Histochem Cell Biol (1999) 111:405–410

ORIGINAL PAPER

Roeland W. Dirks · Sabine Snaar

Dynamics of RNA polymerase II localization during the cell cycle

Accepted: 3 March 1999

Abstract Mitosis is characterized by condensation of chromatin, cessation of RNA transcription, and redistribution of nuclear proteins. We investigated the distribution of the hypo- and hyperphosphorylated forms of RNA polymerase II in mitotic cells from different cell lines by immunofluorescence. In interphase cells, the hyperphosphorylated RNA polymerase II (Pol IIO) is present in speckles and diffusely throughout the nucleoplasm. In prophase, when speckles disappear, Pol IIO concentrates at the surface of chromosomes and, in addition, localizes in small spots throughout the cytoplasm. The association of Pol IIO with the surface of chromosomes is visible until the chromosomes start to decondense during late anaphase/early telophase. In telophase cells, Pol IIO is absent in newly formed nuclei but present in the cytoplasm, while Pol IIO disappears nearly completely in late telophase cells. In early G1 cells, when cell nuclei increase in size, Pol IIO becomes present in the nucleus, first in small spots and later diffusely and in speckles. The hypophosphorylated form of RNA polymerase II (Pol IIA) is nearly absent in mitotic cells suggesting that Pol IIA is hyperphosphorylated at the onset of mitosis. Because Pol IIO, unlike Pol IIA, cannot assemble in transcription preinitiation complexes, the conversion of Pol IIA to Pol IIO and the lining of chromosomes with Pol IIO might be underlying a mechanism by which mitotic cells repress their transcriptional activity.

Introduction

An important characteristic of mitosis is that RNA transcription and processing stops (Prescott and Bender 1962). This transcriptional inactivation is associated with redistribution of transcription and processing factors and extensive chromatin condensation, making gene sequences inaccessible for transcription factors. Concerning the redistribution of nuclear factors in mitosis, most of our current knowledge is derived from immunocytochemical studies of nucleolar components. Concomitant with the stop of RNA polymerase I (Pol I)-mediated transcription, responsible for the synthesis of rRNAs, nucleoli start to disintegrate in early prophase. Interestingly, cessation of rRNA synthesis proved not to be the result of the dissociation of Pol I from the transcriptionally active rRNA genes. Notably, Pol I was shown to remain associated with the condensed rRNA genes throughout mitosis in a state of arrested transcription (Scheer and Rose 1984; Matsui and Sandberg 1985; Gilbert et al. 1995). Also, some nucleolar proteins, including transcription initiation factor UBF (Roussel et al. 1993), TATA-binding protein/SL1 (Jordan et al. 1996), TATA-binding protein-associated factor for Pol I (Roussel et al. 1996), and the nucleolar organizer region protein pp135 (Pfeifle et al. 1986), remain associated with the rRNA genes. However, other nucleolar proteins involved in rRNA processing or ribosome assembly redistribute throughout the cytoplasm at the onset of mitosis. Many of these proteins, including for example fibrillarin (Yasuda and Maul 1990), the small nucleolar RNAs U3 and U14 (Beven et al. 1996), Ki-67 (Verheijen et al. 1989; Isola et al. 1990; Starborg et al. 1996), and major nucleolar phosphoprotein B23 (Zatsepina et al. 1997), were shown to accumulate at the surface of all chromosomes at late prophase/metaphase until early telophase while a few others remained uniformly distributed in the cytoplasm (Jiménez-García et al. 1994; Pai et al. 1995). In late telophase/early G1 all these factors were shown to move from the chromosome periphery or cytoplasm into forming nucleoli along a number of different pathways. Equal partitioning of factors between daughter cells, a reassembly of nuclei and nucleoli, as well as maintenance of chromosome integrity have been suggested as possible functions for the perichromosomal compartmentalization of nucleolar proteins.

R.W. Dirks (✉) · S. Snaar
Department of Molecular Cell Biology,
Laboratory for Cytochemistry and Cytometry,
Leiden University Medical Center, Wassenaarseweg 72,
2333 AL Leiden, The Netherlands
e-mail: Dirks@rullf2.LeidenUniv.nl
Tel.: +31-71-5276026

In contrast to rRNA processing factors, those needed for mRNA processing have not yet been found associated with specific chromosomal sites or with the surface of chromosomes. Immunofluorescence studies revealed that splicing factors, including snRNPs, are localized in speckle domains and more diffusely throughout the nucleoplasm excluding nucleoli in interphase cell nuclei (Spector 1993). This organization appeared highly dynamic and dependent on the transcriptional activity of genes (Dirks et al. 1997; Misteli et al. 1997). A more drastic reorganization of splicing factors was observed when cells entered mitosis. In metaphase and anaphase cells, splicing factors are found distributed throughout the cytoplasm and, in addition, concentrated in a few cytoplasmic foci. During telophase, splicing factors redistribute to daughter cell nuclei and when cells progress to G1 speckle domains are formed (Ferreira et al. 1994).

At this moment there is no detailed information available about the localization pattern of other mRNA processing factors in the mitotic phase of the cell cycle. The only non-nucleolar factors that are found associated with the perichromosomal compartment in mitosis are bcl-2 (Willingham and Bhalla 1994) and possibly RNA polymerase II (Pol II). Early biochemical work on isolated chromosomes provided evidence that Pol II is present in mitotic chromosomes as a structural component (Matsui et al. 1979). Furthermore, it was shown that the amount of Pol II associated with mitotic chromosomes was nearly the same as the amount associated with chromatin in interphase. These studies did not, however, provide morphological information nor did they make a distinction between the hypo- and hyperphosphorylated forms of Pol II. The hypophosphorylated form of Pol II (Pol IIA) is required for the formation of transcription preinitiation complexes and immunocytochemical studies revealed that it is widely distributed in interphase cell nuclei as small foci (Bregman et al. 1995). Concomitant with transcription initiation, Pol IIA gets hyperphosphorylated at the carboxy-terminal domain which is needed for the recruitment of RNA processing factors (Neugebauer and Roth 1997).

In interphase cell nuclei, hyperphosphorylated Pol II (Pol IIO) is found localized in speckle domains, which also contain splicing factors, and in small foci throughout the nucleoplasm which colocalize with sites of active gene transcription (Zeng et al. 1997). How Pol II localizes in mitotic cells is not completely understood yet because the limited number of studies on this subject are not conclusive. Thibodeau and Vincent (1991) found a 255-kDa phosphoprotein, identified as Pol IIO (Vincent et al. 1996), to be present in the cytoplasm of metaphase cells. In contrast to this observation, Kim et al. (1997) reported the presence of Pol IIO at the surface of metaphase chromosomes though they did not report its localization in other stages of mitosis. Because of this controversy and lack of knowledge, we studied the distribution of Pol IIA and Pol IIO in the different stages of mitosis using specific antibodies directed against Pol IIO and Pol IIA. Our results show that during prophase Pol IIO starts to accumulate at the periphery of chromosomes and remains associated with the chromosome periphery until anaphase. At late anaphase/early telophase when chromosomes decondense, the Pol IIO staining around chromosomes disappears and becomes visible in the cytoplasm. When cells proceed to telophase and when daughter cells are formed, the Pol IIO staining disappears almost completely. Finally, Pol IIO reappears in early G1 cells, first in dispersed foci and later in speckle domains. The amount of Pol IIA appears to be strongly reduced in the different stages of mitosis and Pol IIA never accumulates at the surface of chromosomes. The possible implications of these observations are discussed.

Materials and methods

Cell lines

Rat fibroblast 9G, human osteosarcoma U2OS, and HeLa (X1) cells were cultured on non-coated microscope glass slides in Dulbecco's modified Eagle medium supplemented with 0.03% glutamine, 1000 U/ml penicillin/streptomycin, and 10% fetal calf serum (all from Gibco BRL, Gaithersburg, Md., USA) in a 5% CO_2 atmosphere. To inhibit transcriptional activity of Pol II, cells were incubated for 0.5–2 h with 25 µg/ml 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB; Sigma, St. Louis, Mo., USA) or 0.5 µg/ml actinomycin D (Sigma).

Pretreatments and fixation of cells

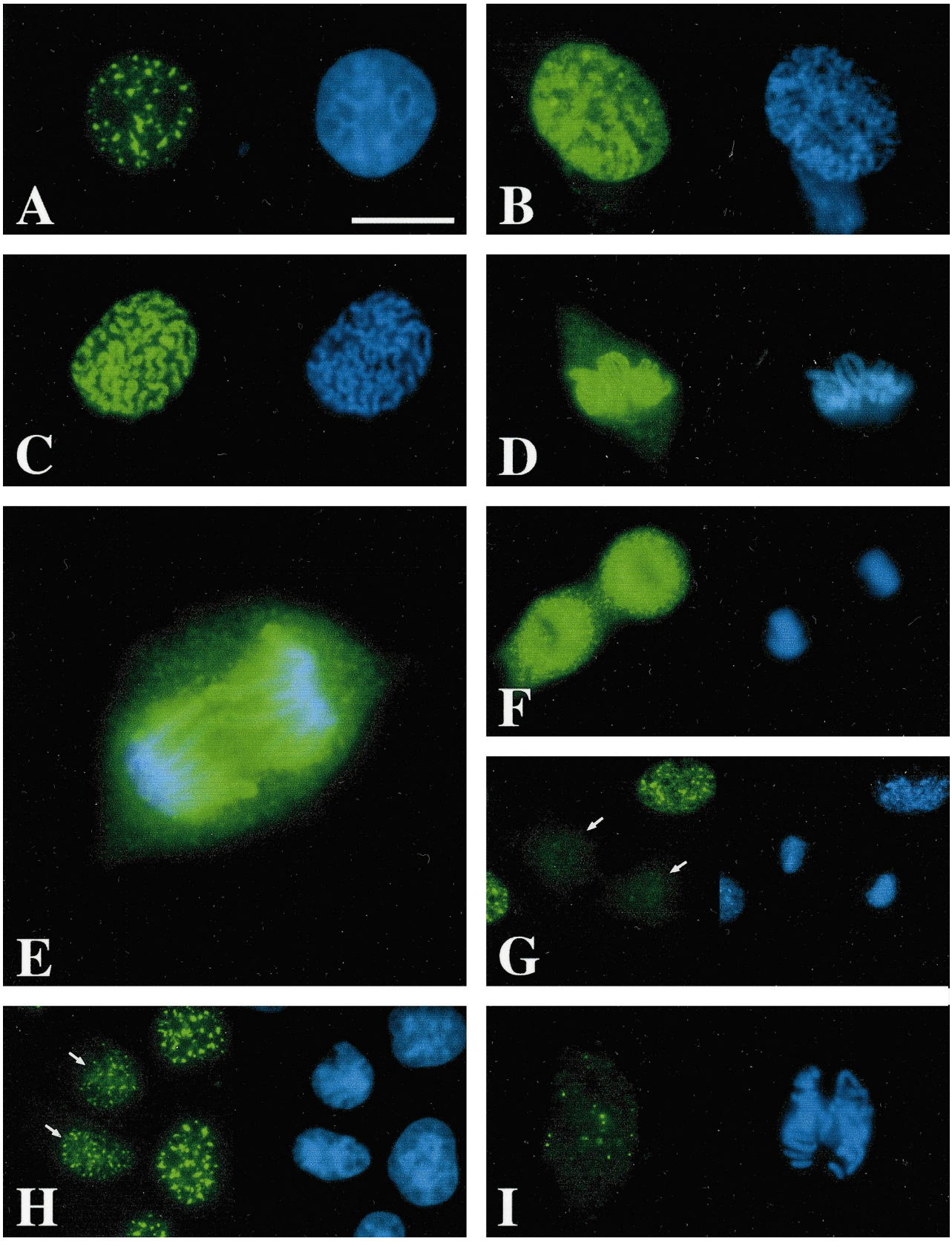
Slides with cells were briefly washed in PBS and fixed in one of the following fixatives for 15 min: 3.7% formaldehyde (Merck, Darmstadt, Germany), 5% acetic acid in PBS; 3.7% formaldehyde in PBS; 50% methanol/50% acetone (v/v); methanol only. After fixation in formaldehyde-containing fixatives, cells were briefly washed in PBS and stored in 70% ethanol at 4°C. Cells fixed in methanol/acetone or methanol were washed and stored in 70% ethanol at 4°C.

Some slides with cells were incubated in 0.1% Triton X-100 for 5 min at 4°C before fixation essentially as described (Dirks et al. 1995). Following this extraction, cells were either washed in PBS and fixed or incubated with 200 µg/ml RNase A in PBS for 30 min before fixation.

Immunocytochemical detection

Cells fixed in formaldehyde/acetic acid or in formaldehyde and stored in 70% ethanol were briefly washed in deionized water and pretreated with 0.1% (w/v) pepsin (Sigma) in 0.01 M HCl for

Fig. 1A–I Localization of RNA polymerase II in rat 9G cells ▶ which are in different stages of the cell cycle. Hyperphosphorylated RNA polymerase II (Pol IIO; **A–H**) and hypophosphorylated RNA polymerase II (Pol IIA; **I**) were localized by immunofluorescence (*green*) using mAb CC-3 and mAb 8WG16, respectively. **A** Localization of Pol IIO in an interphase cell. In early prophase (**B**)/prophase (**C**), Pol IIO redistributes and is found localized near the condensing chromosomes. **D,E** Metaphase and anaphase cells revealed a clear staining of Pol IIO lining the chromosomes. The numerous small cytoplasmic spots are not in focus. In telophase cells, Pol IIO is only detectable in the cytoplasm (**F**) and in late telophase cells there is no Pol IIO detectable anymore (**G**, *arrows*). When cells are in early G1, a fine punctate distribution of Pol IIO is observed in the nucleus (**H**, *arrows*). **I** Metaphase cells stained with mAb 8WG16 revealed a few spots but no association of Pol IIA with chromosomes. Cells are counterstained with DAPI (*blue*). *Bar* 15 µm


A
B
C
D
E
F
G
H
I

1 min at 37°C. Then, cells were briefly washed in deionized water, washed for 3 min in 70% ethanol to inactivate the pepsin, and washed for 3 min in TRIS-buffered saline (TBS: 150 mM NaCl, 100 mM TRIS-HCl, pH 7.4). Cells were then incubated with the first antibody which may consist of mAb CC-3 (Vincent et al. 1996) for the detection of Pol IIO, mAb 8WG16 (Thompson et al. 1989; Promega, Madison, Wis., USA) for detection of Pol IIA, or mAb anti-m3G (Oncogene Science, Cambridge, Mass., USA) for the detection of splicing factors. Methanol/acetic acid- and methanol-fixed cells were washed in TBS and incubated with one of these antibodies. After the first antibody layer, cells were washed 3×5 min in TBS and then incubated with rabbit anti-mouse FITC (Sigma). All antibodies were diluted in TBS containing 0.5% (w/v) blocking reagent (Boehringer Mannheim, Mannheim, Germany) and incubations were done for 30 min at room temperature. Finally, cells were washed 3×5 min in TBS, dehydrated in a graded ethanol series, and mounted in Vectashield (Vector Laboratories, Burlingame, Calif., USA) containing 4′,6-diamidino-2-phenylindole (DAPI) as a DNA counterstain.

Microscopy

A Leica epifluorescence microscope (DM; Leica) equipped with a 100-W mercury arc lamp, single bandpass filters for fluorescein and DAPI, and a 63× oil objective with a 1.3 numerical aperture was used to examine the cells. Digital images were collected with a cooled CCD camera (Photometrics) and processed on a Macintosh computer using SCIL-image (Multihouse, The Netherlands).

Results

Using mAb CC-3, which has previously been shown to react with a phosphoepitope on the carboxy-terminal domain of RNA polymerase (Vincent et al. 1996), we have analyzed the distribution of Pol IIO in rat fibroblast 9G as well as in human U2OS and HeLa (X1) cells. Interphase cells of these cell types revealed a speckled distribution of Pol IIO in addition to a more diffuse staining throughout the nucleoplasm excluding nucleoli (Fig. 1A). These patterns were very similar to those described previously (Mortillaro et al. 1996; Neugebauer and Roth 1997). In early prophase, when chromosomes start to condense and nucleoli disassemble, the pattern of Pol IIO localization changed. The staining of speckles gradually disappeared while Pol IIO started to accumulate near the condensing chromatin (Fig. 1B). As prophase proceeds, the association of Pol IIO with the boundaries of chromosomes became more clear and speckles were no longer visible (Fig. 1C). In approximately 20% of the HeLa (X1) cells some bright foci were observed in addition to the staining around chromosomes. Metaphase and early anaphase cells of all cell lines tested revealed an intense staining of chromosome boundaries in addition to punctate cytoplasmic signals (Fig. 1D,E). The cytoplasmic signals, which are out of focus in Fig. 1D and E, were most abundant and most intense in HeLa (X1) cells and obscured the signals derived from the chromosome boundaries. Judged visually, the fluorescence staining of metaphase cells with mAb CC-3 appeared much more intense than the staining of interphase cells. In late anaphase/early telophase cells, when chromosomes start to decondense, the Pol IIO staining of chromosomes disappeared while the cytoplasmic staining remained present (Fig. 1F). Interestingly, no or hardly any Pol IIO was observed in late telophase/early G1 cells (Fig. 1G). This was consistently found in all cell lines used in this study. At the next stage, when daughter cell nuclei increased in size, a spot-like Pol IIO staining pattern was observed (Fig. 1H).

Cells were also stained with antibodies specific for splicing factors while the same secondary antibody was used by which mAb CC-3 was detected. In accordance with previous studies (Ferreira et al. 1994), these antibodies did not reveal any staining of chromosome boundaries in mitotic cells. Instead, splicing factors were located diffusely and in a few foci in the cytoplasm of these cells (result not shown).

To exclude the possibility that the observed staining patterns are the result of a fixation artifact, rat 9G and U2OS cells were fixed using different fixatives. All fixatives tested revealed identical localization patterns of Pol IIO though strongest fluorescence intensities were observed in cells fixed with methanol/acetone followed by cells fixed in formaldehyde/acetic acid. However, when cells were fixed in methanol/acetic acid, the chromosomes appeared to be swollen.

To learn more about the nature of Pol IIO association with the chromosomal periphery, cells were treated with Triton X-100 before fixation. After such a treatment the intensity of Pol IIO staining around chromosomes did not diminish, not even when this treatment was followed by an incubation with RNase A. The number and staining intensity of speckle domains in interphase cells, however, was found strongly reduced. Also, when cells were cultured in the presence of the transcription inhibitors DRB or actinomycin D, the Pol IIO staining pattern in mitotic cells appeared unaffected while speckle domains in interphase cells showed characteristic changes such as rounding-up and an increase in fluorescence intensity (result not shown).

We also investigated the distribution pattern of Pol IIA with mAb 8WG16. Pol IIA revealed a punctate staining pattern throughout the nucleus in interphase cells, which is in accordance with previous studies (Bregman et al. 1995). Mitotic cells, however, revealed much less Pol IIA staining compared to interphase cells and no staining of chromosome boundaries (Fig. 1I).

Discussion

Pol IIA and Pol IIO have been shown to have different localization patterns in interphase cells. Pol IIA is present in numerous small foci throughout the cell nucleus while Pol IIO is concentrated in speckle domains and diffusely localized in the nucleoplasm. In this study, we show that the different forms of Pol II also have different localization patterns in mitotic cells. A subset of Pol IIO is shown to associate with the periphery of chromosomes when cells enter mitosis and to line the periphery of chromosomes until late anaphase is reached and when

chromosomes start to decondense. Another subset of Pol IIO was observed in the cytoplasm of mitotic cells in a punctate pattern. In telophase cells, Pol IIO was detected only in the cytoplasm of cells, while at late telophase and just after cytokinesis Pol IIO could not be detected anymore. As the daughter cell nuclei increase in size, Pol IIO staining was observed again in the nucleus, first in small spots and later in speckles. That the strong staining of Pol IIO at the surface of chromosomes has not been noticed in previous studies could be due to the strong, punctate cytoplasmic staining of Pol IIO in metaphase cells of some cell types (Thibodeau and Vincent 1991). Only recently, Pol IIO was shown present at the periphery of chromosomes using a different mAb specific for Pol IIO (mAb H5) than we used in this study (Kim et al. 1997). Previous studies, however, did not reveal a staining of chromosomes with mAb H5 (Warren et al. 1992).

Our microscopic observations support early biochemical studies showing that Pol II is present in isolated mitotic chromosomes and we show now that it is Pol IIO that decorates the chromosomes. However, because of the limited resolution of the light microscope it is not possible to conclude whether Pol IIO associates only with the chromosome surface or forms a structural component of chromosomes. Staining of cells with mAb 8WG16 revealed that the amount of Pol IIA staining is greatly reduced in mitotic cells compared to interphase cells and that Pol IIA is not associated with the periphery of chromosomes. Previously it has been suggested that the increase of Pol IIO staining in mitotic cells is due to a better accessibility of Pol IIO for antibodies in mitotic cells and we can also reason that the decrease in Pol IIA staining is caused by masking of Pol IIA in mitotic cells rendering them inaccessible for antibody detection. It may, however, also be true that the decrease of Pol IIA staining is directly related to the increase in Pol IIO staining. Because it has been shown that the total amount of RNA polymerase in metaphase cells remains the same as in interphase cells, this could mean that Pol IIA is hyperphosphorylated at the start of mitosis. This would make sense because Pol IIA is required for the formation of transcription initiation complexes. Because Pol IIO cannot be assembled into preinitiation complexes (see for example Chesnut et al. 1992), the transition from Pol IIA to Pol IIO might be a mechanism by which cells block their transcriptional activity during mitosis.

Phosphorylation of Pol IIA can be mediated by a number of kinases including the basal transcription factor TFIIH and Cdc2. Cdc2 kinase is activated when cells enter mitosis and it has recently been shown that phosphorylation of the carboxy-terminal domain by Cdc2 kinase can inhibit transcription (Gebara et al. 1997). Gebara et al. (1997) also observed an increase in the abundance of Pol IIO during mitosis which would be in accordance with our observation that the fluorescence intensity of Pol IIO staining in mitotic cells is much higher compared to that in interphase cells. However, it is also possible that the mAb CC-3 recognizes, in addition to phosphoepitopes on Pol II, phosphoepitopes on other proteins which are formed during mitosis and are present around chromosomes. This possibility is currently being investigated by Dr. Michel Vincent's group (Université Laval, Canada). On the basis of our observations and those of Gebara et al. (1997), we can postulate that at the onset of mitosis Pol IIA, which is present near preinitiation complexes, gets hyperphosphorylated and can, therefore, be detected at the surface of condensed chromosomes as Pol IIO with mAb CC-3. Whether Pol IIO, which is present in speckles and diffusely distributed throughout the nucleoplasm at interphase, also associates with the surface of chromosomes or moves together with splicing factors to the cytoplasm cannot be answered here.

In addition to Pol IIO, many other factors, mostly derived from nucleoli, are found associated with mitotic chromosomes (see Introduction). It has been suggested that nuclear factors line the chromosomes to be equally distributed among daughter cells. In the case of Pol II this seems less likely because at the end of telophase there was hardly any Pol II staining observed in the cytoplasm and nucleus of cells. Alternatively, it has been suggested that nuclear factors form a coat around the chromosomes to preserve their integrity during cell division. Like the nucleolar factors, Pol IIO is firmly associated with chromosomes because it cannot be removed by Triton X-100 or RNase treatment. Therefore, we cannot exclude the possibility that Pol IIO plays a role in preserving chromosome integrity in addition to ensuring transcriptional repression. Future studies may tell us how Pol IIO is bound to the surface of mitotic chromosomes and whether some of the nucleolar components, also shown to line the chromosomes in mitosis, interact with Pol IIO and play a role in preventing transcription.

Acknowledgements We thank Michel Vincent for kindly providing the anti-Pol IIO antibody CC-3 and Anton K. Raap for critical reading of the manuscript.

References

Beven AF, Lee R, Razaz M, Leader DJ, Brown JWS, Shaw PJ (1996) The organization of ribosomal RNA processing correlates with the distribution of nucleolar snRNAs. J Cell Sci 109:1241–1251

Bregman DB, Du L, Van der Zee S, Warren SL (1995) Transcription-dependent redistribution of the large subunit of RNA polymerase II to discrete nuclear domains. J Cell Biol 129: 287–298

Chesnut JD, Stephens JH, Dahmus ME (1992) The interaction of RNA polymerase II with the adenovirus-2 major late promoter is precluded by phosphorylation of the C-terminal domain of subunit IIa. J Biol Chem 267:10500–10506

Dirks RW, Daniël KC, Raap AK (1995) RNAs radiate from gene to cytoplasm as revealed by fluorescence in situ hybridization. J Cell Sci 108:2565–2572

Dirks RW, Pauw ESD de, Raap AK (1997) Splicing factors associate with nuclear HCMV-IE transcripts after transcriptional activation of the gene, but dissociate upon transcription inhibition: evidence for a dynamic organization of splicing factors. J Cell Sci 110:515–522

Ferreira JA, Carmo-Fonseca M, Lamond AI (1994) Differential interaction of splicing snRNPs with coiled bodies and inter-

chromatin granules during mitosis and assembly of daughter cell nuclei. J Cell Biol 126:11–23

Gebara MM, Sayre MH, Corden JL (1997) Phosphorylation of the carboxy-terminal repeat domain in RNA polymerase II by cyclin-dependent kinases is sufficient to inhibit transcription. J Cell Biochem 64:390–402

Gilbert N, Lucas L, Klein C, Menager M, Bonnet N, Ploton D (1995) Three-dimensional co-localization of RNA polymerase I and DNA during interphase and mitosis by confocal microscopy. J Cell Sci 108:115–125

Isola J, Helin H, Kallioniemi O-P (1990) Immunoelectron-microscopic localization of a proliferation-associated antigen Ki-67 in MCF-7 cells. Histochem J 22:498–506

Jiménez-García LF, Segura-Valdez ML, Ochs RL, Rothblum LI, Hannan R, Spector DL (1994) Nucleogenesis: U3 snRNA containing prenucleolar bodies move to sites of active pre-rRNA transcription after mitosis. Mol Biol Cell 5:955–966

Jordan P, Mannervik M, Tora L, Carmo-Fonseca M (1996) In vivo evidence that TATA-binding protein/SL1 colocalizes with UBF and RNA polymerase I when rRNA synthesis is either active or inactive. J Cell Biol 133:225–234

Kim E, Du L, Bregman DB, Warren SL (1997) Splicing factors associate with hyperphosphorylated RNA polymerase II in the absence of pre-mRNA. J Cell Biol 136:19–28

Matsui S, Sandberg AA (1985) Intranuclear compartmentalization of DNA-dependent RNA polymerases: association of RNA polymerase I with nucleolar organizing chromosomes. Chromosoma 92:1–6

Matsui S, Weinfeld H, Sandberg AA (1979) Quantitative conservation of chromatin-bound RNA polymerases I and II in mitosis. J Cell Biol 80:451–464

Misteli T, Cáceres JF, Spector DL (1997) The dynamics of a pre-mRNA splicing factor in living cells. Nature 387:523–527

Mortillaro MJ, Blencowe BJ, Wei X, Nakayasu H, Du L, Warren SL, Sharp PA, Berezney R (1996) A hyperphosphorylated form of the largest subunit of RNA polymerase II is associated with splicing complexes and the nuclear matrix. Proc Natl Acad Sci USA 93:8253–8257

Neugebauer KM, Roth MB (1997) Transcription units as RNA processing units. Genes Dev 11:3279–3285

Pai C-Y, Chen HK, Sheu H-L, Yeh N-H (1995) Cell cycle-dependent alterations of a highly phosphorylated nucleolar protein p130 are associated with nucleogenesis. J Cell Sci 108:1911–1920

Pfeifle J, Boller K, Anderer AF (1986) Phosphoprotein pp135 is an essential component of the nucleolus organizer region (NOR). Exp Cell Res 162:11–22

Prescott DM, Bender MA (1962) Synthesis of RNA and protein during mitosis in mammalian tissue culture cells. Exp Cell Res 26:260–268

Roussel P, André C, Masson C, Geraud G, Hernandez-Verdun D (1993) Localization of RNA polymerase I transcription factor hUBF during the cell cycle. J Cell Sci 104:327–337

Roussel P, André C, Comai L, Hernandez-Verdun D (1996) The rDNA transcription machinery is assembled during mitosis in active NORs and absent in inactive NORs. J Cell Biol 133: 235–246

Scheer U, Rose KM (1984) Localization of RNA polymerase I in interphase cells and mitotic chromosomes by light and electron microscopic immunocytochemistry. Proc Natl Acad Sci USA 81:1431–1435

Spector DL (1993) Macromolecular domains within the cell nucleus. Annu Rev Cell Biol 9:265–315

Starborg M, Gell K, Brundell E, Höög C (1996) The murine Ki-67 cell proliferation antigen accumulates in the nucleolar and heterochromatic regions of interphase cells and at the periphery of the mitotic chromosomes in a process essential for cell cycle progression. J Cell Sci 109:143–153

Thibodeau A, Vincent M (1991) Monoclonal antibody CC-3 recognizes phosphoproteins in interphase and mitotic cells. Exp Cell Res 195:145–153

Thompson NE, Steinberg TH, Aronson DB, Burgess RR (1989) Inhibition of in vivo and in vitro transcription by monoclonal antibodies prepared against wheat germ RNA polymerase II that react with the heptapeptide repeat of eukaryotic RNA polymerase II. J Biol Chem 264:11511–11520

Verheijen R, Kuijpers HJH, Driel R van, Beck JLM, Dierendonck JH van, Brakenhoff GJ, Ramaekers FCS (1989) Ki-67 detects a nuclear matrix-associated proliferation-related antigen II. Localization in mitotic cells and association with chromosomes. J Cell Sci 92:531–540

Vincent M, Lauriault P, Dubois M-F, Lavoie S, Bensaude O, Chabot B (1996) The nuclear matrix protein p255 is a highly phosphorylated form of RNA polymerase II largest subunit which associates with splicieosomes. Nucleic Acids Res 24:4649–4652

Warren SL, Landolfi AS, Curtis C, Morrow JS (1992) Cytostellin: a novel, highly conserved protein that undergoes continuous redistribution during the cell cycle. J Cell Sci 103:381–388

Willingham MC, Bhalla K (1994) Transient mitotic phase localization of Bcl-2 oncoprotein in human carcinoma cells and its possible role in prevention of apoptosis. J Histochem Cytochem 42:441–450

Yasuda Y, Maul GG (1990) A nucleolar auto-antigen is part of a major chromosomal surface component. Chromosoma 99: 152–160

Zatsepina OV, Todorov IT, Philipova RN, Krachmarov CP, Trendelenburg MF, Jordan EG (1997) Cell cycle-dependent translocations of a major nucleolar phosphoprotein, B23, and some characteristics of its variants. Eur J Cell Biol 73:58–70

Zeng C, Kim E, Warren S, Berget S (1997) Dynamic relocation of transcription and splicing factors dependent upon transcriptional activity. EMBO J 16:1401–1412