RNA polymerase II is released from the DNA template during transcription-coupled repair in mammalian cells

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The genome is not repaired uniformly. DNA-binding proteins including histones inhibit repair, likely by blocking access of repair factors to the damage (4–6). Also, there is an enhanced rate of repair of the template strand of actively transcribed genes, called transcription-coupled repair (TCR) (7–10).

An emerging consensus is that the enhanced rate of TCR compared with global repair results from RNAPII revealing to repair factors transcription-blocking adducts that would otherwise be blocked by components of chromatin and actively participating in recruiting repair factors to damage sites. RNAPII is blocked by template but not coding strand lesions and remains stably bound at the damage site (11–13). Evidence for efficient excision where RNAPII is stalled has been reported (11, 13, 14). To access a transcription-blocking adduct, the transcription-repair factor TFIIF likely has a central role, because TFIIF interacts with RNAPII during transcription initiation and because during global repair, TFIIF is involved in forming the repair bubble, it interacts with repair factors, and following repair, excision products released from chromatin are found bound to TFIIF (15–17). In addition, Cockayne syndrome complementation group B (CSB) protein, which is required for TCR (18) and which induces the forward translocation of RNAPII (14, 19), likely positions stalled RNAPII so as to allow excision of the transcription-blocking adduct in the presence of RNAPII. The presence of the transcription bubble likely facilitates repair as well. Excision repair occurs more rapidly in vitro when the adduct is located in a region of nonhomology resembling a transcription or repair bubble (20).

In an alternative model of TCR, it was suggested that TFIIS acts as a transcription-repair coupling factor by causing RNAPII stalled at an adduct to “back up,” thereby revealing the adduct to the repair enzyme assembly. It was suggested that following repair, the backed-up RNAPII may resume elongation (12). The backing-up effect of TFIIS has been demonstrated; however, it is reversed by CSB in vitro (14, 19). Furthermore, TFIIS is not required for TCR in yeast (21) or human cells (22).

An interesting aspect of TCR is the fate of RNAPII stalled at a template strand lesion. We have addressed the fate of RNAPII in vivo using methods to control transcription, to locate elongating and stalled transcription complexes genome-wide (native elongation transcript sequencing (NET-seq)) (23), and to locate excision repair sites genome-wide (XR-seq) (8). Our
results suggest that RNAPII that has participated in transcription-coupled repair does not continue transcribing the template and does not engage in additional rounds of TCR. This is consistent with the dissociation of RNAPII following incision of a transcription-blocking lesion.

Results

Control of transcription with DRB

Our experiments utilized the transcription inhibitor 5,6-dichlorobenzimidazole 1-β-d-ribofuranoside (DRB) to control RNAPII elongation. Under physiological conditions, transcription proceeds through the formation of a paused, promoter-proximal RNAPII complex before entering into a uniform elongation mode. DRB prevents the conversion of this paused complex to a fully elongating complex by inhibiting the positive transcription elongation factor complex (24). The DRB effect is reversible, and to create a discrete “wave” of RNAPIs transcribing along the template, we used a pulse–chase–pulse scheme termed double DRB (DRB2) treatment illustrated in Fig. 1A, in which cells were first incubated in DRB for 2 h, to prevent new, actively elongating complexes and to permit RNAPII complexes already elongating during treatment with DRB to complete and terminate transcription. After 2 h, which is sufficient for the completion of even the longest transcripts, the DRB was washed off, and the cells were incubated without DRB for 10 min. Then DRB was added again. During the 10-min DRB-free period, RNAPII elongation begins from promoter proximal pause sites in a synchronized manner. Fig. 1B illustrates RNA levels produced in unirradiated XPC mutant cells at various times during the DRB2 procedure. RNA levels were measured by RT-qPCR using primers located at +2, +10, +50, and +100 kb in the OPA1 gene, which is 200 kb in length. The increase in progressively distal RNA levels with time illustrates a discrete wave of RNAPII transcribing along the gene at ~1 kb/min during the DRB2 treatment, in agreement with reported rates of RNAPII elongation (24).

Overall effects of controlled transcription on repair

To measure repair under various transcription conditions, we used the in vivo excision assay, described previously (25). In this assay, the 26–27–nt excision products are isolated from UV-irradiated cells, radiolabeled, and resolved on sequencing gels as shown in Fig. 2B. The irradiation produces two bulky adducts in DNA, cyclobutane pyrimidine dimers (CPDs), and pyrimidine-pyrimidone (6-4) photoproducts. Both are substrates for excision repair. However, because the (6-4) photoproducts are efficiently recognized by the core excision repair factors, their rates of repair are only modestly affected by transcription, and we analyzed only the repair of CPDs to gain insight into TCR (8). This was done by selectively immunoprecipitating the excision products with an anti–CPD-DNA antibody prior to radiolabeling. Excision products generated within the cell are vulnerable to degradation in vivo, and the repair signal at any time point reflects the combination of formation and degradation of the excision product. XPC mutant cells were used in all of our experiments; these cells are incapable of “global” excision repair, and thus the repair signal from these cells arises solely from TCR (26, 27).

Repair was measured following the three treatment regimens shown in Fig. 2A. In the DMSO control, the cells were irradiated and then incubated in culture medium. In the DRB regimen, the cells were irradiated and then cultured in DRB. In the DRB2 regimen, the cells were incubated in a DRB-containing medium for 2 h, after which the medium was replaced with DRB-free medium for 10 min to enable elongation to begin from the 5’ end of genes. After 10 min, the cells were irradiated with UV, and DRB was added to the medium. The raw repair data in Fig. 2B and the quantitative values in Fig. 2C show that compared with the DMSO control, the DRB and DRB2 treatments changed the excision kinetics. These observed changes in transcription-coupled repair levels are consistent with decreased repair associated with decreased transcription caused by DRB. In the case of single DRB after UV treatment, the amount of RNAPII elongating at the time of irradiation is sufficient to initiate nearly normal levels of TCR at early time points, but repair at 60 min is lower than control because of a lack of newly initiated transcription. During the DRB2 regimen,
the low repair at all time points reflects the single wave of transcription. Importantly, the relatively high repair in control cells at 60 min presumably results from continued formation of newly elongating transcripts at the 5’ end of genes.

**Global analysis of individual elongation complexes and repair events**

XR-seq experiments were performed with DRB2-treated cells to locate specific sites of repair throughout the genome. In XR-seq, excision products with CPD adducts are isolated, sequenced, and mapped to the genome (8). In addition, NET-seq (23) was performed to identify where elongating RNAP is located during the experiments. In NET-seq, nascent RNA that is bound to RNAPII and thus protected from RNase digestion is isolated, sequenced, and mapped to the genome. Fig. 3 shows a scatter plot in which each gene is represented by a dot, plotted along the x axis based upon its relative transcription level (NET-seq value) and along the y axis based upon its repair level (XR-seq value). The extent of repair and transcription are expressed as excision products or nascent RNA sequencing reads per kilobase per million total reads (RPKM). The results are shown for both coding (Fig. 3, NTS, left panels) and template (Fig. 3, TS, right panels) strands. Overall, the positive association between transcription and repair of the TS exhibited in Fig. 3 by these DRB2-treated cells indicates that the greater the likelihood of a gene being transcribed, the greater the likelihood that it will undergo transcription-coupled repair.

The left panels of Fig. 3 illustrate TCR of the NTS of genes. Compared with the signals from the TS (right panels), the dots are more concentrated in the lower left quadrants of the NTS panels, which indicates reduced transcription and repair levels. Earlier studies demonstrated the existence of transcription-coupled repair of the coding strand (NTS) of genes in the vicinity of the promoter, and it was attributed to antisense transcription from bidirectional promoters that occurs in this region (8–10). Our results demonstrate that this antisense transcription and transcription-repair coupling are produced by the
DRB2 procedure and indicate that levels of antisense transcription and TCR are uniformly low among genes.

To further assess the distribution of repair along the template, the scatter plots in Fig. 4 were generated to illustrate the relative number of reads per gene (y axis) as a function of gene length (x axis). The top two rows illustrate repair of the template strand (TS), and repair of the coding strand is shown in the bottom two rows (NTS). Plots of template strand repair clearly illustrate two major populations (high RPKM and low RPKM) in all conditions, representing genes with high and low transcription-coupled repair levels. As in Fig. 3, predominantly low TCR is seen from the NTS, which is due to bidirectional transcription from the promoter/enhancer regions.

Interestingly, the distributions of high RPKM template strand repair groups as a function of gene length (Fig. 4, top two rows) were different between control, DRB, and DRB2 conditions. Linear regression lines drawn on the plot and slope values illustrate trends in TCR versus gene length. In the control, at 30 min (top left panel) there were fewer repair reads per kb in long genes compared with short genes, and this negative correlation was stronger at 60 min (Fig. 4, second row, left panel). Relevant to this observation, earlier studies (8) (also see below) showed an elevated level of transcription-repair coupling at the 5’ end of genes compared with repair within gene bodies. In the control (DMSO) cells in Fig. 4, it is likely that this higher level of repair at the 5’ end more strongly influences the overall repair level of short genes compared with long genes and is responsible for the negative correlation. Consistent with this interpretation, the negative correlation between gene size and number of reads is absent in the single DRB-treated cells (middle panels), in which there is no new transcription after UV and thus reduced repair at the 5’ end of genes. In the DRB2 condition (Fig. 4, right column), there was no obvious difference between 30 and 60 min, but there was a negative correlation between RPKM and gene length. This is because, during the 10-min DRB-free incubation before UV, the likelihood that a discrete wave of elongating RNAPII complexes is generated depends upon promoter activity (Fig. 3), not gene length. When the level of transcription-coupled repair is then expressed as a function of gene length, longer genes exhibit less repair per kb than short genes (Fig. 4, right column).

Analysis of a discrete wave of repair

To address whether a single elongating RNAPII could induce multiple transcription coupled events on one template, we selected for further analysis 1646 genes based upon length over...
100 kb and exhibiting higher repair levels (RPKM over 0.1). These criteria increase the likelihood that multiple repair events per gene would be detected if they occurred. If one RNA-P II could induce multiple repair events (transcription resumes following TCR), we expect to see the repair signal shift to the 3’ end of the template with time following DRB treatment. We plotted the repair signals from the first 100 kb of the template strand of these genes in Fig. 5A after normalizing to the sequencing depth and the relative repair levels we observed from the excision assay (Fig. 2). Repair of the non-transcribed coding strands (NTS) are plotted for reference.

In control DMSO-treated cells (Fig. 5A, left column), most of the signal is distributed evenly on the gene body, and there is an elevated signal close to the transcription start sites (TSSs) at 30 min after UV. At 60 min, the signal from the gene body overall decreased, and the signal near the TSS increased. The increased repair near the TSS is consistent with earlier studies (8) as mentioned above and consistent with repair associated with transcription initiated after UV. The decreased repair in the gene bodies at 60 min is consistent with a single round of TCR, initiated by RNA-P II complexes transcribing at the time of irradiation, and largely complete by ~30 min. These results are also consistent with the results in Fig. 4. The stronger negative correlation between repair and gene length at 60 min compared with 30 min in Fig. 4 reflects the reduced repair in gene bodies, which, for longer genes, had a more profound effect than the increased repair near the TSS.

When DRB was added after UV (Fig. 5A, middle column), repair signals again were relatively evenly distributed on the gene bodies but were in this circumstance reduced near the TSS. The reduced repair levels near the TSS support the conclusion that in control cells (left column), the elevated repair near the TSS is due to transcription elongation that began following UV. As in control cells, repair in the gene bodies was lower at 60 min compared with 30 min, again suggesting that each transcription-coupled repair event is not followed by elongation and additional TCR.

In the DRB2 regimen (Fig. 5A, right column), the cells exhibited very little repair in the gene bodies, as expected from the depletion of elongating RNA-P II complexes during the 2-h pretreatment with DRB. The cells incubated for 30 min following UV exhibited repair as a peak within 10 kb of the TSS, resulting from the wave of elongating RNA-P II that was produced during the 10 min of DRB-free incubation before UV. Most importantly, the peak of repair near the TSS decreased at 60 min but did not shift dramatically in the 3’ direction. Some repair was
seen up to approximately +25 kb; however, this distance is rather small considering the elongation rate of RNAPII (24, 28), and this modest extension of repair in the 3′ direction may reflect the elongation of multiple RNAPII complexes during the 10-min DRB-free incubation, with the first polymerase repairing the first lesion encountered and dissociating and then the second polymerase repairing the next lesion downstream following repair of the first. In addition, the lesion distribution is expected to affect the observed repair pattern. In these experiments, the 20 J/m² dose is expected to produce ~1 lesion/strand per 10 kb, and ~90% of the photoproducts are CPDs.

To examine whether multiple RNAPII complexes begin elongation during the 10-min DRB-free incubation, we compared the XR-seq patterns following 5- and 10-min DRB-free incubations, under the DRB2 condition and with 60-min repair. The results in Fig. 5B show that the shorter 5-min RNAPII release condition produced a slightly narrower repair peak. The broader repair peak following the 10-min release is exclusively due to more repair in the 3′ direction and is consistent with release of multiple RNAPII complexes during the DRB-free incubation time only in a fraction of the templates. Thus the results in Fig. 5A (right column), in which the peak of repair decreases from 30 to 60 min, indicate that following transcription-coupled repair, the RNAPII that targeted the transcription-blocking adduct for repair does not proceed to initiate repair at the next lesion downstream in the template strand.

Analysis of a discrete wave of transcription

To further characterize the disposition of RNAPII in transcription-repair coupling, we used NET-seq to map the loca-
Fate of RNAPII in transcription-coupled repair

Figure 6. Distribution of elongating RNAPII. This figure shows the NET-seq data for the same genes as in Fig. 5. RNAP-associated RNA was isolated from XPC mutant cells treated with the DRB2 regimen either in the absence of UV (Ctl) or with UV (UV) treatment at the end of the DRB-free incubation. Mammalian NET-seq signals of the first 1 kb to 100 kb from the TSS were analyzed (bin size, 250 nt) as nascent transcript reads per kb per million total reads (RPM). Relative transcription was plotted in an aggregate view (top panel) or as gene-by-gene heat maps (middle panels). In the aggregate view, the dots illustrate the data for each bin, and lines show the smoothed data trend. The aggregate view also shows elongation sites in both the TS and the NTS (coding strand). The difference between control and + UV is shown as the UV/control (Ctl) ratio at 30- or 60-min repair time in the two panels on the right. The difference between the 30- and 60-min repair times is shown as the 60/30-min ratios for + UV and − UV treatments in the bottom two panels. To calculate the ratio between any two different conditions, signals of individual genes were smoothed (80 bins).

The NET-seq results in Fig. 6 (top and middle panels, left column) show that in the minus UV control (Ctl) at 30 min there was a discrete concentration of RNAPII located within 25 kb of the TSS, and the distribution of RNAPII was similar among genes. After 60 min, RNAPII elongated, as indicated by the loss of the concentrated elongation complexes near the TSS, and the distance traversed in the 3’ direction was genespecific. Following UV and 30-min repair (Fig. 6, top and mid-
transcription and TCR was completed and the repair products were degraded. The repair signal peak near the TSS, and this distribution is similar to the distribution of repair under the same condition (Fig. 5A, right column). Importantly, following 60 min of repair (Fig. 6, top and middle panels, right column), the density of RNAPII near the TSS decreased but was not associated with an increase of RNAPII downstream (Fig. 6, compare bottom left and right panels). These data are consistent with dissociation of RNAPII from the template after it participates in a single round of TCR.

**Transcription and TCR in the OPA1 gene**

The effects of UV damage on transcription elongation and repair kinetics are visually illustrated in Fig. 7 with screenshots showing locations of both RNAPII (NET-seq, blue) and excision repair (XR-seq, red) within the OPA1 gene (108 kb) under the DRB2 condition. Quantitative values for transcription shown on the plot were obtained by calculating total NET-seq reads from 1 to 20 kb and from 40 to 60 kb (red horizontal bars at top). In the absence of UV, the nascent transcripts, representing elongation complexes, are concentrated near the TSS 30 min after release from the promoter proximal pause site. By 60 min, they are seen to have elongated further into the gene body. Following UV, elongation complexes are more highly concentrated near the TSS at 30 min, and at 60 min, there is a striking loss of elongation complexes and failure to elongate into the gene body. Repair events are also concentrated near the TSS at 30 min. After 60 min, there is a striking reduction in repair near the TSS, and there is only a modest increase in repair in the 3′ direction because of limited release of more than one elongating RNAPII during the 10-min DRB-free incubation period. Thus the sites of repair coincide with the sites of elongation complexes. The distribution of elongation complexes and repair events as a function of time indicates that individual RNAPII complexes are unable to continuously progress downstream and engage in multiple rounds of TCR and are apparently dissociated from the template in association with TCR.

**Discussion**

Our findings support the concept that RNAPII dissociates from the template during transcription-coupled repair. Our methods appear reliable because they showed that DRB did reduce transcription in a controllable manner, and reduction of transcription with DRB produced an expected overall reduction in TCR as measured in XP-C mutant cells. Our data show blockage of elongation where elongating RNAPII is expected to encounter the first CPD in the template. We detect, under a controlled, discrete wave of transcription, essentially no additional repair or transcription further downstream following repair of the first adduct encountered. Our findings extend those of Andrade-Lima et al. (29) obtained by different methods that focused on recovery of transcription following UV.

In light of our findings combined with numerous reports on the fates of RNAPII and the transcript upon encountering a photoprotected basepair, we propose the model shown in Fig. 8. In this illustration, the three template strand adducts that undergo TCR require three different RNAPIIs that each initiate transcription at the upstream promoter. The polymerases each
undergo transcription blockage, TCR, and RNAPII dissociation, with the successive removal of lesions from the template in the 5’ to 3’ direction and eventual clearance of adducts from the template enabling synthesis of full-length transcripts.

The point at which RNAP is dissociated during TCR remains unclear. Regarding the possibility of dissociation before repair, it is difficult to envisage how RNAP II could target a transcription-blocking adduct for repair after it dissociates. Dissociation of RNAPII independently of repair assisted by RNAP-interacting factors has been observed in vitro with cell extracts (11, 13, 16, 30) but may be artifactual for various reasons, such as loss of compartmentalization in preparation of cell extracts. In our experiments, TCR-independent RNAPII dissociation may have occurred in the irradiated cells but was undetected by our methods. However, this cannot be a major pathway of RNAPII transcriptional termination because in that case there would not have been TCR targeted to the template strand.

In light of these findings and considerations, we propose that RNAPII dissociation occurs in some way in conjunction with repair. One mechanism for RNAP dissociation in conjunction with repair has been described in *Escherichia coli*. Mfd, the transcription-repair coupling factor in *E. coli* (31, 32), binds simultaneously to the upstream face of stalled RNAP and to the upstream template. The translocation activity of Mfd is activated by this interaction, and the translocation action of Mfd dissolves RNAP from the template (31–33). The dissociated RNAP remains tethered to the template via the template-bound Mfd in a semi-stable complex that has a half-life of ~10 min in isolation. Mfd in this conformation reveals its UvrA-binding site and exhibits a high affinity for UvrA, the damage recognition subunit of the *E. coli* excision repair enzyme. Binding of UvrA induces dissociation of Mfd and RNAP from the DNA and delivery of the repair enzyme to the damage, with formation of a damaged DNA–UvrA–UvrB preincision complex, which makes dual incisions in the presence of UvrC (31, 32).

The mammalian CSB protein has been considered analogous to Mfd because CSB is required for transcription-coupled repair (18, 34) and because, like Mfd, CSB is a large protein with a centrally located helicase motifs region (35, 36). However, unlike Mfd, CSB alone does not remove RNAP stalled on a DNA template, although similar to Mfd, CSB induces forward translocation of RNAP (14, 16, 19). It is conceivable that, by analogy with Mfd, removal of RNAPII by CSB would occur in the presence of repair factors; however, this was not observed (14).

Other relevant differences exist between excision repair in humans and *E. coli*. During repair in *E. coli*, there is no melting of the adducted DNA substrate duplex, no repair bubble is formed, and after dual incisions are made, the excision product remains annealed to the chromosome together with the UvrB-UvrC repair factors. These are dissociated by the action of DNA helicase II (UvrD gene product) and DNA polymerase I (37, 38). Furthermore, in *E. coli*, in the absence of Mfd, RNA polymerase stalled at an adduct inhibits repair of the adduct (39). In contrast, in humans, the dual incisions are made in a repair bubble (1–3) or, in the case of coupled repair, in a transcription-repair bubble, apparently in the presence of the stalled RNAPII (11, 13, 14). Thus, whereas both prokaryotes and eukaryotes perform dual incision-based excision repair and both perform global and transcription-coupled repair, the corresponding repair proteins in prokaryotes and eukaryotes are not homologous, their reaction mechanisms are different, and the mechanism for dissociation of mammalian RNAPII during TCR must be different from the corresponding mechanism in *E. coli*.

The scenario most consistent with the presently available information is that in mammalian cells, dissociation of adduct-blocked RNAPII occurs following dual incision. Eukaryotic dual incision sites are the same in global and transcription-coupled repair (25), and in both repair pathways, the excision product is released and degraded, although before degradation, the released product can be temporarily isolated either complexed with TFIIH or in partially degraded form complexed with RPA (25). Removal of the damaged section of template within the transcription/repair bubble may precede or occur together with dissociation of RNAPII. Dissociation of RNAPII from the template may be facilitated by the forward translocation action of CSB (19) and/or by repair synthesis. RNAPII in elongation mode is known to remain bound to the template in active form even under harsh conditions, and CSB translocase or proteins associated with repair synthesis may provide the force needed to dissociate RNAPII from the template following dual incision. The results presented in this paper are not consistent with RNAPII resuming transcription either before repair synthesis, by “jumping the gap” in the template, or after repair synthesis, by remaining in a paused, transcription-competent, template-bound state during repair synthesis.

**Experimental procedures**

**Cells culture and DRB treatment**

XP-C (GM15983) human skin fibroblast cells were purchased from Coriell Institute. All of the cells were grown in DMEM-high glucose (ThermoFisher) supplemented with 10% (v/v) fetal bovine serum (Sigma) and maintained at 37 °C in 5% CO₂. DRB or DMSO was added to the medium when cells were ~80% confluent. The final concentrations of DRB and DMSO were 100 µM and 0.1% (v/v). For post-UV DRB treatment, medium was collected, and DRB was added to the medium just before irradiation. The cells were irradiated with UVC (20 J/m²). Then the DRB containing medium was added back, and the cells were incubated for 30 or 60 min at 37 °C in 5% CO₂. For DRB2 regimen, the cells were first incubated with DRB at 37 °C in 5% CO₂ for 2 h. After collecting the medium with DRB, cells were washed two times with PBS and then incubated in fresh medium for 10 min. Then medium was removed, and the cells were irradiated with UVC. After irradiation, medium with DRB was added back, and the cells were incubated for 30 or 60 min at 37 °C in 5% CO₂.

**Real-time quantitative PCR analysis of OPA1 transcription**

Total RNA of XP-C cells was purified using TRIzol reagent (Ambion). First-strand synthesis was performed using an iScript cDNA synthesis kit (Bio-Rad). Real-time PCR was performed using iTaq™ Universal SYBR®Green Supermix (Bio-Rad). Primers to detect different regions of the OPA1 gene and 18S rRNA were based on the published sequences (24, 40).
Excision assay and excision repair sequencing (XR-seq) library preparation

The cells were irradiated with 20 J/m² UVC and were incubated at 37 °C in 5% CO₂ for 30 or 60 min. Excision assays were performed according to the published procedures (25). In brief, excision products were first extracted by the Hirt procedure. After phenol-chloroform extraction and ethanol precipitation, CPD-containing excision products were purified by immunoprecipitation. Purified excision products were radiolabeled with ³²P-cordycepin and resolved on 10% urea-polyacrylamide sequencing gels. The signals were detected by phosphorimaging and quantified by normalizing to the internal control 50-mer “spike” DNA added to each reaction before the labeling reaction. Signals for treated cells were normalized to the signals from control cells (DMSO) and repaired for 60 min in each experiment.

Library generation for XR-seq was based on the published procedure (8). The cells were lysed in hypotonic buffer with Nonidet P-40. Then excision products were purified using antibodies against TFIH components (anti-p89, SC-293 and anti-p62, SC-292 from Santa Cruz). After adaptor ligation, CPD excision products were purified again by immunoprecipitation using an anti-CPD-DNA antibody (Cosmo Bio USA). After repair by CPD photolyase, the fragments were amplified by PCR, and the libraries were sequenced by Hiseq 2500 (1 × 50).

XR-seq experiments were performed two times, and the data presented in text figures are from averaging of the two experiments. To illustrate the concordance of the two experiments, Fig. S1 shows individually the results of the two experiments that were averaged to obtain the values plotted in the critical XR-seq experiment in Fig. 5.

Mammalian NET-seq library preparation

Library generation for NET-seq followed the published procedure with some modification (23). The cells were lysed, and nuclei were purified. The nuclei were resuspended in NUN1 buffer (20 mM Tris-Cl, pH 7.9, 75 mM NaCl, 0.5 mM EDTA, 50% (v/v) glycerol) and then were lysed with NUN2 buffer (20 mM HEPES-KOH, pH 7.6, 300 mM NaCl, 0.2 mM EDTA, 7.5 mM MgCl₂, 1% (v/v) Nonidet P-40, and 1 mM urea). After centrifugation, the chromatin fraction containing elongating RNAPII was collected. RNAPII–RNA–DNA complexes were released by Micrococcal nuclease digestion and were enriched by the immunoprecipitation with the antibody recognizing the C-terminal domain of RNAPII (MAB10601 from Medical & Biological Laboratories). Phosphorylation at the 5’ end of fragments was performed by T4-PNK. Fragments were used for the generation of libraries using a kit for small RNA library generation (NEBNext Small RNA Prep Set for Illumina), and the libraries were sequenced by Hiseq 2500 (2 × 50).

NET-seq experiments were done twice, and the data presented in text figures represent the average of the two experiments. To illustrate the concordance of the two experiments, Fig. S2 shows individually the results of the two experiments that were averaged to obtain the values plotted in the critical NET-seq experiment in Fig. 6.

Analysis of data from high-throughput sequencing

For XR-seq, adaptor sequences were first removed using BBduk tool of BBMap with the options: ktrim = r k = 21 hdist = 2 minlen = 1. Duplicated sequences were then removed using Collapser tool of FASTX. After removing 50-mers (contaminant), unique reads were mapped to the human genome (hg19) using bowtie2 with the option “very sensitive.” Gene structure annotations were downloaded from University of California, Santa Cruz Table Browser. Annotations were modified for genes with multiple isoforms or overlapping regions to generate longest non-overlapped genes annotation (25,839 genes). Aligned reads were strand-specifically assigned to the genes using Intersect tool of Bedtools with the option “c.” For intragene distribution, genes with RPKM ≥ 0.1 in all conditions and the length ≥100 kb were selected (1646 genes). Genes were separated into the 250 bases/bin, and the aligned reads were assigned to each bin using Intersect tool of Bedtools with the options “c” and “F 0.5.” The number of reads was first normalized to the number of total aligned reads and then was normalized to the relative CPD repair levels from Fig. 2 (DMSO 30 min: 1.07, DMSO 60 min: 1, DRB 30 min: 1.06, DRB 60 min: 0.56, DRB2 30 min: 0.52, DRB2 60 min: 0.31).

For NET-seq, adaptor sequences were first removed using BBduk tool of BBMap with the options: ktrim = r k = 21 hdist = 2 minlen = 1 tpe tbo. Reads were mapped to the human genome (hg19) using BBMap with the options: maxindel = 100,000 intronlen = 10 ambig = random qin = 33. After mapping, reads with proper pair were selected, and the locations of RNAPII were defined as the position of first base from the 3’ end of the isolated RNA. Positions of RNAPII were assigned to each bin of the same gene sets used in XR-seq in the same manner that XR-seq sites were assigned. The number of reads was normalized to total reads and was aligned to the 1–100-kb region of the gene sets. For comparing the ratio between different conditions, data from each gene was smoothed by loess methods to decrease variations caused by bins without any reads.

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