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 µg) of the indicated Tpt1 preparations were analyzed by SDS-PAGE. Polypeptides were visualized by staining with Commissie 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 µl) containing 100 mM Tris-HCl (pH 7.5), 0.2 µM (2 pmol) 5'-32P-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 µl) containing 100 mM Tris-HCl (pH 7.5), 0.2 µM (2 pmol) 5'-32P-labeled 10-mer pDNA substrate (shown at bottom), 1 mM NAD+, and 0.5 µM (5 pmol) ApeTpt1 were incubated for 30 min at the temperatures specified. The reactions were initiated by adding 1 µl of Tpt1 to 9 µl 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 x ADP-pDNA/(ADPR-pDNA + 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 µl) containing 100 mM Tris-HCl (pH 7.5), 0.1 µM (1 pmol) $^{32}$P-labeled ADPR-pDNA (shown at bottom), 1 mM nicotinamide (where indicated by +), and 1 µM (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.