Hum Genet (1981) 57:93–95

Human Genetics

Short Communications

High Resolution R- and G-Banding on the Same Preparation

Bernard Dutrillaux[1,2] and Evani Viegas-Pequignot[2]

[1] Commissariat à l'Energie Atomique, Institut de Protection et de Sûreté Nucléaire, Département de Protection, Services de Pathologie et de Protection Sanitaire, B.P.n°6, F-92260 Fontenay-Aux-Roses, France
[2] Institut de Progenèse, 15, rue de l'Ecole de Médecine, F-75270 Paris Cedex 06, France

Summary. A simple method using bromodeoxyuridine (BrdU), for both cell synchronization and incorporation into replicating DNA is described. Many prophasic and prometaphasic mitoses were observed, and due to the probable blocking at different times of the cell cycle, very good R-banding and G-banding were obtained simultaneously on the same preparation.

Introduction

Since 1975 many cell synchronization techniques have been developed using thymidine (Dutrillaux 1975a; Viegas-Péquignot and Dutrillaux 1978) or amethopterin (Yunis 1976; Pai and Thomas 1980; Camargo and Cervenka 1980) to obtain R- or G-banded prophasic and prometaphasic chromosomes. We report on a technique using bromodeoxyuridine (BrdU) to synchronize cell divisions, and obtain excellent R-banding, G-banding, and H-banding on different cells of the same preparation, and at various stages of chromosome condensation. Compared with the method previously published, using thymidine and BrdU (Dutrillaux 1975a), a higher ratio of prophasic mitoses, with about 1,000 bands per haploid set of chromosomes is more easily obtained.

Material and Methods

After three days of growth of peripheral lymphocytes, BrdU is added at a final concentration of 200 µg per ml, for approximately 15–17 h (overnight). Then the cells are washed twice with TC 199, and reincubated with complete medium (TC 199 + human serum + 0.3 µg thymidine per ml) for 6–7 h. The rest of the technique is the same as the usual technique (Dutrillaux 1975b), except that several rinses in fixative improve cell spreading.

The following stains may be used: (a) acridine orange staining (Couturier et al. 1973): 20 min in 0.05 mg per ml of phosphate buffer pH 6.5, followed by rinsing and mounting in buffer. (b) Giemsa after acridine orange staining: the mitoses, observed in UV light, are treated in Earle's BSS pH 6.5 at 87°C for 10–60 s and stained with Giemsa (Dutrillaux and Viegas-Péquignot 1979). (c) Giemsa after Hoechst staining, modified from the method of Perry and Wolff (1974) 15 min in Hoechst 33258 at a concentration of 1 µg per ml of SSC × 2, buffer or water. The slides, mounted in SSC × 2 are exposed for 30 to 90 min under a HBO 200 mercury vapour lamp, at a distance of approximately 30 cm. The slides are rinsed and stained for 7 min in 1.5% Giemsa. Exposure to a bactericidal UV lamp for 24 h may also be used. Without other treatment good banding is observed. However, for a better contrast, it may be necessary to treat before staining: (a) with SSC × 2 for 1 h at 60°C, for better G-banding; (b) with Earle's BSS for 5–20 s at 87°C, for better R-banding. Several successive treatments and stainings may be carried out to obtain more details on certain chromosome segments.

Offprint requests to: B. Dutrillaux (address see above)

Results

A high percentage of mitotic cells is found without using colchicine. The cells are at various stages of chromosome contraction, but prophases and prometaphases are predominant. G-banded cells (Fig. 1) are the most numerous, around 70–80% of mitoses. R-banded cells (Fig. 2) represent 10–30% of mitoses. Among them the percentage of metaphases is relatively higher than for G-banded cells. H-banded cells represent the rest of the mitoses.

The percentages of these different bandings vary according to the duration of culture after removing BrdU, 6–7 h being an efficient time. The quality of the banding is about equivalent using acridine orange or Giemsa staining. However, the latter is obviously more convenient.

Discussion

Among the different synchronizing agents, thymidine and amethopterin are the most widely used for cytogenetic purposes. In previous work we prefered the former because it is less clastogenic. However, synchronization using high doses of thymidine makes it difficult to obtain G-banding, after BrdU incorporation. When BrdU is used during the last 6–7 h, all banded metaphases show R-banding, which can be explained by the incorporation of BrdU into late replicating segments (G-bands + heterochromatin). It indicates also that the thymidine block occurs mostly in the middle of the S phase (Viegas-Péquignot and Dutrillaux 1978).

To obtain BrdU incorporation into early replicating bands (R-bands), the thymidine block must be used at the preceding cell cycle, since BrdU and thymidine are competitors; and then synchronization is not very good. Another possibility is to use amethopterin and BrdU together, but the mixture appears to be relatively toxic, and we could not obtain good quality mitoses. Therfore, we tried to use BrdU itself as the synchronizing agent.

0340-6717/81/0057/0093/$01.00

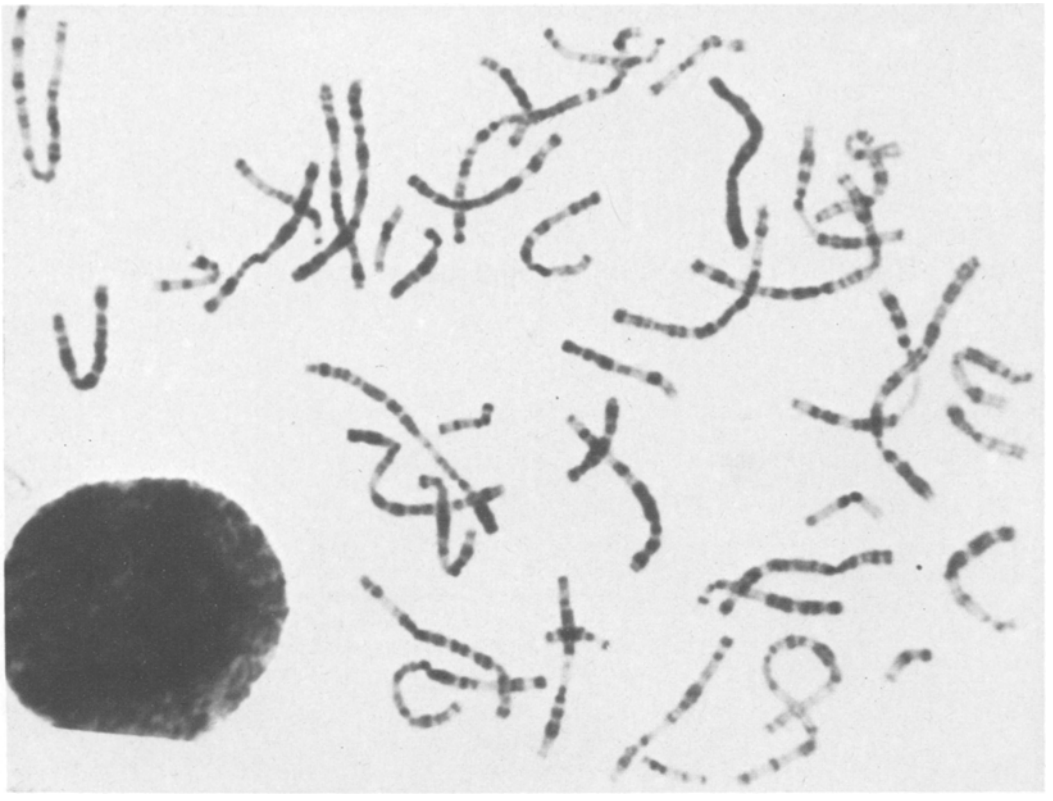

Fig. 1. Partial G-banded prometaphase, obtained simultaneously to the R-banded one of Fig. 2

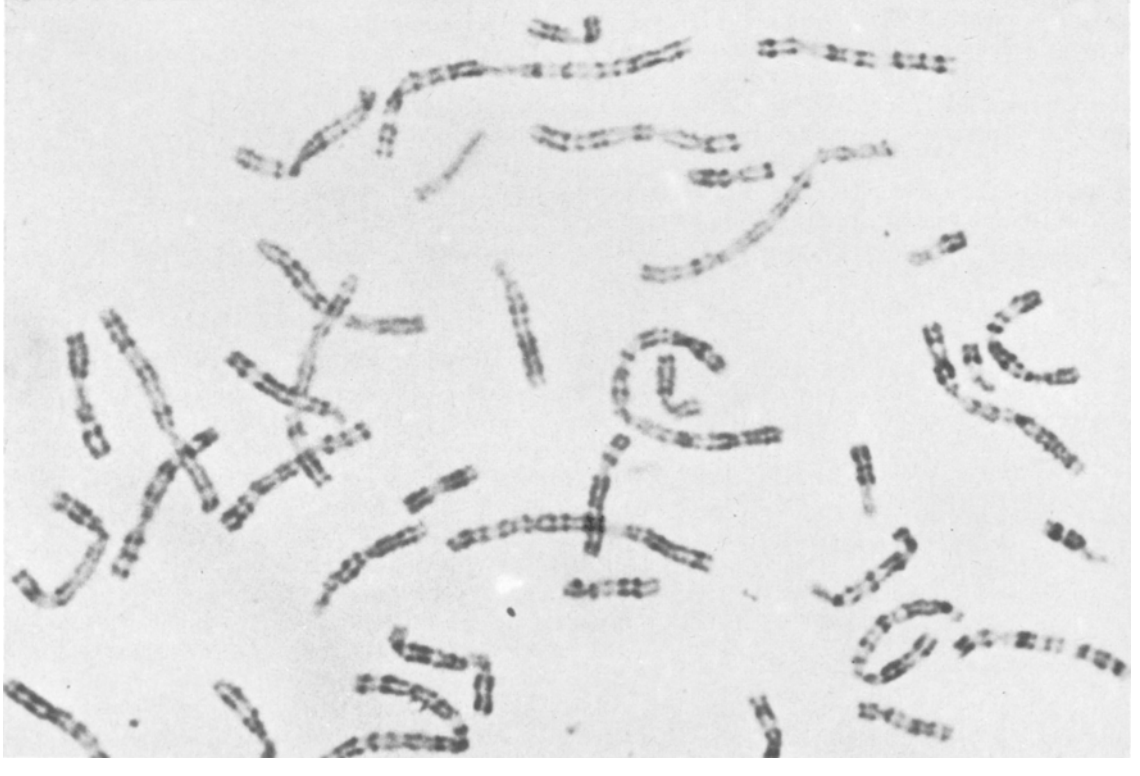

Fig. 2. Partial R-banded prometaphase

At a final dose of 200 µg per ml, it appears to block the cell cycle at the middle of the S phase. Then it is incorporated at the beginning of the S phase (into R-bands) and very good G-banding is obtained, if BrdU is eliminated at the end of the culture. The mechanism of BrdU block is still unknown, but it is assumed that similar to the mechanism of thymidine block, it inhibits the synthesis of deoxycytidine (Xeros 1962; Meuth and Green 1974; Schempp et al. 1978).

Surprisingly, a relatively high percentage of the mitoses, between 10 to 30%, show a typical R-banding. This indicates that the BrdU-induced block is not complete, or operates at different moments. The fact that typical R-, G-, and H-bandings are observed, and not intermediary bandings, like those obtained after BrdU pulses at low concentrations (Dutrillaux 1975c) favours the hypothesis that several blocks exist. One would take place at the middle of S phase, after DNA replication in R-bands; one at the end of S phase or in G_2; and another more difficult to demonstrate would take place after G-band replication but before H-band replication. In contrast, no block seems to occur during the replication of these structures. This may reflect the existence of different enzymatic reactions acting separately at the different periods of replication, the excess of BrdU, or of thymidine preventing the beginning of these reactions. A more precise study of these blocks is in progress.

The Hoechst-Giemsa stain is a real improvement since it may be completed by gradual treatments, by SSC × 2, or Earle's BSS to obtain a better definition of G- or R-bands. A correct handling of the method, by varying UV irradiation and heat treatments permits one to focus specifically on a certain chromosome, or chromosome segment, depending on its richness in G- or R-bands.

Acknowledgements. This research was supported by C.N.R.S. (E.R.A. no. 47) and by C.E.A. (Contract no. BC 1862).

References

Camargo M, Cervenka J (1980) Pattern of chromosomal replication in synchronized lymphocytes. I. Evaluation and application of methotrexate block. Hum Genet 54:47–53

Couturier J, Dutrillaux B, Lejeune J (1973) Etude des fluorescences spécifiques des bandes R et des bandes Q des chromosomes humains. CR Acad Sci [D] (Paris) 276:339–342

Dutrillaux B (1975a) Obtention simultanée de plusieurs marquages chromosomiques sur les mêmes préparations, après traitement par le BrdU. Hum Genet 30:297–306

Dutrillaux B (1975b) Sur la nature et l'origine des chromosomes humains. Monographie des Annales de Génétique. L'expansion Scientifique, Paris

Dutrillaux B (1975c) Traitement discontinus par les BrdU et coloration par l'acridine orange: obtention de marquages R, Q et intermediaires. Chromosoma 52:261–273

Dutrillaux B, Viegas-Péquignot E (1979) Thymidine synchronization and chromosome labeling. In: Routine use of chromosome analysis in radiation protection. Proc IAEA Meeting, 1978 IAEA-TEC DOC, 224, p 123

Meuth M, Green H (1974) Induction of a deoxycytidineless stade in cultured mammalian cells by bromodeoxyuridine. Cell 2:109–112

Pai GS, Thomas GH (1980) A new R-banding technique in clinical cytogenetics. Hum Genet 54:41–45

Perry P, Wolff S (1974) New Giemsa method for the differential staining of sister chromatids. Nature 251:156–158

Schempp N, Sigwarth I, Vogel W (1978) Demonstration of replication patterns corresponding to G- and R-type banding of chromosomes after partial synchronization of cell cultures with BrdU or dT surplus. Hum Genet 45:199–202

Viegas-Péquignot E, Dutrillaux B (1978) Une méthode simple pour obtenir des prophases et des prométaphases. Ann Genet (Paris) 21:122–125

Xeros N (1962) Deoxyriboside control and synchronization of mitosis. Nature 194:682–683

Yunis JJ (1976) High resolution of human chromosomes. Science 191: 1268–1270

Received August 19, 1980

Note Added in Proof

After an extensive use of the technique, it appears that an intermediary banding (showing earliest replicating R-bands and latest replicating Q-bands) is observed in some cultures. A short analysis of the banding pathern of chromosomes 21 and 22 immediately indicates the type of the banding of the whole cell.