208. A Preliminary Note on the DNA Replication Pattern of Sex Chromosomes of the Rat

By Nobuo Takagi and Sajiro Makino


Notwithstanding that the rat has long been a favorite experimental animal in both biological and medical fields, the karyotype of this animal has remained rather unsatisfactory, particularly on the sex chromosomes. Recent studies of DNA replication patterns based on tritiated thymidine uptake and autoradiography have facilitated the identification of chromosomes and have shown in several mammals that a single X chromosome in female cells and the Y chromosome are late replicating. The present study was undertaken in an attempt to characterize the sex chromosomes of the rat, using current techniques.

Materials and methods: Cultured lung cells from male and female newborn rats of the Wistar-King A strain were admitted to incorporate H3--thymidine (specific activity 2.3 c/mM; the Radiochemical Centre, Amersham, England) for the final 3 or 4 hours of incubation. Slides were prepared following a schedule of colchicine-treatment, waterpretreatment, glacial acetic acid-methanol (1:3) fixation and air-drying. Modified basic fuchsin staining method after Carr and Walker (1961) was adopted. After taking photographs of suitable metaphase cells, slides were coated with Sakura NRM--2 emulsion* and development was made with Kodak D-19, or Sakura Konidol-X. Moderately labeled metaphase cells were re-photographed. The photographs taken first were used as the guide in karyotyping the autoradiographs of the same cells (Fig. 1). Karyotypes were analysed according to the system of Hungerford and Nowell (1963).

Observations: The amount of grains associated with the metaphase chromosomes was highly variable. There were many mitotic cells which showed a little or no label, while some displayed heavy labeling of all chromosomes. Approximately 50 per cent of female metaphase cells examined showed that one chromosome was relatively late labeling in its entirety (Fig. 2). The late-replicating chromosome is characterized by a remarkable short arm and nearly corresponds in length to the satellited chromosome 3: it was undoubtedly identified as the X chromosome, referring to the results

* Generously supplied by Konishiroku Photo, Ind. Co. Ltd., Tokyo, through Mr. Akira Hirata. Aided by a grant from Ministry of Education for the cooperative Research (Cancer), No. 956105, 1965.
of karyotype analysis in untreated cells and to the information obtained in other mammals. Grain counts in 19 adequate metaphase cells revealed that the late-replicating X incorporated approximately twice as much H3–thymidine as the other one.

In contrast to female cells, male cells showed no late replicating X, but there was a single prominently late-replicating chromosome invariably occurring in male cells. This particular chromosome was of the smallest one amongst 15 acrocentric chromosomes and in most cases was heteropycnotic. On account of this invariable late-replicating pattern, the small element was therefore taken as the Y chromosome.

Like the situation obtained in man and other mammals, the termination process of DNA replication in the rat was not synchronous among individual chromosomes as well as along the chromosomes. Detailed analysis of late-replicating patterns in chromosomes may be essential for the characterization of chromosomes in general.

Comments: Following the discovery that one of the X chromosomes of female cells of Chinese hamster is late-replicating by means of autoradiography (Taylor 1960), a similar feature has been offered in several species of mammals including man (Morishima et al. 1962, Gilbert et al. 1962, German 1962, 1964, Schmid 1963, Moorhead and Defendi 1963, Kikuchi and Sandberg 1964, and others), domestic cows (Mukherjee and Shinha 1964, Gartler and Burt 1964, Evans 1965), mules (Mukherjee and Shinha 1964), Syrian hamsters (Galton and Holt 1964), dogs (Fraccaro et al. 1965), cats (Hsu and
Bearden 1965), mice (Galton and Holt 1965, Evans et al. 1965), zebra-donkey hybrids (Benirschke et al. 1964), goats (Evans 1965) and swines (Evans 1965). Further, the Y chromosome was reported to be late labeling in most mammals.

The present experiment has demonstrated rather consistently the characteristic late-replicating X and Y chromosomes in the rat. The X chromosome identified here is one of acrocentric chromosomes, having the prominent small short arm which is in all probability of the Xat-type described by Hungerford and Nowell (1963), and corresponding in length to the chromosome 3 without heterochromatic tendency. The Y chromosome is the smallest of all acrocentric elements, ranking between no. 18 and 19 in length and heteropycnotic in nature.

Studies by Ohno and Makino (1961) and Ohno et al. (1959) revealed that the sex chromatin of mammalian female cells was of a single X-chromosome origin. Further extensive autoradiographic studies in human subjects have shown that the single sex-chromatin forming X corresponds to the late-replicating one (Morishima et al. 1962, etc.).

Though the late-replicating X is detectable by means of careful autoradiography, it is not very prominent. In other words, termination of the replication in the late-replicating X is not late enough from the other chromosomes as has been recognized in other mammalian females. Similar situation was reported to occur in the mouse X chromosome by several recent authors (Hsu and Witt cited from Hsu et al. 1964, Evans et al. 1965).

Detailed accounts involving those of autosomes will be published elsewhere in the near future in reference to current data.

References

No. 10] DNA Replication Pattern of Sex Chromosomes of Rat