Mitotic Transcription Repression In Vivo in the Absence of Nucleosomal Chromatin Condensation

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Abstract. All nuclear RNA synthesis is repressed during the mitotic phase of the cell cycle. In addition, RNA polymerase II (RNAP II), nascent RNA and many transcription factors disengage from DNA during mitosis. It has been proposed that mitotic transcription repression and disengagement of factors are due to either mitotic chromatin condensation or biochemical modifications to the transcription machinery. In this study, we investigate the requirement for chromatin condensation in establishing mitotic transcription repression and factor loss, by analyzing transcription and RNAP II localization in mitotic cells infected with herpes simplex virus type 1. We find that virus-infected cells enter mitosis and that mitotic viral DNA is maintained in a nucleosome-free and noncondensed state. Our data show that RNAP II transcription is repressed on cellular genes that are condensed into mitotic chromosomes and on viral genes that remain nucleosome free and noncondensed. Although RNAP II may interact indirectly with viral DNA during mitosis, it remains transcriptionally unengaged. This study demonstrates that mitotic repression of transcription and loss of transcription factors from mitotic DNA can occur independently of nucleosomal chromatin condensation.

Key words: transcription • RNA polymerase II • chromosomes • mitosis • chromatin

Introduction

In higher eukaryotes, mitosis is accompanied by global repression of nuclear RNA synthesis. Studies performed over 30 years ago reveal that incorporation of radioactive precursors into RNA ceases from mid-prophase until late telophase (Taylor, 1960; Prescott and Bender, 1962; Gottesfeld and Forbes, 1997). More recent data from run-on transcription and in situ hybridization assays indicate that transcription on specific RNA polymerase (RNAP) I, II, and III transcribed genes is profoundly repressed during M-phase (Shermoen and O’Farrell, 1991; Weisenberger and Scheer, 1995; White et al., 1995; Martinez-Balbas et al., 1995; Gebrane-Younes et al., 1997; Parsons and Spencer, 1997). The global repression of RNAP II transcription is coupled to the dissociation of transcription activators, RNAP II and nascent RNA molecules from condensing mitotic chromatin (Shermoen and O’Farrell, 1991; Martinez-Balbas et al., 1995; Segil et al., 1996; Kim et al., 1997; Parsons and Spencer, 1997). RNAP II, TFIID and activators such as HSF1, Sp1 and C/EBP disengage from condensing chromosomes and enter the cytoplasm after breakdown of the nuclear membrane in mid-prophase (Martinez-Balbas et al., 1995; Parsons and Spencer, 1997; Segil et al., 1996). At the end of telophase, RNAP II and transcription factors reassociate with decondensing chromatin and transcription resumes.

Several hypotheses have been proposed to explain how transcription might be repressed during mitosis. These fall into two general categories: chromosome condensation hypotheses and transcription factor inactivation hypotheses (Gottesfeld and Forbes, 1997). Chromosome condensation hypotheses suggest that compaction of mitotic chromatin may physically block the association of transcription factors with promoters, thereby repressing transcription initiation. Similarly, mitosis-specific modifications to core histones or binding of condensation factors such as condensins and XCAP proteins to mitotic DNA may interfere with transcription initiation or elongation. Although chromosomal condensation has been proposed as a potential repression mechanism, in vitro biochemical data support the transcription factor inactivation hypotheses.
In contrast, cells exhibit high levels of protein phosphorylation directly or indirectly triggered by cdc2-cyclin B kinase (Saharabuddhe et al., 1984). As a result, RNA Pol II and many basal and activator transcription factors acquire mitosis-specific phosphorylations (Gottesfeld and Forbes, 1997). In vitro transcription assays using purified factors show that cdc2-cyclin B activity inhibits RNA Pol II transcription. Cdc2-cyclin B–dependent phosphorylation of the cdk7 subunit of TFIIH or the carboxy-terminal domain of the large subunit of RNA Pol II is sufficient to repress transcription initiation on naked DNA templates in vitro (Leresche et al., 1996; Gaboria et al., 1997; Koulitchev and Reinhberg, 1998; Long et al., 1998). In addition, mitosis-specific phosphorylation of some RNA Pol II transcription activators, such as Oct-1 and Sp1, interferes with their DNA binding in vitro (Martinez-Balbas et al., 1995; Segil et al., 1996; Gebara et al., 1997; Akoulitchev and Reinberg, 1998; Lons et al., 1998). In sum, data from in vitro transcription and factor binding assays suggest that hyperphosphorylation of RNA Pol II transcription factors may be sufficient to prevent transcription initiation on RNA Pol II promoters during mitosis.

A thorough mitosis-specific phosphorylation of RNA Pol II transcription factors may be sufficient to repress transcription initiation in vitro, it is not clear whether the same mechanisms lead to transcription repression and to displacement of RNA Pol II elongation complexes, transcription factors and nascent RNA from mitotic chromatin in vivo. In this study, we explore the question of how RNA Pol II transcription is repressed during mitosis in vivo, and what role chromosome condensation plays in triggering either mitotic transcription repression or dissociation of transcription factors from mitotic DNA. To do this, we have developed a system that allows us to separate nucleosomal chromatin condensation from other biochemical effects triggered by cdc2-cyclin B activity as cells enter mitosis. We find that RNA Pol II transcription is repressed on DNA that is either condensed or noncondensed during mitosis. In addition, loss of RNA Pol II and basal transcription factors can occur independently of nucleosomal chromatin condensation. We conclude that mitotic repression of RNA Pol II and displacement of transcription elongation complexes may be triggered by events distinct from mitotic chromosome condensation. Although our data are consistent with the hypothesis that mitosis-specific biochemical modifications to the RNA Pol II transcription apparatus may be sufficient to bring about mitotic transcription repression, they do not exclude a role for template modification independent from chromosome condensation.

**Materials and Methods**

**Cells, Viruses, and Infections**

HeLa S3 (human epithelioid cervical carcinoma), U2OS (human osteosarcoma), and SK-N-SH (human neuroblastoma) cells were obtained from American Type Culture Collection and grown as monolayers in DME + 10% fetal bovine serum. Herpes simplex virus type 1 (HSV-1) wild-type strain KOS1.1 and the ICP0 mutant strain n212 (kindly provided by D. Priscilla Schaffer, University of Pennsylvania School of Medicine) were propagated and titers were obtained as previously described (Rice et al., 1994). Cells were infected at a multiplicity of infection (MOI) of 10 plaque-forming units per cell as described (Rice et al., 1995).

**Antibodies and Immunostaining**

The following monoclonal antibodies were used for immunostaining: anti-ICP4 (H1101, Goodwin Institute) at a 1:2,000 dilution, anti–phosphohistone H3 (Upstate Biotechnology) at 1:1,000, anti-RBDU (Boehringer) at 1:50, 8WG16 (Thompson et al., 1989) at 1:200 and ARNA-3 (Research Diagnostics) at 1:2 dilution. Anti–polycyclon antibodies H4 penta and H4 K8 (Upstate Biotechnology) were used at a 1:500 dilution. Monoclonal antibody anti-ICP4 was conjugated to either Texas red or Oregon green fluorochrome (Molecular Probes) and used at a dilution of 1:200. Secondary antibodies were: goat anti-mouse A Alexa 488 and goat anti-rabbit A Alexa 488 (Molecular Probes), goat anti-rabbit Cy3, goat anti–mouse Cy3 and goat anti–mouse lissamine rhodamine (Jackson ImmunoResearch Laboratories). For Western blots, the A RNA-3-3 antibody was used at a dilution of 1:50 and H1101 at a dilution of 1:5,000.

Immunostaining was performed on cycling populations of HeLa S3 cells. Cells were either grown on coverslips or deposited onto coverslips using a cytospin centrifuge. Cells were fixed for 5 min in 1% paraformaldehyde in PBS, then washed in PBS. A tetrafix fixation, cells were permeabilized by immersing coverslips in 0.5% Triton X-100/PBS for 5 min, then washing in PBS. Coverslips were incubated for 30 min at 37°C in dilutions of primary antibodies in PBS/0.5% BSA. A tetrafix washing in PBS, cells were incubated for 30 min at 37°C in dilutions of secondary antibodies in PBS/0.5% BSA. To visualize DNA, secondary antibody dilutions contained 5 µg/ml of 4’,6-diamidine-2-phenylindole (DAPI). A nα-ICP4-Texas red and anti–ICP4-Oregon green conjugates were used as tertiary stains after secondary antibody staining. A tetrafix washing in PBS, coverslips were mounted in glycerol and cells were visualized with a Zeiss LSM 510 confocal microscope using sequential laser scans for each fluorochrome. Images were converted to TIFF files and figures were assembled and labeled using Adobe Photoshop software. Control stains included: secondary antibody only, secondary plus tertiary antibodies only, single primary antibody and secondary antibodies only. Bleedthrough staining and cross-reactions were undetectable using confocal microscopy and sequential laser scans.

**BrdU Labeling and HSV In Situ Hybridization**

HeLa S3 cells were synchronized in early S-phase by incubating in 2.5 mM thymidine for 24 h. Thymidine was removed and cells were infected 3 h later with HSV-1, at an MOI of 10.

For BrdU labeling, 5 mM BrdU was added at 4 h postinfection (7 h after thymidine washout). At this time point, cells were predominantly in G2-phase. Cells were harvested at 6 h postinfection. During the 2-h labeling period, ~30% of the G2-phase-infected cells progressed into mitosis. Cytosins were prepared and cells were fixed, permeabilized, and stained as described above, using antibodies that recognize BrdU (Boehringer) and ICP4.

For HSV in situ hybridization, the synchronized infected cells were harvested at 6 h postinfection and cytopsins were prepared. Cells were fixed with 3% paraformaldehyde for 10 min, washed, and permeabilized with 0.5% Triton X-100/PBS for 10 min. Cells were biotinylated by a biotin-labeled HSV DNA probe following instructions provided by ENZO Diagnostics (PathoGene HSV DNA Probe A ssay). In situ hybridization was performed at 85°C for 5 min. Hybridized probe was visualized using the Fluorescent Streptavidin In Situ Detection System (ENZO Diagnostics). Cells were then stained with anti–ICP4-Texas red and DAPI.

For both BrdU labeling and in situ hybridization experiments, control cytopsins were treated with 1 mg/ml DNase I in PBS for 10 min at 37°C before staining or in situ hybridization.

**Whole-Cell Run-on Transcription Assays**

U2OS cells were synchronized and harvested for run-on transcription assays as follows. Monolayers at ~20% confluency were grown for 24 h in the presence of 2.5 mM thymidine, to block cells in early S-phase. Thymidine was washed out and cells were incubated for 7 h in DME medium. Cells were either mock infected or infected with HSV-1 strain n212 at an MOI of 10, in the presence of 0.5 µg/ml nocodazole. At 7 h postinfection (14 h after thymidine washout), mitotic cells were removed by mitotic shake-off. At this time point ~16% of cells in the culture had progressed into M-phase (Table I). The remaining adherent cells (mid- to late-G2) were harvested by trypsinization. Miotic shake-offs yielded populations that were >95% mitotic as assessed by propidium iodide staining and visual scoring. G2-phase cells were scored at <3% mitotic. Miotic and G2 populations were stained with anti–ICP4-Oregon green in order to assess
Table I. Mitotic Index of Cells Mock Infected or Infected with HSV-1

| Hours postinfection | HeLa Mock infected* | KOS1.1 infected‡ | U2OS Mock infected³ | n212 infected³ |
|---------------------|---------------------|-------------------|--------------------|----------------|
| 0                   | 2.3%                | ND                | 1.5%               | ND             |
| 3                   | 26.3%               | 18.0%             | ND                 | ND             |
| 5                   | 63.5%               | 40.1%             | ND                 | ND             |
| 7                   | ND                  | ND                | 20%                | 16%            |
| 9                   | ND                  | ND                | 44%                | 42%            |

*HeLa S3 cells were synchronized in S-phase by incubating for 24 h in the presence of thymidine. 3 h after thymidine washout, cells were infected with wild-type virus KOS1.1. At the times indicated, cells were fixed, stained with propidium iodide, and scored for mitotic index.

‡U2OS cells were blocked with thymidine and infected with n212 virus 7 h after thymidine washout. Nocodazole was added to block cells in mitosis. Harvesting was done as for HeLa S3 cells.

the efficiency of infection. Greater than 80% of mitotic and G2 cells showed clearly visible viral replication compartments. A first, cells were resuspended in DEME10% glycerol and frozen at -70°C.

Whole-cell run-on transcription assays were performed as described (Parsons and Spencer, 1997). In brief, cells were thawed, adjusted to equal numbers of cells per sample, and permeabilized using lysolecithin. Permeabilization was measured by trypan blue exclusion and was >95%. Permeabilized cells were incubated in buffer containing 1.2 mM ATP, GTP, and CTP, as well as radioactive α-32P]UTP (3,000 mCi/ml). The reaction was terminated by addition of DNAase I, and proteins were removed using Proteinase K digestion. Run-on transcription RNA was purified and hybridized to filters bearing single-stranded DNA probes. Radioactivity hybridizing to each probe was quantitated with a Fuji XA 1100 Bioimage analyzer with Maccas Imaging software. Probes have been described (Parsons and Spencer, 1997; Spencer et al., 1997) and detect either sense or antisense transcription from the following cellular genes: c-myc exon 1, c-myc intron 1, γ-actin 5′ region (exons 1–4), γ-actin 3′ region (exons 4–6), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), and histone H2b. Probes that detect sense or antisense transcription from HSV-1 genes included: ICP27 (an immediate-early gene), ICP8 (a delayed-early gene), gC, and UL36 (late genes). A autoradiographs were scanned and images were saved in A dobe Photoshop software as TIFF images. Images were assembled and labeled using Quark X Press or A dobe Photoshop software.

Immunodetection of Nascent RNA and Viral Replication Compartments

In vivo labeling with fluorouridine was performed as follows. SK-N-SH cells were cultured directly on glass coverslips under conditions recommended by A merican T ype Culture Collection. Cells were incubated for 20 min in fresh medium containing 2.5 mM thymidine. 3 h after thymidine washout, cells were infected with wild-type virus KOS1.1. At the times indicated, cells were fixed, stained with propidium iodide, and scored for mitotic index.

Integrated intensities of defined regions of the nucleus were calculated and the numbers were normalized to background values. The net phosphorus and nitrogen values were computed as described (Locklear et al., 1990; Hendzel and Bazett-Jones, 1996; Bazett-Jones and Hendzel, 1999; Hendzel et al., 1999; Boisvert et al., 2000). Net phosphorus and nitrogen maps were formed by subtracting the 120 eV image from the 155 eV image and net nitrogen maps by subtracting the 385 eV image from the 415 eV image using Digital M ic rograph v. 2.5 software. R esultant images, both I F and E M, were processed and aligned using A dobe Photoshop 5.0.

In Vivo DNA-Protein Cross-linking

M itotic and interphase U2OS cells were harvested as described above for run-on transcription assays. Proteins were cross-linked to DNA as described previously (Parsons and Spencer, 1997). In brief, 1 × 107 cells were incubated in 0.1% formaldehyde in DEME 10 min at room temperature. A fter cross-linking, cells were washed in PBS, resuspended in nuclear lysis buffer and lysed by passing through a 26-gauge needle. Lysates were loaded onto cesium chloride gradients and centrifuged at 77,000 g in a SW 60Ti rotor for 20 h at 20°C. Fractions were collected and those fractions containing DNA cross-linked to protein were pooled, dialyzed, and precipitated. DNA –cross-linked proteins were digested with DN Ase I, R N Ase A, and S1 nuclease to remove nucleic acids. The proteins from DNA –cross-linked fractions and proteins from the free protein fractions at the top of the gradients were analyzed by SDS-PAGE and Western blotting. Western blots were probed with A R N A-3, stripped, and re-probed with anti-ICP4. A autoradiographs were scanned and images were assembled and labeled using A dobe Photoshop software, as described above.

Online Supplemental Material

Three-dimensional (3-D) animated confocal images were generated as follows. Cycling HeLa S3 cells were fixed and stained with anti-ICP4 antibody, lissamine rhodamine-conjugated secondary antibody and D A PI to visualize DNA. A stack of 30 images, from top to bottom of each cell, was assembled with an L SM 310 confocal microscope using two laser wavelengths. Z-resolution was 0.2 µm. The fluorescence signals from both fluorochromes were superimposed. 3-D images were rendered from z-stack data using 3-D for L SM 310 software. Movies were created from 3-D images using Metamorph software. Videos are available at http://www.jcb.org/cgi/content/full/150/1/113/D1.
**Results**

**Viral DNA Remains in a Nucleosome-free, Noncondensed State during Mitosis**

Herpes simplex virus type 1 (HSV-1) is a >150-kD nuclear DNA virus whose genome is transcribed by host R N A P II (Smiley et al., 1991). Within 3–4 h postinfection, R N A P II and host transcription factors are recruited from the host genome onto the viral genome, and become concentrated in subnuclear foci known as viral replication compartments which are sites of viral DNA replication and transcription (de Bruyn K ops and K niepe, 1988; Rice et al., 1994; Phelan et al., 1997). BrD-U is rapidly incorporated into viral replication compartments and viral DNA remains within replication compartments after its synthesis (de Bruyn K ops and K niepe, 1988; Phelan et al., 1997). Formation of replication compartments requires viral DNA synthesis and compartments are absent when viral DNA synthesis is inhibited by DNA polymerase inhibitors or when cells are infected with viruses mutant in genes encoding one of the seven essential virus-encoded DNA replication proteins (G odowski and K niepe, 1983; U prichard and K niepe, 1997). Replication compartments disperse after treatment with DN Ase I (Rixon et al., 1983; Quinlan et al., 1984).

HSV replication compartments are also sites of viral and cellular regulatory protein accumulation. In keeping with their role in viral gene transcription, these structures recruit host R N A polymerase II, TBP and basal transcription factors, as well as the viral transcription activator protein ICP4 (R andall and D inwoodie, 1986; K niepe et al., 1987; R ice et al., 1994; de Bruyn K ops et al., 1998; Spencer, C., unpublished data). ICP4 is a sequence-specific and nonspecific DNA-binding protein, and DNA binding is essential for its gene activation function (K attar-Cooley and W ilcox, 1989; M ichael and R oziman, 1989; A llen and E verrett, 1997). Treatment with DN Ase I or inhibition of viral DNA replication eliminates recruitment of ICP4 into replication compartments. Consistent with the involvement of replication compartments in viral DNA replication, viral replication compartments accumulate virus-encoded replication proteins including DNA polymerase and the DNA-binding protein, ICP8 (K niepe and S pagan, 1982; Quinlan et al., 1984). ICP8 is required for viral DNA replication and for replication compartment formation and it binds preferentially to single-stranded and double-stranded DNA, holding it in an extended conformation (L einbach and C asto, 1983; R uyechan, 1983; L ee and K niepe, 1985; G ourves et al., 2000). ICP8 and the other six essential viral DNA replication proteins colocalize in viral replication compartments at sites of BrD-U labeling (Quinlan et al., 1984; de Bruyn K ops and K niepe, 1988). DN Ase I treatment abolishes recruitment of these proteins to replication compartments. Electron microscopic immunocytochemical analysis of viral DNA Miller spreads shows that ICP8 comprises a major component of ~17-nm thick viral double-stranded DNA molecules that are nucleosome free (M unner et al., 1980; P uvion-D utilleul et al., 1985). In addition, nascent viral RNA s have been detected in replication compartments, and more prominently in interchromatin granule clusters and cytoplasm (B esse et al., 1995).

Interestingly, subnuclear structures resembling viral replication compartments form in uninfected cells after transfection with plasmids encoding the seven essential H SV-1 replication proteins, along with a plasmid containing the H SV-1 origin of replication. These replication compartment structures are sites of DNA synthesis and do not form in the presence of H SV DNA polymerase inhibitors or in the absence of one of the viral DNA replication proteins (L ukonis and W eller, 1997; Z hong and H ayward, 1997). Hence, H SV replication compartments are comprised of viral DNA, nascent RNA, cellular and viral proteins.

A virus infection proceeds, host transcription is repressed and viral gene transcription is activated in a controlled temporal order (G odowski and K niepe, 1986; R ice et al., 1994; S pencer et al., 1997). Because viral and host cell genes contain similar R N A P II promoter elements and use the same transcription machinery, this global change in transcription may be due, in part, to nucleotide sequence-independent mechanisms, such as differences between host and viral chromatin structure (Smiley et al., 1991). In support of this idea, data from fractionation and nuclease digestion experiments suggest that parental and newly replicated H SV-1 DNA are free of nucleosomes during lytic infection (M outtet et al., 1979; L einbach and S ummers, 1980; M ullen et al., 1980; P ignatti and C assai, 1980; S inden et al., 1982; P uvion-D utilleul et al., 1985; M uggeridge and F raser, 1986; L entine and B achenheimer, 1990). Electron microscopic studies of Miller spreads show a preponderance of DNA in non-nucleosomal form in H SV-infected cells (M ullen et al., 1980; P uvion-D utilleul et al., 1985). This DNA takes three forms: 3–5-nm protein-free strands, strands with sparse 10–20-nm large granules different from nucleosomes and ~17-nm uniformly thick heavily stained strands.

We reasoned that if H SV-1 DNA remains nucleosome free and virus infection does not interfere with entry of cells into mitosis, viral DNA may be present in a noncondensed state during mitosis. The presence of both noncondensed and condensed chromatin in vivo would allow us to determine whether chromosome condensation was required for either transcription repression or dissociation of transcription factors during mitosis in intact mitotic cells.

We first confirmed that human cells infected with H SV-1 progress through G2-phase and enter mitosis. Hela S 3 cells were synchronized in early S-phase by growth for 24 h in the presence of thymidine. 3 h after thymidine washout, cells were infected with H SV-1. 6 h after infection, cells were fixed, stained with propidium iodide, and scored for mitotic index. Table I shows that Hela S cells entered mitosis when infected with H SV-1, although the rate of mitotic entry was slowed compared with mock infected cells. The rate of mitotic entry was similar in mock infected U 20 S cells and in U 20 S cells infected with the H SV-1 mutant virus, n212 (Table I).

We next confirmed that infected mitotic cells contained viral replication compartments. Fig. 1 a shows interphase and mitotic Hela cells, fixed and stained with the DNA stain D A P I and an antibody that recognizes the viral immediate-early protein ICP4. ICP4 binds to viral DNA and is the main transcriptional activator protein encoded by H SV-1 (K attar-Cooley and W ilcox, 1989; S miley et al.,
We next wanted to establish the spatial relationships between host chromatin and viral replication compartments in interphase and mitotic cells. To do this, we fixed and stained cells as in Fig. 1a, then generated 3-D animated images from confocal microscope stacks. Fig. 1b shows four frames from a 3-D animated movie of an interphase cell infected with HSV-1 for 6 h. (Video 1 is available at http://www.jcb.org/cgi/content/full/150/1/13/DC1.) The interphase cell contained two large viral replication compartments that occupied most of the cell nucleus. Viral and cellular DNA appeared to occupy discrete intranuclear regions, with cellular DNA concentrated at the nuclear periphery, as seen previously (de Bruyn Kops and Knipe, 1994; Besse et al., 1995). Fig. 1c shows four frames from a 3-D animated image of a mitotic infected cell. (Video 2 is available at http://www.jcb.org/cgi/content/full/150/1/13/DC1.) The mitotic cell contained a large viral replication compartment that appeared spatially separate from host chromosomes. ICP4 staining occurred throughout viral replication compartments, as did staining with antibody against the viral DNA-binding protein ICP8 (data not shown). Mitotic cells infected with wild-type HSV-1 were arrested in pro-metaphase, as seen previously (Lomonte and Everett, 1999). This mitotic arrest is due to the actions of the viral immediate-early protein ICP0, which triggers degradation of the CENP-C kinetochore protein (Everett et al., 1999). In cells infected with viruses containing null mutations in the gene encoding ICP0 (n212), mitosis proceeds normally through metaphase, anaphase, and telophase.

In all transcription and cross-linking experiments described in this report, >80% of mitotic infected cells contained clearly visible replication compartments. Together, Table I and Fig. 1 indicate that human cells infected with HSV-1 enter mitosis, condense host chromatin, and contain discrete extrachromosomal viral replication compartments.

Although it has been established that viral replication compartments in interphase cells contain newly replicated viral DNA (de Bruyn Kops and Knipe, 1988; Knipe, 1990; Phelan et al., 1997), we wanted to determine whether viral replication compartments in mitotic cells also contain newly replicated viral DNA. To do this, we used two methods: BrdU incorporation and HSV in situ hybridization.

For BrdU labeling experiments, HeLa S3 cells were arrested in early S-phase, released from arrest for 3 h, and infected with HSV-1. The synchronized infected cells were incubated in the presence of BrdU for 2 h, beginning at 4 h postinfection. At 4 h postinfection (7 h after thymidine washout), cells were predominantly in G2-phase; therefore, incorporation of BrdU into nascent DNA would be due to the actions of the HSV-1 DNA polymerase. Viral DNA polymerase is necessary for formation of viral replication compartments (Quinlan et al., 1984; de Bruyn Kops et al., 1998). Mitotic and G2 cells were harvested at 6 h postinfection and cytospins were prepared. Cells were fixed and stained with anti-BrdU and anti-ICP4 antibodies. Both interphase and M-phase cells contained replication compartments that reacted with anti-BrdU and anti-ICP4 antibodies (Fig. 2a). The BrdU and DAPI signals were undetectable in cells that were treated with DNase I before staining. These data indicate that viral replication compartments in both interphase and mitotic cells contain newly replicated DNA.

For HSV in situ hybridization experiments, HeLa S3 cells were arrested in early S-phase, released from arrest for 3 h and infected with HSV-1. The synchronized infected cells were harvested at 6 h postinfection and cytospins were prepared. Cells were fixed and hybridized to a biotin-labeled HSV DNA probe. HSV DNA hybridization was detected with streptavidin-fluorescein. Both interphase and mitotic cells contained replication compartments that hybridized to the HSV DNA probe (Fig. 2b). Replication
compartment hybridization and DAPI staining were undetectable in cells that were treated with DNase I before in situ hybridization. Together, the BrdU and in situ hybridization experiments show that both mitotic and interphase replication compartments contain newly replicated viral DNA.

To extend previous reports that HSV-1 DNA is nucleosome free, we examined infected cells using two cellular imaging methods. First, we immunostained interphase and mitotic infected cells with antibodies that recognize histone H4 and a mitosis-specific epitope on histone H3. Viral replication compartments in both interphase and mitotic cells showed no detectable staining with antibodies that recognize histones H4 (Fig. 3 a) or H3 (Fig. 3 b). This absence of staining is in sharp contrast to the pronounced replication compartment staining seen for other host cell DNA-binding proteins such as RNA Pol II, basal transcription factors, p53, Rb, and DNA ligase, as well as for viral DNA.

Figure 2. HSV-1 replication compartments contain newly replicated viral DNA. HeLa S3 cells were synchronized in S-phase, released for 3 h and infected with wild-type HSV-1. (a) At 4 h postinfection (when the majority of cells were in G2-phase), 5 mM BrdU was added to the culture medium. Cells were harvested at 6 h postinfection, fixed, and stained with anti-BrdU and anti-ICP4 antibodies, as indicated. (b) Synchronized infected cells were harvested at 6 h postinfection, fixed, and subjected to in situ hybridization using a biotin-labeled HSV-1 DNA probe and streptavidin-fluorescein. Cells were then stained with anti-ICP4 antibody and DAPI. Bars, 10 μm.

Figure 3. Viral replication compartments contain little if any histone H4 or H3. Cycling HeLa S3 cells were infected with HSV-1 strain KOS1.1 for 6 h, fixed, and immunostained with antibody that recognizes histone H4 (a) or a mitosis-specific phospho-epitope on histone H3 (b). Cells were also stained with anti-ICP4 conjugated to Texas red and DAPI, to visualize DNA. Anti-histone H3 recognizes histone H3 predominantly in mitotic cells. Bars, 10 μm.
replication and regulatory proteins such as ICP4, ICP8, ICP27, and viral replication proteins (Wilcock and Lane, 1991; Rice et al., 1994; Zhong and Hayward, 1997; de Bruyn Kops et al., 1998). These data suggest that histones H3 and H4 do not contribute significantly to the protein complement of HSV nucleoprotein fibers.

Second, we examined replication compartments at the ultrastructural level using correlative immunofluorescence microscopy and electron spectroscopic imaging (ESI). The latter technique, based on transmission electron microscopy, detects electron energy loss events in a specimen. Such losses are characteristic of the elements present in the specimen, and, thus, can reveal the distributions of elements such as nitrogen and phosphorus. Measurement of the relative concentrations and mapping of these elements permit the delineation of protein- and nucleic acid–based structures (Hendzel and Bazett-Jones, 1996; Bazett-Jones and Hendzel, 1999; Hendzel et al., 1999). Images collected at 155 eV are enhanced in phosphorus, whereas images collected at 120 eV contain predominantly mass information. Subtracting the 120-eV image from the 155-eV image produces a net phosphorus image, characteristic of nucleic acids. Similarly, images collected at 415 eV are enhanced in nitrogen. Subtracting the reference 385-eV image from the 415-eV image produces a net nitrogen image, characteristic of both proteins and nucleic acids. With this method, nucleic acid fibers of 2–5 nm in diameter can be visualized, and regions of decondensed chromatin can easily be distinguished from chromatin compacted beyond the 30-nm fiber (Hendzel et al., 1999).

HeLa S3 cells were infected, stained with anti-ICP4 antibodies, and embedded for electron microscopy. Thin sections were prepared by ultramicrotomy and deposited onto lettered EM specimen grids. The sections were first imaged in the fluorescence microscope to find replication compartments and host chromatin (Fig. 4a). The regions of interest were then found in the electron microscope (with the aid of the lettered grids) and imaged by ESI to obtain high resolution structure and analytical information. The light and EM images were then superimposed in order to identify the regions of interest in the electron images (Fig. 4b). The viral replication compartment in this cell occupied a large volume of the nucleus, with host chromatin (arrows in Fig. 4, c–f) forced to the nuclear periphery, as seen previously (Besse et al., 1995). Fig. 4, c and d, show the boxed region in Fig. 4b imaged at higher magnification. Similarly, Fig. 4, e and f, show the boxed region in Fig. 4d imaged at an even higher magnification. At these higher magnifications, the replication compartments appeared to contain both fibers and granules composed of both nitrogen and phosphorus. The phosphorus signal in these fibers appeared to be qualitatively close to the detection limit, significantly lower than that of 10-nm nucleosomal DNA fibers (Boisvert et al., 2000). On the other hand, the nitrogen content of these fibers was significantly higher, indicating that the DNA fibers associate with protein.

These data confirm previous conclusions derived from biochemical and electron microscopic assays that show HSV DNA in a non-nucleosomal extended conformation in interphase infected cells (Muller et al., 1980; Puvion-Dutilleul et al., 1985).

Mitotic cells were also imaged using the correlative ESI technique (Fig. 5). Host mitotic chromosomes were visible as bright regions with high phosphorus and nitrogen signals (arrows in Fig. 5, c and d). Again, the replication compartment appeared as a well-delineated cluster of fibers that were low in phosphorus and high in nitrogen, consistent with fully extended DNA fibers associated or coated with protein (Fig. 5, e–h). Similar to interphase replication...
compartment fibers, the phosphorus signal in mitotic replication fibers appeared to be close to the detection limit, lower than that of 10-nm nucleosomal chromatin and consistent with 2-nm diameter DNA strands. To compare viral fibers with host 10- and 30-nm chromatin, see Boisvert et al. (2000). A iso present were occasional punctate clusters of phosphorus accumulation, similar to structures previously observed in interphase infected cells (Muller et al., 1980; Puvion-Dutilleul et al., 1985). These 2-nm fibers coated with protein were the main structural features within replication compartments. In conclusion, replication compartments in both interphase and mitotic cells contain newly replicated viral DNA and this DNA does not form the >10-nm fibers characteristic of nucleosomal DNA. In addition, the degree of compaction of viral DNA fibers in interphase and mitosis does not vary significantly on a qualitative level, indicating that viral DNA does not undergo detectable mitotic condensation.

To further investigate the characteristics of viral replication compartment DNA during interphase and mitosis, we calculated the phosphorus to nitrogen (P/N) ratios of host and viral nucleoprotein fibers imaged by ESI. The integrated intensities for P and N within defined regions of the nucleus were calculated, as described in Materials and Methods. The P/N ratio of 10- and 30-nm nucleosomal chromatin has been previously described (Locklear et al., 1990; Bazett-Jones et al., 1999). Host chromatin in infected interphase and mitotic cells contained average P/N ratios of 0.605 and 0.612, respectively (Table II). In contrast, the P/N ratios of viral chromatin were 0.125 and 0.186 in interphase and mitotic cells, respectively. The observation that the P/N ratios of viral replication compartment fibers differ from those of host chromatin by factors of three- to fivefold indicates that viral nucleoprotein fibers are not organized as nucleosomal chromatin. The high protein/DNA ratio is atypical of folded or open chromatin or of RNA and indicates that viral DNA is likely associated with large amounts of protein, as seen by others (Muller et al., 1980; Puvion-Dutilleul et al., 1985). The protein composition of HSV replication compartments is known to include viral DNA-binding and replication proteins as well as cellular and viral gene regulatory proteins (Rice et al., 1994; de Bruyn Kops et al., 1998). The increase in P/N ratio for viral nucleoprotein fibers during mitosis is interesting, and may indicate either that viral DNA undergoes a degree of compaction during mitosis or that viral nucleoprotein fibers lose protein content. The latter explanation is supported by our immunofluorescence data that show the loss of some viral and cellular

Table II. Quantitation of Phosphorus and Nitrogen Content in Host Chromatin and HSV-1 Nucleoprotein Fibers

| Sample          | Average P/N ratio | Number of areas measured | P/N ratio host: P/N ratio viral |
|-----------------|-------------------|--------------------------|--------------------------------|
| Host chromatin  |                   |                          |                                |
| Interphase      | 0.605             | 20                       | 4.83 ± 0.14                    |
| Mitosis         | 0.612             | 20                       | 3.28 ± 0.63                    |
| HSV nucleoprotein |                  |                          |                                |
| Interphase      | 0.125             | 62                       |                                |
| Mitosis         | 0.186             | 83                       |                                |

Figure 5. Viral replication compartment DNA is non-nucleosomal and noncondensed during mitosis. HeLa S3 cells were infected, harvested, and processed for immunofluorescence and ESI, as described in the legend to Fig. 4. A viral replication compartment in a mitotic cell was visualized by ICP4 staining and immunofluorescence (a). The same cell as seen in a was imaged by electron microscopy and the two images were superimposed (b). In this instance the immunofluorescence image was superimposed on the nitrogen map EM image. The box in b, encompassing part of a replication compartment, was imaged for net phosphorus (c) and net nitrogen (d). Arrows indicate host chromosomes and RC indicates replication compartment. The box in c was imaged for net phosphorus (e) and net nitrogen (f). The boxed area in d was imaged at higher magnification (50 k) for net phosphorus (g) and net nitrogen (h). The phosphorus and nitrogen maps (c-h) show mitotic replication compartments as diffuse noncondensed areas containing both phosphorus- and nitrogen-containing fibers of <10 nm, similar to the pattern seen in interphase replication compartments (Fig. 4, c-f). Bars: (b) 5 µm; (h) 50 nm.
proteins from viral replication compartments during mitosis (Spencer, C.A., unpublished data and Figs. 8–10). In any case, data derived from quantitation of phosphorus and nitrogen show that viral DNA is not organized into nucleosomal chromatin in either interphase or mitosis and that it does not condense similarly to host chromatin during mitosis.

**Transcription Is Repressed on Viral and Cellular Genes during Mitosis**

We first examined transcription of viral and cellular genes in interphase and mitotic cells using whole cell run-on transcription assays. We have used these assays previously to show that RNA polymerase II transcription is repressed on specific cellular genes during mitosis (Parsons and Spencer, 1997). Synchronized U2OS cells were infected with the n212 virus for 7 h, mitotic and G2-phase cells were collected from the same flasks and assays were performed on equal numbers of cells per sample, as described in Materials and Methods. We chose to infect U2OS cells with the n212 virus, which contains a null mutation in the gene encoding the viral immediate-early protein ICP0 (Cai et al., 1993; Sacks and Schaffer, 1987; Yao and Schaffer, 1995). The absence of ICP0 enhances the progression of infected cells into mitosis (Lomonte and Everett, 1999), which in turn facilitated our enrichment of mitotic populations of infected cells. Virus infection and transcription patterns are similar in U2OS cells infected with either the wild-type virus or the n212 virus at an MOI of 10 plaque-forming units per cell (Sacks and Schaffer, 1987; Spencer et al., 1997).

In uninfected cells (Fig. 6 a), transcription of cellular genes was repressed during mitosis, as reported previously...
Mitotic transcription levels declined from 17% to 0% of interphase (G2) levels, as assessed by PhosphorImager analysis. In infected interphase cells (Fig. 6 b, left panels), host gene transcription levels were lower than those in uninfected cells, as seen previously (Spencer et al., 1997). In contrast, viral gene transcription levels were high in infected interphase (G2) cells. When infected cells entered mitosis (Fig. 6 b, right), transcription of both host and viral genes was repressed to less than 10% of interphase (G2) levels. Data from these assays indicate that transcription of both host and viral genes is repressed in mitotic cells.

To verify repression of viral gene transcription during mitosis using a different assay, we pulse labeled intact infected cells with fluorouridine in vivo, fixed and stained cells to detect fluorouridine-containing nascent RNA. Fluorouridine is incorporated rapidly into nascent RNA and is used as a sensitive indicator of transcriptional activity in intact living cells (Haider et al., 1997; Boisvert et al., 2000). Fluorouridine was incorporated into nascent RNA efficiently in interphase cells early in infection (Fig. 7 a), before formation of discrete viral replication compartments. After the appearance of viral replication compartments, fluorouridine staining was less intense (Fig. 7 b). In interphase cells, little or no fluorouridine was incorporated into nascent RNA during the 10-min labeling period (Fig. 7 c). Interestingly, fluorouridine labeled RNA did not accumulate in viral replication compartments during interphase or mitosis, in contrast to results from RNA labeling studies in permeabilized infected cells (Phelan et al., 1997). As replication compartments are the sites of viral transcription, these data suggest that nascent viral RNA may be rapidly processed or transported in intact cells. In conclusion, the fluorouridine labeling data support the whole cell run-on transcription results showing repression of viral and cellular gene transcription during mitosis. Hence, transcription appears to be repressed during mitosis, on DNA that is condensed into chromosomes and DNA that remains free of nucleosomal chromatin condensation.

**RNAP II Disengages from Viral DNA during Mitosis**

In uninfected cells, RNAP II and many transcription factors are absent from chromatin between prophase and telophase (Gottesfeld and Forbes, 1997; Parsons and Spencer, 1997). To determine whether chromatin condensation is necessary for exclusion of transcription factors from mitotic DNA, we examined the location of RNAP II in interphase and mitotic infected cells. Cycling HeLa cells were infected with HSV-1 for 6 h, fixed, and immunostained with anti-RNAP II antibody, 8WG16. Cycling HeLa cells were either uninfected (a and b) or infected with HSV-1 for 6 h (c-f), fixed, and stained with 8WG16 and anti-ICP4-Texas red conjugate. DNA was stained with DAPI. (a and b), RNAP II staining was nuclear with exclusion from nucleoli in uninfected cells. (c-f), RNAP II localized to viral replication compartments in interphase nuclei, as seen previously (Rice et al., 1994, 1995). Mitotic replication compartments stained weakly with 8WG16. Bar, 10 μm.
whether RNA Pol II was engaged on viral DNA or was indirectly associated with viral replication compartments. To do so, we analyzed RNA Pol II using in vivo cross-linking assays. We have used formaldehyde cross-linking previously to show that RNA Pol II is not transcriptionally engaged on mitotic chromatin in uninfected cells (Parsons and Spencer, 1997). At low formaldehyde concentrations, cross-linking is selective for proteins that are in direct contact with DNA (Wrenn and Katzenellenbogen, 1990). Infected or uninfected U2OS cells were separated into mitotic and interphase populations, treated with 0.1% formaldehyde for 10 min, and the cross-linked and non–cross-linked proteins fractionated on cesium chloride gradients. Cross-linked and non–cross-linked proteins were treated with nucleases, formaldehyde cross-links were reversed and proteins analyzed by Western blotting. Fig. 10 (top) shows cross-linked and non–cross-linked RNA Pol II large subunits in infected and uninfected cells. As seen previously (Parsons and Spencer, 1997), the non–cross-linked large subunit of RNA Pol II in both interphase and mitotic uninfected cells displayed two phosphorylation variants: IIo, which is hyperphosphorylated, and IIa, which is nonphosphorylated (Fig. 10, lane 2; Dahmus, 1994, 1996). In interphase cells, the transcriptionally engaged form of the RNA Pol II large subunit is hyperphosphorylated and the nonengaged form is nonphosphorylated. In mitotic cells, the IIo form may result from mitosis-specific phosphorylatory and may be distinct from the IIo form present in interphase cells (Kim et al., 1997; Parsons and Spencer, 1997). In interphase uninfected cells, the IIo form of the RNA Pol II large subunit cross-linked to DNA. Fig. 10, lane 3, consistent with IIo being transcriptionally engaged on DNA. In mitotic uninfected cells, little if any RNA Pol II large subunit cross-linked to DNA (lane 1), as seen previously (Parsons and Spencer, 1997). In HSV-1–infected interphase cells, a hyperphosphorylated form of the RNA Pol II large subunit also cross-linked to DNA (lane 6), but in mitotic infected cells little if any RNA Pol II large subunit cross-linked to DNA (lane 4). In contrast, the viral immediate-early protein ICP4 cross-linked to DNA in both interphase and mitotic infected cells (Fig. 10, lanes 4 and 6).

Taken together, the immunofluorescence localization and in vivo cross-linking assays suggest that RNA Pol II is

Figure 9. Mitotic replication compartments stain with anti-RNA Pol II antibody, ARNA-3. Cycling infected HeLa cells were fixed and stained with ARNA-3 and anti-ICP4-Oregon green conjugate. DNA was stained with DAPI. ARNA-3 antibody stained viral replication compartments and cytoplasm in mitotic cells. Bar, 10 μm.

Figure 10. In vivo cross-linking of RNA Pol II large subunit and ICP4 to uninfected and infected DNA. Uninfected or HSV-1–infected synchronized U2OS cells were separated into mitotic and interphase populations and treated with 0.1% formaldehyde to cross-link proteins to DNA. Cross-linked proteins were fractionated from non–cross-linked proteins, treated with nucleases and analyzed by Western blotting. (a) Blot probed with ARNA-3 antibody, which recognizes all phosphorylation variants of the large subunit of RNA Pol II. IIo is the hyperphosphorylated variant and IIa is nonphosphorylated. X-link denotes proteins cross-linked to DNA; Protein denotes proteins not cross-linked. (b) The same blot as in a, reprobed with anti-ICP4 antibody. Lane 1, protein cross-linked to DNA from 1.1 × 10⁷ mitotic uninfected cells. Lane 2, free protein from 1.3 × 10⁷ mitotic uninfected cells. Lane 3, protein cross-linked to DNA from 1.2 × 10⁷ interphase uninfected cells. Lane 4, protein cross-linked to DNA from 1.0 × 10⁷ mitotic HSV-1–infected cells. Lane 5, free protein from 1.3 × 10⁷ mitotic HSV-1–infected cells. Lane 6, protein cross-linked to DNA from 1.1 × 10⁷ interphase HSV-1–infected cells.
Discussion

A remarkable feature of mitosis is the cessation of all nuclear RNA synthesis and the loss of RNA II transcription factors from condensing mitotic chromatin. Mitotic transcription repression and loss of DNA-binding factors may have important cell cycle regulatory consequences. It is possible that removal of large protein complexes such as RNA II elongation complexes may be necessary in order to condense DNA into mitotic chromosomes. In addition, the cyclic disengagement of transcription factors from DNA at each cell cycle could have consequences for gene regulation. It has been shown that the rapid mitotic cycles of early Drosophila embryos cause premature termination of transcription on long transcription units, thereby preventing expression of full-length mRNA until cell cycles lengthen (Shermon and O’Farrell, 1991; O’Farrell, 1992). There is also evidence that exit from mitosis may be a cell cycle checkpoint. Growth-related genes such as c-fos, c-jun, c-myc, and p53 are sequentially induced after exit from mitosis in patterns similar to those in cells entering the cell cycle from quiescence (Cosenza et al., 1991, 1994). Cells require the presence of serum growth factors as they exit mitosis in order to proceed to the next cell cycle (Zetterberg and Larsson, 1985).

It is possible that mitotic transcription repression could facilitate changes in gene expression as cells exit mitosis.

In this study, we have exploited HSV-1 infection of human cells in order to assess the requirement for nucleosomal chromatin condensation in bringing about mitotic transcription repression and loss of transcription complexes from mitotic DNA. Data from this study indicate that during lytic infection, HSV-1 DNA is nucleosome free, is devoid of 10–30-nm chromatin structure, and does not undergo mitotic chromosome condensation. The reasons why HSV-1 DNA remains free of histones and nucleosomes are unknown. It is possible that the cellular pool of histones is not sufficient to interact with the large amount of newly synthesized viral DNA after infection. It is also possible that viral DNA is maintained in a nucleosome-free state due to its strong binding to viral regulatory proteins such as ICP4 and ICP8 (de Bruyn Kops and Kniite, 1988; Allen and Everett, 1997). We have not examined whether DNA within viral replication compartments binds condensins or other SMC proteins. However, as the presence of nucleosomes appears to be a prerequisite for chromatin condensation, HSV-1 DNA may remain noncondensed simply due to the absence of nucleosomes (Newport, 1987).

Our data show that transcription is repressed on both condensed and noncondensed genomes during mitosis. Although this suggests that mitotic transcription repression can occur in the absence of chromosome condensation, it is possible that the interaction of transcriptionally active DNA with template modifying factors could contribute to cessation of transcription, even in the absence of chromosome condensation. Such template modifying factors could include condensins, SM C proteins, or topoisomerases. Similarly, mitotic loss of chromatin remodeling factors such as SWI/SNF (Sif et al., 1998) or interaction of chromatin with negative regulatory proteins (Knopfer and Eissenman, 1999; Maldonado et al., 1999) could be involved in repressing transcription from either condensed or noncondensed templates. In addition, mitotic modifications to viral DNA-binding proteins such as ICP4 or ICP8 could contribute to mitotic transcription repression of the HSV-1 genome. Our conclusions support earlier in vitro studies that show repression of RNA III transcription in mitotic Xenopus extracts in the presence of topoisomerase inhibitors or in the absence of normal nucleosome structure (Hartl et al., 1993).

Our experiments do not address the question of what biochemical mechanisms lead to mitotic transcription repression in the absence of nucleosomal chromatin condensation. However, our data are consistent with the hypothesis that mitosis-specific modifications to transcription proteins may be sufficient to bring about mitotic transcription repression. Biochemical studies show that mitosis-specific phosphorylations on members of the RNA II and RNA III transcription factors mediate transcription repression in vitro. Similarly, mitotic phosphorylation of RNA I and RNA III transcription factors mediate transcription repression in vitro (Hartl et al., 1993; Gottesfeld et al., 1994; Heix et al., 1998; Kuhn et al., 1998; Klein and Grummt, 1999). These in vitro studies suggest that mitotic phosphorylation alone could repress transcription of all three nuclear RNA polymerases during mitosis. Similarly, phosphorylation of transcription proteins after their removal from DNA early in mitosis may keep these proteins from reassociating with promoters, leading to loss of transcription factors from mitotic chromatin (Segil et al., 1991; Martinez-Balbas et al., 1995; Gottesfeld and Forbes, 1997).

Our data show that RNA II remains disengaged from both condensed and noncondensed DNA during mitosis. This suggests that chromosome condensation is not required for removal of transcription complexes from DNA or for keeping these complexes off mitotic chromosomes. However, it is possible that interactions of mitotic chromatin with condensation proteins such as condensins, SM C proteins and topoisomerases could be involved. Similarly, mitotic modifications to viral or host chromatin templates could make significant contributions to either establishing or maintaining transcription factor loss during mitosis, even in the absence of chromosome condensation. Our study does not address the question of how transcription complexes and activator proteins are lost from mitotic chromatin. It is possible that RNA II elongation complexes may simply complete a round of transcription and terminate within the 15–30 min of prophase. Therefore, RNA II may be rendered incapable of reinitiating due to either mitosis-specific phosphorylations or to template modifications that occur after normal transcription termination. In this case, transcription complexes are not removed from mitotic chromatin, but are prevented from reinitiating a new round of transcription. Although this may be the case for RNA II elongation complexes, it seems likely that other mechanisms operate to remove...
DNA binding factors such as TBP, Oct-1, and HSF1, as well as nascent RNA.

Our findings on H SV-1 mitotic transcription repression have interesting parallels with recent studies of mitotic repression of R NAP I transcription (Siri et al., 2000). Although R NAP I transcription is repressed during mitosis, R NAP I and its transcription regulators remain localized to some nuclear organizing regions during mitosis (Zatsepina et al., 1993; Weisenberger and Scheer, 1995; Jordon et al., 1996; Roussel et al., 1996; Gebrane-Younes et al., 1997; Siri et al., 1999). Nuclear organizing regions that remain associated with the RNA P I transcription machinery during mitosis also appear to contain noncondensed chromatin. Recent studies (Siri et al., 2000) show that rRNA transcription can be restored in mitotic cells after treatment with the cdc2-cyclin B kinase inhibitor roscovitine, although R NAP P I transcription is not restored. It will be of interest to determine whether specific cdc2-cyclin B inhibitors can re-establish H SV-1 transcription in mitotic cells, on viral templates that remain noncondensed during mitosis.

In summary, our study suggests that nucleosomal chromatin condensation is not required for mitotic repression of R NAP P I transcription or for dissociation of transcription factors from mitotic DNA. These conclusions are consistent with previously hypothesized roles for mitotic modifications to the R NAP P I transcription machinery in effecting mitotic repression, but do not exclude the possibility that template modifications may contribute to mitotic repression in vivo.

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