Involvement of Sulfhydryl Groups of Chromosomal Proteins in Sister Chromatid Differentiation

Charles H.C.M. Buys and Stef Stienstra
Department of Human Genetics, State University of Groningen, The Netherlands

Abstract. The fluorescence of human lymphocyte chromosomes stained with sulfhydryl group-specific fluorochromes is markedly enhanced by a mild near-ultraviolet irradiation pretreatment, indicating breakage of protein disulfide bonds. When metaphase preparations of cells cultured in the presence of BrdU during two cell cycles are irradiated and subsequently stained with the sulfhydryl group-specific fluorescent reagents used in this study, a differential fluorescence of sister chromatids is observed. After staining with the DNA-specific fluorochrome DAPI an opposite pattern of lateral differentiation appears. It can be concluded that the chromatid containing bifilarly BrdU-substituted DNA has a higher content of sulfhydryl groups than the chromatid containing unifilarly BrdU-substituted DNA. This implies a more pronounced effect of breakage of disulfide bonds in the chromatid with the higher degree of BrdU-substitution. BrdU-containing chromosomes pretreated with the mild near-ultraviolet irradiation procedure used by us, do not show any differentiation of sister chromatids after Feulgen staining. Using sulfhydryl group-specific reagents, differential fluorescence of sister chromatids could still be induced by irradiation with near-ultraviolet light after the complete removal of DNA from the chromosomes by incubation with DNase I. Thus, the protein effect of irradiation of BrdU-containing chromosomes takes place independently of what occurs to DNA.

Our results indicate that subsequent to the primary alteration of chromatin structure caused by the incorporation of BrdU into DNA, breakage of disulfide bonds of chromosomal proteins might play an important role in bringing about differential staining of sister chromatids, at least for those procedures that use irradiation as a pretreatment or prolonged illumination during microscopic examination.

Introduction

Culturing cells in the presence of 5-bromodeoxyuridine (BrdU) for two rounds of replication results in chromosomes having one chromatid containing DNA
with BrdU in a single strand, and another containing DNA with both strands substituted (Latt, 1973). A number of procedures leading to differential staining of sister chromatids in metaphases of cells subjected to treatment with this thymidine analogue have in common an exposure to light of short wave length (Perry and Wolff, 1974; Holmquist and Comings, 1975; Sugiyama et al., 1976; Scheid, 1976). Light might produce alterations in the nucleic acid and protein components of chromatin. Both DNA (Latt and Wohlleb, 1975; Goto et al., 1978) and chromosomal proteins (Perry and Wolff, 1974; Ikushima and Wolff, 1974) have been held responsible for differential staining of sister chromatids of BrdU-incorporated chromosomes. Wolff and Bodycote (1977) reported the induction of differential staining of sister chromatids by irradiation with near-ultraviolet light as well as by prolonged incubation with reducing thiol compounds in the dark. They suggested a differential loosening of the chromosomal structure by breakage of protein disulfide bonds as an underlying mechanism of sister chromatid differentiation after incorporation of BrdU. Using sulfhydryl group-specific fluorochromes we have attempted to test their hypothesis. Here we communicate a direct demonstration of the involvement of chromosomal proteins by means of the sulfhydryl groups of their cysteine residues in sister chromatid differentiation under the influence of near-ultraviolet radiation without the complicating presence of photosensitive dyes.

**Materials and Methods**

*Cell Culture and Chromosome Preparation Methods.* Cells were cultured from heparinized whole blood (20% v/v) in RPMI 1640 medium (Gibco, Glasgow, Scotland, U.K.) supplemented with 2 mM L-glutamine, penicillin (100 U/ml) and streptomycin (100 µg/ml). For lymphocyte stimulation leucoagglutinin (Pharmacia, Uppsala, Sweden) was used in a final concentration of 4 µg/ml. BrdU (Sigma, St. Louis, Missouri, U.S.A.) was added to a final concentration of $10^{-4}$ M. After 66-68 h in a moisturized 5% CO$_2$-incubator cells were arrested in metaphase by addition of vinblastine sulfate (obtained as Velbe from Lilly, Indianapolis, Ind., U.S.A.) to a final concentration of 0.04 µg/ml for 2 h. Cells were harvested by centrifugation and washed in 0.15 M NaCl, 0.01 M K-Na-phosphate buffer, pH 7.35. Hypotonic treatment was carried out in 0.060 M KCl at 37°C for 10 min. After centrifugation this solution was replaced by fixative (methanol/acetic acid, 3:1, v/v). The fixative was changed twice for fresh one. Metaphase preparations were made by dropping the fixed cells from a height of 1 m onto slides and subsequent air-drying. All manipulations were carried out under dim light.

*Near-ultraviolet Irradiation Procedure.* Preparations were mounted under coverglass in a 50 mM acetate buffer, pH 3.8, and subjected to a simple irradiation procedure essentially according to Wolff and Bodycote (1977). Preparations were irradiated during 6 min at a distance of 17 cm from an HBO 200 W mercury burner in a Wild lampholder with a quartz collector. A fan prevented heating above 30°C. After irradiation the coverglasses were removed and the chromosome preparations were stained.

*Enzymic Degradation of DNA.* Preparations were incubated with bovine pancreatic deoxyribonuclease (DNase I, EC 3.1.4.5., obtained from Serva) in a concentration of 45 Kunitz units per ml 50 mM Tris/HCl-buffer, pH 7.5, containing 3 mM magnesium chloride.

*Fluorescence Staining Methods.* For staining with N-(3-pyrene)maleimide (Fluka, Buchs, Switzerland) slides were flooded with a 1 mM solution of this sulfhydryl reagent in pyridine, covered with