Precocious detection on amphibian oocyte lampbrush chromosomes of subtle changes in the cellular localisation of the Ro52 protein induced by in vitro culture

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Abstract

Subterminal lampbrush loops of one of the twelve bivalents of the oocyte karyotype of *Pleurodeles waltl* (Amphibian, Urodele) underwent prominent morphological changes upon *in vitro* culture. These loops exhibited a fine RNP granular matrix, which evolved during culture into huge structures that we have named « chaussons » (slippers). This phenomenon involved a progressive accumulation of proteins in the RNP matrix without protein neosynthesis. One of these proteins, which is homologous to the human Ro52 E3 ubiquitin ligase, translocated into the nucleus during the culture. These results emphasise the considerable value of the lampbrush chromosomes system for a direct visualisation of modifications in gene expression. They also raise the question whether such a nuclear accumulation of Ro52 occurs also in human oocytes in the course of *in vitro* fertilization procedures for overcoming infertility problems.

Introduction

Over the last five decades, the amphibian oocyte has been widely used as an experimental system to study complex biological processes. Because of its large size and easy manipulation, in vitro translated RNA, plasmid DNA, proteins or somatic nuclei can be easily injected into the cytoplasm or the large nucleus also called germinal vesicle (GV) (for a review, see Halley-Stott et al., 2010). The fate of these exogenous elements can be investigated by further in vitro culture of the injected oocyte, in which RNA transcription and protein synthesis may continue for up to several weeks.

The germinal vesicle contains lampbrush chromosomes (LBCs), which are giant in size and actively transcribed. They are considered as a prime model for the analysis of transcriptional and post-transcriptional processes (for a review, see Morgan, 2002, Gall et al., 2004, Sommerville, 2010 and Macgregor, this issue). At the diplotene stage, these chromosomes are associated in bivalents whose axes are comprised of a succession of highly condensed chromatin regions, the chromomeres, from which lateral loops unfold that are the sites of intense RNA synthesis. The nascent RNA transcripts associate with proteins to form the ribonucleoprotein (RNP) matrix of the loops with some proteins common to all lateral loops and others specific of a subset. Almost all loops share a common basic organization with transcripts appearing as fibrils comprised of 30 nm RNP particles, but they differ by their size and the degree of aggregation of the RNP fibrils. While the majority of the loops conform to a standard type in which the RNP matrix exhibits a fine fibrous texture, others such as the granular, globular and giant

fusing loops display distinct morphologies. They are always observed at the same chromosomal loci and constitute obvious landmarks which enabled LBCs maps to be drawn up in many newt species (for a review, see Callan, 1986) and in the anurans *Xenopus laevis* (Callan et al., 1987) and *Xenopus tropicalis* (Penrad-Mobayed et al., 2009). LBCs have also been used as a powerful system for a direct visualization of changes in gene expression in the oocyte. Their architecture is dynamic and directly related to transcriptional and post-transcriptional processes any modifications of which are reflected by concomitant morphological variations in chromosomal structure. Thus, analysis of LBCs after cold or heat shock treatment revealed a morphological evolution of specific loops, which occurred at constant and reproducible loci (for a review, see Angelier et al., 1990).

We report here the observation of such a prominent morphological development affecting the subterminal loops of bivalent XII in full-grown (stage VI) oocytes (Bonnanfant-Jaïs and Mentré, 1983) of the newt *Pleurodeles waltl* (Amphibian, Urodele) when cultured *in vitro*. Using a polyclonal antibody we provide evidence that this major structural change was concomitant with an accumulation of the Ro52 E3 ubiquitin ligase on these loops.

Materials and methods

Oocytes culture. *P. waltl* were raised in our laboratory at 18°C. Ovarian biopsies were performed on adult females anesthetized in 0.15% Tricaine methane sulfonate (MS222, Sigma Chemical, St. Louis, MO). Oocytes were defolliculated for 2-3 hours in saline buffer OR2 containing 0.15% collagenase type II (Sigma Chemical, St. Louis, MO). Full grown oocytes (stage VI) (Bonnanfant-Jaïs and Mentré, 1983) were selected and incubated at 18°C either in MBS Buffer (Modified Barth's Solution) (Gurdon, 1977) or in the culture medium 199 with 25 mM Hepes (Gibco) supplemented with Gentamycin (5μg/mL). Oocytes were incubated with cycloheximide (200 μg/ml) during 24h as indicated.

Nuclear spreads and immunofluorescence. Germinal vesicles from stage V-VI oocytes were manually isolated in 75mmol/L KCl, 25mmol/L NaCl, 0.01mmol/L MgCl2 and 0.01mmol/L CaCl2, pH7.2 and lampbrush chromosomes were prepared as previously described (Penrad-Mobayed et al., 2010). Nuclear spread preparations were fixed for 30 min at 4°C in Phosphate Buffer Saline (PBS) containing 2% paraformaldehyde and 1 mM $MgCl_2$, washed with PBS and blocked for 10 min with Horse serum at 10% dilution. They were then incubated with a rabbit polyclonal anti-Ro52 antiserum (Ricchiuti et al., 1997) (kindly supplied by S. Muller, I.B.M.C, Strasbourg) at 1:250 dilution. After washing with PBS, preparations were incubated with the secondary antibody (Alexa 488 goat anti-rabbit IgG, Invitrogen Corp., or biotinylated goat anti-rabbit diluted 1:50 and Texas red-

streptavidin diluted 1:50). Preparations were post-stained either with DAPI (4', 6-diamidino-2-phenylindole dihydrochloride) at a concentration of 0.5 μg/mL or Hoechst 33342 (1:1,000). Fluorescence microscopy was performed using a Leitz DMRB CDD camera and images were assembled using Adobe Photoshop.

Incubation conditions for in vitro RNA synthesis and autoradiography

Oocytes were incubated in MBS Buffer which contained 250 μCi/mL of 5,6-3H-uridine (aqeuous solution, sp. act., 42 Ci/mMol) and 250 μCi/mL of 5,6-3H-cytidine (aqueous solution, sp. act. 30 Ci/mMol). Incubation time ranged from 24 to 48 h. Chromosomes preparations were fixed for 30 min in 2% paraformaldehyde and processed for autoradiography as previously described (Penrad-Mobayed et al., 1986). Chromosomes were stained as described by Gall (1981) with Coomassie blue (0,1% in 50% methanol, 10% acetic acid) for 10 mn.

Protein extraction and SDS-polyacrylamide gel electrophoresis and western blots

Oocytes were incubated in MBS containing 2% TCA (w/v) for 20 min to precipitate proteins, washed with PBS and kept at 4°C overnight before dissection. Air-dried germinal vesicles were resuspended in Laemmli buffer and treated for electrophoresis. Cytoplasms from enucleated oocytes were homogeneized in Tris-EDTA buffer containing 0.1 mg/mL pancreatic ribonuclease A and 10mmol/L Pefabloc (Interchim, USA). The homogenate was centrifuged for 10 min at 10,000g. Proteins of the supernatant were

precipitated overnight at -20°C. The precipitate was recovered by 30 min centrifugation at 5000 g and treated for electrophoresis. Samples were denatured by heating in Laemmli buffer and separated in a 4-12% NuPage gels (In vitrogen). After transfer PVDF membranes were incubated overnight in PBS + 0.1% Tween 20 (PBST) + 5% fat-free dry milk at 4°C and further incubated with either the primary rabbit polyclonal anti-Ro52 at a 1/500 dilution or the monoclonal anti-alpha actine antibody (MP Biomedicals) at a 1/10,000 dilution in PBS + 0.5% BSA for 1h. They were then washed in PBST, incubated with either an HRP conjugated goat anti-rabbit or an HRP conjugated rabbit anti-mouse (Jackson ImmunoResearch) diluted to 1/10,000 in PBS + 0.5% BSA for 1h and washed in PBST. Detection was performed using the ECL “SuperSignal West Femto” (Thermo Scientific). Membranes were scanned with the LAS-3000 imaging system (FUJI).

Results

Effects of in vitro culture on lampbrush chromosomes structure

The mapping of *P. waltl* LBCs had been achieved thanks to their different lengths and the presence of conspicuous landmarks on 10 out of the 12 bivalents of the oocyte karyotype (Lacroix, 1968). LBCs were oriented according to their centromere location with the short arm to the right (Lacroix, 1968). Bivalent XII is the smallest of the *P. waltl* karyotype and lacks any regular identifying landmarks. However, several double-bridges of the chromosome axis at the right extremity constitute a useful recognition feature (Fig.1 a). These split regions, which correspond to the separation of the two halves of the chromomere (Macgregor, this issue), are fragile and often break during the LBCs spreading procedure resulting in the tearing off of the right end of the bivalent. (Fig. 1b, 2a). Of note, these double-axis regions are also observed in the smallest chromosome of the newts *Triturus cristatus* and *Triturus marmoratus* (for a review see Callan, 1986).

In full-grown stage VI oocytes subterminal loops at the left and right extremities of bivalent XII exhibited a fine granular matrix with a distinguishable polarity (Fig. 2a-d). In oocytes incubated *in vitro* from 24 to 72h, these subterminal lampbrush loops remained well developed but underwent prominent morphological modifications (Fig. 2e-h). The matrix of the left loops thickened and their axis coiled up in a pronounced spiral in the thickest regions (Fig. 2g). Changes affecting the right loops were spectacular. After 24h of culture they became covered with a thick matrix dotted with granules of progressively increasing size along their length (fig. 2h and fig.

5a'). After 48h, these granules were fused in a continuous sheet around the loop axis (Fig. 5b'). This huge development of the left and right subterminal loops of bivalent XII were still observable after 10 days of culture. Similar morphological changes were recorded when the oocytes were cultured in the MBS solution or in the 199 medium with or without cycloheximide (data not shown). Active RNA transcription in the subterminal loops was not affected by the culture as shown by the incorporation of RNA precursors into their matrix (Fig. 1b'-d'). In contrast, the subterminal loops of the other chromosomes did not undergo similar changes (Fig 3c' and 4c'). As for other loops of the karyotype (Fig. 4c', 4d'), they became somewhat shortened and less extended similarly to what had been reported for LBCs from cultured full-grown oocytes of *T. cristatus* (Flannery and Hill, 1988) and *X. tropicalis* (Penrad-Mobayed et al., 2010).

Immunolocalization of Ro52 on LBCs spreads of *P.waltl* oocytes

Morphological changes occurring on one loop may be due to variations in the composition of proteins that form its RNP matrix. We investigated the possibility that the Ro52 protein might be one of them because Vishnyakova et al., (2004) had reported previously the labelling of one loop near the right extremity of bivalent XII of *P. waltl* using a polyclonal anti-Ro52 antiserum. We first checked the specificity of this antiserum by immunoblotting using nuclear extracts of *P. waltl* oocytes. The antiserum reacted with one major band of the expected 52 kDa molecular weight (Fig. 6a). When applied to LBCs from freshly isolated control oocytes, the anti-Ro52 antibody labelled less than ten lampbrush loops on 5 different bivalents including bivalent XII. As reported by

Vishnyakova et al. (2004), we have also observed that some loops on bivalents I, II, V, VIII were intensely labelled, as shown for bivalent V (Fig. 3d), while subterminal loops at the right extremity of bivalent XII were weakly labelled (Fig. 3b). However, we have detected in addition other lightly labelled loci not described by Vishnyakova et al. (2004) such as the subterminal loops at the left extremity of bivalent XII (Fig. 3a) and lateral loops near its right extremity (Fig. 3b), as well as the subterminal loops of other bivalents such as the ones shown in Fig. 3c. After 24h of culture, both the left and right subterminal loops of bivalent XII became strongly labelled (Fig 4.a, b and Fig. 5a, b) while the subterminal loops of other chromosomes remained only lightly labelled (fig. 4c). In contrast no change in Ro52 staining of the other chromosomal sites was observed during the same culture periods as shown for the labelled loops of bivalent V (fig. 4d).

Changes in nucleocytoplasmic distribution of Ro52 during the in vitro culture

The nucleocytoplasmic distribution of the Ro52 protein in the course of a 3 days culture was compared to that of freshly isolated controls. In the control oocyte, a Ro52 immunoreacting band was observed in both the nuclear and cytoplasmic extracts. During the in vitro culture, the intensity of this band increased in the nuclear extract whereas it decreased markedly in the cytoplasmic extract (Fig. 6b).

Discussion

Prolonged *in vitro* culture of full-grown *P. waltl* oocytes led to a huge development of the RNP matrix of the subterminal loops of bivalent XII and the right ones in particular. The development of this atypical structure that we propose to refer to as “chaussons” (slippers) is probably due to an accumulation of proteins that interact with the RNP transcripts of these loops. One of these proteins is immunologically related to the human Ro52 protein as shown with the anti-Ro52 antibody, which revealed one major band corresponding to the expected molecular mass in GV extracts. It is noteworthy that the immunostaining intensity of this band increased in parallel with that of the RNP matrix of the bivalent XII subterminal loops during the *in vitro* culture. The question arises whether these culture–induced modifications of bivalent XII are specific of *P. waltl* or could be observed in other amphibians. The use of in vitro-cultured amphibian oocytes as an experimental system was initiated by Gurdon et al. (1971). They demonstrated that oocytes cultured in a simple saline solution could translate any injected mRNA over a long period with a high efficiency and no species specificity. Since then in vitro culture for 1 to 3 days of injected oocytes has been used extensively to analyse with LBCs as a readout the effect on mRNA translation, chromatin condensation and nucleo-cytoplasmic trafficking of a variety of molecules, be they oligonucleotides, RNAs, plasmid DNAs or antibodies (for a review, see Morgan, 2002). The vast majority of these studies has been carried out with *Xenopus laevis* oocytes whose LBCs display lateral loops with a small size compared to that of the newt LBCs. This may be the reason why no culture-induced modification of the morphology of *Xenopus* LBCs lateral loops has been

reported to date. To our knowledge however no detailed study of such modifications has been reported either in newt species in which LBCs have been mapped.

Similarly to Vishnyakova et al. (2004), we have observed that less than ten loops distributed on 5 bivalents including bivalent XII, some of them of giant size, were stained with the anti-Ro52 antibody in *P. waltl* oocytes before culture. All of these loops exhibited a morphologically similar RNP matrix in phase microscopy. It is remarkable that only the sub-terminal loops of bivalent XII underwent a modification into a “chausson” structure upon in vitro culture. This implies that the RNP matrix of these loops is different from that of other Ro52-specific loops. As mentioned above loops are comprised of a DNA axis and an RNP matrix composed of RNA and proteins either common to all transcribed loops or specific of a subset of them. Proteins in the loops can bind either directly to DNA or nascent RNAs or indirectly through protein-protein interactions. This may be the case for the Ro52 protein, also denoted 52KDa Ro/SSA, RING-finger protein 81(RNF81) or TRIM21, which is a RING-finger protein and a member of the tripartite motif (TRIM) family of proteins that are characterized by a RING finger domain, one or two b-box motifs and a coiled-coil domain containing a leucine zipper motif. In Ro52, the coiled-coil domain is followed by a B30.2 (PRYSPRY) motif in the C-terminal end. The coiled-coil domain is important for cytoplasmic localisation of the protein, while the b30.2 region is essential for its nuclear translocation (for a review, see Oke & Wahren-Herlenius, 2012). It has been suggested that Ro52 functions as a transcription factor (Frank, 1999, Wang et al., 2001) but it has been clearly demonstrated that it has an E3 ligase activity and acts in the process

of ubiquitinylation (Wada & Kamitani, 2006, Espinosa et a., 2008). It may interact with both the cytoplasmic UBE2D1 and the nuclear UBE2E1 ubiquitin conjugating enzymes (for a review, see Oke & Wahren-Herlenius, 2012). It has also been suggested that Ro52 is part of the Ro Ribonucleoprotein particles (Ro-RNPs), which are also composed of Ro 60, La proteins and hYRNA small cytoplasmic RNA molecules, although this association has not been confirmed yet (Sim and Wolin, 2011).

We have observed that in the *P. waltl* oocyte, Ro52 was initially detected equally in the cytoplasm and GV and accumulated in the GV during culture. This nuclear translocation could explain in part the immunostaining of granules accumulated in the RNP matrix of the “chaussons”. Simple diffusion is probably not involved in this translocation because Simons et al., (1994) have shown that the human Ro52 protein microinjected into *X. laevis* oocyte is translocated into the nucleus via an active transport mechanism. A parallel can be drawn between the nuclear translocation of Ro52 during the in vitro culture of the *P. waltl* oocyte and its similar translocation reported in mammals where it has been shown to be expressed in most tissues and cells and predominantly cytoplasmic or nuclear according to the cell type (for review, see Oke and Wahren-Herlenius, 2012). Ro52 has also been demonstrated to translocate from the cytoplasm to the cell surface or into the nucleus in apoptotic cells or stressed cells as in the case of HeLa cells exposed to extrinsic factors such as nitric oxide (NO) (Espinosa et al., 2008, for review, see Oke and Wahren-Herlenius, 2012). One possibility is that the dissociation of the amphibian oocyte from the environing maternal tissues can induce a stress to the oocyte, which may trigger the nuclear translocation of Ro52 and

its accumulation at the level of some transcripts and RNPs. The biological significance of this observation remains to be assessed. It can be stressed that Ro52 is the focus of numerous studies because it is one of the major auto antigens targeted in the Sjögren's syndrome (SS), an autoimmune disorder. Ro52 shows also the highest correlation with the autoimmune disease Neonatal Lupus Erythematous (NLE) that can affect foetuses of mothers positive for Ro autoantibodies (for review, see Oke and Wahren-Herlenius, 2012). In this context our results raise the question whether the nuclear accumulation of Ro52 may also occur in human oocytes in the course of in vitro fertilization procedures and what might be the consequences on foetal development.

Acknowledgements

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References

Angelier N, Bonnanfant-Jaïs ML, Herberts Ch, Lautredou N, Moreau N, N'Da E, Penrad-Mobayed M, Rodriguez-Martin ML, Sourrouille P (1990). Chromosomes of amphibian oocytes as a model for gene expression: Significance of Lampbrush loops. Int. J. Dev. Biol 34: 69-80.

Bonnanfant-Jaïs ML, Mentré P (1983) Study of oogenesis in the different stages oocyte development. J Submicrosc Cytol 15: 453-478.

Callan HG (1986) Lampbrush Chromosomes Springer-Verlag , Berlin, Heildelberg, New-york, Tokyo.

Callan HG, Gall JG, Berg CA (1987) The lampbrush chromosomes of Xenopus laevis: preparation,identification and distribution of 5S DNA sequences. Chromosoma 95: 236-250.

Espinosa A, Oke V, Elfving A, Nyberg F, Covacu R, Wahren-Herlerius M (2008) The autoantigen Ro52 is an E3 ligase resident in the cytoplasm but enters the nucleus upon cellular exposure to nitric oxide. Exp Cell Res 314: 3605-3613.

Flannery AV, Hill RS (1988) The effect of heat shock on the morphology of amphibian lampbrush chromosomes. Exp Cell Res 177: 9-18.

Frank MB (1999) Characterization of DNA Binding Properties and Sequence specificity of the Human 52 kDa Ro/SS-A (Ro52) Zinc Finger Protein. Biochem. Biophys. Res. Commun 259: 665–670.

Gall JG, Stephenson EC, Erba HP, Diaz MO, Barsacchi-Pilone G (1981) Histones genes are located at the sphere loci of newt lampbrush chromosomes. Chromosoma: 84, 159-171.

Gall JG, Wu Z, Murphy C, Gao H (2004) Structure in the amphibian germinal vesicle. Exp Cell Res 296: 28-34.

Gurdon J.B 1977 Methods for nuclear transplantation in amphibia. Methods Cell Biol. 16:125-39.

Gurdon JB, Lane CD, Woodland HR, Marbaix G (1971) Use of frog eggs and oocytes for the study of messenger RNA and its translation in living cells. Nature 233: 177-182.

Halley-Stott RP, Pasque V, Astrand C, Miyamoto K, Simeoni I, Jullien J, Gurdon JB (2010) Mammalian nuclear transplantation to Germinal Vesicle stage Xenopus oocytes - a method for quantitative transcriptional reprogramming. Methods 51:56-65.

Lacroix JC (1968) Etude descriptive des chromosomes en écouvillon dans le genre pleurodeles (Amphibien, Urodèle). Ann Embryol Morphogen 1:179-202.

Macgregor HC (2012) Chromomeres revisited. Chromosome Res: in press.

Morgan GT (2002) Lampbrush chromosomes and associated bodies: new insights into principles of nuclear structure and function. Chromosome Res 10:177-200.

Oke V, Wahren-Herlenius M (2012) The immunobiology of Ro52 (TRIM21) in autoimmunity: a critical review. J Autoimmun 39:77-82.

Penrad-Mobayed M, Bonnanfant-Jaïs ML, N'Da E, Angelier N (1986) Evidence for a particular mode of transcription in globular loops of lampbrush chromosomes of the newt Pleurodeles waltlii. Chromosoma. 94:319-328.

Penrad-Mobayed M, El Jamil A, Kanhoush R, Perrin C (2009) Working map of the lampbrush chromosomes of Xenopus tropicalis: a new tool for cytogenetic analyses. Dev Dyn 238:1492-1501.

Penrad-Mobayed M, Kanhoush R, Perrin C (2010) Tips and tricks for preparing lampbrush chromosome spreads from Xenopus tropicalis oocytes. Methods 51:37-44.

Ricchiuti V, Pruijn GJ, Thijssen JP, van Venrooij WJ, Muller S (1997) Accessibility of epitopes on the 52-kD Ro/SSA protein (Ro52) and on the RoRNP associated Ro52 protein as determined by anti-peptide antibodies. J Autoimmun 10:181-91.

Sim S, Wolin SL (2011) Emerging roles for the Ro 60 kDa autoantigen in noncoding RNA metabolism. RNA. 2: 686- 699.

Simons FHM, Pruijn GJM, van Venrooij WJ (1994) Analysis of the intracellular localization and assembly of Ro ribonucleoprotein particles by microinjection into Xenopus laevis oocytes. J Cell Biol 125:981-988.

Sommerville J (2010) Using oocyte nuclei for studies on chromatin structure and gene expression. Methods 51:157-164

Vishnyakova NM, Lacroix JC, Rodionov AV (2004) Cytogenetic maps of lampbrush chromosomes of newts of the genus Pleurodeles: an algorithm of lampbrush chromosome identification in Pleurodeles waltl by immunocytochemical marker-loop staining with polyclonal anti-Ro52 antisera. Genetika 40: 614-623.

Wada K, Kamitani T (2006) Autoantigen Ro52 is an E3 ubiquitin ligase. Biochem. Biophys. Res. Commun 339: 415–421.

Wang D, Buyon JP, Yang Z, Di Donato F, Miranda-Carus ME, Chan EK (2001) Leucine zipper domain of 52 kDa SS-A/Ro promotes protein dimer formation and inhibits in vitro transcription activity. Biochim Biophys Acta 1568:155-161.

Legends to figures

Fig.1. a: *P. waltl* bivalent XII observed in phase contrast. This LBC is identifiable by its small size and the double-axis regions at its right extremity, as visible here on one chromosome (arrowheads). Arrows indicate left (LE) and right (RE) extremities of the bivalent. b : light microscope autoradiograph of part of bivalent XII isolated from an oocyte incubated for 48 h with tritiated cytidine and uridine. Two photographs had to be juxtaposed because bivalent XII is often stretched on nuclear spreads due to the frequent presence of double-axis regions (arrowheads) at the right extremity. c and d : enlarged images of the left (SLLs) (c) and right (SLRs) subterminal loops (d) shown in b. Note that the conspicuous SLLs and SLRs together with some other loops of a standard type are strongly labelled. Bar represents 15μm in a, 30μm in b and 10μm in c and d.

Fig. 2. Bivalent XII from full-grown (stage VI) oocytes of the same female, before (a-d) and after 24 h (e-h) in vitro culture in the MBS solution. a and b: general view of bivalent XII before culture: (a) phase contrast, (b) Hoechst staining. Arrowheads point to the double-bridges of one chromosome axis, the right extremity (RE) of the other chromosome is broken (open arrowhead). c, d: enlarged images of the SLLs (c) at the the left extremity (LE) and SLRs (d) at the right extremity of the bivalent XII shown in (a). c: subterminal loops (SLLs) show an obvious polarity. Their thinnest part is close to the resolving limit of the light microscope. d: subterminal loops at the right extremity (SLRs). One SLR shows a light zigzag arrangement of its RNP matrix and its sister

loop is completely folded. e-h: bivalent XII after 24h in vitro culture. e: phase contrast. f: Hoechst staining. g, h: enlarged images of the SLLs (g) and SLRs (h) shown at the left and right extremities in (e). Note the important development of the RNP matrix of the SLRs. Bar represents 60 μm in a, b, e, f and 15μm in c, d, g, h.

Fig. 3. Immunostaining of Ro52 loci on LBCs from control oocyte before in vitro culture. Chromosomes were immunostained with the polyclonal Ro52 antibody/ Alexa 488 (pseudo-colored in red)) and counterstained with Hoechst (blue) to show the DNA axis (left panels). The corresponding phase-contrast images are shown on the right panels. Arrows point to the loop loci where the correspondence with their matching phase-contrast is obvious. a and a', b and b': SLLs and SLRs of bivalent XII shown in Fig. 2c and 2d are lightly labelled. Note that other loops near the SLRs are also lightly labelled (arrowheads). c, c': detailed region of an other bivalent of the karyotype showing subterminal loops lightly labelled (arrow). d, d': detailed region of bivalent V showing a pair of lateral loops strongly labelled (arrows). Bar represents 15μm in a-d and a'-d'.

Fig 4. Immunostaining of Ro52 loci in LBCs from oocytes after 24 h in vitro culture. Chromosomes were immunostained with the polyclonal Ro52 antibody/ Alexa 488 (pseudo-colored in red) and counterstained with Hoechst (blue) to show the DNA axis (left panels). The corresponding phase-contrast images are shown on the right panels. a,a', b, b': SLLs and SLRs of bivalent XII strongly labelled. c, c': detailed region of an other bivalent showing

subterminal loops lightly labelled (arrows). d, d': detailed region of bivalent V (similar to that shown in Fig 3 d-d') exhibiting a pair of strongly labelled lateral loops (arrows). Bar represents 15μm in a-d and a'-d'.

Fig. 5. Immunostaining of Ro52 loci on bivalent XII after 24 h (a, a') or 48 h (b,b') of in vitro culture in the 199 medium. Chromosomes were immunostained with the polyclonal Ro52 antibody/ Texas Red-streptavidin (red) and counterstained with DAPI (blue) to show the DNA axis. Note the presence of large granules in the RNP matrix of the SLRs after 24 h of culture and the huge development of the SLRs after 48 h of culture. Note that the chromosome axes became shortened after in vitro culture. Bar represents 25 μm in a, a' and b, b'.

Fig. 6. Nucleocytoplasmic distribution of the Ro52 protein. Western blot of nuclear and cytoplasm extracts from control and in vitro incubated oocytes from 24 to 72 h using the polyclonal anti-Ro52 antibody. Anti alpha-actine was used as a control of the input amount. Each slot corresponds to 30 GVs or 3 cytoplasms. a: control of the specificity of the polyclonal anti-Ro52 antibody. Note the major 52 kDa band detected with the Ro52 antiserum (arrow) in the nuclear extracts (N). b: nuclear import of Ro52 during in vitro culture. In control oocytes the 52 kDa immunoreacting band is present both in the nucleus and the cytoplasm. The level of the immunoreacting band increased in nuclear extracts whereas it decreased markedly in the cytoplasm during the in vitro culture.

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Figure 1

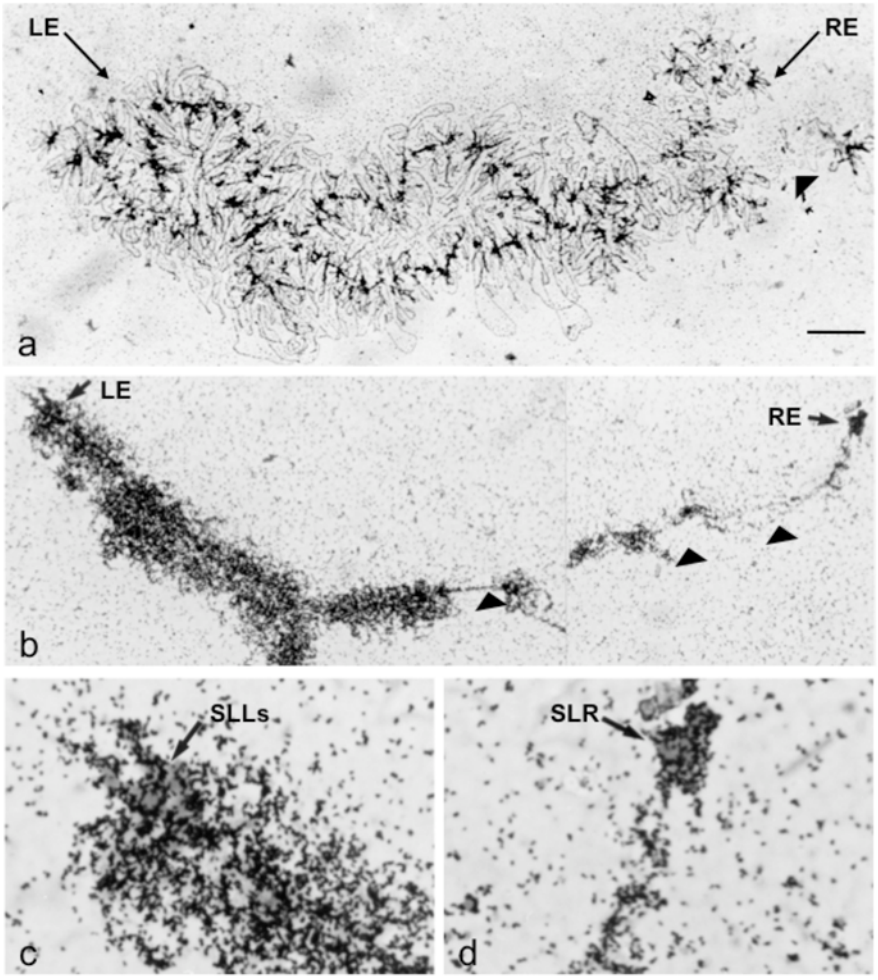

Figure 2

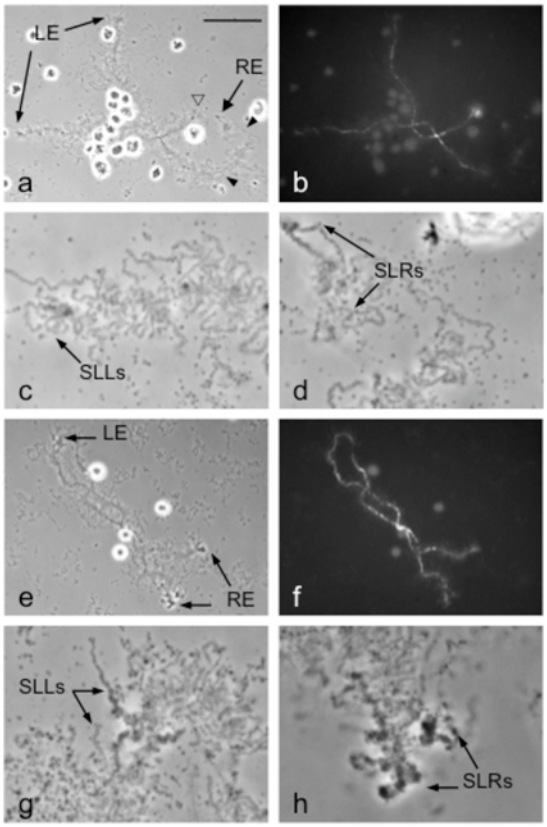

Figure 3

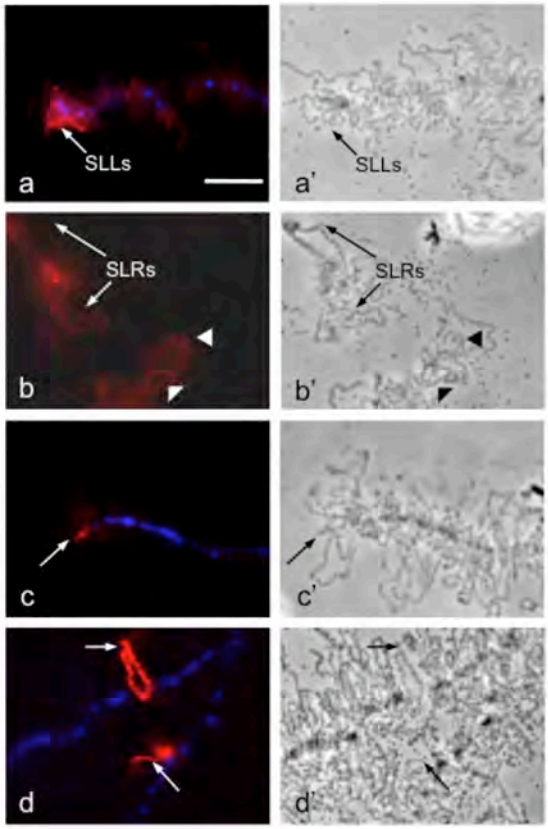

Figure 4

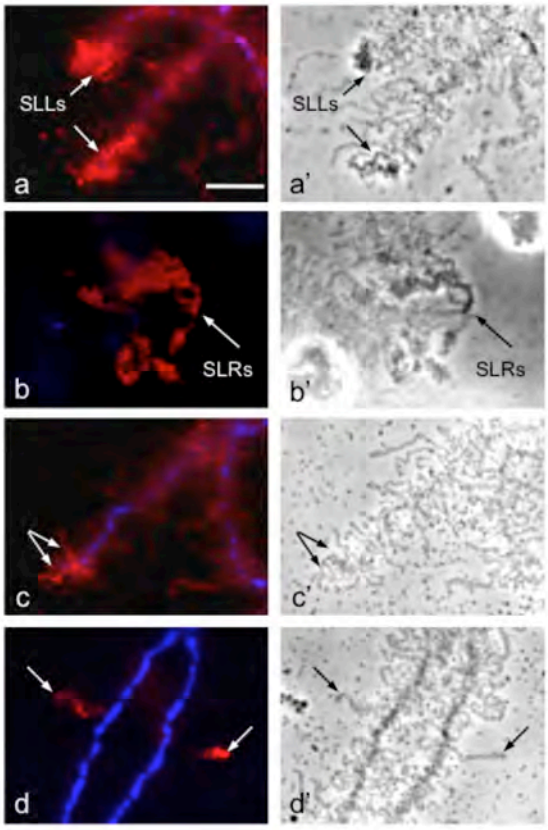

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Figure 5

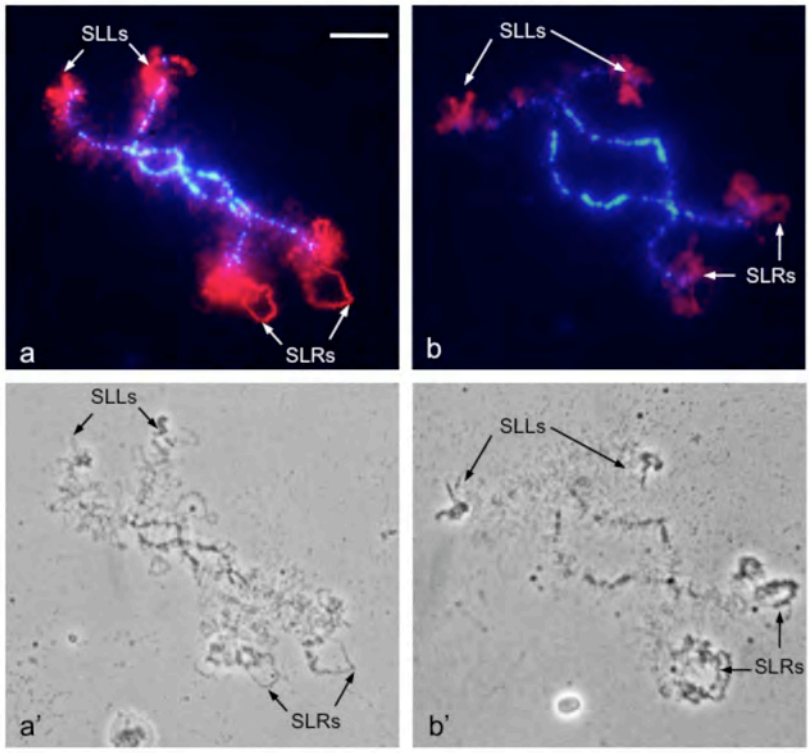
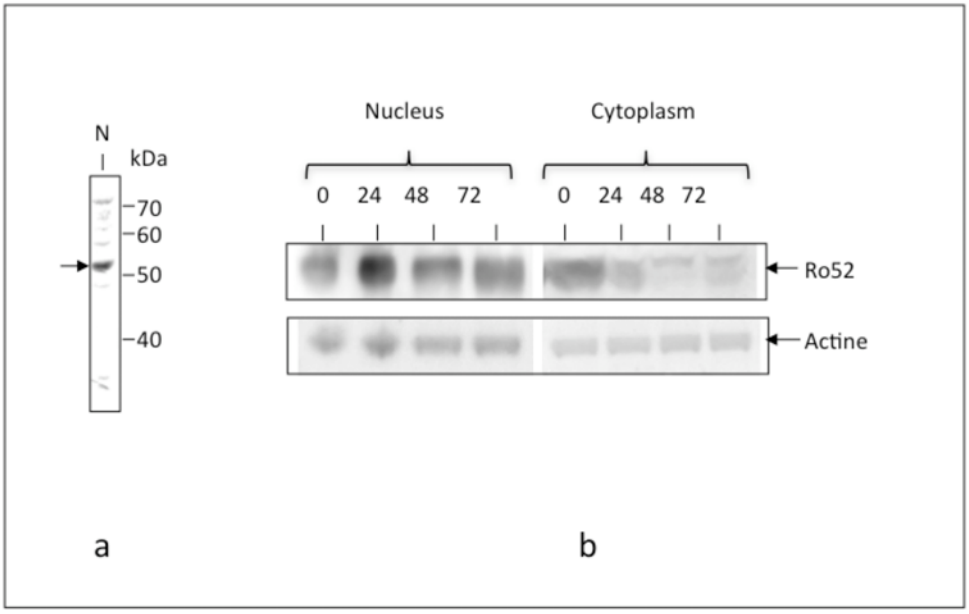


Figure 6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kDa
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