Crystal structure of a reverse polymerase

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The primary transcripts of transfer RNA (tRNA) molecules undergo extensive processing and chemical modification to become functional components of the protein synthesis apparatus. Some of these reactions are common to all tRNAs in a particular cell, whereas others are specific to a subset of the species or even to just a single amino-acid–accepting class (1). In some organisms the maturation of histidine-specific tRNAs ($tRNA^{His}$) includes the unique addition of guanosine (G_{-1}) to the 5′ terminus of tRNA (2). This G_{-1} addition step is a phosphodiester bond formation reaction that operates in a unique 3′-5′ direction, opposite to all conventional DNA and RNA polymerases. In PNAS, Hyde et al. (3) unveil crystal structures of a Thg1 enzyme that catalyzes G_{-1} addition. The structures show that the catalytic domain of Thg1 shares both a common architecture and a two-metal ion-dependent mechanism with canonical 5′-3′ DNA polymerases. These remarkable findings open new vistas in tRNA enzymology and the evolution of nucleic acid polymerases.

The addition of G_{-1} at the 5′ terminus of $tRNA^{His}$ is essential to protein synthesis and cell viability (4), because this guanosine is a key determinant required for recognition by histidyl-tRNA synthetase (HisRS)—the enzyme responsible for the covalent attachment of histidine to the $tRNA^{His}$ 3′ terminus (5, 6). The His-$tRNA^{His}$ product of the HisRS reaction is then a substrate for elongation factor-mediated protein synthesis on the ribosome. Interestingly, in most bacteria and some archaea, G_{-1} is not added by Thg1 but is instead encoded at the 5′ end of the $tRNA^{His}$ gene. The elongated 5′ end is then generated via cleavage at a distinct position by the endonuclease RNase P (7) (Fig. 1). By contrast, in all eukaryotes, some archaea, and a few groups of bacteria, RNaseP-mediated cleavage of the primary $tRNA^{His}$ transcript produces the usual 5′ end found in all other tRNAs, generating a common 7-bp acceptor stem (2, 8). The Thg1-catalyzed G_{-1} addition at the $tRNA^{His}$ 5′ end in these organisms is in the 3′-5′ direction, in contrast to the 5′-3′ polymerization reaction catalyzed by tRNA nucleotidyltransferase (tRNA NTase), which adds (or repairs) the CCA trinucleotide at the 3′ end of all tRNAs (9) (Fig. 1).

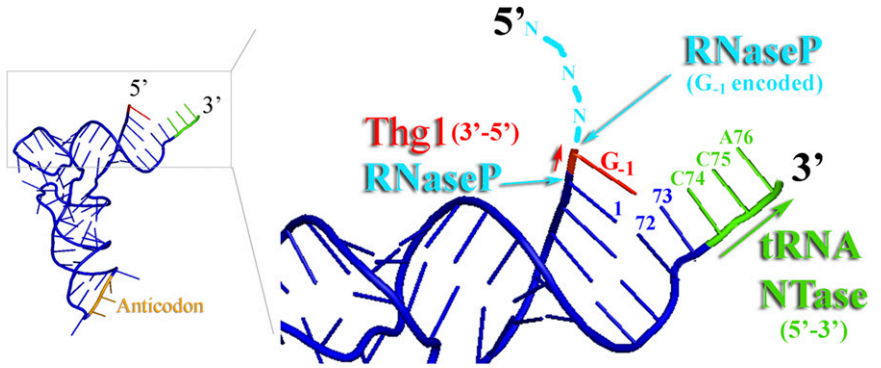

Fig. 1. Unusual RNA polymerase reactions at the 5′ and 3′ termini of tRNA: Thg1 and tRNA nucleotidyltransferase.

In all eukaryotes, $tRNA^{His}$ possesses an unpaired A73 nucleotide at the position opposite to G_{-1} (Fig. 1). In this domain, Thg1 catalyzes nontemplated G_{-1} addition by a three-step reaction that first features ATP-mediated adenylylation of the 5′ monophosphorylated tRNA to generate an Ap-p-tRNA intermediate, with release of pyrophosphate. The 3′ ribose hydroxyl of GTP then attacks the phosphate proximal to the tRNA, forming 5′ triphosphorylated G_{-1}-$tRNA^{His}$, which is further processed by a Thg1-associated pyrophosphatase activity to generate the mature product (3, 4, 10, 11). However, if the 5′ tRNA terminus is triphosphorylated, Thg1 adds G_{-1} by a different mechanism that does not require ATP: the 3′ OH of GTP directly attacks the α-phosphate group, and the reaction is powered instead by the energy liberated upon generation of pyrophosphate (4). This reaction is similar to that catalyzed by conventional 5′-3′ polymerases, except that the attacking oxygen nucleophile is the GTP 3′ OH moiety instead of the 3′ OH group of the elongating primer strand (Fig. 2). Strikingly, both yeast and archaeal Thg1 enzymes are able to catalyze in vitro template-directed addition of multiple nucleotides to the tRNA 3′ end, with native or variant tRNA 3′ terminal sequences serving as the template (12, 13) (Fig. 1). This 3′-5′ polymerase activity also functions in vivo in yeast, but only if the $tRNA^{His}$ substrate is mutated to contain C73 (14). Thus, eukaryotes may conserve A73 in $tRNA^{His}$ to prevent this polymerization reaction, possibly enhancing the performance of His-$tRNA^{His}$ on the ribosome.

The Thg1 sequence had revealed no detectable homology or motifs common to other nucleic acid enzymes. The crystal structure of human Thg1 now solved by Hyde et al. (3) therefore provides a remarkable dénouement by showing that Nature accomplishes 3′-5′ nucleic acid polymerization using a structural fold homologous to the palm domain of canonical 5′-3′ DNA polymerases (15). Moreover, superpositions of Thg1 with DNA polymerase locate three common carboxylate groups in the predicted active site. Using a cocrystal structure of Thg1 with bound dGTP together with metal ion soaking experiments, Hyde et al. (3) further show that these catalytic residues bridge to the substrate α- and β-phosphates via two divalent metal ions that are oriented similarly to those bound in substrate complexes of T7 DNA polymerase (16). Thus, both the palm domain fold and classic two-metal ion mechanism for 5′-3′ nucleotide addition by DNA polymerases appear to be conserved in the 3′-5′ phosphodiester bond formation reaction catalyzed by Thg1.

Based on mutational experiments, Hyde et al. (3) suggest that the dGTP nucleotide bound to human Thg1 may correspond to the position of ATP used in the initial activation step that produces the adenylylated intermediate. The triphosphate portion of a second bound nucleotide is also observed in the active site (3), where it coordinates a number of basic residues important to catalytic efficiency in the yeast homolog (17). Thus, several pieces

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of the overall reaction scheme may already be in place, but a full understanding of the stereochemical mechanism of catalysis will require additional structures, including a tRNA-bound complex. Thg1 is known to recognize the GUG anticodon of $tRNA^{His}$, located at the opposite end of the L-shaped molecule with respect to the site of G_{-1} addition (Fig. 1) (18). A small, helical subdomain located 20–30 Å from the nucleotide binding site also contains highly conserved residues critical to catalysis (3, 17), and may be a key determinant of tRNA binding.

The finding that 5′-3′ and 3′-5′ nucleic acid polymerization reactions are catalyzed by the same protein fold and metal-dependent mechanism may ultimately offer additional perspective on why 5′-3′ polymerases predominate in Nature even though the two schemes are equally favored on thermodynamic grounds (Fig. 2) (3). Interestingly, archaeal Thg1 enzymes are highly inefficient in G_{-1} addition opposite the A73 eukaryote-specific determinant, yet the yeast homolog catalyzes template-dependent reverse polymerization opposite C73 with efficiency comparable to ATP-dependent nucleotidyl transfer (12, 13). Thus, it appears that reverse polymerization is the more deeply rooted mechanism, and that the ATP-dependent reaction is a recent eukaryotic innovation coevolving with the transition from C73 to A73 in $tRNA^{His}$. Further, a different 3′-5′ polymerization catalyzed by a still-unknown enzyme is responsible for a remarkable 5′ tRNA editing mechanism occurring in the mitochondria of lower eukaryotes, in which the 3′ portions of tRNA acceptor stems function as templates (19). These editing reactions mirror the repair function of tRNA NTase (Fig. 1) in those organisms in which the 3′ terminal CCA is encoded (13). The pioneering work of Hyde et al. (3) now offers a landmark structural paradigm by which 3′-5′ polymerization in these and perhaps other as-yet uncharacterized systems will be evaluated.

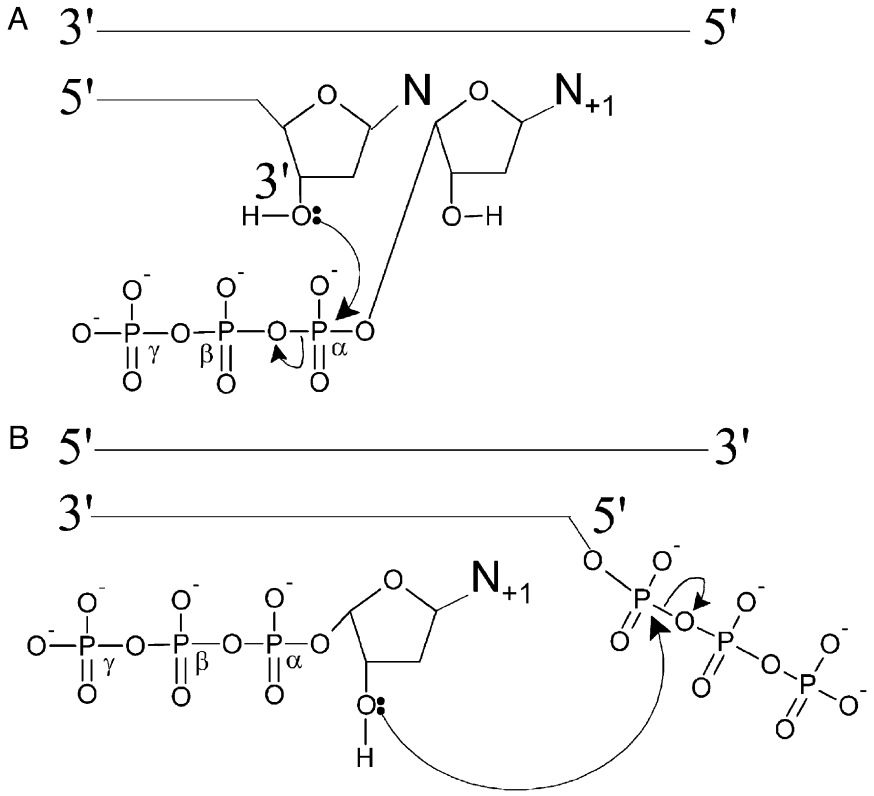

Fig. 2. Isoenergetic mechanisms for canonical 5′-3′ nucleic acid polymerization (*A*) and for polymerization in the reverse 3′-5′ direction (*B*).

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1. Phizicky EM, Hopper AK (2010) tRNA biology charges to the front. *Genes Dev* 24:1832–1860.
2. Cooley L, Appel B, Söll D (1982) Post-transcriptional nucleotide addition is responsible for the formation of the 5′ terminus of histidine tRNA. *Proc Natl Acad Sci USA* 79:6475–6479.
3. Hyde SJ, et al. (2010) $tRNA^{His}$ guanylyltransferase (THG1), a unique 3′-5′nucleotidyl transferase, shares unexpected structural homology with canonical 5′-3′ DNA polymerases. *Proc Natl Acad Sci USA* 107: 20305–20310.
4. Gu W, Jackman JE, Lohan AJ, Gray MW, Phizicky EM (2003) $tRNA^{His}$ maturation: An essential yeast protein catalyzes addition of a guanine nucleotide to the 5′ end of $tRNA^{His}$. *Genes Dev* 17:2889–2901.
5. Himeno H, et al. (1989) Role of the extra G-C pair at the end of the acceptor stem of tRNA(His) in aminoacylation. *Nucleic Acids Res* 17:7855–7863.
6. Rudinger J, Florentz C, Giegé R (1994) Histidylation by yeast HisRS of tRNA or tRNA-like structure relies on residues −1 and 73 but is dependent on the RNA context. *Nucleic Acids Res* 22:5031–5037.
7. Orellana O, Cooley L, Söll D (1986) The additional guanylate at the 5′ terminus of *Escherichia coli* $tRNA^{His}$ is the result of unusual processing by RNase P. *Mol Cell Biol* 6:525–529.
8. Heinemann IU, Randau L, Tomko RJ Jr., Söll D (2010) 3′–5′ $tRNA^{His}$ guanylyltransferase in bacteria. *FEBS Lett* 584:3567–3572.
9. Vörtler S, Mörl M (2010) tRNA-nucleotidyltransferases: Highly unusual RNA polymerases with vital functions. *FEBS Lett* 584:297–302.
10. Pande S, Jahn D, Söll D (1991) Histidine tRNA guanylyltransferase from *Saccharomyces cerevisiae*. I. Purification and physical properties. *J Biol Chem* 266: 22826–22831.
11. Jahn D, Pande S (1991) Histidine tRNA guanylyltransferase from *Saccharomyces cerevisiae*. II. Catalytic mechanism. *J Biol Chem* 266:22832–22836.
12. Jackman JE, Phizicky EM (2006) $tRNA^{His}$ guanylyltransferase catalyzes a 3′–5′ polymerization reaction that is distinct from G_{-1} addition. *Proc Natl Acad Sci USA* 103: 8640–8645.
13. Abad MG, Rao BS, Jackman JE (2010) Template-dependent 3′–5′ nucleotide addition is a shared feature of $tRNA^{His}$ guanylyltransferase enzymes from multiple domains of life. *Proc Natl Acad Sci USA* 107:674–679.
14. Preston MA, Phizicky EM (2010) The requirement for the highly conserved G_{-1} residue of *Saccharomyces cerevisiae* $tRNA^{His}$ can be circumvented by overexpression of $tRNA^{His}$ and its synthetase. *RNA* 16:1068–1077.
15. Steitz TA (1999) DNA polymerases: Structural diversity and common mechanisms. *J Biol Chem* 274:17395–17398.
16. Doublié S, Tabor S, Long AM, Richardson CC, Ellenberger T (1998) Crystal structure of a bacteriophage T7 DNA replication complex at 2.2 A resolution. *Nature* 391:251–258.
17. Jackman JE, Phizicky EM (2008) Identification of critical residues for G_{-1} addition and substrate recognition by tRNA(His) guanylyltransferase. *Biochemistry* 47: 4817–4825.
18. Jackman JE, Phizicky EM (2006) $tRNA^{His}$ guanylyltransferase adds G_{-1} to the 5′ end of $tRNA^{His}$ by recognition of the anticodon, one of several features unexpectedly shared with tRNA synthetases. *RNA* 12: 1007–1014.
19. Lonergan KM, Gray MW (1993) Editing of transfer RNAs in Acanthamoeba castellanii mitochondria. *Science* 259:812–816.