PALA is that it inhibits an enzyme coded for at the locus under study and should not select for overproducers of enzymes catalyzing later steps in the pathway which 6-AU might do. 6-AU could be used as a secondary selection agent in retesting and maintaining stocks of PALA resistant flies. However, the most important advantage of PALA stems from the fact that it is a transition state analogue of ATCase. Mutations leading to modification of ATCase so that it does not interact with PALA will probably also eliminate ATCase activity. Increased ATCase levels thus are a likely means for obtaining resistance to PALA. In conclusion, these preliminary results indicate that PALA could be an excellent selection agent for overproducers.

The nucleolar chromosome in embryos of Rana pipiens[1]

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Summary. Karyotype analyses of prometaphases from medullary plate cells derived from mid-neurulae of Rana pipiens have led to the identification of the nucleolar chromosome and nucleolar organizing region, which is located on the longer arm of a small sub-metacentric chromosome (No. 10).

Nucleolar chromosomes have been identified in several systems. Direct identification has been made in pachytene chromosomes, e.g.[4] and occasionally in late prophases and early prometaphases of somatic cells when the nucleoli persisted[5]. Both approaches to identifying the nucleolar chromosome of an organism are extremely time consuming and difficult due to the lack of spreading of the chromosomes at these stages and also due to the rarity of persistent nucleoli in mitotic chromosomes. Attempts to induce the persistence of nucleoli in mitotic chromosomes have been reported[6,7]; however, these approaches have not yielded wide spread application in different systems. It is also possible to identify nucleolar chromosomes in mitotic chromosomes through an indirect approach. For example, in the Xenopus mutant, heterozygous for the nucleolus, one can correlate the absence of a prominent secondary constriction in one homologue of a pair of metaphase chromosomes with the absence of one nucleolus in interphase nuclei[8,9]. We report here the direct identification of the nucleolar chromosome and the nucleolar organizing region in embryos of Vermont Rana pipiens, revealed microscopically in prometaphases of medullary plate cells containing a large number of persistent nucleoli.

Materials and methods. Adult Rana pipiens used in these studies were purchased from J. M. Hazen Co., Alburg, Vermont, and are derived from 5 different shipments obtained over a period of 4 years. Ovulation was induced by intraabdominal injection of pituitary glands and artificial insemination was performed with sperm suspensions derived from macerated testes, based on original procedures of Rugh[10]. When the embryos attained the stage of mid-neurula (stage 14[11]), the medullary plate along with the underlying mesoderm and archenteron roof was excised in Steinberg's salt solution[12]. Next, the medullary plate was either mechanically separated from the underlying tissues in Steinberg's solution, or transferred to 0.5% trypsin dissolved in modified Steinberg's solution[13] for 2–3 min, during which time the medullary plate was separated from the other tissues. The medullary plate was then rinsed in Steinberg's solution for 2–3 min, and immediately fixed and stained in acetic orcein. Subsequently, the tissue was squashed and processed for permanent preparations.

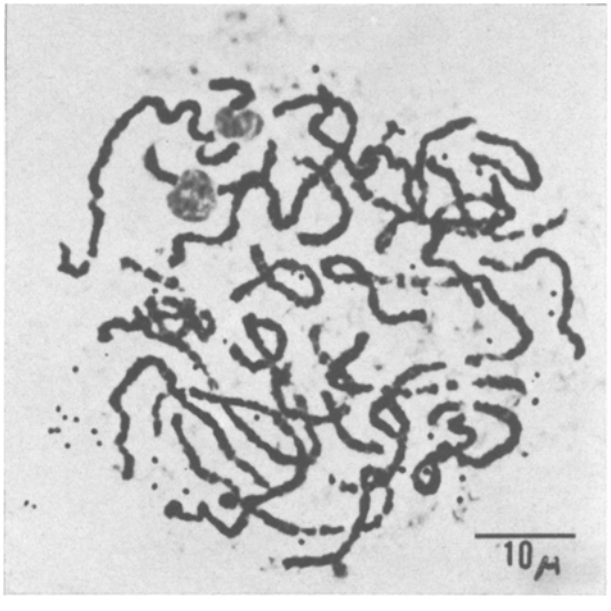

Fig. 1. Prometaphase from a medullary plate cell derived from a diploid embryo at the mid-neurula stage. 1 pair of small homologous chromosomes contains 2 nucleoli.

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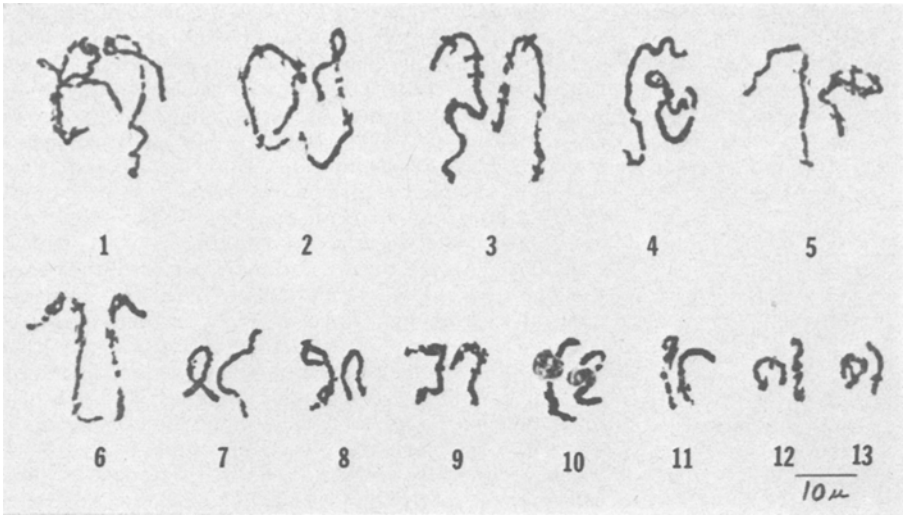

Fig. 2. Karyotype of prometaphase chromosomes shown in figure 1. The pair of chromosomes in the tenth position contains nucleoli.

Results. Microscopic examination of medullary plate cells revealed numerous prometaphases, containing in each diploid complement 1 or 2 chromosomes bearing a large nucleolus (figure 1). Occasionally, even early metaphases (polar) displayed persisting nucleoli; however, at this stage, the nucleoli were quite small. The frequent appearance of prometaphases with nucleoli was consistently observed in all 80 embryos examined and these individuals resulted from 10 parental crosses. 9 prometaphases, obtained from 5 embryos resulting from 3 different parental crosses, were subjected to karyotype analysis. The chromosomes were arranged in order of decreasing length determined by means of a linear map measure. In 7 of 9 karyotypes, the pair of nucleolar chromosomes appeared in the number-10-position (figure 2), and in 2 karyotypes, the nucleoli were associated with a pair of homologues in the number-11-position. On the basis of chromosome length, the nucleolar chromosomes from prometaphase stage correspond to metaphase chromosomes numbers 10 and 12 (nomenclature according to centromere position[14]), which are also similar in length. Figure 3 depicts a metaphase plate obtained from a bone marrow cell of an adult female in which the chromosomes have been arranged according to centromere position (figure 3a) and also according to decreasing length (figure 3b).

It is now generally accepted that the location of a prominent secondary constriction visible in metaphase chromosomes corresponds to the site of the nucleolus[5, 9, 15]. In order to determine conclusively whether the prominent secondary constriction present in the longer arm of the small submetacentric chromosome corresponds to the site of the nucleolus, a series of measurements were made with a linear map measure, using the constriction, instead of the centromere, as a reference point to separate the chromosome into long and short portions. By dividing the length of the longer portion by the length of the entire chromosome, we obtained a percentage value which represents the relative site of the constriction in the chromosome. The position of the nucleolus in prometaphases was determined in an identical manner (figure 4). In

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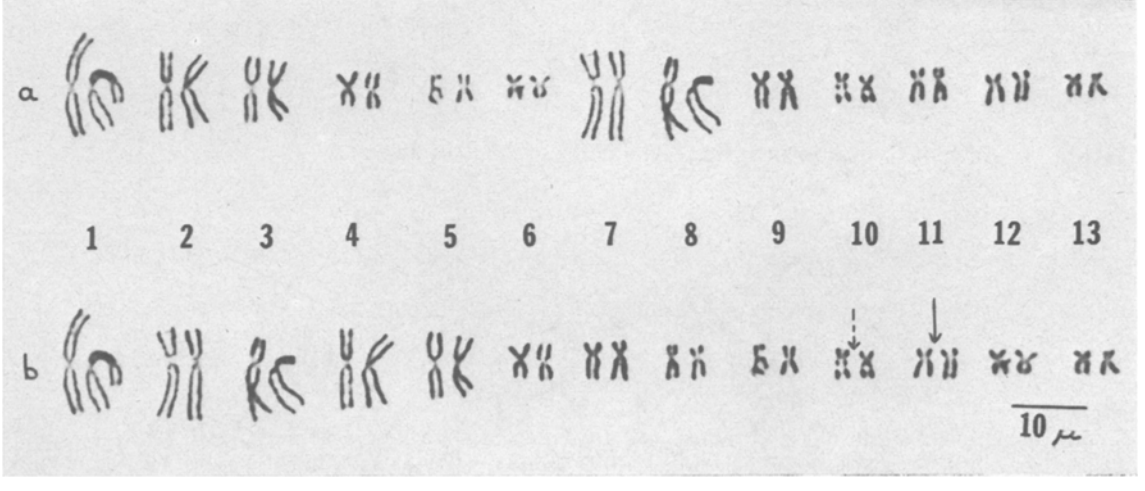

Fig. 3. *a* Karyotype of metaphase plate derived from a bone marrow cell of an adult female in which chromosomes have been arranged according to centromere position. *b* Metaphase chromosomes shown in figure 3a, but arranged according to decreasing length. Arrows indicate No. 10 (- - →) and No. 12 (——►) chromosomes from karyotype based on centromere classification; these chromosomes occupy positions 10 and 11 when arranged according to decreasing length.

measuring the chromosomes in prometaphases and metaphases, the variable distance occupied by the nucleolus and the secondary constriction was not included in the measurements. The results of these measurements are summarized in the table. In all cells analyzed, the range of relative values for the location of the secondary constriction in chromosome number 10 of metaphases correspond to the range of relative values for the site of the nucleolus in chromosome numbers 10 or 11 of prometaphases. An analysis of variance test of these relative values indicated that the nucleolus in prometaphases and the secondary constriction in metaphases are contained within the same region of the chromosome ($p \geq 0.5$). Thus, the number-10-chromosome in metaphase cells of Rana pipiens has been identified as the nucleolar chromosome, and the nucleolar organizing region is located on the longer arm of the small sub-metacentric chromosome in the region of the secondary constriction.

Position of nucleolus and secondary constriction

	Range (relative values)
Nucleolus from prometaphase chromosome No. 10 or No. 11	
Medullary plate cells (stage 14)	0.59–0.65
Secondary constriction from metaphase chromosome No. 10	
Animal hemisphere cells (stage 8)	0.60–0.62
Medullary plate cells (stage 14)	0.60–0.63
Tail tip cells (stage 25)	0.60–0.66

Each range of relative values was derived from measurements made on 20 chromosomes from 10 pairs of homologous chromosomes.

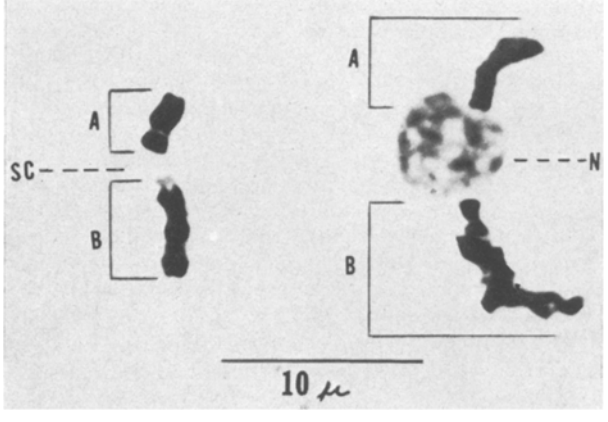

Fig. 4. Nucleolar chromosomes (No. 10): The chromosome on the left is derived from a metaphase and that on the right is derived from a prometaphase. Linear measurements were determined as indicated in lines A and B. The relatives sites of the secondary constriction (SC) and the nucleolus (N) were determined in 10 cases of each type by the formula B/(A+B). Data are presented in the table.

Discussion. An interesting finding from these studies was the high frequency of prometaphases with large persistent nucleoli in the mitotic cells of the medullary plates. A major constituent of the nucleolus is ribosomal RNA, and ribosomal cistrons are located within or adjacent to the chromatin of the nucleolar organizer[16–18]. The frequent persistence of large nucleoli in prometaphases of medullary plate cells may be a reflection of intense synthesis of ribosomal RNA. Preliminary studies on other cell types of Rana indicate that the frequency of prometaphases in larval gut, brain and kidney is quite low; furthermore, when prometaphases are present, nucleoli are rarely observable and only as 2 small bodies (Newman, unpublished). Further studies are needed to determine conclusively whether the frequent persistence and large size of nucleoli in prometaphases of medullary plate cells is in fact a reflection of differential transcription occurring in vivo.

The persistence of these nucleoli appears to be a normal event during embryogenesis of this species, since siblings derived from the same batch of inseminated eggs, which were not sacrificed for cytological studies, exhibited normal larval development. The nucleoli observed in prometaphases of medullary plate cells are extremely large (range from 3 μm to 6 μm) and should be amenable to dissection, manipulation and further analyses. Also, studies similar to these reported here could be extended to other organisms in which the nucleolar chromosomes and nucleolar organizer regions have not yet been identified.

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Orientation of Giemsa C-bands in interphase cells of Allium cepa L.

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Summary. Orientation of Giemsa C-bands in Allium cepa was studied in both mitotic and interphase cells. It has been shown that telophase orientation of the chromosome is maintained throughout the interphase and early prophase. It has been assumed that this non-random orientation is due to anchorage of the telomeres with the nuclear membrane. Contrary to earlier observations, 2 by 2 pairing of the telomeres could not be traced in this species.

It is a common belief that interphase chromosomes are randomly arranged and may occupy any position within the nucleus[1], although as early as in 1902 Sutton[2] suggested that interphase chromosomes maintain their arrangement at telophase until the next prophase. Such definite and nonrandom arrangement of interphase chromosomes have been reported by several workers in different plant and animal species. In Allium cepa, it has been shown that interphase chromosomes have nonrandom orientation[3–6]. A. cepa is an excellent material to study the interphase orientation of chromosomes, as they show Giemsa C-bands only at the telomeric regions. In