Journal club

THE (CHAIN) TERMINATORS

'It's in my DNA' is an expression we hear often nowadays. Nucleic acids were discovered by the chemist Friedrich Miescher in 1869, but the secrets of DNA were largely hidden until the DNA of the human genome, which comprises about 3.0×10^9 base pairs, was sequenced 132 years later, in 2001. Now, DNA sequencing is routine and underpins many biological research programmes, including those aimed at understanding human evolution, development and disease.

Fred Sanger (1918–2013) was the man who made this possible. In 1975, he described an elegant method — the plus and minus method — of sequencing DNA based on the position of DNA molecules copied by a DNA polymerase from the DNA of a simple bacteriophage under conditions of limiting different dNTPs. In this method, not only did Sanger introduce the idea of separating individual DNA molecules that differed by only a single residue according to their length on polyacrylamide gels, he also found a way of defining the 3′ end of each molecule, thereby deciphering the DNA sequence. Before publication of this method, DNA sequencing was extremely laborious and only short lengths of DNA had been sequenced, usually by degradative methods.

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In 1977, Sanger improved and simplified his method by using the DNA-chain terminators, 2′,3′ -dideoxynucleoside triphosphates (ddNTPs), three of which — ddATP, ddGTP and ddCTP — had never been synthesized before. Sanger described his 1975 paper as his 'most important paper', but I judge his 1977 paper to be even more important, because it was the basis of all the early genome sequencing projects — the human, nematode, yeast and *Escherichia coli* projects. Even now, one of the popular high-throughput genome sequencing methods uses chain terminators, although modified to be reversible. Thus, Sanger developed a method that still has great impact today.

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ORIGINAL ARTICLES Sanger, F. & Coulson, A. R. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J. Mol. Biol.* **94**, 441–448 (1975) | Sanger, F., Nicklen, S. & Coulson, A. R. DNA sequencing with chain-terminating inhibitors. *Proc. Natl Acad. Sci. USA* **74**, 5463–5467 (1977)

MEMBRANE TRAFFICKING

Sorting it out at the Golgi

The Golgi apparatus is the main sorting hub of the secretory pathway. Here, secreted proteins and proteins targeted to post-Golgi locations (such as the plasma membrane) move through the Golgi stacks and eventually leave the Golgi (anterograde transport), whereas Golgi-resident and endoplasmic reticulum (ER)-resident proteins are recycled and retained (retrograde transport). How these sorting events are regulated is poorly understood. Rothman and colleagues now show that anterograde transport is driven by protein palmitoylation.

Early cell-free studies in the Rothman lab suggested a role for palmitate in intra-Golgi transport. The authors now show that palmitate labels the Golgi in HeLa cells. This labelling was dependent on the active palmitoylation machinery and occurred at the cis-Golgi, where several DHHC palmitoyltransferases are also enriched. Thus, the cis-Golgi is a site of protein palmitoylation.

Overexpression of two cis-Golgi-localized palmitoyltransferases, DHHC3 and DHHC7, increased palmitate incorporation at the Golgi, which was accompanied by increased levels of palmitoylated proteins at the plasma membrane. This result suggests that protein palmitoylation at the Golgi promotes anterograde protein transport. Accordingly, plasma membrane proteins were identified as important DHHC substrates by bioinformatics analysis. Furthermore, mutation of palmitoylation sites impaired protein trafficking to the plasma membrane, and intra-Golgi transport was specifically affected.

Palmitoylated proteins were found to be enriched at highly curved regions at the cisternal rims of the Golgi. Palmitoylation also induced the relocalization

EPIGENETICS

Guardians of the oocyte methylome

The methylation state of the oocyte genome differs from that of most other cell types; for example, it is hypomethylated in regions that are transcriptionally inactive. In *Nature*, Li et al. now report that STELLA — a factor important for female fertility — prevents de novo DNA methylation in growing mouse oocytes and that this activity is important for embryonic development.

The authors found that during oogenesis, STELLA regulates the subcellular distribution of the E3 ubiquitin ligase UHRF1, which is known to recruit DNA and chromatin modifiers. Although UHRF1 is usually mostly cytoplasmic, it was predominantly found in the nucleus in growing oocytes from 5- to 20-day-old (D5–D20) postnatal *Stella*-knockout female mice. STELLA is usually evenly distributed between the nucleus and the cytoplasm but ectopic overexpression of a mutant STELLA that cannot be exported from the nucleus but that can still interact with UHRF1 failed to rescue UHRF1 mislocalization, indicating that STELLA transports UHRF1 out of the nucleus.

Whereas oocytes from D5 mice lacking STELLA had a largely normal hypomethylated genome, there was an approximately twofold increase in global methylation in oocytes from adult mice. Hypermethylation occurred in a wide range of genomic areas. Of note, regions that are transcriptionally inactive in the oocyte were aberrantly methylated. Furthermore, DNA methylation at differentially methylated regions of maternally or paternally imprinted genes was normal, indicating