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## *IN VIVO* SISTER CHROMATID DIFFERENTIATION IN AN AMPHIBIAN MODEL—APPLICATION OF REVERSE BANDING

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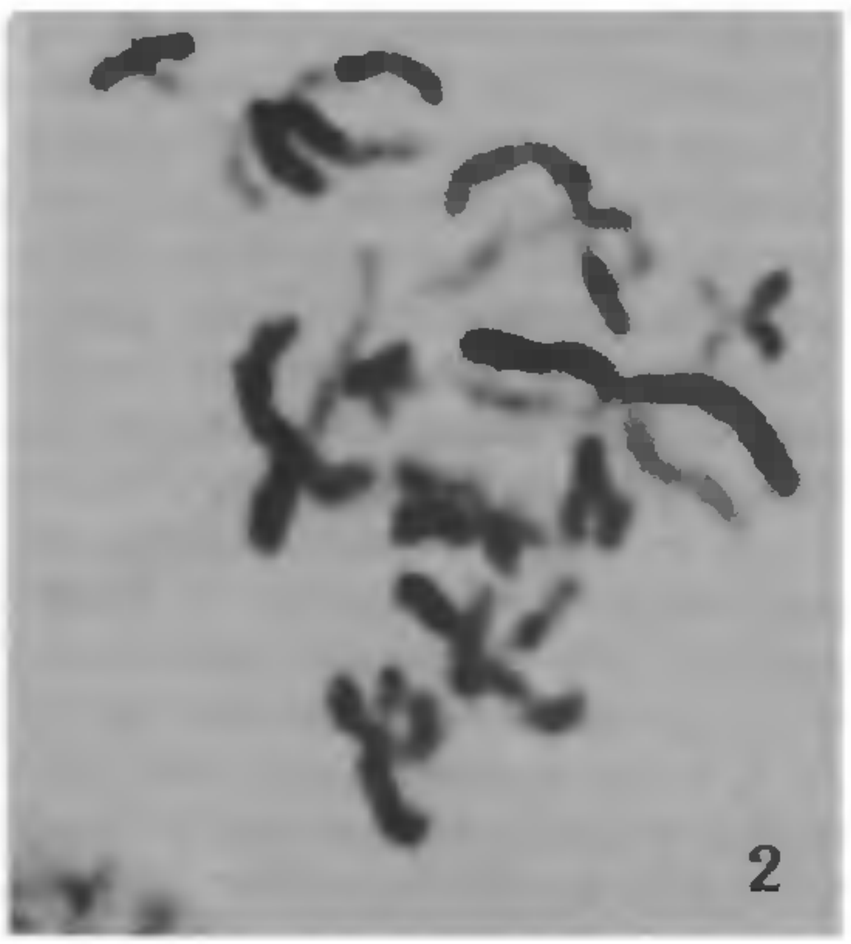
COMBINING 5-bromo-2-deoxyuridine (BUdR) incorporation with Hoechst 33258 staining[1], it is now possible to visualize sister chromatid differentiation (SCD) at the metaphase stage of cell division in which unifilarly BUdR-substituted (TB) chromatid fluoresces more intensely than its bifilarly (BB)-substituted counterpart. The mechanism of this SCD was explained as a result of quenching effect of BUdR on the fluorescence of 33258 Hoechst chromosome complex[2,3]. To avoid technical disadvantages, fluorescence plus Giemsa-stained preparation (FPG) was introduced[4]. This modified technique gives differentially Giemsa-stained chromosomes with darkly-stained TB chromatids and faintly-stained BB chromatids. The mechanism of Giemsa differential staining by FPG technique is based on the differential photolysis of BUdR-substituted DNA. Considering its importance in mutagenicity/carcinogenicity bioassay, various attempts were made to simplify the technique. Takayama and Sakanishi[5] obtained good SCD in the BUdR-incorporated chromosomes using acid extraction technique which showed reverse results in their preparation, viz. the TB chromatids stained faint while the BB chromatids stained dark. The reverse differential staining of sister chromatids has also been reported[6]. Subsequently direct staining of BUdR-substituted chromosomes has been reported[7] in a $Na_2HPO_4$-Giemsa solution without any fluorochrome pretreatment resulting in a reverse, i.e. B-dark type of SCD. The B-dark type SCD was also obtained[8] by immersing BUdR-substituted chromosomes into 4-Na EDTA-Giemsa solution at room temperature. But all these studies were confined to mammalian cell systems. On the other hand, the application of *in viro* SCE by differential staining in amphibian system has extended the horizon of mutagenicity and carcinogenicity study; this has special significance because of their dual habitats—land and water. The successful application of FPG technique in amphibian system was earlier reported[9,10]. Recently, we have succeeded in producing the SCD of a B-dark type in an amphibian specimen by direct staining of *in vivo* BUdR-substituted chromosomes. The use of *in vivo* SCD and SCEs in Amphibia, and its present staining modification will be of value in cytogenetic monitoring of environmental mutagens.

Specimens of *Bufo melanostictus* (Schneider; Anura; Amphibia) of either sex and weighing about 25 g received intraperitoneal injection of BUdR (Sigma, St. Louis, USA) at 100 mg/kg/h and exposed *in vivo* for 15, 20, 25, 30, 35, 40 and 45 h to allow the cells to pass at least two consecutive replication cycles. Metaphase chromosomes were prepared from intestinal epithelial cells by mitotic division inhibition technique[11] already in practice in our laboratory. Three-day-old air-dried slides were used for staining. For reverse banding, air-dried slides were trypsinized in 0.001% trypsin (Difco, Michigan, USA) solution at 30–32°C for 30–40 sec and rinsed briefly in alkaline solution of 0.1 M $Na_2HPO_4$ at pH 9. The slides were then stained in Giemsa stain diluted in 10% alkaline solution (0.1 M $Na_2HPO_4$, pH 9) for 10 min. The staining time, however, was variable depending on the age of the slide, the temperature and the quality of preparation.

The conventionally-stained metaphase complement of *B. melanostictus* consists of 22 biarmed chromosomes (NF = 44) (figure 1). SCD was clearly visible after direct staining of trypsinized air-dried slides in $Na_2HPO_4$-Giemsa solution (figure 2). The frequency of second cycle cells with SCD was highest (~60%)


**Figures 1 and 2.** **1.** Conventional stained somatic metaphase chromosomes of *Bufo melanostictus* showing $2n = 22$ long biarmed chromosomes, and **2.** BUdR-exposed metaphase complements of *B. melanostictus* showing SCD.

at 35 h (figure 3). Darkly stained chromatids were always in a condensed state than lightly stained ones. Maximum accumulation of second cycle cells was recorded at 35 h of *in vivo* BUdR exposure and therefore the sample at 35 h was analysed. Several factors seem to affect the production of SCD: the quality of air-dried preparation, the age of the slide, temperature, etc. The chromosomes show a fuzzy appearance with no sign of SCD in freshly prepared

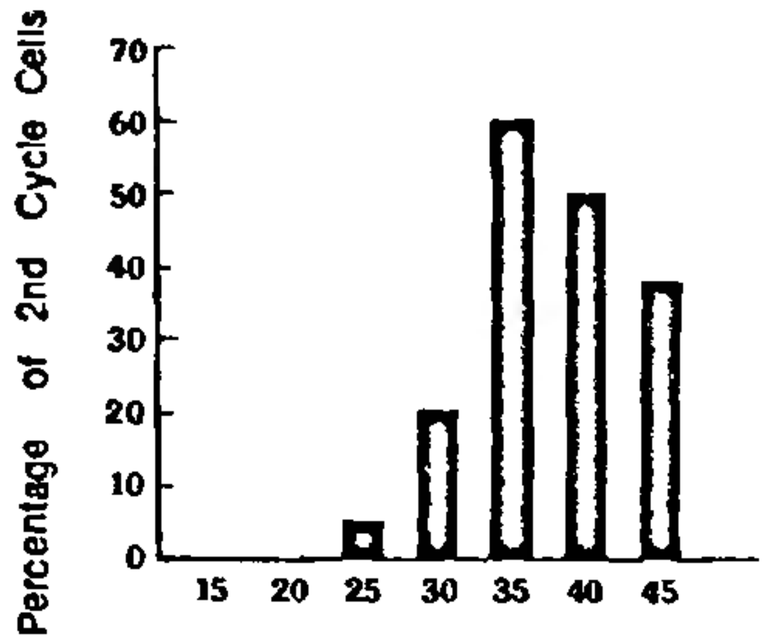



**Figure 3.** Histogram representing percentage of second cycle cells in different hours of BUdR exposure in *B. melanostictus*.

air-dried slides while 3–5-day-old slides constantly show good differentiation. To avoid temperature effect for better SCD, the slides should preferably be kept at 30–32°C during staining. Right concentration of trypsin solution and the time of trypsinization are two other factors. Increased concentration of trypsin solution used or excessive exposure to trypsin solution causes undesirable swelling of the chromosomes. Again, chromosomes treated only with trypsin solution and stained in Giemsa stain diluted in distilled water show no SCD. Therefore, the $Na_2HPO_4$-Giemsa solution (pH 9) seems to be an essential step for producing reverse SCD in amphibian chromosomes.

The mechanism underlying the preferential binding of BB chromatids is not clear. According to earlier reports[12,13] the undespiralized state of BB chromatids make them more accessible to Giemsa dye which can act as an agent to recondense collapsing chromosomes by its cationic nature. Another possible explanation is that the high alkalinity of Giemsa staining solution plays an essential role in the induction of such reverse phenomenon or B-dark type SCD.

Differential staining of sister chromatids by reverse banding was reported earlier in Chinese hamster *in vitro* cells and other mammalian cells in culture[7,13]. This technique has not been applied to Amphibia so far. Its importance in mutagenicity and carcinogenicity bioassay of environmental agents needs special mention not only because of the easy application of this technique and easy availability of the specimen in our country but also for having the much elongated metaphase chromosomes with a remarkably low $2n$ number[9,14,15]. Moreover, since the technique needs

no Hoechst 33258 treatment and photolysis, it is an additional advantage to those where availability or cost of the chemical is a criterion.

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# NEWS

## CHANDLER WINS 1988 WORLD FOOD PRIZE

Dr Robert F. Chandler, Jr., first director general of IRRI, is the 1988 winner of the World Food Prize. Chandler's selection was announced by General Foods Corporation, sponsor of the prize, on 7 June.

The World Food Prize is the foremost international award to recognize, encourage, and reward outstanding individual achievement in improving and increasing the world food supply.

Chandler was cited for his decades of work toward the alleviation of hunger.

Dr M. S. Swaminathan, IRRI director general from 1982 to 1987, received the first World Food Prize in 1987 for his role in bringing the Green Revolution to India.

Chandler established IRRI in 1960 and headed it until 1972. He defined its research directions, built its physical plant, and assembled its international staff. He molded IRRI into an international centre of excellence to help alleviate hunger worldwide.

Chandler also founded the Asian Vegetable Research and Development Centre in Taiwan, China, and served as its director general from 1972 to 1975.

Born in Columbus, Ohio, and raised in New Gloucester, Maine, Chandler received a BS degree in Horticulture from the University of Maine and a Ph.D. in Pomology from the University of Maryland. He spent his early career as an educator.

Since his formal retirement, Chandler has been a member of the program committee of the Near East Foundation and has continued to further the cause of world food production through professional consultancies with many agricultural research institutions.

In recognition of his work, he received the 1986 Presidential End Hunger Award from US President Ronald Reagan.

Chandler has written two books, *Rice in the tropics—a guide to national development* and *An adventure in applied science—a history of the International Rice Research Institute*. He has donated his rice library to the National Cereals Research Institute in Badeggi, Nigeria. (*The IRRI Reporter*, July 1988, 2/88; Published by the International Rice Research Institute, P.O. Box 933, 1099, Manila, Philippines.)