subjected plasmids encoding the prokaryotic DNA methyltransferase M.HaeIII to an *in vivo* selection test that required functional methyltransferase activity for the plasmid to survive and maintain its ability to be transformed into cells.

First they connected two copies of M.*Hae*III to create a fused dimer and showed that this dimer was functional. They then created a library with start codons at random locations in the 5′ coding region of the first copy and another library with stop codons at random locations in the 3′ coding region of the second copy. *In vivo* selection applied to each library resulted in clones of different N- and C-terminally truncated intermediates. Permutants that structurally resembled known classes of DNA methyltransferases were created when secondary libraries of the intermediates were generated with random truncations at the opposite termini and the selection test was applied.

The permutants had a lower level of activity than the original M.HaeIII; however, random mutation of the permutants followed by selection greatly improved their activity. Interestingly, not all possible permutants were recovered, indicating that structural constraints restrict the function of intermediates. Some of the most active permutants didn't resemble any known class of methyltransferase. Remarkably, a search of sequenced prokaryotic genomes identified several naturally occurring members of this newly described class of enzyme.

By putting to rest the supposition that intermediates are likely to be non-functional, this work shows experimentally that new protein classes can evolve gradually through permutation by duplication.

Emily Niemitz, Associate Editor,
Nature Genetics

ORIGINAL RESEARCH PAPER Peisajovich, S. G. *et al.* Evolution of new protein topologies through multistep gene rearrangements. *Nature Genet.* 15 January 2006 (doi:10.1038/ng1717)

studied by Rauen's group had sporadic missense mutations in *BRAF* (18/23) or *MAP2K1/MAP2K2* (3/23) — two transducers of MAPK signalling. Aoki and colleagues also found *BRAF* mutations (in 16/43 patients), with three cases having mutations in *KRAS*.

The fact that most cases of CFC were caused by mutations in Ras–MAPK signalling establishes a role for the pathway in human development, as well as aiding the diagnosis of CFC. The connection of CFC with such a notorious pathway might bring unexpected benefits, as drugs that are being targeted against cancerous mutations in this pathway could be used to alleviate or even cure the symptoms of CFC. These studies also highlight another important issue — given the predominance of rare disorders among the unmapped monogenic diseases, there is an urgent need for incentives to create biobanks of the kind that were so crucial in these studies.

Tanita Casci

ORIGINAL RESEARCH PAPERS
Rodriguez-Viciana, P. *et al.* Germline mutations in genes within the MAPK pathway cause cardio-facio-cutaneous syndrome. *Science* 26 January 2006 (doi:10.1126/science.1124642) | Niihori, T. *et al.* Germline *KRAS* and *BRAF* mutations in cardio-facio-cutaneous (CFC) syndrome. *Nature Genet.* 12 February 2006 (doi:10.1038/ng1749)
FURTHER READING Tartaglia, M. *et al.* Exclusion of *PTPN11* mutations in Costello syndrome: further evidence for distinct genetic etiologies for Noonan, cardio-facio-cutaneous and Costello syndromes. *Clin. Genet.* **63**, 423–426 (2003)

EPIGENETICS

The open secret of pluripotentiality

The differentiation of embryonic stem (ES) cells is accompanied by activation and/or silencing of specific loci and global chromatin reorganization. Tom Misteli and colleagues now show that hyperdynamic association between the chromatin and the major proteins that determine its architecture is a hallmark of pluripotency, and that it is required for differentiation to occur.

To investigate the chromatin changes that occur during lineage restriction, Meshorer *et al.* followed mouse ES cells as they differentiated into neural progenitor cells (NPCs). Changes in the chromatin architecture were studied using combinations of antibodies against HP1α and Oct4 (a stem-cell marker) or nestin (an NPC marker), and against histone H3 trimethylated on lysine 9 (a marker of heterochromatin). Immunostaining results, which were later confirmed by FISH analysis, revealed that heterochromatin is diffuse in ES cells and that it becomes more compact only in later stages of differentiation. Using fluorescence recovery after bleaching (FRAP), the authors showed that the exchange rates of GFP-labelled HP1α in heterochromatin were much higher in ES cells than in the NPCs, indicating that, in ES cells, a fraction of HP1 is only loosely associated with the heterochromatin. Faster exchange rates were also seen for subpopulations of the linker histone H1° and the H2B and H3 core histones, which is consistent with previous reports that heterochromatin domains are established as differentiation proceeds.

The hyperdynamic association of chromatin-associated proteins is functionally important. Removal of nucleosome assembly factor HirA exacerbates the looseness of chromatin-associated protein binding, making it even more dynamic. It turns out that HirA$^{-/-}$ cells undergo rapid early differentiation, indicating that increased availability of histones facilitates this process. Conversely, cells that express a variant of the H1° linker histone that binds chromatin with increased affinity fail to differentiate.

By examining the dynamic behaviour of histones in undifferentiated but lineage-committed cells, the authors showed that the hyperdynamic binding of chromatin-associated proteins is a hallmark of pluripotent cells and not just of an undifferentiated state. The authors propose that the loosely bound and soluble pool of structural proteins is crucial for maintaining pluripotency and for early differentiation. According to their appealing model, the dynamic and plastic chromatin state is the key to keeping all differentiation options open.

Magdalena Skipper

ORIGINAL RESEARCH PAPER Meshorer, E. *et al.* Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. *Dev. Cell* **10**, 105–116 (2006)