Short Communication

The DNA topoisomerase I inhibitor camptothecin blocks postmitotic reformation of nucleoli in mammalian cells

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Introduction

During mitotic prophase of most eukaryotic cells, transcription of the rRNA genes ceases and nucleolar structure breaks down. While some nucleolar protein components remain associated with the chromosomal NORs, others bind to the surface of the chromosomes or become dispersed throughout the cytoplasm of dividing cells (see Tab. I in [1]). During telophase, nucleoli reform progressively at the NORs in a process termed nucleologenesis (see [7, 21]). Experimentally, nucleologenesis can be divided into two steps: *i*) assembly of prenucleolar bodies (PNBs) from dispersed nucleolar material derived from the mother cell and *ii*) subsequent fusion of PNBs around the NORs in a process which is dependent on the resumption of rDNA transcription [3]. This conclusion was based on microinjection experiments in which antibodies to RNA polymerase I were introduced into mitotic cells in order to prevent reactivation of the rRNA genes. Daughter cells formed normally under such conditions with the notable exception that they lacked nucleoli since nucleologenesis did not proceed beyond the PNB-stage [3, 4].

Previous studies have shown that the enzyme DNA topoisomerase I is required for transcript elongation of protein-encoding as well as rRNA genes [6, 8, 10, 14, 16, 20, 23]. Further studies indicated that DNA topoisomerase I activity can be selectively blocked by camptothecin, a drug with potential applications in tumor therapy (for review, see [13]). In the present study we have investigated the effects of camptothecin on postmitotic nucleolar reorganization.

Abbreviations. DMSO Dimethyl sulfoxide.—FITC Fluorescein isothiocyanate.—NOR Nucleolus organizer region.—PNB Prenucleolar body.—PBS Phosphate buffered saline.

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Materials and methods

Camptothecin lactone (NSC-94600) was a gift of the Drug Synthesis and Chemistry Branch of the National Cancer Institute (Bethesda, MD/USA). A stock solution of camptothecin was prepared (10 mM in DMSO) and stored at −20°C.

In order to investigate the effects of camptothecin on nucleologenesis, protocols previously developed for similar studies with the DNA-intercalating drug actinomycin D were followed [3]. PtK_2 cells grown on coverslips were labeled at metaphase by microinjecting non-immune mouse IgGs. Camptothecin was then added to the culture medium at a final concentration of 20 μM for at least 2 h. Control experiments were performed by adding an equal volume of DMSO alone to the culture medium (final concentration 0.2%).

Coverslips were fixed at various times after addition of the drug in methanol (−20°C) for 10 min, dipped for a few seconds in acetone (−20°C) and air dried. The cells were then processed for double-label immunofluorescence microscopy. The distribution of the microinjected mouse IgGs was visualized by incubating the coverslips with anti-mouse IgG antibodies conjugated to Texas Red (Dianova, Hamburg/Germany) for 15 min. Then the specimens were washed for 15 min in PBS and incubated with human autoantibodies against either the nucleolar protein fibrillarin [17], DNA topoisomerase I (kindly provided by Dr. H. Ponstingl, German Cancer Reserarch Center, Heidelberg/Germany) or RNA polymerase I [19] for 15 min. Finally, the coverslips were washed in PBS and incubated with secondary anti-human IgG conjugated to FITC (Dianova; for further details, see [5]).

Results and discussion

In order to follow the time course of nucleologenesis, individual PtK_2 cells were labeled at metaphase by microinjection of non-immune mouse IgGs. After various times cells were then processed for immunofluorescence microscopy.

Mitosis proceeded normally under these experimental conditions. Daughter cells had entered interphase within 2 h as judged by the decondensed state of their chromatin and the presence of distinct nucleoli (Fig. 1; see also [2]). Daughter cells derived from an injected mother cell could be clearly identified by the presence of mouse IgGs in the cytoplasm (Fig. 1a). When probed with antibodies to the nucleolar protein fibrillarin, the newly formed nucleoli exhibited a bright fluorescence which was indistinguishable from the nucleolar fluorescence of adjacent non-injected cells (Fig. 1a′). Addition of 0.2% DMSO to the culture at the time of injection had no noticeable effects on postmitotic reformation of the nuclei and nucleoli of daughter cells (Fig. 1).

In contrast, in the presence of camptothecin at concentrations known to inhibit DNA topoisomerase I activity (see, e.g. [15]) nucleologenesis was severely inhibited. Metaphase cells exposed to the drug completed mitosis and divided normally. Two hours later, the nuclei of the resulting daughter cells appeared "empty" when viewed in phase contrast (Figs. 2a″, b″, c″). However, antibodies to fibrillarin revealed a finely punctate pattern of their nuclei resembling PNBs of early telophase (Fig. 2a′). Antibodies to DNA topoisomerase I stained the nucleoplasm and a single prominent dot-like structure in each daughter nucleus which most probably corresponds to the NOR (Fig. 2b′; the male PtK_2 cells have only one NOR per chromosome set located on the X chromosome). The NOR was also positive for RNA polymerase I (Fig. 2c′). Earlier studies have shown that both types of enzyme molecules remain, at least in part, bound to the chromosomal NORs during normal mitosis [11, 22].

Treatment of interphase cells with camptothecin for 2 h caused a segregation of nucleolar components. This structural rearrangement and spatial separation of the nucleolar components could be readily visualized by phase contrast optics in all non-injected cells (e.g., Fig. 2a″). Ultrastructural aspects of this process have already been described (e.g., [9] and references therein). Antibodies to fibrillarin stained selectively the phase-light (i.e., dense fibrillar) component (Figs. 2a′, a″; see also [18]) whereas RNA polymerase I and DNA topoisomerase I were present in "caps" of the segregated nucleoli (Figs. 2b′, b″; c′, c″; see also [20, 22]). Segregation of nucleolar components can also be induced by other drugs such as actinomycin D which inhibit transcription (for review, see [12]). We therefore conclude that camptothecin-induced nucleolar segregation is a consequence of blocked rDNA transcription.

The results of the present study demonstrate that camptothecin not only causes nucleolar segregation but also inhibits postmitotic nucleolar reformation at the PNB stage. Since the activity of DNA topoisomerase I is required for transcription of the rRNA genes [6, 8, 10, 14, 16, 20, 23], the inability of PNBs to fuse around the NORs appears to be related to the inability of the rRNA genes to activate transcription.

In a previous study we have shown that when postmitotic transcription of the rRNA genes was selectively blocked by microinjection of antibodies to RNA polymerase I into metaphase cells, nucleologenesis was also arrested at the PNB-stage [3]. This finding in conjunction with those presented above allow us to conclude that formation of multiple PNBs in telophasic cells is independent

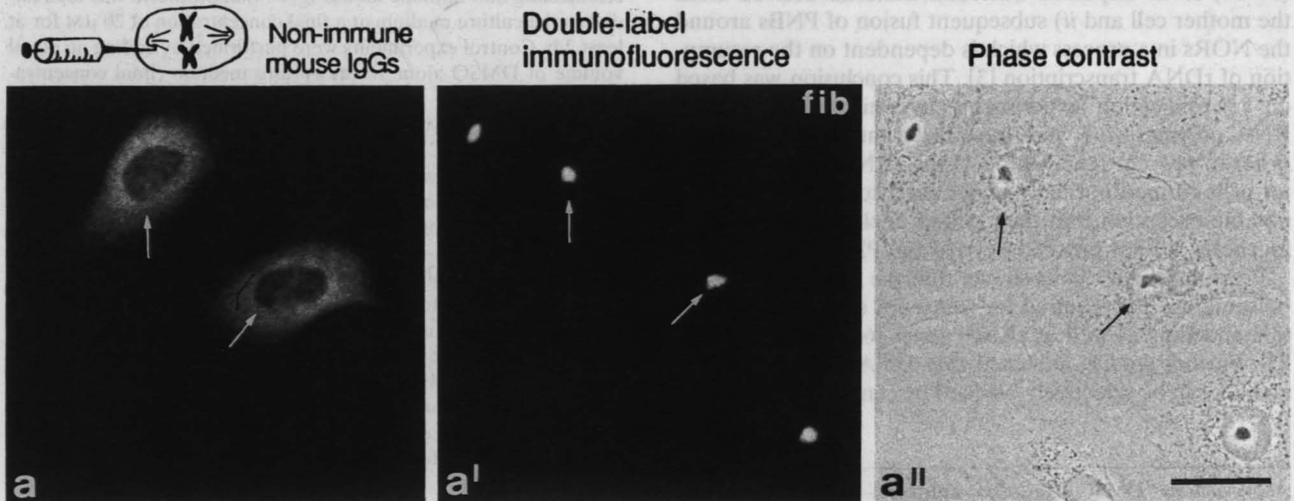

Fig. 1. Daughter cells reach interphase no later than 2 h after microinjection of non-immune IgGs into a dividing cell. To identify postmitotic cells, a metaphase PtK_2 cell was microinjected with mouse IgGs and examined 2 h later by phase contrast (**a″**) and double-label immunofluorescence microscopy. The distribution of the mouse IgGs is shown in **a** (only the daughter cells are labeled; note the exclusion of the IgGs from the nuclei). The nuclei of the daughter cells contain well developed nucleoli as seen by phase contrast (**a″**) and immunofluorescence microscopy using antibodies to fibrillarin (**a′**). *Arrows* denote the daughter cells.—Bar 20 μm.

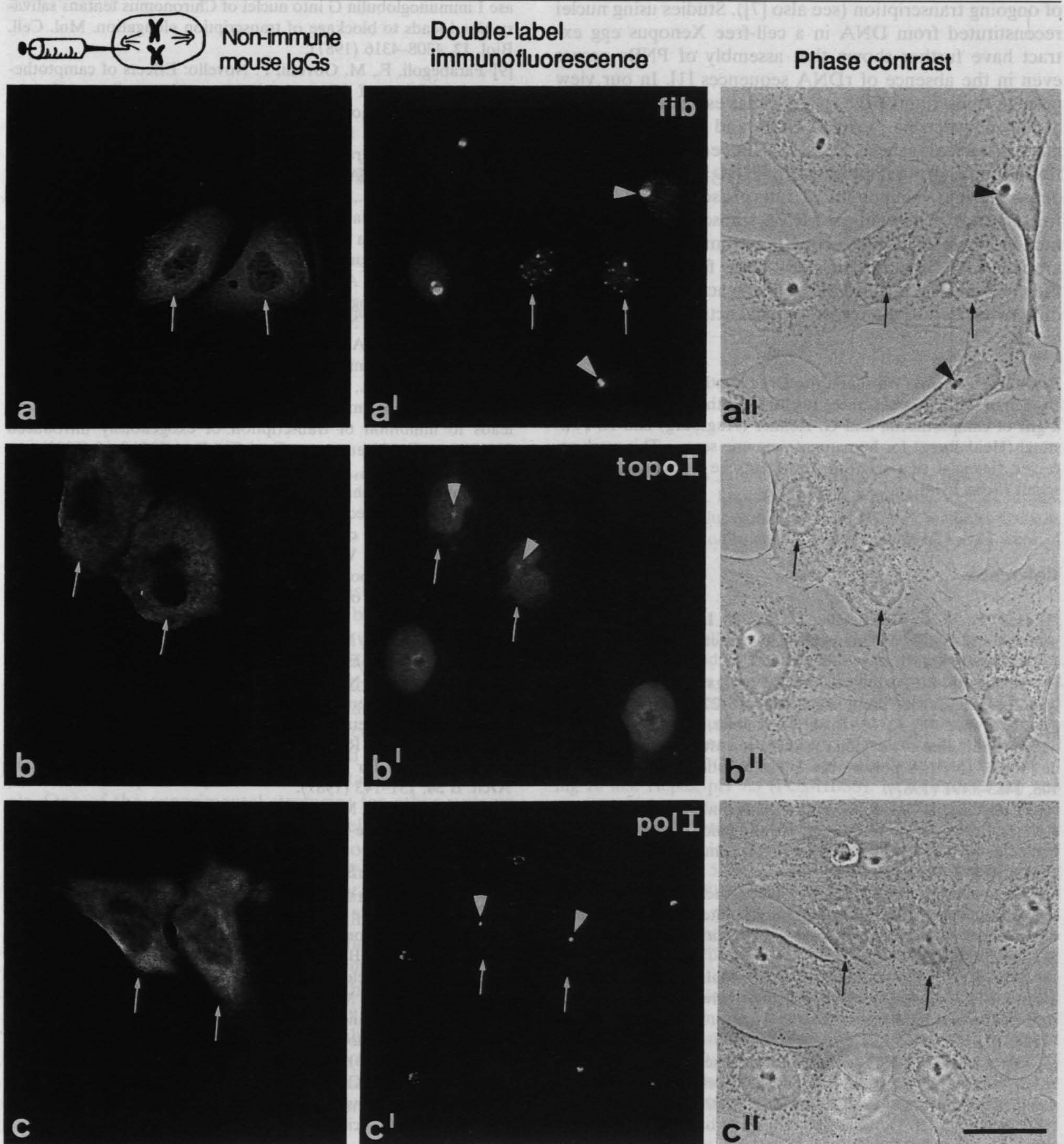

Fig. 2. Effect of camptothecin on nucleolar reformation. Dividing PtK_2 cells were injected with non-immune mouse IgGs and cultured for 2 h in the presence of camptothecin. Double-label immunofluorescence reveals the distribution of the mouse IgGs (**a–c**; *arrows*) as well as fibrillarin (**a'**), DNA topoisomerase I (**b'**) and RNA polymerase I (**c'**). *Arrowheads* point to the putative NORs (**b', c'**). Note the absence of nucleoli in the postmitotic daughter cells as revealed by phase-contrast microscopy (**a"–c"**). Nucleoli of non-injected cells are segregated as a result of the camptothecin action (some are indicated by *arrowheads* in **a', a"**). — Bar 20 μm.

of ongoing transcription (see also [7]). Studies using nuclei reconstituted from DNA in a cell-free Xenopus egg extract have further shown that assembly of PNBs occurs even in the absence of rDNA sequences [1]. In our view nascent transcripts of the rRNA genes establish a structural link between an "active" NOR and the PNBs during normal nucleologenesis [3, 4, 21]. Indeed we could locate fibrillarin, a protein present in PNBs as well as in the dense fibrillar component of interphase nucleoli, to the free 5′-ends of nascent pre-rRNA transcript fibrils by immunogold electron microscopy of chromatin-spread preparations [21]. The present study lends further support to the view that the nucleolus organizing activity of a NOR is directly related to the transcriptional activity of the rRNA genes.

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References

[1] Bell, P., M. C. Dabauvalle, U. Scheer: In vitro assembly of prenucleolar bodies in Xenopus egg extract. J. Cell Biol. **118**, 1297–1304 (1992).

[2] Benavente, R.: Postmitotic nuclear reorganization events analyzed in living cells. Chromosoma **100**, 215–220 (1991).

[3] Benavente, R., K. M. Rose, G. Reimer, B. Hügle-Dörr, U. Scheer: Inhibition of nucleolar reformation after microinjection of antibodies to RNA polymerase I into mitotic cells. J. Cell Biol. **105**, 1483–1491 (1987).

[4] Benavente, R., M. S. Schmidt-Zachmann, B. Hügle-Dörr, G. Reimer, K. M. Rose, U. Scheer: Identification and definition of nucleolus-related fibrillar bodies in micronucleated cells. Exp. Cell Res. **178**, 518–523 (1988).

[5] Benavente, R., U. Scheer, N. Chaly: Nucleocytoplasmic sorting of macromolecules following mitosis: fate of nuclear constituents after inhibition of pore complex function. Eur. J. Cell Biol. **50**, 209–219 (1989).

[6] Brill, S. J., S. Di Nardo, K. Voelker-Meiman, R. Sternglanz: Need for DNA topoisomerase activity as swivel for DNA replication and for transcription of ribosomal genes. Nature **326**, 414–416 (1987).

[7] De la Torre, C., G. Giménez-Martín: The nucleolar cycle. In: E. G. Jordan, C. A. Cullis (eds.): The Nucleolus. pp. 153–177. Cambridge University Press. Cambridge 1982.

[8] Egyhazi, E., E. Durban: Microinjection of DNA topoisomerase I immunoglobulin G into nuclei of Chironomus tentans salivary gland leads to blockage of transcription elongation. Mol. Cell. Biol. **12**, 4308–4316 (1987).

[9] Farabegoli, F., M. Govoni, F. Novello: Effects of camptothecin, an inhibitor of DNA topoisomerase I, on ribosomal gene structure and function in TG cells. Biol. Cell **74**, 281–286 (1992).

[10] Garg, L. C., S. Di Angelo, S. T. Jacob: Role of DNA topoisomerase I in the transcription of supercoiled rRNA gene. Proc. Natl. Acad. Sci. USA **84**, 3185–3188 (1987).

[11] Guldner, H. H., C. Szostecki, H. P. Vosberg, H. J. Lakomek, E. Penner, R. A. Bautz: Scl 70 autoantibodies from scleroderma patients recognize a 95 kDa protein identified as DNA topoisomerase I. Chromosoma **94**, 132–138 (1986).

[12] Hadjiolov, A. A.: The nucleolus and ribosome biogenesis. Cell Biology Monographs. Vol. 12, pp. 1–268. Springer-Verlag. Berlin, Heidelberg, New York 1985.

[13] Liu, L. F.: DNA topoisomerase I poisons as antitumor drugs. Annu. Rev. Biochem. **58**, 351–375 (1989).

[14] Matsumoto, K., I. Sugawara, T. Takahashi, T. Andoh: Injection of anti-topoisomerase I IgG into nuclei of mouse L929 cells leads to inhibition of transcription of exogenously introduced SV40 sarge T antigene gene. Exp. Cell Res. **204**, 6–10 (1993).

[15] Mattern, M. R., S. M. Mong, H. F. Bartus, C. K. Mirabelli, S. T. Crooke, R. K. Johnson: Relationships between the intracellular effects of camptothecin and the inhibition of DNA topoisomerase I in cultured L1210 cells. Cancer Res. **47**, 1793–1798 (1987).

[16] Muller, M. T., W. P. Pfund, V. B. Metha, D. K. Trask: Eukaryotic type I topoisomerase is enriched in the nucleolus and catalytically active on ribosomal DNA. EMBO J. **4**, 1237–1243 (1985).

[17] Reimer, G., K. M. Pollard, C. A. Penning, R. L. Ochs, M. A. Lischwe, H. Busch, E. M. Tan: Monoclonal antibody from a (New Zealand black and New Zealand white) F1 mouse and some human scleroderma sera target a M_r 34,000 protein of the U3 RNP particle. Arthr. Rheum. **30**, 793–800 (1987).

[18] Reimer, G., I. Raska, E. M. Tan, U. Scheer: Human autoantibodies: probes for nucleolus structure and function. Virchows Arch. B **54**, 131–143 (1987).

[19] Reimer, G., K. M. Rose, U. Scheer, E. M. Tan: Autoantibody to RNA polymerase I in scleroderma sera. J. Clin. Invest. **79**, 65–72 (1987).

[20] Rose, K. M., J. Szopa, F. S. Han, Y. C. Cheng. A. Richter, U. Scheer: Association of DNA topoisomerase I and RNA polymerase I: a possible role for topoisomerase I in ribosomal gene transcription. Chromosoma **96**, 411–416 (1986).

[21] Scheer, U., R. Benavente: Functional and dynamic aspects of the mammalian nucleolus. BioEssays **12**, 14–21 (1990).

[22] Scheer, U., K. M. Rose: 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 (1984).

[23] Zhang, H., J. C. Wang, L. Liu: Involvement of DNA topoisomerase I in transcription of human ribosomal RNA genes. Proc. Natl. Acad. Sci. USA **85**, 1060–1064 (1988).