Conditional Expression of RNA Polymerase II in Mammalian Cells

DELETION OF THE CARBOXYL-TERMINAL DOMAIN OF THE LARGE SUBUNIT AFFECTS EARLY STEPS IN TRANSCRIPTION

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Mark Meininghaus, Rob D. Chapman, Manuela Horndasch, and Dirk Eick‡

From the Institute for Clinical Molecular Biology and Tumor Genetics, GSF-Research Center for Environment and Health, Marchioninistrasse 25, D-81377 Munich, Germany

The carboxyl-terminal domain (CTD) of the large subunit of mammalian RNA polymerase II contains 52 repeats of a heptapeptide that is the target of a variety of kinases. The hyperphosphorylated CTD recruits important factors for mRNA capping, splicing, and 3′-processing. The role of the CTD for the transcription process in vivo, however, is not yet clear. We have conditionally expressed α-amanitin-resistant large subunit with an almost entirely deleted CTD (LSΔ5) in B-cells. These cells have a defect in global transcription of cellular genes in the presence of α-amanitin. Moreover, pol II harboring LSΔ5 failed to transcribe up to the promoter-proximal pause sites in the hsp70A and c-fos gene promoters. The results indicate that the CTD is already required for phosphorylation at steps that occur before promoter-proximal pausing and maturation of mRNA.

Eukaryotic mRNA synthesis is catalyzed by the multisubunit RNA polymerase II (pol II). The large subunit of pol II (LS) is highly conserved among eukaryotic RNA polymerases and also shows striking homology to the large subunit of Escherichia coli RNA polymerase (1). The LS has evolved a particularly structured carboxyl-terminal domain (CTD) that is not present in other RNA polymerases (2). This CTD comprises multiple copies of a heptapeptide repeat with the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. The number of repeats varies from 26/27 in yeast to 52 in mouse and human cells (3). Deletion of more than half of the repeats in yeast and mouse interferes with cell viability (4–6). Mice homozygous for a deletion of 13 repeats are smaller than wild-type littermates (7), suggesting that the CTD is important in regulating growth during mammalian development. In cells, two forms of pol II are detectable containing either a hypophosphorylated (pol IIA) or hyperphosphorylated CTD (pol IIα). Although pol IIA is consistently found in the initiation complex, pol IIα is associated with elongating complexes.

An increasing number of genes have been shown to be regulated by promoter-proximal pausing of pol II. These genes include Drosophila hsp70 and hsp26 genes, as well as the mammalian c-myc, c-fos, and immunoglobulin κ genes (8–15). The passage of the paused pol II into a processive mode coincides in vivo with hyperphosphorylation of the CTD (11, 16).

Recent studies suggest that the hyperphosphorylated CTD functions as a platform for the assembly of complexes that cap, splice, cleave, and polyadenylate pre-mRNA (2, 17, 18). Capping of mRNA occurs shortly after transcription initiation (19), preceding other mRNA processing events such as mRNA splicing and polyadenylation. The capping enzyme is not stably associated with basal transcription factors or the RNA pol II holoenzyme but is directly and specifically recruited to the hyperphosphorylated form of CTD (20–22, 24). Similarly, several components of the splicing machinery (25, 26) and related factors such as SR proteins and SR-like proteins (27–29) are recruited to pol II by the hyperphosphorylated CTD. The cleavage-polyadenylation factors CPSF and Cst2′ specifically bind to the hyperphosphorylated CTD and copurify with pol II in a high molecular mass complex (21), suggesting that polyadenylation factors are recruited to an RNA 3′-processing signal by pol II, where they dissociate from the polymerase and initiate polyadenylation. In an extension of this model, pol II is required for 3′-processing in vitro in the absence of transcription (30, 31).

In addition to pre-mRNA maturation, hyperphosphorylation of CTD appears to play an important role in rendering pol II processive. A positive and a negative elongation factor, implicated in 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) inhibition of transcription elongation, have been identified. DSIF (DRB sensitivity-inducing factor) is a negative elongation factor that renders elongation sensitive to DRB (32–34). It consists of p14 and p160 subunits, which have homology to the yeast Sp44-Spt5 complex (35). p160 shows homology to the bacterial elongation factor NusG (32, 33) and interacts with another negative transcription factor, NELF (36). The state of CTD phosphorylation determines the negative action of DSIF on RNA elongation and provides a direct link between DSIF and the positive elongation factor P-TEFb, a CTD-specific kinase (cyclin T/Tdk9), which is inhibited by DRB (37, 38). Other factors, e.g. the Elongator complex, may also bind to pol II in a CTD-dependent manner (39). Taken together, a huge body of evidence suggests an important role for CTD in activation of RNA elongation and maturation of pre-mRNA in vivo.

However, it is not yet clear whether the CTD of pol II is always required for transcriptional initiation in vivo. Several studies showed that pol II with a deleted CTD is transcriptionally active in vitro (Serizawa et al. (40)) and initiates and transcribes transiently transfected genes (21, 41) as well as the CUP1 gene in yeast (42). Here, we show evidence that pol II with a deleted CTD is defective in global gene transcription and
is unable to transcribe up to promoter-proximal pause sites on chromosomal templates.

MATERIALS AND METHODS

DNA Constructs—A multiple cloning site with the restriction sites BglII/NotI/SfiI/GoMI (5’-GATCCGCGGGCCAGAGCGGCGG-3’) was inserted into the BglII site, and a neomycin resistance gene (Stratagene) was inserted between the NotI site of SfiI mock (MM172–2/1). Subsequently, the 3’-part of the recombinant large subunit of pol II with wild-type CTD (HAwt), a CTD deletion mutant with 31 repeats (HA331), or with 5 repeats (HA5) were inserted as an SfiI fragment into the SfiI site of MM172–2/1. Vectors were designated LS*I wt, LS*I Δ31, and LS*I Δ5, respectively. HAwt, HA331, and HA5Δ5 were all kindly provided by W. Schaffner (41).

Cell Lines and Culture—All cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (Life Technologies, Inc.), which is the following is referred to as growth medium. Stable cell lines lacking the presence of 1 μg/ml G418 or 0.1 μg/ml tetracycline. Raji is a Burkitt’s lymphoma B-cell line carrying a t(8,14) chromosomal template. Isolation of nuclei and nuclear run-on experiments the cells were treated for 25 min with TPA. The 3’-untranslated region of hsp70A and human c-myc (48) were amplified by polymerase chain reaction. DNA fragments were amplified with primers carrying the T7 RNA polymerase promoter for in vitro transcription. The primers for the polymerase family were designed as recommended by the manufacturer in the presence of α-32P-CTP. Full-length transcripts were isolated by polyacrylamide gel electrophoresis and used for hybridization to DNA oligonucleotides as described above.

Southern Blotting—DNA purification, digestion with suitable restriction enzymes, and Southern blotting were performed by standard methods (46). The following probe was used in Fig. 1B, a 1.2-kb BamHI–HindIII fragment of PMc1ino Poly(A) (Stratagene). The description of the plasmids used in Fig. 4 for nuclear run-on analysis is as follows: S471–1, a PUC12 vector containing a 1.5-kb SacI fragment of c-myc, was digested with SacI and gave rise to fragments 1 (PUC12) and 4 (α-myc) (49). BT34–8, a SP65 vector containing a 1.2-kb EcoRI fragment (μα) of the Ig μ-heavy chain, was digested with EcoRI and gave rise to fragments 2 (SP65) and 5 (μα) (49). BT20–21, a SP65 vector containing a 0.9-kb EcoRI/SacI fragment (μα) of the Ig μ-heavy chain, was digested with EcoRI/SacI and gave rise to fragments 3 (SP65) and 6 (μα) (49).

RESULTS

Conditional Expression of the Large Subunit of pol II—The gene for the α-amanitin-resistant, large subunit of pol II (LS*Iwt) and CTD deletion mutants thereof (Δ31 consisting of only 31 repeats; Δ5 of 5 repeats) were cloned under the control of a tetraacycline (Tc)-regulated promoter into an episomal, Epstein-Barr virus-derived vector (LS*I mock) (Fig. 1A). Plasmids were stably transfected into the Burkitt lymphoma Raji cells, and subsequently digested with the Ig μ-heavy chain, was digested with EcoRI/SacI and gave rise to fragments 3 (SP65) and 6 (μα) (49).

Te-regulated expression of the LS was checked for each cell line in the presence and absence of α-amanitin. Expression of
the recombinant LS was observed as early as 6 h after removal of Tc (Fig. 2, B–D) and was maximal after 24 h. At this time, α-amanitin was added. The complete inhibition of transcriptional activity of the endogenous pol II was achieved after an additional 24 h (51, data not shown) and was accompanied by a substantial degradation of the endogenous LS (Fig. 2A). LS*wt and LS*31 were well expressed at day 2 and day 3 in the presence of α-amanitin (Fig. 2, B and C). In contrast, expression of LS*Δ5 was high at day 2 but declined at day 3 in the presence of α-amanitin (Fig. 2D), suggesting that LS*Δ5 cannot support its own expression. Sequence analysis revealed no additional mutations in LS*Δ5 except the deletion of CTD (data not shown).

The CTD-less subunit has previously been reported to display transcriptional activity on transiently transfected reporter gene constructs (21, 41). Its transcriptional activity in RajiLS*5 cells was tested after transient transfection of plasmid DNA carrying a c-myc promoter cloned upstream of the luciferase gene. Cells expressing LS*Δ5 (–Tc) showed an ~20-fold higher luciferase activity as cells non-expressing LS*Δ5 (±Tc) (Fig. 2E). In a similar experiment, a CMV promoter-driven luciferase construct showed a 46-fold higher luciferase activity in cells expressing LS*Δ5 (data not shown). The transfection data indicate that the CTD-less large subunit is assembled into a transcriptionally active complex in RajiLS*Δ5 cells.

Viability of Cells Expressing CTD Deletion Mutants—Truncation of the CTD has been reported to affect viability of cells to various extents (4). Raji cells expressing LS*wt with a full size CTD proliferated in the presence of α-amanitin. At the beginning, the cells run through a crises with reduced viability when they were grown for the first time in the presence of α-amanitin (Fig. 3A). After a period of 3 weeks, however, cells proliferated at a similar rate as RajiLS*mock cells in the absence of α-amanitin (data not shown). Repression of LS*wt after 45 days by addition of Tc resulted in growth inhibition of cells, indicating that LS*wt was still required for growth (Fig. 3A).

Proliferation and viability of RajiLS*31 cells was significantly reduced by α-amanitin. The viability of cells steadily decreased in the presence of α-amanitin (Fig. 3A), whereas the total number of living cells remained relatively constant at the beginning (Fig. 3B). Thus, the cells could still proliferate for a limited time in the presence of α-amanitin. RajiLS*mock and RajiLS*Δ5 cells died between day 4 and day 8 after addition of α-amanitin. In conclusion, expression of LS*wt from a heterologous promoter could fully restore viability and proliferation in the presence of α-amanitin, LS*Δ5 failed, and LS*31 had an intermediate phenotype. Expression of the VP16 fusion protein (TcTA) had no apparent effect on proliferation of RajiLS*mock cells (data not shown).

Dominant-negative Phenotype of LS*Δ5—We next tested whether expression of LS*31 and LS*Δ5 had a dominant-negative effect on transcription of the endogenous pol II. For this purpose, nuclear run-on experiments were performed with nuclei of cells expressing the endogenous LS together with LS*wt, LS*31, or LS*Δ5 (Fig. 4). The transcription rate for two genes was determined. One DNA fragment covering c-myc exon 2 (Fig. 4A, fragment 4) and two fragments corresponding to the Ig heavy chain gene locus (fragments 5 and 6) were separated by agarose gel electrophoresis and blotted onto a nylon membrane. Fragments 1–3 correspond to vector sequences of pBR322 derivatives. Run-on RNA of untransfected Raji cells does not give rise to vector-specific hybridization signals (Ref. 49 and data not shown), whereas run-on RNA derived from RajiLS*mock cells produced clear signals (Fig. 4C). These signals are derived from RNA transcribed from sequences of the episomal vector backbone in transfected cells. The vector-specific signals further increased in RajiLS*wt cells (Fig. 4D) consistent with the observation that LS*wt was strongly expressed at day 1 after induction (Fig. 2B). In contrast, expression of LS*Δ5 almost completely repressed the transcriptional run-on activity for episomal sequences in Raji cells (Fig. 4F). Expression of LS*31 showed an intermediate effect (Fig. 4E). Expression of LS*wt, LS*Δ31, and LS*Δ5 did not affect the transcription rates for the c-myc and Ig μ genes (Fig. 4, D–F). Taken together, LS*Δ5 showed a strong dominant-negative effect on transcription of vector sequences, whereas it showed no effect on transcription of two chromosomal genes.

Treatment of Raji Cells with α-Amanitin Does Not Affect Steady-state mRNA Levels in Raji Cells—Deletion of CTD has been shown to affect mRNA 5' capping and splicing and 3' processing of the primary transcript but appears to have little effect on the production of pre-mRNA (21, 22). This is consistent with the observation that transcription by pol II in in vitro...
Deletion of CTD Affects Global Gene Transcription—Deletion of the CTD has no general effect on the transcriptional activity of chromosomal genes more directly, we performed run-on experiments with nuclei of RajiLS*mock, RajiLS*wt, and RajiLS*Δ5 cells in the presence and absence of α-amanitin. Run-on RNAs were hybridized to 1176 gene and 15 control probes spotted on a nylon filter (Atlas 1.2 DNA array, CLONTECH) (Fig. 6, A–D). With this technique 48% of the gene probes generated a positive and reproducible transcription signal for RajiLS*mock cells in the absence of α-amanitin (Fig. 6A, data not shown). Almost all signals disappeared, if the run-on reaction was performed in the presence of α-amanitin (Fig. 6B, data not shown). RajiLS*wt cells gave a very similar pattern of transcriptional active genes in the presence of α-amanitin as RajiLS*mock cells in the absence of α-amanitin (Fig. 5C, data not shown). It should be noted that cells expressing RajiLS*wt produced reproducibly higher transcription signals for a small number of gene probes (data not shown). This suggests that this polymerase may have lost a negative control. RajiLS*Δ5 cells almost entirely failed to produce specific transcription signals in the presence of α-amanitin. The few observed signals

Transcription assays and in transient transfection experiments is not strictly dependent on the presence of CTD (21, 40–41). We used DNA microarrays to analyze the extent to which LS*Δ5 is able to restore gene expression in Raji cells. For this purpose, expression of LS*mock, LS*wt, LS*Δ31, and LS*Δ5 cells was cultured in the absence of Tc. α-Amanitin was added (+ α-am.) 24 h after removal of Tc. The induction of the recombinant large subunits was analyzed by Western blot analysis at the indicated time points using CTD-specific (A) and HA-specific antibodies (B–D). Transcriptional activity of LS*wt and LS*Δ5 was measured in transient transfection experiments (E). Cells were cultivated in the absence of Tc for 2 days, subsequently α-amanitin was added, and cells were transfected with plasmid DNA of c-myc luciferase reporter construct after additional 24 h. Cellular extracts were prepared 24 h later. Luciferase activity is shown in relative light units. The mean of three independent experiments is shown.

![Fig. 2](image_url) Conditional expression of the large subunit of pol II. RajiLS*mock, RajiLS*wt, RajiLS*Δ31, and RajiLS*Δ5 cells were cultivated in the absence of Tc. α-Amanitin was added (+ α-am.) 24 h after removal of Tc. The induction of the recombinant large subunits was analyzed by Western blot analysis at the indicated time points using CTD-specific (A) and HA-specific antibodies (B–D). Transcriptional activity of LS*wt and LS*Δ5 was measured in transient transfection experiments (E). Cells were cultivated in the absence of Tc for 2 days, subsequently α-amanitin was added, and cells were transfected with plasmid DNA of c-myc luciferase reporter construct after additional 24 h. Cellular extracts were prepared 24 h later. Luciferase activity is shown in relative light units. The mean of three independent experiments is shown.

![Fig. 3](image_url) Growth kinetics of Raji cells expressing LS*mock, LS*wt, LS*Δ31, and LS*Δ5. α-Amanitin was added 24 h after removal of Tc. The numbers of living (Nd) and dead cells (Nd) were determined by trypan blue staining. The percentage of viable cells (V) was calculated using the formula V = 100 × Nd/Nt + Nd) (A). Cumulative living cell numbers were determined by counting living cells and taking into account the splitting procedure (B).

![Fig. 4](image_url) Dominant-negative effect of LS*Δ5 on nuclear run-on experiments. Plasmids containing c-myc exon 2 and two fragments of the Ig µ-heavy chain gene locus were digested with the appropriate restriction enzymes. Fragments were separated by agarose gel electrophoresis and subsequently transferred to a Hybond-N+ membrane by Southern blotting. A schematic drawing of the gel (A) and the ethidium bromide stained gel (B) are shown in the upper part. Fragments 1–3 correspond to vector backbones, fragment 4 to c-myc, and fragments 5 and 6 to Ig µ fragments. Filters were hybridized with [α-32P]CTP-labeled nuclear run-on transcripts from RajiLS*mock (C), RajiLS*wt (D), RajiLS*Δ31 (E), and RajiLS*Δ5 cells (F), which were grown 24 h without Tc. All run-ons were carried out in the absence of Sarkosyl and α-amanitin. For a detailed description of the plasmids see "Materials and Methods."
that were reproducibly detectable could be divided into two classes. To class 1 belong the gene probes that also produced signals in RajiLS\^\text{*}mock cells in the presence of \textit{α-amanitin} (Fig. 5, spot 12/11). Signals for spots of class 2 are observed in RajiLS\^\text{*}Δ5 cells but not in RajiLS\^\text{*}mock cells (spots at position 2/1 and 2/11). The intensity of the signals for these spots is, however, only 5–10% of the signal intensity observed in RajiLS\^\text{*}wt cells, indicating that LS\^\text{*}Δ5 can transcribe these genes only at low rate. We also tested the pol II-specific run-on activity of RajiLS\^\text{*}Δ31 cells in the absence and presence of \textit{α-amanitin}. The results were very similar to the results obtained with RajiLS\^\text{*}wt cells and gave no indication for genes that were significantly altered in the transcription rate (data not shown).

The almost complete disappearance of pol II-specific transcription signals for probes on filters B and D in \textit{α-amanitin}-treated cells is not due to an inefficient labeling of RNAs in the run-on reaction. Nuclei incorporated still a high amount of label, indicating that pol I and pol III transcription were not affected by \textit{α-amanitin} in the nuclei of RajiLS\^\text{*}mock and RajiLS\^\text{*}Δ5 cells (Fig. 6E). In conclusion, deletion of the CTD affects global run-on activity of pol II. We next asked, whether deletion of CTD affected initiation and/or elongation of RNA from promoter-proximal pause sites.

**Deletion of CTD Affects Initiation of pol II**—We have shown previously that transiently expressed LS\^\text{*}wt but not LS\^\text{*}Δ5 is able to induce expression of the \textit{hsp\textsubscript{70A}} and \textit{c-fos} genes in 293 cells after appropriate stimuli (51). We confirmed this observation for Raji cells. LS\^\text{*}wt and LS\^\text{*}Δ31 induced transcription of the \textit{hsp\textsubscript{70A}} gene after heat shock and \textit{c-fos} transcription after phorbol ester activation, whereas LS\^\text{*}Δ5 could not (Fig. 7, A and B). \textit{hsp\textsubscript{70A}} and \textit{c-fos} belong to the class of genes that are regulated by promoter-proximal pausing of pol II (12, 14, 52). In the uninduced stage, pol II pauses in a region approximately 20–40 bp downstream of the initiation site. Upon activation of the gene, the CTD of the paused pol II presumably becomes hyperphosphorylated, rendering pol II processive for transcription and recruiting mRNA maturation factors. The \textit{hsp\textsubscript{70A}} and \textit{c-fos} genes were used to determine if pol II with a deleted CTD is still able to initiate and to transcribe up to the respective promoter-proximal pause site. The distribution of pol II in the promoter-proximal region of the \textit{hsp\textsubscript{70A}} and \textit{c-fos} genes was studied in nuclear run-on experiments. To determine the position from which pol II continues transcription in isolated nuclei, labeled run-on RNAs were hybridized to a set of antisense oligonucleotides, each 50 nucleotides long, spanning the respective promoter region.

In RajiLS\^\text{*}mock, RajiLS\^\text{*}wt, and RajiLS\^\text{*}Δ5 cells, a strong transcription signal is produced for \textit{hsp\textsubscript{70A}} on oligonucleotide C, which corresponds to the previously described paused pol II site at the \textit{hsp\textsubscript{70A}} promoter (Fig. 7C, lanes 1, 5, and 9). Upon heat shock, processivity of pol II is induced, and transcription signals on oligonucleotides D to I strongly increase (lanes 2, 6, and 10). If cells were treated with \textit{α-amanitin}, pausing of pol II was observed in cells expressing LS\^\text{*}wt (lane 7), but not in RajiLS\^\text{*}mock (lane 3), and not in RajiLS\^\text{*}Δ5 cells (lane 11). Consequently, the \textit{hsp\textsubscript{70A}} gene was inducible only in RajiLS\^\text{*}wt cells (lane 8) in the presence of \textit{α-amanitin} but not in RajiLS\^\text{*}mock cells (lane 4) and RajiLS\^\text{*}Δ5 cells (lane 12).

For the \textit{c-fos} gene, similar results were obtained. In the uninduced situation, promoter-proximal pausing of pol II was observed in cells expressing LS\^\text{*}wt in the presence of \textit{α-amanitin} (Fig. 7D, lane 7) but not in RajiLS\^\text{*}mock (lane 1), and not in RajiLS\^\text{*}Δ5 cells (lane 11). Consequently, the \textit{hsp\textsubscript{70A}} gene was inducible only in RajiLS\^\text{*}wt cells (lane 8) in the presence of \textit{α-amanitin} but not in RajiLS\^\text{*}mock cells (lane 4) and RajiLS\^\text{*}Δ5 cells (lane 12).

**DISCUSSION**

We have established human B-cell lines conditionally expressing the large subunit of pol II to study the effect of CTD deletions on transcription initiation and promoter-proximal pausing. Long term cultures could be established in the presence of \textit{α-amanitin} from Raji cells expressing LS\^\text{*}wt but not from cells expressing LS\^\text{*}Δ31 and LS\^\text{*}Δ5. Raji cells expressing LS\^\text{*}wt initially run through a crisis but thereafter proliferated quite normally in the presence of \textit{α-amanitin}, with a doubling time of 30 h. Thus, constitutive expression of LS\^\text{*}wt from a
heterologous promoter does not conflict with proliferation and long term survival of Raji cells. Currently we do not know the reason for the initial crisis. A possible reason for the crisis could be the point mutation in LS*wt that confers α-amanitin resistance. Cells expressing this mutant may have an altered gene expression pattern and require an adaptation phase for growth. In contrast, expression of LS*A51, even though it initially prolonged survival, and LS*A5 failed to replace the endogenous LS in regard to long term survival. This is in line with an earlier report that ratL6 myoblasts expressing LS*A31 showed a limited growth in a colony assay, whereas LS*A5 failed to support growth (4).

In this study we were particularly interested in the question how deletion of the CTD affects global gene expression in Raji cells. LS*A5 has been shown to be defective in enhancer-driven expression of transiently transfected genes (41). The mutant has also been reported to be unable to facilitate pre-mRNA maturation in transient transfection experiments (21). However, LS*A5 is able to transcribe transiently transfected CMV promoter and SP1-driven promoter constructs (41). These results could be confirmed for the CMV promoter and in addition for the c-myc promoter in RajiLS*A5 cells. pol II with a deleted CTD has also been reported to initiate at the Drosophila hsp70 gene promoter and to transcribe to the promoter-proximal pause site in vitro (53). Thus, it was not clear whether LS*A5 has a general defect in initiation and/or elongation.

A first approach to compare steady-state mRNA levels in cells expressing LS*wt and LS*A5 turned out to be unsuccessful, because treatment with α-amanitin for 24 h did not lead to significant changes in steady-state mRNA levels. This unexpected result suggested that inhibition of pol II transcription by α-amanitin may lead to a global stabilization of mRNA in Raji cells. Stabilization of mRNA by protein synthesis inhibitors, like cycloheximide, is a well known phenomenon that most likely results from the inhibition of the synthesis of factors required for mRNA degradation. Stabilization of mRNA by transcription inhibitors has been reported for the transferrin receptor gene (54) and multidrug resistance gene mdr1 (55) but is not yet known to be a general phenomenon. This observation certainly deserves further analysis. Here, this phenomenon made a comparison of mRNAs levels in cells expressing LS*wt and LS*A5 impossible.

As a consequence we measured the transcription rate of genes in nuclear run-on experiments. More than 500 transcriptionally active genes in phorbol ester-stimulated Raji cells were analyzed. These genes were also found to be transcribed in cells expressing LS*wt but were repressed or strongly reduced in transcription in cells expressing LS*A5, indicating that LS*A5 has a severe and general defect in transcription in vivo. We did not detect a single gene whose transcription was not severely affected. Notably, the low but significant transcription signals that were still detectable for a few genes in RajiLS*A5 but not in RajiLS*mock cells in the presence of α-amanitin indicate that the mutant LS*A5 is not transcriptionally dead. This is in agreement with the transient transfection experiments by us and others (21, 22, 41). The ability of LS*A5 to transcribe from some promoters in transfection may rely on the fact that transiently transfected DNA does not establish proper chromatin and may be easily accessible for the transcriptional machinery. Genes packaged in regular chromatin may be less accessible to the transcriptional machinery, particularly if the CTD is deleted. In conclusion, a minimal size of the CTD appears to be required for the transcription of all mammalian genes. However, we cannot rule out that very few of the ~100,000 estimated genes do not require the function of CTD for its transcription. For example, the CUP1 gene in yeast has been reported to be transcribed quite efficiently by pol II with a deleted CTD (42).

What could be the reason that pol II without a CTD is unable to transcribe chromatin-packaged genes? In addition to binding of pre-mRNA maturation factors, CTD may permit recruitment of factors to the transcriptional machinery, either directly or indirectly, that allows transcription of chromatin-packaged DNA templates. Such a factor could be the Elongator complex, for example, which harbors HAT activity and binds to pol II...
only if the CTD is hyperphosphorylated (39). Alternatively, the phosphorylated CTD may be required to release inhibitory factors from pol II, e.g. DSIF, which interfere with elongation (32, 33, 36). Since phosphorylation of the CTD is assumed to be a critical step in activation of promoter-proximal paused pol II, we asked if the transition from a paused to an elongating pol II is affected if CTD is deleted. At the hsp70A and the c-fos promoters, pol II with a deleted CTD was unable to produce a transcription signal in nuclear run-ons, neither in uninduced nor induced cells. The run-on reactions have been carried out in the presence of Sarkosyl, which has been described to release nucleosomes from DNA (23) and activate paused pol II (8). If pol II with a deleted CTD would be present at the pause site, Sarkosyl should induce its transcriptional activity in nuclear run-on reactions were carried out in the presence of Sarkosyl as described under “Materials and Methods.” Labeled nuclear RNA was hybridized to a set of oligonucleotides covering the hsp70A (C) and c-fos (D) untranscribed promoter region and 400 or 300 nucleotides of the transcribed region, respectively. Signals obtained from uniformly labeled T7-RNA are shown on the left-hand side. 7SK is a control oligonucleotide for pol III-specific transcription.

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