Two Ancient Solutions to One Modern Problem: DNA Management in Single-Celled Organisms

Single-celled bacteria and archaea use different but equally clever molecular systems to partition their DNA during cell division; bacteria with their ParABS motor system and archaea with their Seg filament system. These microscopic mechanisms show that even the simplest life forms have complex and elegant survival solutions.

One of the most fundamental challenges in microscopic cellular life is ensuring accurate DNA partitioning during cell division. This process, much like dividing a vast library's contents between two new locations, requires intricate coordination and precise organization. To address this important scientific phenomenon, Yuh-Ju Sun (National Tsing Hua University) and Chwan-Deng Hsiao (Academia Sinica) collaborated by using various bioassays and protein crystallography techniques to reveal fascinating details regarding how single-celled organisms (bacteria and archaea) accomplish this crucial task. All crystallographic data were collected at **TLS 15A**, **TPS 05A**, and **TPS 07A** of the NSRRC.

Researchers have uncovered the sophisticated ParABS (*par* stands for partitioning) system in bacteria, consisting of three key components working in harmony. ParA acts as a molecular motor powered by ATP, ParB serves as a versatile DNA-binding protein, and the *parS* sequences function as specific DNA anchoring points.[1] In this study, the *Helicobacter pylori* ParB (*Hp*ParB) protein demonstrates remarkable adaptability, behaving like a skilled librarian who can both locate specific books and organize entire shelves. This versatility is controlled by CTP, which acts as a molecular switch. When CTP is absent, *Hp*ParB focuses on specific DNA sequences (*e.g.*, *parS*), but in its presence, *Hp*ParB can slide freely along DNA (**Fig. 1(a)**).

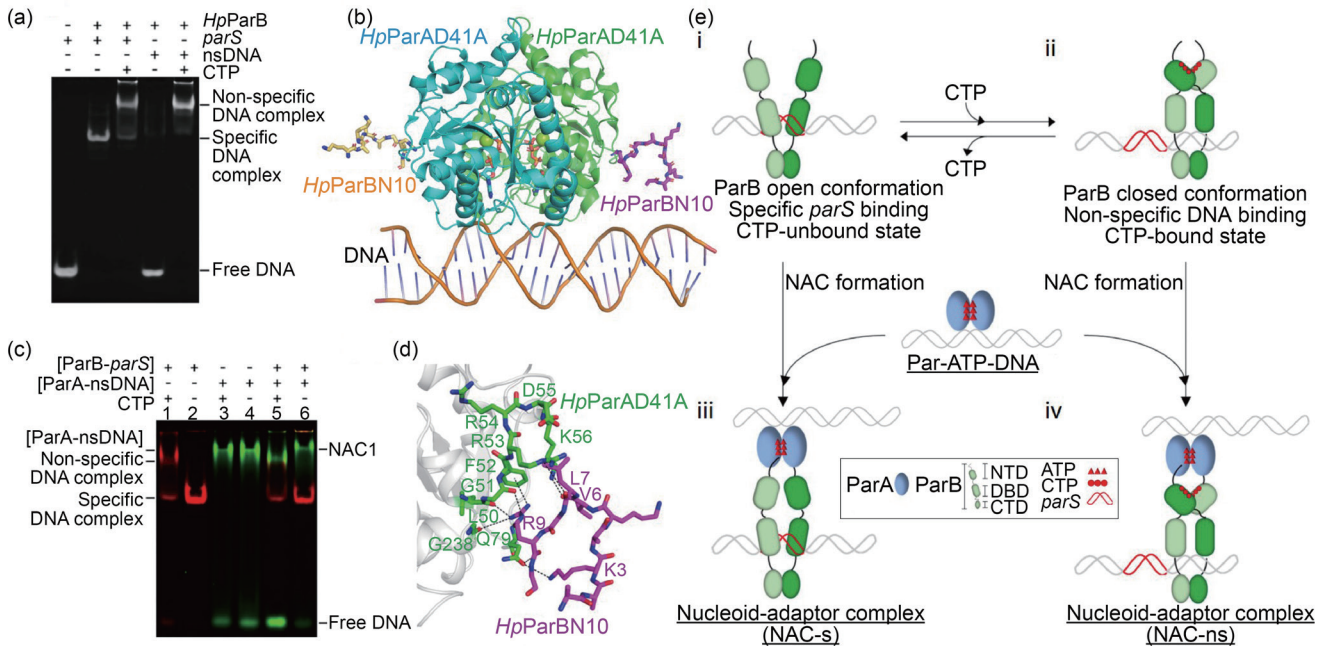

Fig. 1: (a) The electrophoretic mobility shift assay (EMSA) reveals *Hp*ParB's pattern for binding to *parS* and non-specific DNA, both with and without CTP present. (b) The *Hp*ParAD41A–DNA–*Hp*ParBN10 complex structure shows a cyan and green dimer, with orange and magenta *Hp*ParBN10 peptides bound to each monomer, alongside a wheat-colored DNA molecule. (c) EMSA analysis was performed to examine binding interactions between *Hp*ParB–*parS* and *Hp*ParA–nsDNA complexes. Initially, *Hp*ParB and *Hp*ParA were separately incubated with Cy3-labeled *parS* and Cy5-labeled nsDNA, respectively, either with or without CTP. These preformed complexes were then combined and further incubated under CTP-present or CTP-absent conditions before EMSA detection. (d) In the *Hp*ParAD41A–DNA–*Hp*ParBN10 complex, the magenta *Hp*ParBN10 binding site interacts with the grey *Hp*ParAD41A ribbon structure, where green-colored residues form key contacts marked by dashed lines. (e) The ParABS system model shows how green-colored ParB, containing three domains—the N-terminal domain (NTD), the DNA-binding domain (DBD), and the C-terminal domain (CTD)—adopts CTP-regulated open and closed conformations for specific (i) and non-specific (ii) DNA binding. When these Par–DNA complexes interact with purple-blue ParA–ATP–DNA, they form either specific NAC-s (iii) or non-specific NAC-ns (iv) nucleoid–adaptor complexes. [Reproduced from Ref. 1]

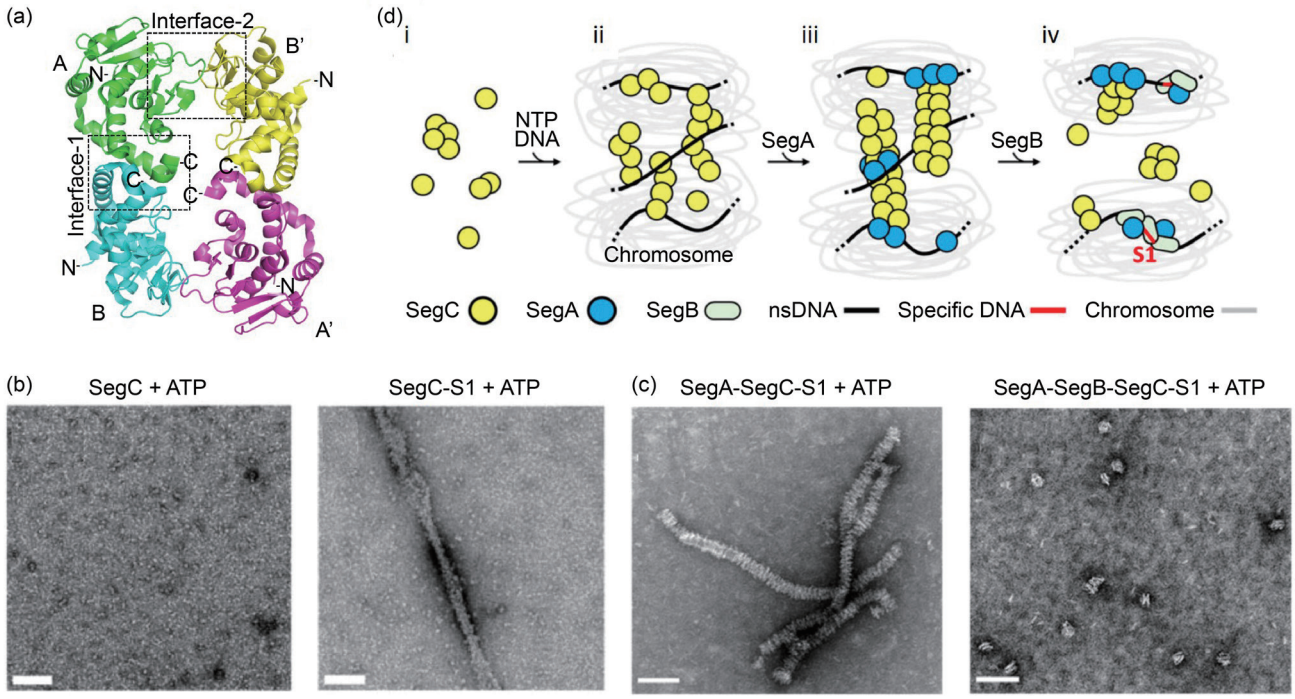

Fig. 2: (a) The SegC tetramer comprises four molecules (A, B, A', B') displayed in green, cyan, magenta, and yellow, respectively, with dimer interfaces marked by dotted squares as Interface-1 and Interface-2. (b) Negative-stain electron microscopy (EM) showing SegC structures with and without DNA and NTP. S1 (site 1) refers to a specific DNA sequence. (c) Negative-stain EM visualization of SegC, SegA, SegB, and DNA interactions. (d) A four-step model for SegC filament function: (i) Random distribution and multimerization of SegC in archaeal cells, (ii) NTP and DNA-dependent filament formation, (iii) SegA-mediated remodeling into higher-order filaments, and (iv) SegB-stimulated SegA ATPase activity leading to filament disassembly. [Reproduced from Ref. 2]

To improve our understanding of this system, researchers employed a clever strategy using a mutant form of *Hp*ParA (*Hp*ParAD41A) that displays a slower turnover rate, similar to using slow-motion photography to capture rapid movements. This approach revealed crucial details about how *Hp*ParA interacts with DNA and uses ATP for energy. *Hp*ParAD41A allowed scientists to observe the step-by-step process of how *Hp*ParA recognizes and binds to DNA, providing unprecedented insights into this fundamental mechanism (**Fig. 1(b)**).

One of the most significant discoveries in the bacterial system involves the formation of the nucleoid–adaptor complex (NAC; this term represents the composition of *Hp*ParA–*Hp*ParB–DNA). The research revealed that *Hp*ParA proteins form dimers when bound to CTP, creating a complex with DNA that can interact with *Hp*ParB through a specialized cation–π interaction. This interaction, particularly between *Hp*ParB's Arg9 and *Hp*ParA's Phe52, proves essential for the entire system's functionality (**Figs. 1(b) –1(d)**).

The bacterial study also unveiled a detailed molecular mechanism where CTP acts as a master regulator. When CTP is absent, ParB maintains an "open" configuration, specifically binding to *parS* sequences and preparing to interact with the ParA–ATP–DNA complex. The introduction of CTP triggers ParB to adopt a "closed" form, enabling it to slide along DNA non-specifically and interact more efficiently with ParA. This CTP-dependent switching mechanism is crucial for promoting ATP hydrolysis by ParA and ensuring proper system function (**Fig. 1(e)**).

Meanwhile, in the ancient world of archaea, researchers have discovered a different but equally fascinating system involving three proteins: SegA, SegB, and the newly identified SegC. Detailed structural analysis revealed that SegC has a unique architectural design that allows it to form both dimers (pairs) and tetramers (groups of four). This molecular architecture, particularly the protein's C-terminal region, proves crucial for its functionality—when researchers removed this tail end, SegC lost its ability to bind DNA and form filaments (**Fig. 2(a)**). The SegC protein shows the remarkable abilities, binding to DNA without sequence specificity and forming thread-like structures (filaments) when it encounters DNA and energy molecules (**Fig. 2(b)**).

The coordination between these components involves SegC working with SegA to form larger filaments in the presence of ATP, while SegB can break these structures apart when needed (**Fig. 2(c)**). The archaeal system's unique feature lies in SegC's ability to break down various energy molecules (NTPs), though the exact role of this capability remains under investigation. The researchers proposed a step-wise process where SegC forms initial filaments, SegA helps

organize these structures, and SegB eventually breaks them down to complete the DNA organization process (**Fig. 2(d)**).

The implications of this research extend far beyond basic science. Understanding these fundamental processes could lead to new strategies for controlling bacterial growth, potentially contributing to antibiotic developments. Furthermore, since archaea are considered ancient relatives of complex organisms, these findings provide valuable insights into how DNA organization evolved over time. (Reported by Chun-Hsiang Huang)

This report features the work of Yuh-Ju Sun and Chwan-Deng Hsiao published in Nucleic Acids Res. ***52****, 7321 (2024) and Nucleic Acids Res.* ***52****, 9966 (2024).*

TPS 05A Protein Microcrystallogrphy
TPS 07A Micro-focus Protein Crystallography
TLS 15A1 Biopharmaceuticals Protein Crystallography

- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

References

1. C.-H. Chu, C.-T. Wu, M.-G. Lin, C.-Y. Yen, Y.-Z. Wu, C.-D. Hsiao, Y.-J. Sun, Nucleic Acids Res. **52**, 7321 (2024).
2. M.-G. Lin, C.-Y. Yen, Y.-Y. Shen, Y.-S. Huang, I. W. Ng, D. Barillà, Y.-J. Sun, C.-D. Hsiao, Nucleic Acids Res. **52**, 9966 (2024).

Targeting DNA Junctions for Anticancer Drug Development

DNA helix–helix junctions form tetraplex base pairs at the junction interface, serving as "hotspots" for bidirectional bis-intercalating agents. This study investigates the structural basis for targeting DNA junctions with acridine bis-intercalators as a potential anticancer strategy.

Biological processes such as recombination or replication can generate DNA juxtaposed helix–helix structures and duplex crossovers.[1,2] These structures require topoisomerases to decatenate the interlinked DNA crossover sites.[3] Within the crossover structures, the base pairs of the duplexes can interact with each other, resulting in novel junctions. Targeting DNA junction sites with bis-intercalating compounds containing bidirectional linkers could inhibit topoisomerase activity, therefore representing an effective anticancer strategy.[4,5] Bidirectional bis-intercalators have the unique ability to insert their chromophores simultaneously into the base pairs of two DNA duplexes. This non-covalent bridging ability of small molecules enables them to cross-link DNA junctions, thereby disrupting biological processes critical for cellular function. However,

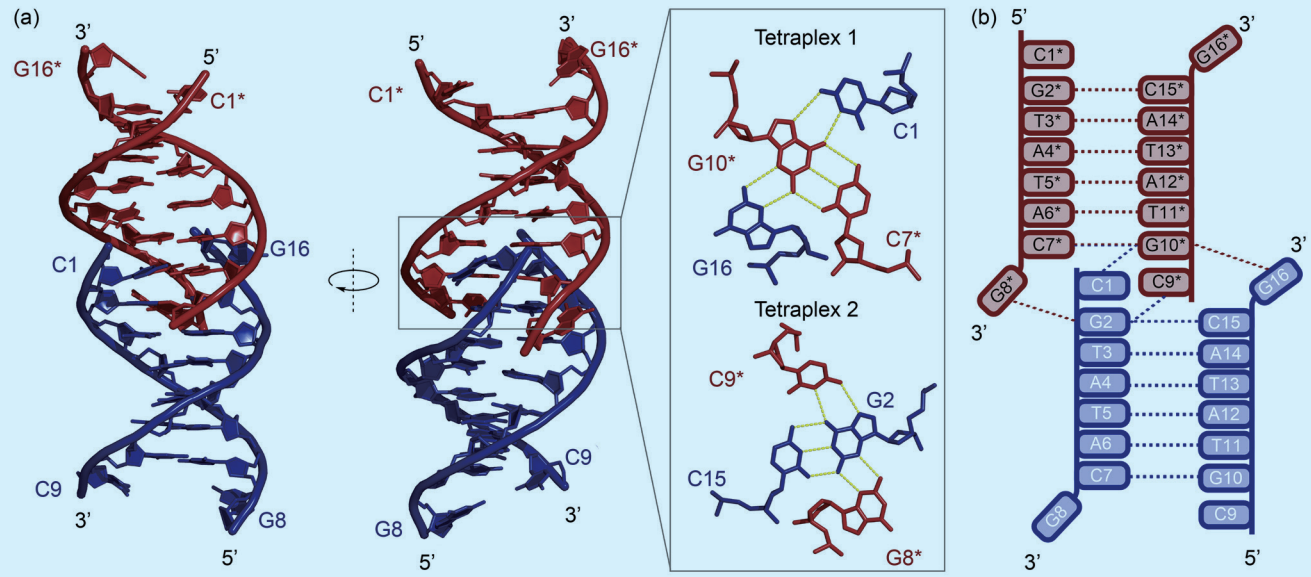

Fig. 1: Structural features of a $d(CGTATACG)_2$ DNA forming junction. (a) Crystal structure assembly of continuous duplexes forming an end-to-end helix–helix junction structure. One DNA duplex is shown in dark blue and the adjacent symmetry-related duplex is in dark red. Asterisks (*) represent residues in the adjacent duplex. Two layered tetraplex base pairings at the junction interface are shown in an enlarged view. (b) Schematic representation of the crystal structure of $d(CGTATACG)_2$, indicating the residues involved in junction formation. [Reproduced from Ref. 6]