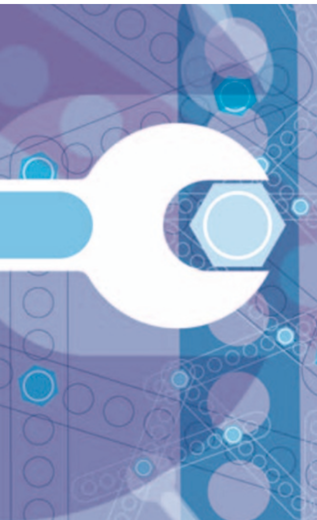
EPIGENETICS

The DNA's fixed, but what about the histones?



Cells have many mechanisms for faithfully repairing DNA after damage, but what about restoring the epigenetic marks that are also crucial for proper function? New research on nucleotide excision repair (NER) after UV damage shows that new histones are incorporated at the damage site instead of recycled histones that would already bear the correct modifying marks. This might have implications for epigenetic stability.

Almouzni and colleagues developed an assay for the incorporation of histone variant H3.1 at sites of UV damage, which involves visualizing local concentrations of epitope-tagged histone in transiently transfected cells that are subject to local UV irradiation. Even at low UV doses they found that new histones were recruited to damage sites about half an hour after irradiation. This effect was not limited to S phase, which would imply that the histones were being incorporated at replication forks, and was also not found in NER-deficient cells.

Having shown that new H3.1 is incorporated at UV-damaged sites in association with NER, the authors investigated whether the histone chaperone chromatin assembly factor 1 (CAF1), which associates with H3.1, was involved. Using RNAi, they showed that CAF1 is necessary after the repair process, which is consistent with a direct role in the incorporation of new histones.

These results raise many questions about the maintenance of epigenetic marks in the face of DNA damage. Are epigenetic marks restored after damage, and, if so, how? Neighbouring regions of chromatin could function as a template to guide the correct histone-modifying enzymes to the new unmarked histones. As the results do not discount some recycling of old histones, an alternative is semi-conservative replication, analogous to that of DNA, in which new histones are used in equal proportion to old histones, from which they obtain their correct marks.

EPIGENETICS

Imprinting links embryogenesis and tumour formation

Genomic imprinting — the epigenetic marking of a gene on the basis of parental origin, which results in monoallelic expression — is thought to control the amount of maternal resources that are allocated to the offspring between conception and weaning. Recent work by Journot and colleagues identifies a network of imprinted genes that are involved in the control of embryonic growth, and potentially in tumour development.

Although the misregulation of imprinted loci leads to developmental abnormalities in humans, biological functions are known for only a handful of imprinted genes. Motivated by this gap in our knowledge, Varrault *et al.* studied a maternally imprinted gene, *Zac1*, which encodes a zinc-finger transcription factor that induces apoptosis and cell-cycle arrest. Knocking out *Zac1* resulted in fetal growth restriction in pups of *Zac1*$^{-/-}$ and *Zac1*$^{+/-}$ mice that received their knockout allele from the father (in the mother, *Zac1* is inactive anyway). Interestingly, this phenotype is counterintuitive from the point of view of the function of *Zac1* as a tumour suppressor gene (its loss should facilitate growth), although it is consistent with a paternally expressed imprinted gene (such genes are thought to promote fetal growth).

But *Zac1* knockouts also had ossification defects and other morphological abnormalities, and many pups died postnatally as a result of lung abnormalities. To understand the underlying mechanisms, the authors performed a meta-analysis of 116 mouse microarray data sets to look for genes that are co-regulated with *Zac1*, assuming that genes that cluster together might share biological function. The set of genes that were most closely linked to *Zac1* was enriched in imprinted genes, including *H19*, insulin-like growth factor 2 (*Igf2*), insulin-like growth factor 2 receptor (*Igf2r*) and others. The phenotypes of mutants in many of these genes, including *Zac1*, indicate that these genes form an imprinted gene network (IGN) that controls embryonic growth and differentiation.

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The authors confirmed the role of *Zac1* in this network; for example, they showed that loss of *Zac1* led to downregulation of *Igf2*, cyclin-dependent kinase inhibitor 1C (*Cdkn1c*) and *H19*. In turn, overexpressing *Zac1* in tissue culture resulted in the robust upregulation of some of the same genes. The effects of *Zac1* on *Igf2* and *H19* are direct — using chromatin immunoprecipitation and an electrolytic mobility shift assay (EMSA), Varrault *et al.* showed that *Zac1* binds directly to an E2 enhancer that lies downstream of *H19* and is shared with *Igf2*. These results indicate that downregulation of genes such as *H19* and *Igf2* might account for the growth impairment of *Zac1*-deficient mice, albeit by an unknown mechanism.

The authors point out that the network has a scale-free architecture; for example, paternally expressed 3 (*Peg3*), necdin (*Ndn*) and guanine nucleotide binding protein, alpha stimulating (*Gnas*) emerge as nodes (they have high connectivity), whereas others, *Igf2* and *H19* among them, are connected to fewer genes. But more work is required to explore the potential implications of this observation.

The authors end on an intriguing note: global loss of imprinting has been previously implicated in tumorigenesis and shown to affect expression of many of the genes within the IGN. So, IGN might not only function in embryonic development but also in safeguarding against tumour formation.

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On the other hand, it is possible that the histone modifications are not restored. Instead, the presence of new histones could form a memory of the damage. This could serve an adaptive role in the recovery process or, instead, be maladaptive, and help to explain the occurrence of radiation-induced genomic instability in the progeny of the damaged cells.

More mechanistic studies are now needed to distinguish between these possibilities and explain how epigenetics interacts with damage repair. The experiments also need to be extended to other histone variants to test their generality.

Patrick Goymer

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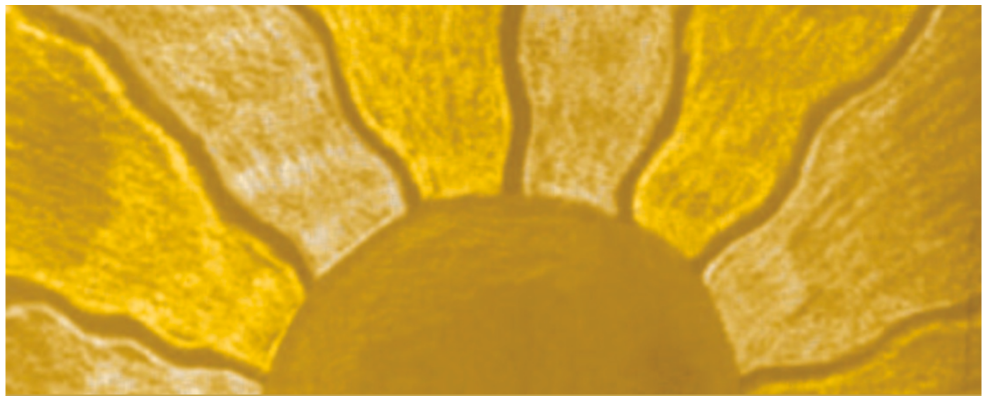
Image courtesy of D. Jones.



GENOMICS

The dawn of Neanderthal genomics

Initial sequencing and analysis of Neanderthal genomic DNA have recently been reported. Neanderthals, who became extinct some 30,000 years ago, are our closest hominid relatives; their genome therefore offers unique opportunities to identify the genetic changes that are specific to modern humans, facilitating our understanding of both human and other hominid biology and evolution.

A group led by Eddy Rubin used metagenomics — an approach previously developed for environmental genomics and for ancient DNA cloning — combined with Sanger sequencing and massive parallel pyrosequencing to obtain ~65,000 bp of Neanderthal genome sequence. At the same time, Svante Pääbo and colleagues used direct high-throughput sequencing to obtain ~752,000 bp of unique Neanderthal nuclear DNA. Both groups used a Neanderthal bone sample from Croatia — a male, as it turns out, as both groups detected Y-chromosome sequences in their reads.

Sequencing DNA from ancient samples is fraught with problems such as contamination with DNA from other sources; for example, from modern humans and organisms that have colonized the ancient remains. Between them, the groups used a number of methods to ensure that what they were analysing was genuine Neanderthal DNA: comparisons of PCR-amplified mitochondrial DNA sequences from the ancient sample and modern humans, analysis of signatures of DNA damage that are characteristic of ancient DNA (deamination of cytosine to uracil is frequently found in ancient DNA) and sequence comparisons to human and chimpanzee orthologous sequences. Comparison with chimpanzee sequences also allowed the authors to identify the number of mutations that are specific to each of these lineages. For example, Green *et al.* estimate that ~8% of the DNA sequence changes in the human lineage have occurred after divergence from the Neanderthal.

Given the current human–chimpanzee divergence, Noonan *et al.* estimate that, on average, modern human and Neanderthal genomes shared a most recent common ancestor ~706,000 years ago, and that the human and Neanderthal ancestral populations split ~370,000 yrs ago. Green *et al.* put human–Neanderthal DNA sequence divergence at ~516,000 yrs.

As late as 30,000 years ago, modern humans and Neanderthals co-existed in Europe. Although there is no archaeological evidence that they lived together, the nature of their interaction and, most intriguingly, the question of whether any interbreeding between the two species took place, are subject to debate. By comparing the frequency of the ancestral and derived alleles in Neanderthals and humans, both groups looked at admixture between the two. Although Noonan *et al.* find little evidence for its existence, Green *et al.* suggest that some gene flow from humans (in particular from human males) to Neanderthals might have occurred.

Ultimately, a definitive answer to this and other questions will require additional sequence data. And more data will be available — given the advances in sequencing technologies and procedures for retrieval of DNA from ancient samples, a project to produce a draft version of the Neanderthal genome has been initiated. The authors promise the draft sequence within two years.

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