RNA SYNTHESIS IN CHROMOSOME CONNECTIVES

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INTRODUCTION

Connections between chromosomes have been observed and described by numerous investigators (1–10). They have been seen with the light microscope (1, 2, 6, 7, 10), the transmission electron microscope (3–5, 8), and the scanning electron microscope (9). In addition, surface specializations (side arms, loops, whiskers) have been observed on chromosomes by a number of researchers who have employed a variety of cytological techniques (10–14). This paper describes inter- and intrachromo-

somal connectives in cultured lymphocytes from the opossum (*Didelphis virginiana*) and demonstrates that in certain instances they are distinctly labeled in autoradiographs of cells incubated with uridine-5-^{3}H.

MATERIALS AND METHODS

Lymphocytes which were obtained from cardiac blood of male and female opossums were cultured with phytohemagglutinin (Difco Laboratories, Detroit, Mich.) according to a previously described method (15). After 36–48 h of incubation, the cultures were labeled for 5–20 min with 5 μCi/ml of uridine-5-^{3}H (Amersham/Searle Corp., Arlington Heights, Ill., sp act 5 Ci/mmol). The cells were placed in a hypotonic solution (1% sodium citrate) for 20 min at 37°C and fixed in a 3:1 solution of absolute methanol-acetic acid followed by 10 min in 50% acetic acid in order to obtain cytoplasm-free chromosomes (16). Slides were coated with Kodak NTB2 liquid emulsion, exposed 3 days, developed, and fixed. Subsequently stained preparations were scanned with a light microscope for labeled chromosome spreads which were photographed and recorded. The slides were then degrained (17), the spreads relocated and photographed again for comparison. This procedure eliminated the possibility of chemographic reduction of the emulsion caused by staining (18). Some labeled preparations were treated with 200 μg/ml of ribonuclease (Mann Research Labs. Inc., New York) in pH 7.0 phosphate buffer for 2 h at 37°C before autoradiography.

RESULTS

One or more interchromosomal connectives (Fig. 1) were observed in approximately 10% of the hypotonic spreads investigated. In addition to distinct connectives, many chromosomes demonstrated generally fluffy borders or surfaces as may be observed by careful analysis of this figure. Fig. 2 *a* shows a connective between the body and telomeric region of an individual chromosome as well as another between this and an adjacent chromosome (arrows). The autoradiograph of this same chromosome spread (prepared from a culture which had been labeled terminally with uridine-5-^{3}H) shows clearly definable bands of grains which have the exact morphology as the connectives (Fig. 2 *b*, arrows). Similar labeling patterns were observed in approximately 30% of recognizable connectives; the remainder were unlabeled.

That the grains represented newly synthesized RNA (and not DNA) is supported by the following observations: (*a*) the cells were labeled a maximum of 20 min, and the minimum G_2 phase (during which time no DNA is synthesized) is 90 min in opossum lymphocytes (19); (*b*) uridine-5-^{3}H cannot be converted to thymidine-^{3}H since the tritium molecule would have to be cleaved from the uridine molecule in order to allow methylation of the 5 position in the pyrimidine ring (20); and (*c*) ribonuclease treatment removed over 97% of the

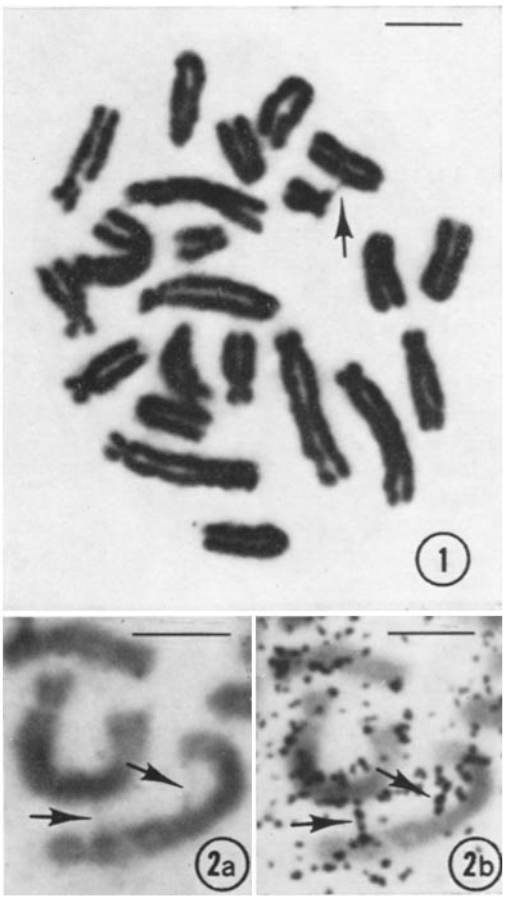

FIGURE 1 An unlabeled hypotonic chromosome spread from a culture of opossum lymphocytes. The arrow indicates a connective between one of the X chromosomes and an autosome. Scale bar equals 5 μm. × 1,700.

FIGURE 2 *a* Portion of chromosome spread from an opossum lymphocyte showing connectives between regions of an individual chromosome and between adjacent chromosomes (arrows). Scale bar equals 5 μm. × 2,200.

FIGURE 2 *b* Autoradiograph of the same chromosome spread labeled with uridine-5-^{3}H. The arrows demonstrate sites of RNA synthesis. Note that the grain patterns correspond exactly to the morphology of the chromosome connectives. Scale bar equals 5 μm. × 2,000.

grains which reduced grain counts to those equivalent to background. Grains were removed equally from all parts of the chromosomes, including the connectives.

DISCUSSION

Speculation exists regarding the composition of interchromosomal connectives, although considerable evidence suggests that they contain DNA (12, 13). Hoskins (5) believes that chromosomal spindle fibers not only contain DNA but are reliant on it for structural integrity, but this idea is not acceptable to DuPraw (12). The latter cites the existence of radial configurations of metaphase chromosomes which are connected by fibrous structures (see particularly the photograph of *Didelphis* chromosomes by Dr. T. C. Hsu) as evidence that all of the chromosomes of a cell may be connected as "parts of a single, circular and enormously long DNA molecule" (12). Yu (14), on the other hand, believes that fibrous materials which emanate from the surfaces of Chinese hamster chromosomes contain protein rather than DNA.

Techniques for the preparation of chromosomes may play a significant role in determining the nature and composition of surface fibrous materials. Hoskins (21) believes that water is generally destructive to chromosome structure. Boss (10) suggested that connectives observed between chromosomes of adult newt fibroblasts were the result of hypotonic treatment which either aggregated already present submicroscopic connectives or produced them by rendering the chromosome surfaces adhesive. Yu (14) reported, however, that the surface fibers of Chinese hamster chromosomes were removed by hypotonic solution.

If chromosome connectives do contain DNA as is strongly suggested, then it is reasonable to consider them capable of directing RNA synthesis as evidenced in this investigation. Diffuse chromatin of isolated thymus nuclei has been shown to be active in messenger RNA synthesis (22). That the RNA synthesis observed in the present investigation might also be largely messenger in nature is supported by the findings of Cooper and Rubin (23, 24, 25) who studied RNA metabolism in phytohemagglutinin-stimulated lymphocytes. They stated, "The precise nature and function of the abundant nonribosomal RNA produced by lymphocytes in the presence of PHA are unknown, but an obvious hypothesis is that it is messenger RNA being produced in large quantity" (24).

Comings (26) believes that because of the lack of intrachromosomal RNA synthetic patterns and because of poor localization of grains to chromosomes, labeling which is observed with uridine-^{3}H is the result of "random, non-specific adherence to metaphase chromosomes of free, labeled RNA." The presence of surface extensions of DNA (which most commonly may be below the resolving power of the light microscope), however, might explain why autoradiographic grain patterns in this and other RNA synthetic studies (27, 28) are often diffusely localized to the chromosome surfaces. Thus, it is possible that these surface extensions may represent the active chromatin fraction of prophase and metaphase chromosomes.

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