. cerevisiae* rDNA array (**Fig. 1**). Logically this organization facilitates reciprocal control of RNA polymerase I and cryptic RNA polymerase II transcription (e.g., TAR1) in the rDNA. Simply, these interactions can be invoked to establish mutually exclusive

RNA polymerase I and II transcription factories between the 25S rDNA, promoter and terminator regions (**Fig. 1**). Other questions, including whether RNA polymerase III transcription factories are promoted by the formation of RNA polymerase I and/or II factories (**Fig. 1.1 and 1.2**) remain unanswered. This is particularly important because *S. cerevisiae* is one of the few organisms in which the 5S rDNA is found within the 35S rDNA repeats, therefore the formation of RNA polymerase III transcription factories may differ from those found in other eukaryotes. Moreover, there is no evidence as to whether the interactions require active RNA polymerase I transcription, despite tantalizing evidence that active RNA polymerase II is required (ref. 6, Mayan and Aragón). Finally, the dynamic nature of the nucleolus implicates systems that prevent and resolve nucleic acid knots in the maintenance of nucleolar structure. How these resolving systems relate to the structures that promote and stabilize spatial proximity within the rDNA remains to be determined. In short, it appears that our understanding of nucleolar structure will remain jumbled until the development and implementation of methods that enable the incremental untangling of individual rDNA repeats, and hence of the nucleolus itself.

References
1. Kempers-Veenstra AE, et al. EMBO J 1986; 5: 2703-2710.
2. Johnson SP, et al. Mol Cell Biol 1989; 9:4986-4993.
3. Mayan M, et al. Cell Cycle 2010; 9:4328-37.
4. Shiue CN, et al. Oncogene 2009; 28: 1833-1842.
5. Nemeth A, et al. EMBO J 2008; 27: 1255-1265.
6. O'Sullivan J, et al. Yeast 2009; 26: 125-138.
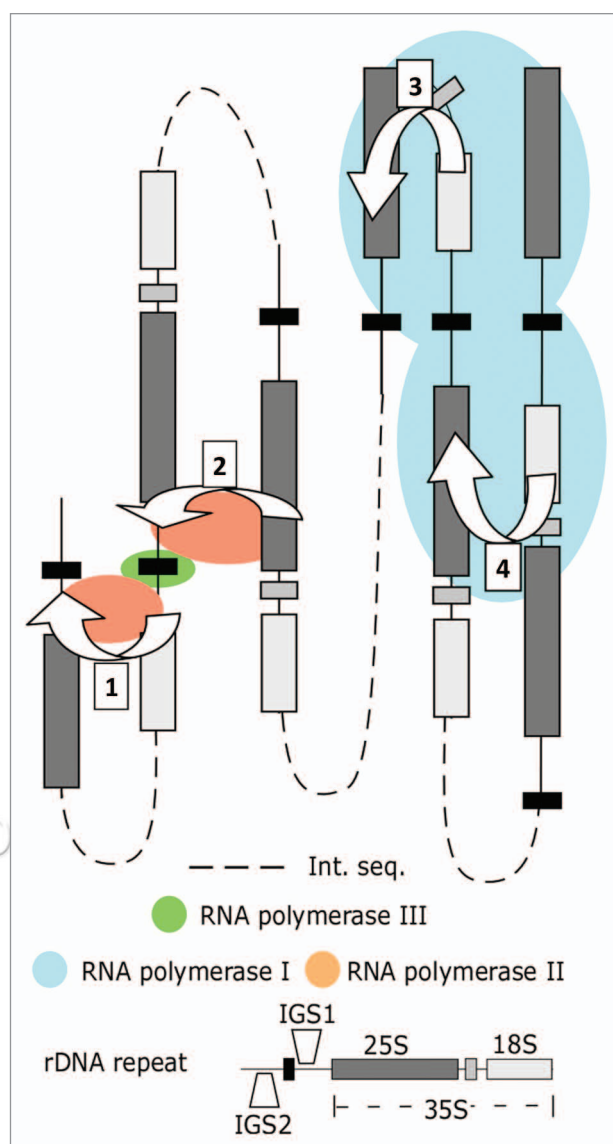
Figure 1. Model for mutually exclusive formation of RNA polymerase I, II and III transcription factories within the yeast rDNA. Interactions bridging the IGS1-IGS2 (1) and the 25S rDNA-IGS1 (2) sequences form part of an RNA polymerase II transcription factory promoting transcription from cryptic RNA polymerase II promoters. These interactions isolate active 5S rDNA in yeast. The 25S - IGS1 interaction is mutually exclusive with the intra-35S (18-25S) rDNA loop (3) that positions IGS1 and IGS2 sequences for rapid re-initiation of RNA polymerase I and requires Rep1p binding and blockage at the replication fork binding site. For simplicity figures are depicted in two-dimensions, in reality the structures would form rosettes that merge the two halves (1, 2) of the polymerase II factories. The rosette structure would also merge the RNA polymerase I factories (3, 4). Proximity-based ligation methods are unable to differentiate interactions occurring within a repeat (3) from those occurring between repeats (4), yet they are structurally different. Int. seq.: intervening sequence that could include no, one or several rDNA repeats.

Old-fashioned genetic screens give new insights to DNA replication

Comment on: Ma L, et al. Cell Cycle 2010; 9:4399–410.

Bik-Kwoon Tye; Department of Molecular Biology and Genetics; Cornell University; Ithaca, NY USA; Email: bt16@cornell.edu; DOI: 10.4161/cc.9.23.13930

There is much to be said about keeping things simple even in this fast paced *omics* age. Just when we thought that for mapping global genetic networks, robotic systematic genetic analysis (SGA)[1] had replaced classical bench top random mutagenesis screens,[2,3]

Ma et al.[4] showed otherwise in a previous issue of *Cell Cycle*. In an effort to identify new players in the initiation of DNA replication, these authors used a phenotypic color-sectoring assay to screen EMS mutagenized cells for *initiation of DNA replication* (*idr*) mutants. A

comparison of the results from this study and from a parallel SGA screen[5] yields an overlapping set of putative *idr* mutants but contrasting conclusions. Both studies identified a number of genes involved in sister chromatid cohesion (SCC) to have synthetic defects

with the Origin Recognition Complex defective mutants, orc2 and orc5. These SCC genes include *DCC1*, *CTF4*, *CTF8* and *CTF18*, which encode subunits of the alternate RFC complex important for sister chromatid cohesion. While the SGA study found that ORC plays a role in SCC, a conclusion supported by another study,[6] it categorically ruled out that the SCC genes, in particular *CTF4* and *CTF18*, play any role in the initiation of DNA replication. By contrast, the Ma study showed convincingly that Ctf1 and Ctf18 are directly involved in DNA replication initiation based on their association with ORC, Cdt1 and MCM proteins, their requirement in S-phase entry, S-phase progression and preRC formation. Furthermore, they showed that Ctf4 plays a role in maintaining the association of Mcm10 and DNA polymerase α.[7] Why are there such discrepancies? The diametrically opposite conclusions stem from the use of deletion mutations in SGA versus point mutations in

the random mutagenesis study. As it turns out, just like ORC, the SCC proteins are involved in both replication initiation and sister chromatid cohesion and that these two functions are genetically separable. In such synthetic defect analyses, the use of deletion mutations that harbor more than one defect may cloud conclusions.

What is satisfying about the Ma et al. study is that along with the *idr*-SCC genes, they have also identified a significant number of other previously unknown *IDR* genes including *CDC14* and *ADK1*. In a separate paper, the same group showed that *CDC14* plays an essential role in dephosphorylating multiple initiation proteins to allow preRC formation during the M-G$_1$ transition.[8] A more surprising finding is perhaps the direct involvement of *ADK1*, which encodes an adenylate kinase, in providing ATP for pre-RC assembly.[9] This finding may revive an old notion that nucleotide

substrates for specific DNA metabolism may be directly channeled by the relevant nucleotide biosynthetic enzymes as suggested for the channeling of hydroxymethyl dCTP for DNA synthesis in the T-even phages.[10] There is still much to be learned about the interconnecting processes that weave the cell division cycle using new and old global genetic analyses. An important lesson from the Ma et al. study is that there is plenty of room for old tricks to turn new profits.

References
1. Tong AH, et al. Science 2001; 294:2364-8.
2. Hartwell LH, et al. Genetics 1973; 74:267-86.
3. Maine GT, et al. Genetics 1984; 106:365-385.
4. Ma L, et al. Cell Cycle 2010; 9:4399-410.
5. Suter B, et al. Genetics 2004; 167:579-91.
6. Shimada K, et al. Cell 2007; 128:85-99.
7. Wang J, et al. Biochem Biophys Res Commun 2010; 395:336-41.
8. Zhai Y, et al. J Cell Sci 2010; 123:3933-43.
9. Cheng X, et al. J Biol Chem 2010; 285:29974-80.
10. Flanegan JB, et al. J Biol Chem 1977; 252:3019-27.

Toxicity and lifespan extension: Complex outcomes of histone overexpression in budding yeast

Comment on: Singh RK, et al. Cell Cycle 2010; 9:4236–44.

Paul D. Kaufman; University of Massachusetts Medical School; Worcester, MA USA; Email: paul.kaufman1@umassmed.edu; DOI: 10.4161/cc.9.23.13931

Histone synthesis is highly regulated in all eukaryotes, and has served as a paradigm for understanding cell cycle regulated-transcription,[1] post-transcriptional RNA processing,[2] and regulated protein decay.[3] Improper histone expression can have deleterious effects on cell-cycle progression,[4] chromosome stability[5] and gene expression.[6] Two recent papers show there are many other effects, describing new phenotypes associated with histone overexpression in budding yeast.

In previous experiments, Akash Gunjan and co-workers observed that budding yeast cells lacking checkpoint kinase Rad53 are very sensitive to histone overexpression. This lead to the discovery Rad53 has a novel, non-checkpoint related role in degradation of excess soluble histones.[3] Specifically, histone phosphorylation by Rad53 triggers proteosome-mediated degradation.[7] In a previous issue of *Cell Cycle*, this laboratory explored multiple ways in which excess histones affect cells. Multiple effects are observed: yeast mutants lacking several different histone modifying enzyme subunits are shown to be sensitive to histone H3 overexperession, suggesting that excess histones might overload modification

pathways. H3 overexpression also results in reduced nucleosomal linker length and altered nucleosome positions in vivo. Upon analysis of steady-state mRNA levels, few genes are altered upon overexpression of H3 alone, a somewhat surprising result given the chromatin structural changes documented above. However, over-expression of an H3/H4 gene pair or all four core histones resulted in >200 loci with >2-fold alterations in mRNA levels. Curiously, several clusters of adjacent genes were among these, suggesting that specific chromatin neighborhoods are particularly sensitive to histone density. Perhaps these observations are related to older findings that overexpression of histone pairs but not individual histones causes chromosome loss.[5] Finally, the investigators show that excess histones can be crosslinked to RNA in vivo, raising the possibility that excess histones could exert some of their effects via sequestration of RNAs.

Those sorts of observations might lead one to predict that histone overexpression would promote reduced viability or shorter lifespans. However, recent work from Jessica Tyler and co-workers[8] suggests just the opposite: histone H3/H4 (but not H2A/H2B)

overexpression lengthens the budding yeast replicative life-span. Conversely, replicatively old cells display reduced histone levels. These data suggest that depletion of histones during aging could lead to undesirable access to DNA, perhaps via cryptic transcription; in this view, extra histones would offset this loss.

How can we consolidate these seemingly contradictory observations that histone overexpression can perturb chromosomes yet still extend lifespan? A comprehensive model would suggest that cells are able to cope with minor perturbations caused by histone overexperssion when they are young (as long as the Rad53-mediated decay pathway is intact, so that grossly toxic occlusion of DNA is avoided). In contrast, in old cells the reduced amount of histones reveals the benefit of a fully packaged genome, such that histone overexpression prevents many of the deleterious effects of age-associated histone loss in old cells. In the future, it will be interesting to determine the extent that the phenomena of kinase-mediated destruction of nascent molecules and age-associated histone loss are conserved in other eukaryotes.

References
1. Osley MA. Annu Rev Biochem 1991; 60:827-861.
2. Marzluff WF, et al. Curr Opin Cell Biol 2002; 4:692-9.
3. Gunjan A, et al. Cell 2003; 115:537-49.
4. Kim UJ, et al. EMBO J 1988; 7:2211-2219.
5. Meeks-Wagner D, et al. Cell 1986; 44:43-52.
6. Wyrick JJ, et al. Nature 1999; 402:418-21.
7. Singh RK, et al. Nat Cell Biol 2009; 11:925-33.
8. Feser J, et al. Mol Cell 2010; 39:724-35.
9. Singh RK, et al. Cell Cycle 2010; 9:4236-44.

A combination of 1α,25-dihydroxyvitamin D3, a plant-derived antioxidant and an inhibitor of the Cot1/Tlp2 oncogene as molecularly targeted weapons in the hematologist's battle against acute myeloid leukemias

Comment on: Wang X, et al. Cell Cycle 2010; 9:4542–51.

Philip J. Hughes and Geoffrey Brown; School of Immunity and Infection; College of Medical and Dental Sciences; University of Birmingham; Birmingham, UK; Email: P.J.Hughes@bham.ac.uk or G.Brown@bham.ac.uk; DOI: 10.4161/cc.9.23.13932

Acute myeloid leukemia (AML) is a heterogeneous disease in which multiple genetic and epigenetic defects lead to abnormal differentiation and unlimited self-renewal of hematopoietic stem/myeloid progenitor cells.[1] AML is a difficult disease to treat, especially in the case of refractory, relapsed and elderly AML patients.[2] Prognosis is highly dependent on age at presentation and the underlying molecular defect. Hence, cytogenetics and molecular biology are increasingly being used in diagnosis and to determinate optimum therapy.[3] In certain subsets of patients mean survival time and overall cure rate have been greatly improved with patient-tailored chemotherapeutic regimens and drugs targeting the molecular defects driving proliferation/ survival of neoplastic cells.[3]

It has been known for ~30 years that 1α,25-$(OH)_2D_3$ induces growth arrest and monocytic differentiation of cultured AML cells. Even so, 1α,25-$(OH)_2D_3$ isn't used therapeutically, largely due to an unacceptable frequency of occurrence of hypercalcemia and a high proportion of non-responding patients.[4,5] Analogs with low calcemic capacity have been developed, but none of these have been shown to be clinical useful. Efforts are under way to decrease the likelihood of 1α,25-$(OH)_2D_3$–mediated toxicity by increasing the sensitivity of AML cells to 1α,25-$(OH)_2D_3$, by co-administration of "differentiation potentiating" agents. Alternatively, specific inhibition of pathways that mediate 1α,25-$(OH)_2D_3$ resistance may produce clinically beneficial effects.

Seminal work on 1α,25-$(OH)_2D_3$–induced monocytic differentiation of AML cells has emanated from the Studzinski lab (UMD-New Jersey Medical School, Newark, NJ). Recent studies have centred on identifying the mechanisms by which 1α,25-$(OH)_2D_3$ activates the p42 ERK, JUN and p38 MAP kinase signaling pathways and unravelling the complex stimulatory and inhibitory interactions between these pathways.[6] In particular, Studzinski's group have been at the forefront of research into the use of novel drug combinations as treatments for AML. This research has shown that silibinin, a plant antioxidant, potentiates 1α,25-$(OH)_2D_3$–mediated differentiation in some AML cell lines and primary cells by augmenting MAP kinase signalling pathways. Unfortunately, several sub-sets of primary AML cells still remained 1α,25-$(OH)_2D_3$–resistant.[5] In a previous issue of *Cell Cycle*, Wang et al.[7] shedded new light on resistance by identifying a 1α,25-$(OH)_2D_3$- and silibinin-activated signalling pathway that negatively regulates monocytic differentiation in several types of AML cells. The Cot1 proto-oncogene, a member of the MAP kinase kinase kinase (MAP3K) superfamily, and its downstream target the serine threonine kinase ERK5 are key components of this pathway. Cot1 has been reported to be involved in regulating activities of the differentiation promoting p42 ERK, JUN and p38 and the differentiation restraining ERK5 MAP kinase signalling pathways. Earlier this year Studzinski's group demonstrated that expression of Cot1, and its kinase activity, was rapidly increased by 1α,25-$(OH)_2D_3$ in HL60 myeloid leukemic cells[7,8] and this disrupts pro-differentiation and growth arresting pathways by interfering with MAPK signalling.[9] In other cellular scenario's activation of Cot1 and ERK5 has pro-proliferative effects by down-regulating the expression of, and signalling from, the cell cycle inhibitory protein p27[Kip1]. Wang et al.[7] show that this also occurs in several different subclasses of AML and confirm that Cot1 can act as an oncogene in these cells.

These findings have important implications for the therapy of AML, since small molecule pharmacological inhibitors of Cot1 are available and could be used to remove the 'molecular brake' that restrains 1α,25-$(OH)_2D_3$–stimulated monocytic differentiation. To this end Wang et al.[7] have shown in a previous issue of *Cell Cycle* that co-administration of silibinin and a Cot1 inhibitor increases the sensitivity of several myeloid leukemic cell lines and primary cells to 1α,25-$(OH)_2D_3$. Most importantly they were able show that inhibition of Cot1 was sufficient to induce growth arrest and differentiation in even the most primitive AML cell lines and primary leukemic blasts which are normally differentiation resistant. Cot1 inhibitors were less effective at inducing differentiation of more differentiated AML blasts and cell lines which indicates that high expression of Cot1 may be a major cause of the failure of primitive AML cell types to respond to differentiation inhibitors. The key point to take from these studies is that by thoroughly understanding the molecular mechanisms by which differentiating agents produce their effects, and perhaps more importantly by appreciating why some cell types do not respond, new therapies for hard-to-treat myeloid leukemias will undoubtedly be uncovered.

References
1. Vardiman JE, et al. Blood 2009; 114:937-51.
2. Motyckova G, et al. Curr Hematol Malig Report 2010; 5:109-17.
3. Döhner H, et al. Blood 2010; 115:453-74.
4. Gocek E, et al. Leuk Res 2010; 34:649-57.
5. Zhang J, et al. Hematol Oncol 2010; 28:124-32.
6. Hughes PJ, et al. Leuk Res 2010; 34:553-65.
7. Wang X, et al. J Cell Cycle 2010; 9:4542-51.
8. Wang X, et al. J Steroid Biochem Mol Biol 2010; 121:395-98.
9. Wang X, et al. J Cell Physiol 2010; DOI:JCP-10-0611.

Cdc25 phosphatases: Differential regulation by ubiquitin-mediated proteolysis
Comment on: Thomas Y, et al. Cell Cycle 2010; 9:4338–50.

Lauren M. Young and Michele Pagano*; Department of Pathology; NYU Cancer Institute; New York University School of Medicine; New York, NY USA;
*Email: michele.pagano@med.nyu.edu; DOI: 10.4161/cc.9.23.13934

Considered the "engines" of cell cycle progression, cyclin-dependent kinases (CDKs) phosphorylate myriad downstream substrates to promote cell growth, replication and division. Tight control of CDK activity results from, among other mechanisms, the opposing actions of inhibitory kinases (Wee1 and Mik1) and activating phosphatases. Cdc25 phosphatases are essential components of this process, reversing inhibitory phosphorylation of CDKs during key cell cycle transitions and peaking in their activity as cell division nears its mitotic end.

Although the three mammalian Cdc25 paralogs—Cdc25A, Cdc25B and Cdc25C—are regulated by multiple mechanisms, degradation of Cdc25A and Cdc25B by the ubiquitin-proteasome system remains a major method of regulation during the cell cycle. Interestingly, Cdc25 genes are considered proto-oncogenes whose excessive activity accelerates proliferation, but the increased levels of Cdc25A and Cdc25B observed in tumors do not generally occur as a consequence of amplification or mutations that lead to overt overexpression. Instead, elevated Cdc25 levels result from their stabilization and persistence during cell cycle periods when they should be absent or degraded.[1]

The functional characteristics that distinguish the Cdc25 paralogs have been increasingly investigated. While the mouse knockout model of *Cdc25A* displays embryonic lethality, deletion of either *Cdc25B* or *Cdc25C* imparts little phenotypic outcome at both the organismal and cellular levels.[2] In fact, cells derived from mice lacking both *Cdc25B* and *Cdc25C* do not show significant defects in their cell cycles or DNA damage responses, with these normal phenotypes perhaps indicating redundancy in their functions and substrates. In contrast to these loss-of-function studies, examining the aberrant persistence of these proteins may reveal more about their particular roles and elucidate more subtle mechanisms of their regulation. Studies investigating the specific conditions for Cdc25A degradation mediated by the Anaphase Promoting Complex/Cyclosome (APC/C^Cdh1) or the SCF^βTrCP ubiquitin ligases have led to a greater understanding of the delicate balance of Cdc25A levels across the cell cycle[3] (**Fig. 1**). Now, by confirming and extending the long-postulated notion that Cdc25B, like Cdc25A, is degraded by the ubiquitin-proteasome system via the F-box protein βTrCP,[4] the work of Thomas et al. in a previous issue of *Cell Cycle* furthered this discussion of distinct roles within the Cdc25 family.[5]

In their report, the authors investigate the unique features of Cdc25B activity during mitosis, showing βTrCP-dependent degradation at the metphase-anaphase transition—in surprising contrast to the nearly simultaneous removal of Cdc25A through APC/C^Cdh1—and raise intriguing questions about novel mechanisms regulating βTrCP-mediated ubiquitylation. In S phase, when the activity of CDKs must be maintained at levels low enough to be permissive for DNA replication, Cdc25A levels are moderated by βTrCP. Following S-phase, CDK activity rises, inhibiting replication (thereby preventing reduplication) and promoting mitotic events.[3] In response to DNA damage in S and G$_2$, βTrCP-dependent degradation of Cdc25A is enhanced. These interphase and DNA damage-responsive interactions between Cdc25A and βTrCP require phosphorylation by a number of kinases—one priming (Chk1),[3] and others processive (CKIα, GSK3β and Nek11),[6,7] with binding dependent upon the final phosphorylation within the protein's βTrCP degron, an inverted variant of the classical DSGxxS motif (STDSG).

The many characterized substrates of βTrCP share some version of this DSGxxS phosphodegron, sometimes with phosphomimetic substitution, but Thomas et al. instead show that Cdc25B uniquely lacks any phosphorylable residue in its degron sequence (DDGFVD). Since regulation of the timely recognition of substrates by βTrCP famously follows a phosphorylation within this motif, how might Cdc25B ubiquitylation be controlled?

The authors speculate that regulation of Cdc25B degradation in the absence of a phosphorylable degron may be due to modification of βTrCP itself, or it may represent a kind of competition for βTrCP, in which occupation of its substrate recognition site by phosphorylated substrates (which are likely to bind βTrCP with high affinity) allows Cdc25B to accumulate, but permits its degradation at the end of mitosis after other substrates have been eliminated. The abundance of βTrCP in the cell and its diverse substrate targets argue that perhaps another mechanism might exist. One way to counteract a 'constitutively active' degron would be to oppose ubiquitylation in a regulated manner, namely using a deubiquitylating enzyme (DUB). Thus, it is possible that a yet undiscovered DUB stabilizes Cdc25B at the G$_2$/M transition. For example, recent evidence indicates that Dub3 (also called USP17) positively regulates Cdc25A levels and has been found to be elevated in human breast cancer cell lines in which Cdc25A is stabilized.[8] Dub3
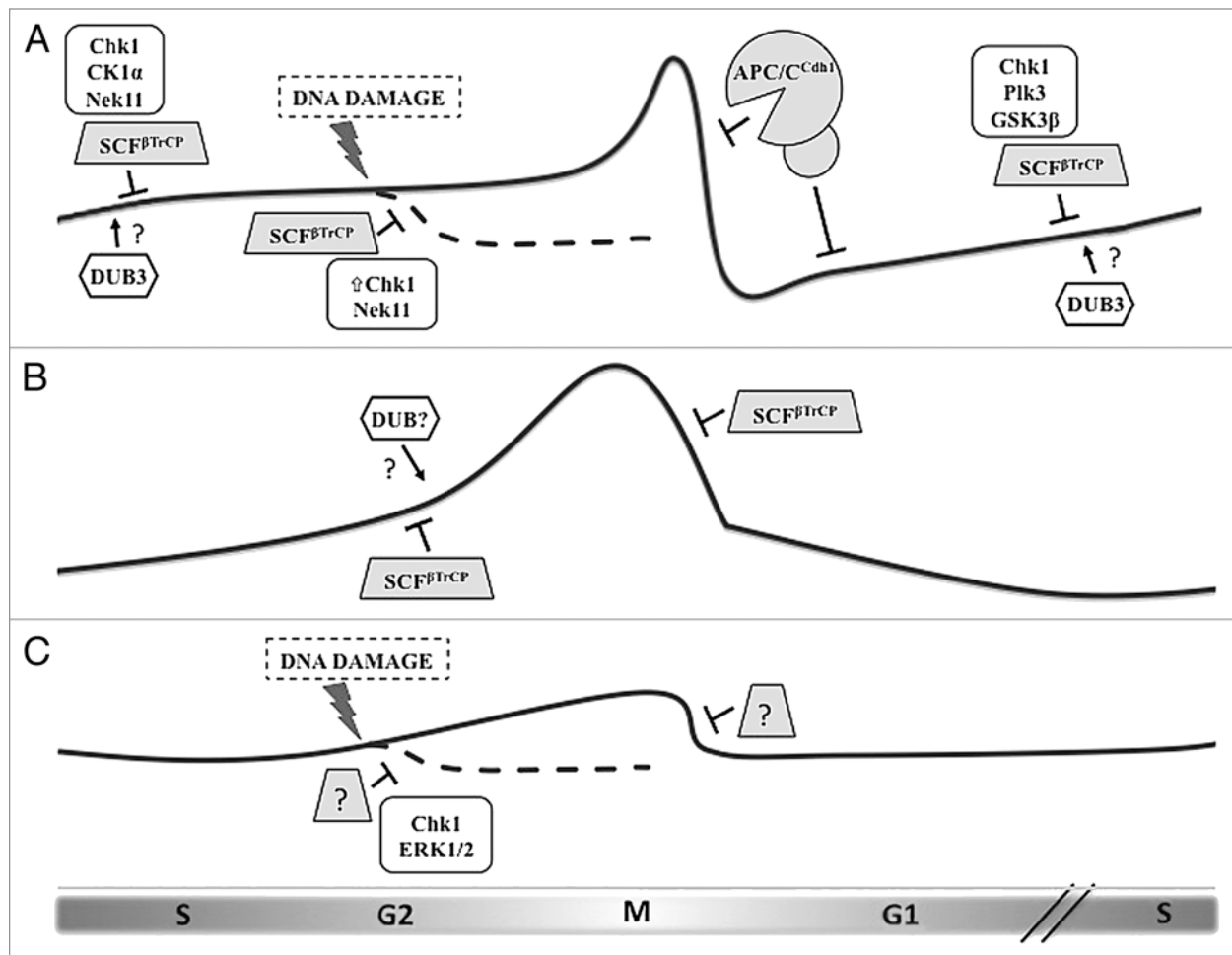
Figure 1. Regulation of the three CDC25 paralogs during the cell cycle by ubiquitin-mediated degradation. (A) Cdc25A protein levels begin to rise in late G_1 and remain modest during S phase and early G_2 by the action of the ubiquitin ligase SCF^{βTrCP}, whose recognition of Cdc25A depends upon phosphorylation by a number of kinases. DNA damage induction of Chk1 activity increases the priming phosphorylation of Cdc25A, leading to greater βTrCP-induced degradation. At the end of mitosis and through G_1, Cdc25A is eliminated via the APC/C^{Cdh1} ubiquitin ligase. Dub3 has been shown to stabilize Cdc25A by deubiquitylation, although the precise timing and conditions for its activity remain unknown. (B) Cdc25B levels are also moderated by βTrCP, but in a phosphorylation-independent manner. Rapid destruction of Cdc25B at the metaphase-anaphase transition is mediated by βTrCP. Since the degron of Cdc25B mimics constitutive phosphorylation, we hypothesize that another mechanism counteracts βTrCP-mediated ubiquitylation during its rise in G_2, possibly the activity of a deubiquitylating enzyme (DUB). (C) Cdc25C is the least-studied protein of the family, and its regulation is determined mostly by phosphorylation events that regulate its phosphatase activity and localization. Evidence exists that Cdc25C is ubiquitylated and degraded following certain forms of G_2 arrest, but, although this event requires Chk1 and ERK1/2, the contributing ubiquitin ligase is unknown.

may therefore contribute to tumorigenesis by inappropriately stabilizing Cdc25A and, conceivably, other substrates.

High expression of exogenous Cdc25B is known to induce mitotic catastrophe, and overexpression of Cdc25B in S phase results in centrosome overduplication.[9] In their report, Thomas et al. show that a stabilized Cdc25B mutant (unable to bind βTrCP) yields discrete cellular consequences, including a delay in mitotic exit, diverse chromosomal and spindle defects and the appearance of micronuclei that accrue in subsequent mitoses. Therefore, the regulation of Cdc25B turnover in the absence of a phosphorylable degron is a subject of interest for future studies of Cdc25 phosphatase activity during the cell cycle, and exploration of alternative regulatory mechanisms may provide a greater overall understanding of regulated proteolysis.

References
1. Löffler H, et al. Oncogene 2003; 22:8063-71.
2. Guardavaccaro et al. Mol Cell 2006; 22:1-4.
3. Frescas D, et al. Nat Rev Cancer. 2008; 8:438-49.
4. Kanemori Y, et al. Proc Natl Acad Sci USA 2005; 102:6279-84.
5. Thomas Y, et al. Cell Cycle 2010; 9:4338-50.
6. Melixetian M, et al. Nat Cell Biol 2009; 11:1247-53.
7. Honaker Y, et al. Oncogene 2010; 29:3324-34.
8. Pereg Y, et al. Nat Cell Biol 2010; 12:400-6.
9. Boutros R, et al. Cancer Res. 2007; 67:11557-64.

Establishment of sister chromatid cohesion: The role of the clamp loaders

Comment on: Maradeo ME, Cell Cycle 2010; 9:4370–8.

Oren Parnas and Martin Kupiec*; Department of Molecular Microbiology and Biotechnology; Tel Aviv University; Ramat Aviv, Israel;
*Email: martin@post.tau.ac.ail; DOI: 10.4161/cc.9.23.13961

Each time a cell divides, the whole genome must be duplicated and the two copies faithfully separated between the mother and the daughter cells. This is achieved by a complex mechanism that involves maintaining the newly replicated and the old DNA molecules (the two sister chromatids) attached while building the separating machinery. Sister chromatid cohesion (SCC) is being actively investigated in yeast.[1] The cohesin complex, composed of the Smc1, Smc3, Scc1/Mcd1 and Scc3 subunits, forms a ring that is essential for cohesion. Two additional protein heteroduplexes are required: the Scc2-Scc4 and the Pds5-Rad61/Wpl1 complexes. Finally, the Ctf7/Eco1 acetyltransferase plays an important regulatory role by acetylating Smc3 during S phase; this acetylation is essential to establish cohesion, by a mechanism that is being actively studied.[2-4]

In the last years, a new family of proteins has been found to affect SCC: the Replication Factor C (RFC) family. The canonical RFC complex plays an essential role in loading the processivity clamp PCNA onto DNA. RFC is composed of four small (Rfc2-5) and a large subunit (Rfc1). Three additional RFC-like factors exist in yeast; in these complexes the large subunit is replaced by the Rfc1-like proteins Rad24, Ctf18 or Elg1.[5] Whereas Ctf18 and Elg1 have been shown to interact with PCNA, Rad24 loads the PCNA-like clamp 9-1-1 that plays an important role in checkpoint induction. Mutations in either Elg1 or Ctf18 cause defects in SCC.[6,7]

Previous results by the Skibbens lab have shown that a deletion of the *ELG1* gene can partially rescue the lethal effects of the *ctf7-eco1* mutation, whereas deletion of *CTF18*, when combined with that mutation, causes lethality.[8] In a previous issue of *Cell Cycle*,[9] Maradeo and Skibbens investigated whether mutations in one of the shared small subunits, Rfc5, may also affect SCC. Being part of both the Elg1 and Ctf18 complexes, it is unclear a priori what phenotype is to be expected.

A double mutant *rfc5-1 eco1* exhibited viability at temperatures at which the single *eco1* mutant is inviable, demonstrating that the mutation at *RFC5* has an effect similar to deleting *ELG1*. Significantly, additional mutation of *ELG1* did not have a further effect, confirming that in these crosses the Rfc5 defect exerts its phenotype through Elg1, and not through Ctf18. Double mutants *rfc5-1 scc1* or *rfc5-1 smc3-5* show a reduction in viability at low temperatures, at which the single mutants are viable, again showing an *elg1*-like effect. Similar results were obtained when combining *rfc5-1* with Scc2. Notably, in all these crosses, the phenotype of the *rfc5-1* mutant was milder than that obtained in the absence of Elg1. It is unclear at the moment if this is due to the fact that the former is a hypomorphic allele, whereas the latter completely lack protein, or whether these results indicate that Elg1 may have Rfc5-independent functions.

The striking effects observed for a mutation in a subunit that is shared by four different complexes are surprising. The authors propose several mechanisms that may explain the role played by Rfc5: It may directly interact with cohesins or cohesin-deposition factors, or may play a role as sensor to allow recruitment of a specific RFC complex, depending on the cell's needs. Alternatives include the possibility that sub-complexes containing only part of the RFC subunits may play additional roles. Such complexes have been shown to be active in vitro.

This paper contributes to the large number of observations recently published, which require fresh models about the mechanism that establishes cohesion. It is becoming increasingly clear that such a mechanism occurs in multiple, regulated steps, which are tightly coordinated with the passage of the DNA polymerase complex during DNA replication, perhaps by PCNA modification.[7] Further genetic and biochemical evidence is necessary to unravel the complexity of this important step in the cell cycle.

References
1. Skibbens RV. Curr Biol 2009; 19:R1126-32.
2. Beckouet F, et al. Mol Cell 2010; 39:689-99.
3. Borges V, et al. Mol Cell 2010; 39:677-88.
4. Xiong B, et al. Curr Biol 2010; 20:1660-5.
5. Aroya SB, et al. DNA Repair 2005; 4:409-17.
6. Parnas O, et al. PLoS One 2009; 4:e5497.
7. Parnas O, et al. EMBO J 2010; 29:2611-22.
8. Maradeo ME, et al. PLoS One 2009; 4:e4707.
9. Maradeo ME, et al. Cell Cycle 2010; 9:4370-8.