Replication pattern of double minutes derived from an insect cell line[1]

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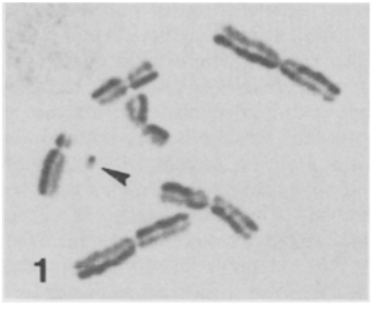
Summary. The DNA replication pattern of double minutes derived from an established cell line of *Aedes albopictus* is described. Although the vast majority of double minutes replicate semiconservatively once during the S phase, some double minutes appear to exhibit different pattern(s). Two theories are suggested as possible explanations of our findings.
Key words. Aedes albopictus; DNA replication; mosquito; double minutes; chromosomal staining; cell line, insect.

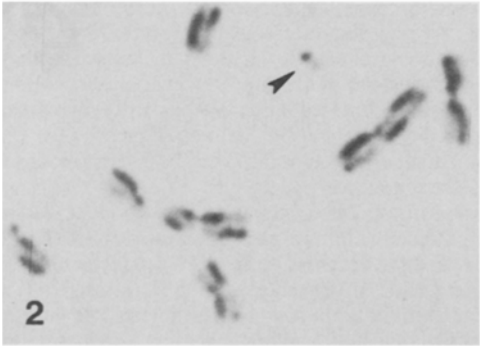
At metaphase, double minutes (DMs) appear as small, often spherical paired chromatin structures[2]. They are found in a wide variety of tumors in man and experimental animals[3]. Recently, we have shown that DMs are not limited to mammalian species but can be present in tumorigenic insect cells as well[4]. With specific cytochemical stains[5,6] and BrdU uptake[5,7], it has been shown that DMs contain DNA and that they replicate once during the early S phase of the cell cycle[8]. When cells containing DMs are cultured in presence of BrdU for two cell cycles, the DMs exhibit harlequin staining, typical of the semiconservative pattern of DNA replication. The purpose of this investigation was to determine whether DMs derived from insects, a group phylogenetically remote from mammals, maintain the same basic pattern of DNA replication as is found in mammalian species[7,8].

Materials and methods. Cells from an established cell line of the mosquito, *Aedes albopictus* (C6/36)[9] were grown at 28 °C in MEM (minimal essential medium) supplemented with 10% fetal bovine serum (GIBCO) and without an antibiotic. Asynchronous log-phase cells were cultured in media containg 20–80 μM BrdU for two replication cycles and subsequently harvested for metaphase chromosome preparations[10]. Control cultures were treated the same way except for the exposure to BrdU. The slides were then rinsed in distilled water before staining in Hoechst 33258 (0.5 μg/ml dH_2O) for 20 min. Following a second wash in distilled water, the preparations were wet-mounted in McIlvaine's buffer (pH 8), sealed with nail polish, exposed to black light and stained in 2% Giemsa solution. A minimum of 50 metaphases from each set of preparations was examined for differential staining, either directly under the microscope or by photomicrography.

Results and discussion. The diploid chromosome number of *A. albopictus* is 6. However, the chromosomal constitution of this clonal line (C6/36) is 90% diploid and 10% heteroploid with various degrees of chromosomal abnormalities. About 4.2% of all metaphases examined contained both chromosomes and double minutes. The frequency of DMs/metaphase ranged from 1 to 3. Figures 1 and 2 show metaphases stained for harlequin chromosomes after two replication cycles in presence of BrdU. Both chromosomes and DMs have one darker and one lighter chromatid. This is the typical staining shown by the semiconservative DNA replication pattern. This study, therefore, proves that the DMs derived from an insect cell line replicate their DNA semiconservatively once during the S phase of the cell cycle, just as has already been reported for a human carcinoma cell line[8]. None of the metaphases prepared from control cultures exhibited differential staining of sister chromatids, whereas 100% of the metaphases contained harlequin chromosomes after two replication cycles in BrdU. No single minutes were observed in any of the metaphases examined.

Rarely, some metaphases showed typical harlequin staining of chromatids in all the chromosomes, and the DMs appeared without differential staining, so that both chromatids exhibited dark staining (fig. 3). Two theories might be suggested to explain this staining pattern of the DM. The first is that some DMs may replicate their DNA much earlier than other DMs in the S phase and, therefore, might escape incorporation of BrdU during the first cycle. If this is true, the up-take of BrdU by the DM in the second cycle alone would not produce harlequin staining. Therefore, the DNA replication pattern of all DMs derived from this insect cell line might not be synchronous, although up-take is synchronous in mammals[7]. The sec-





Figures 1 and 2. Metaphase spreads from the cell line, *Aedes albopictus* (C6/36) exhibiting harlequin staining of both chromosomes and double minutes (arrows).

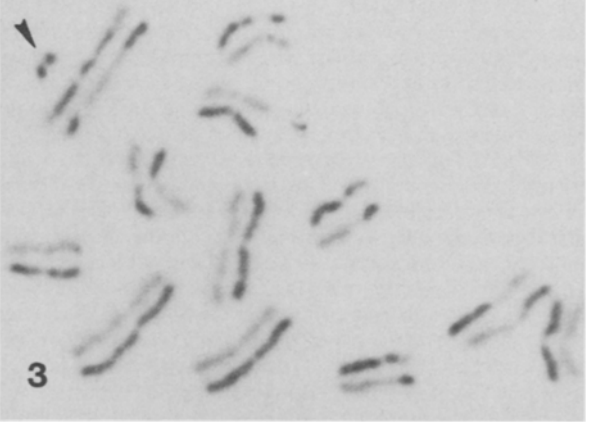


Figure 3. Metaphase spread from the cell line, *Aedes albopictus* (C6/36) exhibiting harlequin staining of chromosomes but no differential staining of the double minute (arrow).

ond theory is that, in the presence of BrdU, some DMs may not replicate their DNA at all or replicate only once during the two cell cycles. For harlequin chromatin staining to appear, BrdU must be incorporated in the DNA of DMs for two consecutive cycles. Consequently, this research indicates for the first time that the DM-DNA may have two modes of replication. Usually, it may replicate once during S phase of the cell cycle concurrently with chromosomal DNA. Sometimes, however, it might escape replication during S phase, while chromosomal DNA does replicate. The differential DNA replication patterns of DMs might produce uneven numbers of DMs in subsequent cell generations and might cause numerical heterogeneity of DMs in a cell population undergoing anomalous mitotic segregation.

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Glucagon and pancreatic polypeptide immunoreactivities co-exist in a population of rat islet cells

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Summary. In mammalian pancreas, glucagon and pancreatic polypeptide have been shown to be present in distinct cell types. The present communication reports that, in rat pancreas, in addition to glucagon and pancreatic polypeptide cell populations, there is a small population of cells which contain both glucagon and pancreatic polypeptide immunoreactivities.
Key words. Co-existence of glucagon; pancreatic polypeptide.

Immunocytochemical studies have shown that, in mammals and many other vertebrates, the four major pancreatic hormones, insulin, glucagon, somatostatin and pancreatic polypeptide, are located in four distinct cell types[1–3]. We reported earlier that, in frog pancreas, glucagon and pancreatic polypeptide overlap in distribution, i.e. a majority of glucagon containing cells also contain pancreatic polypeptide[4,5]. The present communication reports that in one mammalian species, rat, there is also a small degree of overlap in cellular distribution of glucagon and pancreatic polypeptide. In the rat pancreas, in addition to glucagon containing cells and pancreatic polypeptide containing cells, there is another population of cells which contain both glucagon and pancreatic polypeptide immunoreactivities. This observation suggests that glucagon and pancreatic polypeptide may be phylogenetically and biochemically closely related.

Materials and methods. Pancreases from five normal Sprague-Dawley adult rats were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer and embedded in Epon 812. Plastic sections of 1–1.5 μm were collected on glass slides. Before immunohistochemical staining, the plastic sections were deplasticized with alcoholic sodium hydroxide solution according to Lane and Europa[6]. The nature of the hormonal content of the pancreatic islet cells was demonstrated by staining the deplasticized section of pancreas with rabbit antiserum to porcine glucagon[7] or rabbit anti-serum to bovine pancreatic polypeptide[8] by the preroxidase anti-peroxidase (PAP) technique of Sternberger[9]. The anti-glucagon serum was used at 1:2000 dilution and the anti-pancreatic polypeptide serum was used at 1:5000 dilution. Specificity of each antiserum was demonstrated by staining adjacent sections with the antiserum absorbed with homologous or heterologous antigen (100 μg/ml diluted antiserum) prior to staining.

The distribution of glucagon and pancreatic polypeptide immunoreactivities in pancreas were studied by the following two experiments: (1) Two adjacent plastic sections were deplasticized and stained for glucagon and pancreatic polypeptide, respectively. The staining patterns of islets of these two adjacent sections were photographed and compared. (2) A plastic section was first stained with glucagon antiserum. The positively stained cells were visualized using 4-chloro-1-naphthol as the reducing agent in the PAP method. The staining pattern of islets was photographed. The same section was then treated with alcohol and $KMnO_4/H_2SO_4$ solution according to the method of Tramu et al.[10] to remove the reaction product of 4-chloro-1-naphthol and tissue-bound antibodies. The section was then restained with a second antibody, anti-bovine pancreatic polypeptide by the PAP method. The stained islets were again photographed. The staining patterns of the same islet in the two staining sequences were compared. To test the effectiveness of the antibody removal procedure in this experiment, a control section was run simultaneously with the experimental section. The control section was stained for glucagon identically as for the experimental section during the first staining sequence. After being subjected to procedure for removal of the bound antibodies, this section was stained with normal rabbit serum instead of the anti-pancreatic polypeptide serum in the second staining sequence. The control section was negatively stained after the second staining sequence of PAP procedure, indicating complete removal of bound antibodies of first staining sequence.

Results and discussion. Comparison of adjacent sections, respectively stained with anti-glucagon and anti-pancreatic polypeptide sera, and sequential staining of a same section with the two antisera showed that a small number of islet cells are immunoreactive to both antisera in addition to sole glucagon immunoreactive cells and pancreatic polypeptide immunoreactive cells.

Figure 1 shows part of an islet in two adjacent sections stained respectively with antiglucagon (fig. 1A) and antipancreatic