Materials and Methods.

NPH-II was expressed in baculovirus infected insect cells and purified as described (1). DED1 was expressed in *E. coli* as described (2), except that bacteria were grown at 28°C to prevent formation of inclusion bodies and that adsorption to Phosphocellulose resin (P11, Whatman) and elution with NaCl (1) were added as purification step. Homogeneity (>95%) and concentration of DED1 and NPH-II were assessed by SDS PAGE and subsequent Coomassie staining of the peptide. TRAP protein was expressed in *E. coli* and purified as described (3).

RNA oligonucleotides were either purchased from DHARMACON or transcribed *in vitro* from linearized DNA plasmids using T7 polymerase. The 78 nucleotide RNA used for TRAP-RNP remodeling (53 nt TRAP binding site (underlined) and 24 nt single strand overhang 5'-GAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAUGAGAGGUACACACUACACAUAGCCACC) was prepared by template-directed ligation of two RNA pieces using T4 DNA ligase (4). Bipartite control RNA duplexes were formed as described (5).

The EJC was assembled through splicing of radiolabeled RNA *in vitro* (6). The internal radiolabel (position –21 relative to the 5’ splice site) was introduced by template-directed ligation using T4 DNA ligase (4). This RNA was then spliced *in vitro* at 30°C for 60 minutes using HeLa cell extract (6), followed by fractionation using glycerol gradient sedimentation (Fig.1c). Fractions containing the radiolabeled RNA with bound EJC were identified by digestion of the RNA with micrococcal nuclease (7) and subsequent analysis on denaturing PAGE (Fig.1c). Fractions enriched for EJC containing RNAs were pooled and desalted by size exclusion chromatography (Biorad P6 spin columns). Tailless EJC-RNA complexes were prepared by treatment of spliced mRNAs with micrococcal nuclease prior to desalting (Fig.1c).

**TRAP-RNA remodeling**

TRAP-RNP remodeling reactions were performed at room temperature in a buffer containing 40 mM Tris-HCl pH 8.0, 0.01% (v/v) Nonidet P40, 0.5 nM radiolabeled RNA,
and 30 mM NaCl. The following concentrations of applicable components were used in reactions for NPH-II: 3.5 mM ATP, 4 mM MgCl$_2$, 20 nM NPH-II, 20 nM TRAP (monomer), 600 nM TRAP RNA scavenger DNA, 0.5 nM RNA control duplex, 10 µM tryptophan. Higher tryptophan concentrations did not significantly change the remodeling rate, lower tryptophan concentrations significantly increased the rate of spontaneous TRAP dissociation.

TRAP-RNA complexes were formed prior to the reaction for 10 minutes. Subsequently, NPH-II (or DED1) was added and incubation was continued for at least 5 minutes. Longer incubation times did not change the observed results. Remodeling reactions were started by adding a mixture of ATP and RNA scavenger. The latter consists of DNA oligonucleotides that hybridize to the TRAP binding site and thus prevent re-binding of TRAP to its cognate RNA once it has been displaced. Aliquots were removed at appropriate times and reactions were stopped by EDTA (5 mM final) and NPH-II (DED1) scavenger RNA, that prevents NPH-II (or DED1) from binding to the RNA from which TRAP has been displaced. The aliquots were applied to 8% non-denaturing PAGE (1.5 mm thick, run at 10 V/cm at 4ºC). Gels were dried and bands corresponding to free and complexed RNA were visualized using a PhosphorImager (Molecular Dynamics). Radioactivity in the respective bands was quantified using the ImageQuant software (Molecular Dynamics). The fraction of displaced TRAP (frac [P]) was calculated from the amount of radioactivity in the TRAP species ([TRAP]) and the amount of radioactivity in the free RNA ([RNA]) according to frac [P] = [RNA]/([RNA] + [TRAP]). Dissociation and displacement rate constants were determined from plots of frac [P] vs. time by least square fitting to the integrated rate law of a first order reaction using Kaleidagraph (Synergy software).

**EJC remodeling reactions**

EJC-RNP remodeling reactions were performed at room temperature in a buffer containing 40 mM Tris-HCl pH 8.0, 0.01% (v/v) Nonidet P40, roughly 0.1 nM EJC bound to radiolabeled RNA, and 40 mM NaCl. The following concentrations of applicable components were used in reactions for NPH-II: 10 mM ATP, 2 mM MgCl$_2$, 30
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nM NPH-II. RNA control duplex, where applicable, was at 0.5 nM. (Note that the ATP concentration exceeds the Mg\(^{2+}\) concentration, which leads to a decrease in the rate constant for duplex unwinding, compared to previously published results (5)). The high ATP concentration was necessary to accomplish a significant reaction amplitude. Lower ATP concentrations resulted in lower reaction amplitudes, but identical rate constants for EJC displacement. ATP concentrations above 10 mM were technically impractical. ATP regenerating systems were also not a practical choice because of the exceptionally high rate by which NPH-II hydrolyzes ATP (8, 9). For reactions involving DED1 conditions were as follows: 10 mM ATP, 2 mM MgCl\(_2\), 600 nM DED1, 0.5 nM RNA control duplex (where applicable).

EJC bound to RNA was incubated for 10 min with DED1 (NPH-II), or the respective control solutions corrected for all salt concentrations. Remodeling reactions were started by addition of ATP, where applicable. Reactions were stopped at appropriate times with EDTA solution (2 mM final concentration) and by placement on ice. Subsequently, ATP was removed by size exclusion chromatography (Biorad P6 spin columns). Samples were then subjected to digestion with micrococcal nuclease (10 minutes), followed by phenol extraction and ethanol precipitation (7). Then, a 22 nucleotide DNA standard was added to normalize for loading and other deviations during the subsequent analysis on denaturing PAGE (10 %). Gels were dried and bands corresponding EJC specific RNA fragments were visualized using a PhosphorImager (Molecular Dynamics). Radioactivity in the respective bands was quantified using the ImageQuant software (Molecular Dynamics). The amount of displaced EJC (frac [P]), was calculated from the ratio of radioactivity in the standard to the radioactivity in the EJC bands ([STD]_R/[EJC]_R), normalized for the same ratio in control reactions ([STD]_0/[EJC]_0) according to: frac [P] = 1-([STD]_0/[EJC]_R/[EJC]_0/[STD]_R). Dissociation and displacement rate constants were determined from plots of frac [P] vs. time by least square fitting to the integrated first order rate law using Kaleidagraph (Synergy software).
- scavenger
+ scavenger

-duplex

unwound

Time
No ATP

Time
No ATP
Figure S1.

Non-processive RNA helicase activity by DED1.

Unwinding of a radiolabeled 16 bp duplex containing a 24 nucleotide single stranded RNA overhang (0.5 nM) by 600 nM DED1. DED1 and RNA were pre-incubated for 5 minutes at room temperature (40 mM Tris/HCl, pH 8.0, 50 mM NaCl). The reaction (conducted at room temperature) was started by addition of a mixture of ATP, MgCl₂, and the scavenger RNA (final concentrations: 2 mM ATP, 2 mM MgCl₂, and 1 μM scavenger RNA (right part, + scavenger). The scavenger RNA traps all excess DED1 as well as DED1 that dissociates during the course of the reaction, and thus allows only a single cycle of RNA unwinding by DED1. Aliquots were removed from the reaction at increasing times, and the reaction was stopped by addition of EDTA (10 mM final and glycerol (10% v/v). Duplex and single stranded product were separated on non-denaturing PAGE (5) and visualized by a PhosphorImager.

Without scavenger RNA (- scavenger), appreciable unwinding was observed, whereas in the presence of scavenger RNA (+ scavenger) no significant product formation was detected. This observation indicates that DED1 lacks the ability to remain on the substrate for subsequent unwinding steps, i.e., DED1 is not processive. RNA helicases that act processively such as NPH-II or HCV NS3 are capable of unwinding RNA in the presence of scavenger RNA (5, 10).

Caption Figure S1

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References