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Latt S A. Microfluorometric detection of deoxyribonucleic acid replication in human metaphase chromosomes. *Proc. Nat. Acad. Sci. USA* 70:3395-9, 1973.
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This paper describes the first method for fluorometric detection of DNA synthesis as a high-resolution, versatile, and broadly applicable alternative to autoradiography. Incorporation of the DNA base analogue, BrdU, into DNA in place of thymidine is detected by a reduction in the intensity of fluorescence of the bisbenzimidazole dye, 33258 Hoechst, bound to DNA containing this base analogue. Detection, by fluorescence microscopy, of the late-replicating human X chromosome, documentation of the semiconservative distribution of newly replicated DNA between sister chromatids, and simple detection of sister chromatid exchanges (SCEs) was accomplished. [The *SCI*® indicates that this paper has been cited in over 630 publications.]

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My initial research in human genetics utilized fluorescent dyes to study the mechanism of the "banding" of human chromosomes, discovered by Torbjorn Caspersson and associates. I wanted to devise chromosome staining sensitive to DNA replication with a known, deliberately designed specificity and then to apply it to the interpretation of chromosome staining patterns.

I hypothesized that a heavy atom, such as the bromine in BrdU, might quench, via the promotion of intersystem crossing, the fluorescence of a dye bound to DNA near the incorporated BrdU. Since the timing of BrdU administration to cells could be controlled, different incorporation patterns could be produced and detected. The problem was to find the appropriate dye. I tried quinacrine mustard, then used for standard banding, without success. While assembling other candidate dyes, I read a paper by I. Hilwig and A. Gropp[1] on using a blue benzimidazole dye, 33258 Hoechst, to highlight the A-T rich centromeres of mouse chromosomes. H. Loewe, a colleague of Hilwig, kindly provided me with some 33258 Hoechst, as well as related dyes.

I screened dyes by comparing their fluorescence when bound to poly(dA-BrdU) with that when bound to poly(dA-dT) and found that of the bisbenzimidazoles to be the most BrdU-sensitive (nearly 80 percent quenching), with partial quenching of acridine orange and negligible quenching of ethidium or quinacrine. I designed protocols using BrdU and 33258 Hoechst to detect early and late-replicating regions of human lymphocyte chromosomes, as well as the segregation of newly replicated DNA, the latter revealing at striking resolution the sister chromatid exchanges (SCEs) initially described by J. Herbert Taylor, who had used tritiated thymidine autoradiography. We used 3HdT, 3HBrdU, autoradiography, and a microspectrofluorometer to characterize the basis and to quantitate the extent of 33258 fluorescence quenching in cytological preparations of metaphase chromosomes. In the process, we noted an apparent light-exposure-dependent reduction in Giemsa staining that we attributed to complex photochemistry.

We then characterized the dye-DNA interactions, the replication characteristics of human chromosomes, the preferential localization of SCEs in human chromosomes, the lateral asymmetry of dA and dT distribution in mouse centromeres and the human Y, and the ability of SCEs to detect the impact of submicrogram per ml quantities of alkylating agents on human chromosomes. I proposed that this last feature, soon extended to *in vivo* conditions, would serve as a useful means of assessing genetic damage.[2]

By the July 1974 Leiden Chromosome Conference, it became apparent that others, using acridine orange, had empirically developed comparable cytological techniques,[3] while P. Perry and S. Wolff had exploited the "complex photochemistry" referred to above to follow dye staining and light exposure with hot salt treatment and to produce a beautiful negative Giemsa image of the BrdU incorporation.[4] J. German's laboratory[5] had discovered a dramatic increase in SCEs in lymphocytes from patients with Bloom's syndrome.

The very high DNA specificity of 33258 (and 33342) Hoechst fluorescence led to additional uses, in flow cytometry, in which I, Joe Gray at the Lawrence Livermore Laboratory, and Tom Jovin and Donna Jovin did the initial work.[6] While much of the work at a cellular level can be done with greater sensitivity by the anti-BrdU antibody approach pioneered by Howard G. Gratzner,[7] the bisbenzimidazoles remain very useful for metaphase chromosome flow sorting and "cloning." Characterization of the dye-DNA interactions has progressed from our early optical studies to recent, elegant X-ray crystallographic work by Richard E. Dickerson and associates.[8]

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3. **Kato H.** Spontaneous sister chromatid exchanges detected by a BudR-labelling method. *Nature* 251:70-2, 1974. (Cited 260 times.)
4. **Perry P & Wolff S.** New Giemsa method for differential staining of sister chromatids. *Nature* 251:156-8, 1974. (Cited 1,640 times.)
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6. **Rabinovitch P S, Kubbies M, Chen Y C, Schindler D & Hoehn H.** BrdU-Hoechst flow cytometry: a unique tool for quantitative cell cycle analysis. *Exp. Cell Res.* 174:309-18, 1988.
7. **Gratzner H G, Pollack A, Ingram D J & Leif R C.** Deoxyribonucleic acid replication in single cells and chromosomes by immunologic techniques. *J. Histochem. Cytochem.* 24:34-9, 1976. (Cited 30 times.)
8. **Pjura P E, Grzeskowiak K & Dickerson R E.** Binding of Hoechst 33258 to the minor groove of B-DNA. *J. Mol. Biol.* 197:257-71, 1987.

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