A Monoclonal Antibody That Recognizes a Phosphorylated Epitope Stains Lampbrush Chromosome Loops and Small Granules in the Amphibian Germinal Vesicle

Mark B. Roth,*† Christine Murphy,* and Joseph G. Gall*

*Department of Embryology, Carnegie Institution, Baltimore, Maryland 21210; and †Department of Basic Sciences, The Fred Hutchinson Cancer Center, Seattle, Washington 98104

Abstract. An mAb library was produced against proteins from the germinal vesicle (GV) of the frog *Xenopus laevis*; mAb 104 was selected from this library on the basis of its immunofluorescent staining of lampbrush chromosome loops. Chromosomes from several species of frogs and salamanders stained equally well. The antibody also stained the surface of numerous small granules in the GV nucleoplasm. The interior of the same granules was stained by antibodies against small nuclear ribonucleoproteins (snRNPs). mAb 104 also stained somatic nuclei from many vertebrate and invertebrate species, usually in a finely punctate pattern similar to that described for anti-snRNP and other antinuclear antibodies. The staining of somatic nuclei was much stronger during the mitotic stages than during interphase. Immunoblot analysis showed that mAb 104 recognizes a phosphorylated epitope.

Lampbrush chromosomes from amphibian oocytes provide a useful system for studying proteins associated with nascent RNA transcripts. Each chromosome has a central axis consisting of transcriptionally inactive chromatin (chromomeres) from which loops of active chromatin extend laterally. The bulk of a loop consists of nascent transcripts with associated ribonucleoprotein (RNP) still attached to the DNA template. The RNP matrix of a loop is so abundant that individual transcription units are readily visible by light optical microscopy (Scheer et al., 1976; Gall et al., 1983). Some loops consist of a single transcription unit, whereas many contain two or more units in various orientations.

Antibodies have been used to identify proteins associated with the nascent transcripts on lampbrush loops (Scott and Sommerville, 1974; Sommerville et al., 1978; Martin and Okamura, 1981; Lacroix et al., 1985; Roth and Gall, 1987; Gall and Callan, 1989; Piñol-Roma et al., 1989). The majority of loops are morphologically similar and contain a set of common proteins, including both heterogeneous nuclear RNPs (hnRNPs) and small nuclear RNPs (snRNPs). A few “landmark” loops are morphologically distinct (reviewed in Callan, 1986), and in several cases their protein composition is known to be unusual.

We have produced a number of mAbs from mice injected with germinal vesicle (GV) proteins from the frog *Xenopus laevis* and the newt *Notophthalmus viridescens*. Several of these bind strongly to lampbrush chromosome loops (Roth and Gall, 1987) or to other intranuclear structures. Most are relatively species-specific; for instance, most mAbs raised against *Xenopus* react with *Xenopus* and other anurans (e.g., *Rana*) but not with urodeles or other vertebrates. One mAb, designated 104, has proved of unusual interest because it cross-reacts with a wide variety of vertebrate and invertebrate species. In immunofluorescence assays mAb 104 stains most lampbrush loops and numerous small granules in the GV nucleoplasm. In somatic cells it stains similar but smaller nuclear granules in a strongly cell cycle–dependent manner. Immunoblot analysis shows that the epitope recognized by the antibody contains a phosphate residue. Here we describe our studies on mAb 104, with emphasis on its reaction with lampbrush loops and its usefulness in defining a population of intranuclear granules.

Materials and Methods

mAbs

The production of mAbs and their use in immunoblot analysis and immunofluorescence are described in Roth and Gall (1987).

Lampbrush Chromosome Preparations

GVs were isolated by hand from oocytes of *X. laevis* and *N. viridescens*. Lampbrush chromosome preparations were made essentially as described in Roth and Gall (1987) and Callan et al. (1987), except that 1 mM Mg²⁺ was included in both the nuclear isolation and dispersal media. Mg²⁺ is essential for good morphological preservation of nucleoli, spheres, and small granules, especially in the dispersal medium. After centrifugation to attach the nuclear contents to the slide, preparations were placed directly into 70% ethanol.
**Immunofluorescence**

Lampbrush chromosome preparations were transferred from ethanol to PBS, and then into a "blocking" solution consisting of 10% horse serum in PBS with 0.02% NaN₃ as preservative. After a few minutes, the blocking solution was replaced by the primary antibody, usually undiluted cultured supernate from a hybridoma line. After 1 h the antibody was washed away with 10% horse serum in PBS and the secondary antibody was applied for 1 h. For mAb 104 this was rhodamine-conjugated goat anti-mouse IgM (Cappel 2,211-0201); in other cases rhodamine-conjugated goat anti-mouse IgG (Cappel 2,611-0081) or goat anti-human IgG (Cappel 2,201-0081) was used. Preparations were mounted in 50% glycerol containing 1 mg/ml phenylenediamine to prevent fluorescence fading.

**Phosphatase Treatment of Proteins**

Proteins were treated with phosphatase either before or after electrophoresis and transfer to nitrocellulose membranes. 20 GVs were isolated in 20 µl of a buffer consisting of 64 mM KCl, 16 mM NaCl, 10 mM MgCl₂, 10 mM MOPS, pH 7.2, 1 mM EDTA, 1 mM DTT, and 100 µM PMSE. 10 U of calf intestinal alkaline phosphatase (Boehringer Mannheim Diagnostics, Inc., Houston, TX) were added and the solution was incubated at 37°C for 1 h. Cytological preparations and immunoblots were treated with phosphatase by placing them directly into a solution of phosphatase (0.5 U/µl) in 50 mM Tris, pH 8.0, 1 mM EDTA for 1 h at room temperature.

**Results**

**Production of mAbs**

To obtain antibodies against various nuclear antigens, we immunized mice with proteins from *Xenopus* GVs according to the procedure used previously for *Notophthalmus* (Roth and Gall, 1987). Hybridoma cell lines were produced from the spleens of the mice, and conditioned medium from 1,300 lines was tested against GV proteins in a solid-phase radio-immune assay. All positive media were then tested for their ability to bind to *Xenopus* lampbrush chromosomes by indirect immunofluorescence.

**Immunofluorescent Staining of GV Contents by mAb 104**

In our first experiments with mAb 104, we detected binding on *Xenopus* lampbrush chromosomes with a fluorescent secondary antibody directed against mouse IgG. The intensity of staining was quite variable, and we thought that binding was primarily to a small subset of prominent landmark loops near the middle of chromosome 14 (Callan et al., 1987). After learning that mAb 104 was an IgM, we carried out subsequent experiments with a secondary antibody against mouse IgM. We still found staining of the landmark loops, but now we saw staining of nearly all other loops, as well as numerous small granules in the nucleoplasm. Because mAb 104 cross-reacted with proteins from many different species, we studied the staining pattern in GVs of the newt *N. viridescens*, whose lampbrush chromosomes are far superior to those of *Xenopus* for detailed morphological analysis. In the newt, mAb 104 stained almost all typical lampbrush loops (Fig. 1). Typical lampbrush loops consist of one or more "thin-to-thick" regions that represent individual transcription units (reviewed in Gall et al., 1983; Callan, 1986). The same thin-to-thick pattern was visible by immunofluorescence and by phase contrast or differential in-

![Figure 1](https://example.com/figure1.png)

**Figure 1.** (A) Segment of a lampbrush chromosome, six B granules and a small sphere (S) with two B granules on its surface, from a GV of the newt, *N. viridescens*. Differential interference contrast shows loops extending laterally from the chromomere axis; the loops consist primarily of nascent RNA transcripts and associated protein, whereas the chromomeres are regions of condensed chromatin. The loops and B granules are known from an earlier study to contain snRNPs (Gall and Callan, 1989). (B) Immunofluorescence of the same area after staining with mAb 104 and rhodamine-labeled goat anti-mouse IgM. The lampbrush loops stain uniformly, but the chromomeres are unstained. Only the periphery of the B granules is stained. The body of the sphere is at background level. (C) A short segment of a lampbrush chromosome, an extrachromosomal nucleolus (N) and two spheres (S) with attached B granules, from a GV of *N. viridescens*. Differential interference contrast. (D) Immunofluorescence image of the same region after staining with mAb 104. The chromosome loops and the periphery of the B granules are stained; the nucleolus and the sphere bodies are unstained. Bars, 10 µm.
Figure 2. A single B granule from a GV of the frog *Rana temporaria*, stained with mAb 104 and rhodamine-labeled goat anti-mouse IgM. A through-focus series taken with a confocal laser scan microscope at 0.5-μm intervals. Stain is limited to irregular patches on the surface of the granule. Bar, 10 μm.

terference contrast, suggesting that the antigen recognized by mAb 104 is uniformly distributed over the transcription unit. Among the few unstained or weakly stained loops were the "sequentially labeling" loops on chromosome 11 and the giant loops on chromosome 2 (Callan, 1986). These landmark loops fail to stain with other antibodies that stain most loops (Roth and Gall, 1987; Gall and Callan, 1989), including mAb iD2, which is directed against several hnRNPs (Leser et al., 1984), and mAb Y12, which recognizes the Sm epitope common to the major snRNPs (Lerner et al., 1981). The chromomere axis of the chromosomes (Fig. 1, A and B) and the multiple extrachromosomal nucleoli (Figs. 1, C and D; 3, A and B) also failed to stain.

In addition to the chromosome loops, mAb 104 stained thousands of small granules, ~1–4 μm in diameter, scattered throughout the preparation (Figs. 1–3). These "B" granules, as we call them, were suspended in the nucleoplasm before the GV was spread for cytological analysis. Staining was limited to minute patches that cover the surface of the granules, as seen most convincingly by confocal laser scan microscopy (Fig. 2). B granules contain snRNPs, as shown by their reaction with antibodies against the Sm antigen and against the trimethylguanosine cap of the major snRNAs (Gall and Callan, 1989). Additional evidence for snRNPs in the B granules is provided in Fig. 3, C and D, which shows staining with serum 361, a human autoimmune serum that immunoprecipitates U1 snRNPs (David Wassarman, unpublished observations). It is clear that the snRNP antigen(s) detected by serum 361 are in the center of the B granules, in contrast to the peripheral localization of 104 antigen.

Although mAb 104 stains thousands of small granules in the nucleoplasm, others remain unstained. Many of the unstained granules have a distinctive "doughnut" appearance (Fig. 3, A and C). This second type, which we call "A" granule, stains intensely with serum 361 (Fig. 3 D).

One other prominent nuclear structure stained by mAb 104 is the so-called sphere organelle. A GV from a mediumsized oocyte of the newt contains several dozen spheres, of which the largest are 8–10 μm in diameter (Gall and Callan,
They usually have smaller spherical or subspherical protuberances on their surface (Fig. 1, A and C). The body of the sphere is not stained by mAb 104, but the protuberances stain exactly like the B granules just described; that is, staining is in minute patches on the surface of the protuberances (Fig. 1, B and D).

Antigens Recognized by mAb 104 Do Not Coprecipitate with hnRNPs

A few other antibodies stain the periphery of the B granules in the same pattern as mAb 104 (data not shown). Of particular interest is mAb iD2, which recognizes several abundant hnRNPs (Leser et al., 1984). We asked whether the antigen(s) recognized by mAb 104 might be coprecipitated with hnRNPs. hnRNPs were immunoprecipitated from HeLa cell supernatant using mAb 4F4 (Pifiol-Roma et al., 1988), electrophoresed on a polyacrylamide gel, and transferred to a nitrocellulose filter. mAb 104 did not react with any protein in the immunoprecipitate, although it readily recognized a protein when total HeLa cell proteins were tested at the same time (data not shown).

Staining of Other Cell Types

We tested for cross-reaction of mAb 104 with cells of other organisms. Formaldehyde-fixed tissue culture cells from several animals were stained by indirect immunofluorescence; these included human (HeLa), African green monkey (Vero), mouse (L cells and NIH 3T3), Drosophila (Schneider), and Xenopus (Xla). We also tested tissues from a variety of organisms, including nematode (Caenorhabditis), earthworm, grasshopper (Melanoplus), mouse, and amphibians (Rana, Plethodon). In all cases punctate nuclear staining was detected. Fig. 4, A–C shows labeling of a single mouse L cell. There was no detectable labeling of nucleoli or of condensed chromatin, as can be seen by comparing the immunofluorescence and DAPI-stained images. The major differences among the different organisms and cell types were in the number and size of the punctate regions in the nucleus.

Mitosis

We noted that the intensity of staining was strongly dependent on the cell cycle (Fig. 4, D–F). As cells entered

Figure 4. (A) Phase-contrast view of a mouse L cell, fixed briefly with formaldehyde and double stained with mAb 104 (rhodamine-labeled second antibody) and the DNA-specific dye 4′,6-diamidino-2-phenylindole (DAPI). (B) DAPI stain shows heterochromatic masses (primarily satellite DNA) against a generalized background stain. (C) Immunofluorescence shows numerous bright dots against a lightly stained background. The clumps of antigen do not correspond to the heterochromatic regions. (D) Differential interference contrast view of cultured mouse L cells. Dividing cells have rounded up. (E) DAPI stain of the same cells. P, M, A, and T indicate cells in prophase, metaphase, anaphase, and telophase, respectively. (F) The same cells after immunofluorescent staining with mAb 104 and rhodamine-labeled goat anti-mouse IgM. Staining is intense in cells in mitosis but drops off abruptly at the beginning of interphase (compare the well-stained telophase cell at upper left with the unstained early interphase at lower right). Stain is excluded from the chromosome area. Many but not all of the interphase nuclei in the background are actually stained as in C, but a photographic exposure long enough to show this fact would obliterate all detail in the intensely stained mitotic cells. Bars: (C) 10 μm; (F) 20 μm.
prophase, nuclear staining with mAb 104 became more prominent. After breakdown of the nuclear envelope, intense staining was seen throughout the cytoplasm, and this persisted through metaphase and anaphase. Early in telophase cytoplasmic staining disappeared and granules reappeared in the newly formed daughter nuclei. At the same time, the general intensity of nuclear staining decreased, reaching a minimum during interphase.

**Sedimentation of Granules Reacting with mAb 104**

Indirect immunofluorescence of many different animal cells suggested that much of the antigen recognized by mAb 104 was organized into microscopically visible granules. To determine whether this property could be used to enrich the corresponding antigen(s), we sonicated hand-isolated GVs and centrifuged them at 12,000 g for 2 min. Analysis of the proteins in the pellet and supernate by immunoblotting showed that all of the immunoreactive material was in the pellet. Magnesium (10 mM) in the isolation medium was essential for this effect; without it the antigen remained suspended, even after centrifugation at 70,000 g for 1 h. We examined some of the sedimented material by centrifuging sonicated GV contents onto microscope slides, which were then fixed in formaldehyde and stained with mAb 104. The antibody stained an array of minute granules, many of which were smaller than the B granules in our cytological preparations.

**mAb 104 Recognizes a Phosphorylated Epitope**

To further characterize the antigen(s) recognized by mAb 104, we used the antibody on immunoblots of *Xenopus GV* proteins. The antibody bound to a sharp band at 43 kD and to a poorly resolved smear above 100 kD (Fig. 5, lane 1). On immunoblots of proteins extracted from *Drosophila* tissue culture cells, the antibody bound strongly to a band at 55 kD and weakly to a few other bands.

Because mAb 104 stained mitotic cells more intensely than interphase cells, we reasoned that either the protein was turning over during the cell cycle, or the epitope recognized by the antibody was altered by a posttranslational modification in a cell cycle–dependent manner. Since many proteins are transiently phosphorylated during mitosis, we tested for antibody binding after dephosphorylation. Nitrocellulose strips that carried GV proteins were incubated with calf intestinal alkaline phosphatase before immunostaining. Nitrocellulose membranes in which GVs are isolated and in which the nuclear matrix of the loops expands, and the multiple nucleoli partially dissolve. Both features are helpful when attention is directed specifically to the chromosomes. In order to preserve nonchromosomal elements of the GV in our more recent experiments, we have added 1 mM Mg ++ to the solutions in which GVs are isolated and in which the nuclear contents are spread for cytological observation (in Mg ++ concentrations of 5 mM or more, GV contents will not spread). In 1 mM Mg ++, the nucleoli are preserved intact, as are thousands of smaller granules present in the nucleoplasm. Spheres are retained in spread preparations either with or without Mg ++, but the protuberances on their surface are better preserved in Mg ++. With the good morphological preservation attained in Mg ++ preparations, we now find that several antigens associated with nascent transcripts are also present in the spheres and granules. This first became clear when we studied two antibodies specific for snRNPs: mAb Y12 that recognizes the Sm epitope (Lerner et al., 1981) and mAb K121 that recognizes the trimethylguanosine cap of snRNAs (Krainer, 1988). These two antibodies stain the spheres and granules (both A and B types), but they also stain most lampbrush loops (Gall and Callan, 1989). In the present study we found that mAb 104, detected originally on the basis of its loop staining, also stains B granules and the...
protuberances on spheres; it does not stain A granules. The
detailed staining pattern of mAB 104, however, is quite
different from that of mAbs Y12 and K121. mAB 104 stains
the periphery of the B granules and the periphery of the pro-
tuberances on the spheres (Figs. 1–3), whereas Y12 and
K121 stain the body of the sphere and the interior of the gran-
ules and protuberances. Other anti-snRNP antibodies, such
as human serum 361 (Fig. 3, C and D), similarly stain the
protuberances on the spheres; it does not stain A granules. The
staining pattern on the chromosome loops is quite
similar for all the antibodies we have discussed (mAbs 104,
SE5, iD2, Y12, K121, and serum 361). All stain the majority
of typical loops, but leave certain prominent landmark loops
unstained; chief among the latter are the “sequentially label-
ing” loops on chromosomes 11 and the giant loops on chromo-
some 2. Staining of typical loops is more or less proportional
to the mass of RNP matrix, as shown by the general similar-
ity of the immunofluorescent and phase contrast or differen-
tial interference contrast images (Fig. 1). Certain morpho-
logically identifiable loops may be stained relatively more
intensely by one antibody, but we have not seen cases where
antibody staining was limited to one part of a transcription
unit. A possible interpretation of these observations is that
most nascent transcripts are associated along their whole
length with a common set of hnRNPs and snRNPs proteins.
Certain loops lack these proteins and a few have them in al-
tered relative proportions, but within a given transcription
unit their distribution is uniform. Our observations are less
compatible with models in which hnRNPs and snRNPs pro-
teins or protein complexes go on and off the nascent tran-
scripts independently.

mAB 104 has been useful in characterizing various com-
ponents in the GV, particularly the granules that contain
snRNPs. mAB 104 also stains somatic nuclei from a variety
of vertebrate and invertebrate species. The staining pattern
in somatic nuclei is similar to that described as speckled or
punctate for several anti-snRNP antibodies (Lerner et al.,
1981; Reuter et al., 1984; Nyman et al., 1986; Spector
and Smith, 1986; Spector, 1990) and a non-snRNP splicing
factor (Fu and Maniatis, 1990).

Staining by mAB 104 is strongly dependent on the cell cy-
cle. Although weak staining is evident in most nuclei that we
have observed, the intensity of stain is dramatically en-
hanced from prophase through late telophase of the cell cycle
(Fig. 4, D–F). After breakdown of the nuclear envelope,
staining occurs throughout the cell, as is common for many
nuclear antigens.

Immunoblots of proteins from several sources (mouse, hu-
man, newt, and \textit{Drosophila}) show a strong immunoreactive
band in the range of 40–55 kD, suggesting that the
immunofluorescence assay may recognize related proteins in
these different species. We have shown that the epitope rec-
ognized by mAB 104 includes a phosphate residue. We do
not know whether the 104 protein fluctuates in amount dur-
ing the cell cycle or is phosphorylated and dephosphorylated
in a cell cycle–dependent fashion (or both). Many proteins
are phosphorylated during mitosis including lamin (Otta-
viano and Gerace, 1985), pp60–160k (Chackalaparampi and
Shalloway, 1988), and proteins involved in the control of mi-
tosis (Draetta and Beach, 1988). Some antibodies raised
against mitotic cells react with phosphorylated groups on a
variety of proteins only during mitosis (Davis et al., 1983).
Whether the antigen(s) recognized by mAB 104 play an es-
dential role in mitosis, or merely undergo secondary changes
during the cell cycle remains to be determined.

We have used mAB 104 to screen cDNA expression
libraries of \textit{Xenopus} and \textit{Notophthalmus} ovary RNA, but so
far have failed to recover positive clones. A likely problem
is that the phosphorylated epitope recognized by the anti-
body is not present on the protein produced by lambda
phage. As an alternative approach we have purified enough
of the 55-kD protein from \textit{Drosophila} cells to begin microse-
quencing. We hope eventually to study the production of
the 104 antigen in oocytes and somatic cells, and to examine
its regulation during the cell cycle.

We thank S. Piñol-Roma for help with immunoprecipitation of hnRNP pro-
teins. We thank Joe Craft and Joan Steitz for human serum 361.

This work was supported by grant 33397 from the National Institute of
General Medical Sciences. M. B. Roth was supported by a postdoctoral fel-
lowship from the Jane Coffin Childs Memorial Fund. J. G. Gall is Ameri-
can Cancer Society Professor of Developmental Genetics.

Received for publication 30 May 1990 and in revised form 2 August 1990.

References

Callan, H. G. 1986. Lambrush Chromosomes. \textit{In Molecular Biology, Bio-
chemistry and Biophysics}. M. Solioz, editor. Springer-Verlag, Berlin,
Heidelberg. 1–254.

Callan, H. G., J. G. Gall, and C. A. Berg. 1987. The lambrush chromosomess
of \textit{Xenopus laevis}: preparation, identification, and distribution of SS DNA
sequences. \textit{Chromosoma} (Berl.). 95:236–250.
Chackalaparampil, I., and D. Shalloway. 1988. Altered phosphorylation and activation of pp60 census during fibroblast mitosis. *Cell.* 52:801-810.

Davis, F., T. Y. Tsao, S. K. Fowler, and P. N. Rao. 1983. Monoclonal antibodies to mitotic cells. *Proc. Natl. Acad. Sci. USA.* 80:2926-2930.

Draetta, G., and D. Beach. 1988. Activation of cdc2 protein kinase during mitosis in human cells: cell cycle-dependent phosphorylation and subunit rearrangement. *Cell.* 54:17-26.

Fu, X.-D., and T. Maniatis. 1990. Factor required for mammalian spliceosome assembly is localized to discrete regions in the nucleus. *Nature (Lond.)*. 343:437-441.

Gall, J. G., and H. G. Callan. 1989. The sphere organelle contains small nuclear ribonucleoproteins. *Proc. Natl. Acad. Sci. USA.* 86:6635-6639.

Gall, J. G., M. O. Diaz, E. C. Stephenson, and K. A. Mahon. 1983. The transcription unit of lampbrush chromosomes. In *Gene Structure and Regulation in Development.* Symp. Soc. Dev. Biol. S. Subtelny and F. C. Kafatos, editors. Alan R. Liss, Inc., New York. 137-146.

Krainer, A. 1988. Pre-mRNA splicing by complementation with purified human U1, U2, U4/U6 and U5 snRNPs. *Nucleic Acids Res.* 16:9415-9429.

Leser, G. P., J. Escara-Wilke, and T. E. Martin. 1984. Monoclonal antibodies to heterogeneous nuclear RNA-protein complexes: the core proteins comprise a conserved group of related polypeptides. *J. Biol. Chem.* 259:1827-1833.

Martin, T. E., and C. S. Okamura. 1981. Immunocytochemistry of nuclear hnRNP complexes. In *The Cell Nucleus.* Busch, H., editor. Academic Press, New York. 119-144.

Nyman, U., H. Hallman, G. Hadaczky, I. Petterson, G. Sharp, and N. R. Ringerz. 1986. Intranuclear localization of snRNP antigens. *J. Cell Biol.* 102:137-144.

Ottaviano, Y., and L. Gerace. 1985. Phosphorylation of the nuclear lamins during interphase and mitosis. *J. Biol. Chem.* 260:624-632.

Pifiol-Rosina, S., Y. D. Choi, M. J. Matunis, and G. Dreyfuss. 1988. Immunopurification of heterogeneous nuclear ribonucleoprotein particles reveals an assortment of RNA-binding proteins. *Genes & Dev.* 2:215-227.

Reuter, R., B. Appel, P. Bringmann, J. Rinke, and R. Lührmann. 1984. 5'-Terminal caps of snRNAs are reactive with antibodies specific for 2,2,7-trimethylguanosine in whole cells and nuclear matrices. *Exp. Cell Res.* 154:548-560.

Roth, M. B., and J. G. Gall. 1987. Monoclonal antibodies that recognize transcription unit proteins on newt lampbrush chromosomes. *J. Cell Biol.* 105:1047-1054.

Schoer, U., W. W. Franke, M. F. Trendelenburg, and H. Spring. 1976. Classification of loops of lampbrush chromosomes according to the arrangement of transcriptional complexes. *J. Cell Sci.* 22:503-520.

Scott, S. E. M., and J. Sommerville. 1974. Location of nuclear proteins on the chromosomes of newt oocytes. *Nature (Lond.)*. 250:680-682.

Sommerville, J. C., C. Crichton, and D. Malcolm. 1978. Immunofluorescent localization of transcriptional activity on lampbrush chromosomes. *Chromosoma (Berl.)*. 66:99-114.

Spector, D. L. 1990. Higher order nuclear organization: three-dimensional distribution of small nuclear ribonucleoprotein particles. *Proc. Natl. Acad. Sci. USA.* 87:147-151.

Spector, D., and H. C. Smith. 1986. Redistribution of U-snRNPs during mitosis. *Exp. Cell Res.* 163:87-94.