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GENERAL GENETICS

Cytogenetic Maps of Lampbrush Chromosomes of Newts of the Genus *Pleurodeles*: An Algorithm of Lampbrush Chromosome Identification in *Pleurodeles waltl* by Immunocytochemical Staining of Landmark Loops with Polyclonal Anti-Ro52 Antisera

N. M. Vishnyakova[1, 2], J.-C. Lacroix[2], and A. V. Rodionov[1]

[1] *Biological Institute, St. Petersburg State University, Staryi Peterhof, St. Petersburg, 198504 Russia; e-mail: biocyt@rednet.ru*

[2] *Universite Pierre et Marie Curie, Paris, France*

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Abstract—Our work was aimed at developing a simple and effective method of identification of most or all chromosomes of *Pleurodeles* newts. To this end, we used DAPI staining of the chromomeres of newt lampbrush chromosomes and immunochemical reactions between the ribonucleoproteins of landmark lateral loops and polyclonal antibodies against human zinc-finger protein Ro52 (52-kDa Ro/SS-A). A method has been developed to obtain lampbrush chromosome preparations in newts of the genes *Pleurodeles*. Cytological maps of *P. waltl* chromosomes (Spanish population/subspecies) showing distributions of chromomeres and marker landmark loops along the chromosome length were constructed.

INTRODUCTION

The newts of the genus *Pleurodeles* are a traditional model for developmental biology, because of their high reproductive ability and easy maintenance under laboratory conditions. These newts are also the classic model for studying the lampbrush (lampbrush) chromosomes [1]. In nature, *Pleurodeles* newts inhabit a wide area from Portugal and Spain to Marocco, Algeria, and Tunisia. Two species of these newts are distinguished: *P. waltl* Michaelles and *P. poireti* Gervais, which can be crossed to yield fertile hybrids [2].

Lacroix was the first to describe *Pleurodeles* lampbrush chromosomes and to construct their first working maps, which represent distribution of the landmark structures: nucleolus, some chromomeres (granules), and the marker landmark loops differing from normal in morphology of RNP matrix [3, 4]. These studies have shown that the *Pleurodeles* karyotype has only few of unique landmark loops differing from others in size and particular staining, which are chromosome-specific markers of lampbrush chromosomes in urodele amphibians [1]. In fact, their number was insufficient to identify unambiguously all bivalents in the karyotype of *Pleurodeles*. Because of high size variatibility in newt chromosomes, chromosome 1 was difficult to distinguish from chromosome 5, and chromosome 3 was morphologically similar to chromosome 2 on the preparations. Unambiguous identification of *Pleurodeles* chromosomes is possible with only sex chromosome 4, because it has unique chromosomal markers (the so-called sphere and *M*-structure [3–5]).

The study of Scott and Sommerville [6] inspired hope for to finding new chromosomal markers, because these authors found proteins specific for individual transcription units. Afterwards, Lacroix *et al.* [5, 7–9] and Gall *et al.* [9, 10] applied monoclonal antibodies against nuclear amphibian proteins to show that some of them indeed bind to a few of transcription units or recognize only a single transcription unit on lampbrush chromosome landmark loop. Hence, a number of unique marker landmark loops may be identified on the newt lampbrush chromosomes using antibodies capable of binding definite transcription units, which would be helpful in constructing the chromosome cytological maps.

In this study, using immunocytochemical staining of lampbrush chromosomes we have found that several lateral landmark loops on *P. waltl* lampbrush chromosomes specifically bind antibodies against human Ro52 protein. Due to a specific distribution of these landmark loops, all 12 chromosomes were accurately identified and oriented in the genome and detailed cytogenetic maps were constructed for all bivalents in *P. waltl*.

MATERIALS AND METHODS

We examined lampbrush chromosomes of newts *P. waltl* from a Spanish population. Lampbrush chromosome preparations were made using a procedure adapted for *Pleurodeles* [2, 3]. The animals were maintained in

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water at 20°C. An experimental female newt was anesthetized with 1% aqueous solution of MS222 (Sigma). A longitudinal section (1–1.5 cm) was made using surgical scissors between the central line of the ventral side and lateral line. A fragment of an ovary was withdrawn and washed three times in solution *A* (82.5 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM Na_2HPO_4, 0.125 mM polyvinylpirrolidon PVP 40 000, 5 mM HEPES). Afterwards, follicles were separated with pincers under a binocular microscope to form groups of three to four oocytes. The ovary piece were incubated in 1 mg/ml collagenase solution (*solution B*: 10 mg type 1 collagenase, Sigma C0130, diluted in 10 ml of solution A) for 4 h at 25°C with periodic stirring. Next, oocytes were treated three times with fresh portions of solution A and defolliclized for 15 min in 1 mM EDTA solution at room temperature with permanent stirring. The oocytes were again washed three times in solution A. Undamaged oocytes were selected, graded by size in Petri dishes with solution A, and placed into an incubator (19°C), where they remained from several days to eight weeks. Twenty hours after defolliclization, the oocytes could be used for isolation of lampbrush chromosomes.

Nuclear isolation was made with two thin-tip pincers under a binocular microscope in a salt cellar containing *solution C* (10 mM Tris, 75 mM KCl, 25 mM NaCl; after pH adjustment to 7.2 with HCl, the solution was autoclaved). The oocyte envelope was disrupted with two pincers and a nucleus was separated from cytoplasm by retraction it into a pipette and pushing out. A purified nucleus was transferred into a special chamber containing *solution D* (25 mM NaCl, 0.05 mM $CaCl_2$, 10 mM Tris, 75 mM KCl, 1 mM $MgCl_2$; after pH adjustment to 7.2 with HCl, the solution was autoclaved). The chamber represented a slide with a 5–7-mm hole; a cover glass was stuck with melted paraffin to one side of the slide. The chamber was presterilized under a UV lamp.

The nuclear envelope was removed with a pincer and a fine needle and carefully withdrawn from the chamber. The slide was placed on ice for 10 min to precipitate chromosomes and then covered with the cover glass. An excess of solution D was sucked out under a cover glass with filter paper and the preparation was examined for quality on a phase-contrast microscope. The chamber was centrifuged at 500 *g* for 5 min to attach lampbrush to the chamber bottom; then the velocity was increased to 3000 *g* and the preparation was centrifuged for 25 min at 6–8°C. Next, chromosome fixation was conducted for 20 min at 6–8°C in 4% paraformaldehyde in a Ringer solution, pH 7.4–7.5 (113 mM NaCl, 2 mM KCl, 0.7 mM $CaCl_2 \cdot 2H_2O$; pH 7–7.4 adjusted with a solution of $NaHCO_3$; the autoclaved solution was stored at 4°C). The preparations were washed in three fresh portions of 1 × TBS (10 mM Tris, 100 mM NaCl; pH 7.5–7.6 adjusted with 1 n HCl) for 10 min each time, and afterwards, chromosomes were ready to analysis (morphological analysis or immunochemical staining).

In this study, we used polyclonal antibodies against zinc-finger human leukocyte protein Ro52 (52 kDa, Ro/SS-A) [11], which were kindly provided by S. Muller (UPR 9021 CNRS Immunochimie des Peptides et des Virus, Strasbourg, France). Chromosomes were treated with antibodies at a temperature of 6–8°C. The solutions were removed from the chamber using an air pump and filter paper, because a contact between air and chromosomes (chamber drying) leads to chromosome disruption. The preparation was washed in TBS (three washings for 10 min each). TBS was replaced for 10% horse serum (in TBS); 10 min after that, the serum was partially removed and the first antibodies diluted 1/250 in TBS were added. Incubation continued from 30 min to 1 h; next, chromosomes were washed with three fresh portions of TBS. The biotinylated or fluorochrome-bound second antibodies (Texas Red or FITC) diluted 1/50 in TBS were added for 30 min after partial removal of the washing buffer; and the preparation was again washed. In case of biotinylated antibodies, the preparation was treated with streptavidin for 30 min (Texas Red, 1/50), which was followed by washing three times with TBS. Lampbrush chromomeres were detected by additional DAPI (Sigma) staining (0.5 μg/ml in TBS). After washing and partial removal of TBS, the preparations were embedded into a TBS–glycerol mixture (1 : 2), pH 7.8. The chamber was covered with a cover glass and stuck with transparent nail varnish.

Chromosomes were photographed on the Kodak 400 transparency film. The image was projected onto a screen, and chromosome measurement along two axes was performed with a curvimeter. The data were processed using a specially developed Excel program.

RESULTS AND DISCUSSION

Most lateral landmark loops of *P. waltl* lampbrush are uniform in size and indistinguishable in matrix morphology; only some of them differ from the others in density of the RNP matrix. Consequently, only a few of these landmark loops can be identified on a phase-contrast microscope (Fig. 1a). To reveal molecular heterogeneity of *P. waltl* lateral lampbrush landmark loops, the antibodies against chromosomal proteins were used. As shown previously [5–10], in urodele amphibians, some lampbrush landmark loops are unique in their antigen determinants. Sometimes a landmark loop indistinguishable in morphology and outlines from other ones on a phase-contrast microscope could exhibit an unusual antibody-stained pattern. In this study, about 350 monoclonal and polyclonal antibodies specific and non-specific to amphibian nuclear proteins have been tested. We have found that polyclonal antibodies against zinc-finger human protein Ro52 from Algera series bind specifically to large landmark loops on five pairs of *Pleurodeles* chromosomes (Figs. 1b and 2). The marker landmark loops that carry epitopes interacting with anti-Ro52

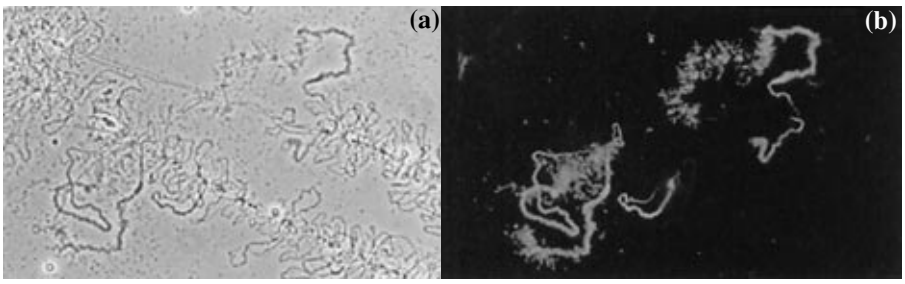

Fig. 1. A fragment of lampbrush chromosome of *Pleurodeles waltl.* (a) Chromosome examination on a phase-contrast microscope; the lateral landmark loops and chromomeres are observed. (b) A fragment of the same chromosome after treatment with fluorescent antibodies (Texas Red dye) against Ro52 zinc-finger human protein (*Algera series*).

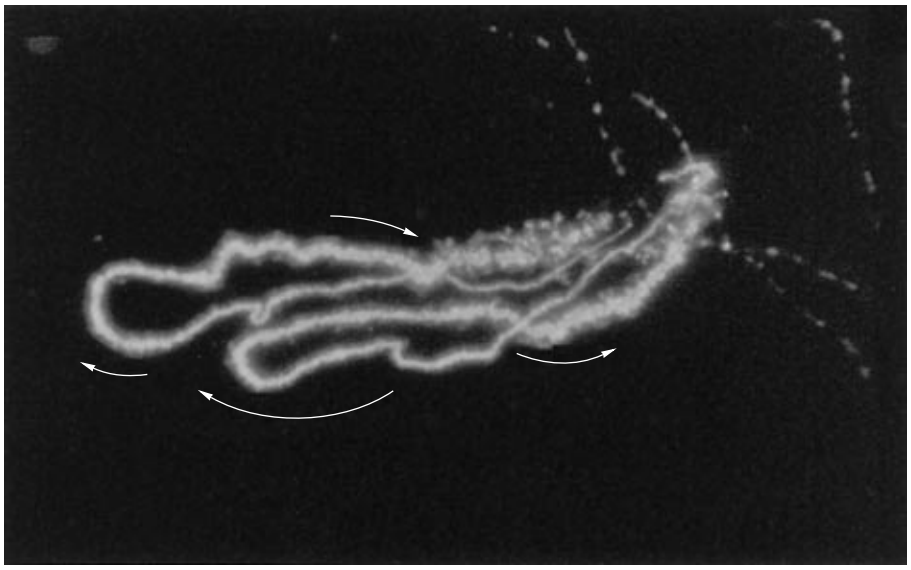

Fig. 2. A Ro52-positive landmark loop on lampbrush chromosome 2 of *Pleurodeles waltl.* Texas Red dye. The gradient of matrix is clearly visible. Arrows indicate direction of transcription. A single transcription unit is in the lateral landmark loop.

antibodies were designated *Ro52L* (i.e., *Ro52*-positive landmark loops). To map these landmark loops to *P. waltl* lampbrush chromosomes, the latter were stained with a fluorescent AB-specific dye DAPI. Along the axis of lampbrush DAPI-stained bivalents the bright fluorescent chromomeres are seen, some of which are unique in size or brightness (Figs. 3, 4). As traditionally done with chromosome description in newts of the genus *Pleurodeles* [3–5], chromomeres were designated using letters *DC* (DAPI-positive chromomere), chromosome number, and individual number determined by chromomere coordinates. For example, *DC1.60* is a chromosome 1 chromomere positioned at a 60% distance from the q-arm telomer.

After DAPI staining, the lampbrush lateral landmark loops are almost indiscernible (Figs. 3–5). However, a combination of DAPI-staining and treatment with anti-Ro52 antibodies makes it possible to identify all bivalents and to saturate lampbrush-chromosome maps with the internal markers (Fig. 5). Location of these markers is unambiguous and they can be used for further analysis of chromosome rearrangement in *Pleurodeles* evolution and for solving other cytogenetic problems (see, e.g., [1, 4, 8, 12, 13]).

In *P. waltl* from the Spanish population (subspecies), large *Ro52L* landmark loops were recorded on chromosomes 1 and 5; three of these landmark loops were on chromosome 2 (Figs. 5, 6). The minor sites binding anti-*Ro52* antibodies (small *Ro52*-positive landmark loops) were on chromosomes 2, 8, and 12 (in Fig. 5, minor landmark loops are designated as ml). Identification of each *Ro52L* landmark loop was reproducible in all examined animals.

Position and coordinates of each *Ro52L* landmark loop were determined relative to other chromosome markers on *P. waltl* chromosome maps, previously constructed by Lacroix [3]. The landmark loops were designated according to principle described for chromomeres. For example, *Ro52L1.39* is a *Ro52*-positive landmark loop on chromosome 1, which is located 39% of bivalent length away from q-arm telomere at the lampbrush stage. The *Ro52L* landmark loop coordinates were not determined on chromosomes 8 and 12,

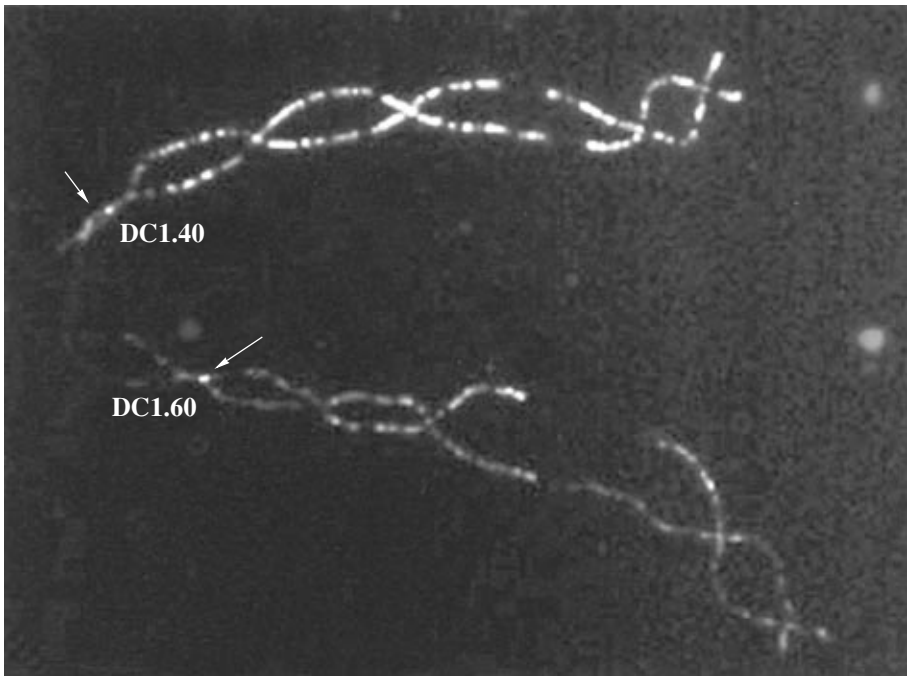

Fig. 3. DAPI-stained lampbrush chromosome 1 of *Pleurodeles waltl.* Chiasmata or pseudochiasmata are in the regions of DAPI-positive chromomeres *DC1.40* and *DC1.60*.

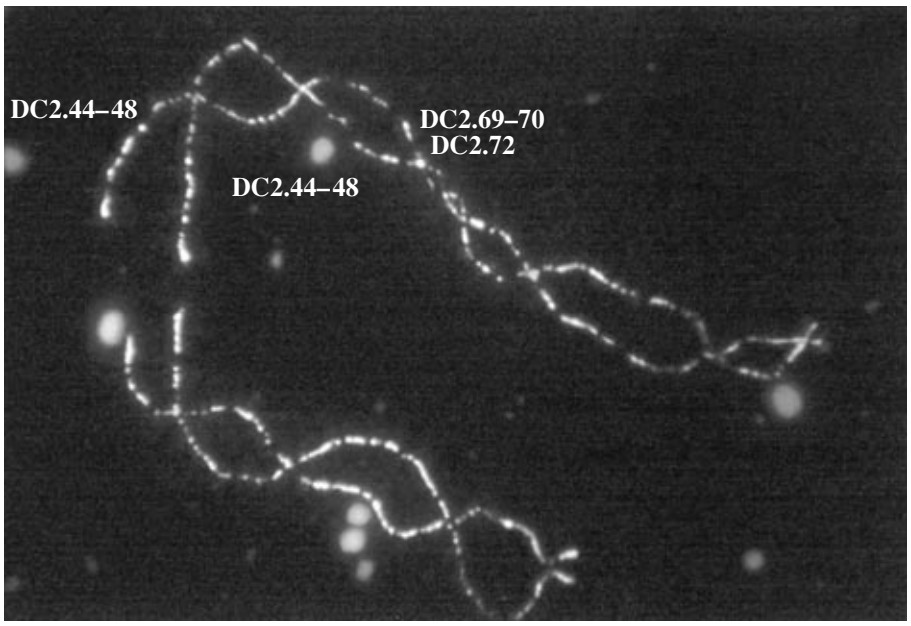

Fig. 4. DAPI-stained lampbrush chromosome 2 of *Pleurodeles waltl.* Some DAPI-positive chromomeres are marked.

though these landmark loops were used to identify the latter chromosomes.

Due to the unique pattern of *Ro52L* landmark loop, all lampbrush chromosomes of *P. waltl* were identified and their maps were constructed (Fig. 6). On all schematic maps, bivalent location is consistent with their relative sizes in a decreasing order from I to XII. The most probable centromere location on chromosomes of *P. waltl* was previously determined by Lacrois [3] on the basis of centromere indices of mitotic chromosomes and analysis of lampbrush chromosomes with chromosomal rearrangements. On these schemes, chromosomes are oriented with their short arms (p) to the right. All measurements and calculations of the relative position of

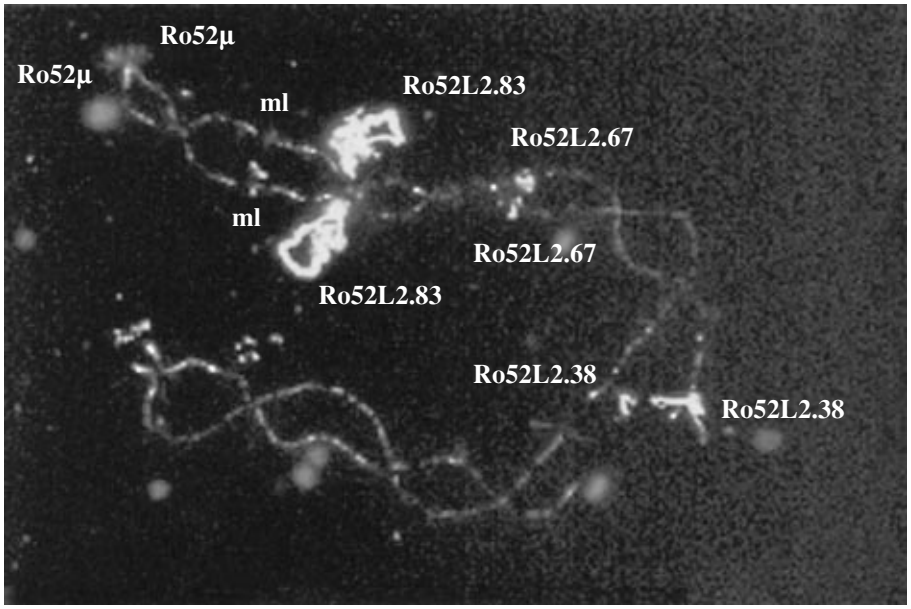

Fig. 5. Lampbrush chromosome 2 of *Pleurodeles waltl*, which is DAPI-stained and treated with antibodies against Ro52 zinc-finger protein. See text for description of the markers.

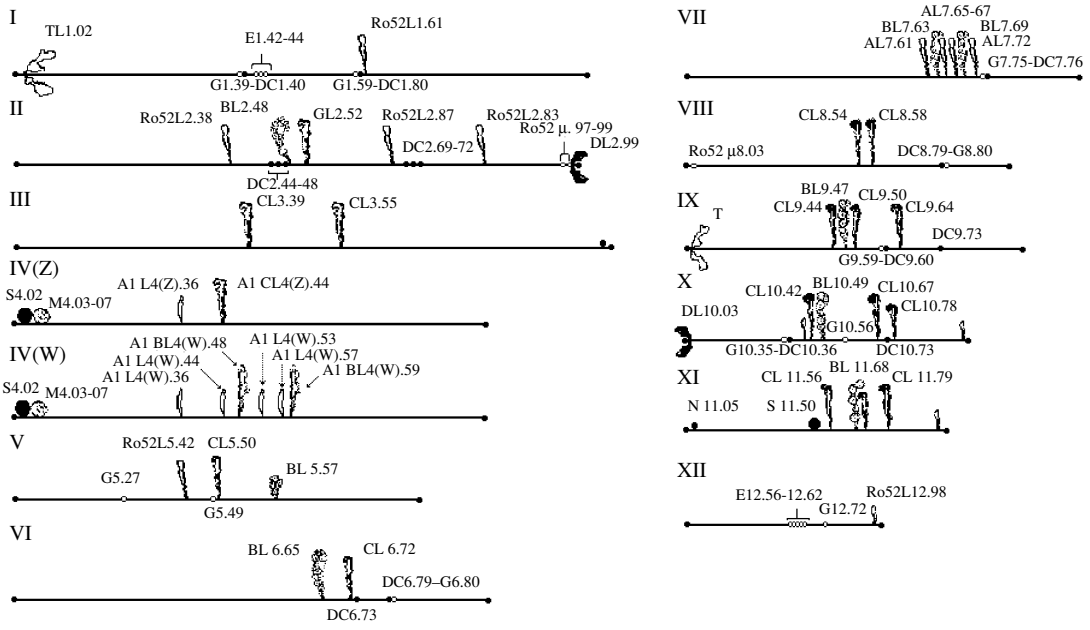

Fig. 6. Lampbrush chromosome maps for *Pleurodeles waltl* (a Spanish population). See text for description of the markers.

all *Pleurodeles* markers (Flqter indices) (Fig. 6) begins from the left end of each chromosome (i.e., from the q-arm telomere).

Ro52-positive landmark loops of *Pleurodeles* lampbrush include a single transcription unit and exhibit a clear gradient of the RNP matrix, the start and the end of a transcription unit (Figs. 1a, 1b, 2, and 5). The nature of *Ro52L* landmark loops in *Pleurodeles* lampbrush and of the related transcripts need to be specially studied. Note however, that Ro52 is a member of zinc-finger protein family, many of which bind to DNA and regulate gene expression [14, 15]. We have previously shown that some zinc-finger proteins are present in nuclei of amphibian oocytes [7–10, 16–18] and, in particular, in lampbrush landmark loops of both anurous (*Xenopus*) and urodele amphibians (*Triton, Notophthalmus*, and *Pleurodeles*) [7–10, 18].

Anti-Ro52 antibodies bind to ribonucleic and deoxyribonucleic complexes involved in transcription [14–16]. Interaction of RNP matrix of *Ro52L* landmark loops with antibodies against human zinc-finger protein Ro52 suggests that *P. waltl* genome contains at least five transcription units. Their RNP complexes are similar in composition to the evolutionary conserved nuclear and cytoplasmic Ro-RNP complexes found in all studied multicellular organisms, whose function remains unknown [11, 14, 15, 19–21]. These landmark loops may be places of synthesis of nuclear precursors of low-molecular hY RNAs or other RNA whose secondary structure might be similar to that of hY RNA and/or mutant 5S rRNA [22].

Algorithm of Chromosome Identification in Pleurodeles waltl

Chromosome 1. In *P. waltl* genome, the bivalent, which is second in size (relative length 122 a.u.) corresponds to chromosome 1 of the meiotic karyotype [3]. This chromosome is readily identified by two large symmetrical axial chromomeres, which are well-discernible after DAPI-staining and on a phase-contrast microscope (each granule is 1–2 μm in size). Coordinates of these chromomeres (Elqter indices) are 0.40 and 0.60; individual marker designations are *DC1.40* and *DC1.60.* On lampbrush chromosome preparations, *DC1.40* and *DC1.60* markers of homologous chromosomes are often fused since chiasmata and pseudochiasmata are observed in these very regions (Fig. 3). If crossovers in chromomeres *DC1.40* and *DC1.60* are true chiasmata, these are recombination hot spots in *Pleurodele* genome. Interestingly, that the axial "granules" of *Pleurodeles* lampbrush are enriched with topoisomerase II [23].

So-called phase-contrast granules that we designated as G-structures (*G1.39* and *G1.59*) are immediately adjacent to the DAPI-positive granules *DC1.40* and *DC1.60*. The G-structures are well discernible on a phase-contrast microscope and they are not DAPI-stainable. Because of symmetrical positioning of these granules (Fig. 6), they cannot be used for chromosome 1 orientation during phase-contrast microscopy [3]. This, however, can be done using a specific cluster of small granules, which Lacroix [3] termed the E structure. The E structure is asymmetrical relative to the mid-chromosome region (Fig. 6).

The *Ro52L1.61* landmark loop located near the bright chromomere *DC1.60* is another asymmetrical marker of bivalent 1 in *P. waltl.* The latter is a marker for the right chromosome part, whereas the E structure with granules *G1.39* and *DC1.40* is a marker for the left chromosome part.

Note that it is convenient to use tubular landmark loops (*TL1.02*) near the q-arm telomere for the light optic identification of chromosome 1 orientation [2, 3]. These landmark loops develop after a long-term cultivation of the newt oocytes (during four–six days) before chromosome isolation.

Chromosome 2 ranges third in size in the *P. waltl* karyotype at the lampbrush stage. The relative length of chromosome 2 is 120 a.u., it corresponds to mitotic chromosome 2 [2, 3]. An unusual D-landmark loop (*DL2.99*) with a strongly stained matrix and internal cavities, which is located in subtelomere region of bivalent p-arm, is a bright marker of this chromosome [3]. The D-landmark loop is characterized by a dense packed RNP matrix, which consists of RNP particles 30 nm in diameter and supercoiled DNA axis of the landmark loop [24–26]. Chromosome 2 D-landmark loops (*DL2.99*) are observed almost in every oocyte, but chromosome 10 contains a landmark loop of the same type. Identification and orientation of chromosome 2 is determined by positioning of the D-landmark loop, axial granules, and *Ro52*-positive landmark loops (Fig. 6, chromosome 2).

In *P. waltl*, two groups of large DAPI-positive chromomeres (*DC2.44–48* and *DC2.69–72*) are usually seen on the chromosome 2 axis. When these chromomeres are not clearly discernible, accurate identification and orientation of this chromosome is possible from the *Ro52L2.38 Ro52*-positive landmark loops, which is closely followed by a cluster of axial "granules" *DC2.44–DC2.48*), and from *Ro52L2.67* and *Ro52L2.83* landmark loops.

A special *Ro52*-positive structure μ is located near the p-arm telomere of chromosome 2 [2]. The nature of μ structure remains unclear; it may represent the landmark loops folded around the bivalent axis. After DAPI-staining, a gap is observed in this region: chromosome axis looks interrupted at this site.

The presence of globular B-landmark loops (BL), *BL2.48,* and granular C-landmark loops (CL), *CL2.52* in the bivalent central portion is another particular feature of chromosome 2. BL landmark loops with RNP globules of about 0.7 μm in diameter consist of several transcription units [27], which were found to include scattered repeats [28]. The assemblage of low-molecular nuclear RNP (snRNP, [29]) seems to occur on transcripts of BL landmark loops. In *P. waltl*, the immunochemical properties of BL-landmark loop granules are similar to B-snurposomes [29]. Centromeres are assumed to be located exactly in the region *DC2.44–CL2.52* [3, 4].

Chromosome 3 is the longest bivalent (126 a.u.) in *P. waltl* karyotype at the lampbrush stage. This bivalent corresponds to chromosome 3 of the mitotic karyotype. At the right end of this bivalent, a nucleolus is often observed (N structure). If the N marker is present, identification of this chromosome causes no difficulties. However, in most cases, the bivalent contains no nucleolus. In this case, chromosome 3 can be identified only after the determination of bivalents 1 and 2. Lacroix has described two particular granular C-landmark loops (*CL3.39* and *CL3.55*) of this chromosome, which are usually difficult to identify against the background of

other normal landmark loops. We have not found *Ro52L* landmark loops on bivalent 3.

Chromosome 4 (sex bivalent ZW). The relative size of this chromosome is 100 a.u. It carries two particular markers, so-called M structure (*M4.03–07* and a sphere *S4.02* [3, 6, 29] and, therefore, this bivalent can be identified in all oocytes of *P. waltl*. In large oocytes, *S4.02* sphere sometimes reaches 1.5 mm in diameter. The spheres of two homologs are fused into a single large structure in 98% of cases. In *P. waltl*, chromosomes 4 and chromosome 11 interact with antibodies against a factor of transcription termination of RNA-polymerase III La11G11 and Sm-antigen of snRNPsome (snurposomes) [30]. They also interact with antibodies against B24 protein, which is, presumably, involved in replication [31]. Gall *et al.* [29, 32] believe that the mechanisms of transcription and processing are formed in the spheres. The M structures contain a very small amount of RNA, interact with A1, La11G11, B71, and B24 antibodies [6, 29, 30], and are assumed to play a role in accumulation of proteins, utilized by a cell in late oogenesis and early embyogenesis [30]. We have observed animals both homo- and heterozygous for the M marker. In homozygotes, M^+/M^+, M markers of the homologs are often fused into a single large globule. Figure 6 shows the detailed map of sex bivalent 4 of *P. waltl*, which has been developed by Lacroix *et al.* [5]. We failed to detect large *Ro52L* landmark loops on bivalent 4. However, a small *Ro52*-positive landmark loop is often detectable between the sphere and M structure. Because of it ephemerality, this structure is not represented in our maps.

Chromosome 5. Relative size of this chromosome is 87 a.u. In *P. waltl*, bivalent 5 have a few markers and, therefore, is not readily identifiable. Like chromosome 1, it carries two particular axial granules, which are well discernible under a phase-contrast microscope (*G5.27* and *G5.49*). The well-developed C landmark loops (*CL5.50*) as well as a small but clearly discernible B landmark loop (*BL5.57*) are often observed near the last locus on chromosome 5. Because of the presence of a pair of large axial granules, chromosome 5 is difficult to differentiate from chromosome 1 both by phase-contrast and after routine DAPI staining. Therefore, antibodies were used to identify this chromosome. *Ro52L* landmark loop is detected on bivalent 5 in site 0.420 (landmark loop *Ro52L5.42*) (Fig. 6, chromosome 5). The bivalent orientation is determined from the latter landmark loop. On the scheme, this bivalent is oriented so that *Ro52L5.42* is in the left part, and the landmark loops C and B are in the right part of this chromosome.

Chromosome 6 has a relative size of 102 a. u. This chromosome is identified from a group of specific, bright globular B landmark loops *BL6.65* at the right end of the bivalent. To the right of these landmark loops, the permanent long granular C landmark loops (*CL6.72*) are followed by two axial granules (*DC6.73* and *DC6.79*) clearly discernible after DAPI staining (Fig. 6, chromosome 6) and by a DAPI-unattainable axial granule (*G6.80*), which is clearly discernible by phase-contrast microscopy.

Chromosome 7 has a relative size of 85 a.u. It is readily identified from a group of globular B-landmark loops (*BL7.63* and *BL7.69*), which are banded from both sides with giant A landmark loops (*AL7.61* and *AL7.72*). The latter are closely followed by an axial granule (*G7.75*) readily discernible under a phase -contrast microscope and by a large axial brightly DAPI-stainable chromomere (granule) (*DC7.76*).

Chromosome 8 has a relative size of 69 a.u. This bivalent can be identified from the two structures that mark the right chromosome end: the largest DAPI-positive granule (*DC8.79*) and adjacent clearly discernible axial granule (*G8.80*), which is observed in phase-contrast. In addition, chromosome 8 can be identified from the μ structure (relative position 0.03), which is located near the left end of this chromosome [2, 3]. Chromosome 8 can be readily identified in *Pleurodeles* genome after bivalent treatment with anti-Ro52 antibodies, which bring out the μ structure (Fig. 6, chromosome 8).

In the center of bivalent 8, the giant C landmark loops (*CL8.54–CL8.58*) are detectable, which may serve as additional marker for this chromosome.

Chromosome 9. The relative size is 72 a.u. The chromosome is identified from B landmark loops forming two blocks in the center of bivalent, which are in turn surrounded by C-type landmark loops (coordinates of the entire B and C landmark loop cluster are 0.44 to 0.50). Near the right telomere, a C landmark loop (*CL9.64*) is detectable somewhat a part of the above cluster. Between these two clusters, the phase-contrast and DAPI-positive granules are often detected (relative position of the entire cluster *G9.59–DC9.60*). C landmark loop *CL9.64* is followed by DAPI-positive granule *DC9.73*. After a long-term cultivation of the newt oocytes before chromosome isolation, T landmark loops (*TL0.1*) appear on chromosome 9 near the q-arm telomere.

Chromosome 10. The relative size is 61 a.u. The D landmark loop similar to D landmark loop of bivalent 2 marks the left telomere of this chromosome. In the absence of D landmark loops, chromosome 9 can be identified from two clusters of giant landmark loops: *CL10.42; BL10.49* and *CL10.67*; *CL10.78.* Several phase-contrast and DAPI-positive granules were revealed on chromosome 10, which is also helpful in identifying this chromosome (Fig. 6, chromosome 10).

Chromosome 11. The relative size is 55 a.u. Like bivalents 4 and 7, this chromosome is the most readily identifiable, because a sphere similar to that on chromosome 4 is present in central portion of chromosome 11. Orientation of the latter is determined from the granular landmark loops *CL11.56* and *CL11.79* and from the globular landmark loop *BL11.68.* Both clusters of landmark loops are to the right from the sphere. According to Lacroix [3], the centromere region of bivalent 11 is between the landmark loops *CL11.56* and *BL11.68.*

Chromosome 12. The relative size is 42 a.u. It is the smallest chromosome in *P. waltl* karyotype, in the right part of which double bridges (lateral landmark loops extended at the bottom) are often formed. This bivalent contains axial granules discernible only on a phase-contrast microscope (*G12.72*) and an array of dense chromomeres (from eight to eleven) carrying small dense landmark loops (E structure) in the center of the bivalent [2, 3]. The small landmark loop *Ro52L12.98* near the right telomere of chromosome 12 is an additional marker of the latter.

Thus, a combination of lampbrush DAPI-staining and treatment with polyclonal antibodies against Ro52 protein makes it possible to identify unambiguously all lampbrush chromosomes in karyotype of *P. waltl*. In our further study, this approach will be used to compare chromosomes of *P. waltl* of a Spanish population (subspecies), *P. waltl* of a Moroccan population (subspecies), and of *P. poireti*.

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