

of increasing sample sizes to detect a greater number of disease-associated SNPs, and served as a cogent reminder that even more associations could be expected if studies were performed using samples that were larger still.

And the larger sample sizes came! Inspired by the WTCCC, international consortia rapidly formed to pool data. Sample sizes well into the tens of thousands became routine (Fig. 1), and at least 30 GWAS exceeding 100,000 individuals are now available online (www.ebi.ac.uk/gwas). The first study involving roughly 500,000 participants will soon be released (www.ukbiobank.ac.uk).

The WTCCC also helped to propel a revolution in data distribution. The study was one of the first GWAS to provide information about each participant's genotype and associated traits for use by the scientific community. Although access to these data were subsequently controlled to ensure participant confidentiality¹⁰, the tradition of open data-sharing and collaboration pioneered by the WTCCC has continued.

Where do we go from here? The flood of GWAS continues unabated, despite predictions that it was a transitional technology that would soon be supplanted by techniques to sequence either entire genomes or all protein-coding regions. Such sequencing studies have certainly identified many rare variants and

polymorphisms involving more than a single nucleotide (such as inserted or deleted sections of chromosomes), which GWAS have difficulty detecting. But the low cost and straightforward analytics of GWAS seem likely to ensure its longevity.

A crucial gap in the GWAS spectrum remains to be filled, because ancestrally diverse, non-European populations have been appallingly under-studied¹¹. Notable GWAS in these populations include studies of cardiac conduction in African Americans¹² and sleep apnoea in Hispanic and Latino Americans¹³. One of the next steps will be to identify associations in under-studied populations such as those in Africa and Latin America, and in isolated and indigenous peoples such as those in the Arctic, Pacific islands and Americas. Another outstanding opportunity lies in studies of adverse reactions to drug or other treatments, in which effect sizes are often large and may be directly relevant to clinical care¹⁴.

Despite the thousands of studies and millions of genomes examined, associations identified by GWAS still explain only a small fraction of the heritability of complex diseases, and the overwhelming majority fall in regions of the genome that have no known function⁴. These gaps in knowledge are major challenges and must be overcome if we are to develop

effective treatments and improve clinical care¹⁵. Ten years on, we are clearly on the right path, as set for us by the WTCCC. But, as with everything in science, the more we know, the more we have to learn. ■

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1. The Wellcome Trust Case Control Consortium. *Nature* **447**, 661–678 (2007).
2. Hirschhorn, J. N., Lohmueller, K., Byrne, E. & Hirschhorn, K. *Genet. Med.* **4**, 45–61 (2002).
3. Klein, R. J. et al. *Science* **308**, 385–389 (2005).
4. Hindorf, L. A. et al. *Proc. Natl Acad. Sci. USA* **106**, 9362–9367 (2009).
5. Manolio, T. A., Brooks, L. D. & Collins, F. C. *J. Clin. Invest.* **118**, 1590–1605 (2008).
6. Mossman, K. *Sci. Am.* **298**, 42 (2008).
7. Summerskill, W. *Lancet* **371**, 370–371 (2008).
8. Warren, H. R. et al. *Nature Genet.* **49**, 403–415 (2017).
9. Marchini, J., Howie, B., Myers, S., McVean, G. & Donnelly, P. *Nature Genet.* **39**, 906–913 (2007).
10. Homer, N. et al. *PLoS Genet.* **4**, e1000167 (2008).
11. Popejoy, A. B. & Fullerton, S. M. *Nature* **538**, 161–164 (2016).
12. Evans, D. S. et al. *Hum. Mol. Genet.* **25**, 4350–4368 (2016).
13. Cade, B. E. et al. *Am. J. Respir. Crit. Care Med.* **194**, 886–897 (2016).
14. Chan, S. L., Jin, S., Loh, M. & Brunham, L. R. *Pharmacogenomics* **16**, 1161–1178 (2015).
15. Price, A. L., Spencer, C. C. A. & Donnelly, P. *Proc. R. Soc. B* **282**, 20151684 (2015).

MOLECULAR BIOLOGY

Local metabolites linked to memory

Production of the metabolite acetyl-CoA near specific regions of DNA modulates gene expression in mouse neurons during cellular differentiation and memory formation. SEE ARTICLE P.381

L. ASHLEY WATSON & LI-HUEI TSAI

In the cell nucleus, DNA is wrapped around histone proteins to form a complex called chromatin. Histones can be modified by acetyl groups, leading to the modulation of gene expression. Such acetylation requires a nuclear pool of the metabolite acetyl coenzyme A (acetyl-CoA)^{1–3}, which can be generated through two pathways, involving either acetate metabolism by the enzyme acetyl-CoA synthetase 2 (ACSS2) or citrate metabolism by the enzyme ATP-citrate lyase (ACL)¹. Proliferating cells predominantly use the ACL pathway, although the invoked pathway can vary depending on tissue type or stage of development, and can be altered during disease progression³. On page 381, Mews *et al.*⁴ identify a major role for the ACSS2 pathway in mouse neurons, in which it regulates histone

acetylation and transcriptional dynamics during differentiation *in vitro* and in response to behavioural training *in vivo*.

Histone acetylation is regulated by the opposing actions of two classes of enzyme: histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs transfer acetyl groups from acetyl-CoA to the lysine amino-acid residues of histones. Acetylation weakens the electrostatic interactions between DNA and histones, effectively relaxing the structure of chromatin to create a more permissive environment for transcription. By contrast, HDACs remove acetyl groups, making the environment less permissive for transcription.

Researchers rarely question the source of the acetyl-CoA that provides acetyl groups for histones. For instance, despite the fact that the differentiation and function of neurons depend on histone acetylation⁵, the

mechanism by which acetyl-CoA is produced in neurons has been largely unknown. Investigation of the links between the ACL and ACSS2 pathways and chromatin regulation holds promise for increasing our understanding of the molecular mechanisms that integrate environmental stimuli with gene expression.

Enter Mews and colleagues. Their work stems from the keen observation that the gene that encodes ACSS2 is highly expressed in mature neurons in the hippocampus of the mouse brain⁶. This prompted the authors to test the relative contributions of the ACSS2 and ACL pathways to histone acetylation in neurons.

Using an *in vitro* model of neuronal differentiation, they demonstrated that ACSS2 undergoes a dramatic change in subcellular location as cells differentiate, moving from the cytoplasm in progenitor cells to the nucleus in mature neurons. ACL, however, remains in the cytoplasm throughout. The altered location of ACSS2 correlates with an increase in histone acetylation at DNA sequences called promoters that drive the expression of neuron-specific genes, and Mews *et al.* showed that ACSS2 is necessary for the transcriptional induction of key neuronal genes and for the final stage of neuron differentiation.

The authors next demonstrated that ACSS2 binds to chromatin directly. Isolation and sequencing of DNA regions that were bound by ACSS2 revealed that the enzyme is enriched in regions of chromatin that overlap

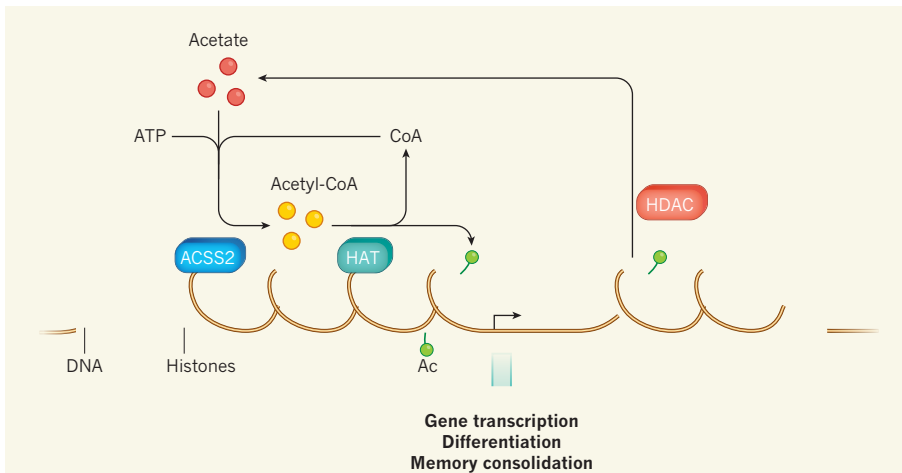


Figure 1 | From metabolic signalling to gene regulation through chromatin. The metabolite acetyl coenzyme A (acetyl-CoA) is produced by the enzyme acetyl-CoA synthetase 2 (ACSS2) from molecules of acetate, coenzyme A (CoA) and ATP. Mews *et al.*⁴ show that, in mice, ACSS2 binds to chromatin (a complex of DNA wrapped around proteins called histones) in differentiating or mature neurons. Binding occurs close to DNA sequences that drive the expression of neuron-specific genes that regulate differentiation or the consolidation of long-term memory. Histone acetyltransferase (HAT) enzymes transfer an acetyl group (Ac) from acetyl-CoA to histones, producing CoA as a by-product. Histone acetylation leads to chromatin unwinding and gene transcription. The authors speculate that the presence of ACSS2 on chromatin results in a local increase in the level of acetyl-CoA that is available to HATs. By contrast, enzymes known as histone deacetylases (HDACs) remove acetyl groups from histones, probably to counteract the histone-acetylation process. This action provides a source of recycled acetate for use in the further production of acetyl-CoA.

or are close to regions of dense histone acetylation. Levels of acetyl-CoA and histone acetylation in the nucleus were reduced when Mews *et al.* inhibited the activity of ACSS2 or reduced expression of the gene that encodes it. Together, these data led the authors to propose a model in which the localized production of acetyl-CoA by ACSS2 supplies HATs with a readily available substrate at specific promoters, leading to the rapid and efficient upregulation of neuronal transcripts during differentiation (Fig. 1).

Neuronal differentiation occurs during embryonic development. Previous studies⁵ suggest that, after birth, histone acetylation in mature neurons is associated strongly with memory formation. Chromatin becomes acetylated in specific regions of the brain, such as the hippocampus, in response to neuronal activity or behavioural training in rodents⁷. Such acetylation correlates with the increased expression of a set of 'immediate early' genes⁸, which encode proteins that broadly mediate changes in the strength of connections between neurons, therefore facilitating memory consolidation⁹. Mews *et al.* extended their *in vitro* findings by demonstrating that ACSS2 is required in the mouse hippocampus for the induction of immediate early genes and for long-term spatial memory. Together, their *in vitro* and *in vivo* results provide the first evidence, to our knowledge, for metabolic signalling to chromatin in the brain and show that this signalling has a crucial role in memory consolidation.

Despite the strength of Mews and colleagues' findings, some questions remain. For instance, what is the source of the acetate required by ACSS2 during the differentiation of neurons or memory formation? ACSS2 seems to bind to the promoters of immediate early genes before memory consolidation, implying that acetate, rather than ACSS2, is the limiting factor for histone acetylation at these genes. Furthermore, the authors suggest that the pool of available acetate might be governed by the activity of HDACs, which produce acetate as they deacetylate histones. This possibility is yet to be tested.

The relationship between the activity of ACSS2 and the level of histone acetylation is now clear, but the exact specificity of ACSS2 activity on chromatin is not. Mews *et al.* observed that the regions of chromatin in which ACSS2 was enriched corresponded directly to only 13–16% of histone acetylation marks, and many ACSS2-dense sites were located up to 20 kilobases away from regions that became more heavily acetylated following differentiation. ACSS2 is one of three members of the ACSS family of enzymes, another of which (ACSS1) is also expressed in neurons¹⁰. Other acetate-dependent enzymes might therefore act in different memory contexts, or in cooperation with ACSS2 in the spatial memory that is tested by the current study.

Finally, the contribution of ACL-dependent production of acetyl-CoA to memory has not yet been characterized fully. This enzyme might also have key roles in histone acetylation

in neurons, in certain contexts. Although the importance of ACSS2 in long-term spatial memory has now been demonstrated, we still lack a complete understanding of the relative roles of the ACL and ACSS2 pathways in the different types of histone-acetylation-dependent memory storage.

Many disease states, including neurodegeneration and cancer, are characterized by alterations in histone acetylation and acetate dependence. Manipulation of the activity or levels of ACSS2 could therefore provide a therapeutic approach to restoring appropriate histone acetylation. Moreover, alterations in acetyl-CoA metabolism, histone acetylation and gene expression are implicated in ageing¹¹. Mews and colleagues' findings might also provide insight into the mechanisms that link ageing- and disease-related metabolic changes to an altered chromatin landscape and transcriptional output, paving the way for further discoveries in this exciting field of research. ■

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- Wellen, K. E. *et al.* *Science* **324**, 1076–1080 (2009).
- Cai, L., Sutter, B. M., Li, B. & Tu, B. P. *Mol. Cell* **42**, 426–437 (2011).
- Pietrocola, F., Galluzzi, L., Bravo-San Pedro, J. M., Madeo, F. & Kroemer, G. *Cell Metab.* **21**, 805–821 (2015).
- Mews, P. *et al.* *Nature* **546**, 381–386 (2017).
- Gräff, J. & Tsai, L.-H. *Nature Rev. Neurosci.* **14**, 97–111 (2013).
- Lein, E. S. *et al.* *Nature* **445**, 168–176 (2007).
- Schmitt, M. & Matthies, H. *Acta Biol. Med. Ger.* **38**, 683–689 (1979).
- Peixoto, L. & Abel, T. *Neuropsychopharmacology* **38**, 62–76 (2013).
- West, A. E. & Greenberg, M. E. *Cold Spring Harb. Perspect. Biol.* **3**, a005744 (2011).
- Zhang, Y. *et al.* *J. Neurosci.* **34**, 11929–11947 (2014).
- Peleg, S., Feller, C., Ladurner, A. G. & Imhof, A. *Trends Biochem. Sci.* **41**, 700–711 (2016).

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CORRECTION

The News & Views article 'Palaeoanthropology: On the origin of our species' by Chris Stringer and Julia Galway-Witham (*Nature* **546**, 212–214; 2017) stated that at least five individuals were identified from human fossils uncovered in excavations reported by Hublin *et al.* (*Nature* **546**, 289–292; 2017) and Richter *et al.* (*Nature* **546**, 293–296; 2017). However, fossil samples uncovered during previous excavations were also used to identify the individuals. The text of the News & Views article has been amended online.