ments (figures 1 and 2). A TSP is defined as the interval between the earliest time of shift down (from 22 °C to 17 °C) at which the mutant phenotype is observed, and the latest time of shift up (from 17 °C to 22 °C) at which the mutant phenotype occurs. Figure 1 shows that the roller phenotype TSP is entirely situated in the first larval stage (L_1). It begins a few hours after hatching and is extending from 18 to 25 h of development at 22 °C after fertilized egg deposition. Figure 2 shows that the period for the dumpy phenotype is more extended. It includes: later L_1 stage, all the L_2 stage, and the beginning of the L_3 stage (from 18 to 42 h of development at 22 °C, after fertilized egg deposition).

Mutant expression at 22 °C. f48ts length growth curves at 17 °C and 22 °C (figure 3) show that dumpiness expression starts at the beginning of the L_3 stage, near the end of the dumpy phenotype TSP. Roller phenotype is never seen in the larvae (from L_1 through L_4) and is only expressed in the adult stage.

Mode of inheritance. Genetic studies prove that f48ts carries a single autosomal recessive mutation. Homozygous 48/48 hermaphrodites reared at 22 °C are mated, at this same temperature, with wild-type males (+/+).

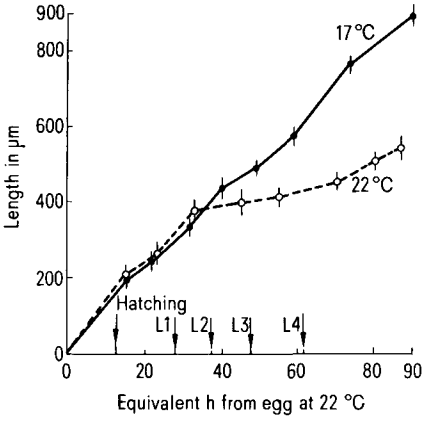

Fig. 3. Length growth curves of f48ts. Evolution of the f48ts length during development is determined at 17 °C (●—●) and 22 °C (○---○). Eggs during early embryogenesis are deposited on fresh medium. At various times, worms are killed with a moderate heat and their lengths measured.

Heterozygotic hermaphrodites (+/48), easily recognized by their wild-type phenotype, are picked, and roller or dumpy recombinants are selected from their self-fertilizing progeny. We did not find any recombinant among a total of 8.093 progeny. This means that if 2 closely linked mutations were involved, they should be distant by less than 0.02 C.M. Thus it is justified to assume that 1 gene is involved. As yet no data on localization and allelism are available. The fact that a single pleiotropic mutation can lead to both roller and dumpy phenotypes was already observed in *C. elegans* Bristol, by Brenner[7] with non-temperature-sensitive mutants (alleles of dpy-2 gene, on linkage group II).

The A and B tests defined by Hirsh et al.[8] prove non-maternal inheritance of the 2 temperature-sensitive phenotypes. These 2 tests show that the mutant allele of the gene studied must be expressed in the zygote in order that the mutant phenotype be realized. This is not suprising since the TSPs are both late, and indicates that the roller and dumpy phenotypes of f48ts are not due to an oocyte component.

Conclusions. The fact that the roller phenotype is only seen in the adult was already noted with non-temperature-sensitive roller mutants[7] which include the only temperature-sensitive roller previously isolated[9]. These observations show that if the adult cuticle is modified, and recent studies of Higgins and Hirsh[9] agree with this conception, it would only alter the adult cuticle. The fact that we find for the f48ts roller phenotype, a temperature-sensitive period earlier than the formation of the adult cuticle is not incompatible with this hypothesis, since the time at which the restrictive temperature affects the gene product can precede the moment of the phenotypic expression of the mutation. Thus it would be interesting to compare the biochemical composition of the larval cuticles and the adult cuticle in this roller mutant.

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Circles in spermatocyte chromatin loops. Electron microscopy and AgAs-NORs studies[1]

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Summary. We describe the production of circles in chromomeric loops during the pachytene stage of the spermatocytes. These circles are found attached to chromatin or already free in the nucleoplasm. Each circle measures an average of 3700 Å in circunference. We suggest that such circles might indicate the presence of tandem repetitions.

Thomas et al.[3] demonstrated that repeated sequences of the DNA in the eukaryotes can produce rolling circles by denaturation and annealling. According to the rolling helix model[4], a single copy of a repeated sequence may form a circle. This circle migrates along the DNA helix, maintaining the base-pairing. During the migration, repairs could be made enzymatically and excision of these circles could be the 1st step of replication. Again, the findings of Hourcade et al.[5] demonstrated that the rolling circles could account for the amplification in the nucleoplasm. In this article we describe the origin of

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circles in chromatin loops during male meiosis. We assume that the circles are produced by direct replication of repeating sequences.

Material and methods. The preparations were obtained from spermatocytes of the frog *Odontophrynus americanus* 4 n = 44 (Ceratophrydidae).

Electron microscopy. The seminiferous tubules were dissected in 0.7% NaCl solution and squashed on a slide. The cell suspension obtained was spread over distilled water (pH 7.0, 5 min) in a plastic tray with Teflon bars and collected on Parlodium (1.5%) covered grids. The specimen was positively stained in 95% ethanolic uranyl acetate (1%, 1 min) and then washed in 95% ethanol. Following this procedure, some specimens were shadowed with Paladium (10°C angle). The frequency of spermatocytes along the tubules is variable. A 0.5 cm fragment has about 1–3 pachytenes in each grid. The grids are previously selected under the phase microscope for identification of the stage.

AgAs-NORs technique. This method was used for spermatocytes obtained by the conventional squash preparations and treated according to Goodpasture et al.[6].

Results and discussion. During the pachytene stage of the spermatogenesis, we observed the occurrence of circles attached to some chromatin loops. These circles are tandemly distributed along the fibre (figure 1). They were also observed free in the nucleoplasm (figure 2) or packed. Each circular element measures an average of 3700 Å in length. The extreme values are 2900 Å and 4000 Å in a total of 10 rings. The chromatin fibre as well as the circular filament are slightly granular having each particle 60–80 Å (figures 1 and 2). Some circles show a dense point in the circumference and a tail-like prolongation. This is a inner filament, its size reaching half of each circle (figures 2–4).

In our study we utilized 3 pachytene nuclei with 13, 24 and 50 rings respectively. Packed rings were not counted. The presumptive chromatin nature of the rings is based on morphological observations. According to Olins and Olins[7], the DNA of each particle with an average diameter of 70 Å would have a length of about 400 Å and a packing ratio (DNA length: particle diameter) of about 6:1. By this approach, each circle with ~ 3700 Å of length (~ 53 particles) would have a DNA length of ~ 21,200 Å or ~ 2.1 μ. This is close to the 2–3 μm length measured for the *Xenopus* rDNA cistron[8].

By using the AgAs-NORs technique, we looked for active NORs in the spermatogenesis of the same species. In the 4 n somatic cells, there are 4 homologue chromosomes (11th group) with secondary constrictions[9] which present N-band by the technique using Giemsa[10] or AgAs[11] to stain the NORs. The analysis of the spermato-

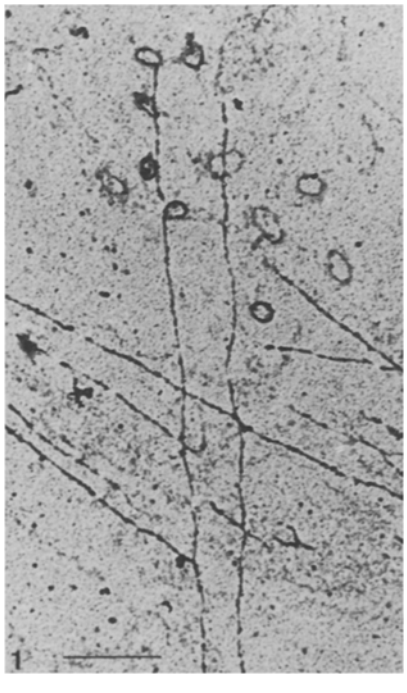

Fig. 1. Circles at chromatin loops of a amphibian spermatocyte. (Bar = 0.5 μm.) Alcoholic uranyl acetate.

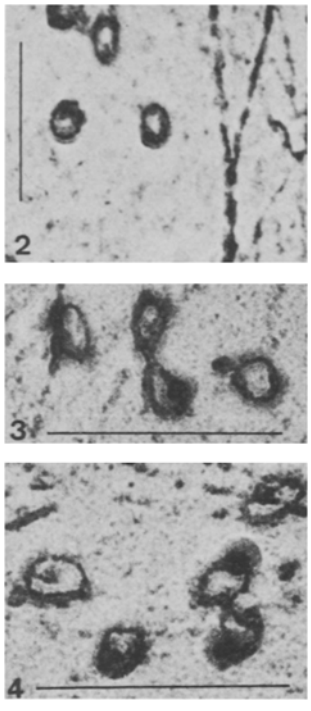

Fig. 2. Free circles in the nucleoplasm of a spermatocyte in pachytene. 2 circles show tail-like prolongation. (Bar = 0.5 μm.) Alcoholic uranyl acetate, Pa shadowing.

Figures 3 and 4. Chromatin attached circles showing each a dense point in the circumference. (Bar = 0.5 μm.) Alcoholic uranyl acetate, Pa shadowing.

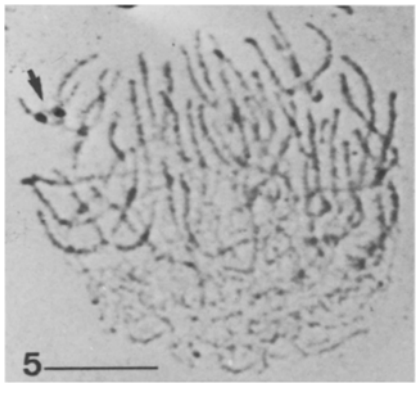

Fig. 5. Bouquet configuration of a 4 n spermatocyte showing AgAs bands (arrow) at the smallest chromosomes (11th group). (Bar = 10 μm.)

cytes evidenced the occurrence of 2 silver bands in the pachytene bouquet configuration. These bands correspond to the pairing of the 4 homologues as 1 quadrivalent or 2 bivalents (figure 5).

Our observations on the chromosomal origin of 'circular' DNA fit well with the repetition models proposed by Thomas[4,12]. It implies the formation of circles in tandem and in intermittent repetition[12]. The distribution of the circles we describe supports the occurrence of tandem repetition. Evidence of ring formation in intermittent repetition was previously reported[13]. We still ignore the DNA base composition of these circles, as well as its possible involvement in RNA synthesis. The hypothesis that the rings might be copies of rDNA is attractive but obviously deserves further proof. Therefore the question whether the mechanism of gene amplification found in oocytes has its counterpart in spermatocytes is still speculative.

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Filter characteristics of appendicularian food catching nets

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Summary. Scanning electron micrographs reveal extensive filter surfaces in the external food catching net of planktonic appendicularia. This filter consists of crossing arrays of filaments about 0.04 μm thick and pores about 0.24 × 0.07 μm wide. The open area fraction is above 50%. The filter probably enables the appendicularia to feed efficiently on particles much smaller than bacteria.

Appendicularia (Chordata, Tunicata) are tiny planktonic animals of abundant occurrence and world-wide distribution. They feed in a most unusual way by secreting an external gelatinous house containing an elaborate feeding filter (figure 1). Undulatory movements of the tail forces water through the house, and any particle present in the water will be trapped by the filter and sucked into the mouth of the animal[2].

In this way the appendicularia is one of the few zooplankton organisms that feed in a very efficient way on phytoplankton and other nanoplankton. As this is a very important step in the food chain from primarily produced organic material to nectonic fishes etc. in the sea, the appendicularia has been studied with increasing interest during recent years.

The gelatinous house and feeding filter is secreted by cells covering the trunk of appendicularia[3]. A previously

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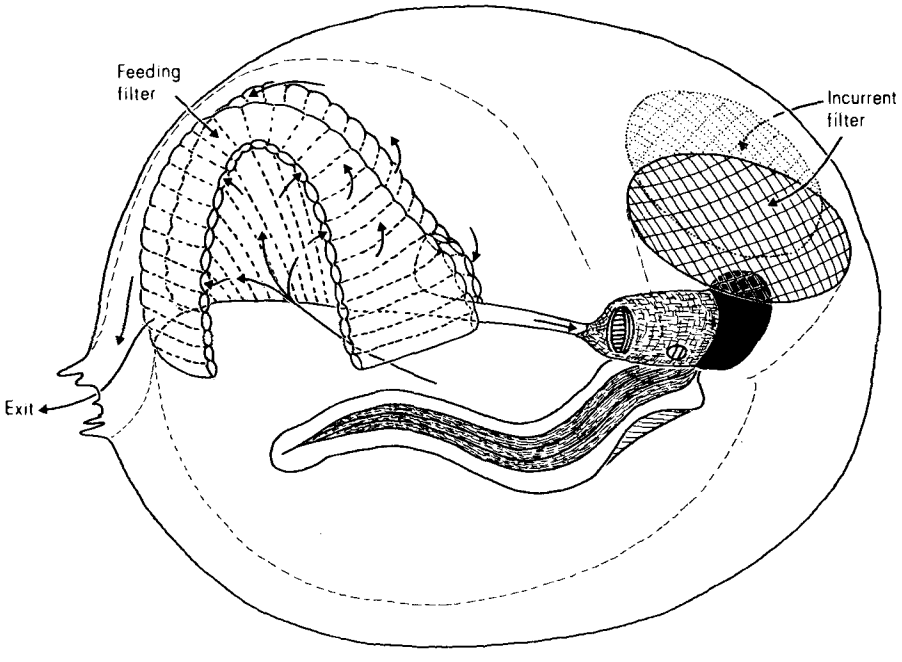

Fig. 1. Schematic diagram of *O. dioica* in its house. Lateral view.