Supporting Information

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SI Discussion

To evaluate the effect of human facilitates chromatin transcription (hFACT) on the rate of transcription, we analyzed the kinetics of transcription through the 603 nucleosome in the presence or absence of hFACT (Fig. 3*A*) using three different models (Fig. S9). In the "minimal" model 1, RNA polymerase II (Pol II) can progress only to the next position at each position on the nucleosomal DNA. In model 2, Pol II can either progress to the next position on the nucleosomal DNA or irreversibly partition into nonproductive complexes. In model 3, Pol II can either progress to the next position on the nucleosomal DNA or reversibly partition into nonproductive complexes.

Although it is formally possible that transcription in the presence of FACT can occur without formation of the nonproductive complexes (model 1), multiple nonproductive complexes containing stalled and arrested elongation complexes (ECs) are formed during Pol II transcription in the absence of FACT (1, 2). Accordingly, the obtained experimental data did not fit the "minimal" sequential model 1 (data not shown).

Analysis of the more complex and more realistic model 2, involving formation of irreversible nonproductive complexes, shows a reasonably good fit of the experimental data to the model (data not shown). However, model 3, involving formation of reversible nonproductive complexes, shows a considerably better fit of the data (Fig. S10), suggesting that this model better describes the process of transcription through the nucleosome. Furthermore, analysis of an extended (10-min) time course of transcription through the nucleosome indicates that the majority of nucleosome-specific paused complexes are eventually extended into the run-off transcripts (data not shown), suggesting that formation of the nonproductive complexes is a reversible process.

In summary, our analysis of models 1–3 (Fig. S9) suggests that only model 3 adequately describes the process of transcription through the nucleosome. Accordingly, we used this model for data analysis (Fig. 3).

SI Materials and Methods

Preparation of Proteins, DNA Templates, and Nucleosomes. Site-directed mutagenesis was used to create the point mutations in the *Xenopus laevis* WT histone plasmids with the QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies). H2B containing T112C mutation was labeled with Alexa Fluor 488 and refolded into dimer as described previously (3). A typical labeling efficiency of ~75% was routinely obtained for the H2A/H2B dimer. $(H3\text{-}H4)_2$ tetramers were refolded using WT or mutant H3 (I51A, I51N) and WT H4 or mutant H4 (Y98H) and purified as described previously (4). Note that mutant H3 and H4 did not refold into octamers.

Nucleosomes were assembled on a 147-bp 601 positioning sequence (5) using the salt gradient method (4). Assembly was carried out in two steps. In the first step, the amount of DNA was kept constant, and tetramer (WT or mutant) was titrated in to obtain an ideal ratio of tetrasomes (containing no free DNA). Using this ratio, in the next step, labeled dimer was titrated in to obtain nucleosomes. Nucleosomes were run on a native PAGE. The bands were visualized by scanning on a Typhoon Imager at 488-nm excitation and 520-nm emission, followed by staining for DNA with ethidium bromide and for protein with Imperial stain (Pierce).

Nucleosomes for transcription by Pol II and nucleosome fate assays were assembled on 227-bp and 148-bp DNA templates, respectively. Transcription by *Escherichia coli* RNA polymerase (RNAP) was conducted on 271-bp template containing a strong bacteriophage T7A1 promoter (6). Nucleosomes and subnucleosomes for transcription were prepared as described previously (6).

Dimethyl Suberimidate Cross-Linking of Reconstituted Nucleosomes and Histone Octamers. For cross-linking of reconstituted nucleosomes, the 603 nucleosomes were dialyzed for 90 min against buffer containing 0.1 M sodium borate (pH 10), 0.1 M NaCl, and 0.1 mM EDTA. Dimethyl suberimidate (DMS) was then added to a final concentration of 0.5 mg/mL, and the reaction was incubated at room temperature for 60 min. The reaction was then dialyzed for 90 min against buffer containing 25 mM Tris·HCl (pH 6.9) and 1 mM EDTA and then for 90 min against buffer containing 10 mM Tris·HCl (pH 7.5), 10 mM NaCl, 0.1% Nonidet P-40, 0.2 mM EDTA, and 5 mM 2-mercaptoethanol.

For cross-linking of histone octamers, the histones were incubated in the presence of indicated concentrations of DMS (Fig. S8) in buffer containing 0.1 M sodium borate (pH 10), 0.1 M NaCl, 0.2 mM EDTA, and 2 M NaCl at room temperature for 60 min. The reaction was terminated by adding 0.25 M Tris·HCl (pH 6.9) to a final concentration of 50 mM. The reactions were dialyzed for 90 min against buffer containing 20 mM Tris·HCl (pH 7.5), 50 mM NaCl, 1 mM EDTA, and 1 mM 2-mercaptoethanol. The cross-linked octamers were then used for nucleosome reconstitution. The cross-linked nucleosomes and protein complexes were analyzed by 15% SDS/PAGE after staining with R-250 Coomassie blue. The cross-linked nucleosomes were also analyzed by 4.5% native PAGE.

Transcription and Kinetics Assay. Yeast Pol II ECs were assembled on a 50-bp DNA fragment from the nine-nucleotide RNA and separate template and nontemplate strands. These complexes were immobilized on nickel-nitrilotriacetic acid-agarose beads, washed, eluted from the beads, and ligated to nucleosomal templates or corresponding DNA fragments. Pol II was advanced to position −5 using [α-^{32}P]NTPs to label the RNA. Transcription was resumed from position −5 by the addition of a large excess of unlabeled NTPs in the presence of 150 mM KCl at final concentrations of 100 μM hFACT and 3.3 μM nucleosomes. hFACT was added to the −5 complexes simultaneously with the NTPs. Transcript elongation reactions with yeast Pol II were run for the indicated time periods. For nucleosome fate experiments, the nontemplate DNA strands of the EC-5 complexes were end-labeled by [α-^{32}P]ATP, and transcription was carried out in the presence of unlabeled NTPs in the presence of indicated concentrations of KCl.

For transcription by *E. coli* RNAP, all of the steps were similar to those for Pol II, but with the DNA templates containing the T7A1 promoter, which allows formation of the pre-ECs directly on the nucleosomal templates.

1. Izban MG, Luse DS (1991) Transcription on nucleosomal templates by RNA polymerase II in vitro: Inhibition of elongation with enhancement of sequence-specific pausing. *Genes Dev* 5(4):683–696.
2. Kireeva ML, et al. (2005) Nature of the nucleosomal barrier to RNA polymerase II. *Mol Cell* 18(1):97–108.
3. Winkler DD, Luger K (2011) The histone chaperone FACT: Structural insights and mechanisms for nucleosome reorganization. *J Biol Chem* 286(21):18369–18374.
4. Dyer PN, et al. (2004) Reconstitution of nucleosome core particles from recombinant histones and DNA. *Methods Enzymol* 375:23–44.

5. Lowary PT, Widom J (1998) New DNA sequence rules for high-affinity binding to histone octamer and sequence-directed nucleosome positioning. *J Mol Biol* 276(1):19–42.

6. Kulaeva OI, et al. (2009) Mechanism of chromatin remodeling and recovery during passage of RNA polymerase II. *Nat Struct Mol Biol* 16(12):1272–1278.

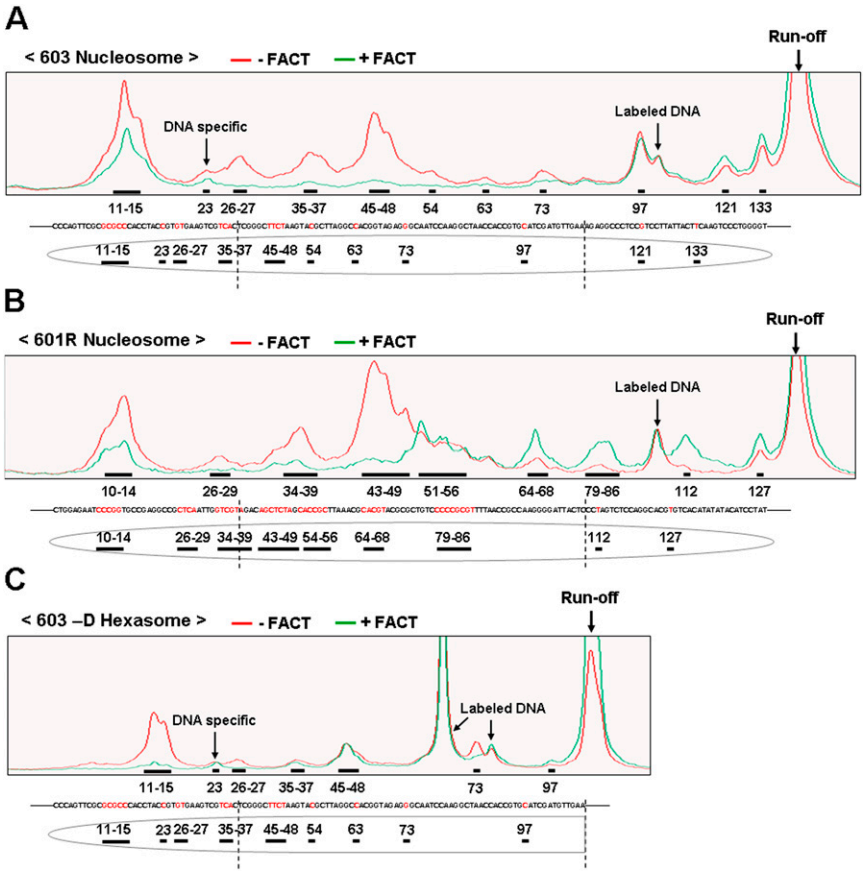

Fig. S1. Quantitative analysis of Pol II pausing during transcription through 603 and 601R nucleosomes in the absence (–FACT) or presence (+FACT) of hFACT. Lanes 4 and 7 in Fig. 1*B* (*A*), lanes 11 and 14 in Fig. 1*B* (*B*), and lanes 4 and 7 in Fig. 1*D* (*C*) were scanned with a Cyclone Phosphor Imager (PerkinElmer). Corresponding lanes are shown in red and green, respectively. Vertical dotted lines indicate the boundaries between H2A/2B dimers and H3/H4 tetramers. The positions of DNA-specific pausing and labeled DNA are indicated. The positions of nucleosomes are designated as described previously (1).

1. Morozov AV, et al. (2009) *U*sing DNA mechanics to predict in vitro nucleosome positions and formation energies. *Nucleic Acids Res* 37(14):4707–4722.

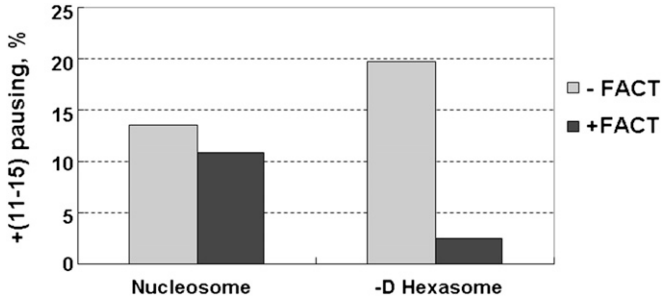

Fig. S2. Quantitation of +(11–15) pausing (calculated as molar fraction of all transcripts present in the lane) after transcription of 603 nucleosome and -D hexasome by Pol II in the absence or presence of hFACT. Lane 5 in Fig. 1*B* and lane 8 in Fig. 1*D* were quantified using a Cyclone Phosphor Imager.

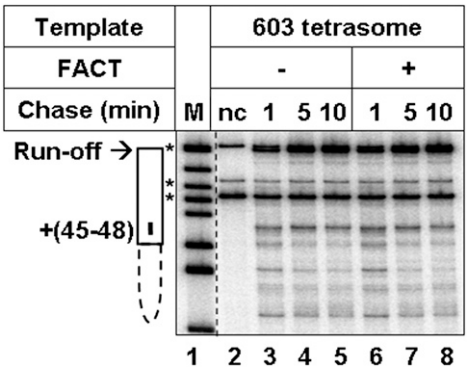

Fig. S3. hFACT does not affect transcription of 603 tetrasomes (DNA-bound H3/H4 histone tetramer). The 603 tetrasomes were transcribed at 150 mM KCl in the absence or presence of hFACT for the indicated times, and pulse-labeled RNA was analyzed by denaturing PAGE. Black asterisks indicate the labeled DNA fragments.

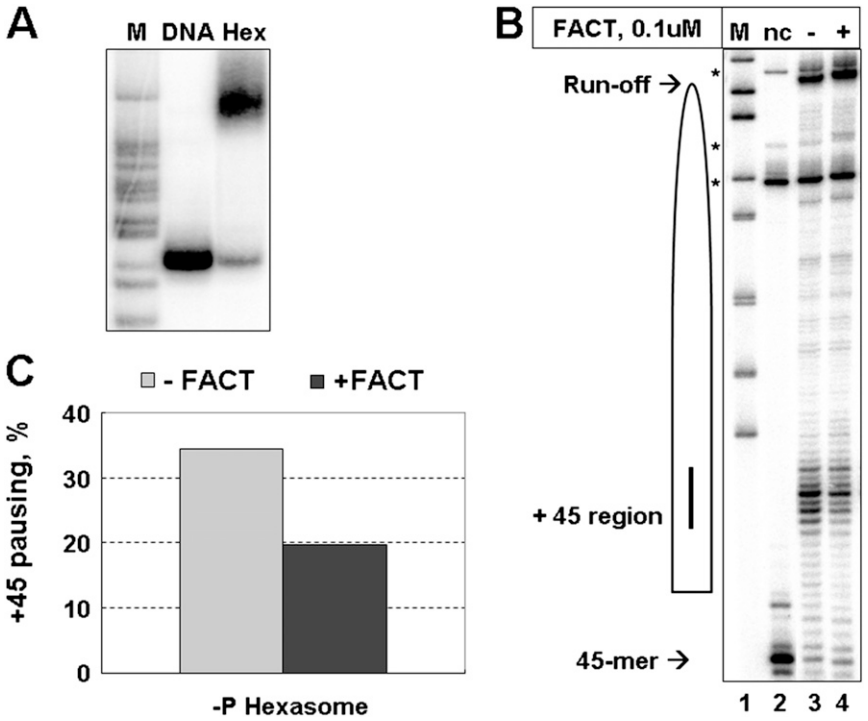

Fig. S4. hFACT relieves the +45 pausing during transcription of 603-P hexasome. (*A*) Analysis of 603-P hexasomes by 4.5% native PAGE. M, molecular weight standards. (*B*) The 603-P hexasomes were transcribed by Pol II at 150 mM KCl in the absence or presence of hFACT for 10 min, followed by analysis of pulse-labeled RNA by denaturing PAGE. Black asterisks indicate labeled DNA fragments. (*C*) Quantitation of +45 pausing after transcription of 603-P hexasomes by Pol II in the absence or presence of hFACT. Lanes 3 and 4 in *B* were quantified using a Cyclone Phosphor Imager.

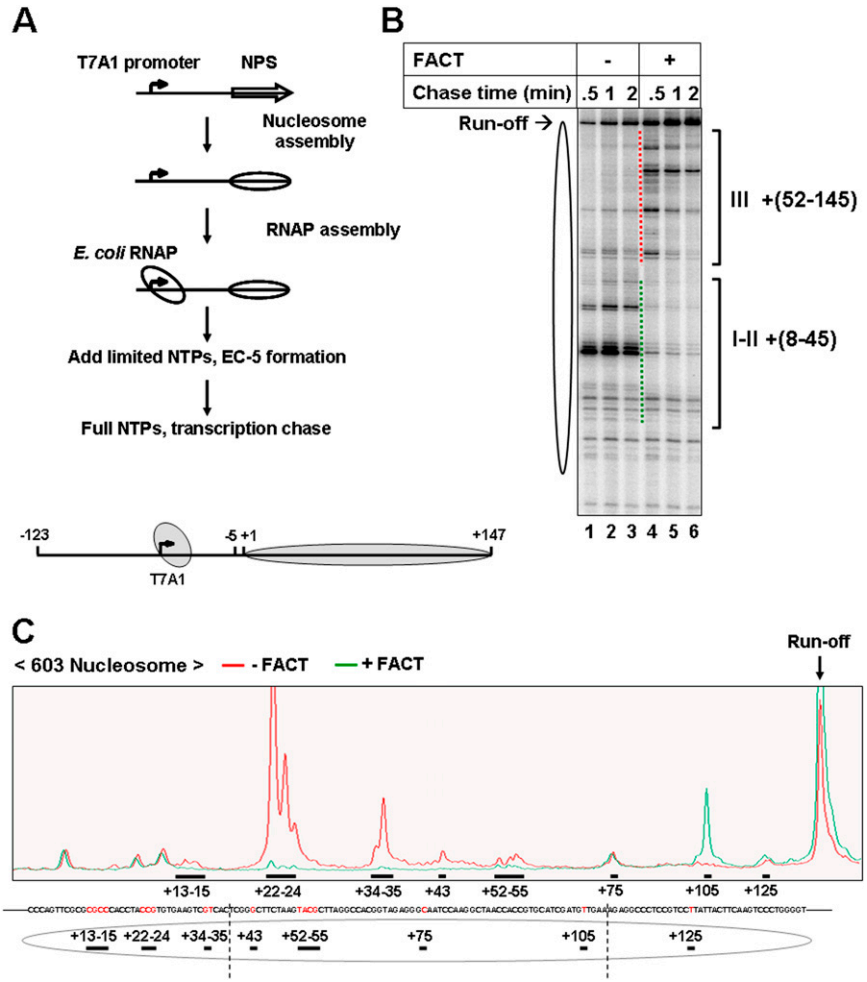

Fig. S5. FACT facilitates transcription through chromatin by *E. coli* RNA polymerase. (*A*) (*Upper*) The experimental approach (see text for details). (*Lower*) Diagram of the nucleosomal template for *E. coli* RNA polymerase transcription. (*B*) 603 nucleosomes were transcribed by *E. coli* RNA polymerase at 150 mM KCl in the absence or presence of FACT, followed by analysis of pulse-labeled RNA by denaturing PAGE. Other designations are as in Fig. 1*B*. (*C*) Quantitative analysis of pausing. Lanes 3 and 6 in *B* were scanned using a Cyclone Phosphor Imager (shown in red and green, respectively). Designations are as in Fig. S1.

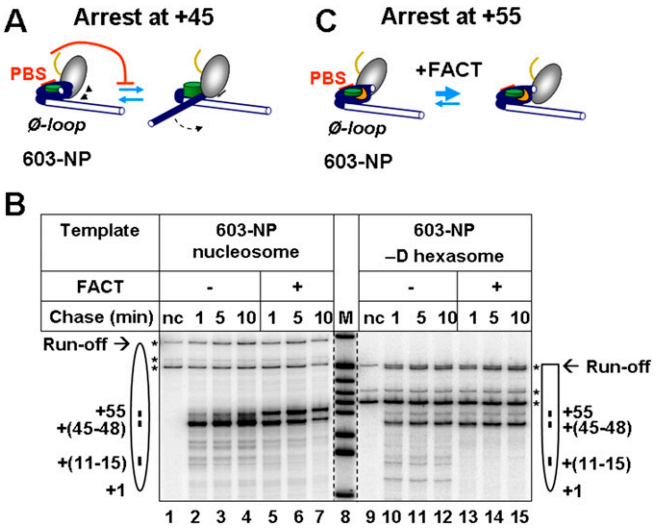

Fig. S6. Strong DNA–histone interactions prevent FACT-dependent transcription through a nucleosome. (*A*) Presence of the DNA sequence with high affinity to H3/H4 tetramer [polar barrier sequence (PBS)] prevents DNA uncoiling from the surface of the octamer and further transcription through the nucleosome (6). (*B*) The 603-NP nucleosomes and -D hexasomes with PBS regions were transcribed by Pol II at 150 mM KCl in the absence or presence of FACT, followed by analysis of pulse-labeled RNA by denaturing PAGE. (*C*) The PBS sequence allows only limited FACT-dependent uncoiling of DNA from the octamer and thus inhibits transcription past the +55 region.

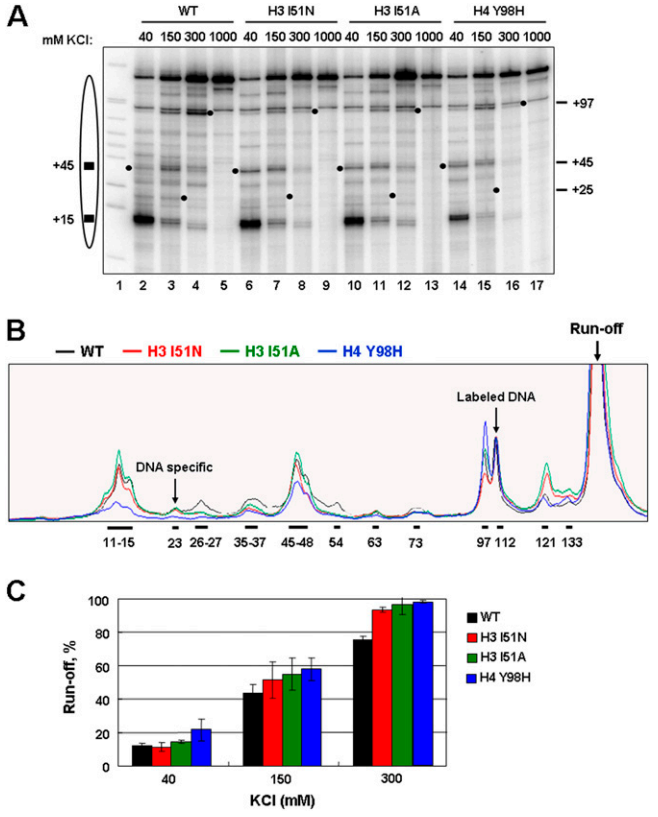

Fig. S7. Effect of mutations disrupting the dimer–tetramer interface on transcription through the 603 nucleosome by Pol II. (*A*) 603 nucleosomes containing various mutations in the dimer–tetramer interface were transcribed by Pol II at different KCl concentrations for 10 min, followed by analysis of pulse-labeled RNA by denaturing PAGE. The changes in pausing caused by the mutations are indicated by dots. (*B*) Quantitative analysis of Pol II pausing during transcription through the 603 nucleosomes containing various mutations in the dimer–tetramer interface. Scans of lanes 3, 7, 11, and 15 in *A* are shown in black, red, green, and blue, respectively. Other designations are as in Fig. S1. (*C*) Quantitation of run-off transcripts shown in *A*.

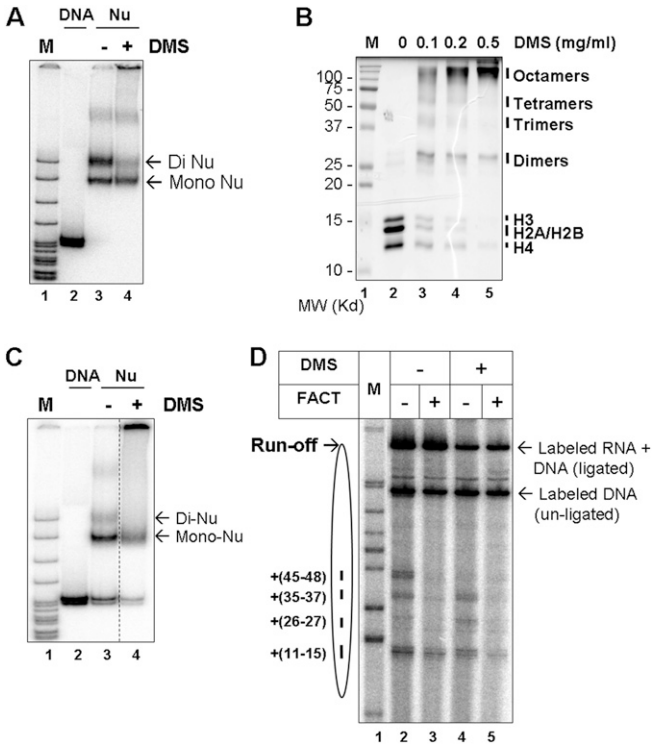

Fig. S8. Analysis of DMS cross-linked 603 nucleosome. Cross-linking was conducted in the presence of various concentrations of DMS before and after nucleosome assembly. (*A* and *C*) Analysis of 603 DNA intact and cross-linked nucleosomes (Nu) by 4.5% native PAGE. The positions of mononucleosomes and dinucleosomes are indicated. (*B*) Analysis of intact and cross-linked proteins by 15% SDS/PAGE (R-250 Coomassie blue staining). (*D*) Effect of cross-linking of histone octamers on transcription through the nucleosomes in the presence or absence of hFACT. 603 nucleosomes were transcribed by Pol II at 150 mM KCl before and after cross-linking of the octamer by DMS and subsequent nucleosome assembly, followed by analysis of pulse-labeled RNA by denaturing PAGE.

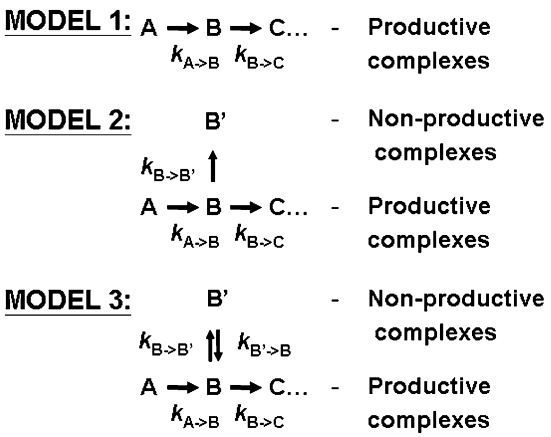

Fig. S9. Elongation models used to analyze the quantified data. Model 1 (minimal): At each position on the nucleosomal DNA, Pol II can progress only to the next position. Model 2: At each position on the nucleosomal DNA, Pol II can progress to the next position or irreversibly partition into nonproductive complexes. Model 3: At each position on the nucleosomal DNA, Pol II can progress to the next position or reversibly partition between productive and nonproductive complexes.

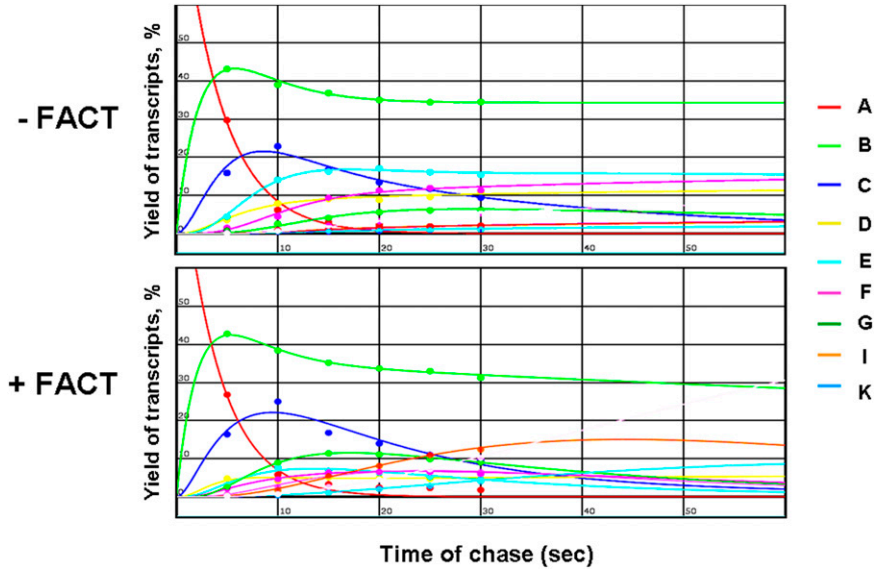

Fig. S10. Analysis of time courses of transcription through chromatin in the absence (*Upper*) or presence (*Lower*) of hFACT using KinTek software, fit with the model.

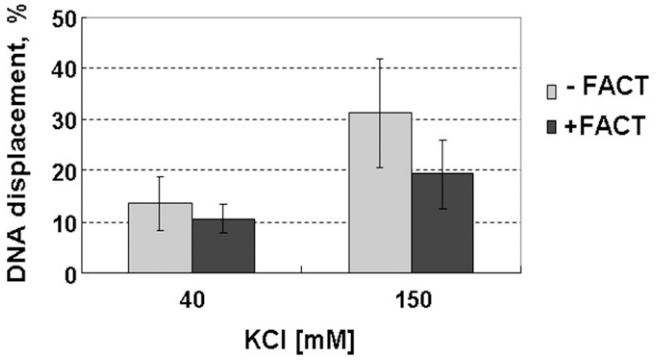

Fig. S11. Quantitation of DNA displaced from the 603 nucleosome after transcription by *E. coli* RNA polymerase at different concentrations of KCl in the absence or presence of hFACT.