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SISTER CHROMATID EXCHANGE

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The phenomenon of sister chromatid exchange (SCE) which represents interchange of DNA between sister DNA molecules at apparently homologous sites, has been known for more than 20 years but the real breakthrough in study of this phenomenon occurred only in the early 70s when simple techniques based on 5-bromodeoxyuridine (BrdU) labelling and fluorochrome or Giemsa staining of metaphase chromosomes were developed (1-6). Soon after the development of these simple but extremely sensitive techniques, it was found that the incidence of SCEs, in general, is an efficient indicator of genetic damages caused by chemical carcinogens and/or mutagens. The simplicity of the technique and its high degree of sensitivity have made the SCE test an integral part of mutagen screening systems.

Basic Principles of the Technique

The basic technique involves labelling the cells for two replication cycles with BrdU so that at metaphase, the two chromatids of a chromosome are uni- and bi-filarly substituted with BrdU. When such chromosomes are examined for their fluorescence patterns (with Hoechst 33258, Acridine Orange etc), the bifilarly substituted chromatid fluoresces dully while the other one is brightly fluorescing (1,4,7,8). Subsequently it was found that a combination of fluorescence and Giemsa staining gives a very good and permanent differential staining of the bi- and uni-filarly substituted sister chromatids (4). In these "harlequenized" chromosomes, any exchange between sister chromatids is easily identifiable by a reciprocal exchange in the staining intensities of sister chromatids at any point (see fig. 1). In later studies, it has been further observed that if cells are labelled for one cycle with BrdU and then allowed to replicate for the 2nd cycle in normal thymidine containing medium, the unsubstituted and unifilarly-BrdU substituted sister chromatids in 2nd cycle metaphase show dark and light Giemsa staining, respectively. This later observation is particularly useful for *in vivo* studies (see below).

BrdU-Labelling Procedures

Nearly all mutagen testing studies using SCE test system have been done with mammalian cells either *in vitro* or *in vivo*. A brief description of the the methods of BrdU labelling in these cases is given below.

In vitro systems

1. Leucocyte Culture System : Phytohaemagglutinin-stimulated lymphocytes are labelled with BrdU for 2 cycles. BrdU is added to the medium at the same time as the cultures are initiated and the culture bottles are covered with a black paper to protect them from light. Since it is now known that the PHA-stimulated lymphocytes complete 2 cell cycles by about 40-48h (9,10), the BrdU-labelled cultures may be harvested (after 1-2h colcemid treatment) at 48h to yield a high frequency of 2nd cycle metaphases. The test chemical may be added at different times of the culture depending upon the nature of the chemical. Generally, however,

the cells must pass through one S-period after treatment with the test chemical for expression of the induced SCEs. Therefore, G_2 treatment may give a negative result.

2. Established Cell Lines : A large variety of established cell lines are available and they may be conveniently used for labelling with BrdU. The cell lines may also be synchronised by suitable methods so that the test chemical may be applied at known periods of cell cycle.

3. In vitro culture of Bone Marrow Cells : Bone marrow contains a variety of rapidly proliferating cells which can be used for short-term cultures without necessitating PHA stimulation. The marrow from femur etc can be flushed out into TC 199 medium supplemented with 15-20% serum : the marrow from femur of an average size rat may provide enough cells for setting up 2-4 cultures (each with 5ml medium). BrdU is also added to the medium at the beginning of the culture and after incubation at 37°C for 24 to 30h, large number of 2nd cycle metaphases may be obtained. The bone marrow cultures may thus provide a simpler and rapid alternative to the peripheral blood lymphocyte culture system.

4. Concentration of BrdU used in the medium in vitro : The success in getting clear differential staining of uni- and bi-filarly BrdU substituted sister chromatids primarily depends upon optimal level of BrdU incorporation into the chromosomal DNA. For in vitro studies, 1 to 10µg BrdU/ml of culture medium is usually used. In a detailed study, Sharma and Das (11) have recently shown that the amount of BrdU required in the medium for an optimal differential staining of sister chromatids depends upon the chemical composition of the medium as well as on the species being used. Since higher concentrations of BrdU themselves induce SCEs and may have other effects, it is imperative that a minimal concentration of BrdU is used. Depending upon the species and the medium used, 0.1µg/ml to 1µg/ml BrdU concentration may be satisfactory in most cases (11).

In vivo Systems

After the successful studies by Bloom and Hsu (12) for examining SCEs in a developing hen's egg by giving a single injection of BrdU in ovo, in vivo techniques for laboratory mammals have been developed. The availability of suitable techniques for in vivo labelling of chromosomes of laboratory mammals with BrdU (13,14) for one or two cycles has made the SCE technique very useful for mutagen screening studies since now the test chemical can be monitored in vivo and thus the question of metabolic activation can also be considered. Besides, this system permits analysis of somatic as well as meiotic cells. Several techniques have been used for in vivo BrdU labelling.

1. Intravenous Perfusion : In this method, the animal is continuously perfused with BrdU in phosphate buffered saline (50mg/Kg/h) for a period enough to cover 2 cell cycles (see ref. 15).

2. BrdU Tablet Implantation : A 55mg tablet of BrdU is implanted subcutaneously in a 30g rat (see ref. 16) : a slow but constant release of BrdU from this tablet occurs to the blood stream of animal and results in a satisfactory BrdU substitution for 2 cycles in dividing cells.

3. Multiple Injection Method : Hourly intraperitoneal injections of BrdU to the animal also give satisfactory substitution. For studying SCEs in bone marrow or other somatic cells of mice, 50-60mg BrdU/Kg body

weight is injected every hour for 9-10h and the animal is given colchicine (or colcemid) 12-15h after the last BrdU injection and sacrificed 2h later. For studying SCEs in spermatogonial cells, 14 hourly injections of BrdU are given and about 40h after the last BrdU injection, colchicine is administered (see ref. 13,14). The test chemical is injected after the last BrdU injection. In this method, BrdU substitution occurs only in the first cycle while in the 2nd cycle BrdU is no more available and thus in the 2nd cycle metaphase, the chromosomes have non-substituted (dark) and unifilarly substituted (light) sister chromatids.

Differential Staining of Sister Chromatids

Fluorescence staining with Hoechst 33258 or Acridine Orange etc directly reveals differential fluorescence of uni- and bi-filarly substituted sister chromatids. However, the fluorescence technique has limitations for routine analysis and therefore, Giemsa staining is the method of choice. Many different methods are described in literature for obtaining differential Giemsa staining of BrdU-substituted sister chromatids. In our laboratory, the following method modified after Goto et al (17) is routinely used :

i. Air-dry chromosome preparations are stained with Hoechst 33258 (50ug/ml) for 15 min, rinsed twice in water and dried.

ii. A few drops of 2x SSC (saline-sodium-citrate) are put on the slide and covered with a cover glass. The slides are now put in bright sunlight for 15 to 60min depending upon the ambient temperature.

iii. After the sunlight exposure, the coverglass is floated off in dist. water and slides rinsed well in dist water. The slides may be dehydrated through ethanol series or may be directly air-dried.

iv. The slides are now stained with 2% Giemsa solution for 2-5min and rinsed well in dist. water to remove excessive stain. Overstaining can obscure the differential staining of sister chromatids. If excessively stained, the slides may be washed longer in dist. water or they may be destained dy dipping in acetomethanol (1:3), rinsed in absolute alcohol, dried and restained in Giemsa for optimal staining.

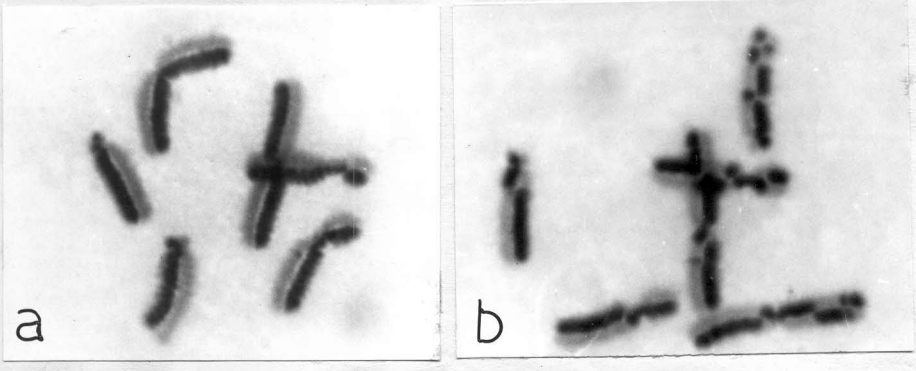

Fig. 1. BrdU-Giemsa differential staining of sister chromatids in PHA-stimulated lymphocytes of Indian Muntjac labelled with BrdU for 2 cycles (48h). a - An untreated cell; b - a treated cell with a high incidence of SCEs (microphotographs kindly provided by Dr. Mercy Jacob).

SCEs as Indicators of Genetic Damage

Since the pioneering studies by Latt (18) and Perry and Evans (19), the SCEs have been considered to be very sensitive indicators of the damaging effects of chemical mutagens and carcinogens on chromosomes since dosages which are not mutagenic can also induce a higher incidence of SCEs (5,6). The development of *in vivo* SCE assay system has greatly enhanced the utility of this technique since now not only the question of metabolic activation of the test substance can be taken into consideration, but spermatogonial and meiotic cells too can be directly analysed for any induced damage.

Since the beginning of the BrdU-SCE methodology it has been realised that not all chromosome damaging agents cause a high incidence of SCEs. For example, X-irradiation, certain human genetic disorders (Fanconi's anaemia, Ataxia Telangiectasia, Xeroderma Pigmentosum etc) and certain chemicals like Bleomycin, EMS etc, cause a high incidence of chromosome damage or gene mutations but do not show a parallel increase in SCE frequencies (see ref. 5,6,20). This aspect is very important for consideration in any programme of mutagen and/or carcinogen screening by SCE technique since a negative result by SCE assay system may be misleading in terms of genetic hazards posed by the test substance. Moreover, in some recent studies the incidence of spontaneous and/or induced SCEs has been shown to vary in different cell types of an animal or species (13,14,21-24). This aspect too is important in evaluating the mutagenic and/or carcinogenic potential of a test substance.

Mechanism of SCEs

For a proper appraisal of the SCE assay system in the screening programmes, an understanding of the mechanism of SCE induction is essential. In recent years, several studies have been done to understand this aspect and a few models have been proposed (24-26). It is believed that the spontaneous as well as induced SCEs result from the same mechanism which may involve some kind of disturbances in the progression of replication along the chromosome.

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