The initiation of DNA synthesis in molecular detail

Life on Earth depends on the ability of cells to duplicate their genetic material, encoded in DNA molecules, and pass this information on to the next generation. Elucidation of the molecular mechanism underlying the priming step of this copying process provides insights into how DNA replication begins.

This is a summary of:
Li, A. W. H. *et al.* Molecular basis for the initiation of DNA primer synthesis. *Nature* https://doi.org/10.1038/s41586-022-04695-0 (2022).

Cite this as:
Nature https://doi.org/10.1038/d41586-022-01141-z (2022)

The question

A cell's genetic blueprint is encoded in its genome, which comprises DNA polymers that are in turn made up of nucleotide building blocks. During cell division, this genome must be accurately duplicated and passed on to the daughter cells. DNA replication is performed by enzymes called polymerases, which move along an unzipped DNA molecule and synthesize complementary strands by stringing nucleotides together with speed and fidelity.

But polymerases cannot initiate this synthesis process from scratch, and require a short polynucleotide primer to start it[1]. Most organisms rely on enzymes known as primases to catalyse the formation of a dinucleotide bond between two nucleotides, and then to extend this to generate the primer. In eukaryotic cells (those with a nucleus), DNA replication is primed by members of the primase–polymerase (Prim–Pol) superfamily, which also have roles in other DNA metabolic processes, from DNA repair to adaptive immunity (through the CRISPR–Cas system)[2,3]. However, the molecular basis for the dinucleotide-synthesis step of DNA replication has not been determined.

The discovery

To elucidate the mechanism of primer synthesis, we conducted crystallographic studies of a bacterial primase called CRISPR-associated Prim–Pol (CAPP). We first shortened the enzyme to a minimal catalytic domain that retained efficient priming activity and was tractable for structural studies. Using X-ray crystallography, we captured the main intermediates during the enzyme's catalytic cycle. We were then able to design *in vitro* studies to establish the roles of specific amino acids of CAPP during primer initiation and extension. We coupled these analyses with extensive biochemical and biophysical studies to develop a mechanistic model of primer synthesis.

We successfully elucidated the structures of a number of catalytic intermediates, including the elusive primer initiation complex. We were able to 'trap' the truncated enzyme in the process of forming the dinucleotide bond, revealing how the first bond between nucleotides that form the new primer strand is synthesized (Fig. 1a). These structural models revealed, in unprecedented detail, the molecular interactions between the enzyme, nucleotides, metals and DNA involved in primer synthesis, uncovering a network of interactions that stabilize the incoming nucleotides before dinucleotide bond formation. Coupling this information with functional studies allowed us to propose a more comprehensive model for the priming mechanism (Fig. 1b). A striking observation was that there are overt structural similarities between the evolutionarily distant CAPPs and human Prim–Pol enzymes (Pri1 (ref. 4) and PrimPol[5]). Functional studies of the human enzymes strongly supported a unified model and suggested that a similar priming mechanism is at work during the initiation of DNA synthesis in eukaryotic cells.

The implications

Our findings provide a molecular model that explains how primers are synthesized, initiating DNA replication. The results suggest a unified model in which the fundamental aspects of primer synthesis are conserved throughout evolution and across all domains of life. The applicability of this model to other primases, including eukaryotic Prim–Pols, opens up the possibility of developing targeted drugs to influence their activities, which could prove effective in treating diseases such as cancer.

However, although our results imply that the human enzymes work in a similar way to their bacterial 'cousins', we must acknowledge that further structural studies are required to strengthen this conclusion. Furthermore, our work has focused on the core catalytic domain of primases, and does not explain the roles that auxiliary domains and subunits might have in primer synthesis or in regulating this process in a cellular context.

The next steps would be to obtain structural information on eukaryotic replicative primases caught in the act of synthesizing dinucleotides. Elucidating structures of intact (untruncated) primases bound to DNA templates would also assist greatly in identifying the roles of the other domains and subunits.

Lewis J. Bainbridge and **Aidan J. Doherty** are at the Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Brighton, UK.

EXPERT OPINION

"The diversity and biological importance of the Prim–Pol enzyme family have become evident only in recent years, and this paper provides an important advance in this area of research. The findings will be of considerable interest not only to molecular and structural biologists in the field of DNA replication and repair, but also to a wider audience — because Prim–Pol enzymes are implicated in a broad range of biological functions."

Adele Williamson is at the University of Waikato, Hamilton, New Zealand.

FIGURE

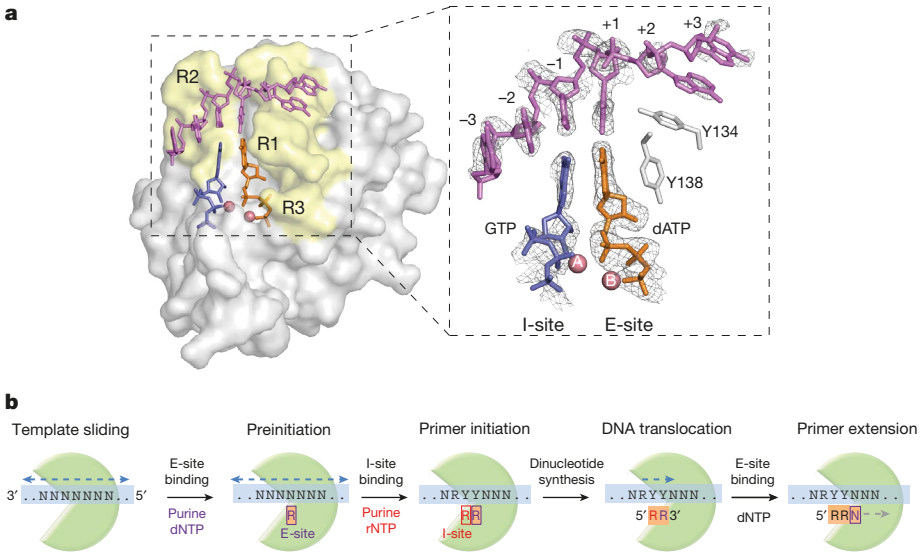

Figure 1 | Crystal structure of a primase initiating DNA synthesis. a, Left, the overall structure of a primer initiation complex comprising CAPP (grey) bound to DNA (pink) and metal ions, showing the initiating nucleotides (blue and orange) that will form the first dinucleotide of the new primer strand. Prominent regions on the protein's surface (R1 to R3) that interact with the DNA and nucleotides are shown in yellow. Right, magnification of the nucleotides (GTP and dATP in this case), the initiation (I) and elongation (E) sites, metal ions (spheres A and B), and two key amino acids of CAPP (Y134 and Y138). **b**, Model showing Prim–Pol's mechanism of primer synthesis and elongation. Green crescent, Prim–Pol; blue rectangle, the DNA template; peach rectangle, the newly synthesized primer; dashed blue arrows, enzyme movement. dNTP, deoxyribonucleotide triphosphate; N, any nucleotide; R, purine; rNTP, ribonucleotide triphosphate; Y, pyrimidine.

BEHIND THE PAPER

This story developed from the chance discovery, more than 20 years ago, that eukaryotic-like 'primases' are widespread in prokaryotes[1]. Unexpectedly, some of these enzymes are involved in DNA repair and function as polymerases, but have no overt primase activity. This led to a proposal to rename this primase superfamily to primase–polymerase (Prim–Pol), to better reflect their wider biological roles and origins[2]. Subsequent studies led to the discovery of other Prim–Pols that can prime, including eukaryotic PrimPol that reprimes stalled replication, and the CAPPs involved in the synthesis of CRISPR–Cas spacers[3]. This 'primed' our interest in how Prim–Pols catalyse primer synthesis. At the time, the dogma was that replicative Prim–Pols require further domains or subunits to facilitate priming. However, while truncating CAPP, we found that the catalytic domain alone was primase proficient. This discovery completely changed our perspective on how DNA primers are synthesized and provided us with a tractable model with which to address and, finally, resolve this elusive mechanism.

A.J.D.

REFERENCES

1. Bell, S. D. *Biochem. Soc. Trans.* **47**, 351–356 (2019).
2. Guilliam, T. A., Keen, B. A., Brissett, N. C. & Doherty, A. J. *Nucleic Acids Res.* **43**, 6651–6664 (2015).
3. Zabrady, K., Zabrady, M., Kolesar, P., Li, A. W. H. & Doherty, A. J. *Nature Commun.* **12**, 3690 (2021).
4. Holzer, S. *et al. ACS Chem. Biol.* **14**, 1904–1912 (2019).
5. Rechkoblit, O. *et al. Nature Commun.* **12**, 4020 (2021).

FROM THE EDITOR

For me, it's always a thrill to see a new structure — especially one that I know researchers have been trying to solve for some time — that provides crucial insights into the molecule's biochemical mechanisms. In this work, the unique properties of this enzyme, and how it initially incorporates a ribonucleotide and deoxyribonucleotide side by side and then extends the strand from the primer itself, come into much clearer focus.

Angela Eggleston, Biological Sciences Senior Editor, *Nature*