Friday 22 November

PLENARY SESSION 8

Chromatin modelling

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Histone methylation in transcriptional control

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Histone modifications are able to regulate transcription positively and negatively. Our ongoing analysis of the mechanism by which such modifications regulate transcription has focused on lysine and arginine methylation. Three distinct sites of methylation on histone H3 will be discussed, involving residues K4, K9 and R17. Methylation at K9 is a repressive event for transcription at both heterochromatic sites and at promoters regulated by the RB repressor protein. We can now show that K9 methylation is involved in the repression of DNA methylated promoters by MeCP2. A K9 H3 methyltransferase associates with MeCP2 and is delivered to the differentially methylated domain of the H19 promoter. Methylation at K4 results in transcriptional activation. Using antibodies raised specifically against the di- or tri-methylated state of K4, we show that only tri-methylated K4 correlates with activation of transcription in yeast. This result demonstrates a new rule for histone modifications in which the methyl state of a lysine is a consideration for activity. Given that any lysine can be mono-, di- or tri-methylated, the complexity of the code on histones is clearly much larger than previously suspected. Methylation at R17 correlates with activity of estrogen regulated genes such as pS2. Using chromatin immunoprecipitations we have investigated the ordered appearance of modifications on the pS2 promoter following estrogen stimulation. We can show that acetylation by CBP takes place before methylation of R17 by CARM1 and that an acetylated H3 substrate augments methylation by CARM1. These results provide evidence for a "cross-talk" between CBP acetylation and CARM1 methylation.

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Histone deacetylase inhibitors in acute promyelocytic leukemia

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Abstract not received.

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Chromosome structural changes in human cancer and their reversal by DNA methylation inhibitors

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Silencing of tumor suppressor genes by hypermethylation of promoter CpG islands is well documented in human cancer. We have analyzed the methylation status and chromatin structure of three CpG islands in the p14(ARF)/p16(INK4A) locus in a series of normal and cancer cell lines using nucleage digestion and chromatin immunoprecipitation assays. We have found an altered chromatin structure associated with the silencing of tumor suppressor genes in human cancer cell lines which involves CpG island methylation, chromatin condensation, histone deacetylation and MeCP2 binding as well as increased methylation of the lysine 9 residue of histone H3. CpG islands in cancer cells exhibiting the epigenetic profile of heterochromatin do not resist transcript elongation by RNA polymerase II, even though this profile is incompatible with transcriptional initiation. Treatment with 5-aza-2'-deoxycytidine induced the formation of a hemimethylated state in treated cells and a rapid and substantial remodeling of heterochromatic domains in T24 bladder cancer cell lines, drastically reducing levels of H3-K9 methylation and increasing levels of H3-K4 methylation. Thus, drugs that inhibit DNA cytosine methylation cause rapid changes in the state of chromatin modification presumably resulting in the activation of expression of silenced genes. We have also begun work on other cytosine analogs which have the ability to induce cytosine demethylation but which are more stable than aza nucleosides and which show promise as orally effective agents.

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The role of secondary DNA structures in silencing transcription

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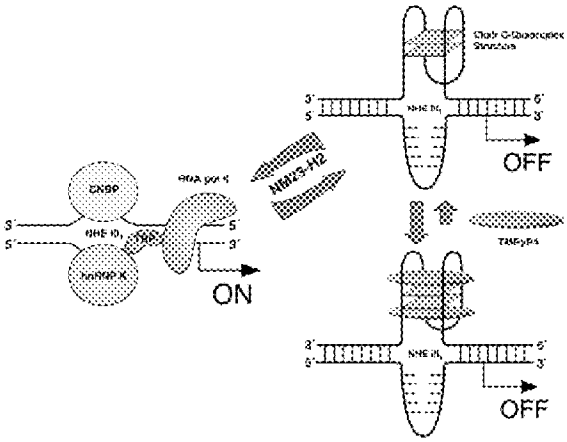
The role of secondary DNA structures in control of gene expression has long been debated. In this presentation I provide direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. The nuclease hypersensitivity element III1 upstream of the P1 promoter of c-MYC controls 85–90% of the transcriptional activation of this gene. We have demonstrated that the purine-rich strand of the DNA in this region can form two different intramolecular G-quadruplex structures, only one of which appears to be biologically relevant. This biologically relevant structure is the kinetically favored chair-form G-quadruplex, which when mutated with a single G to A transition is destabilized, resulting in a 3-fold increase in basal transcriptional activity of the c-MYC promoter. The cationic porphyrin TMPyP4, which has been shown to stabilize this G-quadruplex structure, is able to further suppress c-MYC transcriptional activation. These results provide compelling evidence that a specific G-quadruplex structure formed in the c-MYC promoter region functions as a transcriptional repressor element. Furthermore, we establish the principle that c-MYC transcription can be controlled by ligand-mediated G-quadruplex stabilization (see Figure 1).



The formation of similar G-quadruplexes in other promoters of growth regulatory genes (unpublished results), such as PDGF-A, c-myb, and Ki-ras, suggest that this will be a more general phenomenon in genes associated with growth and proliferation. The sequestration of the active form of the promoter as a G-quadruplex rather than in a nucleosome may have advantages for a rapid response required for genes involved in proliferation and may have been an ancient mechanism for controlling gene expression.

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PLENARY SESSION 9

Cell cycle modulation

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Cyclin D1 and Cancer

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The product of the PRAD1 oncogene, cyclin D1, is a key regulator of G1 progression where it binds and to and activates the cyclin-dependent kinases CDK4 and CDK6. These kinase complexes then in turn phosphorylate and contribute to inactivation of the pRb tumour suppressor protein, a requirement for transit from G1 into S phase. Deregulated expression of cyclin D1 is regularly observed in human tumours and is thought to confer accelerated G1 progression, mitogen-independence and anti-oestrogen resistance in a variety of cell types. The relevance of this cell cycle protein to