AUTISM

A long genetic explanation

Topoisomerase enzymes facilitate gene transcription by resolving DNA tangles. Malfunction of these enzymes seems to compromise the expression of very long genes, potentially mediating neurodevelopmental disorders. SEE ARTICLE P.58

ROBERT N. PLASSCHAERT
& MARISA S. BARTOLOMEI

Topoisomerases are a family of enzymes that catalyse the unwinding and unknotting of DNA sequences. By introducing transient 'nicks', these enzymes can relieve the topological pile-up of DNA that is caused by processes such as replication and transcription. On page 58 of this issue, King *et al.*[1] provide evidence that topoisomerases are required for the proper expression of extremely long genes in neurons. This insight has implications for our understanding of the fundamentals of both transcription and neurodevelopmental disorders*.

Genomic imprinting is an evolutionarily conserved mammalian phenomenon in which expression of a gene occurs preferentially from one parental chromosome. In human neurons, for example, the *UBE3A* gene is expressed only from the maternal chromosome, and deletion or mutation of this allele (gene copy) causes a severe neurodevelopmental disorder called Angelman syndrome[2].

In a previous study[3], King and colleagues screened for small molecules that, when applied to neurons in culture, activated the normally silenced paternal allele of *UBE3A*. Surprisingly, they found that topoisomerase inhibitors activated this allele. This ability of the enzymes to correct gene 'dosage' by derepressing silent alleles seemingly held much promise for the treatment of disorders that involve imprinted genes or that map to the X chromosome in females. But the mechanism underlying this activation remained unclear.

The earlier report also showed that topoisomerase inhibitors reduced the expression of *UBE3A-ATS*, a very long transcript expressed in neurons. *UBE3A-ATS* overlaps with paternal *UBE3A* on the opposite strand and is associated with the silencing of this allele (Fig. 1a). Repression of *UBE3A-ATS* by topoisomerase inhibitors, therefore, suggested that topoisomerases are involved in maintaining the expression of extremely long genes.

King *et al.* now report that, indeed, treatment of mouse and human neurons with topoisomerase inhibitors results in the widespread silencing of very long genes (those longer than 67 kilobases). This repression depends on the dose of the inhibitor and is highly correlated with increased gene length. Sustained repression of topoisomerase expression using short hairpin RNA (shRNA) sequences also resulted in reduced expression of long genes, excluding the possibility of off-target effects of the inhibitors.

To investigate the mechanism of topoisomerase action, King and colleagues mapped genome-wide binding sites of RNA polymerase II (Pol II) — the enzyme that catalyses DNA transcription — before and after treatment with topoisomerase inhibitors. The authors noted a significant enrichment of Pol II in promoter regions after treatment and a corresponding paucity of Pol II in the body of long genes (Fig. 1b). For short genes, however, Pol II density across the gene body was slightly increased. These results suggest that topoisomerases are specifically involved in the elongation step of transcription during the expression of long genes.

King *et al.* also found that topoisomerase inhibitors decreased the expression of an impressive proportion (27%) of long genes that are candidates for an association with autism spectrum disorders (ASDs). The authors show that the inhibitors significantly downregulate the collective expression of such ASD candidate genes, further supporting the link between topoisomerase mutations and reduced expression of long ASD genes. Consistently, recent work[4,5] has uncovered rare *de novo* mutations in topoisomerases from patients with ASD.

Notably, these findings suggest a possible role for topoisomerases in other genetic disorders in which the causal gene is exceptionally long. It is plausible that mutations that reduce the expression of these enzymes could allow the appropriate expression of all but a few very long genes. For instance, *CFTR*, the

*This article and the paper under discussion[1] were published online on 28 August 2013.

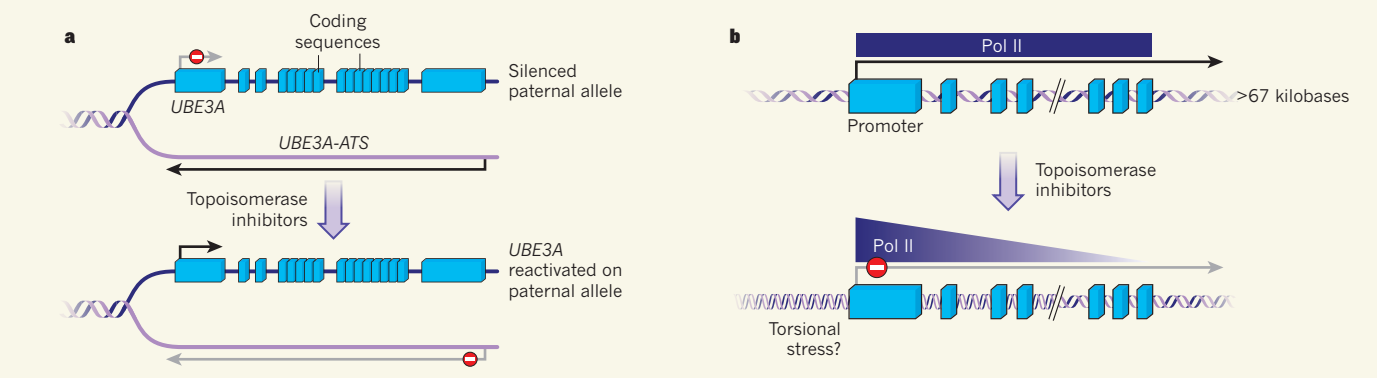

Figure 1 | Topoisomerase inhibitors and transcription. **a**, Treating neurons with topoisomerase inhibitors results in activation of the normally silent paternal allele of the *UBE3A* gene. It also represses transcription of the long transcript *UBE3A-ATS* in the antisense direction. **b**, King *et al.*[1] show that these inhibitors repress the transcription of all genes that have very long sequences (more than 67 kilobases), increasing the binding of the Pol II enzyme at their promoter sequences while decreasing its binding to their coding sequences. The authors hypothesize that topoisomerase inhibition causes a build-up of torsional stress associated with the transcription of long genes, resulting in their repression.

gene mutated in cystic fibrosis, spans more than 200 kilobases. And *DMD*, the causal gene in many forms of muscular dystrophy, spans a staggering 2.2 megabases. It would be surprising if topoisomerases did not contribute to these disorders in certain rare cases at least.

Nevertheless, the work also shows that topoisomerase inhibitors are not a panacea for disorders that would benefit from the activation of a normally silent allele. As these inhibitors are likely to have a widespread effect on the expression of all long genes, even appropriate targets such as *UBE3A* are unlikely to be activated without nonspecific effects. Furthermore, the delicate interplay between gene dosage and traits associated with such disorders makes a broad regulator of transcription such as topoisomerase a less attractive target for drug design.

Despite these concerns, King and colleagues' paper presents an intriguing and fundamentally novel role for topoisomerases in gene regulation. Although these enzymes were known to be required for the expression of longer transcripts in yeast[6], the present work cements their importance in mammals. Moreover, the studies show a requirement for both topoisomerase I and II enzymes in the expression of long genes, whereas only topoisomerase II is required in yeast. Such observations reveal an increased significance for topoisomerases in the regulation of the expanded and much more complex mammalian genome.

By excluding other possibilities, this work also strongly indicates that topoisomerases modify DNA topology during the expression of long transcripts. In prokaryotes (bacteria and archaea), transcription causes dynamic DNA supercoiling, which acts as both a negative and a positive regulator of transcription[7]. Transcription-associated supercoiling also occurs in mammals[8], but its functional relevance is unclear. King and co-authors' findings point towards the possible importance of overcoming supercoiling during the transcription of long genes. Thus, modifying the action or recruitment of topoisomerases should provide a way of exploring how the expression of long mammalian genes is modulated. ■

Robert N. Plasschaert *and* **Marisa S. Bartolomei** *are in the Department of Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania 19104, USA.*
e-mail: bartolom@mail.med.upenn.edu

1. King, I. F. *et al. Nature* **501,** 58–62 (2013).
2. Kishino, T., Lalande, M. & Wagstaff, J. *Nature Genet.* **15,** 70–73 (1997).
3. Huang, H.-S. *et al. Nature* **481,** 185–189 (2011).
4. Neale, B. M. *et al. Nature* **485,** 242–245 (2012).
5. Iossifov, I. *et al. Neuron* **74,** 285–299 (2012).
6. Joshi, R. S., Piña, B. & Roca, J. *Nucleic Acids Res.* **40,** 7907–7915 (2012).
7. Peter, B. J. *et al. Genome Biol.* **5,** R87 (2004).
8. Kouzine, F., Sanford, S., Elisha-Feil, Z. & Levens, D. *Nature Struct. Mol. Biol.* **15,** 146–154 (2008).

QUANTUM INFORMATION

Sharing quantum secrets

A cost-effective architecture for quantum cryptography has been demonstrated in which a single receiver positioned at a network-hub node is shared by many end users to exchange secret encryption keys. **SEE LETTER P.69**

RUPERT URSIN & RICHARD HUGHES

Keeping a secret has never been easy. Throughout history, ideas for encrypting messages have spurred people to come up with ways of breaking the keys that encrypt the messages. Perhaps the most famous example is the race that occurred during the Second World War between the German Enigma cipher machines and the British Colossus — the world's first electronic, digital computer. In the 1980s, the game changed with the invention of a cryptographic technique known as quantum key distribution (QKD)[1] that uses the laws of quantum physics to guarantee secure communication. So far, however, QKD has been demonstrated only for point-to-point communications and relatively simple networks. On page 69 of this issue, Fröhlich *et al.*[2] describe a method that brings the advantages of QKD to as many as 64 end users, who can share a quantum key, and thus a secret. The results are illustrative of the worldwide research progress towards a practical 'quantum Internet'.

Through the seminal research of Auguste Kerckhoffs in the nineteenth century[3] and Claude Shannon in the twentieth century[4], it is possible to reduce the problem of secure communication to the secure transfer of a secret encryption key. Unfortunately, neither Kerckhoffs nor Shannon explained how secure key distribution could be performed. Today, it relies on the unproven difficulty of cracking certain hard mathematical problems, such as factoring large integers. But factoring methods are continually improving, making the security lifetime of this method hard to predict.

Quantum cryptography avoids these issues. In this technique, the key is encoded into quantum states, such as the polarization of a series of single photons that are passed between two parties trying to share secret information. Heisenberg's uncertainty principle dictates that a third party trying to decode the key cannot look at these photons without changing or destroying the information they carry. In this case, it does not matter what technology the third party has: it will never be able to break the laws of quantum physics and decrypt the key.

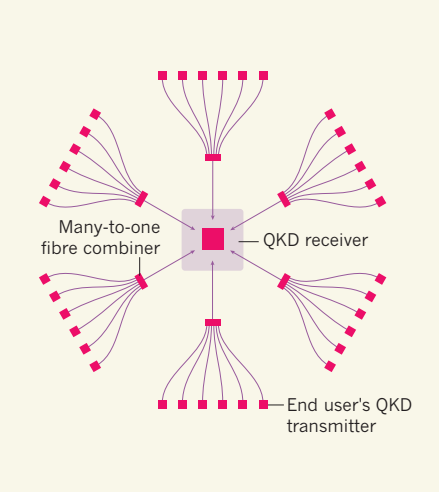

Figure 1 | A quantum access network. Fröhlich and colleagues[2] have developed a cost-effective quantum key distribution (QKD) architecture in which a single QKD receiver is placed at a trusted network-hub node and is shared by many end users' QKD transmitters. The QKD single-photon signals from the users are sent over optical fibres, through a many-to-one combiner, and routed to the network hub.

More-recent research[5] shows that QKD is secure, even if quantum mechanics turns out to be only an approximate theory describing the world. QKD is the first quantum-information application to reach the level of a commercial technology[6,7]. Present-day commercial QKD systems have been developed with a view to incorporating them into existing telecommunication infrastructures at the metro-area scale.

But we live in a networked world and QKD is intrinsically a point-to-point protocol. Several research groups have previously investigated how the advantages of QKD could be brought to multi-party networks (for example, see refs 8 and 9). These 'trusted QKD networks' amount to a mesh of point-to-point QKD links between nodes within which QKD-generated keys must be physically secured against adversaries (hence the need for the nodes to be trusted). However, this approach involves tremendous duplication of resources, with each node requiring QKD receivers to accept incoming photons, and QKD transmitters to send keys on to other nodes. The resulting high