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Protein-Templated DNA Synthesis: A Paradigm Shift in Molecular Biology

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INTRODUCTION

For nearly seven decades, the Central Dogma of molecular biology stipulated that nucleic acid synthesis strictly requires a preexisting nucleic acid template (DNA or RNA). Recent breakthrough discoveries have fundamentally challenged the central dogma principle. Scientists have now characterised bacterial Defence-Associated Reverse Transcriptases (DRTs) specifically the DRT3 and DRT7 systems that bypass canonical Watson-Crick base pairing entirely. Instead of reading a nucleic acid strand, these enzymes utilize the precise three-dimensional geometry and electrostatic properties of their own amino acid active sites as a physical template to guide sequence-specific DNA synthesis.

This discovery establishes a novel avenue for biological information transfer, demonstrating that proteins can act as direct structural scaffolds for the de novo synthesis of repetitive, double-stranded DNA during antiviral immune responses. The discovery of protein-templated DNA synthesis represents a significant departure from classical molecular biology fundamental principles. For decades, the tenet that nucleic acid templates were essential for DNA synthesis remained unchallenged.

The emergence of DRT systems in bacterial antiviral defence mechanisms now provides compelling evidence that enzymatically active sites can function as precise molecular molds, directing nucleotide incorporation without any nucleic acid blueprint.

Structural & Molecular Architecture

Advanced cryo-electron microscopy (cryo-EM) has resolved the atomic-level architecture of these enzyme systems, revealing how their precise spatial organization enables DNA synthesis without nucleic acid templates.

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The DRT3 Macromolecular Complex

The *Escherichia coli* DRT3 system operates as a massive, highly symmetrical ribonucleoprotein (RNP) complex. This complex exhibits D3-symmetry, forming a 6:6:6 heterooligomeric architecture composed of six copies each of Drt3a (Reverse Transcriptase A) and Drt3b (Reverse Transcriptase B), along with six strands of a specialized non-coding RNA (ncRNA). This trimeric assembly of catalytic subunits working in concert represents a remarkable example of evolutionary organization for a novel enzymatic function.

The DRT7 Dimer Architecture

The DRT7 system, belonging to the UG10 family of prokaryotic retroelements, exhibits a distinctly different architecture compared to DRT3. Unlike the larger DRT3 complex, DRT7 comprises a single polypeptide chain containing both an RT-like domain and a primase polymerase (PP) domain. A key feature of DRT7 is its remarkable conformational flexibility: the enzyme naturally exists as an inactive closed dimer. Upon phage-triggered activation, it undergoes a dramatic spatial rearrangement to transition into an active open-dimer configuration, enabling its enzymatic activity.

Biochemical Mechanisms of DNA Synthesis

The synthesis of repetitive duplex DNA by these bacterial defense systems depends on a highly coordinated and unconventional enzymatic handoff between different catalytic subunits. The process begins with phage infection and proceeds through conformational changes that activate the DRT complexes. The first catalytic unit synthesizes a poly(GT) or poly(T) strand using either a nucleic acid template or a protein-pocket recognition site. The second catalytic unit then synthesizes the complementary strand—remarkably— using the protein active site itself as a molecular template mold, with no nucleic acid template required. Finally, these single strands assemble into repetitive duplex DNA networks that serve as a decoy system to disrupt viral replication.

The DRT3 System: Cooperative Duplex Synthesis

The DRT3 system employs a sequential two-stage mechanism for DNA synthesis. First, the system is maintained under tight regulation to prevent cellular toxicity, but becomes selectively activated when the bacterium detects a specific viral invader, such as the phage T1 protein ST61. In the first synthetic stage, Drt3a initiates synthesis by building a short primer using the highly conserved ACACAC template sequence nestled within the companion ncRNA. This stage produces a poly(GT) single-stranded DNA segment using conventional template directed synthesis. In the second stage, the complementary poly(AC) strand is synthesized by Drt3b in the complete absence of any nucleic acid template. The amino acids in the Drt3b active site fold into a precise three-dimensional configuration that physically mimics the grooves and charge distributions of an RNA strand. Highly specific, conserved active-site residues enforce strict dinucleotide periodicity, ensuring that only alternating Adenine and Cytosine bases can fit and bind properly. Drt3b uses its own protein structure—likely involving tyrosine residues—to prime and extend the complementary sequence through this protein-based molecular template.

The DRT7 System: Conformational Switching and Handoff

The DRT7 system employs a different strategy involving conformational switching and inter-domain communication. Upon activation by a phage-encoded transcriptional regulator, the RT domain of DRT7 initiates protein-primed, sequence-specific poly(T) synthesis without any template strand, relying instead on a specialized arginine-rich recognition pocket. This initial poly(T) tract then triggers the conformational transition from the inactive closed dimer to the active open-dimer state. The poly(T) product is subsequently handed off to the linked primase-polymerase (PP) domain, where it serves as a conventional template for poly(A) extension. Through iterative feedback cycles between these two domains, the enzyme

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generates massive, palindromic alternating poly(A)/poly(T) tracts that spontaneously fold back into duplex-like structures.

Biological Function: Antiviral Defense

The primary biological purpose of protein-templated DNA synthesis is to mount a robust defense mechanism against viral predation. Because this process is highly energetic and inherently toxic to the cell, it typically forces the infected bacterium into abortive infection (Abi)—a programmed self-sacrifice that protects the surrounding bacterial colony from further viral spread. The defensive strategy operates through two complementary mechanisms. The ”molecular sponge” hypothesis proposes that massive accumulation of repetitive poly(GT/AC) or poly(A/T) duplex DNA serves as a decoy system. These repetitive tracts form intricate, non-canonical higher-order networks and non-B-form DNA conformations such as slipped-strand structures and Z-DNA. These complex networks sequester essential phage-derived DNA-binding proteins, effectively starving the virus of the replication machinery needed to copy its own genome. Additionally, the rapid synthesis of untemplated DNA creates large, sprawling DNA tangles that physically disrupt the viral life cycle within the cytoplasm. These accumulated DNA networks impede viral assembly and genome replication, providing a multi-layered defensive strategy against phage infection.

Implications for the Central Dogma

This discovery necessitates a nuanced reassessment of the Central Dogma. Specifically, the findings challenge the biochemical requirement that nucleic acid synthesis must proceed from a nucleic acid template. Protein-templated DNA synthesis demonstrates that biological information can flow structurally from a protein scaffold to DNA sequence, establishing a fundamentally new mode of information transfer. However, it is important to note what remains unchanged by this discovery. The DRT systems do not translate arbitrary protein sequences back into genomic blueprints. The synthesized DNA consists entirely of simple, repetitive dinucleotide or homopolymer blocks—poly(AC), poly(GT), or poly(A/T) repeats. Critically, this mechanism does not overwrite the permanent, heritable bacterial chromosome or establish a new system of heritable genetic inheritance. Therefore, while the strict requirement for nucleic acid templates has been disproven, the Central Dogma regarding the heritability and stability of genomic DNA remains fully intact.

Future prospects and Biotechnological Applications

The ability of an enzyme to synthesize highly predictable, sequence-specific repeating DNA without external templates opens major opportunities in synthetic biology.

Application	Impact & importance
Enzymatic DNA Synthesis	Replacing harsh, chemical-heavy phosphoramidite methods with green, highly efficient, protein-driven enzymatic synthesis for industrial DNA printing.
DNA Data Storage	Utilizing the precise dinucleotide alternation of enzymes like Drt3b to design novel digital-to-biological data encoding schemes.
Biomedical Therapeutics	Reprogramming these protein molds to manufacture structured, repetitive DNA architectures (like DNA origami or aptamers) directly inside targeted human cells to neutralize viral infections or cage oncogenes.

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CONCLUSION

The discovery of protein-templated DNA synthesis in bacterial DRT systems fundamentally expands our understanding of enzymatic catalysis and biological information transfer. By demonstrating that proteins can function as precise molecular templates for nucleotide incorporation without any nucleic acid blueprint, this work establishes a novel principle in molecular biology while maintaining the essential truths of the Central Dogma. The continuing investigation of these systems and their biotechnological application represent a fertile frontier at the intersection of molecular biology, synthetic biology, and biomedical engineering.