Supplementary Information for

Cockayne Syndrome B protein acts as an ATP-dependent processivity factor that helps RNA polymerase II overcome nucleosome barriers

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Materials and Methods

Protein expression and purification

Overexpression and purification of Rhp26 wild type and mutants were performed essentially as previously described(1). Rad26 expressed and purified as previously reported(2), briefly, Rad26 was expressed as a fusion protein with the *Escherichia coli* trigger factor chaperone and a hexa-histidine tag in the N-terminal. Recombinant Rad26 proteins were expressed in *E. coli* strain Rosetta 2(DE3) (Novagen) and purified by an Ni-NTA agarose (Qiagen), Hi-Trap Heparin HP (GE Healthcare), Hi-Trap SP (GE Healthcare), and Superdex 200 10/300 GL columns (GE Healthcare). The hexa-histidine tag and the trigger factor tag was removed by precision protease digestion. Rad26 mutants were purified in the same manner as for wild-type proteins.

S. cerevisiae 12-subunit Pol II was purified essentially as previously described(3, 4). Briefly, Pol II (with a recombinant protein A tag in the Rpb3 subunit) was purified by an IgG affinity column (GE Healthcare), followed by Hi-Trap Heparin (GE Healthcare) and Mono Q anion exchange chromatography columns (GE Healthcare).

Mono-nucleosome Remodeling Assay

The remodeling activity of Rad26/Rhp26 was characterized by the mono-nucleosome sliding assay. Briefly, the 216-bp DNA fragment containing 146-bp Widom601 sequence in the middle was amplified from the pGEM-3Z-601 vector (forward primer 5'GGCACCGGCAAGGTCGCTGTTCAATACAT 3' and reverse primer 5' TGTCATCCCTTATGTGATGGACCCTATAC 3') and 5' end labeled with T4 PNK (New England Biolabs) and γ-^{32}P rATP(5). *Xenopus laevis* histones octamers were purified as previously described(6). Mono-nucleosomes were assembled onto the labeled template by salt

serial dilution method as previously described(7). Cold mono-nucleosome (2 uM) was assembled using unlabeled DNA fragment by the same method, which is then mixed with sonicated calf thymus DNA (1mg/ml, Sigma). 1 ul purified Rhp26/Rad26 remodeler (5 uM) was incubated with 3 ul ^{32}P labeled mono-nucleosome (10 nM) at 30ºC in final 15 ul remodeling buffer (20 mM Tris-HCl pH7.5, 50 mM NaCl, 2.5 mM $MgCl_2$, 2 mM DTT, 100 ug/ml BSA, 5% Glycerol, 0.01% NP-40, 0.01% Triton X-100, and 1 mM ATP). After 60 minutes, 1.5 ul cold nucleosomes was added to extract Rad26/Rhp26 from binding to p32 labeled nucleosome or naked DNA, and quenched the reaction for 30 min at room temperature. The reaction products were loaded onto 5% native PAGE gels and nucleosome and naked DNA bands are resolved by electrophoresis at 4ºC for 2.5 h at 200 V. The p32 signal on the nucleosome and naked DNA was visualized by phosporimaging and quantified using Image Laboratory software (BioRad). The naked DNA band is the product of nucleosome remodeling/histone octamer evicted by Rad26/Rhp26.

Chromatin Remodeling by Restriction Enzyme Accessibility Assays

Restriction enzyme accessibility assays were performed as previously described with modifications (1). Basically, chromatins were reconstituted by the gradient salt dialysis method by using *Xenopus laevis* core histones and an ~3-kb plasmid DNA in which there are 13 HaeIII digestion sites. Then, 200 ng of chromatin were gently mixed with Rhp26/Rad26 of in 1X NEB cut smart buffer containing 3 mM ATP and 5 mM MgCl2. The remodeling reaction was performed at 30ºC for 1h followed by adding 15 U of HaeIII restriction enzyme (NEB) to digest the remodeled chromatin. The remodeling activity of Rad26/Rhp26 changes the accessibility of HaeIII restriction sites by sliding or eviction of histones. After digestion for 1 h at 27 °C, samples were deproteinized by proteinase K, and DNA was purified by phenol/chloroform extraction, resolved by 0.8%

agarose gel, and visualized by Gel Red (Biotium) DNA staining. The reduction of upper bands (and enrichment of lower bands) in a remodeler and ATP dependent manner reflects an increase of restriction enzyme accessibility, an indicative of active chromatin remodeling activities of Rhp26/Rad26.

Generation of nucleosomal transcription templates

Transcription of nucleosomal and DNA templates by yeast Pol II was performed as described (8). Pol II elongation complex (EC10) was assembled on a 77 bp DNA fragment. Briefly, RNA10 (5' AUCGAGAGGA) or TS (5' GGTGTCTCTTGGGTTGGCTCTTCGCCTTCTGTCCTCTC GATATCTGAATTCGTATAGGGTCCATCACATAAGGGATG) were labeled at the 5' end as described (Sidorenkov et al., 1998). If RNA was to be labeled, TS was phosphorylated by T4 PNK with cold ATP. TS and RNA were annealed and assembled with Pol II, and followed by adding NTS (5'Biotin-TGTCATCCCTTATGTGATGGACCCTATACGAATTCAGATATCG AGAGGACAGAAGGCGAAGAGCCAACCCAAGAGACACCGGCACTGGG). The EC10 was immobilized on Streptavidin magnetic beads (NEB), ligated to TspRI digested Widom601 DNA or nucleosomal templates.

In vitro transcription on chromatin templates

In vitro transcription was initiated by adding rNTPs to a final concentration of 1 mM each. Additional 3mM dATP was also included to support Rhp26/Rad26 ATPase activity. Rhp26/Rad26 (final concentration of 200 nM) was added to the reaction mix and kept at 30ºC. Reactions were allowed to continue for the desired time and then quenched by adding an equal volume of formamide loading buffer (90% formamide, 50 mM EDTA, 0.05% xylene cyanol and 0.05%

bromophenol blue). Samples were boiled for 10 min at 95 °C in formamide loading buffer, and the extended RNA was separated from the unextended RNA by denaturing PAGE (6 M urea). The gel was visualized by phosporimaging and quantified using Imagelab software (Bio-Rad).

Competitive EMSA.

Binding reactions were performed with 10 nM elongation complex with 15 bp upstream dsDNA or nucleosome with 15 bp flanking DNA. 200 nM of Rad26 was incubated with EC or nucleosome at room temperature for 15 min before adding the competitor DNA. After adding different concentrations of competitor DNA, the mixture was incubated at room temperature for another 15 min. The final buffer was composed of 20 mM Tris, pH 7.5, 150 mM NaCl, 40 mM KCl, 5 mM DTT, 0.1 mg/ml BSA, 5% glycerol. The mixtures were loaded onto a 20 cm 4.5% native polyacrylamide gels and electrophoresed at 200 V for 2.5 h. The gel was visualized by phosporimaging and quantified using Imagelab software (Bio-Rad).

EMSA of Pol II and transcribed nucleosome

Pol II complexes of free DNA and nucleosomes were formed as described for in vitro transcription on chromatin templates. After transcription, the templates were release from the the beads by EcoRV digestion for 30 min at 30 ºC. The mixtures were loaded onto 20 cm 4% native polyacrylamide gels and electrophoresed at 200 V for 2.5 h. The gel was visualized by phosporimaging and quantified using Imagelab software (Bio-Rad).

Structural modeling of two potential binding sites for Rad26/CSB on Pol II-Nucleosome complex

The structure model was generated as follows: First, the structure of Pol II EC-Rad26 complex (5VVR) was aligned with the structure of Pol II EC-Nucleosome complex (stalled at SHL-1, 6A5V) on Pol II. Second, the structure of Snf2-Nucleosome (5X0Y) was aligned with Pol II EC-Nucleosome complex (6A5V) on the nucleosome. Third, the core domain of Rad26 (5VVR) was aligned with Snf2 (5X0Y) and replaced Snf2 at position 2.

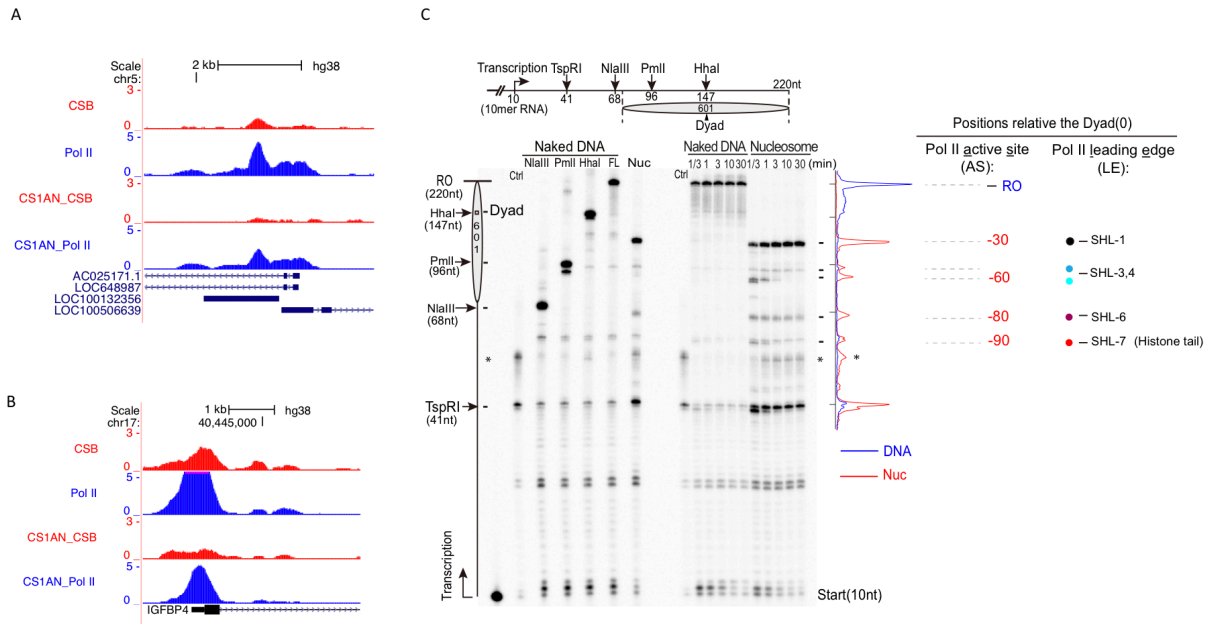

Fig. S1: (A, B) Examples of CSB colocalize with Pol II at specific genes. (C) Mapping the transcription pausing sites on nucleosome. Top: Schematic of the nucleosome template and restrict enzyme digestion sites. The numbers show the total length of transcript RNA. Bottom: Full urea PAGE gel to show the pausing sites of Pol II on nucleosome. The positions of the Pol II active site (AS) and leading edge (LE) are indicated on the right. In the control (ctrl) lane, there was no downstream DNA or nucleosome or ligated.

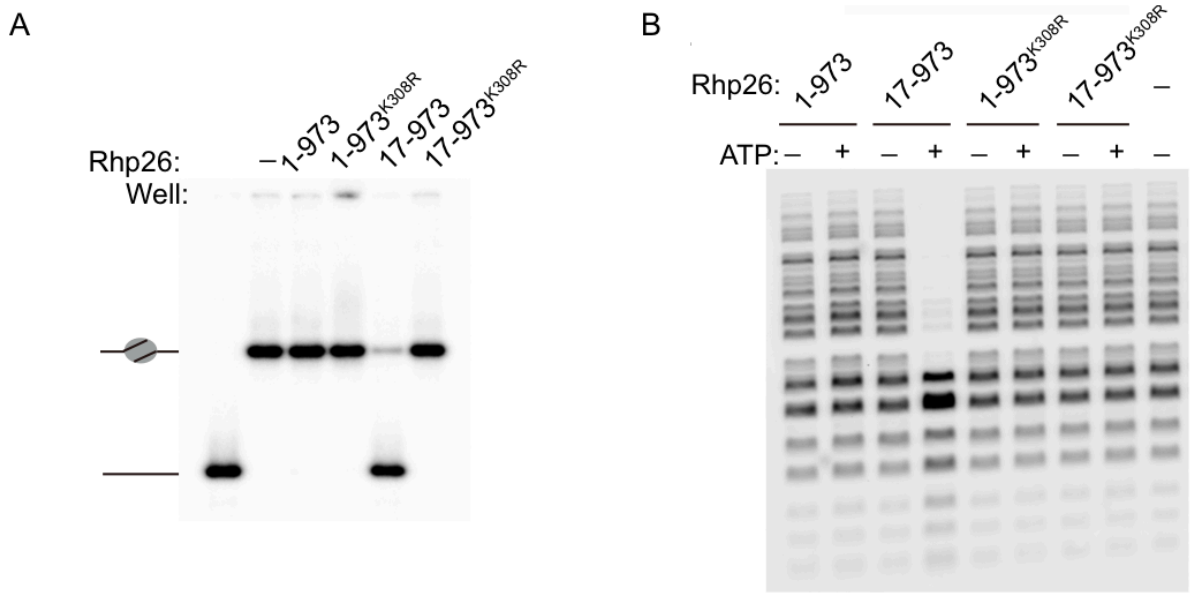

Fig. S2. (A) Mono-nucleosome remodeling by Rhp26. (B) Remodeling by Rhp26 on a nucleosome array.

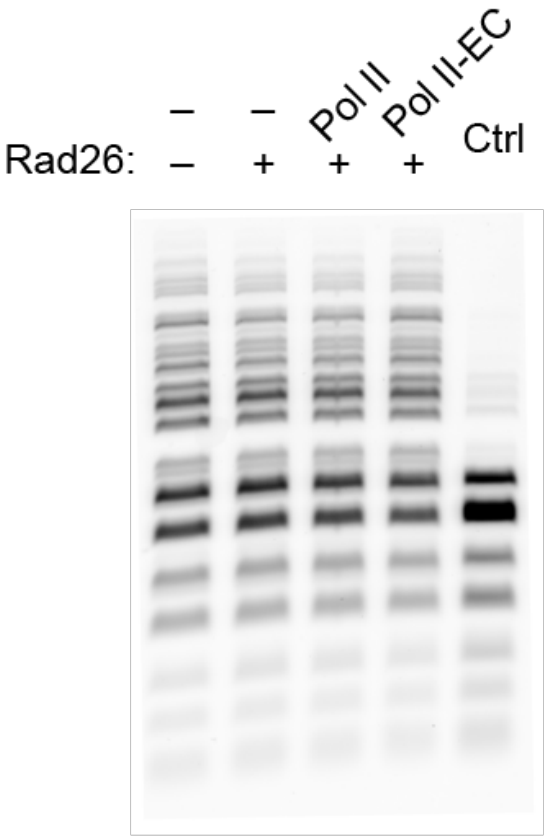

Fig. S3. The remodeling activity of full-length Rad26 cannot be activated by Pol II or Pol II-EC *in trans.* 200nM of Pol II or Pol II-EC was added into the reaction. The positive control lane (Ctrl) shows robust chromatin remodeling by constitutively active Rad26 variant Rad26$^{28\text{-}1085}$.

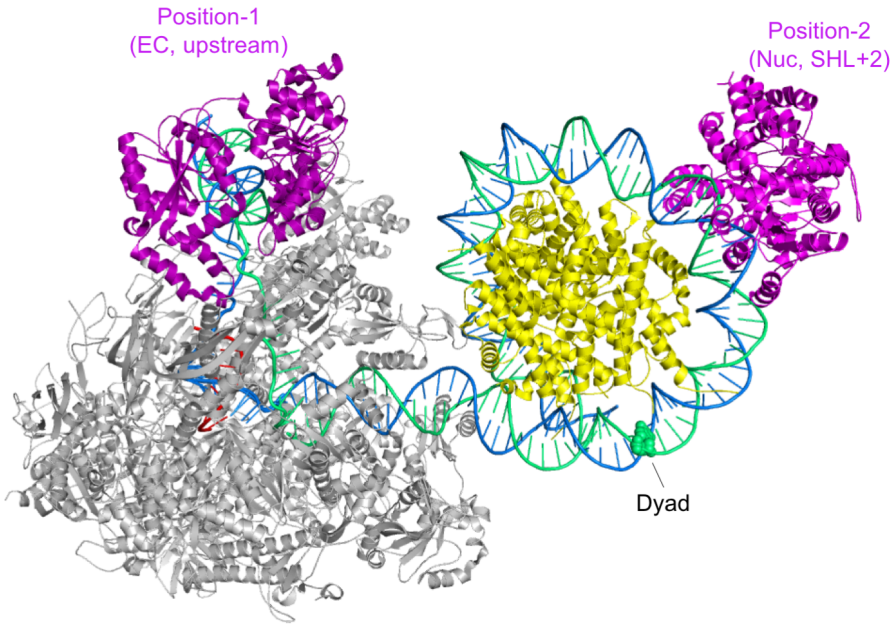

Fig. S4. Two potential working modes and binding sites for Rad26/CSB. At position-1, Rad26/CSB binds to the upstream fork region of the Pol II elongation complex and works as a processivity factor that promotes nucleosome bypass by Pol II (noncanonical mode). At position-2, activated Rad26/CSB may bind nucleosome at canonical SHL 2 position and work as a canonical chromatin remodeler to facilitate nucleosome bypass by Pol II (canonical mode). Pol II, Rad26 and the histone octamer are shown in gray, magenta, and yellow, respectively. Template DNA, non-template DNA, RNA are shown in blue, green, and gray, respectively. The nucleosome dyad is highlighted in green. Please see SI method section for structural modeling details.

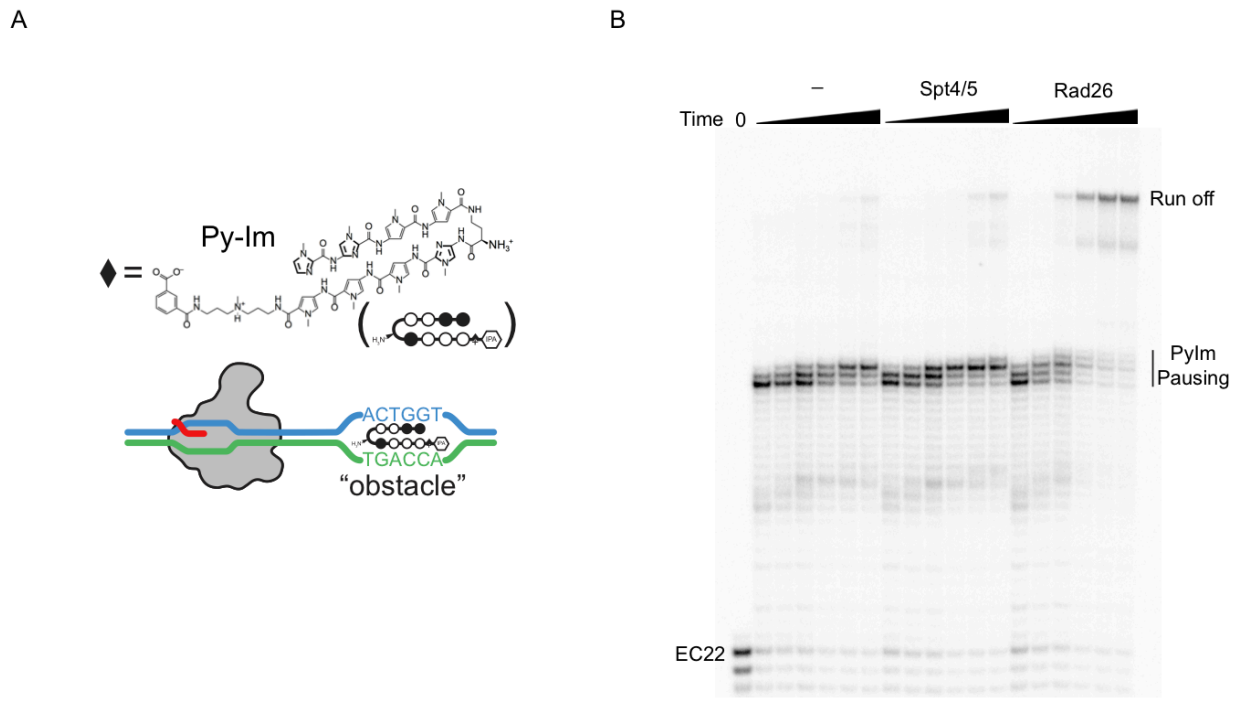

Fig. S5. Rad26, but not Spt4/5, promotes Pol II bypass the Py-IM induced transcription pausing.

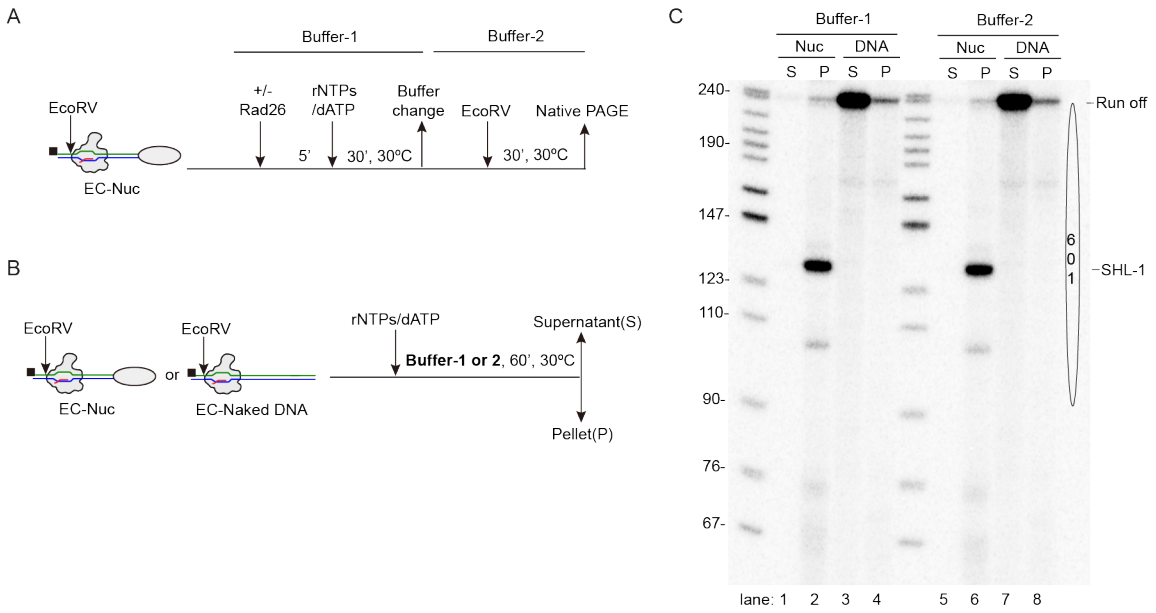

Fig. S6. Stability of nucleosome paused Pol II EC. (A). Experiment setting used in Figure 5 with two different buffers highlighted. Buffer-1 is the buffer for transcription elongation and Rad26 reaction, which is composed of 20 mM tris, pH 7.5, 50 mM NaCl, 40 mM KCl, 5 mM DTT, 5 mM $MgCl_2$, 0.1 mg/ml BSA. Buffer-2 is the EcoRV digestion buffer (Cutsmart buffer of NEB), which is composed of 50 mM potassium acetate, 20 mM tris-acetate, 10 mM magnesium acetate,100 µg/ml BSA, pH 7.9. (B). Experimental setting to test the stability of nucleosome paused Pol II EC. The supernatant and pellet fractions are separated on a urea-PAGE gel and the radioactive signal from the transcript was detected. (C). Nucleosome paused Pol II EC was not released from the streptavidin resin. In our settings, the template strand (TS) and non-template strand (NTS) are fully matched, once Pol II dissociated from the DNA template, TS and NTS will anneal together. So RNA is displaced from the template and released into the supernatant if Pol II is dissociated from the template. In the naked DNA template control, the run off RNA transcript was released into the supernatant (lanes 3 and 7), however, the paused transcripts of nucleosome paused Pol II EC (SHL-1, lanes 2 and 6) are bind to the resin after 60 min incubation, indicating the nucleosome paused Pol II EC is stable and RNA and Pol II are not dissociated from the DNA templates.

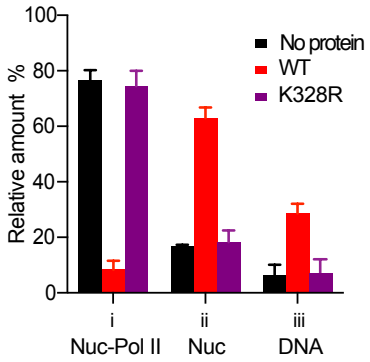

Fig. S7. Quantitation of the native PAGE assay in Figure 5B. Lane 4, 5, 6 from native gel were compared here. The experiments were performed three times and shown as means with standard deviation error bars.

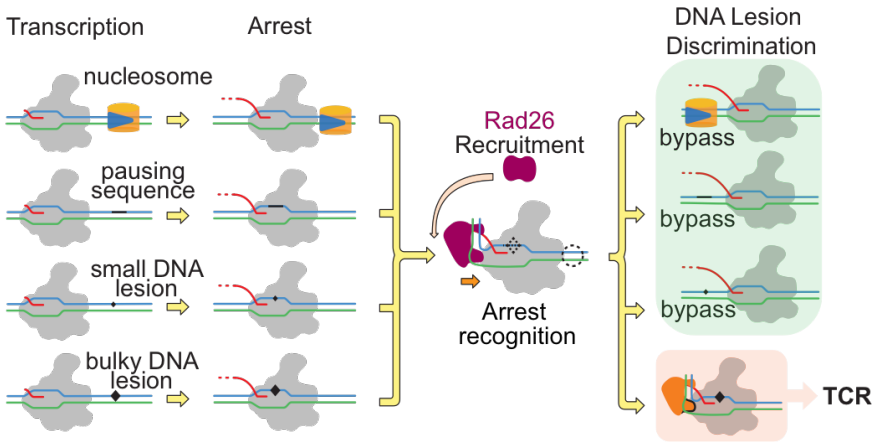

Fig. S8. Model for the function of Rad26 in Pol II transcription and repair. Nucleosome is the most abundant barrier for Pol II transcription elongation. Rad26 recognizes a stalled Pol II and promotes Pol II forward translocation, which helps Pol II bypass the nucleosome barrier and maintain the nucleosome structure. Rad26 also promotes Pol II bypass pausing inducing sequences and less bulky DNA lesions. However, Rad26 fails to promote efficient transcriptional bypass of bulky DNA lesions (such as CPD lesions) and leads to the initiation of TCR.

Reference

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