

The Cell Cycle Revisited: Cdk1/cyclin E Complexes Regulate the G1/S Phase Transition of the Cell Cycle *in vivo*

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The transition between phases of the cell cycle is regulated by cyclin-dependent kinases (Cdks) and their cyclin regulatory subunits. In yeast, a single Cdk is able to regulate diverse cell cycle transitions by associating with multiple stage-specific cyclins. The evolution of multicellular organisms brought additional layers of cell cycle regulation in the form of numerous Cdks, cyclins and Cdk inhibitors to reflect the higher levels of organismal complexity, but how many of these cyclin-Cdk complexes are essential? According to the current model of mammalian cell cycle regulation, Cdk2/cyclin E was thought to be the only Cdk, which controls entry into S phase. Cdk2/cyclin A controls progression through S phase and transition into G2 phase. However, mice lacking Cdk2 develop normally and *Cdk2*^{-/-} MEFs show minimal defects in proliferation and some delay in S phase entry. The surprising fact that the *in vivo* loss of Cdk2 has no effect on mitosis raises two important questions: (1) which kinase compensates the loss of Cdk2 and regulates S phase entry *in vivo*? (2) Considering that Cdk2 is the only known kinase, which binds cyclin E, what is the function of cyclin E in the absence of Cdk2? A major inhibitor of Cdk2 is p27, which is also a tumor suppressor. Overexpression of p27 results in G1 arrest in cell lines. *p27*^{-/-} mice have enlarged body size, increased cell proliferation, pituitary gland tumors, and a 10-fold increase in Cdk2 activity in thymocytes. These findings support the role of Cdk2 as a major target of p27. In this study, we hypothesized that loss of Cdk2 should modify the *p27*^{-/-} mouse phenotypes. We generated *p27*^{-/-}*Cdk2*^{-/-} double knockout (DKO) mice and found no functional complementation in these mice. One hundred percent of the female DKO mice develop ovary tumors, which are not found in *p27*^{-/-} mice. Interestingly, we observed elevated levels of S phase and mitosis in tissues of DKO mice similar to that observed in *p27*^{-/-} tissues. Cdc2 activity was significantly elevated in DKO extracts and p27 binds to Cdc2, cyclin B1, cyclin A2, or sucl complexes in wild-type (WT) and *Cdk2*^{-/-} extracts. We also observed that cyclin E binds and activates Cdc2 and cyclin B1 binds Cdk2. Therefore, both cyclin E and p27 regulate Cdc2 *in vivo*. We silenced Cdc2 using shRNA in *Cdk2*^{-/-} and WT MEFs, measured proliferation and S phase entry after quiescence. We found *Cdk2*^{-/-} MEFs expressing shCdc2 proliferate at a lower rate and enter into S phase with delayed kinetics, compared to the WT MEFs with shCdc2. Our results uncover a critical role for Cdc2 in the regulation of G1/S transition and provide evidence that Cdc2 compensates the loss of Cdk2 function. We suggest a new cell cycle model with high levels of redundancy among the molecular players.

ATP-dependent remodeling by ISWI- containing complexes

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ATP-consuming nucleosome remodelling machines are indispensable for the functioning of eukaryotic genomes. They contribute to the assembly and maintenance of chromosomes, the detection and repair of DNA damage, the replication of the genomes, the recombination of chromosomal segments as well as faithful execution of gene expression programs. Nucleosome remodeling ATPases reside in large multiprotein complexes in association with regulators and targeting subunits. Their deregulation frequently correlates with malignant tumour phenotypes. Subunits of the human BRG1 complex are tumour suppressors and the remodelling ATPase SNF2H is expressed to higher levels in tumour cells.

The best-known functions of remodelling machines are their actions on individual nucleosomes. One of the current models for nucleosome remodelling assumes that these machines act as 'anchored DNA translocases'. Bound to the histone moiety of the nucleosome they translocate on either the adjacent linker DNA or on internal DNA, or both. This leads to a displacement of a DNA segment from the histone surface, which may be propagated around the histone octamer for nucleosome movement, or remain trapped on the surface of the nucleosome to generate a state of increased accessibility of nucleosomal DNA. Given that remodelling complexes have several chromatin binding domains, one has to consider the possibility that these machines have non-catalytic, structural roles in certain chromatin environments.

ATP-dependent nucleosome remodelling synergises with covalent histone modifications to alter chromatin structure. The nucleosome remodelling ATPase ISWI, which is part of several remodelling complexes in *Drosophila*, catalyses the sliding of histone octamers on DNA. We now found a novel principle, by which these two activities may be integrated. ISWI is acetylated at lysine (K) 753 by the acetyltransferase GCN5 *in vivo* and *in vitro*. The sequence surrounding K753 resembles the N-terminus of histone H3, where the corresponding lysine, H3K14 is also a prominent substrate of GCN5. This finding provokes the exciting possibility that properties of nucleosomes and remodelling enzymes may be regulated in concert by acetylation.

We visualised ISWI^{K753ac} during *Drosophila* development and found this protein to be particularly abundant in the earliest developmental stages. Remarkably, ISWI^{K753ac} was found concentrated on mitotic chromosomes of synchronously dividing preblastoderm nuclei and on the prophase I - arrested meiotic karyomer of the germinal vesicle, in contrast to bulk ISWI, which is excluded from condensed chromatin. Thus, K753 acetylation marks an ISWI form with novel, unprecedented properties and functions.

Lymphoid development in mouse and zebrafish

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T cell differentiation has so far been primarily studied in mammalian species. Although these studies have yielded a fairly detailed picture of these processes, many questions remain. To address these problems, we have recently begun to study T cell differentiation in a non-mammalian model through the use of genetic screens after ENU mutagenesis. I will discuss the design of the screen, its overall results and present some initial findings on the role of the Ikaros transcription factor on haematopoietic development in the zebrafish. The presentation will highlight similarities and differences between mouse and zebrafish lymphocyte development.

Cell fate decisions of blood cells

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We are interested in how hematopoietic stem cells in the bone marrow differentiate into either macrophages or lymphocytes. These specialized cells have very different functions in the body: while macrophages provide a first barrier of protection against infecting viruses and bacteria and remove dead cells, lymphocytes attack invaders through the production of antibodies and direct cell killing. Lineage decisions are mediated by transcription factors that govern cell type specific gene expression programs, and that give macrophages and lymphocytes their unique properties. To study how this works at the molecular level we use a gain of function approach, consisting of the enforced expression of macrophage associated transcription factors in committed lymphoid precursors.

Our results show that it is possible to reprogram fully committed B and T cell progenitors into functional macrophages by the expression of C/EBP alpha. The induced up-regulation of macrophage genes and the extinction of lymphoid genes are separable processes, the first one, but not the latter, requiring endogenous PU.1. The extinction of lymphoid genes is mediated by direct antagonisms between C/EBP alpha on the one hand, and Pax5, Notch1 and GATA-3 on the other. We also found that over-expression of PU.1 in T cell precursor reprograms them to acquire a dendritic cell fate. Our experiments re-establish in differentiated cells, to an extent, the transcription factors expression pattern of early progenitors and thus restores plasticity lost during lineage commitment. Understanding the transcription factor antagonisms and synergisms that are operative in this process allows to model cell fate decisions in the blood cell system.

Molecular characterization of circulating haematopoietic stem cells

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CD34+haematopoietic stem cells are used to support cytotoxic therapy. During steady-state, small amounts of these cells are present in the peripheral blood. Reconstitution after chemotherapy or administration of haematopoietic growth factors such as G-CSF facilitates the mobilization of CD34+ cells into the peripheral blood. Still, the exact molecular mechanisms of stem cell mobilization are not yet clear. In order to identify the molecular basis for the functional differences between sedentary and circulating haematopoietic stem and progenitor cells we compared CD34+ cells from bone marrow with those from peripheral blood during G-CSF-induced mobilization using cDNA arrays. We found a specific gene expression profile of CD34+ cells from both compartments. Circulating CD34+ cells showed a higher expression of proapoptotic genes and genes involved in transcription, whereas genes involved in cell cycle were lower expressed in peripheral blood CD34+ cells. This supports the view that circulating CD34+ cells consist of a higher number of quiescent and early stem and progenitor cells as well as a higher proportion of apoptotic cells.

A novel approach for mobilization of CD34+ cells is the use of pegylated G-CSF which has a longer serum half-life than G-CSF. Therefore, only one subcutaneous injection is necessary for mobilization. Comparing G-CSF- with pegylated G-CSF-mobilized CD34+ cells we found a distinct gene expression pattern during either of the two mobilization modalities which is associated with different functional characteristics and compositions of the mobilized cells. This indicates that the type of mobilization such as pulsatile or continuous G-CSF application can influence the gene expression pattern of circulating progenitor cells, which might be relevant for the clinical use.

Recent studies suggest that haematopoietic stem cells might be able to transdifferentiate into non-haematopoietic cells and might therefore serve as a cellular source for non-haematopoietic tissue. Moreover, the presence of sensory and autonomic nerves in the bone marrow could be morphologic correlates of neural regulation of haematopoiesis. This is supported by a recent study showing the G-CSF-induced mobilization of haematopoietic stem cells in mice is mediated by the sympathetic nerve system. We examined human CD34+ cells for expression of genes involved in neurobiologic functions using specialized cDNA arrays and flow cytometry. We found expression of ligand- and voltage-gated ion channels as well as of G protein-coupled receptors of neuromediators such as for example GABA, CRH and orexin receptors in CD34+ cells. Expression was higher in more immature CD34 negative CD34+ cells suggesting that those receptors play a role in developmentally early progenitor cells. Alterations of intracellular cAMP concentrations following stimulation of CRH and orexin receptors showed that these receptors are functionally active. Our findings suggest a molecular interrelation of neuronal and haematopoietic signalling mechanisms and might be the molecular basis for a neural regulation of haematopoiesis and mobilization.

Histone deacetylase inhibitors in cancer therapy

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The modulation of signaling events by histone deacetylase inhibitors can induce apoptosis or differentiation of carcinoma cells. Thus, HDAC inhibitors are candidate drugs for the treatment of cancer. We discovered that the drug valproic acid (VPA) is a powerful inhibitor of HDAC enzymatic activity which also induces proteasomal degradation of one specific isoenzyme, HDAC2. Our functional studies on the molecular mechanisms of HDAC inhibitor therapy show that Stat1 and NF- κ B, two key regulators of signal transduction, gene expression and apoptosis are linked via lysine acetylation of Stat1. Stat1 acetylation is a prerequisite for the interaction with NF- κ B p65. As a consequence, p65 DNA-binding, nuclear localization and expression of anti-apoptotic NF- κ B target genes are decreased. Alterations of the crosstalk between signaling pathways are likely to play an important role in the response of tumor cells to HDAC inhibitor therapy.

Quality control of RNA: new roles for new poly(A) polymerases

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Until recently the prevailing belief has been that the polyadenylation of RNAs has completely different functions in prokaryotes compared to eukaryotes. Polyadenylation of RNAs in bacteria is a prelude to degradation whereas it protects RNAs against destruction in eukaryotic cells. We and several other groups have identified a new protein complex with poly(A) polymerase activity from *Saccharomyces cerevisiae* that contains the Trf4 protein as catalytic subunit. In accordance with earlier *in vivo* studies (1), we could show that aberrantly folded tRNAs are preferentially polyadenylated by the Trf4 complex *in vitro* (2). Moreover, the Trf4 complex stimulates the degradation of unmodified tRNAs by purified nuclear exosome fractions. Degradation is most efficient when coupled to the polyadenylation activity of the Trf4 complex, indicating that the poly(A) tails serve as signals for the recruitment of the exosome. This polyadenylation-mediated RNA surveillance resembles the role of polyadenylation in bacterial RNA turnover.

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MOLECULAR GENETICS OF AN ONCO-DEVELOPMENTAL GENE SWITCH

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CCAAT/enhancer binding proteins (C/EBP) and the c-Myb protein are transcription factors that synergistically control cell fate in the hematopoietic system. On the molecular level, C/EBP plus c-Myb interact with each other, with multiple transcription co-factors, and with chromatin remodeling machines. How these interactions are spatiotemporally coordinated has to be revealed in detail, yet, C/EBPs were found to be involved in the remodeling of chromatin and in the recruitment of large, so called “Mediator” complexes that activate or that silence genes. Disruption of discrete C/EBP functions are associated with the development of acute leukemia.

Distinct mutations in the solvent exposed surface of the Myb DNA binding domain turn Myb into a leukemia protein. Wild type Myb but not its leukemic counterpart bind to the N-terminal tail of Histone H3 and catalyze its modification. The Myb – H3 interaction and the subsequent histone modification are essential for cell differentiation whereas abrogation of the H3 interaction is correlated with leukemogenicity. Thus, the Myb – C/EBP team acts as a molecular switchboard to orchestrate enzymatic conversion of chromatin and cell differentiation. Abrogation of several of its functions are tumorigenic.

JAK2 Mutation in the Pathogenesis of Myeloproliferative Disorders

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Myeloproliferative disorders (MPD) are a group of blood diseases characterized by aberrant proliferation of the myeloid lineages. They represent clonal stem cell disorders with an inherent tendency towards leukemic transformation. A mutation in the Janus kinase 2 (JAK2) gene that substitutes a valine to phenylalanine at position 617 of the Jak2 protein (JAK2-V617F) was found in the majority of MPD patients. This mutation results in a constitutively active JAK2 protein and represents a potential drug target for developing a specific inhibitor. Remarkably, the mutation occurred in the same codon in all MPD patients studied to date. The implications for the diagnosis, prognosis and treatment of MPD will be discussed.

Epigenetics of active chromatin

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Until recently, epigenetic mechanisms have been equated with the inheritance of heterochromatin, exemplified by DNA methylation in mammals. Evidence that transcriptionally active states of chromatin can also be epigenetically maintained is now accumulating. This paradigm shift began with the realization that DNA methylation is secondary to a more universal mechanism for epigenetic silencing based on methylation of histone 3 lysine 9 (H3 K9) for constitutive heterochromatin and H3 K27 for facultative heterochromatin.

Evidence that active chromatin states may also be epigenetically maintained came from the realization that other histone lysine methylations, mainly at H3 K4, characterize active chromatin and preclude H3 K9 methylation. The functional opposition of these two classes of histone lysine methylation was further supported by linkage to the antagonism between Polycomb- and trithorax-Group (PcG and trxG) action. The association of trxG action with H3 K4 methylation and PcG action with H3 K27 methylation provides a molecular explanation for this antagonism.

The silencing methylations at both H3 K9 and H3 K27 are epigenetically maintained via positive feedback loops. The relevant methyltransferases (SUV39 and E(Z), respectively) associate with a protein (HP1 and Polycomb, respectively) that recognizes the methylated epitope. The enzyme is thereby associated with the chromatin that it has methylated, to methylate it further and propagate the silent state. Similarly, recent evidence indicates that epigenetic maintenance of active chromatin occurs in the same way, because a constituent protein of all H3 K4 methyltransferase complexes, WDR5/SWD3 binds to methylated H3 K4. These observations support a polarization model of chromatin, which is based on opposing positive feedback loops. Both active and heterochromatic states involve several positive feedback loops that reinforce the local status quo. Hence each state has an implicit epigenetic status that adds stability and reduces the chances of inadvertent transitions to the other state. Epigenetic mechanisms are thought to play important roles in lineage commitment during development. The simplest model suggests that the epiblast is pluripotent because there are relatively few epigenetic marks in chromatin. Lineage commitment restricts pluripotency because epigenetic marks are imposed on chromatin. These marks are likely to include histone methylations, which can direct inheritable states of gene silencing or activation. This model also suggests that cellular identity in the adult is maintained, in part, by epigenetic mechanisms.

We are studying these issues by examining the function of histone lysine methyltransferases in yeast and the mouse.

Computational Methods in Gene Expression and Gene Regulation

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Abstract

The availability of complete genome sequences as well as functional genomics data like, e.g. large scale gene-expression data has revived the interest in computational prediction of cis-regulatory elements. This talk will introduce computational methods for visualizing associations between genes and conditions in DNA-microarray data. These techniques will also be applied for establishing associations between gene expression data and transcription factor binding sites. While for yeast this can be done based on published transcription factor binding data, for human data we draw on a comparative analysis with mouse data in search for binding sites.

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