A SENSITIVE METHOD OF DETECTING 5-BROMODEOXYURIDINE INCORPORATED DIFFERENTIALLY INTO MAMMALIAN CHROMOSOMES

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Methods of revealing the 5-bromodeoxyuridine (BUdR) label in chromosomes in current use [3, 6-8] allow working with a concentration of BUdR in the culture medium of not less than 3-5 μg/ml. If the BUdR concentration falls to 2-3 μg/ml the reliability of its detection in chromosomes is reduced [5, 9].

A procedure of differential staining of chromosomes by which BUdR labeling can be reliably detected in chromosomes during cell culture in medium with a reduced concentration of the agent is described in this paper. The method offered is a modification of fluorochrome staining of chromosomes with acridine orange used previously [3, 5].

Experiments were carried out on chromosome preparations obtained from a culture of Chinese hamster cells (line 237) and a human peripheral blood culture. Standard methods of cell culture and of obtaining chromosome preparations were used. BUdR (from Calbiochem, USA) was added to the nutrient medium in different concentrations 5-7 h or 24-48 h before fixation of the cells. The presence of BUdR in the chromosomes was determined by testing for longitudinal differentiation (5-7-h labeling) or for differential staining of sister chromatids in mitoses which have gone through more than one replication cycle (24-48-h labeling). In the latter case, the quality of differential staining was evaluated by analysis of the distribution of mitoses according to the degree of differences in staining of sister chromatids, and with determination of four types of these differences: 1) very marked differences, 2) satisfactorily expressed differences, adequate for analysis of sister chromatid exchange (SCE), 3) indistinct differences, making detection of all SCE difficult, 4) no differences. The composition of the dye used was: 100 ml tap water, 1 g sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$), 1.5 ml 10% NaOH solution, and 1.4-1.8 ml of a 0.1% aqueous soltuion of acridine orange (from Serva, West Germany). The original time for staining the preparations was 15-20 min, after which each preparation was mounted under a coverslip without rinsing in a thin layer of dye. Airtight fitting of the coverslip was ensured with resin glue. For different series of preparations (depending on the times and conditions of their keeping, and other factors) either the concentration of acridine orange in the staining solution or the duration of staining had to be changed. With optimal staining, a predominantly orange or orange-red fluorescence of the cell nuclei and chromosomes is observed, with clear differentiation by color and brightness of sister chromatids differing in their BUdR content. A predominantly red fluorescence indicates overstaining of the preparation, yellowish green indicates understaining. The use of this proposed staining method was based on the results of previous experiments with fluorochrome staining of chromosome preparations [1, 2] showing that an alkaline medium increases the contrast of differences between regions of chromosomes with different BUdR content, and that the presence of small quantities of fluorochrome in the mounting medium increases the light resistance of the dye. The concentrations of acridine orange mentioned above only very slightly increase the background "illumination" of the microscopic image, but on the other hand they enable the period of fluorescence of the object to be increased to 15-20 min. Under these conditions photomicrography can be carried out successfully with the use of fine-grain low-sensitivity film of the "Mikrat-200" type, which satisfies the conditions for photographic recording of the differential stain described above as regards its spectral properties and gamma. The metaphase plate of the Chinese hamster, with differential staining of sister chromatids, can be studied after culture of the cells in medium with 0.1 μg/ml BUdR.

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TABLE 1. Percentage Distribution of Mitoses by Quality of Differential Staining (types 1-4) Depending on BUdR Concentration in Culture Medium, Using Different Staining Methods

Method	BUdR concentration, μg/ml	Type			
		1	2	3	4
Latt [4]	10	29	59	9	3
	2	11	42	38	9
	0,5	2	37	52	9
	0,2	0	13	44	33
Kato [6]	10	0	54	34	12
	2	4	42	47	7
	0,5	0	20	59	21
	0,2	0	13	33	54
Proposed modification	10	90	2	0	8
	2	93	3	0	4
	0,5	87	8	1	4
	0,2	5	56	38	11

Legend. In each case 100 metaphases were analyzed.

With such a low concentration of reagent the characteristic side effect of BUdR, namely curving of the chromosomes (usually the chromatid with the higher BUdR content is located on the outer side of the curve), is absent. The results of comparative analysis of the quality of differential staining of chromosomes by different methods during culture of the cells with different concentrations of BUdR are given in Table 1. Data in the table show that with a decrease in the BUdR concentration the percentage distribution of the cells as a whole is shifted toward type 3, or even type 4, i.e., more and more of the metaphases become unsuitable for SCE analysis. If the applicability of the method is restricted by the presence of not more than 50% of differentially stained metaphases in type 3, the threshold BUdR concentration for methods described by Latt and Kato [4-6] will be about 2 μg/ml, whereas for the method which we now describe it is under 0.2 μg/ml. It has been shown in the case of human chromosomes that sufficiently clear differentiation of sister chromatids is observed if the BUdR concentration falls to 0.2-0.5 μg/ml.

By the method described it is possible to use much smaller quantities of BUdR than are customarily used, and the method may also find application in the study of the kinetics of cell populations during long periods of time, the study of the fine structural-functional organization of chromosomes, determination of frequencies of spontaneous SCE, and in some investigations of mutagenesis. The method can also be used to detect BUdR label.

LITERATURE CITED

1. V. A. Benyush, Byull. Éksp. Biol. Med., No. 9, 122 (1973).
2. V. A. Benyush and L. P. Tupitsyna, Byull. Éksp. Biol. Med., No. 4, 108 (1975).
3. B. Dutrillaux, A. M. Fosse, M. Prieur, et al., Chromosoma, 48, 327 (1974).
4. S. A. Latt, Proc. Natl. Acad. Sci. USA, 70, 3395 (1973).
5. S. A. Latt, Proc. Natl. Acad. Sci. USA, 71, 3162 (1974).
6. H. Kato, Nature, 251, 70 (1974).
7. J. R. Korenberg and E. F. Freedlender, Chromosoma, 48, 355 (1974).
8. P. Perry and S. Wolff, Nature, 251, 156 (1974).
9. R. Tice, E. L. Schneider, and J. M. Rary, Exp. Cell Res., 102, 232 (1976).