

Supplemental Material

NAD⁺-dependent synthesis of a 5'-phospho-ADP-ribosylated RNA/DNA cap by RNA 2'-phosphotransferase Tpt1

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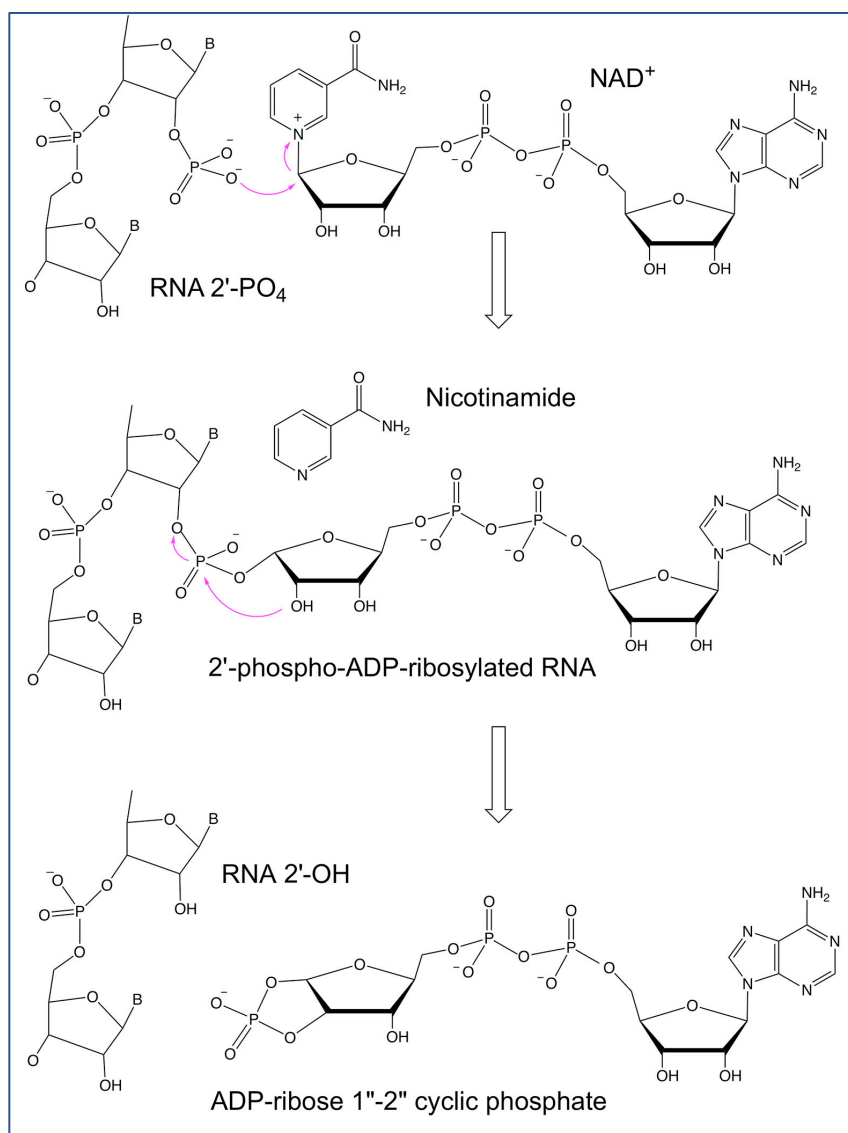


Figure S1. Two-step Tpt1-catalyzed mechanism of 2'-PO₄ removal from a 2'-PO₄, 3'-5' phosphodiester RNA junction.

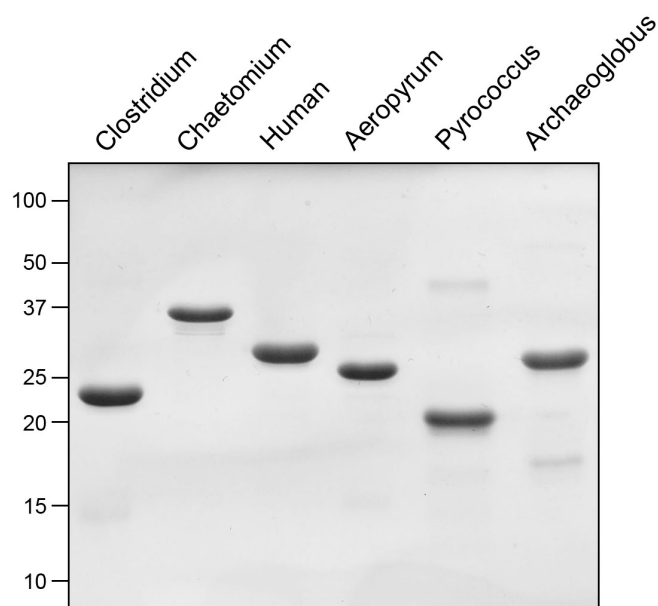


Figure S2. Recombinant Tpt1 proteins. Aliquots (5 μ g) of the indicated Tpt1 preparations were analyzed by SDS-PAGE. Polypeptides were visualized by staining with Coomassie blue dye. The positions and sizes (kDa) of marker polypeptide are indicated at left. The Tpt1 proteins are from the following taxa: bacterium *Clostridium thermocellum* (182-aa); fungus *Chaetomium thermophilum* (322-aa); human *Homo sapiens* (253-aa); and archaea *Aeropyrum pernix* (220-aa), *Pyrococcus horkoshii* (177-aa), and *Archaeoglobus fulgidus* (216-aa).

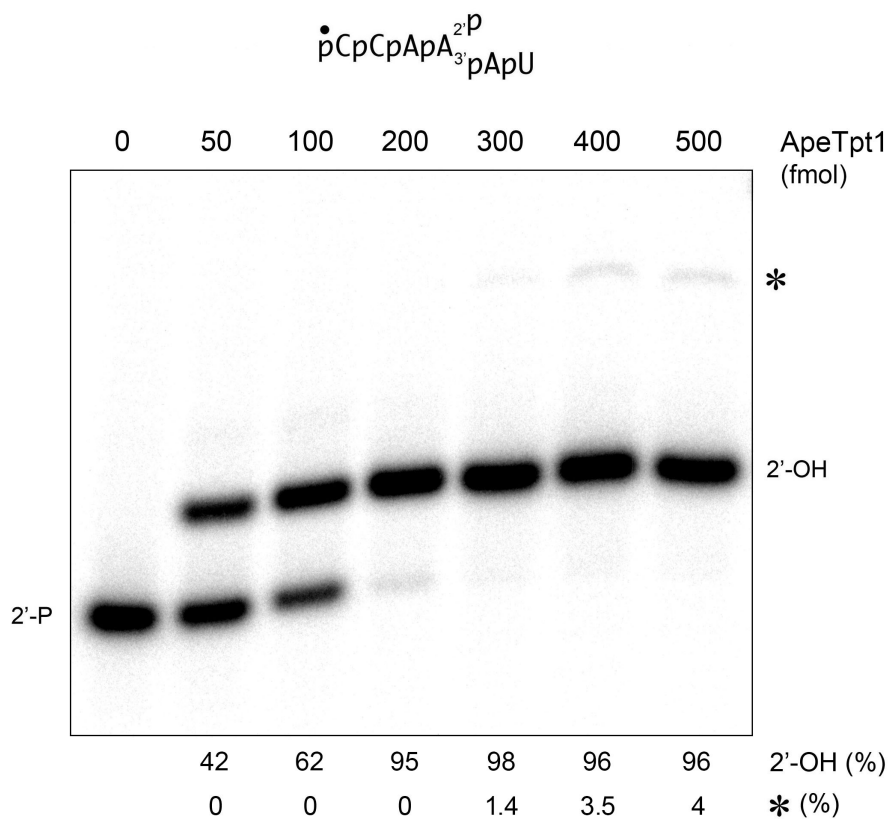


Figure S3. ApeTpt1 titration. Reaction mixtures (10 μ l) containing 100 mM Tris-HCl (pH 7.5), 0.2 μ M (2 pmol) 5' 32 P-labeled 6-mer 2'-PO₄ RNA (shown at top), 1 mM NAD⁺, and 0, 50, 100, 200, 300, 400 or 500 fmol ApeTpt1 as indicated (corresponding to 5, 10, 20, 30 40 or 50 nM ApeTpt1) were incubated at 37°C for 30 min. The reaction products were analyzed by urea-PAGE and visualized by scanning the gel with a Fujifilm FLA-7000 imaging device. The extents of formation of the 2'-OH product and the RNA species were quantified by analyzing the scan with ImageQuant software and are indicated as percent values (of total labeled RNA) below the lanes.

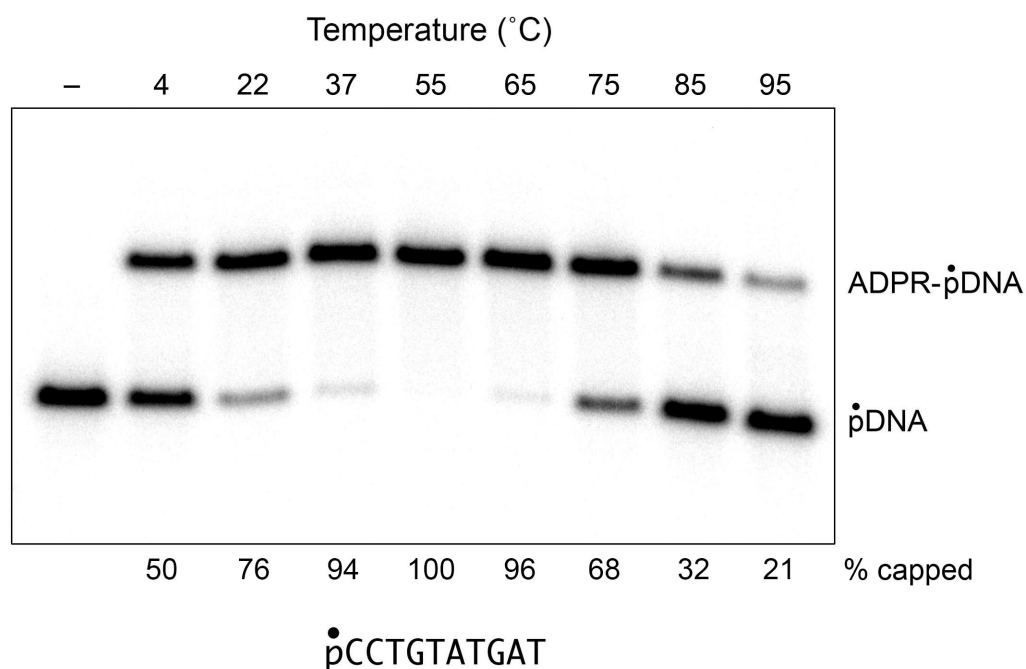


Figure S4. Effect of reaction temperature on pDNA capping by ApeTpt1. Reaction mixtures (10 μ l) containing 100 mM Tris-HCl (pH 7.5), 0.2 μ M (2 pmol) 5' 32 P-labeled 10-mer pDNA substrate (shown at bottom), 1 mM NAD^+ , and 0.5 μ M (5 pmol) ApeTpt1 were incubated for 30 min at the temperatures specified. The reactions were initiated by adding 1 μ l of Tpt1 to 9 μ l of reaction mix that had been pre-incubated at the intended reaction temperature. The reaction products were analyzed by urea-PAGE and visualized by scanning the gel with a Fujifilm FLA-7000 imaging device. The extents of pDNA capping, calculated as $100 \times \text{ADP-pDNA}/(\text{ADPR-pDNA} + \text{pDNA})$, were quantified by analyzing the scan with ImageQuant software and are indicated as percent values below the lanes.

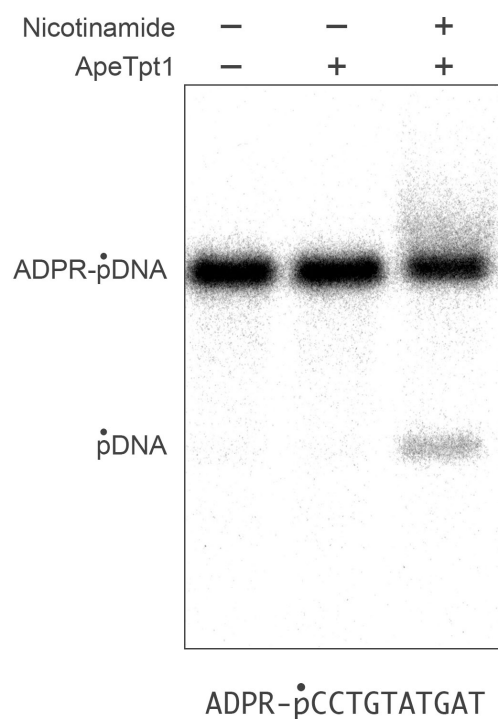


Figure S5. De-capping of ADPR-pDNA in the presence of nicotinamide. Reaction mixtures (10 μ l) containing 100 mM Tris-HCl (pH 7.5), 0.1 μ M (1 pmol) 32 P-labeled ADPR-pDNA (shown at bottom), 1 mM nicotinamide (where indicated by +), and 1 μ M (10 pmol) ApeTpt1 (where indicated by +) were incubated at 37°C for 60 min. The reaction products were analyzed by urea-PAGE and visualized by scanning the gel with a Fujifilm FLA-7000 imaging device.