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Tips and tricks for preparing lampbrush chromosome spreads from *Xenopus tropicalis* oocytes

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ABSTRACT

Due to their large size and fine organization, lampbrush chromosomes (LBCs) of amphibian oocytes have been for decades one of the favorite tools of biologists for the analysis of transcriptional and post-transcriptional processes at the cytological level. The emergence of the diploid *Xenopus tropicalis* amphibian as a model organism for vertebrate developmental genetics and the accumulation of sequence data made available by its recent genomic sequencing, strongly revive the interest of LBCs as a powerful tool to study genes expressed during oogenesis. We describe here a detailed protocol for preparing LBCs from *X. tropicalis* oocyte and give practical advice to encourage a large number of researchers to become familiar with these chromosomes.

1. Introduction

Anyone who has observed a cytological preparation of amphibian lampbrush chromosomes (LBCs) with a phase-contrast microscope has immediately fallen under the spell of these giant chromosomes. LBCs are observed in the oocytes of vertebrates and invertebrates but they are particularly developed in amphibians [1]. They are not confined to the animal kingdom and lampbrush-type chromosomes have been described in the green alga, *Acetabularia mediterranea* [2] and in characean algae [3]. They were first described by Flemming [4] in the oocyte nucleus or germinal vesicle (GV) of the salamander *Ambystoma mexicanum* but the name "lampbrush" was given to them by Rückert [5] for their resemblance to oil-lamp brushes used in the 19th century. As shown in Fig. 1, the two homologues are associated in bivalents and several thousand pairs of lateral loops unfold along their axis, giving them a characteristic feathery aspect. Each loop is the site of intense transcriptional activity and the nascent RNA transcripts together with the bound proteins form the RNP fibrils. The main feature of LBCs is their dynamic architecture directly related to transcriptional and post-transcriptional processes. Any modification of these processes is reflected by concomitant morphological variations of their structure. This is why LBCs have been used for several decades as a powerful model for cytological and biochemical analyses of transcriptional processes (for a review see [6]). More recently, LBCs were also used as a tool for the dissection of complex chromosomal processes such as the cohesion of sister chromatids [7].

The recent sequencing of the diploid genome of *Xenopus tropicalis* makes it an attractive model organism for developmental genetics studies in amphibians. LBCs of *X. tropicalis* provide a valuable tool for studying the molecular organization and function of genomic sequences, which are expressed during oogenesis. *In situ* hybridization of specific probes to nascent transcripts of lateral loops consistently yields very strong signals because the probes bind to numerous closely packed RNA transcripts in these loops (Fig. 1). The localization of the hybridizing loops can be defined accurately using the available LBCs maps of the ten bivalents of the oocyte karyotype [8].

Duryee [9] was the first to demonstrate that LBCs can be hand-isolated from the living oocytes of a frog. In 1960s, Gall [10] and Callan and Lloyd [11] developed methods for preparing newt lampbrush chromosomes for observation by phase-contrast microscopy. Thereafter, this technique with some variations was applied successfully to the mapping of LBCs of different urodelan and anuran species (reviewed by Callan [1]), the latest of these studies to date being the establishment of working maps of *X. laevis* [12] and *X. tropicalis* LBCs [8].

LBCs are observed throughout oogenesis from the early diplotene stage, i.e. Dumont stage I [13] [14]. It is well known from studies of LBCs of different amphibians species that their size and the

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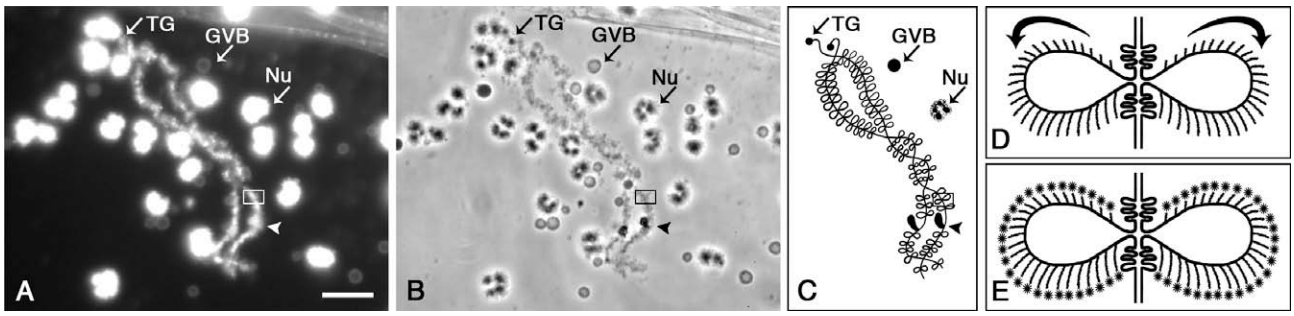

Fig. 1. A GV spread. Fluorescent (A) and corresponding phase contrast (B) micrographs of the content of a GV spread from paraformaldehyde-fixed and propidium iodine-stained preparation observed under a Leica microscope using a Leitz DMRB CCD camera. Bivalent VII, one of the 10 bivalents of the *X. tropicalis* karyotype, and numerous nucleoli (Nu) and GV bodies (GVB) are shown. This bivalent can be identified by the presence of lateral loops of fibrillar matrix (arrowhead) located near its right end and by terminal granules at its left extremity (TG) (for details, see [8]). (C–E) Schematic representations of the organization of LBCs. (C) Each bivalent is formed by two homologues remaining associated at the chiasmata. Each homologue display several hundred lateral loops which exhibit the same "standard type" structure while a few others exhibit distinctive structures considered as "landmarks" (arrowhead). (D) Schematic representation of one pair of lateral loops. The chromatid axes unfold in paired loops extending in opposite directions. The unfolded DNA is a site of intense transcriptional activity. The increasing size of the nascent transcripts indicates the progress of transcription (arrows). (E) Schematic representation of the enhancement of the *in situ* hybridization signal by the binding of specific probes (stars) to the closely packed RNA transcripts.

degree of development of their lateral loops are directly related to their transcriptional activity. LBCs reach their optimal size at Dumont stages III–IV when transcription is at its maximum. At Dumont stage VI, when transcription slows down, chromosomes become shorter and their lateral loops regress. Although LBCs are observed as early as Dumont stage I, LBCs spreads are usually performed with stages IV–VI oocytes. Preparation of satisfactory GV spreads from stages I to III oocytes is considered difficult because their GVs are too small to handle easily. This is the reason why Gall speaking of *X. laevis* stated that only the most intrepid lampbrushlogist would attempt a conventional GV spread from an oocyte with a diameter less than 0.5 mm [15]. *X. laevis* stage V oocytes which are 1 mm in diameter are considered to have an optimal size. *X. tropicalis* oocytes and their corresponding GVs have a smaller size than those of *X. laevis* but we found that this was not a limiting factor for preparing LBCs spreads including those from GVs of stage III oocytes. We describe here a detailed protocol, which allows good quality LBCs spreads to be obtained from *X. tropicalis* oocytes in a routine fashion.

2. A protocol for *X. tropicalis* lampbrush chromosomes spreads

The protocol we used for the construction of the working map of the 10 LBCs of *X. tropicalis* [8] was modified from that established for *X. laevis* LBCs by Callan et al. [12] and detailed further by Gall et al. [15]. A comparison of the main steps of these two protocols is provided in Table 1 and the different steps of our protocol for LBCs spreads of *X. tropicalis* are illustrated in Fig. 2. The composition of the working solutions and the description of the material necessary for preparing LBCs spreads are reported below (Appendix).

2.1. The different stages of oocyte development in the X. tropicalis ovary

On the basis of the classification criteria introduced by Dumont [13] for *X. laevis* and according to the size of the oocyte and pigment distribution, six stages of oocyte development can be distinguished in the *X. tropicalis* ovary (Fig. 3). *X. tropicalis* oocytes are smaller than those of *X. laevis* (Table 2). Stage I oocytes (50–100 μm in diameter) are transparent while those at stage II (200–250 μm in diameter) are of white color. Stage III oocytes (350–400 μm in diameter) appear uniformly grey. At stage IV (400–500 μm in diameter) the pigmented animal hemisphere and the yellowish vegetal hemisphere become clearly differentiated. Stage V oocytes (600–700 μm in diameter) can be distinguished from stage VI oocytes (700–800 μm in diameter) by their smaller size. The ovaries of hormonally- stimulated females also contain oocytes at the same stages of development, but the number of stage VI oocytes is dramatically decreased as shown in Fig. 3.

2.2. Detailed protocol

2.2.1. Preparation of females

Mature females are maintained unfed for two days before surgery. Hormonally stimulated females are injected with human Chorionic Gonadotropin (100 IU/100 μl) into the left or right lymph sac to induce ovulation, which occurs normally 12–24 h later. Ovary biopsy is performed within 2 days following ovulation.

2.2.2. Ovary biopsy

Females are anesthetized for 30–40 min by immersion into 0.1% MS222 (Amino-benzoic Acid Ethyl, Fluka) until they are completely immobile. Because the anesthetic solution is acidic (pH 4–5), it is important to rinse the animals with tap water before surgery. The females are placed on their back and a longitudinal 0.5 cm incision is made on the left or the right side in the posterior half of the trunk using a surgical blade. The use of a surgical blade is preferable to that of iridectomy scissors in order to make a sharp cut through the skin and the underneath muscular wall at the same time. Using forceps with rounded ends, a small piece of ovary is removed and placed into a Petri dish in MBS Buffer (modified Barth's solution) [16] or OR2 medium (oocyte Ringer's medium) [17] at room temperature (RT). The incision is closed with thread silk or cotton suture. Females are maintained under observation in a small aquarium in the laboratory for the next two days before

Table 1
Comparison of the main steps used to prepare LBCs from *X. tropicalis* and *X. laevis*.

	X. tropicalis	*X. laevis*
Oocyte stages used for LBCs spreads	III–VI	IV–V
Extraction of the GVs in the "Isolation Medium"	Embryo dish	Small petri dish
Recovery of the GV content	Directly in the dispersal chamber	Indirectly after transfer in small Petri dish of "Dispersal" medium
Duration and speed of centrifugation	10 min 300*g* + 30 min 3100*g*	60 min 3100–4800*g*