100 YEARS AGO

The recent tube operations in London have brought to the surface specimens of the London Clay from different districts. Samples of this clay taken from such different points as Hyde Park Corner, Brompton Road, and Haverstock Hill have been tested in the physical laboratory of the South-western Polytechnic for the presence of a radio-active gas by Mr. H. Cottam, and he has been unable to detect with his apparatus any marked quantity of active gas from the clays. With the same apparatus he has detected quite easily the radio-active gas from the water of a deep well… which goes below the clay to the greensand. We have come to the conclusion that the London Clay forms a floor through which the radio-active gas does not penetrate; or it may be said that the radio-active substance only travels when the water with which it is associated can travel. This is an argument in support of Prof. J. J. Thomson's view, that the radio-active gas, which he found in deep well waters, arises from the splitting up of a trace of soluble radium salt which comes up with the water.

From *Nature* 6 October 1904.

50 YEARS AGO

Little more than a hundred years have passed since hunters such as Gordon Cumming were at their work among the vast herds of big game in the southern quarter of Africa, and little more than fifty since Selous was making a living by ivory hunting somewhat farther to the north. All those countless thousands of animals have now disappeared for ever… The opening-up and white settlement of East Africa did not come until later, but although the same process of decimation began, and many parts of the country have been cleared of game, it is still possible to see great herds of wild animals that recall the accounts of conditions in South Africa given by the early travellers. Nevertheless, the presence of enormous herds of game animals is quite incompatible with the economic exploitation of the country and the rapid expansion of the native population; the game must go, and there can be no hope of its survival outside the National Parks and game reserves… As yet, however, there is practically no information available on the biology of these mammals, information that is essential for successfully managing such parks and reserves.

From *Nature* 9 October 1954.

residues can only be mono- or di-methylated.

Histone methylation has been linked to biological processes ranging from the regulation of gene transcription, to the inactivation of one copy of the X chromosome in females, to RNA-mediated gene silencing[2,6,7]. One way in which it works is by serving as a docking site for other proteins[2]. The nature of a specific methylation determines the protein that it recruits, which in turn dictates the biological outcome (see, for example, ref. 6).

Unlike other histone modifications, methylation has generally been regarded as stable[3] — a notion that comes from early studies showing that histone proteins and methylated lysine or arginine residues within them have similar turnover rates[8]. But although a non-reversible methyl mark would fit with a role for histone methylation in long-term gene silencing, it is not compatible with situations in which rapid reversal of gene expression takes place. To solve this paradox, mechanisms including enzyme-catalysed demethylation, replacement of methylated histones by unmodified histones, and clipping of methylated histone 'tails' have been proposed[3,9] (Fig. 1a) — but none has yet been demonstrated experimentally. Now, however, Wang *et al.*[4] and Cuthbert *et al.*[5] have found that the human enzyme peptidylarginine deiminase 4 (PAD4/PADI4) can catalyse the conversion of methylated arginines to citrulline, providing yet another mechanism by which histone methylation levels could be controlled (Fig. 1b).

How is it that two groups independently identified the same enzyme and came to similar conclusions? Previous studies[10] established that arginine residues in other proteins can be converted to citrulline by enzymes of the peptidylarginine deiminase family. Of this family, only PAD4/PADI4 is found in the nucleus; moreover, its expression correlates with the appearance of citrulline in histones[11]. These facts made it a good candidate for a histone arginine demethylase.

So Wang *et al.* and Cuthbert *et al.* carried out direct tests of PAD4/PADI4 *in vitro*. They found that it 'deiminated' numerous unmethylated arginines — converted them to citrulline — in histones H3 and H4. It also decreased the amount of arginine methylation that is catalysed by PRMT1 and CARM1 in both histones. Notably, at the same time that the amount of this methylation decreased, the quantities of citrulline in both histones increased. The detection of methylamine as a released product[4] supports the idea that methylated arginine is a genuine substrate of this enzyme. So, PAD4/PADI4 can catalyse both the deimination of unmethylated arginine and the 'demethylimination' of methylated arginine *in vitro*. As Wang *et al.* find, it can also do so in granulocyte cells.

What are the consequences of these reactions? First, as Cuthbert *et al.* show, the conversion of histone arginines to citrullines actually prevents histone methylation by CARM1. Second, Wang *et al.* find that demethylimination of methylated histone arginines reverses the effects associated with methylation. So, PAD4/PADI4 presumably antagonizes the functions of the methylating enzymes CARM1 and PRMT1. For instance, previous studies have linked histone arginine methylation by these enzymes to transcriptional activation by nuclear hormone receptors[12–15] — so PAD4/PADI4 is likely to repress such transcription. Indeed, both groups link the recruitment of PAD4/PADI4 to an oestrogen-responsive gene, pS2, with the appearance of citrullinated histones and the downregulation of this gene.

These papers[4,5] provide convincing evidence that the methylation of arginine amino acids in histone proteins can be reversed enzymatically. But, as ever, the findings raise questions. For example, it seems that PAD4/PADI4 has a very loose substrate specificity *in vitro*. It works on both methylated and unmethylated arginines. And the arginine residues it deiminates are not limited to the sites that are targeted by CARM1 and PRMT1; indeed, arginine 8 in histone H3, a site not known to be methylated by either enzyme, is the preferred target[4]. There might be an interplay between the citrullination of H3 arginine 8 and the methylation of H3 lysine 9, but for now the significance of this citrullination remains unknown.

The second issue worth noting is that, *in vitro*, PAD4/PADI4 cannot demethyliminate H4 or H3 peptides containing di-methylated arginines[4,5]. This presents a puzzle. Although mass-spectrometry analysis[14,15] identified mono-methyl groups as the major methyl form on arginine 3 in histone H4, most arginines 17 and 26 in histone H3 become di-methylated after incubation with CARM1 *in vitro*[16]. How, then, can this methylation be removed? Although an unidentified enzyme might be required, the available evidence suggests that PAD4/PADI4 is responsible: for instance, in granulocytes, activation of this enzyme led to less methylation on H4 arginine 3 and H3 arginine 17, as analysed using site-specific antibodies that recognize di-methylated arginine[4]. The most likely solution is that PAD4/PADI4 can work only on intact chromatin substrates. Alternatively, it might need to work with a partner.

As well as revealing that arginine methylation can be reversed, the new papers[4,5] show that a new form of histone proteins — containing citrulline residues — exists in cells. This finding, too, raises questions. How stable are citrullinated histones? Do they have any effects on chromatin structure? Can other proteins recognize and bind to them? And what is the fate of these histones? With regard to the last question, the transient presence of citrullines in the pS2 gene[5] suggests

©2004 Nature Publishing Group