AN AFRICAN GREEN MONKEY CELL LINE RAMT RESISTANT SIMULTANEOUSLY TO 8-AZAGUANINE, 6-MERCAPTOPURINE, AND 6-THIOGUANINE

V. I. Stobetskii, V. P. Grachev,
L. L. Mironova, and V. G. Chernikov

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One of the most widely used biochemical selective schemes for hybridization of somatic cells is that of Littlefield, based on the use of cells deficient in thymidine kinase (TK^-) and hypoxanthine phosphoribosyltransferase ($HPRT^-$). In this case, THAG medium, containing thymidine, hypoxanthine, aminopterin (which blocks endogenous synthesis of DNA precursors), and glycine, is used to select hybrid cells. Death of the original TK^- and $HPRT^-$ cells takes place because of their inability to utilize exogenous thymidine and hypoxanthine, and growth of the hybrid cells is due to genetic complementation [4].

Under certain experimental conditions cells of only one of the partners taken for hybridization carry either the TK^- or the $HPRT^-$ mutation. In that case, normal cells growing in suspension, most frequently blood leukocytes, are used as the other partner for hybridization, and are removed when the medium is changed.

In view of the relative simplicity of the hybridization scheme in this last case, several rodent cell lines carrying the TK^- or $HPRT^-$ mutation, the defect of which is expressed as resistance to the thymidine analog 5-bromodeoxyuridine or the guanine analogs 8-azaguanine (8-AG) and 6-thioguanine (6-TG), and also the hypoxanthine analog 6-mercaptopurine (6-MP), have now been obtained and described in the literature.

It is also known that resistance to one of the hypoxanthine and guanine analogs is not always accompanied by cross resistance to another [6], and sometimes in the case of resistance to 8-AG, the HPRT defect is absent [7]. Furthermore, some transplantable cell lines with HPRT deficiency, such as mouse RAG cells, are contaminated by mycoplasmas [5].

The object of this investigation was to obtain a line of African Green monkey cells deficient in hypoxanthine utilization, i.e., with the $HPRT^-$ mutation, and correspondingly resistant to analogs of hypoxanthine and guanine.

EXPERIMENTAL METHOD

The original cell line was 4647, obtained by subculture of a primary culture of adult African Green monkey kidney cells, followed by spontaneous transformation. The 4647 cells were cultured in rectangular flasks 0.5 liter in volume in Eagle's medium with 10% bovine serum. The 4647 cells could not be cloned initially.

Cells of the 4647 line were seeded in a dilution of 1:3 in Eagle's medium containing 8-AG, 6-MP, and 6-TG, each in a concentration of 10 μg/ml, for 24 h. After this the flask was washed and filled with medium not containing toxic analogs. The colonies of cells thus formed multiplied and the experiment was repeated. In the process of selection, the cells were not treated with mutagens. After frequent repetition of the procedure as described, long-lasting resistance (up to 10 days) of the cells could be achieved to medium containing 8-AG, 6-MP, and 6-TG, but the cells died after 20 days. A colony of cells actively dividing in selective medium was found in one of the flasks 19 months after the beginning of the experiments, and this colony gave rise to the RAMT line.

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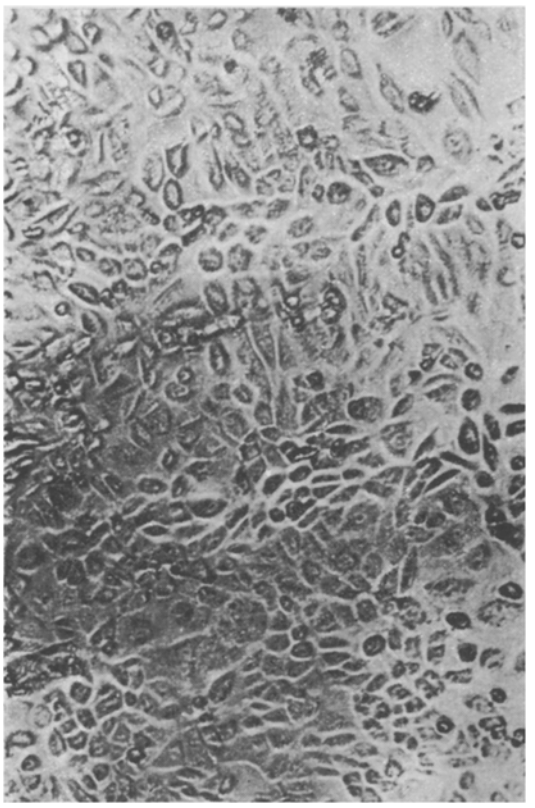

Fig. 1. Culture of African Green monkey RAMT cells. Monolayer of RAMT cells. Living culture. Photographed in transmitted light, 40×.

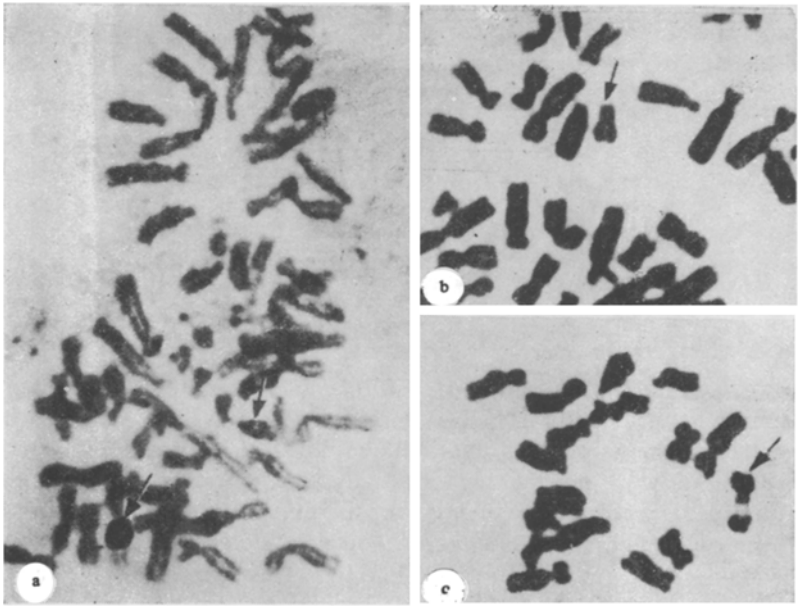

Fig. 2. Nucleolus-forming chromosomes of RAMT cells: a) metaphase plate: stained with silver nitrate; b, c) segments of metaphases: stained with azure-eosin. 770×.

EXPERIMENTAL RESULTS

Cells of the RAMT line are epithelioid in shape, both when cultured in Eagle's medium with 8-AG, 6-MP, and 6-TG, and also when cultured in their absence (Fig. 1). When the cells were seeded in a dilution of 1:3 on THAG medium made up according to Littlefield's formula (thymidine 12.5 μg/ml, hypoxanthine 13.6 μg/ml, aminopterin 0.18 μg/ml, glycine 7.5 μg/ml), they died en masse on the 7th-10th day, and the frequency of revertants after culture for 1 month under nonselective conditions was 1×10^{-6} to 2×10^{-6}. The cloning efficiency of the RAMT cells when seeded on glass petri dishes 10 cm in diameter was about 26%, but when seeded on USSR-manufactured plastic petri dishes 4 cm in diameter it was 10 times less. Cells of the RAMT line grew well on Eagle's medium with 30 μg/ml of 6-MP. On Eagle's medium with 1 μM ouabain, cells of the RAMT line did not multiply but died en masse on the 4th-6th days.

In cytological preparations stained with azure-eosin after fixation in Carnoy's mixture, round granules of different sizes could often be seen in the cytoplasm of the RAMT cells. These formations did not exhibit metachromasia after staining with azure A and showed marked eosinophilia. They also stained well with silver nitrate, evidently because of their protein nature. When stained with the fluorescent dye Hoechst 33258, which specifically binds with DNA and is used for testing for mycoplasmas [3], no inclusions were found in the cytoplasm, and it can accordingly be concluded that cells of the RAMT line were not contaminated by these microorganisms.

Analysis of 100 metaphases at the level of the 3rd subculture showed that 40% of cells had 57-58 chromosomes in their set, and about 20% of cells were tetraploid.

The normal karyotype of the African Green monkey is known to contain only two chromosomes with nucleolar organizer regions (NOR), which can be detected by silver impregnation. During routine staining these regions consisted of well-marked secondary constrictions, with a block of condensed chromatin in their center [1]. On silver impregnation with AgI [2] a considerable difference was found in the degree of silver impregnation of the NOR in the chromosomes of RAMT cells (Fig. 2a). One chromsome with typical morphology had very small point NOR, not always staining with silver. In the second chromosome this region was characterized by a large area of precipitated silver, and on ordinary staining, by a large secondary constriction. In addition, the short arm of this chromosome had an additional segment (Fig. 2b, c). These distinguishing features of the nucleolus-forming chromosomes are clear markers serving to distinguish cells of the RAMT line from other transplantable African green monkey cell lines.

The properties of cells of the RAMT line described above thus permit their use in somatic cell hybridization.

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