a series of patient samples — such as biopsies or tumour DNA isolated from blood samples — during the course of therapy might help to reveal whether crucial DNA alterations arise during treatment or were already present in a subset of tumour cells before treatment. Quigley and colleagues' work with a large number of patient samples only partially addresses this. Analysis of patients over time might also help to determine when therapy needs to be altered to try to prevent the development of treatment-resistant disease. Third, the technologies available for detecting genomic changes are rapidly improving, and the sequencing approaches used in the current studies can detect complex DNA alterations that were particularly challenging to determine using earlier techniques.

The genomic revolution that started with the Human Genome Project is reaching the cusp of a wave of detailed genomic studies that investigate how cancer evolves during treatment. Such progress represents another step closer to an era of precision medicine for cancer therapy. ■

Kellie A. Cotter *and* **Mark A. Rubin** *are in the Department for BioMedical Research, University of Bern, CH-3008 Bern, Switzerland, and at Inselspital, Bern, Switzerland.* **M.A.R.** *is also at Weill Cornell Medicine, New York, USA.*
e-mail: mark.rubin@dbmr.unibe.ch

1. International Human Genome Sequencing Consortium. *Nature* **409**, 860–921 (2001).
2. Venter, J. C. *et al. Science* **291**, 1304–1351 (2001).
3. Takeda, D. Y. *et al. Cell* **174**, 422–432 (2018).
4. Viswanathan, S. R. *et al. Cell* **174**, 433–447 (2018).
5. Quigley, D. A. *et al. Cell* **174**, 758–769 (2018).
6. Weinhold, N., Jacobsen, A., Schultz, N., Sander, C. & Lee, W. *Nature Genet.* **46**, 1160–1165 (2014).
7. Taplin, M.-E. *et al. N. Engl. J. Med.* **332**, 1393–1398 (1995).
8. Visakorpi, T. *et al. Nature Genet.* **9**, 401–406 (1995).
9. Armenia, J. *et al. Nature Genet.* **50**, 645–651 (2018).
10. Kumar, A. *et al. Nature Med.* **22**, 369–378 (2016).
11. Aggarwal, R. *et al. Eur. Urol. Focus* **2**, 469–471 (2016).
12. Holmes, M. G. *et al. J. Vasc. Interv. Radiol.* **28**, 1073–1081 (2017).
13. Glodzik, D. *et al. Nature Genet.* **49**, 341–348 (2017).
14. Herranz, D. *et al. Nature Med.* **20**, 1130–1137 (2014).
15. Shi, J. *et al. Genes Dev.* **27**, 2648–2662 (2013).
16. Zhang, X. *et al. Nature Genet.* **48**, 176–182 (2016).
17. Baca, S. C. *et al. Cell* **153**, 666–677 (2013).

PRIMATE BIOLOGY

Embryonic role for a longevity protein

Monkeys genetically engineered to lack the gene *SIRT6* die a few hours after birth, displaying severe growth defects. This finding reveals a previously unknown role for the SIRT6 protein in primate development. SEE LETTER P.661

SHOSHANA NAIMAN & HAIM Y. COHEN

For decades, biologists using model organisms such as mice and fruit flies have faced concerns about the relevance of their findings to humans. Using a model that is more evolutionarily similar to humans, such as another primate, could potentially close this frustrating gap. On page 661, Zhang *et al.*[1] use CRISPR–Cas9 gene-editing techniques to generate macaque monkeys lacking the gene *SIRT6*. Strikingly, they show that the SIRT6 protein has a role in embryonic development in macaques that was not previously uncovered in mice.

Mammalian SIRT6 removes acetyl groups from histone proteins. DNA is packaged around histones in the nucleus, and this deacetylation condenses the packaged DNA, suppressing gene expression[2]. In mice, SIRT6 is known to be a longevity protein that regulates many factors that alter during ageing, including genome stability, inflammation and metabolism[2]. Indeed, overexpression of *SIRT6* in male mice leads to health improvements and extends lifespan[3], whereas *SIRT6*-deficient mice die a few weeks after birth, displaying features of premature ageing[4].

It is unknown whether SIRT6 is involved in longevity in humans. However, recent data show[5] that an inactivating mutation in human *SIRT6* causes overexpression of embryonic stem-cell genes, which leads to abnormal development and severe brain defects, resulting in embryonic death. These findings suggest a previously unappreciated role for SIRT6 in embryonic development, which should be considered separately from its role in ageing.

Zhang and colleagues used CRISPR–Cas9 to create one male and three female macaque embryos that did not express *SIRT6*. The females died shortly after birth and the male died in the middle of gestation. The absence of SIRT6 caused severe, whole-body developmental delays. Compared with wild-type newborns, the mutants showed lower bone density, lower levels of subcutaneous fat and immature intestines and skeletal muscle. The authors also found that *SIRT6*-deficient monkeys had smaller brains owing to delayed neuronal maturation and an increase in the number of immature neural progenitor cells. Overall, the *SIRT6*-mutant animals were born much smaller than controls and showed gene-expression and morphological profiles closer to those of a typical three-month-old fetus than a full-term animal born after six months of gestation (Fig. 1).

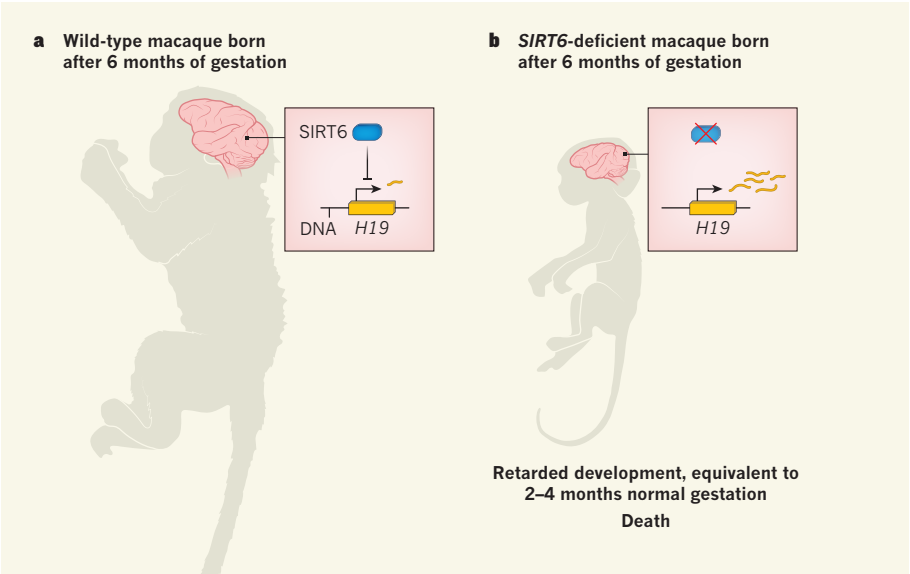

Figure 1 | A role for the *SIRT6* gene in primate development. Zhang *et al.*[1] used gene-editing tools to generate macaque embryos that did not express *SIRT6*. **a**, Wild-type macaques are born after around 6 months of gestation. In these animals, the SIRT6 protein suppresses expression of the gene *H19*. **b**, *SIRT6*-deficient monkeys die a few hours after birth. These animals have major developmental defects and are a similar size to wild-type fetuses at 2 to 4 months of development. Notably, *SIRT6*-deficient animals have small and immature brains — this defect is accompanied by a dramatic increase in *H19* expression.

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. ■

Shoshana Naiman *and* **Haim Y. Cohen** *are at the Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel.*
e-mail: haim.cohen@biu.ac.il

1. Zhang, W. *et al. Nature* **560**, 661–665 (2018).
2. Tennen, R. I. & Chua, K. F. *Trends Biochem. Sci.* **36**, 39–46 (2011).
3. Kanfi, Y. *et al. Nature* **483**, 218–221 (2012).
4. Mostoslavsky, R. *et al. Cell* **124**, 315–329 (2006).
5. Ferrer, C. M. *et al. Genes Dev.* **32**, 373–388 (2018).
6. Abu-Amero, S. *et al. J. Med. Genet.* **45**, 193–199 (2008).
7. Tasselli, L., Zheng, W. & Chua, K. F. *Trends Endocrinol. Metab.* **28**, 168–185 (2017).
8. Peshti, V. *et al. PLoS One* **12**, e0176371 (2017).
9. Hirvonen, K. *et al. BMC Med. Genet.* **18**, 41 (2017).
10. Kaluski, S. *et al. Cell Rep.* **18**, 3052–3062 (2017).
11. Zuo, E. *et al. Cell Res.* **27**, 933–945 (2017).
12. Ma, H. *et al. Nature* **548**, 413–419 (2017).

This article was published online on 22 August 2018.

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]