Expanded View Figures

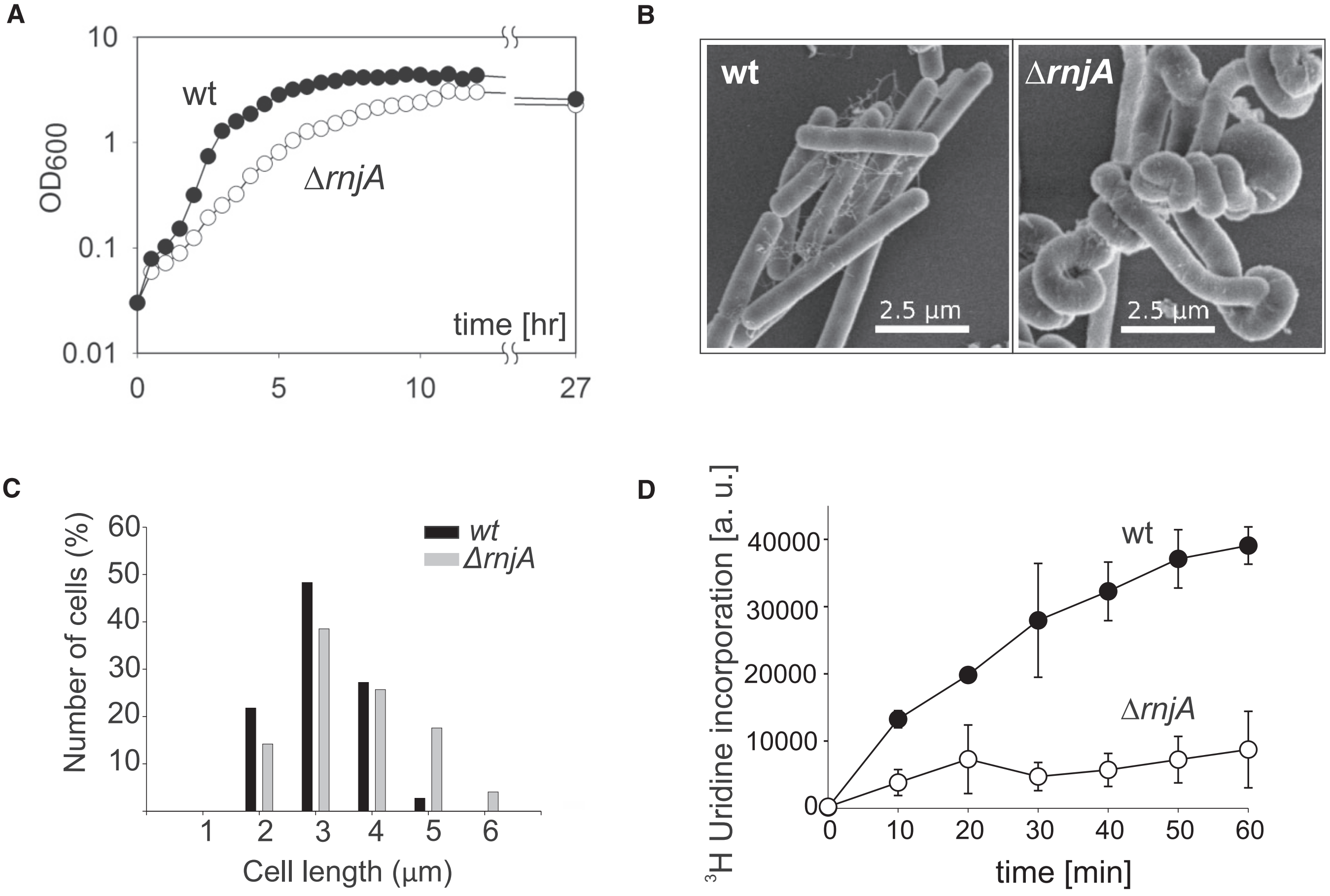

Figure EV1. Phenotypic characterization of the Δ*rnjA* strain.

- A Growth of *Bacillus subtilis* wt (LK1371) and *ΔrnjA* (LK1381) strains in LB medium at 37°C.
- B Morphology of wt (LK1371) and *ΔrnjA* (LK1381) strains as captured by electron microscopy of exponential phase cells (OD_{600} 0.5).
- C Quantification of cells length of exponentially growing (OD_{600} 0.5) wt (LK1371) and *ΔrnjA* (LK1381) cells stained with a membrane dye (NileRed, Sigma-Aldrich). The analysis was performed on two sets consisting of 100 cells each. Due to technical considerations, the length of only non-spiral cells was measured. The length of the cells was analyzed by Fiji ImageJ.
- D Total RNA synthesis wt (LK1371) and *ΔrnjA* (LK1381) strains normalized to cell density (OD_{600}). The data points are averages from two independent experiments (each done in technical duplicates); the error bars show the range.

Source data are available online for this figure.

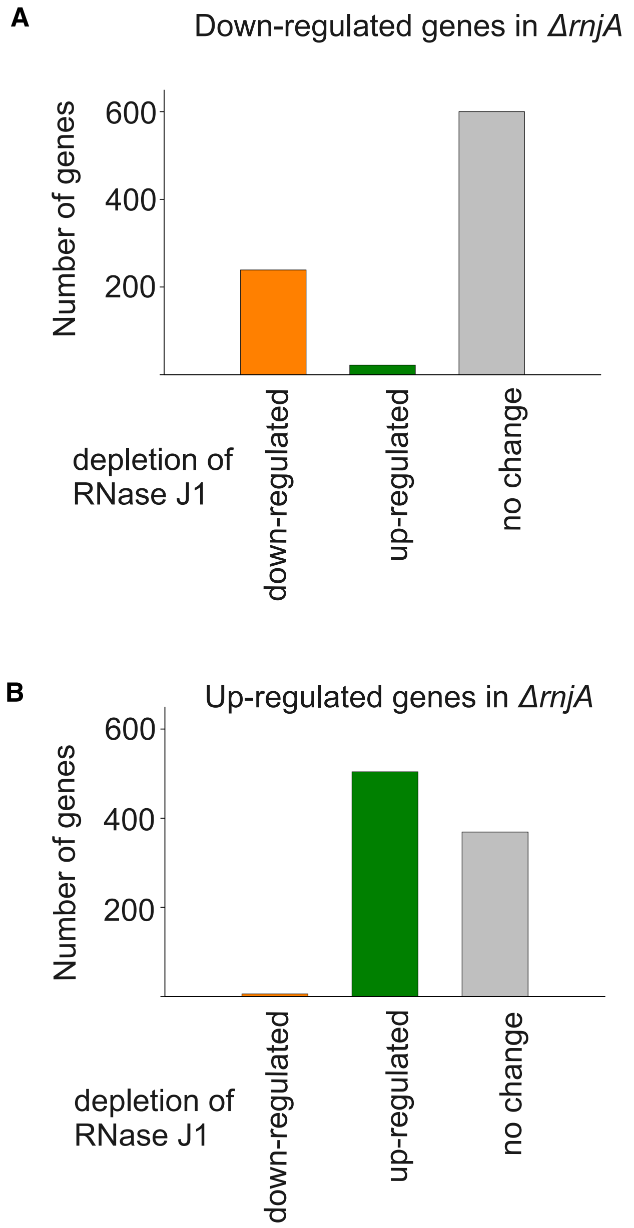

Figure EV2. Comparison of differentially regulated genes in RNase J1 depletion vs. deletion strains.

A Comparison of all downregulated genes in the Δ*rnjA* (LK1381) strain with the RNase J1-depleted strain (Durand *et al*, 2012). A small number of genes from depletion strain had opposite expression patterns. One-third of genes had the same expression pattern. Data from Table EV1 and Durand *et al* (2012).

B Comparison of all upregulated genes in the Δ*rnjA* (LK1381) strain with the RNase J1-depleted strain. A majority of genes had the same expression pattern; only six genes had opposite expression patterns. Data from Table EV2 and Durand *et al* (2012).

Source data are available online for this figure.

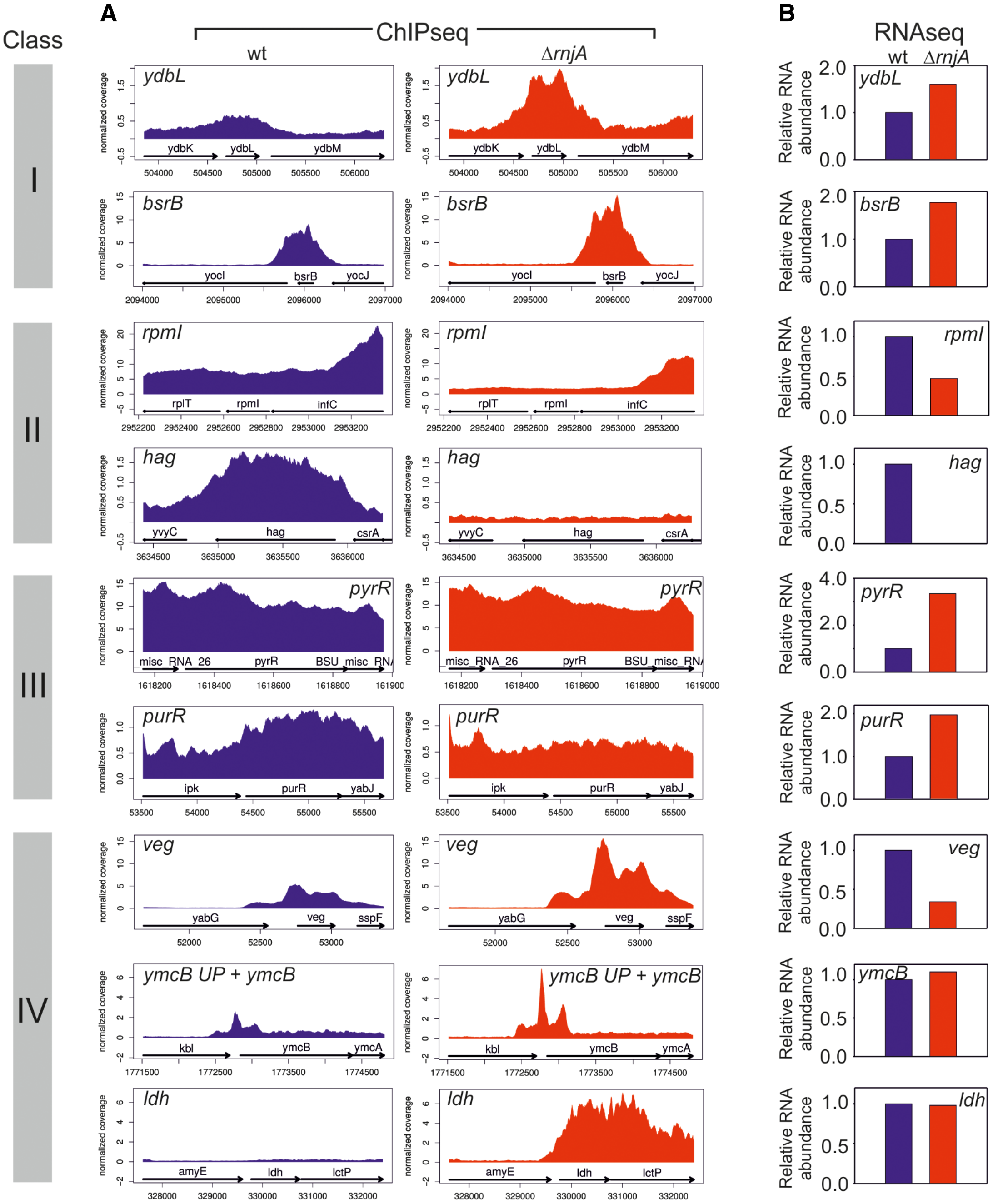

Figure EV3. Detailed comparisons of the RNAseq and ChIPseq data for selected genes.

A RNAP occupancy at selected genes (gene names indicated) in wt (blue) and Δ*rnjA* (red) strains. *ymcB* UP—5′ untranslated region (UTR) of the *ymcB* gene. Data represent mean of normalized RNAP coverage from three independent ChIPseq experiments.

B RNA abundance data for genes shown in (A). The genes are grouped into classes (indicated with colored bars with roman numerals) according to trends they displayed between wt and Δ*rnjA* with respect to gene occupancy and relative expression. Class I: Increased gene occupancy in Δ*rnjA* is accompanied by increased gene expression; class II: decreased gene occupancy in Δ*rnjA* is accompanied by decreased gene expression; class III: decreased or equal gene occupancy in Δ*rnjA* is accompanied by increased gene expression; and class IV: increased gene occupancy in Δ*rnjA* is accompanied by decreased or equal gene expression. The relative RNA level in wt was set as 1. Data represent mean of normalized read coverage from three independent RNAseq experiments.

Source data are available online for this figure.

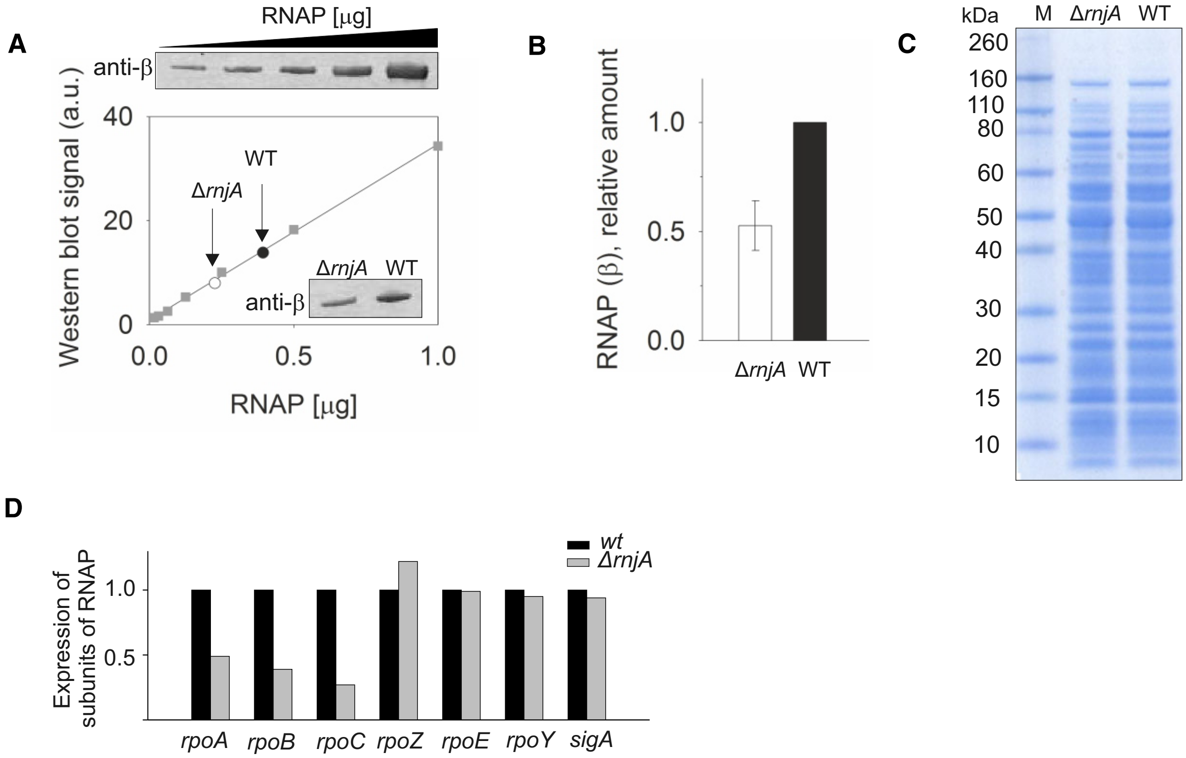

Figure EV4. Amount of RNAP in wt and Δ*rnjA* strains.

A Determination of the amount of RNAP in wt (black circle) and Δ*rnjA* (open circle) strains by Western blotting with monoclonal antibody against the β subunit of RNAP. The data for the calibration curve (squares) are shown above the graph; the data for the two strains are in the inset.

B Quantitation of the data shown in (A). The amount of RNAP in wt was set as 1. The experiment was performed three times on three different days. The bars show the average, the error bars ± SD.

C The amounts of cell lysates used to determine relative amounts of β. 5 μg of total protein was loaded per lane.

D Relative mRNA levels of three main RNAP subunits in wt [black bars, set as 1 (LK1371)] and Δ*rnjA* (gray bars; LK1381) strains. The main RNAP subunits (*rpoA*, *rpoB*, *rpoC*) had lower expression compared to wt. The expression of small subunits (ω, δ and ε) and *sigA* was unchanged.

Source data are available online for this figure.

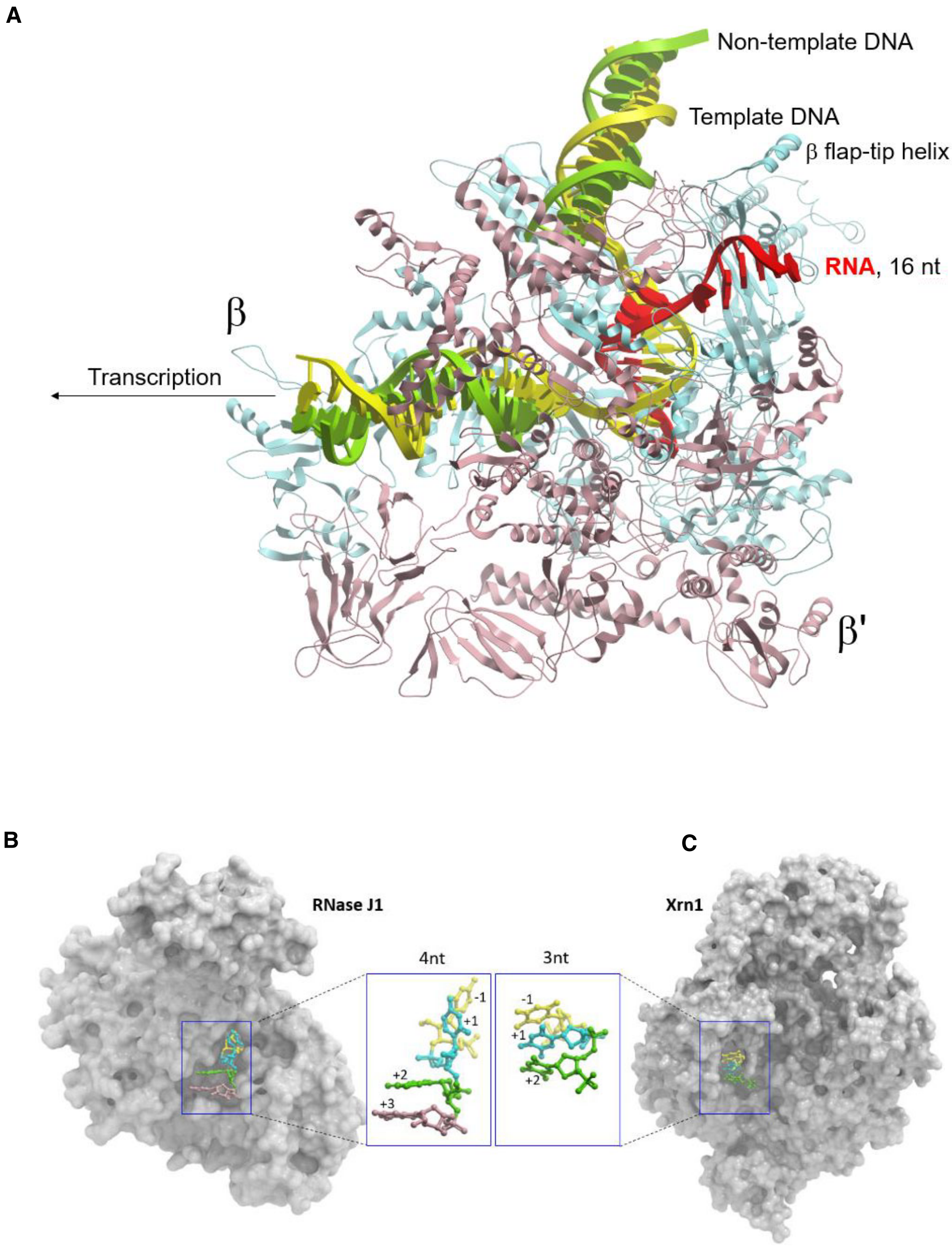

Figure EV5. Paths of RNA in RNAP, RNase J1, and Xrn1.

A Overall view of RNAP-16nt-mRNA (PDB id: 6flq). RNAP and bound nucleic acids are shown as cartoon (β—blue, β′—salmon, template DNA—yellow, non-template DNA—green, mRNA—red).

B Overall view of RNaseJ1-4nt-RNA substrate complex (PDB id: 3t3o).

C Overall view of Xrn1-3nt-RNA substrate complex (PDB id: 2y35). RNaseJ1/Xrn1 are shown as surface representation. The nucleic acid substrates are shown in ball and stick format. The carbon atoms of individual nucleotides are colored relative to the position of the scissile bond: yellow (−1), cyan (+1), green (+2), and pink (+3).