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

Charlotte A. Spencer,* Michael J. Kruhlak,† Heather L. Jenkins,* Xuejun Sun,* and David P. Bazett-Jones‡

*Department of Oncology, University of Alberta, Cross Cancer Institute, Edmonton, Alberta, Canada T6G 1Z2; and ‡Department of Cell Biology and Anatomy, University of Calgary, Calgary, Alberta, Canada T2N 4N1

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 disassociation 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 reassoculate 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 chromosome condensation has been proposed as a potential repression mechanism, in vitro biochemical data support the transcription factor inactivation hypotheses. During mi-
tosis, cells exhibit high levels of protein phosphorylation directly or indirectly triggered by cdc2-cyclin B kinase (Sahasrabuddhe et al., 1984). A s a result, R N A P 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 R N A P II transcription. Cdc2-cyclin B–dependent phosphorylation of the cdk7 subunit of T F I I H or the carboxy-terminal domain of the large subunit of R N A P II is sufficient to repress transcription initiation on naked D N A templates in vitro (L eresch et al., 1996; G ebara et al., 1997; K oulitchev and R einberg, 1998; L ong et al., 1998). In addition, mitosis-specific phosphorylation of some R N A P II transcription activators, such as Oc t-1 and Sp1, interferes with their D N A binding in vitro (M artinez-B albas et al., 1995; S egil et al., 1991). In sum, data from in vitro transcription and factor binding assays suggest that hyperphosphorylation of R N A P II transcription factors may be sufficient to prevent transcription initiation on R N A P II promoters during mitosis.

Although mitosis-specific phosphorylation of R N A P 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 R N A P II elongation complexes, transcription factors and nascent R N A from mitotic chromatin in vivo. In this study, we explore the question of how R N A P 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 D N A. 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 R N A P II transcription is repressed on D N A that is either condensed or noncondensed during mitosis. In addition, loss of R N A P II and basal transcription factors can occur independently of nucleosomal chromatin condensation. We conclude that mitotic repression of R N A P 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 R N A P 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**

H e l a S 3 (human epithelioid cervical carcinoma), U 20 S (human osteosarcoma), and S K- N-S H (human neuroblastoma) cells were obtained from American Type Culture Collection and grown as monolayers in D M E + 10% fetal bovine serum. Herpes simplex virus type 1 (H SV-1) wild-type strain K O S 1.1 and the I C P 0 mutant strain n212 (kindly provided by D r. P riscilla S chaffer, U niversity of Pennsylvania S chool of M edicine) were propagated and titers were obtained as previously described (R ice et al., 1994). Cells were infected at a multiplicity of infection (M O I) of 10 plaque-forming units per cell as described (R ice et al., 1995).

**Antibodies and Immunostaining**

The following monoclonal antibodies were used for immunostaining: anti- I C P 4 (H 110, G oodwin I nstitute) at a 1:1,200 dilution, anti–phospho-histone H 3 (U pstate B iotechology) at 1:1,000, anti-B r d u (S igna) at 1:200, anti-B r d u (B oehringer) at 1:50, B W G16 (T hompson et al., 1989) at 1:200 and A R N A- 3 (R earch D iagnostics) at 1:20 dilution. Rabbit polyclonal antibodies H 4 penta and H 3 (Upstate B iotechology) were used at a 1:500 dilution. Monoclonal antibody anti- I C P 4 was conjugated to either T exas red or O regon green fluorescent Molecule (M olecular P robe) and used at a dilution of 1:200. Secondary antibodies were: goat anti–mouse A l exa 488 and goat anti–rabbit A l exa 488 (M olecular P robe), goat anti–rabbit C y5, goat anti–mouse C y3 and goat anti–mouse lissamine rhodamine (J ackson I mmuno technica). For Western blots, the A R N A- 3 antibody was used at a dilution of 1:50 and H 1101 at a dilution of 1:5,000.

Immunostaining was performed on cycling populations of H e l a S 3 cells. Cells were either grown on coverslips or deposited onto coverslips using a cytospin centrifuge. Cells were fixed for 10 min in 1% paraformaldehyde in PBS, then washed in PBS. A fter fixation, cells were permeabilized by immersing coverslips in 0.5% T riton X-100/P BS 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% B SA. A fter washing in PBS, cells were incubated for 30 min at 37°C in dilutions of secondary antibodies in PBS/0.5% B SA. To visualize D N A, secondary antibody dilutions contained 5 µg/ml of 4 ,6-diamidine-2-phenylindole (D A PI). A nti- I C P 4-T exas red and anti–I C P 4-O regon green conjugates were used as tertiary stains after secondary antibody staining. A fter washing in PBS, coverslips were mounted in glycerol and cells were visualized with a Z eiss L S M 10 confocal microscope using sequential laser scans for each fluorochrome. Images were converted to T I FF files and figures were assembled and labeled using A doe 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**

H e l a S 3 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 H S V-1, at an M O I of 10.

For Brd u labeling, 5 mM B r d u was added at 4 h postinfection (7 h af ter thymidine washout). At this time point, cells were predominantly in G 2-phase. C ells were harvested at 6 h postinfection. During the 2-h labeling period, ~30% of the G 2-phase–infected cells progressed into mitosis. Cytosins were prepared and cells were fixed, permeabilized, and stained as described above, using antibodies that recognize Br d u (B oehringer) and I C P 4.

For HSV in situ hybridization, the synchronized infected cells were harvested at 6 h postinfection and cytopsins were prepared. C ells were fixed with 3% paraformaldehyde for 10 min, washed, and permeabilized with 0.5% T riton X-100/ PBS for 10 min. C ells were hybridized to a biotin-labeled H S V D N A probe following instructions provided by E N Z O D iagnostics (P atho Gene H SV D N A P robe A s a y). In situ hybridization was performed at 85°C for 5 min. Hybridized probe was visualized using the F luorescent S treptavidin In S itu D etection System (E N Z O D iagnostics). C ells were then stained with anti–I C P 4-T exas red and D AP I.

For both Brd u labeling and in situ hybridization experiments, control cytopsins were treated with 1 ng/ml D N Ase I in PBS for 10 min at 37°C before staining or in situ hybridization.

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

U 20 S cells were synchronized and harvested for run-on transcription assays as follows. M onolayers at ~20% confluency were grown for 24 h in the presence of 2.5 mM thymidine, to block cells in early S-phase. T hymidine was washed out and cells were incubated for 7 h in D M E medium. C ells were either mock infected or infected with H S V-1 strain n212 at an M O I of 10, in the presence of 0.5 µg/ml nocodazole. A t 7 h postinfection (14 h after thymidine washout), mitotic cells were removed by mitotic shake-off. A t this time point ~16% of cells in the culture had progressed into M-phase (T able I). T he remaining adherent cells (mid–late G 2) were harvested by trypsinization. M itotic shake-offs yielded populations that were >95% mitotic as assessed by propidium iodide staining and visual scoring. G 2-phase cells were scored at <3% mitotic. M itotic and G 2 populations were stained with anti–I C P 4-O regon green in order to assess
the efficiency of infection. Greater than 80% of mitotic and G2 cells showed clearly visible viral replication compartments. A firter harvesting, cells were resuspended in D M E 10% 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 lysislolethion. Permeabilization was measured by trypsin-blue exclusion and was >95%. Permeable cells were incubated in buffer containing 1.2 mM ATP, GTP, and CTP, as well as radioactive α-[32]PUTP (3,000 Ci/mmol). The reaction was terminated by addition of D n a e 1, and proteins were removed using Proteinase K digestion. Run-on transcription RNA was purified and hybridized to filters bearing single-stranded D N A probes. Radioactivity hybridizing to each probe was quantitated with a Fujix BAS100 bioimaging analyzer with MacBAS 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 H S V -1 genes included: ICP27 (an immediate-early gene), ICP8 (a delayed-early gene), gC, and UL36 (late genes). A uterographs were scanned and images were saved in Adobe Photoshop software as TIFF images. Images were assembled and labeled using Quark Xpress or Adobe 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 American Type Culture Collection. Cells were incubated for 5 min in fresh medium containing wild-type virus, KOS1.1. At the times indicated, cells were fixed, stained with propidium iodide, and visualized using a Leica DMRE epifluorescence microscope and images were saved in Adobe Photoshop software as TIFF images. Images were processed using DigitalMicrograph v. 2.5 software. Resultant images, both IF and EM, were processed and aligned using A dobe Photoshop 5.0.

**Quantitation of Phosphorus and Nitrogen Content of HSV Replication Compartments and Host Chromatin**

Integrated intensities of defined regions of the nucleus were calculated and the numbers were normalized to background values. The net phosphorus and net nitrogen values were computed as described (Locklear et al., 1990; Hendzel and B a z e t t-J ones, 1996; B a z e t t-J ones and Hendzel, 1999; B a z e t t-J ones et al., 1999). Net phosphorus and nitrogen contents 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 DigitalMicrograph v. 2.5 software. Resultant images, both IF and EM, were processed and aligned using Adobe Photoshop 5.0.

**In Vivo DNA-Protein Cross-linking**

Mitotic and interphase U 2 O S cells were harvested as described above for run-on transcription assays. Proteins were cross-linked to D N A as described previously (Parsons and Spencer, 1997). In brief, 1 X 10⁶ cells were incubated in 0.1% formaldehyde in D M E for 10 min at room temperature. After 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 D N A cross-linked to protein were pooled, dialyzed, and precipitated. D N A -cross-linked proteins were digested with D N Ase 1, R N Ase A, and S1 nuclease to remove nucleic acids. The proteins from D N A -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 reprobed with anti-ICP4. A uterographs were scanned and images were assembled and labeled using Adobe Photoshop software, as described above.

**Online Supplemental Material**

Three-dimensional (3-D) animated confocal images were generated as follows. Cycling H e L a S3 cells were fixed and stained with anti-ICP4 antibody, lissamine rhodamine-conjugated secondary antibody and D A P I to visualize D N A. A stack of 30 images, from top to bottom of each cell, was assembled with an L S M 510 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 S M 510 software. Movies were created from 3-D images using Metamorph software. Videos are available at http://www.jcb.org/cgi/content/full/150/1/13/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 RNA polymerase II (Smiley et al., 1991). Within 3–4 h postinfection, RNA polymerase 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 Kops and Knipe, 1988; Rice et al., 1994; Phelan et al., 1997). Brdu is rapidly incorporated into viral replication compartments and viral DNA remains within replication compartments after its synthesis (de Bruyn Kops and Knipe, 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 (Goudowski and Knipe, 1983; U Prichard and Knipe, 1997). Replication compartments disperse after treatment with DNase 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 RNA polymerase II, TBP and basal transcription factors, as well as the viral transcription activator protein ICP4 (Randall and Dinwoodie, 1986; Knipe et al., 1986; Kops et al., 1987; Rice et al., 1994; de Bruyn Kops 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 (Kattar-Cooley and Wilcox, 1988; Michael and Roizman, 1989; Allen and Everett, 1997). Treatment with DNase 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 (Knipe and Spang, 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 (Leinbach and Casto, 1983; Ruthechan, 1983; Lee and Knipe, 1985; Gourves et al., 2000). ICP8 and the other six essential viral DNA replication proteins colocalize in viral replication compartments at sites of Brdu labeling (Quinlan et al., 1984; de Bruyn Kops and K nipe, 1988). DNase 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 (Muller et al., 1980; Puvion-Dutilleul et al., 1985). In addition, nascent viral RNA's have been detected in replication compartments, and more prominently in interchromatin granule clusters and cytoplasm (Besse et al., 1995).

Interestingly, subnuclear structures resembling viral replication compartments form in uninfected cells after transfection with plasmids encoding the seven essential HSV-1 replication proteins, along with a plasmid containing the HSV-1 origin of replication. These replication compartments are sites of DNA synthesis and do not form in the presence of HSV DNA polymerase inhibitors or in the absence of one of the viral DNA replication proteins (Lukonis and Weller, 1997; Zhong and Hayward, 1997). Hence, HSV replication compartments are comprised of viral DNA, nascent RNA, cellular and viral proteins.

As virus infection proceeds, host transcription is repressed and viral gene transcription is activated in a controlled temporal order (Godowski and Knipe, 1986; Rice et al., 1994; Spencer et al., 1997). Because viral and host cell genes contain similar RNA polymerase 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 HSV-1 DNA are free of nucleosomes during lytic infection (Mouttet et al., 1979; Leinbach and Summers, 1980; Muller et al., 1980; Pignatti and Cassai, 1980; Sinden et al., 1982; Puvion-Dutilleul et al., 1985; Muggeridge and Fraser, 1986; Lentine and Bachenheimer, 1990). Electron microscopic studies of Miller spreads show a preponderance of DNA in non-nucleosomal form in HSV-infected cells (Muller et al., 1980; Puvion-Dutilleul et al., 1985). This DNA takes three forms: 3–5-5-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 HSV-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 HSV-1 progress through G2-phase and enter mitosis. HeLa S3 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 HSV-1. 6 h after infection, cells were fixed, stained with propidium iodide, and scored for mitotic index. Table 1 shows that HeLa cells entered mitosis when infected with HSV-1, although the rate of mitotic entry was slowed compared with mock infected cells. The rate of mitotic entry was similar in mock infected U2OS cells and in U2OS cells infected with the HSV-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 DAPI 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 HSV-1 (Kattar-Cooley and Wilcox, 1989; Smiley 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. 1 a, then generated 3-D animated images from confocal microscope stacks. Fig. 1 b 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. 1 c 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. 2 a). 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. 2 b). 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 RNAP 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. Measurements 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 et al., 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 antibody, 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. 4 a). 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. 4 b). 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. 4 b imaged at higher magnification. Similarly, Fig. 4, e and f, show the boxed region in Fig. 4 d imaged at an even higher magnification. A t 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 configuration 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). A gain, 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

![Figure 4](https://example.com/four.jpg)

**Figure 4.** Viral replication compartment DNA is non-nucleosomal and noncondensed during interphase. HeLa S3 cells were blocked in S-phase, released from the block for 3 h and infected with wild-type virus. At 7 h postinfection, mitotic and interphase infected cells were harvested, fixed, and immunostained with anti-ICP4 conjugated to Texas red. Cells were then fixed and sectioned as described in Materials and Methods. Individual cells were first imaged by standard immunofluorescence microscopy, to locate viral replication compartments (a). The same cell as seen in a was then visualized by electron microscopy. The immunofluorescence image was rescaled and superimposed, in this instance, on the phosphorus map EM image (b). The boxed area in b was imaged at a higher magnification for net phosphorus (c) and net nitrogen (d). A rows indicate host chromatin, and the viral replication compartments are indicated by RC. The boxed area in d was imaged at an even higher magnification for net phosphorus (e) and net nitrogen (f). Replication compartment material appears to contain diffuse noncondensed granules and fibers containing both phosphorus and nitrogen, characteristic of nucleic acids and protein. Bar, 5 μm.
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). Also present were occasional punctuate 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**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average P/N ratio</th>
<th>Number of areas measured</th>
<th>P/N ratio host: P/N ratio viral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host chromatin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interphase</td>
<td>0.605</td>
<td>20</td>
<td>4.83 ± 0.14</td>
</tr>
<tr>
<td>Mitosis</td>
<td>0.612</td>
<td>20</td>
<td>3.28 ± 0.63</td>
</tr>
<tr>
<td>HSV nucleoprotein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interphase</td>
<td>0.125</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Mitosis</td>
<td>0.186</td>
<td>83</td>
<td></td>
</tr>
</tbody>
</table>

---

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 boxed area in c 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 Pol 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. 6a), transcription of cellular genes was repressed during mitosis, as reported previously.

Figure 6. Transcription is repressed on both viral and cellular genes during mitosis. Whole cell run-on transcription assays were performed as described in Materials and Methods, on synchronized U2OS cells, either uninfected (a) or infected with HSV-1 for 7 h (b). Cells from the same flasks were separated into interphase (G2) and mitotic populations and assays were done using the same number of cells per sample. Single-stranded run-on transcription probes detect sense (S) or antisense (AS) transcription from cellular genes c-myc, g-actin, GAPDH, and histone H2B, and viral genes ICP27, ICP8, gC, and UL36.

Figure 7. Fluorouridine incorporation in infected interphase and mitotic cells. Cycling SK-N-SH cells were infected with HSV-1, pulse labeled with fluorouridine, fixed, and stained with anti-BrdU- and Alexa 488-conjugated secondary antibody, and anti-ICP4-Texas red conjugate. DNA is stained with DAPI. (a), Infected interphase nucleus before formation of replication compartments. (b) Infected interphase nucleus containing replication compartments. (c) Infected mitotic cell containing viral replication compartment. Bar, 5 μm.
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 infected mitotic cells, little if any 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). A 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-ICP4 and 8WG16, an antibody that recognizes hypophosphorylated forms of the large subunit of RNAP II (Thompson et al., 1989; Rice et al., 1994, 1995). In uninfected cells, RNAP II staining was nuclear with exclusion from nucleoli (Fig. 8, a and b). Mitotic replication compartments stained weakly with 8WG16. Bar, 10 μm.

As the 8WG16 antibody does not recognize hyperphosphorylated forms of the RNAP II large subunit, we also stained infected cells with ARNA-3, an antibody that recognizes all phosphorylation variants of the large subunit (Rice et al., 1994, 1995). In uninfected cells, RNAP II staining was nuclear with exclusion from nucleoli (Fig. 8, a and b). Fig. 8, c-f, shows HSV-1-infected mitotic and interphase cells containing viral replication compartments. Although interphase replication compartments stained strongly with 8WG16, RNAP II staining was low or absent in mitotic replication compartments using this antibody.

A the 8WG16 antibody does not recognize hyperphosphorylated forms of the RNAP II large subunit, we also stained infected cells with ARNA-3, an antibody that recognizes all phosphorylation variants of the large subunit (Rice et al., 1994, 1995). In contrast to the staining patterns observed with 8WG16, ARNA-3 stained replication compartments in both interphase and mitotic cells (Fig. 9).

These immunostaining data suggested that RNAP II may remain engaged on viral DNA during mitosis, but may be hyperphosphorylated and hence unreactive with antibody 8WG16. Therefore, it was important to confirm...
whether RNAP II was engaged on viral DNA or was indirectly associated with viral replication compartments. To do so, we analyzed RNAP II using in vivo cross-linking assays. We have used formaldehyde cross-linking previously to show that RNAP 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 RNAP II large subunits in infected and uninfected cells. As seen previously (Parsons and Spencer, 1997), the non–cross-linked large subunit of RNAP II in both interphase and mitotic uninfected cells displayed two phosphorylation variants: IIo, which is hyperphosphorylated, and IIa, which is nonphosphorylated. In uninfected cells, the transcriptionally engaged form of the RNAP II large subunit is hyperphosphorylated and the nonengaged form is nonphosphorylated. In mitotic cells, the IIo form may result from mitosis-specific phosphorylations 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 RNAP 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 RNAP 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 RNAP II large subunit also cross-linked to DNA (lane 6), but in mitotic infected cells little if any RNAP 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 RNAP II is...
not transcriptionally engaged in either infected or uninfected mitotic cells. It is possible however, that RNA Pol II interacts indirectly with replication compartments during mitosis, perhaps by associating with other proteins that bind to viral DNA, such as ICP4.

Discussion

A remarkable feature of mitosis is the cessation of all nuclear RNA synthesis and the loss of RNA Pol II and 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 Pol 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 (Shenmoen 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 Kipre, 1988; Allen and Verette, 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 chromosomal condensation. Such template modifying factors could include condensins, SMC proteins, or topoisomerases. Similarly, mitotic loss of chromatin remodeling factors such as SW1/SNF (Sif et al., 1998) or interaction of chromatin with negative regulatory proteins (Koepfer and Eisehman, 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 Pol III transcription in mitotic Xenopus extracts in the presence of topoisomerase inhibitors or in the absence of normal nucleosome structure (Hart 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 Pol I and RNA Pol III transcription factors mediate transcription repression in vitro (Hart 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 (Segi et al., 1991; Martinez-Balbas et al., 1995; Gottesfeld and Forbes, 1997).

Our data show that RNA Pol 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, SMC proteins, or 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 Pol II elongation complexes may simply complete a round of transcription and terminate within the 15–30 min of prophase. Thereafter, RNA Pol 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 Pol 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 HSV-1 mitotic transcription repression have interesting parallels with recent studies of mitotic repression of R N A P I transcription (Sirri et al., 2000). Although R N A P I transcription is repressed during mitosis, R N A P I and its transcription regulators remain localized to some nuclear organizing regions during mitosis (Zatsepin et al., 1993; Weisenberger and Scheer, 1995; Jordon et al., 1996; Roussel et al., 1996; Gebrane-Younes et al., 1997; Sirri et al., 1999). Nuclear organizing regions that remain associated with the R N A P I transcription machinery during mitosis also appear to contain noncondensed chromatin. Recent studies (Sirri 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 N A P I II transcription is not restored. It will be of interest to determine whether specific cdc2-cyclin B inhibitors can re-establish HS V-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 N A P I II transcription or for dissociation of transcription factors from mitotic DNA. These conclusions are consistent with previously hypothesized roles for mitotic modifications to the R N A P I II transcription machinery in effecting mitotic repression, but do not exclude the possibility that template modifications may contribute to mitotic repression in vivo.

We are grateful to M ihael Hendzel for helpful advice and discussions and to Dean Jackson for helpful discussion in establishing the protocol for labeling nascent RNA with halogenated nucleotides. We thank Maryse Fillion for preparation of stained cells for SEC analysis and Wei-Xiang Dong for excellent technical assistance during EM imaging. We also thank Priscilla Schaffer for providing the n212 virus and Henry Parker for review of the manuscript.

This work was supported by grants to C.A., Spencer from the Medical Research Council of Canada and the Alberta Cancer Board, and to D.P. Bazett-Jones from the Medical Research Council of Canada and the National Sciences and Engineering Research Council of Canada. C.A. Spencer is a Senior Scholar of the Alberta Heritage Foundation for Medical Research. M.J. Kruhlak is supported by a studentship award from U niversity Technologies International (University of Calgary).

Submitted: 7 February 2000

Revised: 4 May 2000

Accepted: 25 May 2000

References


Knoepf, P.S., and R.N. Eisenman. 1999. Sin meets NuRD and other tails of...
Published July 20, 2000

The Journal of Cell Biology, Volume 150, 2000