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A feedback loop between mTOR and tRNA expression?

Theodoros Kantidakis & Robert J. White

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Cell Cycle News & Views

A spotlight on regulatory networks connecting EMT and cancer stem cells

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

Jiri Zavadil; NYU Langone Medical Center; New York, NY USA; Email: jiri.zavadil@nyumc.org; DOI: 10.4161/cc.9.15.12628

Cellular plasticity enables profound changes in cell phenotypes governed by tightly orchestrated interplay among distinct functional classes of regulatory molecules. This process typically involves the control of specific gene expression programs with distinct functional impacts on cellular behavior. One such manifestation of remarkable cellular plasticity—epithelial-mesenchymal transition (EMT)—has recently attracted broad interest in the field of cancer research, nearly 30 years after the pioneering work by Elizabeth Hay.[1] EMT is a cell phenotype conversion utilized in tissue remodeling during embryonic development, re-enacted in adult tissue homeostasis (e.g. during regeneration upon injury) and in pathological conditions such as development of invasive and metastatic carcinomas or in fibrotic processes affecting vital organs such as the kidney or lungs.[2,3] Cancer EMT has been recently demonstrated to generate breast carcinoma cancer stem-cells (CSCs) characterized by mesenchymal markers and by the capacity of self-renewal and of initiating secondary tumorigenesis.[4] In addition, the process of EMT involves regulatory molecules (e.g., transcription factors Snail, Slug) that confer resistance to therapeutic induction of cell death.

In a previous issue of *Cell Cycle* features a review by Jonas Fuxe and colleagues, which summarizes the latest findings on select signaling and transcriptional regulation of the overlapping EMT and CSC phenotypes. The authors focus on the cross-talk between signaling pathways that determine (or maintain) stem-cells and their niches and also direct the EMT process. The review elaborates on key pathways at the intersection of EMT and cancer: TGF-β/Smad, Wnt and Ras signaling. Next, the authors review the roles of numerous key transcriptional regulators of EMT and of stem cell phenotype maintenance. Fuxe et al. build on the previous work on the Smad/cofactor complex roles in the target gene specificity and the downstream activation/repression outcomes.[5] The authors themselves significantly contributed to this area of research by publishing an elegant study on the co-repressor role of the SNAI1-SMAD3/4 complex in promoting TGF-β-directed cancer-related EMT via repression of key junctional proteins such as Coxsackie- and adenovirus receptor (CAR), occludin and E-cadherin.[6]

In a previous issue of *Cell Cycle*, the authors review the fine-tuning roles of EMT-promoting Smad complexes (EPSCs) that involve many EMT-associated transcriptional regulators as cofactors of the Smad3/4 binding to regulatory chromatin regions—the transcription factors Snail, Zeb1/2, AP-1, β-catenin, LEF/TCF, Twist, Sp1 and chromatin structure regulators p300, HDACs, HMGA2 and pCAF can be parts of the EPSCs and many of them are also linked to upstream signaling events associated with stem-cell phenotypes. The category of EPSCs is then further structured into subsets that provide essentially two types of downstream action—either repression of epithelial components or activation of mesenchymal markers, both essential for EMT. The review is concluded by insights on inflammatory components within the tumor environment and their roles in EMT, and on tumor stem cell niches and the link between the degree of cell differentiation and invasive and metastatic properties of EMT-generated cancer stem cells.

Historically, the primary focus on EMT in cancer has been aimed at signaling pathways, transcriptional regulators and most recently on the roles of microRNAs.[3] The future research directions will likely involve a broader, more comprehensive search for novel molecular regulators of the process, to define prospective targets for therapeutic interventions. The authors propose that new EPSC downstream of TGF-β signals need to be systematically identified, together with their roles in pathological instances of EMT. Indeed, the rapidly evolving high-throughput quantitative techniques such as next-generation sequencing or quantitative proteomics have the capacity to generate catalogs of new EPSC components and to determine the dynamics of their physical distribution on chromatin during EMT, together with chromatin structure changes. With these high-throughput quantitative tools, the aberrant post-transcriptional control of gene expression involving deregulation of microRNAs, mRNA processing and turnover (such as deregulation of differential splicing or of RNA nonsense-mediated decay) will also warrant close attention, together with abnormalities in post-translational protein modifications and in the process of protein turnover. The big picture of the nature and importance of the EMT regulators in tumorigenesis has been already rapidly emerging. The current review by Fuxe et al. is a very timely and focused summary of this exciting and important area of research.

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p38α as an inducer of aneuploidy in p53$^{-/-}$ tetraploid cells

Comment on: Vitale I, et al. Cell Cycle 2010; 9:2823-9.

Laura Carrassa; Istituto di Ricerche Farmacologiche; Milan, Italy; Email: laura.carrassa@marionegri.it; DOI: 10.4161/cc.9.15.12629

Maintenance of genomic stability is fundamental for cells to survive to many rounds of division during their lifetime. To coordinate cell cycle events, surveillance mechanisms known as cell cycle checkpoints have evolved, ensuring that replication and cell division occur correctly. The oncosuppressor p53 plays a crucial role in this process. Depending on the cellular context, transactivation of p53 can stimulate cell cycle arrest in different cell cycle stages as well as apoptosis.[1] It also has a crucial role in the G_1 tetraploidy checkpoint by preventing the survival and propagation of tetraploid cells.[2] Tetraploidy represents an important intermediate between diploidy and aneuploidy and is a common feature of human cancer. Therefore the molecular mechanisms through which tumor cells proceed from diploid to aneuploid are extremely important in cancer research. Tetraploidy can arise when cells aberrantly exit from mitosis, after failure of spindle assembly, chromosome segregation or cytokinesis.[3] The G_1 arrest of tetraploid cells generated by spindle checkpoint failure or by failure of cytokinesis is p53 dependent through the induction of p21.[2] It has been recently described that Chk1 inhibition, which causes spindle checkpoint defects and mitotic aberrations, induces a p53 dependent G_1 tetraploid block.[4,5] The induction of tetraploid cell death triggered by inhibition of Chk1 requires p38α MAPK, which acts as a p53 kinase activated by Chk1 depletion.[6] p38α MAPK is involved in the responses to a variety of stress agents regulating DNA damage repair, cell cycle arrest and apoptosis.[7] In undamaged cell cycle p38α has a role in controlling the entry of cells in mitosis through negative regulation of Cdc25B.[8] The fact that p38 MAPK positively regulates several tumor suppressor (i.e., p53) pathways and attenuates oncogenic (i.e., Cdc25B phosphatase) signals assigns to this protein a role of oncosuppressor.[7] In addition p38α, acting as a p53 kinase in Chk1 depleted tetraploid cells, is fundamental for blocking the re-replication of tetraploid cells, and plays an active part in the maintenance of genomic stability. However, Vitale et al. describe a new role of p38α in controlling proliferation of p53$^{-/-}$ tetraploid cells, in contrast with its function in preventing aneuploidy and avoiding genomic instability.[9] After having generated tetraploid clones from diploid p53$^{+/+}$ and p53$^{-/-}$ HCT-116 cells, they found that p53$^{-/-}$ tetraploid cells showed an increase in phosphorylation of p38α. They noted too that p38α hyperphosphorylation accumulates in mitotic tetraploid cells and behaves like a mitotic passenger protein by localizing in centrosomes in metaphase and in midbody in telophase. To understand the significance of phospho-p38α in mitotic progression, siRNAs or chemical agents were used to inhibit p38α in both p53$^{+/+}$ and p53$^{-/-}$ tetraploid cells. There was a consistent effect in the cell cycle profile in p53$^{-/-}$ tetraploid cells; p38α inhibition resulted in a partial block in the G_2/M phase associated with activation of the spindle assembly checkpoint, which raised the mitotic index and reduced the anaphase-telophase shift. p38α depletion increased monopolar and bipolar metaphases and reduced multipolar mitoses which were common among unstable p53$^{-/-}$ tetraploid cells, generating an aneuploid population (with a near to diploid DNA content). Taken together these results highlight that p38α is a major player in the mitotic progression of p53$^{-/-}$ tetraploid cells and indicate a novel function of p38α in the division of aneuploid cells. According to these data in a p53$^{-/-}$ background p38α would act as promoter of tetraploid cell division, inducing aneuploidy and genomic instability instead of preventing it. It would be interesting to investigate the pathway that in the p53$^{-/-}$ context is involved in the hyperphosphorylation of p38α, contributing to its oncogenic role. These authors in a recent paper showed that in the same p53$^{-/-}$ tetraploid cell system the oncogene MOS is upregulated and is required for multipolar division.[10] MOS may therefore have a part in inhibiting centrosomes coalescence. As both MOS and phosphorylated p38α are more expressed in unstable p53$^{-/-}$ tetraploid cellular clones and can associate with centrosomes, hypothetically the two proteins might reside in the same molecular pathway negatively controlling the ability of supernumerary centrosomes to form clusters, thus increasing aneuploid cell division. Specifically in tumors with this molecular feature (p53$^{-/-}$ tetraploid) p38α may be considered as a target to inhibit to prevent aneuploidy.

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Overeating yeast display fatty acid-induced necrotic cell death

Comment on: Rockenfeller P, et al. Cell Cycle 2010; 9:2836-42.

Paula Ludovico; University of Minho Life and Health Sciences Research Institute; Braga, Portugal; Email: pludovico@ecsaude.uminho.pt;
DOI: 10.4161/cc.9.15.12630

Excess dietary free fatty acids (FA) contribute substantially to obesity and linked metabolic disorders. Overload of the cellular lipid homeostatic mechanisms that normally protect against excess FA leads to the accumulation of lipids in non-adipose tissues, culminating in cell dysfunction and death. This phenomenon, known as lipotoxicity, is believed to be a contributing factor in the etiology of non-insulin dependent diabetes mellitus, cardiomyopathy and other obesity-mediated metabolic disorders.[1] Accumulating evidence points to a close relationship between perturbed lipid homeostasis and endogenous cell death programs, particularly apoptosis.[1] An imbalance of mitochondrial cardiolipin, disruption of mitochondrial function, reactive oxygen species (ROS) generation and endoplasmic reticulum stress are among the frequently described pro-apoptotic effects attributed to saturated fatty acids (SFA) which are also precursors for the production of pro-apoptotic ceramides.[2] Monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), although apparently not as cytotoxic as SFA in mammals, are also now recognized to be part of the repertoire of cellular assassins.[3] However, the multiple cellular mechanisms determining the FA-induced cell death process that depend both on the FA chain length and saturation remain largely unknown.

The novel findings described by Rockenfeller and coworkers in a previous issue of *Cell Cycle*[4] correlate the higher levels of PUFA present in common nutritional cooking oils with increasing sensitivity of wild type yeast cells in the presence of lipases, which mimics the environment of the small intestine. Since the first reports on yeast apoptosis,[5-7] yeast has been established as a powerful model to study the mechanisms and the phylogenetically conserved pathways of programmed cell death.[8] It has been argued that free FA induces apoptosis in both mammalian cells and fission yeast,[1,9] and it has been suggested to induce apoptosis in budding yeast as well.[10] However, challenging the common belief that FA only elicit apoptosis, the results of this study establish that yeast cell death induced by FA is mediated by a necrotic pathway that results in a high percentage of cells with reduced plasma membrane integrity and high ROS generation without phosphatidylserine exposition. Rockenfeller and coworkers strengthened these initial findings using a yeast quadruple mutation (QKO) that genetically ablates the yeast neutral lipid biosynthetic pathway which, combined with the exogenous addition of unsaturated fatty acids (UFA), models acute lipotoxicity.[10] The authors' analysis of the effects of oleic (OA), linoleic (LA) and linolenic (L3A) acids on QKO mutant cells establishes a link between the degree of unsaturation of FA and the primary activation of a necrotic cell death program determined by annexinV/propidium iodide staining and ROS production. Their findings suggest that the level of FA saturation/unsaturation could be a crucial modulator of cell death mode and intensity. The necrotic cell death pathway triggered by UFA is shown to be dependent on mitochondria and most importantly to be associated with the nuclear release of Nhp6Ap, the ortholog of HMGB1, which is a bona fide hallmark of necrotic cell death.[8]

In summary, taking advantage of the conservation of the regulatory processes relevant to establishing cell death and lipid homeostasis in yeast, the authors provide important insights into the specific molecular mechanisms leading to FA-induced cell death, which are still controversial. This study is important because it describes several new groundbreaking insights into lipotoxicity. Not only has it highlighted the potential deleterious effects of PUFA, a major component of nutritional cooking oils, on human health but it also challenges the dogma that lipotoxicity is always related to apoptosis by showing that common dietary UFA leads to a distinct cell death fate, necrosis. The discovery that FA induces necrosis will have potential clinical implications related to the pathophysiological effects of eliciting a pro-inflammatory response in the context of lipotoxicity.

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Fighting of Casein kinase 1 and PP2A/Shugoshin for cohesins during meiosis I

Comment on: Rumpf C, et al. Cell Cycle 2010; 9:2657-62.

José A. Suja; Unidad de Biología Celular; Universidad Autónoma de Madrid; Spain; Email: jose.suja@uam.es; DOI: 10.4161/cc.9.15.12696

Meiosis is a specialized cell division that generates haploid gametes from diploid precursor cells. This is accomplished by two successive rounds of chromosome segregation (meiosis I and meiosis II) after a single round of DNA replication. During prophase I, the homologs, each with two sister chromatids, pair and recombine to form chiasmate bivalents with four chromatids. In metaphase I bivalents, in spite of recombination, segments of sister chromatids remain tightly associated by multisubunit cohesin complexes at the arms, and also at centromeres (**Fig. 1**). Moreover, sister kinetochores must be closely associated to operate as a single functional unit per homolog and then permit the accurate biorientation of bivalents at the metaphase I plate (ref. 1). During the metaphase I/anaphase I transition,

the enzyme separase cleaves the meiosis-specific cohesin's α-kleisin subunit Rec8 along chromosome arms, but not at centromeres, to allow the segregation of recombined homologs to opposite poles. These segregating chromosomes are thus composed of two chromatids that are only associated at their centromeres. The protected cohesin complexes at centromeres are then cleaved by a new round of separase activity during the metaphase II/anaphase II transition (ref. 1).

During recent years it has been demonstrated that centromeric cohesin complexes are protected against degradation by separase during anaphase I by a complex formed by shugoshin (Sgo)/MEI-S332 family members and a phosphatase 2A (PP2A) that is recruited to centromeres (ref. 2 and 3). Different experiments in yeast have revealed that the phosphorylation of Rec8 enhances its cleavability by separase during meiosis,[4] as occurs with its paralog Rad21/Scc1 during mitosis.[5] In this sense it has been shown that the Polo-like kinase phosphorylates Rad21/Scc1 in both yeast[5] and vertebrate mitotic cells (ref. 6). Although it has been suggested that Polo-like kinase could also phosphorylate Rec8 during yeast meiosis (ref. 4), the identity of the kinase(s) responsible for its phosphorylation and then promoting its cleavage by separase remained to be determined.

In a previous issue of *Cell Cycle*, Rumpf et al. reports that in fission yeast Casein kinase 1δ/ε isoforms (CK1δ/ε) are required for Rec8 phosphorylation and then for its efficient removal during meiosis I.[7] The authors first tried to identify the phosphorylated residues of Rec8 around metaphase I by mass spectrometry. They found that up to 17 serine/threonine residues appeared phosphorylated along Rec8. Thereafter, these residues were mutated to alanine (Rec8-17A), which can no longer be phosphorylated, to analyze meiotic chromosome segregation. Surprisingly, cells only showed a mild non-disjunction phenotype. However, when they analyzed chromosome segregation in a mutant version of Rec8-17A with one of the two separase cleavage sites also mutated (Rec8-17A-RD1), chromosome segregation was greatly inhibited. Taking into account this result the authors suggest that, since cleavage of Rec8 by separase at either of two cleavage sites is sufficient for Rec8 degradation, Rec8 phosphorylation is needed to allow an accurate chromosome segregation during meiosis I.

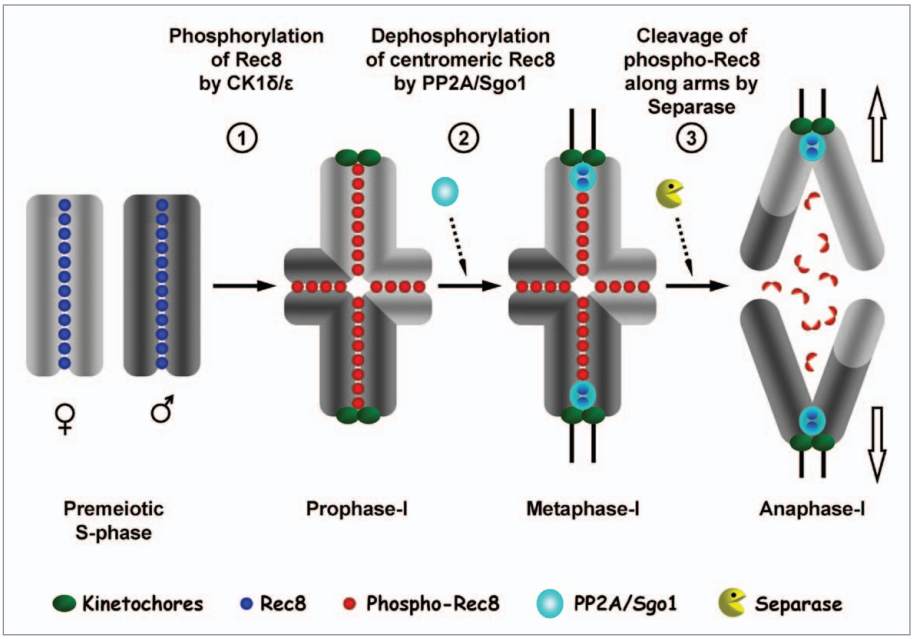

Figure 1. Model of regulation of the phosphorylated state of the cohesin subunit Rec8 at chromosome arms and centromeres, and its differential cleavage by separase during yeast meiosis I.

The authors had previously reported that mutants for Hrr25 and Hhp1/Hhp2, the orthologs of CK1δ/ε in budding and fission yeast respectively, showed abnormal chromosome segregation during meiosis I.[8] With this in mind, they then investigated whether the in vivo phosphorylation of Rec8 was dependent on Hhp1 and Hhp2. The results indicated that in double mutants for these isoforms Rec8 was not phosphorylated and high levels of Rec8-GFP were present during anaphase I. Thus, the authors conclude that Hhp1 and Hhp2 are required for the phosphorylation of Rec8 and for its efficient cleavage from chromosome arms by separase during anaphase I. Accordingly, they found that Hhp1 and Hhp2 tagged with GFP colocalized with Rec8. The authors thus suggest that Rec8 is phosphorylated by CK1δ/ε from premeiotic S-phase up to prophase I all along the chromosome arms and also at centromeres. The subsequent recruitment at centromeres of PP2A/Sgo1 during late prophase I would allow the dephosphorylation of centromeric Rec8. Then, once separase is activated, it would only cleave phosphorylated Rec8 along the arms to trigger the onset of anaphase I **(Fig. 1)**. This same model is also supported by two very recent reports conducted on fission[9] and budding[10] yeast meiosis I. Although the mechanisms controlling the phosphorylated state of Rec8 at the arms and centromeres in yeasts are probably evolutionarily conserved, they need to be validated in other animal and plant model systems.

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PML promotes senescence via JAK/STAT signaling

Comment on: Hubackova S, et al. Cell Cycle 2010; 9:3085-99.

Alwin Krämer; University of Heidelberg; Heidelberg, Germany; Email: a.kraemer@dkfz.de; DOI: 10.4161/cc.9.15.12697

Cellular senescence limits the proliferation of damaged cells that are at risk of neoplastic transformation by imposing an essentially irreversible growth arrest.[1] Traditionally, senescence has been regarded as a strictly intracellular response with the entire signaling taking place within the boundaries of the cell. However, recently senescent cells have been found to secrete a complex mixture of inflammatory proteins and mediators of extracellular matrix remodelling, including key components of the Wnt, IGF1, TGFβ, plasmin and interleukin signalling cascades.[2-4] Current knowledge suggests that signals from these cascades are integrated at the level of the plasma membrane to reinforce the senescence arrest by autocrine or paracrine mechanisms.

The PML protein is an important positive regulator of cellular senescence and a tumor suppressor commonly lost in human cancer.[5] The protein epitomizes the PML nuclear body and is crucially required for its proper assembly. Despite varying PML steady-state levels, in most cell types the number and size of PML nuclear bodies increases in response to soluble factors and cellular stress. Data from many groups clearly show that PML nuclear bodies are involved in the cellular response to DNA damage. One attractive model suggests that PML nuclear bodies mark sites of irreparable DNA lesions and promote signalling to checkpoint complexes.

It has been long kown that PML expression is transcriptionally induced by interferons in various biological settings.[6] However, during oncogene-induced senescence, an increase in number and size of PML nuclear bodies has been described to be caused by p53-mediated transcriptional upregulation of PML.[7] As PML has also been shown to function upstream of p53 in inducing senescence and apoptosis, these data point to the presence of a positive feedback loop.

In spite of this wealth of information, the mechanisms of PML induction during cellular senescence and the links between senescence and senescence-associated cytokine secretion on the one hand and PML expression on the other hand are far from being understood. Clarifyingly, in the current issue of *Cell Cycle,* Hubackova and coworkers now report that genotoxic drugs that induce senescence turn on PML transcription by JAK/STAT-mediated activation of an Interferon Stimulated Response Element (ISRE) within the PML gene promoter.[8] Using a panel of different normal and cancer cell lines they first show that diverse DNA-damaging drugs which are known to induce senescence lead to elevation of the number of PML nuclear bodies as well as PML transcript and protein levels. Importantly and in contrast to what has been reported earlier, this response could be attributed to enhanced PML transcription rather than protein stabilisation. As the PML gene promoter binds transcription factors of the STAT family which are activated by cytokine-stimulated JAK kinases, Hubackova et al. went on to demonstrate that inhibition of JAK signaling via both chemical inhibitors and siRNA prevents PML expression in response to genotoxic drugs. Subsequent gel shift analyses demonstrated the presence of ISRE binding activity in nuclear extracts from DNA-damaging drug-treated senescent cells, thereby clearly implicating the Interferon Stimulated Response Element in genotoxic stress-induced PML transcription. Experiments with both p53-negative cells and a dominant-negative form of p53 showed that transcriptional induction of PML in response to genotoxic stress was independent of p53.

As previously shown for ionizing radiation already, data presented by Hubackova and coworkers confirm that persistent but not acute DNA damage foci induced by genotoxic drugs colocalize with PML nuclear bodies. Since the RecQ helicase BLM was sequestered into these foci together with PML bodies, these results seem to corroborate earlier suggestions on a repressive effect of PML nuclear bodies on DNA repair.

Altogether, the findings reported in this study suggest that the expansion of PML nuclear bodies after genotoxic stress commonly occurs during cellular senescence and is attributable to p53-independent transcriptional activation of PML expression. Most importantly, PML transcription in response to DNA damage is driven by JAK/STAT signaling. Therefore, these findings for the first time make the link between senescence-associated cytokine secretion induced by genotoxic drugs and increased PML expression. Whether or not enhanced PML nuclear body formation during drug-induced senescence serves to sustain the senescence arrest of persistently damaged cells certainly constitutes a rewarding field of further research.

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Cyclin independent role for cdk4/6 during B-cell lymphoma survival

Comment on: Gumina MR, et al. Cell Cycle 2010; 9:446-7.

E. Charles Snow; University of Kentucky Medical Center; Lexington, KY; Email: ecsnow01@uky.edu; DOI: 10.4161/cc.9.15.12708

Cyclin dependent kinases (cdks) are a family of serine/threonine kinases whose activity at discrete cell cycle stages is required for cells to successfully progress into and through the cell cycle. As befits enzymes with such crucial cellular consequences, the cdks are subjected to multiple levels of regulation which include acquisition of a cyclin partner, both positive and negative phosphorylation, and the actions of cyclin dependent kinase inhibitors (cdkis). Two members of this kinase family, cdk4 and cdk6, are essential for movement into and through G1, the cell cycle stage controlled by mitogenic signals capable of driving cells into the cell cycle. Cdk4 and 6 utilize D-type cyclins whose accumulation is likewise regulated by the same external signals. As cycling cells complete the growth factor dependent phase they commit to S phase by activating cdk2 partnered initially with cyclin E. D-type cyclin/cdk4/6 and cyclin E/cdk2 promote cycle progression in large part by regulating the E2F family of transcriptional activators and repressors through phosphorylation of Rb family members.

It is not surprising that cell cycle related proteins operative during G1 to S phase progression are found to undergo dysregulation during transformation of mammalian cells, including B cell lymphomas such as diffuse large B cell lymphomas (DLBCL). Included within the diagnostic category of DLBCL are B cell lymphomas that differ in causative genetic defects and clinical outcomes.[1] These diverse lymphomas can be subdivided into two broad subsets referred to as activated B-cell-like (ABC) DLBCL and germinal centre B-cell-like (GCB) DLBCL, and the B cell lymphomas within each subset are found to display similar biological and clinical features. For example, ABC DLBCL exhibit chronic signaling through their B cell antigen receptors (BCR) while GCB DLBCL do not[2] and targeting the BCR-induced signaling pathways are found to kill only ABC DLBCL. However, a 6p21 amplification responsible for elevated accumulation of cyclin D3 is found in both DLBCL subsets.[3] In a previous issue of *Cell Cycle*, Gumina and colleagues address possible functional consequences of cyclin D3 over expression in DLBCL. For this purpose they select for study the human OCI-LY18 cell line which is derived from a GCB DLBCL. LY18 cells express cyclin D3, cyclin E, cdk2, 4 and 6 and are devoid of cyclin D2 and D3 and the cdkis p21, p27 and p16. The authors indirectly show the presence of active cyclin D3/cdk4 and 6 and cyclin E/cdk2 complexes by showing that Rb is phosphorylated at both cdk4/6 and cdk2 sites. The use of siRNA mediated knock down demonstrates that survival and expansion of LY18 cells along with full phosphorylation of Rb occurs in the absence of either cyclin D3 or cyclin E. This result is not surprising since mammalian cells are know to proliferate normally in the absence of either D-type cyclins[4] or cyclin E[5] exemplifying redundancies that serve as protective mechanisms in key cell cycle events. More interestingly, they find that although single knock downs of cdk4, cdk6 or cdk2 are without affect upon LY18 cell viability and expansion, the double knock down of cdk4 and cdk6 results in reduction of proliferation and an accumulation of viable cells in the G1 cell cycle stage. The first important implication of these findings is the identification of cdk4 and 6 as potential therapeutic targets for DLBCL and this is particularly intriguing based on the availability of inhibitors selective for both cdk4 and cdk6.[6]

The data provided by Gumina and colleagues open a second, and perhaps even more intriguing possibility that cdk4 and cdk6 exert cyclin independent functions critical for LY18 expansion. The LY18 cells are able to proliferate normally in the absence of cyclin D3 (without compensatory expression of either cyclin D1 or D2) and in the absence of cyclin E or cdk2 as long as they continue to express cdk4 and/or cdk6. Roles for cell cycle related proteins outside their normal activities are being uncovered. Of interest in this context is the demonstration that cdk6 enhances the transcriptional activity of androgen receptors in prostate tumor cells independent of the need for a D-type cyclin partner.[7] In fact, the presence of the cyclin partner inhibits this enhancement. Is it possible that the reason cdk4 and cdk6 represent such an attractive therapeutic target in DLBCL is distinct from what we appreciate as the traditional role for these kinases during G1 progression?

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Transcription elongation takes central stage: The P-TEFb connection

Comment on: Moiola C, et al. Cell Cycle 2010; 9:3119-26.

B. Matija Peterlin; University of California at San Francisco; San Francisco, CA USA; Email: matija.peterlin@ucsf.edu; DOI: 10.4161/cc.9.15.12698

Lost between the control of initiation of eukaryotic transcription and effects of chromatin, how RNA polymerase II (RNAPII) elongates and regulates co-transcriptional processing of nascent transcripts, such as splicing and polyadenylation, represented a rather neglected field of investigation. This slight was due mostly to transcriptional studies in vitro, where this regulation was not observed due to the loss of negative and positive elongation factors during the preparation of nuclear extracts. Only whole cell extracts established the importance of post-initiation events, such as RNAPII pausing and release after appropriate signals had been received.[1] Importantly, recent whole genome analyses (WGAs) confirmed that this step in transcription is critical for the expression of most eukaryotic genes.[2]

A great deal of what we know about the control of elongation of transcription came from studies of the human immunodeficiency virus (HIV), which encodes a strong transcriptional transactivator (Tat). Tat not only binds an RNA structure, the transactivation response (TAR) element, but also affects only the elongation step of viral transcription. Nevertheless, in the absence of Tat, abundant RNAPII is already present on the HIV promoter, which leads to the synthesis of short capped but not polyadenylated transcripts that contain TAR.[3] Stalled RNAPII and short transcripts have now been found with most inactive and inducible genes in a variety of cells and organisms. Studies on RNAPII stalling also revealed the negative transcription elongation factor (N-TEF), which contains the DRB-sensitivity inducing factor (DSIF) and negative elongation factor (NELF). They arrest RNAPII near the 5′ end of genes.[4] Next, Tat affinity chromatography revealed its co-activator, the positive elongation factor b (P-TEFb), which is composed of C-type cyclins T1 or T2 (CycT1 or CycT2) and the cyclin dependent kinase 9 (Cdk9).[5] Importantly, Tat recruits P-TEFb to TAR, where Cdk9 phosphorylates serines at position 2 (Ser2) in the C-terminal domain (CTD) of RNAPII and subunits of N-TEF, thus converting DSIF to an elongation factor and removing NELF from double stranded RNA. Although serines at position 5 (Ser5) in the CTD had already been phosphorylated by Cdk7 in TFIIH, which allows for efficient capping of mRNA species, the extensive phosphorylation of Ser2 in the 52 heptad repeats (YSPTSPS) of the human CTD displaces the Mediator complex and recruits the chromatin remodeling, splicing and polyadenylation machineries to the now elongating RNAPII.[4] Thus, P-TEFb reverses effects of N-TEF and insures proper co-transcriptional processing of nascent transcripts.

P-TEFb itself is regulated tightly in cells (**Fig. 1**).[4] It is found in an inactive large complex (LC), where Hexamethylene bisacetamide (HMBA) induced proteins (Hexim1 and Hexim2, Hexim1/2) inhibit the kinase activity of Cdk9 and an active small complex (SC) with Bromo domain-containing protein 4 (Brd4), the Mixed Lineage Leukemia (MLL) complex of elongation factors and/or a plethora of transcriptional activators, such as cMyc and NFκB.[2,4,6] Hexim proteins only inactivate P-TEFb when bound to 7SK snRNA, an abundant non-coding product of RNAPIII. Ratios between inactive and active P-TEFb as well as levels of these C-type cyclins affect the state of proliferation and differentiation of cells. For example, HMBA terminally differentiates leukemic cells by increasing the synthesis of Hexim proteins, which inhibit P-TEFb. 7SK snRNA is itself capped by methylphosphate capping enzyme (MePCE) on its 5′ end and stabilized by La related protein 7 (LaRP7) on its 3′ end.[4] Without these proteins, 7SK snRNA is degraded rapidly and active P-TEFb is released in cells. Thus, it is

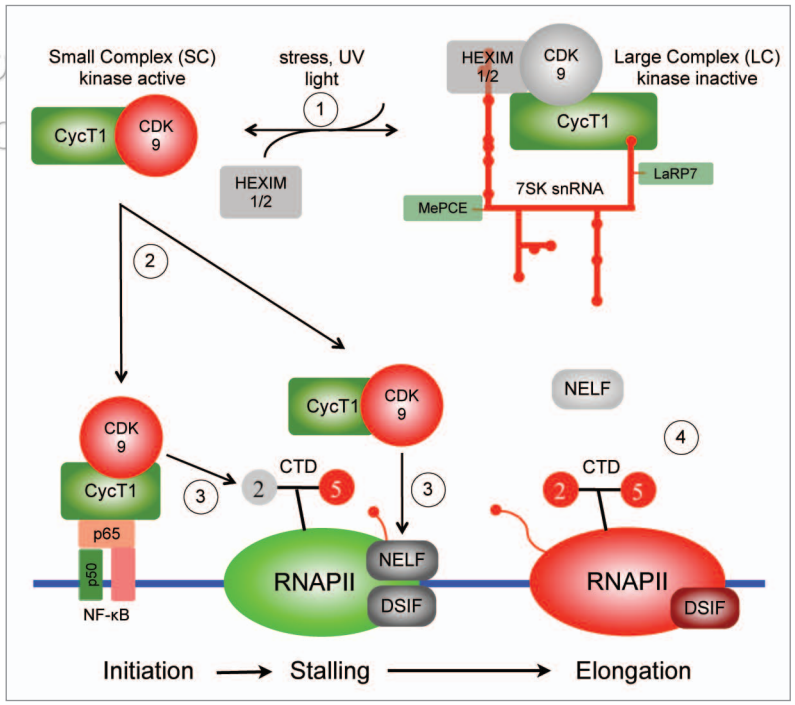

Figure 1. P-TEFb and transcriptional elongation. P-TEFb (CycT1:Cdk9) is found in an inactive large complex (LC) and active small complexes (SC). LC contains two copies of Hexim1/2. MePCE and LaRP7 stabilize 7SK snRNA. Stress, UV light and other stimuli release the active P-TEFb (SC) from the LC (1). P-TEFb is then recruited to transcription units via activators such as NF-κB and cMyc, Brd4 as well as MLL complexes (2). Initiating RNAPII stalls and is phosphorylated on Ser5, which facilitates capping. P-TEFb then phosphorylates Ser2 in the CTD as well as subunits of NELF and DSIF (3). NELF dissociates from RNAPII and DSIF is converted to an elongation factor (4). Highly phosphorylated RNAPII elongates with the chromatin remodeling, splicing and polyadenylation machineries and co-transcriptional processing ensues. P-TEFb can also travel with RNAPII.

not surprising that many cervical, gastric and breast cancers disregulate the LaRP7 gene.[7]

Why would increased levels of P-TEFb lead to malignant transformation? First of all, effects of cMyc and NFκB will be amplified.[2] Levels of cell cycle cyclins, such as CycD1 will increase, since flavopiridol, an inhibitor of P-TEFb, has the opposite effect.[8] Next, a number of anti-apoptotic and early response genes will be induced, some of which code for potent growth factors.[9] Finally, as suggested by this report, the Rb/E2F1 pathway will be disregulated. Further WGAs will reveal additional targets. Importantly, inhibiting P-TEFb should be considered for future therapies of cancer, inflammation and hypertrophic diseases.

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A feedback loop between mTOR and tRNA expression?

Comment on: Huynh LN, et al. Cell Cycle 2010; 9:3112-8.

Theodoros Kantidakis and Robert J. White; Beatson Institute for Cancer Research; Bearsden, Glasgow;
Email: tkantidakis@googlemail.com and r.white@beatson.gla.ac.uk;

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The mTORC1 complex contains mTOR kinase and is known to control the rate of protein synthesis and thereby cell growth in response to nutrient availability.[1] It uses several mechanisms to achieve this, the best understood of which is the direct phosphorylation of 4E-BP, an inhibitor of translation initiation.[1] Levels of tRNA can be rate-limiting for translation in some cell types.[2] Control of tRNA expression might therefore provide an additional potent means to influence protein production.

A flurry of recent papers have established that tRNA synthesis in mammalian cells is controlled by mTORC1.[3-6] This is consistent with previous working establishing similar control of tRNA gene transcription by TORC1 in budding yeast.[7] In both humans and yeast, control is mediated through a repressor called Maf1 that binds and inhibits RNA polymerase III, the enzyme responsible for transcribing tRNA genes. Inactivation of Maf1 by mTORC1 may be direct, as recombinant Maf1 can be phosphorylated in vitro by a recombinant fragment of mTOR or the mTORC1 complex immunoprecipitated from human cells.[5,6] One of the target residues is serine 75, a site that is phosphorylated in vivo and which controls Maf1 function.[4-6]

Inactivation of the transcriptional repressor Maf1 by mTORC1 is strongly reminiscent of its effect on the translational repressor 4E-BP, which binds and inhibits the mRNA cap-binding factor eIF4E.[1] The analogy is strengthened by the fact that in both cases mTORC1 is localised to a nucleic acid template, allowing precise targeting to its substrate. Thus, mTORC1 is recruited to certain transcripts by the sequence-specific mRNA-binding factor SF2/ASF, allowing it to act directly to release 4E-BP.[8] Similarly, mTORC1 is detected at tRNA and 5S rRNA gene promoters, which it can free from Maf1-mediated repression.[3-5] It can be targeted to these sites by the DNA-binding transcription factor TFIIIC, which contains a TOR signalling (TOS) motif like the one present in 4E-BPs.[5] Point mutation of the TOS motif in TFIIIC compromises its association with mTORC1 in vivo.[5]

Huynh et al. have found evidence to suggest that mTORC1 responds to tRNA.[9] This raises the possibility of a feedback loop between tRNA levels and mTORC1 activity (**Fig. 1**). Their discovery is based on the effect of RNAi-mediated depletion of Xpo-t, a karyopherin responsible for export of tRNA from the nucleus into the cytoplasm. When Xpo-t levels are reduced in human fibroblasts, a decrease is observed in the phosphorylation of well-characterised mTORC1 target sites, including phosphoacceptors in 4E-BP.[9] This correlates with the expected nuclear accumulation of tRNA.[9] On the basis of this observation, the authors propose a model in which tRNA contributes to homeostasis by modulating signaling pathways that protect against nutrient limitation. Indeed, they also found that Xpo-t depletion from fibroblasts promotes autophagy, a key survival mechanism under starvation conditions.[9] This response may be ancient, as autophagosomes accumulate in a yeast strain deleted for the tRNA-transporting karyopherin LOS1.[9] A caveat to this appealing model is that the authors have manipulated the karyopherins, rather than tRNAs themselves; the possibility therefore remains that the observed responses are triggered by some tRNA-unrelated function of the karyopherins, that may yet be undiscovered. Nevertheless, the hypothesis that cells

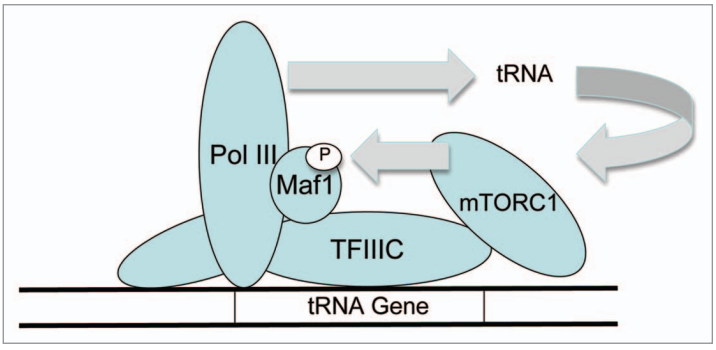

Figure 1. Model in which tRNA expression influences the activity of mTORC1 through some undefined mechanism. In turn, mTORC1 can stimulate synthesis of tRNA by phosphorylating and inactivating Maf1, a repressor of RNA polymerase III. This may occur in the nucleus, where mTORC1 associates with TFIIIC, a transcription factor that binds to tRNA gene promoters. Indeed, mTORC1 can be crosslinked in vivo to tRNA genes.

respond to tRNA as well as to amino acids is exciting and attractive. The TORC1 pathway is ideal to integrate inputs from such signals. Inappropriate levels or localization of tRNA might in this way influence both the activity and production of the translation apparatus, including synthesis of tRNAs themselves. Feedback control of tRNA production would be especially important in cells in which the rate of translation is limited by tRNA availability.[2]

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P-TEFb joins the family of cdks in oncology, promotes cell growth of cancer cells

Comment on: Moiola C, et al. Cell Cycle 2010; 9:3119-26.

Nora M. Navone; University of Texas MD Anderson Cancer Center; Houston, TX USA; Email: nnavone@mdanderson.org; DOI: 10.4161/cc.9.15.12738

Cyclin-dependent kinases (Cdks) are a family of serine/threonine protein kinases involved in cell cycle control and/or regulation of transcription. The enzymatic activity of the Cdks depends on their binding to a regulatory cyclin subunit. Until now, current evidence had indicated that positive transcription elongation factor b (P-TEFb), a heterodimer consisting of Cdk9 and one of the C-type cyclins (T1, T2a, T2b, or K), does not directly regulate the cell cycle.[1] Indeed, the main function of Cdk9 consists of regulating transcription via phosphorylation of the RNA polymerase II (polII) carboxyl terminal domain.[2]

In this issue of *Cell Cycle*, an article by Moiola et al. demonstrates for the first time that cyclin T1, one of the most recently described members of the family of cyclins,[3] induces the transformation of NIH 3T3 cells in vitro and tumorigenesis in vivo. Their studies also suggest that P-TEFb induces NIH 3T3 cell proliferation by CDK4-mediated Rb phosphorylation. Thus, these findings identify cyclin T as an oncogene and suggest that Cyclin T plays a role in controlling the cell cycle, probably via the Rb/E2F1 pathway, previously unknown roles of P-TEFb.

Of note, Moiola and colleagues also report that PTEFb is overexpressed in human head and neck tumors as compared with its expression in normal tissue, thus implicating PTEFb for the first time in the pathogenesis of head and neck cancers. Head and neck cancers account for about 3% of malignancies in the United States,[4] and although advances in therapy in the last decade have provided better local control of advanced cancers, the improvements in overall survival have been modest.[5] In conjunction with early detection, the identification and use of biomarkers of therapy response and disease progression may facilitate disease management. Specifically, these biomarkers could guide in the identification of precancers that could progress to invasive lesions or those that could respond differently to various therapies. The report by Moiola et al. in this issue thus introduces a new player that could be used to bring us closer to individualized disease management for head and neck cancers.

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