TRANSCRIPTION

50th anniversary of transcription portrayal in eukaryotes

Fifty years ago, Robert Roeder and William Rutter reported the isolation of three distinct eukaryotic DNA-dependent RNA polymerases (Pol) — the nucleoplasmic Pol II and Pol III and the nucleolar Pol I — thereby showing that, in contrast to prokaryotes, eukaryotes have more than one type of RNA polymerase. In early 1970, Pierre Chambon and colleagues, and later the Rutter group, used the differential sensitivities of the three RNA polymerases to the mushroom toxin α-amanitin to further characterize the differences between them. The identification and characterization of Pol I, Pol II and Pol III ushered in an era of breakthroughs in our understanding of gene regulation in eukaryotes.

To celebrate the golden anniversary of this discovery, *Nature Reviews Molecular Cell Biology* and *Nature Structural & Molecular Biology* present a special online collection (https://www.nature.com/collections/transcription50), which includes recent Review and Perspective articles that showcase our current understanding of transcription regulation in eukaryotes. The collection includes, among others, articles dedicated to the Pol II complex, transcription initiation and the functional diversity of promoters, transcription elongation, the interplay between transcription and genome organization, and transcription stress responses. We hope you enjoy the read!

Eytan Zlotorynski

ORIGINAL ARTICLES Roeder, R. G. & Rutter, W. J. Multiple forms of DNA-dependent RNA polymerase in eukaryotic organisms. *Nature* **224**, 234–237 (1969) | Kedinger, C. et al. Alpha-amanitin: a specific inhibitor of one of two DNA-dependent RNA polymerase activities from calf thymus. *Biochem. Biophys. Res. Commun.* **38**, 165–171 (1970) | Lindell, T. J. et al. Specific inhibition of nuclear RNA polymerase II by α-amanitin. *Science* **170**, 447–449 (1970)

GENOME EDITING

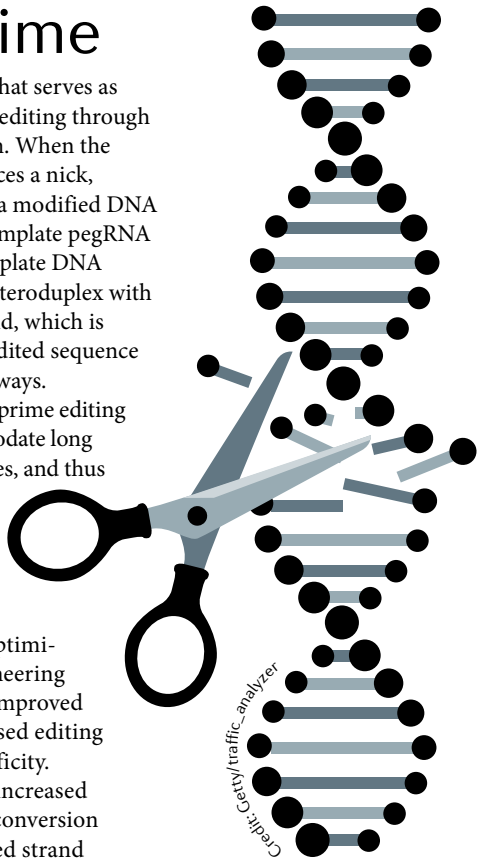
CRISPR–Cas in its prime

The CRISPR–Cas technology has transformed genome editing by relying on nucleic acid base pairing for target specificity. However, most known human disease-relevant mutations remain difficult to correct owing to the formation of insertions and deletions (indels) upon repair of Cas-generated DNA double-stranded breaks (DSBs) and the low efficiency of introducing precise changes through homology-directed repair in most cell types. Anzalone et al. now introduce CRISPR–Cas9-based 'prime editing', which overcomes these obstacles by relying on reverse transcription for editing.

The nuclease Cas9 is targeted to specific sequences by single guide RNAs (sgRNAs) that hybridize with the target DNA. The authors fused a disabled, 'nickase' form of Cas9 that cannot make DSBs to a reverse transcriptase (RT). In addition, they engineered pegRNAs (prime editing guide RNAs), which are sgRNAs that include a sequence that serves as template for precise editing through reverse transcription. When the Cas9 domain produces a nick, the RT polymerizes a modified DNA segment from the template pegRNA directly into the template DNA strand, forming a heteroduplex with the non-edited strand, which is converted into the edited sequence by DNA repair pathways.

Importantly, this prime editing system can accommodate long (≥30 bp) RT templates, and thus its targeting is not constrained by the availability of nearby protospacer adjacent motifs.

Next, pegRNA optimization and the engineering of mutant RT with improved functionality increased editing efficiency and specificity. These were further increased by augmenting the conversion rate of the non-edited strand



PROTEIN AGGREGATION

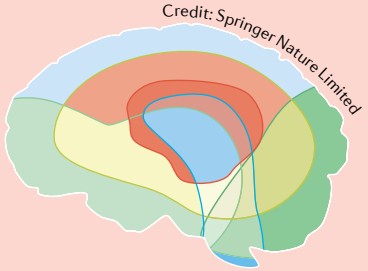
Taking the polyQ load off

The accumulation of toxic protein aggregates is a hallmark of several neurodegenerative diseases. A recent study published in *Nature* indicates that lowering the cellular levels of aggregation-prone mutant proteins, by using autophagosome-tethering compounds that promote their selective elimination via autophagy, might be an effective approach to treating these diseases.

Li et al. set off to identify compounds that simultaneously bind the autophagosome protein LC3 and a mutant form of the huntingtin protein (mHTT), which contains an expanded polyglutamine tract (polyQ) and causes Huntington's disease (HD). A microarray-based screening of 3,375 small molecules led to the isolation of two potential mHTT–LC3 linker compounds that bind mHTT but not wild-type HTT (wtHTT). These two compounds lowered the levels of mHTT in cultured neurons from a HD mouse model. On the basis of structural similarities, two more compounds were identified, which also reduced mHTT levels, but not wtHTT, in HD mouse neurons.

Treatment of mouse neurons with an autophagy inhibitor blocked the mHTT-lowering effect, confirming that the enhanced clearance of mHTT occurred via autophagy. The authors showed that the compounds indeed function by tethering mHTT, LC3 and autophagosomes and, importantly, that they don't interfere with the normal cellular autophagy functions.

Next, the compounds were shown to induce autophagy-dependent lowering of mHTT in human cells from patients with HD — in fibroblasts and in neurons derived from induced pluripotent stem cells (iPSCs). Their efficacy was also validated in vivo: in flies, using transgenic animals that expressed human mHTT, and in the HD mouse model,



> "in principle, targeting disease-causing polyQ proteins to autophagosomes is a valid approach for treatment"