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.

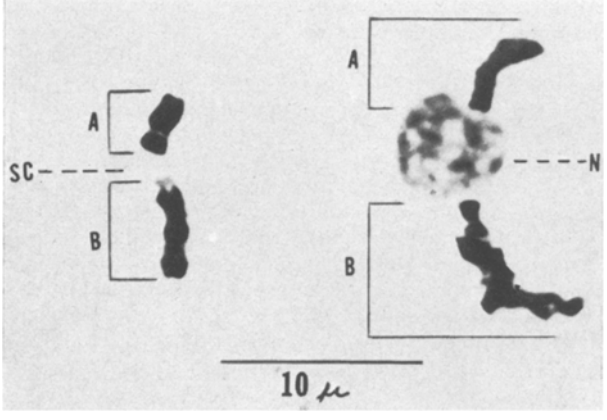

Fig. 4. Nucleolar chromosomes (No. 10): The chromosome on the left is derived from a metaphase and that on the right is derived from a prometaphase. Linear measurements were determined as indicated in lines A and B. The relatives sites of the secondary constriction (SC) and the nucleolus (N) were determined 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

this communication, the orientation of telomeric C-bands in Allium cepa, indicating non-random arrangement of chromosomes in interphase cells, will be presented.

Material and methods. Chromosome and interphase cell preparations were made from roottip meristems of Allium cepa. The preparations were treated with saturated solution of barium hydroxide for 5 min and were allowed to renaturate in 2X SSC at 66 °C for 2 h. The preparations were stained in 5% Giemsa (Merck) solution; washed, air dried and mounted in euparal The method is a slight modification of Vosa and Marchi[7].

Results and discussion. Metaphase chromosomes show deep stained C-bands at telomeric regions of all the chromosomes (figure 1). As there are 16 chromosomes in a cell, 32 bands can be seen in a metaphase plate. At telophase these meta- to submetacentric chromosomes have their telomeres oriented towards the newly formed cell wall (figure 2). Figure 3 presents an interphase cell showing the telophase orientation of the C-bands. Such orientation of the telomeres is also evident in the binucleate cell (figure 4) induced through caffeine treatment (10 mM) and fixed after 20 h at the G_2 phase[8] This orientation is also maintained, even in the early prophase cells (figure 5). The observations on the Giemsa C-bands indicate clearly that the telophase arrangement of the chromosomes is maintained throughout the interphase. The highly reiterated DNA in the telomeres may act as anchor regions to the nuclear membrane[6,9] attributing the definite orientation of the interphase chromosomes.

Giemsa C-bands in interphase cells have been studied in rye too[10,11]. In rye they have observed end to end chromosome attachment in interphase cells. In Allium cepa too, 2 by 2 chromosome association has been reported by Fussel[6] by autoradiography. The present observations, however, do not reveal any regular end-to-end attachment of the C-bands. In interphase cells 24–26 bands (instead of usual 32) can be seen (figures 3 and 5). The lower number may be due either to limitation of the procedure or to ectopic pairing between some of the C-band regions as DNA of similar base compositions and sequence might mediate heterochromatic attraction[12,13]. In no case, however, could 2 by 2 pairing be noticed.

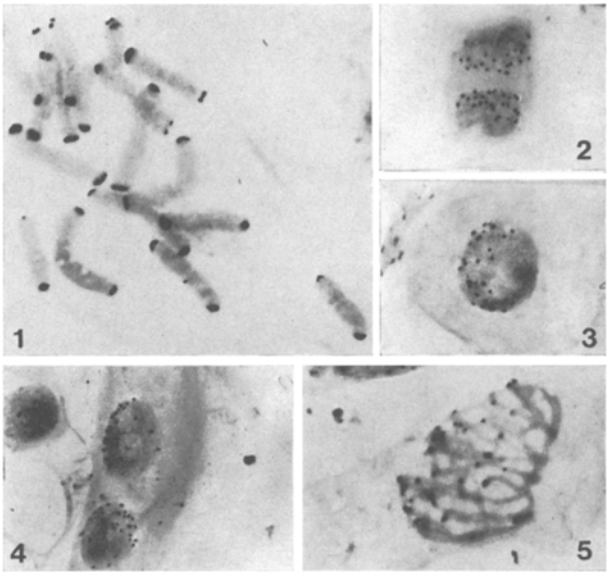

Fig. 1. A metaphase cell of Allium cepa showing 16 chromosomes with C-bands at the telomeric regions. Fig. 2–5. Orientation of C-bands in a telophase cell, or interphase cell, a binucleate G_2 cell and an early prophase cell respectively.

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Effects of Ag^+ on frog skin: Interactions with oxytocin, amiloride and ouabain[1]

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Summary. Different biological effects of Ag^+ (10^{-4} M) were found depending on its presence in the outer or the inner solution bathing the frog skin. A marked increase in the electrical conductance and an interference with the action of oxytocin and amiloride were found only when Ag^+ was added to the outer solution. Results suggest that Ag^+ affects several transport processes, in particular the permeability of the Na entry pathways.

Sensitivity of amphibian epithelia to the presence of minute quantities of heavy metal ions has been reported[2–7]. In particular, Ag^+ induces significant changes in the permeability of frog skin when present in the outer bathing solution[2]. We report here some effects of Ag^+ that have hitherto not been described, and observations on its interaction with the biological effects of oxytocin, amiloride and ouabain.

Materials and methods. The abdominal skin of frogs Rana ridibunda was mounted on Ussing-type conic chambers

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