MOLECULAR BIOLOGY

Antagonizing the neighbours

Joel C. Eissenberg and Sarah C. R. Elgin

Nucleosomes bundle up the DNA in a cell's nucleus, wrapping it around a complex of histone proteins. Studies of histone modifications and the proteins that bind to them reveal a mechanism that may control this packing.

Crack open any cell nucleus and look inside: you'll see what look like beads on a string. The beads are nucleosomes, small protein complexes that help to package the DNA (the strings) into the cramped confines of the nucleus. In the past fifteen years, nucleosomes have graduated in our understanding from being passive spools for DNA to full partners in the control of genetic information in the cell. Diverse chemical modifications of the histone proteins that form the nucleosome core can alter the expression of the associated genes[1]. These modifications make up what is known as the histone code, and a major challenge in molecular biology is to decipher how they affect gene expression.

Two of the most common modifications are phosphorylation and methylation — respectively the addition of a phosphate or a methyl group to the amino acids of which the histones are composed. Allis and colleagues[2] previously proposed that reversible phosphorylation of the amino acids serine or threonine in the 'tail' regions of histones could antagonize the binding of regulatory proteins to neighbouring methylated lysine amino acids, creating a binary control switch. In this issue, Fischle *et al.* (page 1116)[3] and Hirota *et al.* (page 1176)[4] present data that strongly support this model and advance our understanding of how histone modification can control chromosome function.

Among the many histone modifications that have been described, methylation of lysines and phosphorylation of serines and threonines have attracted much attention. Phosphorylation of the serine at the tenth position in the tails of histone H3 (H3S10) occurs during cell division in eukaryotic (higher) cells. Once the cells have replicated their DNA and begin to prepare for division, nearly all of the histone H3 in the nucleus seems to be phosphorylated at this site. Phosphorylation of H3S10 also occurs at other stages in the cell cycle, but only at discrete chromosomal sites that are associated with gene expression.

The methylation of lysines is more complex. Methylation at lysine 9 of histone H3 (H3K9) is found mainly in the heterochromatin — the dense, mostly inactive regions of the genome. Methylation at lysine 4 of histone H3 (H3K4), by contrast, is associated with active genes. The different outcomes of lysine methylation result from the fact that each modification creates a binding target for a distinct protein. Heterochromatin protein 1 (HP1), which promotes heterochromatin formation (and the consequent gene silencing), recognizes and binds to methylated H3K9 using a region called the chromodomain[5,6]. CHD1, an enzyme that may destabilize nucleosomes and expose the DNA for gene expression, recognizes and binds to methylated H3K4 through two tandem chromodomains[7].

Fischle *et al.*[3] and Hirota *et al.*[4] examined cells progressing into the metaphase stage of cell division, where the chromosomes become very tightly packed, or 'condensed', to facilitate their separation into the future daughter cells. Both groups discovered that these cells accumulated histone H3 that is both methylated at lysine 9 and phosphorylated at the neighbouring serine 10. Using antibodies specific for the doubly modified H3, the authors found that this dual modification occurred specifically in heterochromatic regions of chromosomes.

During most of the cell cycle, HP1 is also concentrated in heterochromatin, but with the onset of chromosome condensation, much of the HP1 leaves the chromosomes. Both labs link the dissociation of HP1 with the accumulation of phosphorylated H3S10 by showing that inactivation of an enzyme that phosphorylates H3S10 (Aurora B kinase) results in the retention of HP1. These observations suggest that H3S10 phosphorylation causes displacement of HP1 from heterochromatin with the onset of metaphase. To test this, the ability of HP1 to bind to dually modified H3 tail peptides was measured *in vitro*, using either fluorescence polarization[3] or binding to peptide-coated beads[4]. In both assays, the binding of HP1 to the methylated H3K9 was substantially impaired when the neighbouring H3S10 was simultaneously phosphorylated.

The results demonstrate the need to examine the impact of multiple modifications on a histone tail; using an antibody that recognizes a single modification is clearly not sufficient to infer the histone state. The findings also provide experimental support for the regulatory-switch hypothesis of Allis and colleagues[2]. Methylation at H3K9, and concomitant binding of HP1, is not only found in heterochromatin, but also contributes to inactivation of some genes in euchromatin (the less dense and more active regions of the genome)[8]. If HP1 can be evicted by phosphorylation of H3S10, such repression might be reversed. However, the genomic region where HP1 binds would still be tagged by the methyl groups at H3K9. So if the H3S10 phosphate group were removed (by a protein phosphatase), that would leave the unopposed H3K9 methyl mark available to restore HP1 binding and reconstitute heterochromatin. This model is consistent with genetic studies implicating a histone methylase[9,10] and a protein phosphatase[11] in the control of heterochromatin formation in the fruitfly. Whether it applies to regulation in euchromatic domains remains to be seen.

Another study reported in this issue (page 1181)[7] suggests that the methyl–phospho

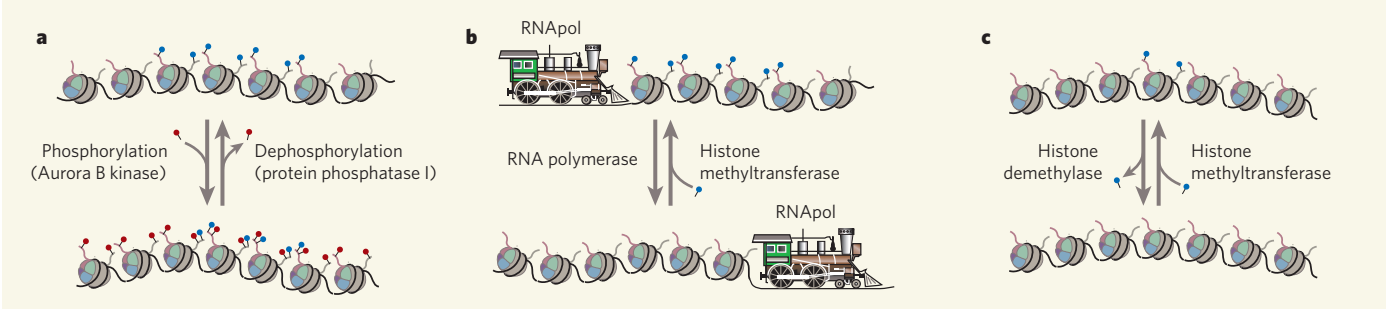

Figure 1 | Three-pronged control. Three contrasting mechanisms to regulate binding of proteins that target methylated histones. **a**, Fischle *et al.*[3] and Hirota *et al.*[4] show that phosphorylation of a serine or threonine amino acid that lies next to a methylated lysine creates a methyl–phospho module. This module can no longer associate with methyl-binding proteins. Phosphorylation is readily reversed by a phosphatase. This mechanism leaves the original pattern of methylation intact. **b**, Passage of RNA polymerase II may result in nucleosome replacement, erasing the original pattern of methylation. **c**, Site-specific patterns of methylated nucleosomes can be erased enzymatically by histone demethylase. In cases **b** and **c**, the pattern of methylation can only be restored by targeted *de novo* histone methyltransferase activity.

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switch mechanism has wider applicability. Flanagan *et al.* describe the crystal structure of the double chromodomains of the mammalian CHD1 protein bound to an H3 peptide containing methylated H3K4. The way in which the CHD1 chromodomains bind to methyl-lysine seems different from how HP1 binds. But binding of the CHD1 chromodomains to methylated H3K4 is antagonized *in vitro* by phosphorylation of a neighbouring threonine (H3T3). CHD1 resembles a helicase, an enzyme capable of loosening nucleosome–DNA contacts. So controlled interaction of CHD1 with H3K4 by phosphorylation of H3T3 might regulate the access of regulatory proteins to DNA to control gene expression.

Using a binary switch to eject proteins that bind to methylated histones essentially reverses the effects of histone methylation. So far, the cell has been found to use two other methods to accomplish this end. First, as RNA polymerase traverses genes to make the encoded messenger RNA, it displaces the nucleosomes from the DNA. Nucleosomes re-form once the polymerase has passed, and the process can replace methylated histones with unmethylated ones[12]. Second, histone demethylase enzymes can directly strip the methyl groups off specific lysines[13,14].

Why use three mechanisms to achieve the same biochemical result? Each has a different overall impact. Eviction of nucleosomes by RNA polymerase can remove all nucleosome modification marks (Fig. 1b). Lysine demethylation can target specific nucleosomes, but will also erase methylation patterns (Fig. 1c). However, by leaving methyl marks intact and ejecting the methyl-lysine binding factors through phosphorylation of neighbouring amino acids, cells can rapidly effect a large-scale reorganization of chromosome structure while preserving the underlying methylation pattern (Fig. 1a); this allows the original structure to be restored on dephosphorylation.

Fischle *et al.*[2] identified 16 instances of lysines flanked by either serine or threonine among the four histone proteins that form the nucleosome, suggesting the possibility of other such binary switches. The monotonous beads-on-a-string conceal a rich variety of ornaments that compete with one another to control gene expression. ■

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ATMOSPHERIC PHYSICS

Reflections on aerosol cooling

Jim Coakley

By changing the composition of Earth's atmosphere, human activity has both a warming and a cooling effect on the planet. According to new calculations, that latter influence is large, but it is likely to be declining.

The Earth is warming because of rising concentrations of greenhouse gases in the atmosphere. But some of the human activities that produce those gases, including burning of fossil fuels and biomass, also produce hazes that partially offset the warming. The extent of this cooling influence is not known. But it occurs because human-generated haze particles scatter and absorb incoming sunlight, an effect known as 'aerosol direct radiative forcing' that reflects solar radiation back into space.

On page 1138 of this issue, Nicolas Bellouin and colleagues[1] provide a new estimate of this aerosol forcing effect. The figure they produce (0.8 ± 0.1 W m^{-2} of incident sunlight) is at the high end of the range (0.2–1.0 W m^{-2}) given in the previous report from the Intergovernmental Panel on Climate Change (IPCC)[2]. Notably, this new estimate comes with an uncertainty of only 10–15%, a far cry from the range cited in the IPCC assessment. For comparison, the warming effect from the build-up of the long-lived greenhouse gases carbon dioxide, methane, nitrous oxide and chlorofluorocarbons is 2.4 ± 0.2 W m^{-2}.

Because of concerns about human health and acid rain, considerable efforts have been and are being made to improve air quality and reduce the amounts of anthropogenic aerosols that arise, for example, from power-plant and manufacturing emissions. So an implication of the figure obtained by Bellouin *et al.* is that such improvements will come at the price of substantial additional warming — as much as half that already experienced from the build-up of greenhouse gases.

Estimating the effect of anthropogenic aerosols on climate is notoriously difficult. Computer models must predict the concentrations, and the physical and chemical make-up of the particles, and the consequent radiative properties. The variability of estimates for the aerosol direct radiative forcing in the IPCC report reflects the wide range of possible outcomes. To constrain this range, Bellouin *et al.* took advantage of new observations of aerosol properties from the Moderate Resolution Imaging Spectrometer (MODIS) instrument now flying on NASA's Terra and Aqua satellites.

A central concept in these studies is 'aerosol optical depth', a measure of the attenuation of sunlight by particles that is proportional to the amount of aerosol. Over oceans, the MODIS observations separate the aerosol optical depths into the fractions contributed by small and large particles[3]. *In situ* measurements and surface-based observations of light attenuation and scattering led Bellouin *et al.* to propose using the separation of the optical depth into contributions made by small, anthropogenic particles and large, natural particles — such as windblown dust and sea spray — to estimate what fraction of aerosols is attributable to anthropogenic causes.

Anthropogenic aerosols over the oceans account for only one-third of the aerosol direct radiative forcing, however. Haze over continents accounts for the rest. But MODIS observations are more uncertain over land, and the separation of aerosol optical depth into fractions contributed by small and large particles is not feasible[3]. As an alternative, Bellouin *et al.* divide the continents into six parts and use predictions from an ensemble of aerosol chemical-transport models to determine the fraction of the total aerosol optical depth contributed by anthropogenic aerosols. They use optical properties for the particles derived from surface-based observations of the scattering and attenuation of sunlight[4], the data coming from one observing site for each of the six continental regions. The particle properties from the surface observations are combined with the aerosol optical depth from the MODIS observations, and the fraction attributed to humans from the models, to predict — through radiative transfer calculations — the effect of anthropogenic aerosols on reflected sunlight.

To estimate the uncertainty in their calculations, Bellouin *et al.* performed a large number of Monte Carlo simulations. In these simulations, the various parameters that affect the aerosol direct radiative forcing — such as the MODIS aerosol optical depth, the model predictions of anthropogenic fractions and the surface-based estimates of the aerosol optical

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