Because of the known role of SIRT6 in suppressing gene expression[2], Zhang *et al.* examined changes in gene expression in the mutants. Among the most upregulated genes was *H19*, which encodes a long non-coding RNA that is known to regulate fetal growth[6]. *H19* expression levels were increased in all tissues examined, with the highest expression in the brain.

Next, the authors used a different gene-editing approach to generate human neural progenitor cells lacking *SIRT6 in vitro*, and showed that the differentiation of these cells into neurons was delayed when compared with wild-type cells. This defect was accompanied by higher levels of H19 RNA. Finally, the group found that SIRT6 removes acetyl groups associated with *H19* transcription, and showed that reducing *H19* expression in human cells lacking *SIRT6* resolved their defects in neuronal differentiation. Thus, SIRT6 inhibits *H19* expression to modulate neuronal development in human cells, as in monkeys.

Several avenues for further work arise from these results. For instance, the absence of SIRT6 altered the expression of thousands of genes in various tissues, and it is unlikely that *H19* is the only gene responsible for the defects observed. Indeed, a human developmental disorder called Silver–Russell syndrome can be caused by increased H19 levels but, in contrast to *SIRT6*-deficient monkeys, people who have this disorder have normal lifespans and less-severe developmental changes[6]. This discrepancy suggests that SIRT6-modulated genes other than *H19* also contribute to the severe effects seen in the authors' mutant monkeys. It will be hard to pinpoint the precise genes that cause developmental defects in *SIRT6*-deficient animals, but this should be investigated in the future.

From an evolutionary point of view, *SIRT6* is fascinating. In all mammals studied, the gene's deletion causes premature death, and the protein has the same enzymatic activity and involvement in glucose metabolism and stem-cell differentiation[7]. However, as we climb the evolutionary ladder from mice to monkeys to humans, some of the traits caused by *SIRT6* deletion become progressively more severe. *SIRT6*-deficient mice die a few weeks to months after birth[8], whereas monkeys die within hours, and humans harbouring a *SIRT6*-inactivating mutation are not even born. This increasing severity could be explained by the acquisition of regulatory roles for *SIRT6* over the course of evolution. In support of this idea, the severe brain defects seen in *SIRT6*-deficient primates have not been reported in mice, and this change correlates well with differences in brain complexity in these species. It will be extremely interesting to further explore the source of this trait enhancement across evolution.

What can we learn about the role of SIRT6 in human ageing from this primate model? At first glance, there is not an obvious connection between the developmental defects seen in the monkeys and ageing, as they are at opposite ends of life's timeline. However, key pathways regulated by SIRT6 are conserved between these species, and genome-wide association studies have found a correlation between *SIRT6* and increased lifespan in humans[9]. These facts, together with data indicating that SIRT6 helps to protect the brain against ageing-related disorders such as Alzheimer's disease[10], strongly suggest that the versatile SIRT6 protein might promote healthy longevity in humans. In the future, developments in CRISPR engineering might enable gene editing in specific tissues, and at chosen time points; if the latter were achieved, it would be fascinating to characterize the role of SIRT6 in primate lifespan.

More generally, genome editing is an exciting future strategy for human therapy. However, the challenge is to induce the desired edits without creating nonspecific mutations or producing mosaic embryos in which only some cells express the edited gene. Promisingly, Zhang and colleagues found no mosaicism or detectable off-target mutations in their mutant animals, and another group that have used CRISPR in monkeys also report no off-target effects[11]. Although there are still many ethical and technical caveats to be considered, the authors' achievement — along with a similar success in human embryos[12] — gives hope that human genetic therapies using CRISPR engineering will be possible in the future. ■

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STRUCTURAL BIOLOGY

Transcriptional speed bumps revealed

The enzyme RNA polymerase II, which transcribes DNA, pauses early in transcription and awaits signals to continue. High-resolution structures reveal how it is stopped and efficiently restarted. **See Articles p.601 & p.607**

KAREN ADELMAN & TELMO HENRIQUES

A first step in gene expression is the recruitment of the DNA-transcribing enzyme RNA polymerase II (Pol II) to a gene, and the assembly of transcriptional machinery around it. Pol II can then initiate RNA synthesis. However, during transcription of most mammalian genes, Pol II does something peculiar — after synthesizing a short RNA molecule usually no longer than 60 nucleotides, it stops, awaiting further instructions before transcribing the remainder of the gene[1]. Such pausing and subsequent RNA elongation is central to gene regulation in animals, yet the mechanisms underlying this process have not been clear. In two papers in this issue, Vos *et al.*[2,3] describe landmark structures that shed new light on Pol II pausing and release.

A heterodimer comprising the proteins SPT4 and SPT5 is crucial for the pausing of Pol II (ref. 4). During transcription initiation, general transcription factors bind and occlude the regions of Pol II recognized by SPT5 — these factors must be released before SPT5 can associate. Thus, SPT5 binding occurs after transcription proper begins, and stable interactions between SPT5 and Pol II require a nascent RNA about 20 nucleotides in length to have formed[5]. Interactions with transcribing Pol II then enable SPT5 to recruit additional factors that govern Pol II activity and RNA processing[4,5]. One such factor is the negative elongation factor (NELF) protein complex, which comprises four subunits (NELF-A, -B, -C and -E)[4].

In contrast to SPT5, which is evolutionarily conserved from bacteria all the way through to humans, no equivalents to the mammalian NELF proteins have been identified in bacteria, yeast, worms or plants[4]. The organisms that do contain a NELF complex are those that exhibit stable pausing of Pol II, implying a role for NELF in this process. Indeed, the release of NELF from Pol II is concomitant with escape from pausing into elongation[1], and acute depletion of NELF both prevents normal pausing[6] and increases premature termination[7]

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(the process whereby Pol II inadvertently releases DNA, ceasing transcription). But the molecular basis of NELF activity has remained obscure. In particular, it has been unclear how NELF interacts with Pol II and how it might stabilize the paused state in a manner that prevents both continued RNA synthesis and transcription termination.

In the first of their papers (page 601), Vos *et al.*[2] used cryo-electron microscopy to resolve the structure of a paused transcription complex at 3.2-ångström resolution. The authors assembled a highly purified structure on an artificial DNA–RNA scaffold that contains sequences known[8] to strongly promote Pol II pausing, using pig Pol II along with human SPT5 and NELF complexes. The Pol II–SPT5–NELF complexes formed on this scaffold showed clear differences compared with previously published Pol II–SPT5 complexes in an actively transcribing conformation[9]. Whereas the DNA–RNA hybrid held within active Pol II has an unpaired DNA base that can be used as a template to direct addition of the next RNA nucleotide, the DNA–RNA hybrid in the paused complex is 'tilted' and lacks unpaired template DNA. Without a free DNA base in its active site, Pol II is unable to carry out RNA elongation.

This non-productive DNA–RNA hybrid conformation alone explains why Pol II pauses. But more importantly, the structure also reveals the role of NELF in this process. The researchers found that a protein lobe comprising the NELF-A and NELF-C subunits binds near a funnel region in Pol II through which nucleotides normally access the active site. The NELF lobe protrudes into the funnel, potentially restricting the entry of nucleotides needed for transcription. In addition, NELF restrains mobile loop domains in Pol II, such as the trigger loop, near the active site. This restraint locks the enzyme in the inactive conformation while simultaneously discouraging Pol II from sliding along the DNA, which can lead to transcription termination.

The NELF binding pocket near the Pol II funnel overlaps with a region that, when not occluded, can be bound by the factor TFIIS to stimulate elongation. Intriguingly, TFIIS has been shown to reactivate Pol II that adopts a non-productive, tilted DNA–RNA hybrid conformation[10]. Thus, Vos *et al.* propose that NELF also prevents Pol II reactivation by blocking TFIIS binding (Fig. 1).

The release of paused Pol II into elongation is triggered by the recruitment of the kinase enzyme P-TEFb, which phosphorylates Pol II and pause-inducing factors, triggering dissociation of NELF (ref. 1). P-TEFb activity is accompanied by the recruitment to Pol II of the SPT6 protein and the polymerase-associated factor (PAF) protein complex. However, whether these elongation-associated factors directly affect Pol II pause release has been unclear. In the second of the papers (page 607), Vos *et al.*[3] examined this possibility

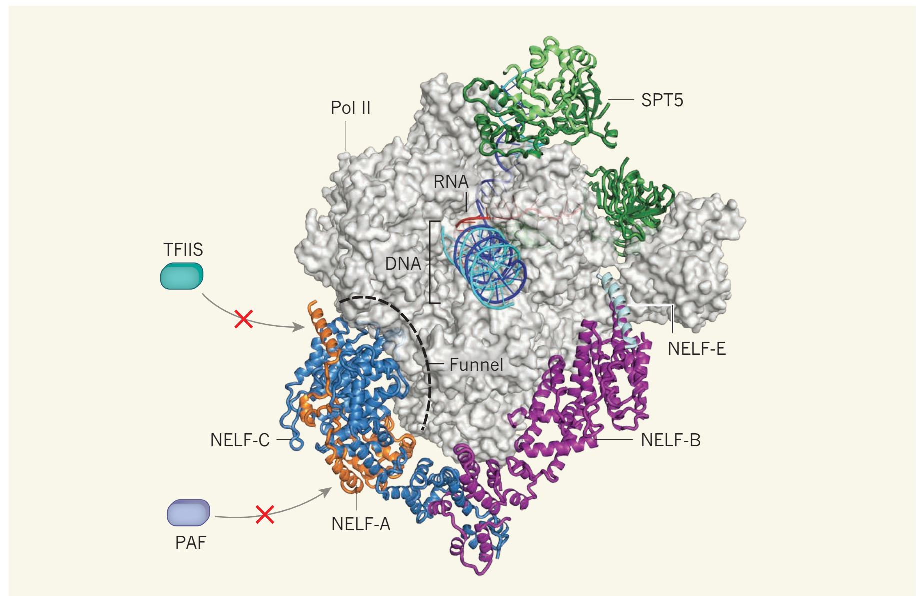

Figure 1 | Visualizing transcriptional pause and release. The DNA-transcribing enzyme RNA polymerase II (Pol II) pauses after initiating RNA synthesis and must be reactivated to continue transcriptional elongation of the nascent RNA. Vos *et al.*[2,3] solved high-resolution structures of Pol II and the transcriptional elongation machinery around it, both in the paused state and after elongation has resumed (the latter is not shown here). In the paused state, nascent RNA and the DNA being transcribed are held by the SPT5 protein and two subunits of the NELF protein complex (NELF-A and NELF-C) in a tilted conformation that prevents further transcription. NELF binds close to a funnel domain in Pol II, and blocks binding of Pol II by the factors TFIIS and PAF, which are needed for efficient elongation. NELF dissociates from Pol II to allow this binding to occur in the reactivated complex. (Figure adapted from Fig. 2b of ref. 2.)

by assembling a structure that included a modified, elongation-permissive nucleic-acid scaffold and these activating proteins.

As anticipated, the DNA–RNA hybrid in the activated elongation complex is no longer tilted and adopts a conformation compatible with RNA synthesis. The authors found multiple sites phosphorylated by P-TEFb in both SPT5 and NELF. Phosphorylation at these sites might aid the opening of the interface between Pol II and SPT5, and lead to dissociation of NELF. Furthermore, the group showed that phosphorylation of SPT6 and a linker region in the carboxy-terminal domain of Pol II aided docking of SPT6 on the enzyme. Most strikingly, the structure revealed that the binding of NELF and PAF to Pol II is mutually exclusive. Thus, dissociation of NELF during pause release enables the binding of PAF as well as TFIIS, allowing transcription to proceed.

Taking these results together, a detailed molecular model of Pol II pausing and release begins to emerge. We note a recurring theme wherein mutually exclusive, overlapping binding sites for a succession of Pol II-associated factors enable an orderly exchange during the transcription cycle. Furthermore, the specificity of each protein's interaction with the Pol II complex is ensured by multiple interaction interfaces, often with scaffold proteins such as SPT5 and the nucleic acids.

Of course, questions remain about the transition from pausing to productive elongation. For example, this work calls into question the roles of RNA-binding domains found in NELF subunits[4]. Surprisingly, Vos *et al.* showed that disruption of one such domain in NELF-E had no effect on pausing. It also remains to be seen whether the tilted DNA–RNA conformation observed by the authors is prevalent *in vivo*, and how the phosphorylation of pause-inducing factors drives pause release.

This work represents a fundamental jump in our understanding of pausing. The structures point to several appealing models for regulated pause release that can be tested in future work. ■

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