Heat shock induces mini-Cajal bodies in the *Xenopus* germinal vesicle

Korie E. Handwerger[1,2,*], Zheng'an Wu[1,*], Christine Murphy[1] and Joseph G. Gall[1,‡]

[1]Department of Embryology, Carnegie Institution of Washington, 115 West University Parkway, Baltimore, MD 21210, USA
[2]Department of Biology, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218, USA
*These authors contributed equally to this study
‡Author for correspondence (e-mail: gall@ciwemb.edu)

Summary

Cajal bodies are evolutionarily conserved nuclear organelles that are believed to play a central role in assembly of RNA transcription and processing complexes. Although knowledge of Cajal body composition and behavior has greatly expanded in recent years, little is known about the molecules and mechanisms that lead to the formation of these organelles in the nucleus. The *Xenopus* oocyte nucleus or germinal vesicle is an excellent model system for the study of Cajal bodies, because it is easy to manipulate and it contains 50-100 Cajal bodies with diameters up to 10 μm. In this study we show that numerous mini-Cajal bodies (less than 2 μm in diameter) form in the germinal vesicle after oocytes recover from heat shock. The mechanism for heat shock induction of mini-Cajal bodies is independent of U7 snRNA and does not require transcription or import of newly translated proteins from the cytoplasm. We suggest that Cajal bodies originate by self-organization of preformed components, preferentially on the surface of B-snurposomes.

Key words: Cajal body, Germinal vesicle, Heat shock, Oocyte, *Xenopus*

Introduction

Cajal bodies (CBs, formerly coiled bodies) and nucleoli share several biological features, including close physical proximity, overlapping protein profiles, disassembly during mitosis, and association with specific chromosomal loci (reviewed by Gall, 2000). Despite these similarities, biogenesis of the two organelles appears to be quite different. Nucleoli normally form on chromosomes at the nucleolus organizers (NOs), which contain actively transcribing genes coding for ribosomal RNA (Hadjiolov, 1985; Shaw and Jordan, 1995; Scheer and Hock, 1999; Olson et al., 2000). The crucial role of these genes in nucleolar biogenesis is illustrated by formation of a small nucleolus at a single transposed rDNA unit in *Drosophila* (Karpen et al., 1988) and by production of multiple nucleoli in oocytes of many species, including *Xenopus*, on amplified extrachromosomal copies of the rDNA (Brown and Dawid, 1968; Gall, 1968; Miller and Beatty, 1969). CBs also preferentially associate with specific chromosomal sites, at least in mammalian cultured cells and amphibian oocytes. These sites are the genes coding for histones (Gall et al., 1981; Callan et al., 1991; Frey and Matera, 1995) and for several small nuclear (sn) RNAs (Frey and Matera, 1995; Smith et al., 1995; Gao et al., 1997; Frey et al., 1999; Jacobs et al., 1999). However, most CBs exist free in the nucleoplasm, and there is no evidence that they are shed there after production at the chromosomal sites. Moreover, observations on nuclei assembled in *Xenopus* egg extract suggest that CBs can arise in nuclei that contain only bacteriophage DNA (Bell et al., 1992). Thus, formation of

nucleoli depends on rDNA, whereas CBs can arise in the complete absence of eukaryotic DNA.

Without compelling evidence that CBs originate at specific genetic loci, emphasis has focused on the possibility that some unique molecule or organelle might act as the seed or scaffold for their formation. In addition to factors found elsewhere in the nucleus, CBs are enriched for the marker protein coilin and the rare U7 snRNA involved in histone pre-mRNA processing (reviewed by Gall, 2000). Coilin and U7 snRNA have thus been considered as strong candidates for nucleators of CB formation. Evidence against such a role for coilin is now strong. CBs form in nuclei assembled in *Xenopus* egg extract, even when the extract has been immunodepleted of coilin (Bauer and Gall, 1997). Likewise, a mouse knockout for the coilin gene displays CB-like structures in its nuclei (Tucker et al., 2001). Evidence that U7 snRNA can play a role in CB biogenesis has been presented in a previous study (Tuma and Roth, 1999). The study showed that injection of U7 snRNA into the *Xenopus* oocyte nucleus (germinal vesicle, GV) was sufficient to induce formation of small 'coiled body-like structures'.

We describe the appearance of mini-CBs (<2 μm) in the GVs of *Xenopus* oocytes that have recovered from heat shock. These mini-CBs are similar in size and number to those described previously (Tuma and Roth, 1999). However, an antisense depletion experiment shows that U7 snRNA is not necessary for their induction by heat shock. Neither transcription of RNA nor import of newly translated proteins into the GV is required, suggesting that mini-CBs assemble from pre-existing components in the GV.

Materials and Methods

Oocytes

Adult female *Xenopus laevis* (Xenopus 1, Ann Arbor, MI) were anesthetized in 0.1% methanesulfonate salt of 3-aminobenzoic acid ethyl ester (MS222; Sigma, St Louis, MO) for approximately 20 minutes, and a piece of ovary was surgically removed. Unless otherwise stated, stage IV-V oocytes were hand-isolated and stored in OR2 saline (Wallace et al., 1973) overnight at 18°C before use.

Fluorescein-U7 snRNA and green fluorescent protein (GFP)-coilin

Fluorescein-U7 snRNA was generated in a 30 µl in vitro transcription reaction containing 1 µg *Sal*I- linearized #401 plasmid DNA (Wu et al., 1996), 166 µM fluorescein-12-UTP (Boehringer Mannheim, Indianapolis, IN), 500 µM ATP and CTP, 250 µM UTP and GTP, 2.5 mM m^{7}G(5′)ppp(5′)G cap (New England Biolabs, Beverly, MA), and 0.4 µM [^{32}P]UTP (Amersham Life Sciences, Arlington Heights, IL) in a buffer consisting of 50 mM NaCl, 40 mM Tris-HCl (pH 8), 30 mM dithiothreitol, 8 mM MgCl$_2$ and 2 mM spermidine. The total amount of RNA synthesized was estimated by measuring the efficiency of [^{32}P]UTP incorporation after trichloroacetic acid precipitation and scintillation counting. The fluorescein-U7 snRNA product was precipitated twice with ethanol to remove unincorporated nucleotides and resuspended in diethylpyrocarbonate-treated H$_2$O at ~0.1 µg/µl. In vitro transcripts of a *Xenopus* GFP-coilin plasmid were synthesized using SP6 polymerase in a similar reaction without fluorescein-UTP. The 1.6 kb open reading frame of *Xenopus* coilin was obtained by PCR from liver cDNA using primers based on the *Xenopus* coilin sequence (Tuma et al., 1993). The PCR product was cloned downstream of GFP in the cloning vector pCS2+GFP (Huang et al., 1999). Transcripts were injected into the cytoplasm of *Xenopus* oocytes, and GVs were isolated 18 hours later for a western blot. A band corresponding to the predicted molecular weight of GFP-coilin was apparent when the western blot was probed with an antibody against GFP.

Oocyte injections

Needles were pulled from capillary tubing as previously described (Gall et al., 1999). Approximately 1 ng fluorescein-U7 snRNA or 1 ng GFP-coilin transcript was injected into the cytoplasm of oocytes before or after heat shock by means of a Nanoject Microinjection Apparatus (Drummond Scientific, Broomall, PA). To deplete oocytes of U7 snRNA, 35 ng of an antisense oligodeoxynucleotide against bases 1-16 of U7 snRNA (Stefanovic et al., 1995) was injected into the oocyte cytoplasm in a volume of 23 nl. Cells were incubated for a minimum of 12 hours at 18°C before further experiments were conducted. U7 snRNA was not detectable by northern blot analysis in nuclei isolated from oligo-treated oocytes.

Heat shock

Oocytes were transferred from OR2 saline at 18°C to pre-warmed OR2 at 31.5-33.5°C for a heat shock lasting 2-4 hours. For recovery, the Petri dish containing heat-shocked oocytes was returned to 18°C for 6-18 hours. In some experiments, actinomycin D (5-10 µg/ml) or cycloheximide (50 µg/ml) was included in the OR2 medium to inhibit transcription or translation. Oocytes were pre-incubated in the drug for 2-3 hours at 18°C, heat shocked while still in the drug, and left in the drug during the recovery period.

GV spreads

GVs were dissected manually from oocytes and their contents were spread and attached to microscope slides by centrifugation as previously described (Gall, 1998). Fixation was in 2% paraformaldehyde in PBS + 1 mM Mg^{2+} for 1-2 hours.

Immunofluorescence

Immunostaining of GV spreads was carried out as described previously (Gall et al., 1999). The following mAbs were used: ARNA-3 against the largest subunit of RNA Pol II (Krämer et al., 1980), H1 against *Xenopus* coilin (Tuma et al., 1993), H14 against the C-terminal domain of human RNA Pol II (Bregman et al., 1995), K121 against trimethylguanosine (Krainer, 1988), Y12 against the Sm epitope (Lerner et al., 1981), anti-SC35 against the SR protein SC35 (Fu and Maniatis, 1990), 17C12 against fibrillarin (Pollard et al., 1997), No114 against *Xenopus* Nopp140 (Schmidt-Zachmann et al., 1984), P7-1A4 against *Xenopus* nucleolin (Messmer and Dreyer, 1993) and 4D11 against human hnRNP L (Piñol-Roma et al., 1989). Affinity-purified rabbit polyclonal sera against human TFIIIA (from R. Roeder) and *Xenopus* coilin (serum C236) were also used. Secondary antibodies were Alexa 488- or Alexa 594-labeled goat anti-mouse IgG (or IgM) or goat anti-rabbit IgG (Molecular Probes, Eugene, OR). GV spreads were observed with a Zeiss Axioplan fluorescence microscope (Carl Zeiss, Thornwood, NY) fitted with a filter set for observing fluorescein or Alexa 488 (excitation filter BP 485/20 and barrier filter BP 520-560) and Alexa 594 (excitation filter BP 515-560 and barrier filter LP 590). Confocal laser scanning microscopy was carried out with the Leica TCS NT system (Leica Microsystems, Exton, PA).

Import assay

Approximately 1 ng fluorescein-U7 snRNA was injected into the cytoplasm of oocytes. After the cells had been heat shocked at 31.5°C for 4 hours, GVs were removed in mineral oil (Paine et al., 1992) and held for a minimum of 4 hours at 18°C. GVs were transferred in 5 µl of oil to a glass microscope slide and gently squashed under a 22 mm^2 glass coverslip. Typical CBs (2-10 µm) and mini-CBs (<2 µm) appeared as intensely fluorescent spherical bodies in the faintly fluorescent nucleoplasm.

[^{35}S]methionine incorporation and protein gels

Oocytes were either held at 18°C (control) or heat shocked at 31.5°C for 1 hour. They were then injected with 23 nl [^{35}S]-methionine (10 mCi/ml, 1175 Ci/mmol; Amersham Life Science, Arlington Heights, IL) and returned to 18°C (control) or 31.5°C for 3 hours (heat shock). GVs were removed immediately after heat shock and after the oocytes had been held at 18°C for a minimum of 12 hours recovery. For each group, 25 GVs were collected in 30 µl of 5:1 isolation medium (83 mM KCl, 17 mM NaCl, 6.5 mM Na$_2$HPO4, 3.5 mM KH$_2$PO4, 1 mM MgCl$_2$, 1 mM dithiothreitol, pH 7.0) and adjusted to a final concentration of 1× SDS buffer (Laemmli, 1970). Samples were split in half and loaded into separate lanes of a 10% discontinuous SDS-polyacrylamide mini-gel (BioRad Laboratories, Hercules, CA). After electrophoresis at 30 mA for 1.5 hours, the gel was split in half. One half was stained with Coomassie Blue and the other half was fixed in 50% methanol+10% acetic acid for 15 minutes, soaked in 7% methanol, 7% acetic acid, 1% glycerol for 5 minutes, dried under vacuum at 80°C for 15 minutes, and exposed to X-Omat film (Eastman Kodak, Rochester, NY) at room temperature for 4 days.

Western blots

Oocytes were either held at 18°C or heat shocked at 31.5°C for 4 hours. Some GVs were removed in 5:1 isolation medium immediately after heat shock and others after overnight incubation at 18°C. For each sample, 40 GVs were placed in 50 µl of 5:1 isolation medium and adjusted to a final concentration of 1× SDS loading buffer. Samples were split in half and loaded into separate lanes of a

10% discontinuous SDS-polyacrylamide mini-gel. After electrophoresis at 30 mA for 1.5 hours, the gel was split in half. One half was stained with Coomassie Blue and the other half was transferred at 4°C onto a polyvinylidene fluoride membrane (Immobilon, Millipore, Bedford, MA) for 1 hour at 100 V. Membranes were briefly immersed in 100% methanol, rinsed in H$_2$O and blocked overnight at 4°C with 5% powdered milk in PBS. Primary antibodies were diluted to working concentrations with 0.025% Tween in PBS. Three mAbs were used: ARNA-3 against the largest subunit of *Drosophila* RNA polymerase II (Krämer et al., 1980), H1 against *Xenopus* coilin (Tuma et al., 1993) and No114 against *Xenopus* Nopp140 (Schmidt-Zachmann et al., 1984). Membranes were incubated with primary antibody for 1 hour at room temperature, washed for 3×5 minutes with 0.05% Tween-PBS and incubated with alkaline phosphatase-linked secondary antibodies for 1 hour at room temperature. Again the membranes were washed for 3×5 minutes with 0.05% Tween-PBS. Finally the blot was exposed to chemiluminescent compound (Amersham Life Science, Arlington Heights, IL) for 5 minutes at room temperature and imaged on a Storm 860 scanner (Molecular Dynamics, Sunnyvale, CA).
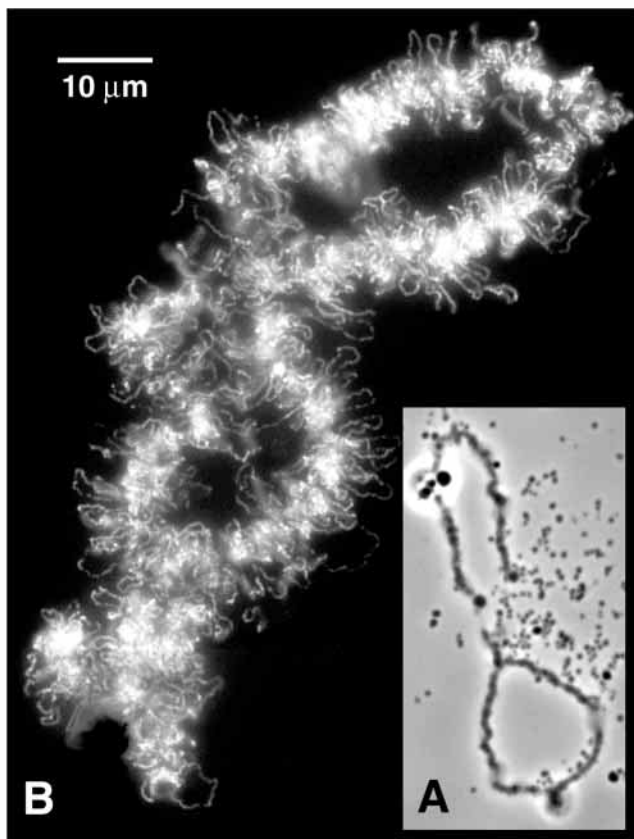
Results

Chromosome and CB structure are affected by heat shock

Several morphological transformations take place in the GV after heat shock, of which the most obvious is a marked overall contraction of the lampbrush chromosomes and loss of their characteristic lateral loops (Flannery and Hill, 1988) (Fig. 1A). These change in chromosome structure demonstrate that RNA polymerase (Pol) II transcription is repressed by heat shock (Callan, 1986) and provide a convenient indication that the heat shock has been effective in any given experiment. If, after heat shock, the oocytes are returned to 18°C for several hours, the lampbrush chromosomes gradually extend in length and recover their loops, signifying the return of transcription (Fig. 1B). In addition, tens to thousands of small spherical particles less than 2 μm in diameter appear in the nucleoplasm. The nature of these particles cannot be deduced from their morphology alone, but antibody staining demonstrates that they contain coilin and therefore might be very small CBs or 'mini-CBs' (Fig. 2). There is no obvious change in the morphology or number of the larger CBs in heat shocked GVs,
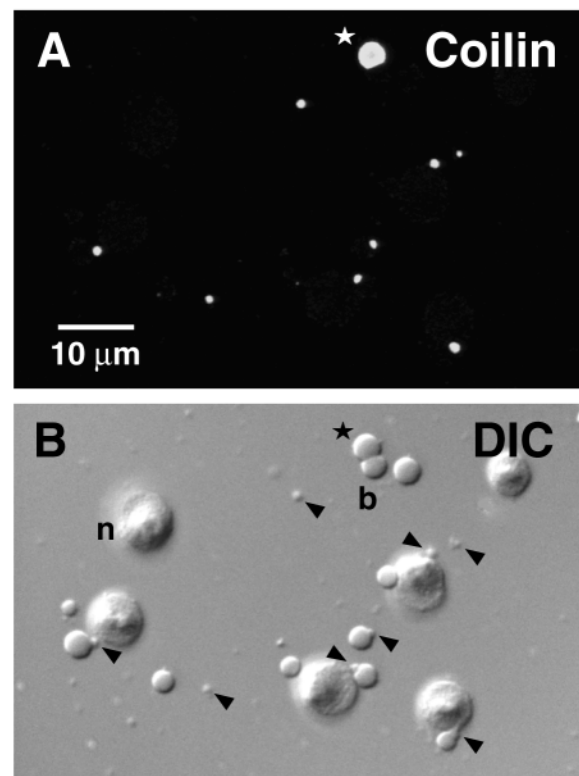

Fig. 1. Effect of heat shock on chromosome morphology. (A) Phase-contrast image of one of the 18 bivalent chromosomes from an oocyte heat shocked for 4 hours at 31.5°C and allowed to recover for 3 hours at 18°C. The overall contraction of the chromosome and loss of lateral loops is typical of transcriptionally inactive chromosomes. (B) Immunofluorescent image of a chromosome from an oocyte heat shocked for 4 hours at 31.5°C and allowed to recover for 21 hours at 18°C. Pol II transcription has returned to normal levels as shown by overall lengthening of the chromosome and re-extension of the lateral loops. As in control preparations, chromosome loops are specifically stained with mAb H14, which recognizes the largest subunit of Pol II when the C-terminal domain is phosphorylated on serine 5.

Fig. 2. A small fraction of the GV contents from a heat-shocked oocyte, spread on a microscope slide and stained with mAb H1 against *Xenopus* coilin. (A) Immunofluorescence to show the distribution of coilin. In this field there is a single small CB (star) and eight mini-CBs. The whole preparation would contain 50-100 large CBs, up to 8-10 μm diameter, and hundreds of additional mini-CBs. (B) The same field by differential interference contrast (DIC). The small CB (star) is associated with an unstained B-snurposome (b) of similar size. The mini-CBs (arrowheads) occur free or attached to the surface of B-snurposomes. Six extrachromosomal nucleoli (n) are also present in this field.

nor do mini-CBs cluster around larger CBs, suggesting that mini-CBs do not arise by simple fragmentation of pre-existing CBs. However, minor changes in size or number of the larger CBs in response to heat shock would be hard to detect because of their normal variability from oocyte to oocyte. Significantly, most mini-CBs are attached to B-snurposomes, which are spherical bodies 2-4 µm in diameter scattered throughout the nucleoplasm of control and heat-shocked GVs. B-snurposomes contain high concentrations of splicing snRNPs and almost certainly correspond to the speckles or interchromatin granule clusters of somatic nuclei (Gall et al., 1999).

Mini-CBs have the same macromolecular composition as larger CBs

Because heat shock can cause aggregation of unfolded proteins (Morimoto et al., 1994), it was possible that mini-CBs were simply precipitates of coilin alone or aggregates of coilin with non-specific proteins. To address this issue, we carried out immunofluorescence on spread preparations of GV contents after recovery from heat shock. Specific staining of mini-CBs was observed with antibodies against the following components: *Xenopus* coilin (mAb H1 and rabbit serum C236), Sm proteins (mAb Y12), the trimethylguanosine cap found on several snRNAs (mAb K121), Nopp140 (mAb No114), TFIIIA (rabbit serum α-TFIIIA) and phosphorylated RNA Pol II (mAb H14) (Fig. 3). Conversely, mini-CBs failed to stain with antibodies against nucleolin (mAb P7-1A4), the SR protein SC35 (mAb α-SC35) and hnRNP L (mAb 4D11). Thus, for all antibodies tested, mini-CBs reacted the same as the larger CBs in the same GV. As mini-CBs have the same or very similar macromolecular composition as CBs of control oocytes, it is unlikely that they represent nonspecific aggregates of unfolded proteins.

GFP-coilin and fluorescein-U7 snRNA target to mini CBs

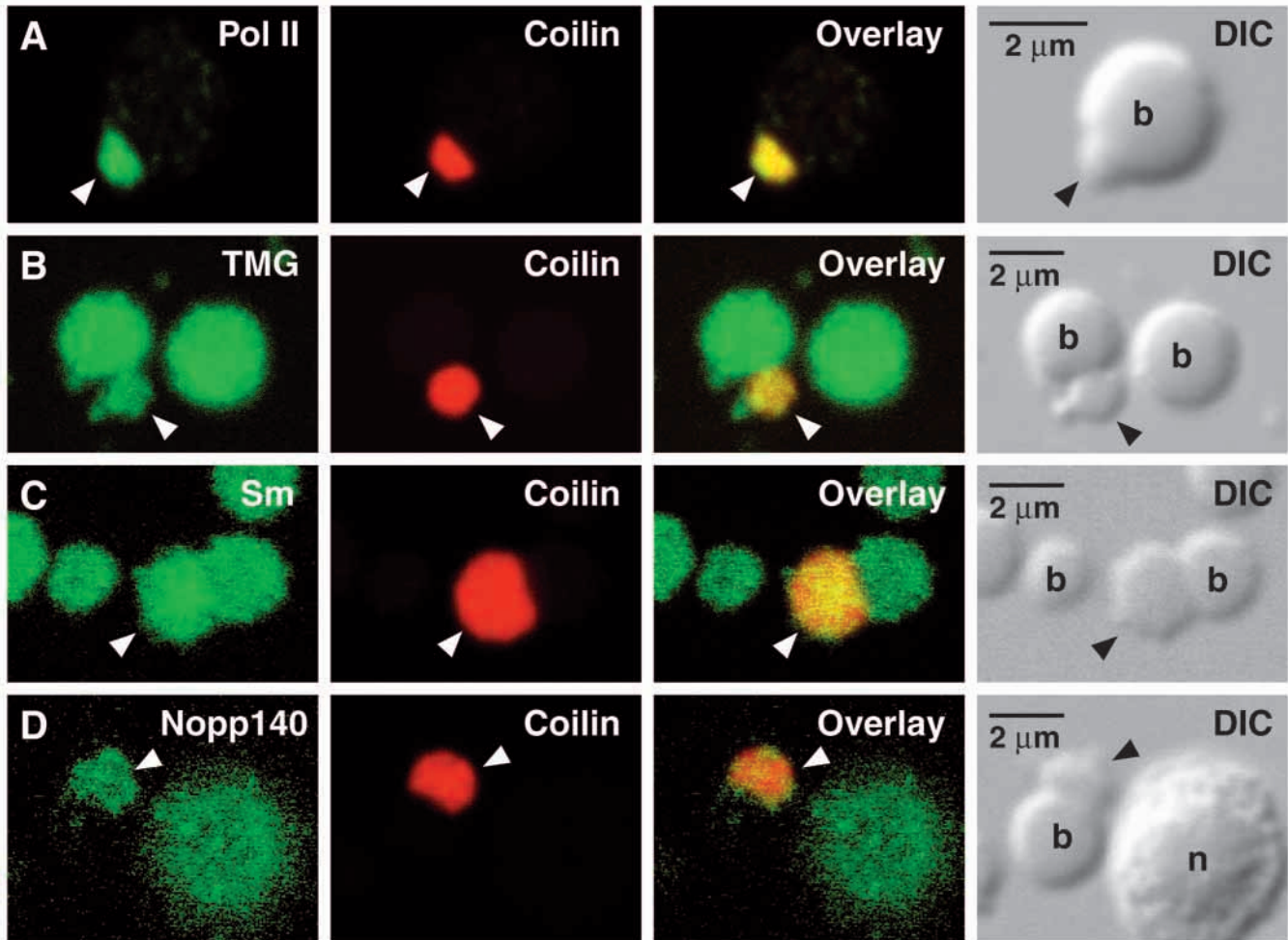A hallmark of CB activity is their rapid uptake of specific

Fig. 3. Confocal images of mini-CBs (arrowheads) stained with four antibodies that recognize typical CB components (first column) and counterstained with α-coilin serum C236 to identify the mini-CBs (second column). The overlay is shown in the third column and a DIC image is shown in the fourth column. In each case, the mini-CB is attached to the surface of a B-snurposome (b). (A) mAb H14, which recognizes the largest subunit of Pol II when the C-terminal domain is phosphorylated on serine 5. Only the mini-CB is stained. (B) mAb K121 against the trimethylguanosine cap found on several snRNAs stains the mini-CB and two B-snurposomes. (C) mAb Y12 against the Sm epitope on snRNPs stains the mini-CB and several B-snurposomes. (D) mAb No114 against *Xenopus* Nopp140 stains the mini-CB and the nucleolus (n) but not the B-snurposome.

RNAs and proteins that have been introduced experimentally into the nucleus (Wu et al., 1994; Wu et al., 1996; Samarsky et al., 1998; Narayanan et al., 1999a; Narayanan et al., 1999b; Sleeman and Lamond, 1999). To test if mini-CBs are functionally equivalent to CBs of control cells, we injected in vitro-synthesized transcripts of GFP-coilin into the oocyte cytoplasm immediately after heat shock. As in control oocytes, GFP-coilin was detectable in CBs within 15-30 minutes of injection and became more evident with time. Mini-CBs formed during overnight recovery from heat shock and they were similarly labeled (Fig. 4). We also injected fluorescein-U7 snRNA into the oocyte cytoplasm prior to or immediately after heat shock. GVs were isolated at various times after injection and the contents examined by fluorescence microscopy. Fluorescein-U7 was targeted to all CBs within a GV, regardless of their size (data not shown). Label could be detected in CBs from GVs isolated only a few minutes after injection, and the intensity of label in CBs continued to rise over the first few hours after injection. Although quantitative studies were not carried out, we saw no obvious differences in the kinetics of uptake between heat-shocked and control oocytes. The experiments with GFP-coilin and fluorescein-U7 demonstrate that mini-CBs not only contain specific CB components but actively recruit them from the nucleoplasm. In this respect they appear to be functionally equivalent to CBs of typical size.

Mini-CB formation does not depend on new protein synthesis

We carried out several experiments to examine the protein composition of GVs before and after heat shock, looking for changes that might be relevant to the formation of mini-CBs. Oocytes were heat shocked for 1 hour at 31.5°C, injected with [^{35}S]methionine, and maintained for an additional 3 hours at the elevated temperature. Some heat shocked oocytes were returned to 18°C for a 12 hour recovery period before GV isolation. GVs were isolated and newly synthesized proteins were examined by polyacrylamide gel electrophoresis and autoradiography. The overall protein composition of GVs, as assessed by Coomassie Blue staining, was similar in all oocytes (Fig. 5A, lanes 1-3). However, GVs from heat-shocked oocytes showed a marked deficiency of newly synthesized proteins relative to control oocytes (Fig. 5B, lanes 4 and 5), consistent with previous studies on whole oocytes (Bienz and Gurdon, 1982; Horrell et al., 1987). During the recovery period (Fig. 5B, lane 6) the level of newly synthesized GV proteins returned to normal. Minor differences were detectable in the lower molecular weight
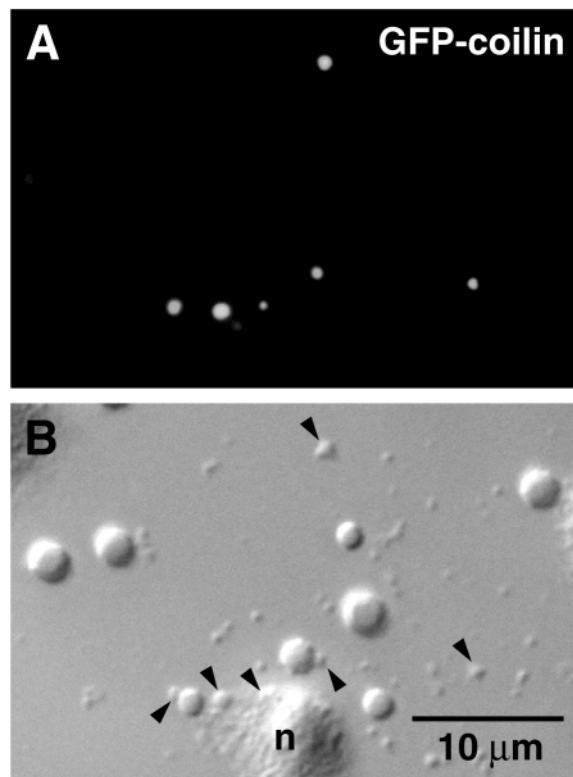

Fig. 4. A small fraction of the contents of a single GV from an oocyte that was heat shocked at 31.5°C for 3 hours, injected with GFP-coilin transcripts (into the cytoplasm) and allowed to recover overnight. (A) Immunofluorescence after staining with an anti-GFP antibody to show the localization of GFP-coilin in mini-CBs. The anti-GFP antibody enhanced the detectable but weak signal from mini-CBs. (B) DIC image of the same field. Mini-CBs are indicated by arrowheads. The larger round objects are B-snurposomes; a single nucleolus (n) is also present.
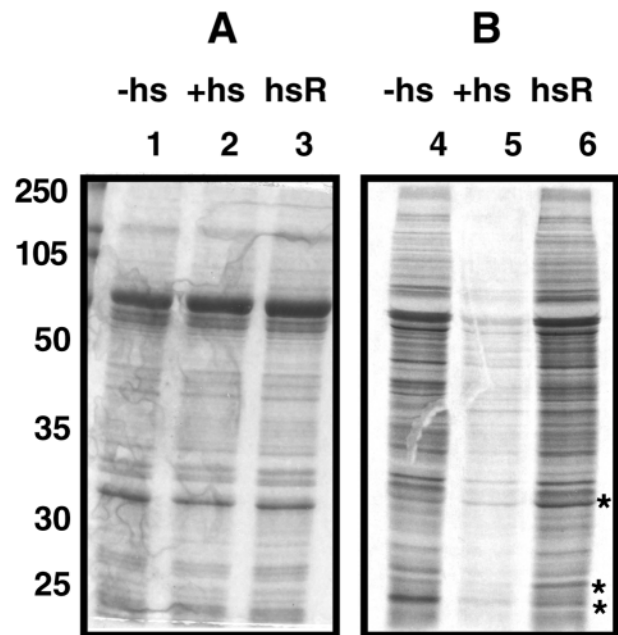

Fig. 5. Polyacrylamide gel electrophoresis of GV proteins from control oocytes (–hs), oocytes heat shocked at 31.5°C for 4 hours (+hs) and oocytes allowed to recover at 18°C for 12 hours after heat shock (hsR). All oocytes were injected with [^{35}S]-methionine to label newly synthesized proteins. (A) Proteins stained with Coomassie Blue to show equal loading of lanes and the absence of obvious changes in overall protein composition. (B) Autoradiograph of the same samples showing marked reduction in new protein synthesis after heat shock (lane 5) compared with control (lane 4), and renewal of protein synthesis during recovery from heat shock (lane 6). Stars indicate regions of the gel where differences are detectable between the control (lane 4) and heat shock recovery (lane 6). Molecular weight markers are in kDa.

range, where a few bands were more intense than in the controls and a few less so (stars, Fig. 5B, lane 6).

Although translation was greatly reduced during heat shock, it was not completely abolished, and new proteins were made during the recovery period when mini-CBs were formed. To test whether these newly synthesized proteins were required for mini-CB formation, we used cycloheximide at 50 µg/ml to inhibit all protein synthesis. In control experiments, no incorporation of [^{35}S]methionine was detected in GV proteins from oocytes incubated for 3 hours at 18°C in the drug. Oocytes were then placed in cycloheximide at 18°C for 3 hours, heat shocked for 4 hours, and allowed to recover at 18°C in the presence of the drug. Mini-CBs formed in these cycloheximide-treated oocytes, demonstrating that no new proteins are required for mini-CB formation. Cycloheximide alone, without heat shock, did not induce mini-CBs, indicating that inhibition of protein synthesis by itself does not cause CB formation.

The cycloheximide experiments ruled out the need for newly synthesized proteins. However, formation of mini-CBs might still depend on import of specific stored proteins from the cytoplasm. To exclude this possibility, we isolated GVs from heat-shocked oocytes and allowed them to recover in the absence of cytoplasm. Oocytes were first injected with fluorescein-U7 snRNA, which served as a visible marker for mini-CB formation, and were then heat shocked for 4 hours. GVs from some oocytes were removed under oil, where they were allowed to recover in the absence of cytoplasm. Other oocytes were held intact in an aqueous medium and their GVs removed under oil after the recovery period. Both populations were viewed by direct immunofluorescence after gentle squashing in oil under a coverslip. Mini-CBs were evident in both sets of GVs (data not shown). This result suggests that protein import during heat shock recovery is not required for mini-CB formation. In other words, the GV contains all components necessary for formation of mini-CBs before the process of recovery begins.

Although import of proteins during recovery from heat shock is not needed for mini-CB formation, modification of pre-existing GV proteins might be required. To look for gross changes, we examined the patterns of three CB proteins by western blotting before, during and after heat shock. We saw no obvious changes in the mobility or amount of coilin, Nopp140, or the largest subunit of RNA Pol II (Fig. 6). All three are phosphoproteins (Young, 1991; Meier and Blobel, 1992; Carmo-Fonseca et al., 1993), and differences in mobility might have been seen, if they had undergone changes in their state of phosphorylation in response to heat shock.

Formation of mini CBs is not dependent on transcription

When transcription is inhibited with actinomycin D or α-amanitin, the lampbrush chromosomes undergo dramatic changes in morphology (Izawa et al., 1963; Schultz et al., 1981) but CBs appear essentially unchanged in size and number. Disruption of transcription by the kinase inhibitor DRB leads to an increase in the number of B-snurposomes associated with the CBs, but does not noticeably affect the size or number of CBs themselves (Morgan et al., 2000). To test whether heat shock would induce mini-CBs in transcriptionally inhibited cells, we incubated oocytes in 10 µg/ml actinomycin
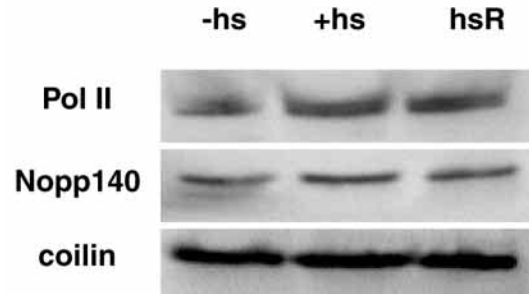

Fig. 6. Western blot of three phosphorylated proteins from GVs of control oocytes (–hs), oocytes heat shocked at 31.5°C for 4 hours (+hs) and oocytes allowed to recover overnight at 18°C after heat shock (hsR). Immunostained with mAb ARNA-3 against the largest subunit of RNA PolII, mAb No114 against *Xenopus* Nopp140 and mAb H1 against *Xenopus* coilin. No obvious differences in mobility were observed that might have indicated a change in phosphorylation state.

D in OR2 saline during heat shock and recovery. As expected, drug treatment caused contraction of the chromosomes and loss of their characteristic loops. Because of the continued presence of the inhibitor, the chromosomes maintained their contracted state during the recovery from heat shock. Nevertheless, mini-CBs formed in such nuclei, demonstrating that their formation can proceed in the complete absence of transcription. The actinomycin results were not entirely unanticipated: we already knew that Pol II transcription on the chromosomes, which is inhibited by the heat shock itself, sometimes does not resume during the recovery period when mini-CBs form. Although heat shock induces mini-CBs in transcriptionally inhibited oocytes, we emphasize that mini-CB formation is separable from transcriptional inhibition, since actinomycin treatment in the absence of heat shock does not cause mini-CBs to form.

Formation of mini-CBs does not depend on U7 snRNA

As in somatic nuclei, U7 snRNA exists at a very low concentration in the GV. Unlike the splicing snRNAs, which are found in other structures, U7 snRNA has been demonstrated only in CBs and at a low concentration in the nucleoplasm (Wu and Gall, 1993; Frey and Matera, 1995; Wu et al., 1996). For this reason U7 snRNA might be limiting for biogenesis of CBs. Consistent with this possibility, Tuma and Roth have shown that injection of exogenous U7 snRNA into the *Xenopus* oocyte nucleus was sufficient to induce formation of small CB-like structures in the nucleoplasm (Tuma and Roth, 1999). Their experiment left unanswered the question whether U7 snRNA is, in fact, necessary for formation of CBs. To examine this issue we used an antisense oligodeoxynucleotide to deplete U7 snRNA from the oocyte. As previously demonstrated, injection of an antisense oligo into the cytoplasm leads to rapid destruction of U7 in the GV, presumably by RNase H-mediated digestion of the RNA-DNA hybrid between endogenous U7 snRNA and the injected oligo (Stefanovic et al., 1995; Bellini and Gall, 1998). GVs from U7-depleted oocytes have morphologically normal CBs, demonstrating that U7 is not required for maintaining the structure of CBs. U7-depleted oocytes were heat shocked and allowed to recover for a minimum of 18 hours. Mini-CBs
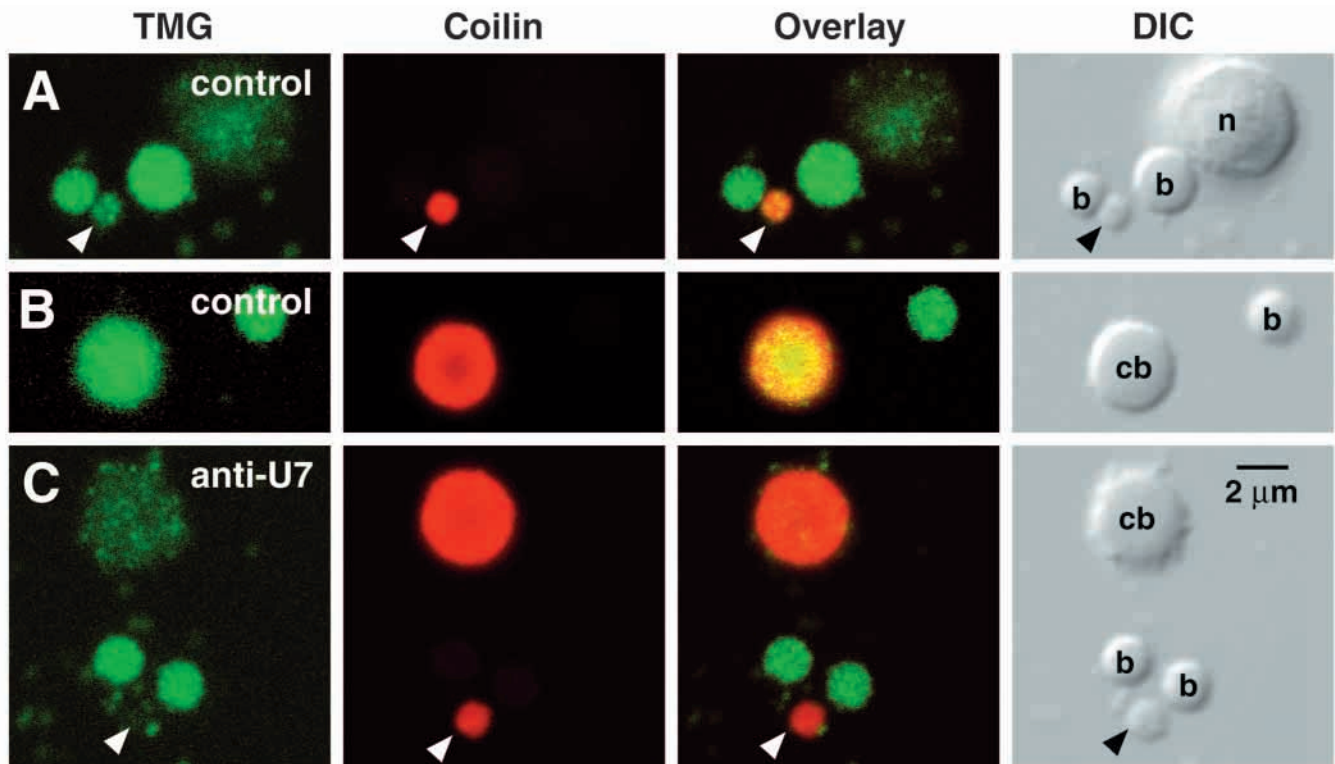
Fig. 7. Formation of mini-CBs in the absence of U7 snRNA. Confocal images of CBs stained with mAb K121 against the trimethylguanosine cap (first column) and counterstained with α-coilin serum C236 to identify the mini-CBs (second column). The overlay is shown in the third column and a DIC image is shown in the fourth column. (A,B) Mini-CB (arrowheads in A) and typical CB (cb in B) from an oocyte that was heat shocked at 31.5°C for 4 hours and allowed to recover overnight. The mini-CB and the typical CB stain strongly for both trimethylguanosine (green) and coilin (red). The mini-CB is closely associated with a B-snurposome (b). Nucleolus (n). (C) Mini-CB (arrowheads) and typical CB (cb) from an oocyte that was heat shocked after injection of an oligodeoxynucleotide against U7 snRNA. Elimination of U7 causes a marked reduction in trimethylguanosine staining of CBs, because U7 is the major capped snRNA in CBs. Mini-CBs are induced by heat shock in U7-depleted oocytes; they too are deficient in trimethylguanosine staining (arrowheads in C). Neither the morphology nor coilin staining of CBs is affected by U7 depletion.

appeared in the GVs of these oocytes as in non-depleted controls (Fig. 7). All CBs in U7-depleted oocytes, whether heat-shocked or control, show much reduced staining with mAb K121 against the trimethylguanosine cap found on several snRNAs, including U7. We interpret this reduced staining as evidence that U7 is the major capped snRNA in oocyte CBs (Bellini and Gall, 1998; Gall et al., 1999). The experiments with U7-depleted oocytes demonstrate that U7 snRNA is not necessary for maintaining pre-existing CBs or for inducing new CBs after heat shock.

Discussion
Mini-CBs and the heat shock response
The experiments reported here demonstrate that heat shock induces the formation of mini-CBs in the *Xenopus* GV, not during heat shock itself, but during a relatively protracted recovery period of 6-18 hours. The classic heat shock or stress response involves generalized repression of both transcription and translation accompanied by transcriptional activation of specific heat shock genes and production of the heat shock proteins themselves (Morimoto et al., 1994). The heat shock response in *Xenopus* oocytes has been studied extensively since the original experiments 20 years ago (Bienz and Gurdon,

1982). It is now generally agreed that the follicle cells surrounding the oocyte mount a typical heat shock response, but that newly synthesized heat shock mRNAs and proteins are not detectable in the oocyte itself after heat shock (Horrell et al., 1987; King and Davis, 1987). However, experiments with injected DNA constructs suggest that the GV is, in fact, competent under some conditions for a heat shock response at the transcriptional level (Landsberger et al., 1995; Gordon et al., 1997; Mercier et al., 1997; Ali et al., 1998). An issue in our experiments is whether the formation of mini-CBs after heat shock is part of the mechanism of the oocyte for responding to stress. We and others have suggested that CBs may be involved in assembling the transcription and/or RNA processing machinery of the cell (reviewed in Gall, 2000). If this is the case, additional CBs might be required to restore full transcriptional activity to the chromosomes during recovery from heat shock. Such an interpretation is not supported by observations on HeLa cells, in which CBs were smaller at 39°C than in controls (Carmo-Fonseca et al., 1993) and lost their content of snRNPs after heat shock at 45°C (Carmo-Fonseca et al., 1992). These somewhat divergent results make it difficult to determine whether the changes seen in CBs after heat shock are part of the stress response itself or represent secondary phenomena that may be specific to certain tissues or cell types.

In any case, a reliable protocol for inducing new CBs in the oocyte permits several useful generalizations about CB biogenesis.

Unlike nucleoli, CBs do not originate on specific DNA sequences

It has been known for many years that some CBs in the amphibian GV are attached to lampbrush chromosomes at the histone gene loci (Gall et al., 1981; Callan et al., 1991), suggesting that histone genes might play a role in CB biogenesis comparable with that of rDNA in the biogenesis of nucleoli. If CBs arise only at histone gene sites, some additional mechanism must account for the large number of unattached or free CBs in the GV. Two hypotheses can be considered. Free CBs might contain amplified copies of the histone genes, similar to the amplified rDNA in the extrachromosomal nucleoli of the GV (Brown and Dawid, 1968; Gall, 1968; Miller and Beatty, 1969), or free CBs might form by successive growth and detachment from the histone gene loci. The first hypothesis, amplification of histone genes, is unlikely, as histone DNA is not detected in free CBs by in situ hybridization during the course of experiments on newt lampbrush chromosomes (Gall et al., 1983). Moreover, amplified rDNA is readily detectable in the extrachromosomal nucleoli of the GV by DAPI staining, whereas CBs are not stained by DAPI. The growth and detachment model would be reasonable for the slow increase in number of CBs that takes place during the many weeks of normal oocyte growth. However, it is more difficult to imagine that hundreds or thousands of mini-CBs arise and detach from the three known histone loci during the course of several hours in our heat shock experiments or in the earlier U7 experiments of Tuma and Roth (Tuma and Roth, 1999). In mammalian cultured cells, CBs associate not only with histone genes (Frey and Matera, 1995) but also with several snRNA genes (Frey and Matera, 1995; Smith et al., 1995; Gao et al., 1997; Frey et al., 1999; Jacobs et al., 1999). Such association with snRNA genes has not been seen in *Xenopus* oocytes, but clearly the snRNA genes raise the same issues as the histone genes. In the case of the oocyte, amplification of the genes for U1, U2, U4, U5, U6 and U7 has been ruled out by quantitative filter hybridization of total GV DNA (Phillips et al., 1992).

More direct evidence that specific genes are not required as nucleating sites for CBs comes from nuclei assembled in *Xenopus* egg extract. Nuclei with prominent CBs can be assembled not only from sperm chromatin but also from prokaryotic DNA (Bell et al., 1992). The latter case effectively rules out the need for any specific eukaryotic gene as a nucleating site for CBs (Bauer et al., 1994; Gall, 2000). Even if CB formation does not require specific DNA sequences, the preferential association of CBs with histone and snRNA gene loci is indisputable. How and why CBs reach these loci, if they do not arise there, are questions that must be addressed by an adequate theory of CB biogenesis (Abbott et al., 1999; Frey et al., 1999).

No component that is unique to CBs or that might be an essential nucleating factor has been identified

Among all CB components, the U7 snRNP comes closest to being CB specific. Roughly 85% of the U7 snRNA in the GV is found in CBs, whereas the rest is in the nucleoplasm (Wu et al., 1996). Tuma and Roth have shown that mini-CBs form after injection of excess U7 snRNA into the *Xenopus* GV, suggesting that U7 might be a central nucleating factor for CBs (Tuma and Roth, 1999). However, by destroying endogenous U7 snRNA with an antisense oligodeoxynucleotide before heat shock, we demonstrated that U7 is not essential for mini-CB formation. Furthermore, in these experiments the removal of U7 has no discernible effect on the morphology of large CBs in control (or heat-shocked) oocytes, showing that U7 is not required for maintaining the structural integrity of CBs (Bellini and Gall, 1998).

Coilin is specifically enriched in CBs and was originally considered as a possible nucleating factor. However, CBs are present in nuclei assembled in coilin-depleted *Xenopus* egg extract (Bauer and Gall, 1997). Even more significant is the fact that coilin knockout mice are viable and exhibit 'residual' CBs in their cells (Tucker et al., 2001). In both the in vitro system and the knockout mouse, CBs contain normal concentrations of fibrillarin and other nucleolar proteins but fail to recruit Sm snRNPs.

Bell and Scheer (Bell and Scheer, 1997) have examined the role of proteins that are shared by CBs and nucleoli in the biogenesis of CBs in *Xenopus* egg extract. They showed that CBs (which they prefer to call prenucleolar bodies) are formed in extracts depleted for any of four nucleolar proteins – fibrillarin, nucleolin, Nopp140 and B23/NO38. Their experiments underscore the difficulty in defining any single component as essential for CB biogenesis or maintenance (Gall, 2000).

Four features of CB biogenesis in the oocyte system seem particularly relevant:

(1) many hundreds of mini-CBs form more or less simultaneously after heat shock or injection of U7 snRNA;

(2) newly formed mini-CBs have the same composition as the larger CBs in the same nuclei;

(3) heat shock induces mini-CBs in the absence of transcription and translation; and

(4) most newly formed mini-CBs are attached to B-snurposomes.

These features suggest a model in which mini-CBs originate over a period of hours by accretion of preformed components from the nucleoplasm, without incorporating newly synthesized RNAs or proteins. As the composition of the smallest and largest CBs is the same, growth in size must involve more or less simultaneous addition of all components. One can imagine that mini-CBs, once initiated, increase in size by addition of relatively large macromolecular complexes from the nucleoplasm.

In our heat-shock experiments and in the earlier U7 experiments (Tuma and Roth, 1999), it was common to see mini-CBs attached to B-snurposomes. In fact, associations between B-snurposomes and CBs are a regular feature of *Xenopus* oocytes. CBs of all sizes, especially those from younger oocytes, may have one or more B-snurposomes attached to their surface or embedded in their matrix. This intimate association between CBs and B-snurposomes suggests that B-snurposomes may themselves be the elusive nucleating factor. If this is the case, the search for a single nucleating molecule may be misplaced – CBs may form in association

with B-snurposomes under a variety of conditions that affect the nuclear environment. Several studies demonstrate the CBs are dynamic structures whose components are in a constant state of flux (Carmo-Fonseca et al., 1993; Wu et al., 1994; Wu et al., 1996; Samarsky et al., 1998; Narayanan et al., 1999b; Speckman et al., 1999; Morgan et al., 2000). Ongoing experiments from our laboratory show that two prominent CB components, U7 snRNA and coilin, equilibrate between the nucleoplasm and CBs with half times of only a few minutes (K. E. H., unpublished). The numbers and sizes of CBs in a given nucleus may thus fluctuate depending on subtle changes in the rates of accumulation and dispersal of their constituent macromolecular complexes. We suggest that heat shock alters the balance in the oocyte toward accumulation, and for reasons that are not understood, B-snurposomes serve as preferential sites for assembly of new CBs. In this model, CBs are an example of the more general class of self-organizing structures (Misteli, 2001).

References

Abbott, J., Marzluff, W. F. and Gall, J. G. (1999). The stem loop binding protein (SLBP1) is present in coiled bodies of the *Xenopus* germinal vesicle. *Mol. Biol. Cell* **10**, 487-499.

Ali, A., Bharadwaj, S., O'Carroll, R. and Ovsenek, N. (1998). HSP90 interacts with and regulates the activity of heat shock factor 1 in *Xenopus* oocytes. *Mol. Cell. Biol.* **18**, 4949-4960.

Bauer, D. W. and Gall, J. G. (1997). Coiled bodies without coilin. *Mol. Biol. Cell* **8**, 73-82.

Bauer, D. W., Murphy, C., Wu, Z., Wu, C.-H. H. and Gall, J. G. (1994). In vitro assembly of coiled bodies in *Xenopus* egg extract. *Mol. Biol. Cell* **5**, 633-644.

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

Bell, P. and Scheer, U. (1997). Prenucleolar bodies contain coilin and are assembled in Xenopus egg extract depleted of specific nucleolar proteins and U3 RNA. *J. Cell Sci.* **110**, 43-54.

Bellini, M. and Gall, J. G. (1998). Coilin can form a complex with the U7 small nuclear ribonucleoprotein. *Mol. Biol. Cell* **9**, 2987-3001.

Bienz, M. and Gurdon, J. B. (1982). The heat-shock response in Xenopus oocytes is controlled at the translational level. *Cell* **29**, 811-819.

Bregman, D. B., Du, L., van der Zee, S. and Warren, S. L. (1995). Transcription-dependent redistribution of the large subunit of RNA polymerase II to discrete nuclear domains. *J. Cell Biol.* **129**, 287-298.

Brown, D. D. and Dawid, I. B. (1968). Specific gene amplification in oocytes. *Science* **160**, 272-280.

Callan, H. G. (1986). *Lampbrush Chromosomes*. Berlin: Springer-Verlag.

Callan, H. G., Gall, J. G. and Murphy, C. (1991). Histone genes are located at the sphere loci of *Xenopus* lampbrush chromosomes. *Chromosoma* **101**, 245-251.

Carmo-Fonseca, M., Ferreira, J. and Lamond, A. I. (1993). Assembly of snRNP-containing coiled bodies is regulated in interphase and mitosis – evidence that the coiled body is a kinetic nuclear structure. *J. Cell Biol.* **120**, 841-852.

Carmo-Fonseca, M., Pepperkok, R., Carvalho, M. T. and Lamond, A. I. (1992). Transcription-dependent colocalization of the U1, U2, U4/U6, and U5 snRNPs in coiled bodies. *J. Cell Biol.* **117**, 1-14.

Flannery, A. V. and Hill, R. S. (1988). The effect of heat shock on the morphology of amphibian lampbrush chromosomes. *Exp. Cell Res.* **177**, 9-18.

Frey, M. R., Bailey, A. D., Weiner, A. M. and Matera, A. G. (1999). Association of snRNA genes with coiled bodies is mediated by nascent snRNA transcripts. *Curr. Biol.* **9**, 126-135.

Frey, M. R. and Matera, A. G. (1995). Coiled bodies contain U7 small nuclear RNA and associate with specific DNA sequences in interphase human cells. *Proc. Natl. Acad. Sci. USA* **92**, 5915-5919.

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

Gall, J. G. (1968). Differential synthesis of the genes for ribosomal RNA during amphibian oogenesis. *Proc. Natl. Acad. Sci. USA* **60**, 553-560.

Gall, J. G. (1998). Spread preparation of *Xenopus* germinal vesicle contents. In *Cells: A Laboratory Manual*, Vol. 1 (ed. D. Spector, R. Goldman and L. Leinwand), pp. 52.51-52.54. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Gall, J. G. (2000). Cajal bodies: the first 100 years. *Annu. Rev. Cell Dev. Biol.* **16**, 273-300.

Gall, J. G., Stephenson, E. C., Erba, H. P., Diaz, M. O. and Barsacchi-Pilone, G. (1981). Histone genes are located at the sphere loci of newt lampbrush chromosomes. *Chromosoma* **84**, 159-171.

Gall, J. G., Diaz, M. O., Stephenson, E. C. and Mahon, K. A. (1983). The transcription unit of lampbrush chromosomes. In *Gene Structure and Regulation in Development*, Vol. 41 (ed. S. Subtelny and F. Kafatos), pp. 137-146. New York: Alan R. Liss.

Gall, J. G., Bellini, M., Wu, Z. and Murphy, C. (1999). Assembly of the nuclear transcription and processing machinery: Cajal bodies (coiled bodies) and transcriptosomes. *Mol. Biol. Cell* **10**, 4385-4402.

Gao, L., Frey, M. R. and Matera, A. G. (1997). Human genes encoding U3 snRNA associate with coiled bodies in interphase cells and are clustered on chromosome 17p11.2 in a complex inverted repeat structure. *Nucleic Acids Res.* **25**, 4740-4747.

Gordon, S., Bharadwaj, S., Hnatov, A., Ali, A. and Ovsenek, N. (1997). Distinct stress-inducible and developmentally regulated heat shock transcription factors in *Xenopus* oocytes. *Dev. Biol.* **181**, 47-63.

Hadjiolov, A. (1985). *The Nucleolus and Ribosome Biogenesis*. Wien: Springer-Verlag.

Horrell, A., Shuttleworth, J. and Colman, A. (1987). Transcript levels and translational control of hsp70 synthesis in *Xenopus* oocytes. *Genes Dev.* **1**, 433-444.

Huang, H., Marsh-Armstrong, N. and Brown, D. D. (1999). Metamorphosis is inhibited in transgenic *Xenopus laevis* tadpoles that overexpress type III deiodinase. *Proc. Natl. Acad. Sci. USA* **96**, 962-967.

Izawa, M., Allfrey, V. G. and Mirsky, A. L. (1963). The relationship between RNA synthesis and loop structure in lampbrush chromosomes. *Proc. Natl. Acad. Sci. USA* **49**, 544-551.

Jacobs, E. Y., Frey, M. R., Wu, W., Ingledue, T. C., Gebuhr, T. C., Gao, L., Marzluff, W. F. and Matera, A. G. (1999). Coiled bodies preferentially associate with U4, U11 and U12 small nuclear RNA genes in interphase HeLa cells but not with U6 and U7 genes. *Mol. Biol. Cell* **10**, 1653-1663.

Karpen, G. H., Schaefer, J. E. and Laird, C. D. (1988). A Drosophila rRNA gene located in euchromatin is active in transcription and nucleolus formation. *Genes Dev.* **2**, 1745-1763.

King, M. L. and Davis, R. (1987). Do *Xenopus* oocytes have a heat shock response? *Dev. Biol.* **119**, 532-539.

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

Krämer, A., Haars, R., Kabisch, R., Will, H., Bautz, F. A. and Bautz, E. K. F. (1980). Monoclonal antibody directed against RNA polymerase II of Drosophila melanogaster. *Mol. Gen. Genet.* **180**, 193-199.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Landsberger, N., Ranjan, M., Almouzni, G., Stump, D. and Wolffe, A. P. (1995). The heat shock response in *Xenopus* oocytes, embryos, and somatic cells: a regulatory role for chromatin. *Dev. Biol.* **170**, 62-74.

Lerner, E. A., Lerner, M. R., Janeway, C. A. and Steitz, J. A. (1981). Monoclonal antibodies to nucleic acid-containing cellular constituents: probes for molecular biology and autoimmune disease. *Proc. Natl. Acad. Sci. USA* **78**, 2737-2741.

Meier, U. T. and Blobel, G. (1992). Nopp140 shuttles on tracks between nucleolus and cytoplasm. *Cell* **70**, 127-138.

Mercier, P. A., Foksa, J., Ovsenek, N. and Westwood, J. T. (1997). *Xenopus* heat shock factor 1 is a nuclear protein before heat stress. *J. Biol. Chem.* **272**, 14147-14151.

Messmer, B. and Dreyer, C. (1993). Requirements for nuclear translocation and nucleolar accumulation of nucleolin of *Xenopus laevis*. *Eur. J. Cell Biol.* **61**, 369-382.

Miller, O. L., Jr and Beatty, B. R. (1969). Visualization of nucleolar genes. *Science* **164**, 955-957.

Misteli, T. (2001). The concept of self-organization in cellular architecture. *J. Cell Biol.* **155**, 181-185.

Morgan, G. T., Doyle, O., Murphy, C. and Gall, J. G. (2000). RNA polymerase II in Cajal bodies of amphibian oocytes. *J. Struct. Biol.* **129**, 258-268.

Morimoto, R. I., Tissières, A. and Georgopoulos, C. (1994). *The Biology of Heat Shock Proteins and Molecular Chaperones*. Plainview, NY: Cold Spring Harbor Laboratory Press.

Narayanan, A., Lukowiak, A., Jády, B. E., Dragon, F., Kiss, T., Terns, R. M. and Terns, M. P. (1999a). Nucleolar localization signals of box H/ACA small nucleolar RNAs. *EMBO J.* **18**, 5120-5130.

Narayanan, A., Speckmann, W., Terns, R. and Terns, M. P. (1999b). Role of the box C/D motif in localization of small nucleolar RNAs to coiled bodies and nucleoli. *Mol. Biol. Cell* **10**, 2131-2147.

Olson, M., Dundr, M. and Szebeni, A. (2000). The nucleolus: an old factory with unexpected capabilities. *Trends Cell Biol.* **10**, 189-196.

Paine, P. L., Johnson, M. E., Lau, Y.-T., Tluczek, L. J. M. and Miller, D. S. (1992). The oocyte nucleus isolated in oil retains in vivo structure and functions. *BioTechniques* **13**, 238-245.

Phillips, S., Cotten, M., Laengle-Rouault, F., Schaffner, G. and Birnstiel, M. L. (1992). Amphibian oocytes and sphere organelles: are the U snRNA genes amplified? *Chromosoma* **101**, 549-556.

Piñol-Roma, S., Swanson, M. S., Gall, J. G. and Dreyfuss, G. (1989). A novel heterogeneous nuclear RNP protein with a unique distribution on nascent transcripts. *J. Cell Biol.* **109**, 2575-2587.

Pollard, K., Lee, D., Casiano, C., Bluthner, M., Johnston, M. and Tan, E. (1997). The autoimmunity-inducing xenobiotic mercury interacts with the autoantigen fibrillarin and modifies its molecular and antigenic properties. *J. Immunol.* **158**, 3521-3528.

Samarsky, D. A., Fournier, M. J., Singer, R. H. and Bertrand, E. (1998). The snoRNA box C/D motif directs nucleolar targeting and also couples snoRNA synthesis and localization. *EMBO J.* **17**, 3747-3757.

Scheer, U. and Hock, R. (1999). Structure and function of the nucleolus. *Curr. Opin. Cell Biol.* **11**, 385-390.

Schmidt-Zachmann, M., Hügle, B., Scheer, U. and Franke, W. W. (1984). Identification and localization of a novel nucleolar protein of high molecular weight by a monoclonal antibody. *Exp. Cell Res.* **153**, 327-346.

Schultz, L. D., Kay, B. K. and Gall, J. G. (1981). In vitro RNA synthesis in oocyte nuclei of the newt *Notophthalmus*. *Chromosoma* **82**, 171-187.

Shaw, P. J. and Jordan, E. G. (1995). The nucleolus. *Annu. Rev. Cell Dev. Biol.* **11**, 93-121.

Sleeman, J. E. and Lamond, A. I. (1999). Newly assembled snRNPs associate with coiled bodies before speckles, suggesting a nuclear snRNP maturation pathway. *Curr. Biol.* **9**, 1065-1074.

Smith, K. P., Carter, K. C., Johnson, C. V. and Lawrence, J. B. (1995). U2 and U1 snRNA gene loci associate with coiled bodies. *J. Cell. Biochem.* **59**, 473-485.

Speckman, W., Narayanan, A., Terns, R. and Terns, M. (1999). Nuclear retention elements of U3 small nucleolar RNA. *Mol. Cell. Biol.* **19**, 8412-8421.

Stefanovic, B., Hackl, W., Lührmann, R. and Schümperli, D. (1995). Assembly, nuclear import and function of U7 snRNPs studied by microinjection of synthetic U7 RNA into Xenopus oocytes. *Nucleic Acids Res.* **23**, 3141-3151.

Tucker, K. E., Berciano, M. T., Jacobs, E. Y., LePage, D. F., Shpargel, K. B., Rossire, J. J., Chan, E. K. L., Lafarga, M., Conlon, R. A. and Matera, A. G. (2001). Residual Cajal bodies in coilin knockout mice fail to recruit Sm snRNPs and SMN, the spinal muscular atrophy gene product. *J. Cell Biol.* **154**, 293-307.

Tuma, R. S. and Roth, M. B. (1999). Induction of coiled body-like structures in *Xenopus* oocytes by U7 snRNA. *Chromosoma* **108**, 337-344.

Tuma, R. S., Stolk, J. A. and Roth, M. B. (1993). Identification and characterization of a sphere organelle protein. *J. Cell Biol.* **122**, 767-773.

Wallace, R. A., Jared, D. W., Dumont, J. N. and Sega, M. W. (1973). Protein incorporation by isolated amphibian oocytes: III. Optimum incubation conditions. *J. Exp. Zool.* **184**, 321-333.

Wu, C.-H. H. and Gall, J. G. (1993). U7 small nuclear RNA in C snurposomes of the *Xenopus* germinal vesicle. *Proc. Natl. Acad. Sci. USA* **90**, 6257-6259.

Wu, C.-H. H., Murphy, C. and Gall, J. G. (1996). The Sm binding site targets U7 snRNA to coiled bodies (spheres) of amphibian oocytes. *RNA* **2**, 811-823.

Wu, Z., Murphy, C. and Gall, J. G. (1994). Human p80-coilin is targeted to sphere organelles in the amphibian germinal vesicle. *Mol. Biol. Cell* **5**, 1119-1127.

Young, R. A. (1991). RNA polymerase II. *Annu. Rev. Biochem.* **60**, 689-715.