# BrdU RELATED DIRECT REVELATION OF TYPICAL DYNAMIC R- AND G-BANDING WITH THE USE OF MONOCLONAL ANTI-BrdU ANTIBODY

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SAVARY J.B., DAUDIGNON A., VASSEUR F., DEMINATTI M.M. — BrdU related direct revelation of typical dynamic R- and G-banding with the use of monoclonal anti-BrdU antibody.

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SUMMARY : Pulse 5-bromodeoxyuridine (5-BrdU) incorporation during the last S-phase is known to produce R- or G-banded chromosomes after photolysis-plus-Giemsa (FPG) staining. The authors applied an immunological staining with monoclonal anti-BrdU antibody instead of the FPG protocol. The results offered banded chromosomes with an immunological typical R-banding (RBI) on the GBG cultivated cells (early pulse incorporation), and an immunological G-banding (GBI) on the RBG cultivated ones (late pulse incorporation). After a further FPG protocol following an immunological treatment, an inverted banding pattern became evident whereas a faint immunological staining remained. Thus the method superimposed a GBG-banding on the RBI-staining or a RBG on the GBI one. This allows a rapid and easy R and G double chromosomal identification on the same metaphase cell, using first the immunological banding then the classical FPG staining. The method allows a reproducible dynamic G-banding with an easy monitored late 5-BrdU pulse incorporation specially attractive

*(Summary continued on next page)*

SAVARY J.B., DAUDIGNON A., VASSEUR F., DEMINATTI M.M. — Révélation directe des banding dynamiques typiques R et G, obtenus par incorporation de la 5-BrdU, au moyen d'un anticorps monoclonal anti-BrdU. *(En Anglais).*
**Ann Génét,** 1992, **35,** n° 1, 27-32.

RÉSUMÉ : L'incorporation de 5-bromodéoxyuridine (5-BrdU) au cours de la dernière phase S produit un marquage chromosomique en bandes R ou G après coloration photolysis-plus-Giemsa (FPG). Les auteurs ont remplacé la coloration FPG par une méthode immunologique qui utilise un anticorps monoclonal anti-5-BrdU. Les résultats ont montré un banding chromosomique R typique (RBI) sur les cellules cultivées selon un protocole d'incorporation précoce qui produit normalement un marquage G (GBG), et un banding chromosomique G typique (GBI) sur les cellules cultivées selon le protocole d'obtention d'un marquage RBG (incorporation en fin de phase S). Sur des chromosomes présentant un banding immunologique un traitement FPG fait apparaître un banding en contretype (marquage GBG sur le marquage RBI et inversement, RBG sur GBI), il subsiste toutefois un faible marquage immunologique. Cette technique permet l'obtention rapide et facile, d'une double identification chromosomique R et G sur la même métaphase, à condition que le marquage immunologique précède la co-

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*(Summary continued)*

in spontaneous dividing cells from bone marrow. This dynamic G-banding protocol should be extended to chorionic villi and malignant cells. Our data are in agreement with a connection between dynamic banding and chromosomal portions containing or not BrdU. The lack of an immunological staining after the FPG protocol has been noticed and assume the photolysis degradation-elution of the DNA in BrdU-substituted areas.

KEY-WORDS : Chromosome. — Dynamic banding. — BrdU. — Monoclonal antibody.

*(Suite et fin du résumé)*

loration FPG. Cette méthode permet un banding dynamique G à partir d'un protocole facile à maîtriser : l'incorporation de 5-BrdU en fin de culture. Elle est particulièrement intéressante dans le marquage dynamique G des tissus présentant des mitoses spontanées comme le trophoblaste et les tissus tumoraux. Nos résultats confirment la relation entre le banding dynamique et les régions chromosomiques substituées ou non par la 5-BrdU. L'impossibilité d'obtention d'un marquage immunologique après un traitement FPG, est un argument en faveur de la dégradation et l'élution du DNA par photolyse dans les portions substituées par la 5-BrdU.

MOTS-CLÉS : Chromosome. — Banding dynamique. — BrdU. — Anticorps monoclonal.

## INTRODUCTION

The incorporation of 5-bromodeoxyuridine (5-BrdU) into chromosomal DNA is well known (Hsu and Somers, 1961 ; Zakharov and Egolina, 1972). BrdU-Giemsa methods were quickly used in cytogenetic analysis, specially for sister chromatid exchanges (SCE) studies on metaphase chromosomes with the photolysis-plus-Giemsa (FPG) method (Perry and Wolff, 1974). Dynamic G (GBG) and R-banding (RBG) using early or late pulse 5-BrdU incorporation during part of the last S-phase before harvesting, have been likewise achieved in human postnatal diagnosis (Dutrillaux and Couturier, 1981 ; Lemieux et al., 1987 ; Lemieux et al., 1990) and prenatal diagnosis (Qu et al., 1989 ; Savary et al., 1991). The development of immunological methods using specific antibody raised against 5-BrdU, to label the replicated DNA in single cells and chromosomes, has also been used (Gratzner et al., 1975). In addition, the antibody was used to detect sister chromatid exchanges (Gratzner et al., 1975 ; Pinkel et al., 1985 ; Natarajan et al., 1986 ; Shiraishi and Ohtsuki, 1987), and the mechanism of differential Giemsa staining of BrdU-substituted chromosomes was extensively studied (Buys and Stienstra, 1980 ; Latt, 1981 ; Buys et al., 1982 ; Speit, 1984). Previous reports showed that the sister chromatid differentiation (SCD) was related with the degradation and elution of BrdU-substituted DNA (Ockey, 1980 ; Webber et al., 1981). Our immunological approach is in agreement with such a mechanism for the FPG disclosure of dynamic banding. Bromodeoxyuridine incorporation and immunological detection has been applied for the demonstration of replication patterns in mammalian chromosomes (Vogel et al., 1986 ; Latos-Bielenska et al., 1987).

In this paper we report results of typical R (RBI) and G (GBI) banding of human metaphase chromosomes, after early or late 5-BrdU pulse incorporation and immunological detection. The comparison between the RBI and the classical RBG banding, or between the GBI and the classical GBG banding showed a complete likeness. A further FPG protocol on immunological stained chromosomes superimposed a reversed banding pattern : GBG on the RBI or RBG on the GBI. This allows a rapid and easy double R and G chromosomal identification on the same metaphase cell, and corroborates that classical dynamic bandings are in connection with specific chromosomal areas containing or not BrdU-substituted DNA. The monitoring of the RBG cultivation protocol (late 5-BrdU pulse labelling) is easier than the GBG one (early pulse labelling). The immunological disclosure of late pulse labelled cells allows a more reproducible dynamic G-banding than the classical GBG protocol. This latter may produce heterogeneous banding patterns related with the various velocity of dividing from one cell to another. In addition, based upon a simple late pulse incorporation, the method may be extended to various tissues, specially those presenting spontaneous mitosis (i.e. : bone marrow – chorionic villi – malignant tissues) although the timing of cell cycle remains often unknown.

## MATERIAL AND METHODS

Peripheral blood lymphocytes and bone marrow cells were cultivated in RPMI 1640 medium supplemented with 20 % fetal calf serum and antibiotics. Early or late pulse 5-BrdU incorporation during part of the last S-phase were as previously described (Savary et al., 1991).

### RBG and GBG banding

Giemsa staining preparations were obtained with an adaptation of the FPG procedure (Perry and Wolff, 1974), as previously described (Savary et al., 1991).

### Immunological banding (RBI, GBI)

The antibodies were diluted in 0.1 M Tris HCl pH 7.4 – 0.1 % Tween 20 – 0.5 % non fat dry milk (buffer 1). The washes were performed with 0.1 M Tris HCl pH 7.4 – 0.1 % Tween 20 (buffer 2) until otherwise stated. Chromosomes preparations from 5-BrdU pulse labelled cells were processed as follow : (1) DNA denaturation with 30 % NaOH 0.07N – 70 % ethanol for 3 min according to Vögel et al. (1986), (2) washing in 95 % ethanol for 5 min, (3) rehydratation in a 70 %-50 % ethanol serie then in 0.1 M. Tris HCl pH 7.4 for 4 min, (4) 90 min incubation at 37 °C in a moist chamber with mouse monoclonal anti-BrdU antibody (Caltag laboratories, San Francisco, CA) diluted 1 : 300 with buffer 1, (5) washing twice in buffer 2, (6) inhibition of endogenous peroxydase at room temperature for 20 min with 0.015 % $H_2O_2$ in methanol, (7) washing twice in buffer 2, (8) 30 min incubation at room temperature in a moist chamber with goat antimouse IgG horseradish peroxydase conjugate (Caltag laboratories, San Francisco, CA) diluted 1 : 100 with buffer 1, (9) washing twice in buffer 2 and once in 0.1 M Tris HCl pH 7.4, (10) staining as described in Graham and Karnovsky (1966), for 3-10 min with 3-3' diaminobenzidine (DAB) solution (Sigma) : 40 mg DAB – 100 ml 0.1 M Tris HCl pH 7.4 – 250 µl 30 % $H_2O_2$, (11) washing in distilled water.

## RESULTS AND DISCUSSION

In lymphocytes, the late 5-BrdU pulse incorporation before harvesting gives, as a rule, a dynamic R-banding (RBG) with the FPG staining but produced a typical G-banding with the immunological staining (GBI). Likewise, the early pulse 5-BrdU incorporation leading to a GBG-banding with the FPG staining, produced typical R-banded chromosomes (RBI) with the immunological procedure. The reversed staining was also achieved when the cells were labelled with 5-BrdU for two rounds of replication and exhibited sister chromatid differentiation (data not shown). The dynamic banding using a monoclonal antibody raised against 5-BrdU point out that the 5-BrdU specific sites into the substituted DNA were not masked and remained accessible to an immunological detection.

Both combined RBG and RBI haploid karyotypes (fig. 1), GBG and GBI haploid karyotypes (fig. 2) exhibited a strict likeness between dynamic banding achieved with the FPG protocol (left) and the immunological method (right) for the global appearance, the detail and the number of bands. The R-immunological banding (RBI) and the G-immunological banding (GBI) patterns were in agreement with the International Classification (ISCN, 1985). When a dynamic FPG-banding was compared with the same immunological one no difference was noticed between euchromatic segments or between heterochromatic portions. Short and long arms of chromosomes exhibited the typical banding pattern with the immunological staining as compared with the FPG-staining. Late replicating segments were as faintly stained with the RBI as with the RBG method and darkly GBI or GBG stained. It was somewhat difficult to distinguish FPG-banding from its immunological counterpart and the protocol appeared as sensitive as the FPG method to reveal chromosomal banding.

Applied on the same metaphase cell, a further FPG protocol following the immunological procedure produced a reversed pattern (fig. 3, fig. 4) in relation to the previous GBI- or RBI-banding. Nevertheless the FPG treatment did not quite removed the immunological staining, and the FPG-banding was superimposed on the immunological banding. This was quite evident with a late pulse incorporation for the late replicating X chromosome. This latter which is heavily BrdU substituted appeared faint with FPG alone (RBG classical X label) and dark with the immunological disclosure alone (GBI X label), but remained slightly stained after a FPG procedure following an immunological staining (fig. 4). Indeed a slight immunological GBI background remained on the inactive X chromosome. Thus in spite of the FPG treatment this X chromosome appeared darker than its classical faint RBG pattern. Antibody elution experiments failed to prevent this immunological background. Only harsh treatments were efficient but damaged the chromosomes and prevented a suitable subsequent identification.

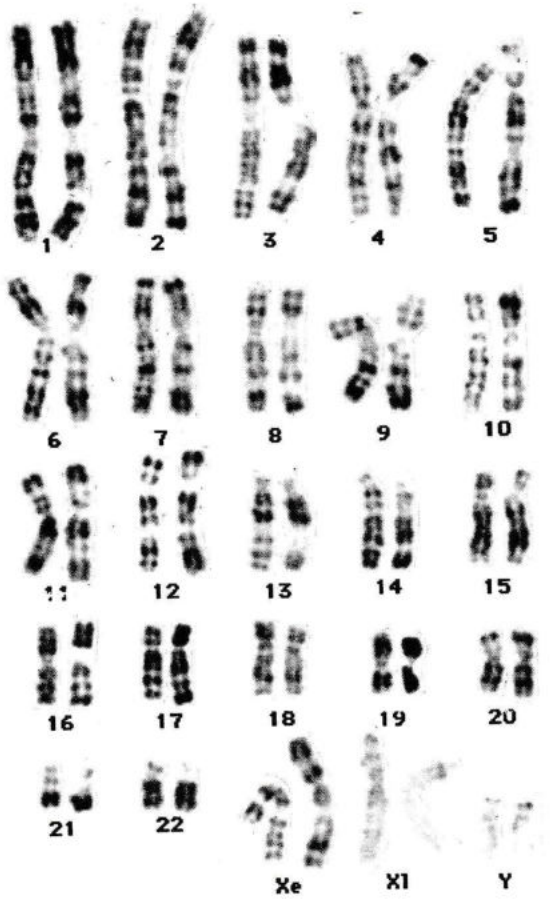


**Fig. 1. — Combined haploid karyotypes from lymphocytes. A complete likeness is noticed between the R-banding patterns (RBG vs RBI) obtained with the FPG procedure (left) and the immunological procedure (right). Xe : early replicating X. XI : late replicating X.**

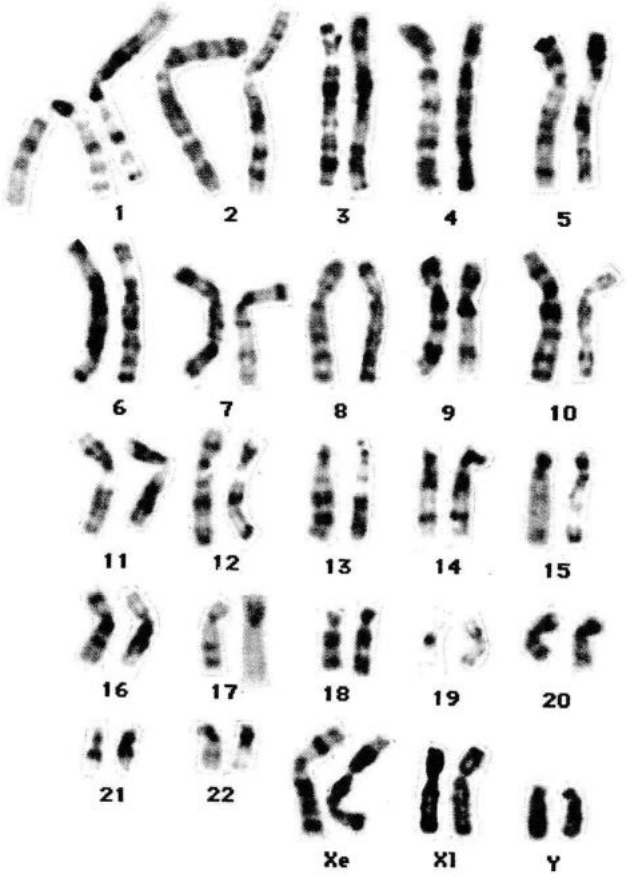


**Fig. 2. — Combined haploid karyotypes from lymphocytes. A complete likeness is noticed between the G-banding patterns (GBG vs GBI) obtained with the FPG procedure (left) and the immunological procedure (right). Xe : early replicating X. XI : late replicating X.**

However this did not disturb a double rapid and easy R and G chromosomal identification on the same metaphase cell with both immunological and FPG staining.

Dynamic GBG-banding requires early pulse 5-BrdU incorporation during the last S-phase before harvesting. Thus it is necessary to have a good knowledge of the cell cycle timing as in the case of stimulated lymphocytes. However the G-banding pattern remains heterogeneous because of the variability in the speed of division from one lymphocyte to another. In opposite late labelling during the last hours of the cultivation allows a better control of the pulse and produces highly reproducible RBG banded chromosomes. Applied on bone marrow cells the immunological staining following a RBG cultivation protocol (late pulse 5-BrdU incorporation) gave a good G-banding appearance (GBI). A further FPG staining always leaded to a RBG pattern on the same chromosomes (fig. 5). The comparison between spontaneous mitoses (bone marrow cells) and stimulated lymphocytes corroborates that dynamic banding either with FPG or immunological disclosure are influenced by the quality of chromosome preparations. This reproducible dynamic double G and R chromosomal identification should be applied to various tissues with spontaneous dividing cells, where both the monitoring of cell division is difficult to perform and the timing of cell cycle is unknown (i.e. chorionic villi cells, malignant cells). Moreover dynamic investigations should be attractive methods in the analysis of chromosomal replication of normal and malignant tissues.

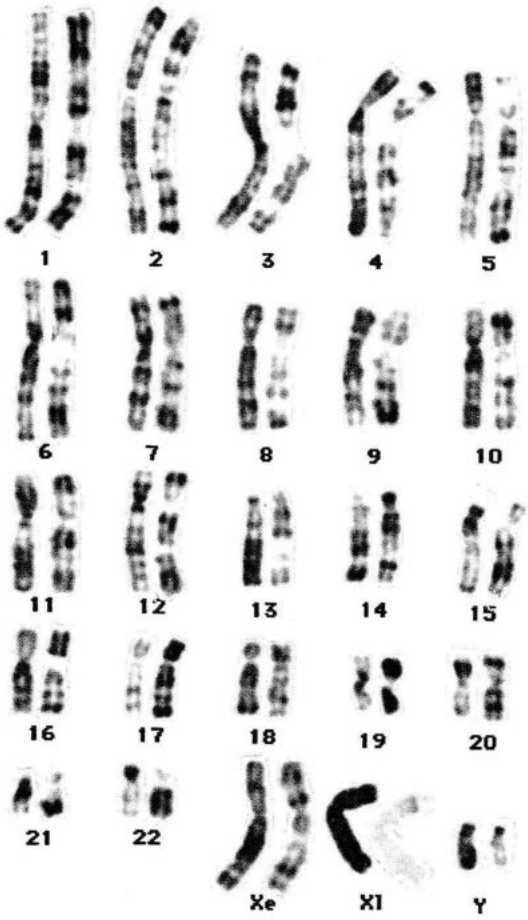


Fig. 3. — Chromosomal dynamic banding obtained after an early pulse 5-BrdU incorporation on lymphocytes. FPG staining (left) produces a GBG banding, immunological staining (right) produces a R banded appearance (RBI) on the same chromosomes. Xe : early replicating X. XI : late replicating X.

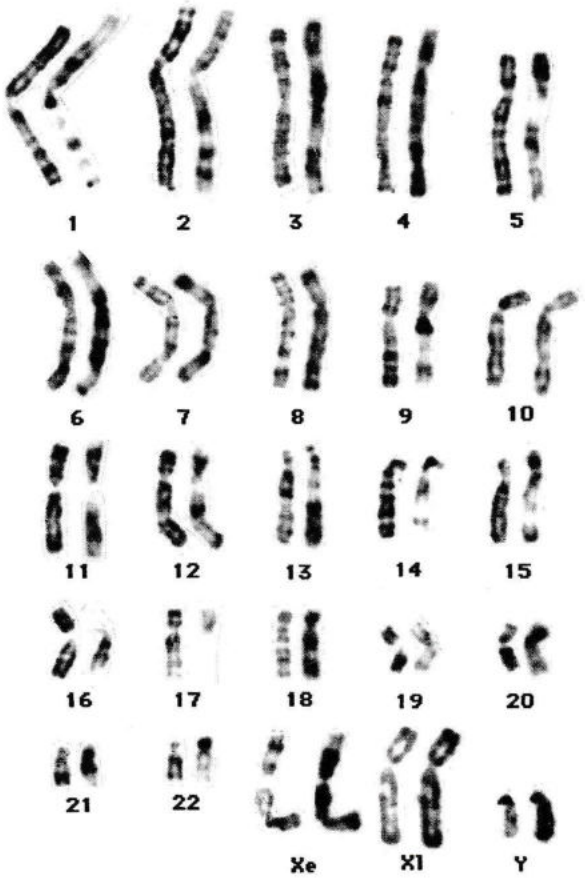


Fig. 4. — Chromosomal dynamic banding obtained after a pulse late 5-BrdU incorporation on lymphocytes (last 7 hours before harvesting). The FPG staining (left) produces an RBG banding, and the immunological staining (right) produces a G banded appearance (GBI) on the same chromosomes. The RBG late replicating X chromosome (XI) is darker than usually because of a background of the previous immunological staining (see text). Xe : early replicating X.

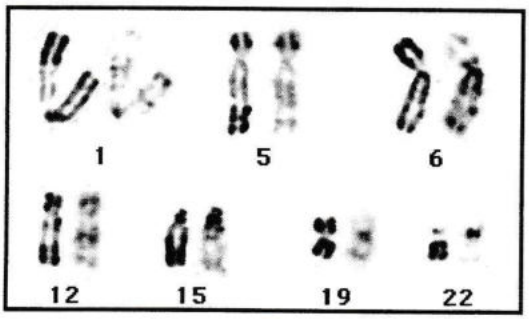


Fig. 5. — Partial haploid karyotype of a bone marrow cell following a late pulse 5-BrdU incorporation. The immunological staining produced a G (GBI) banding (right). A further FPG treatment leaded to a R (RBG) pattern on the same chromosomes (left).

The immunological staining needs neither photolysis nor harsh treatment as in the FPG protocol. Moreover, it is a direct approach of the BrdU substituted DNA : immunological stained areas are BrdU containing. Conversely, the FPG protocol remains an indirect disclosure of the BrdU substitution because the FPG stained portions are BrdU free. The double reversed G and R label on the same preparation, required the immunological procedure before the FPG protocol. After an immunological banding the FPG staining was always obtained. After the disclosure of GBG or RBG banding with the classical FPG (Hoechst 33258 + UV irradiation), the immunological staining was never noticed and the chromosomes remained faint. Following Hoechst 33258 staining without UV

irradiation, immunological banding was obtained. An UV irradiation without Hoechst 33258 staining prevented RBI or GBI banding and even the immunological staining of the chromosomes. There are good evidences in the literature (Ockey, 1980 ; Webber et al., 1981 ; Speit, 1984) indicating that the degradation and elution of BrdU-substituted UV photosensitive areas are responsible for the contrast in sister chromatid differentiation. Thus BrdU-substituted areas are faintly stained with a nucleic acid stain as Giemsa. Such a mechanism may be involved for the dynamic banding disclosure. As following the FPG procedure and specially UV irradiation the chromosomes remained uniformly faint with anti-BrdU antibody staining, our immunological results point out a prime importance part of the UV-irradiation in the FPG protocol specially in the degradation-elution of DNA in BrdU-substituted areas.

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