Late DNA Replication in Male Mouse Meiotic Chromosomes

Abstract. *Parts of the male mouse meiotic complement comprising the Y chromosome, the whole X chromosome, and near-centromeric parts of autosomal bivalents are synthesized late, as judged by tritiated thymidine autoradiography. This confirms the occurrence of end-to-end association between X and Y chromosomes and suggests that paired heterochromatic segments in autosomes must synthesize DNA at the same time.*

The kinetics of spermatogenesis in laboratory rodents have been studied by cell blocking (*1*) or autoradiography. Patterns of labeling of DNA with ^{3}H-thymidine (^{3}H-TdR), however, have been examined in histologic sections (*2*) or on widely time-spaced squashes (*3*) or have been limited to spermatogonial mitosis (*4*). The patterns have been studied over individual meiotic chromosomes only in plants, insects (*5*), or lower vertebrates (*6, 7*). We studied carefully timed tissue samplings containing mouse meiotic chromosome figures and describe here technically good preparations, in terms of chromosome morphology and of autoradiographic resolution.

The percentage of well-spread labeled cells in late diakinesis and early metaphase I (DMI) was recorded as a function of time after injection of ^{3}H-TdR (*8*). It takes a minimum of approximately 9 days for cells to proceed from the last premeiotic phase of DNA synthesis to the observed stages (*9*) (Fig. 1). This is shorter than the 14 to 15 days described in rats (*2*) or the 20 to 21 days observed in newts (*6*). Earlier and later phases of spermatogenesis are correspondingly shorter in the same approximate ratio. The curves of Fig. 1 cannot be taken as supporting or as excluding a low amount of DNA synthesis during the zygotene-pachytene phase (*7, 10*) because of the relative imprecision of autoradiographic data.

Labeling was continuous for at least 2 days (*11*); with time, all DMI figures became labeled, and all chromosome bivalents progressively became uniformly covered with grains. In these conditions, only the regions of chromosomes which are late labeling became apparent in partially labeled cells, and thus only slides in which less than 15 percent of DMI were labeled were selected. This corresponds approximately to less than one DNA premeiotic synthesis time (*2*) between the beginning of injection of ^{3}H-TdR and the entry of cells into nonsynthesizing phases of the mid-leptotene phase (*7*).

Suitable DMI figures (771) exhibiting a partially labeled chromosome complement were analyzed on slides taken from six different animals, according to the above criteria. These figures represent a heterogenous group of cells, because of variability in the speed of progression from the end of the last period of DNA synthesis to the DMI stage.

When only one bivalent was labeled (11 DMI), it was always the sexual XY complex, and label was consistently restricted to one end (Fig. 2a). When grains were also seen over some autosomal bivalents, the XY complex exhibited grains either over one end (Fig. 2b) or over both ends (Fig. 2c). In male mouse mitotic chromosomes the entire Y is uniformly late in replication (*12*). If this holds true for the meiotic Y, these findings confirm earlier morphological observations of an end-to-end association between the X and the Y at meiosis (*13*); the Y can even be particularly recognized in preparations such as Fig. 2b. However, until technically good anaphase figures are obtained it is not possible to determine the actual mode of X-Y association, whether nonchiasmatic or with a chiasma formed between short arms of the X and the Y, as a result of extreme shortness of these arms in the latter case.

When more than 8 to 12 autosomal bivalents became labeled the sexual complex was entirely labeled. In fact, when only one bivalent was entirely labeled, it was always the XY complex (Fig. 3, a and b), as far as it could be morphologically defined in the conditions of heavy labeling.

In autosomal bivalents, asynchrony of DNA-replicating regions was frequently observed (Fig. 3, a and b). In fact, out of 760 DMI with partial labeling of autosomal bivalents, 13 percent of all pooled autosomes had grains localized in only one spot (zero to nine such bivalents per cell). In more than 75 percent of these bivalents, good morphology and autoradiographic resolution enabled localization of the grain spot over or very close to the distal part of an arm. Thirteen percent of

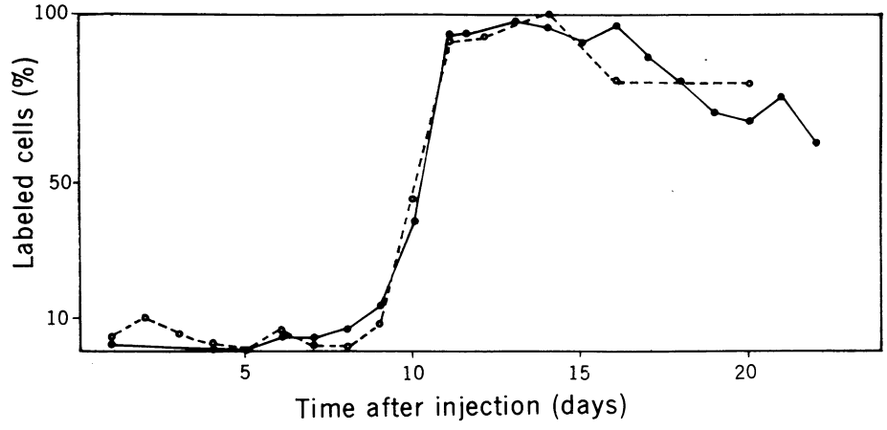

Fig. 1. Percentage of labeled cells in late diakineses and early metaphases I (DMI) as a function of time after an intratesticular injection of tritiated thymidine (0.1 μc). ●——● Slides exposed for 30 days; ○- - - -○ exposed for 70 days.

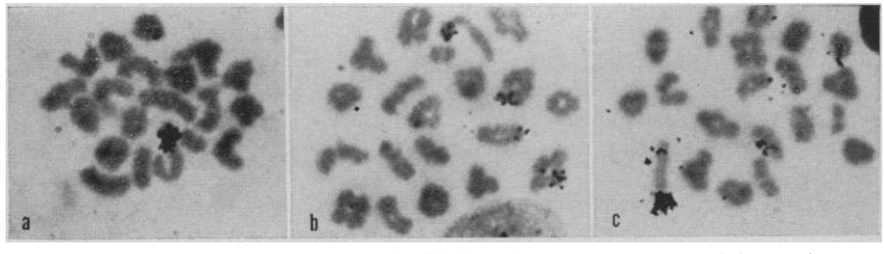

Fig. 2. Meiotic mouse chromosomes in DMI with partial labeling of heterochromosomes. (a) Late labeling of the Y only. (b) Late labeling over the Y and some parts of few autosomal bivalents. (c) Late labeling of the Y, of some autosomes, and of the distal part of the X.

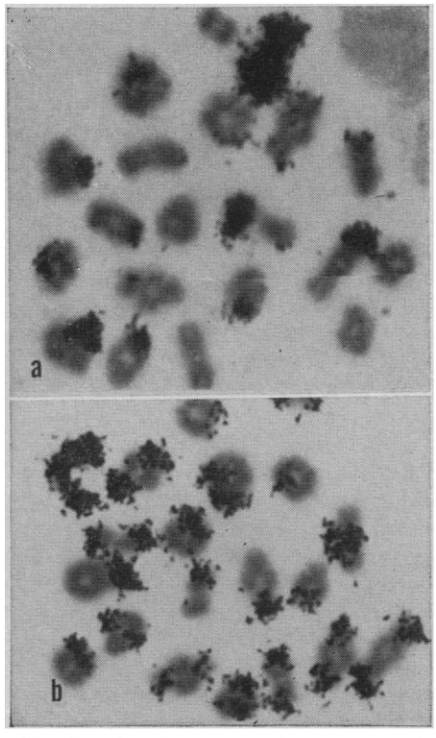

Fig. 3. Meiotic mouse chromosomes in DMI with partial labeling of autosomal bivalents and complete labeling of the sexual complex. (a) Some autosomes partially labeled. (b) All autosomes partially labeled.

all labeled autosomes had grains localized in two spots (zero to ten bivalents per cell) that were also close to arm-ends, with the same reservations as above. The number of grains was more frequently grossly equal in both spots than unequal. A mean of 22 percent of all autosomes were observed to be entirely labeled, whereas 52 percent were devoid of grains.

This overall pattern corresponds well with data for murine mitotic chromosomes (*12*), except for the mitotic X chromosome that has not been observed as being late labeling in males. The distal parts of meiotic bivalents are near-centromeric in mouse telocentric autosomes and generally considered as heterochromatic (*14*). Distal late labeling has also been observed in meiosis in *Triturus* (*6, 7*).

We could not determine an exact relation between termination of DNA synthesis in the two distal parts of a chromatid. Even for those X chromosomes where grains were seen at the end opposite to the Y, possible grains in the proximal end would be undistinguishable from the grain spot of the Y chromosome (Fig. 2c).

Comparison of the length and thickness of mouse meiotic DMI autosomes with the size of grain spots indicates further that synchronously late-synthesizing segments in homologous chromatids do actually pair with each other. Indeed, drawing axes of symmetry through one spot, whenever only one is present in a bivalent, or through the unit constituted by a double spot at both ends of one bivalent, shows that these axes do actually cross the center of the chromatid figures. This observation was technically possible for approximately 70 percent of all autosomal bivalents that were analyzed. In Fig. 3, a and b, this simple geometrical relation applies to an even larger proportion of autosomal chromosomes. Even though it appears quite logical that homologous chromatids pair with a gene-to-gene correspondence and that replication should also be sequentially synchronized, direct proof of the latter point has been lacking. Within the limits of autoradiographic resolution, this is actually the case for the presumably heterochromatic parts of mouse autosomes, owing to their uniformly telocentric configuration in this species and assuming that centromeres are indeed located in the close neighborhood of late-labeling regions.

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References and Notes

1. E. F. Oakberg, *Amer. J. Anat.* **99**, 507 (1956).
2. W. Hilscher and B. Hilscher, *Z. Zellforsch.* **96**, 625 (1969); V. Monesi, *J. Cell Biol.* **14**, 1 (1962).
3. T. Utakoji, *Exp. Cell Res.* **42**, 585 (1966); ——— and T. C. Hsu, *Cytogenetics (Basel)* **4**, 295 (1965).
4. M. Fraccaro, I. Gustavsson, M. Hulten, J. Lindsten, L. Tiepolo, *Cytogenetics (Basel)* **8**, 263 (1969).
5. A. Lima-de-Faria, *Hereditas* **47**, 674 (1961); R. B. Nicklas and R. A. Jaqua, *Science* **147**, 1041 (1965).
6. H. G. Callan and J. H. Taylor, *J. Cell Sci.* **3**, 615 (1968).
7. D. E. Wimber and W. Prensky, *Genetics* **48**, 1731 (1963).
8. A-Swiss albino mice (8-week-old inbred males, free of pathogens) were given injections of sterile thymidine-[^{3}H-methyl] (0.1 μc; specific activity, 6.0 c/mmole) in buffered saline directly into the testicular parenchyma. Mice were killed at 8-hour intervals from the moment of injection up to day 26. Autoradiograms were prepared from air-dried smears [E. P. Evans, G. Breckon, C. E. Ford, *Cytogenetics (Basel)* **3**, 289 (1964)] with Eastman-Kodak NTB-2 liquid emulsion, as described previously [N. Odartchenko, H. Cottier, L. E. Feinendegen, V. P. Bond, *Exp. Cell Res.* **35**, 402 (1964)] and, after exposure times of 30, 70, and 110 days, were stained through the film with Giemsa buffered at *p*H 5.75.
9. This time is not influenced by autoradiographic exposure time (Fig. 1) and appears to be independent of major seasonal variations, since a second group of mice, treated exactly like the first at a 6-month time interval, revealed only an approximate 12-hour prolongation of this time lag.
→ Y. Hotta, M. Ito, H. Stern, *Proc. Nat. Acad. Sci. U.S.* **56**, 1184 (1966).
11. As evidenced by an evaluation of free ^{3}H-TdR in testes tissue at various time intervals after injection.
12. M. Galton and S. F. Holt, *Exp. Cell Res.* **37**, 111 (1965); H. J. Evans, C. E. Ford, M. F. Lyon, J. Gray, *Nature* **206**, 900 (1965).
13. I. Geyer-Duszynska, *Chromosoma* **13**, 521 (1963); S. Ohno, W. D. Kaplan, R. Kinosita, *Exp. Cell Res.* **18**, 282 (1959).
14. S. Ohno, W. D. Kaplan, R. Kinosita, *Exp. Cell Res.* **13**, 358 (1957).
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[8-Arginine]-Vasopressinoic Acid: An Inhibitor of Rabbit Kidney Adenyl Cyclase

Abstract. *Neurohypophyseal hormones and several synthetic analogs stimulate adenyl cyclase prepared from rabbit kidney medullary tissue. [8-Arginine]-vasopressinoic acid inhibits the stimulation of medullary adenyl cyclase by neurohypophyseal peptides but does not influence the action of parathyroid hormone on adenyl cyclase from kidney cortex.*

[8-Arginine]-vasopressin (AVP), the natural antidiuretic hormone of man and most other mammals, stimulates kidney adenyl cyclase, an enzyme responsible for the synthesis of adenosine 3′,5′-monophosphate (cyclic AMP) (*1, 2*) which serves as an intracellular mediator of the action of many hormones. Although a large number of analogs of neurohypophyseal hormones have been synthesized and tested for their antidiuretic activity (*3*), few of these compounds antagonize the antidiuretic action of vasopressin on mammalian kidney (*4*). This is the first report of an antagonist that inhibits the moiety of adenyl cyclase activity which is stimulated by hormone but that does not affect the basal activity of this enzyme.

While studying the action of AVP and some of its congeners on rabbit kidney adenyl cyclase, we found that [8-arginine]-vasopressinoic acid (AVP-acid) (*5*), an analog of AVP in which the carboxamide group of the terminal glycinamide has been replaced by a carboxyl group, inhibits markedly the stimulation of adenyl cyclase by neurohypophyseal peptides.

Medullary and cortical tissues were dissected from rabbit kidney and centrifuged at 600*g*. The sediments were prepared by methods described for the