HETEROCHROMATIN AND LATE REPLICATION
IN THE CHROMOSOMES OF THE RAT*)

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The distribution of heterochromatin in the chromosomes of the rat was
determined by analysing two of its properties: late replication and differential
stain with the DNA d-r method. The presence of late and non-late
replicating c-heterochromatin in the genome of Rattus norvegicus indicates
that this chromatin is an heterogeneous substance exhibiting different
properties. Furthermore, the existence of heterochromatin formed by non-
repeated sequences or by sequences with a low degree of repetitiveness is
suggested by the presence of late replicating areas which do not react with
the DNA d-r method.

Introduction

It has been recently demonstrated that constitutive (c) heterochromatin is formed by highly repeated DNA sequences which stain
differentially when chromosome preparations are treated with DNA
denaturation-renaturation (DNA d-r) techniques (YUNIS & YASMIN,
1971; ARRIGHI & HSU, 1971). Furthermore, it is generally held
that late replication is a property common to c and facultative (f)
heterochromatin (LIMA-DE-FARIA & JAWORSKA, 1968). Thus, it is
possible to predict that late replication and differential stain will
coincide in all those chromosome areas formed by c-heterochromatin.
In this report we have tested this prediction in the chromosome
complement of Rattus norvegicus. The results presented show that the
aforementioned properties do not always coincide and may appear
independently.

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Material and Methods

One male and one female *Rattus norvegicus* weighing approximately 100 gr. were each injected intraperitoneally with 250 μC of 3HTdR (Sp. Act. 6.9 C/mM) and with 0.2 mg. of colchicine. Three hours later the animals were sacrificed and chromosome spreads from bone marrow tissues were prepared. Half of the slides were treated with the DNA d-r technique for c-heterochromatin identification (ARRIGHI & HSU, 1971). The other slides were stained with carbol fuchsin and employed for autoradiography. Exposure time of autoradiograms was 90 days. Previous experiments have shown that partially labelled metaphases obtained from animals treated with 3HTdR three hours before sacrificing are representatives of late replication (BIANCHI, 1966; BIANCHI & BIANCHI, 1966). Therefore, the analysis was limited to 60 metaphases (30 from each animal) which showed half or less than half of the complement labelled. Chromosome identification was performed by matching the autoradiogram with its corresponding unlabelled karyotype.

Results

Late replicating areas in this report were in most pairs coincident with those described previously for rat chromosomes (BIANCHI, 1966; BIANCHI & BIANCHI, 1966; TAKAGI & MAKINO, 1966), they were: the proximal half of the long arm in pair 1, the median region of the arm in pair 2, the median region of the long arm in pair 3, the short arm and kinetochore regions of pairs 3 and 11–13. In the group B (pairs 4–10) most chromosomes showed late replicating areas in the proximal half of the arm; however, a pair tentatively identified as 6 constantly showed late DNA synthesis in the distal half of the arm having the kinetochore free from radioactivity. Pair 14 showed late labelling in the long arm and kinetochore area, in pair 15 the out-of-phase DNA synthesis was limited to the long arm with the kinetochore free from silver grains, pairs 17–19 exhibited labelling over both arms, pairs 16 and 20 had no late replicating areas and were consistently unlabelled when cells had less than half of the complement labelled (Fig. 1). The Y chromosome exhibited a very high silver grain concentration and was one of the last chromosomes in the set to end replication. On the