

genetic contributions to disease risk. Indeed, Zemke and colleagues demonstrate that genetic variants linked to multiple sclerosis, anorexia nervosa and tobacco-use disorder often overlap with regulatory elements that are conserved across species¹⁴. Thus, the work presented by the BICCN has laid a firm foundation for many further discoveries relating to neurobiology and neurological disorders.

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Immunology

DNA is tethered together in immune cells

Zhi Liu & Ye Zheng

A transcription factor in immune cells forms an unexpectedly ladder-like complex with two DNA molecules, allowing the expression of genes that these cells need to suppress harmful immune responses. **See p.433**

The human immune system is meant to defend the body from pathogens such as bacteria and viruses. When the immune system goes awry, however, it can start to attack the body itself, resulting in autoimmune diseases such as type 1 diabetes and rheumatoid arthritis. To prevent this from happening, the immune system has a braking mechanism, in the form of regulatory T (T_{reg}) cells that rein in harmful immune reactions by suppressing the activity of other immune cells. The differentiation of T_{reg} cells from precursor cells (a process referred to as T_{reg} cell development) depends on a transcription factor called FOXP3, which turns genes on or off by binding to DNA. On page 433, Zhang *et al.*¹ describe the ladder-like structure of a complex of ten FOXP3 proteins and two DNA molecules, which could be responsible for bringing remote regions of DNA together to control gene expression.

Most T_{reg} cells arise in the thymus gland along with other T cells; the remainder develop in the body's periphery, such as the small and large intestines. A key step in T_{reg} cell development is the expression of FOXP3, which kick-starts differentiation. This leads to the expression of a suite of genes specific to T_{reg} cells, called signature genes, that allows them to carry out their immune-suppressive function^{2,3}.

The *FOXP3* gene was originally identified by mapping mutations that cause a severe autoimmune disease in humans and a similar condition in a mutant mouse strain^{4,5}. It belongs to a family of at least 40 'forkhead box' proteins, all of which contain a forkhead DNA-binding domain that recognizes a short DNA sequence known as a forkhead motif. When FOXP3 was first crystallized, it was thought to form a type

of dimer in which identical domains from each protein are swapped over⁶. However, a structural study last year revealed that FOXP3 proteins actually form head-to-head dimers when they bind to DNA⁷.

Like other forkhead-box proteins, FOXP3 has been shown to bind to the forkhead motif *in vitro*^{6,7}. However, when researchers sequenced the DNA motifs bound by FOXP3 *in vivo*, the forkhead motifs barely showed up^{8–10}. This result raised the intriguing question of whether FOXP3 functions in T_{reg} cells by binding to DNA sequences other than the forkhead motif.

In the current study, Zhang and colleagues started to tackle this question by looking for sequences in the mouse genome to which FOXP3 was bound. Surprisingly, they found that FOXP3 was strongly associated with DNA fragments containing repeat sequences of several thymine (T) nucleotides, followed by one guanine (G) nucleotide (denoted T_nG), instead of the expected forkhead motif. Notably, repeat sequences such as T_nG are usually considered non-functional and are discarded as junk DNA (sequences with no apparent biological function) by sequence-analysis programs, explaining why previous studies ignored these sequences when looking for fragments of DNA that bind to FOXP3.

Using cryo-electron microscopy, the authors resolved the structure of FOXP3 proteins in a complex with DNA molecules that contained 18 T_nG repeats. The structure of the complex is exquisitely ladder-like: two double-stranded DNA molecules form the 'side rails' of the ladder, which are pulled together by five pairs of FOXP3 proteins that serve as 'rungs'. This represents a new geometry of the FOXP3–DNA assembly, which is in contrast to the simple head-to-head dimer that FOXP3 proteins form on binding to a single DNA molecule (Fig. 1).

The ladder-like structure of the FOXP3–DNA

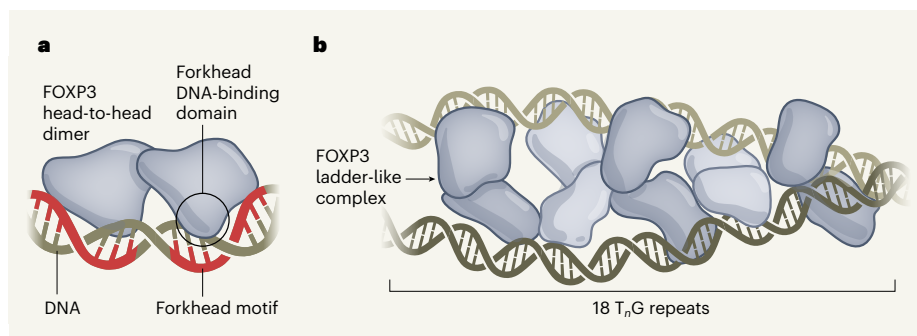


Figure 1 | The transcription factor FOXP3 can control gene expression in regulatory T cells through two distinct mechanisms. **a**, Two FOXP3 proteins form a head-to-head dimer that binds to a region of DNA called the forkhead motif, which directly regulates gene expression. **b**, Zhang *et al.*¹ report that multiple FOXP3 proteins can also bind to regions of DNA called T_nG repeats. This interaction forms a ladder-like complex consisting of two double-stranded DNA molecules held together by five pairs of FOXP3 proteins, which brings together regions of DNA that would otherwise be far apart. Both mechanisms seem to be important for the transcription of genes that allow regulatory T cells to carry out their function of suppressing the activity of other immune cells.

complex suggests that FOXP3 can act as a tether that pulls together two stretches of T_nG repeats that are otherwise far apart, thereby creating and stabilizing long-distance DNA interactions. Indeed, Zhang *et al.* found that T_nG repeats appear frequently in FOXP3-bound long-distance DNA-to-DNA contact sites, many of which are near T_{reg} signature genes. The positions of T_nG repeats also correlate with regions of DNA bound by FOXP3, where ‘enhancer’ and ‘promoter’ DNA sequences are thought to interact to allow gene transcription^{11,12}.

To test whether the ladder-like complex is essential for the function of FOXP3 in T_{reg} cells, Zhang and colleagues introduced mutations into FOXP3 that would disrupt interactions between pairs of proteins that make up the ‘rungs’ of the ladder, and thus prevent DNA bridging. T_{reg} cells carrying these mutations showed a reduction in the expression of T_{reg} signature genes, and so a weaker immune-suppressive function, than did cells in which FOXP3 was not mutated – suggesting that long-distance DNA-to-DNA interactions facilitated by FOXP3 are important for normal T_{reg} cell function. This finding aligns with previous studies, which suggested that DNA-to-DNA interactions fail to emerge during T_{reg} cell development in cells that had been engineered to lack the FOXP3 gene^{11,12}.

Zhang *et al.* also found that FOXP3 in mice, zebrafish (*Danio rerio*), platypus (*Ornithorhynchus anatinus*) and humans all show similar binding to T_nG repeats and DNA-bridging activity, suggesting that the ladder-like assembly of FOXP3 and DNA is evolutionarily conserved – and probably appeared at the same time as T_{reg} cells and FOXP3 evolved in fish¹³. The authors assessed other forkhead-box proteins for their capacity to bind to T_nG repeats and found that three more FOXP family members (FOXP1, FOXP2 and FOXP4) share this ability, suggesting that the function of FOXP proteins as tethers is conserved.

Two roles for FOXP3 are becoming apparent. The first is as a classic transcription factor that turns gene expression on or off. The second is as a tether that brings two molecules of DNA into close proximity. This might help to shape the 3D structure of chromatin (the packaged form of nuclear DNA in a complex with supporting proteins) and thus enable remote enhancer sequences to interact closely with promoter sequences to regulate gene expression. More studies are needed to tease out how each of these roles of FOXP3 contributes to the development and function of T_{reg} cells.

The study by Zhang and colleagues focuses on the structure of FOXP3 itself. However, FOXP3 forms large multi-protein complexes in T_{reg} cells¹⁴. It remains to be determined how other proteins that interact with FOXP3 are involved in the DNA-tethering activity. Moreover, this work would benefit from a further

study using a mouse model carrying mutations that prevent FOXP3 from being able to tether DNA, to explore the consequences for T_{reg} cells and the balance of the immune system *in vivo*.

This study will also rekindle curiosity about the function of DNA repeats. Short DNA sequences that are repeated multiple times (such as T_nG) are called microsatellite sequences, and occupy around 3% of the human genome¹⁵. Because microsatellites have a higher mutation rate than do other regions of DNA (for example, those that code for proteins)¹⁶, differences between the sequences of microsatellites in the genomes of individual humans can be used for genetic fingerprinting in forensics and analyses of genetic variation in populations. The biological functions of microsatellites are not widely studied. Zhang and colleagues’ finding that FOXP3 and other FOXP family members can recognize T_nG repeats to bridge DNA molecules suggests that microsatellites could be key regulators of gene expression.

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Metrology

The trick that could put an optical clock on a chip

Mengxi Tan & David J. Moss

Researchers have made a key breakthrough in how light is used to control time signals from the world’s most precise clocks. The technique marks a crucial step in bringing this technology into everyday life. **See p.267**

As technology has become ever more precise, so too have the tools for measuring time – from the shadows cast by ancient sundials to the digital displays of modern wristwatches. Enter the exciting world of optical clocks, sophisticated devices so accurate and precise that they might soon redefine the second. On page 267, Moille *et al.*¹ report an ingenious method of controlling miniature devices known as optical frequency combs. These combs could be the key to fabricating chip-based optical clocks that are small, ultraprecise and reliable, with low power requirements and transformative real-world applications in timekeeping and navigation.

Any clock must operate in synchrony with an external reference – for example, Earth’s rotation is the reference that marks the length of a day. And a clock’s accuracy is determined

by how precisely it can move in step with this reference. For optical clocks, the reference is the frequency of electromagnetic radiation that is absorbed by electrons when they transition between discrete energy levels in an atom. An optical clock measures time by synchronizing the frequency of a laser with this specific frequency. The higher the frequency, the more precise the time standard.

The problem is that these highly precise optical signals ultimately need to be counted by electronics, and even the most advanced electronic devices operate at frequencies that are thousands of times too slow. Optical frequency combs offer a solution by forming a kind of gear network that converts optical frequencies to electronic ones². These combs are frequency spectra, produced by specialized lasers, that comprise regularly spaced spectral