This Week's Citation Classic®

Wolff S & Perry P. Differential Giemsa staining of sister chromatids and the study of sister chromatid exchanges without autoradiography. *Chromosoma* 48:341-53, 1974. [MRC. Clinical and Population Cytogenetics Unit, Western General Hosp., Edinburgh, Scotland and Lab. Radiobiology and Dept. Anatomy, Univ. California, San Francisco, CA]

Chinese hamster ovary cells grown for two rounds of replication in the presence of bromodeoxyuridine contain chromosomes with sister chromatids that fluoresce differentially when stained with Hoechst 33258. If the fluorescent treatment is followed by Giemsa staining, permanent preparations are made in which sister chromatid exchanges (SCEs) can be seen with great clarity. [The *SCI*® indicates that this paper has been cited in over 420 publications, making it the most-cited paper from this journal.]

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In 1974 Paul Perry and I[1] realized that we could combine S.A. Latt's[2] fluorescent staining technique of chromosomes containing bromodeoxyuridine (BrdUrd) with the Giemsa technique of T. Ikushima and me[3] to produce permanent preparations of differentially stained chromosomes in which it was possible to see sister chromatid exchanges (SCEs) with great precision and clarity. This technique could be used to approach several problems regarding SCE formation and chromosome structure that were controversial because autoradiographic studies, which had hitherto been carried out in this area, lacked the appropriate resolution to provide results that were reproducible in all laboratories.

With the new technique, we were quickly able to show that the number of SCEs was dependent upon the incorporated nucleoside necessary to allow their detection, which we had already postulated to be the case for SCEs determined autoradiographically after the incorporation of tritiated thymidine.[4] By interpolating the results obtained with low concentrations of BrdUrd to those expected at zero concentration, we were able to determine the true spontaneous level of SCE formation in Chinese hamster ovary (CHO) cells. Further, we found that the isolabeling observed in autoradiographic preparations, which had led to the idea that there had to be more than one DNA double helix in a chromosome, was really the result of the formation of multiple SCEs within a very small region and the concomitant production of activated silver grains in the emulsion over both chromatids, which indicated that isolabeling was an artifact of autoradiography.

In endoreduplicated cells, it was shown incontrovertibly that the two outer chromatids of the closely apposed four sister chromatids were lightly stained and that the two inner chromatids were darkly stained. That is, the outer two chromatids were bifilarly substituted with BrdUrd and the two inner ones were unifilarly substituted. Such a result could be obtained only if the newly synthesized polynucleotide strand of a DNA double helix always segregated to the outside of the chromosome, clearly confirming a result that had been noticed in autoradiographic preparations but never given full credence.

In this paper it was also shown that one could distinguish cells that had replicated three times in the presence of BrdUrd from those that had replicated only once or twice. In chromosomes that had replicated once, both sister chromatids were unifilarly substituted and stained darkly. After two rounds of replication with BrdUrd, each chromosome contained one sister chromatid that was unifilarly substituted and one that was bifilarly substituted, and so stained as a harlequin chromosome with one dark and one light chromatid. After three rounds of replication, however, some chromosomes in the cell contained two bifilarly substituted chromatids that stained lightly, whereas others contained harlequin chromosomes. This mixture of chromosome staining in the same cell indicated that at least three rounds of replication had occurred.

An indication of how dramatic these preparations were was brought home forcibly to us by the reaction of the editor of *Chromosoma*. The journal had, and still has, a policy of publishing only original micrographs. When we submitted this manuscript with photographs of harlequin-stained CHO cells, of an endoreduplicated cell, and of a third-division cell in which the chromosomes were dramatically stained, we received an acceptance from the editor with the provision that we supply him with original micrographs. At first I didn't understand the request, but a colleague of mine, upon looking at the photographs, said, "I believe that he thinks you have doctored them with a marking pencil." To assure the editor that this was not the case, we sent him a slide containing synchronized CHO cells in which metaphase after metaphase was stained exactly like the ones in the photographs. By return mail, we received an assurance that the editor never thought we had doctored our photographs, and he requested that we please allow him to keep the microslide we had sent so that he could use it in his demonstration series. He further informed us that he had already sent the paper off to the printer.

I think that this paper has been frequently cited because it showed dramatically how easily obtained cytological preparations could be used to obtain experimental data on a variety of subjects. In fact, the newer techniques drew so many people into the field that in a few short years at least three books were written on SCEs.[5-7]

1. **Perry P & Wolff S.** New Giemsa method for the differential staining of sister chromatids. *Nature* 251:156-8, 1974. (Cited 1,350 times.)
2. **Latt S A.** Microfluorometric detection of deoxyribonucleic acid replication in human metaphase chromosomes. *Proc. Nat. Acad. Sci. USA* 70:3395-9, 1973. (Cited 705 times.)
3. **Ikushima T & Wolff S.** Sister chromatid exchanges induced by light flashes to 5-bromodeoxyuridine- and 5-iododeoxyuridine-substituted Chinese hamster chromosomes. *Exp. Cell Res.* 87:15-9, 1974. (Cited 180 times.)
4. **Wolff S.** Are sister chromatid exchanges sister strand crossovers or radiation-induced exchanges? *Mutat. Res.* 1:337-43, 1964. (Cited 50 times.)
5. ———. *Sister chromatid exchange*. New York: Wiley, 1982. 306 p. (Cited 60 times.)
6. **Sandberg A A.** *Sister chromatid exchange*. New York: Liss, 1982. 706 p.
7. **Tice R R & Hollaender A.** *Sister chromatid exchanges: 25 years of experimental research*. New York: Plenum Press, 1984. 1,033 p.