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THE DISTRIBUTION PATTERNS OF SISTER CHROMATID EXCHANGES IN V79 CHINESE HAMSTER CELLS: RELEVANCE TO GENOTOXICITY STUDIES

G. RAINALDI, T. M'ARIANI, A. PIRAS, M.A. CALIGO, S. SIMI and M. VALDRIGHI

Genetica e Biochimica delle Cellule Somatiche, Istituto di Mutagenesi e Differenziamento del CNR, Pisa

Summary. - *Sister chromatid exchanges (SCEs) are routinely used in genotoxic studies. The observations that some agents induce SCEs without altering the SCE distribution, while others disrupt such distribution, support the use of SCE distribution as a tool to distinguish between agents which damage DNA and agents which interfere with the replication machinery. In addition to that, it was found that compounds belonging to the latter group, are also able to induce gene amplification. Despite the mechanism by which the two phenomena could be associated is undefined, an aberrant induction of SCEs as a basis of gene amplification it must be taken into account.*

Riassunto (Uso della distribuzione degli scambi tra cromatidi fratelli negli studi di genotossicità in cellule di mammifero in coltura). - *Gli scambi tra cromatidi fratelli possono essere indotti da vari composti che interagiscono con la sintesi del DNA sia direttamente, danneggiando il DNA, sia indirettamente, modificando alcuni o molti componenti del complesso replicativo. Nel primo caso tutte le cellule vengono colpite per cui la distribuzione degli SCE rimane confrontabile con quella degli SCE spontanei, anche se il numero degli SCE per cellule è più alto. Nel secondo caso solamente le cellule in S mostrano l'induzione degli SCE. In aggiunta a questo è stato visto che i composti che inducono SCE in maniera anomala inducono anche amplificazione genica. Sebbene il meccanismo con cui i due effetti possono essere collegati non è chiaro, si può avanzare l'ipotesi che l'induzione aberrante di SCE può rappresentare la base per l'induzione di fenomeni di amplificazione genica e che quindi la distribuzione degli SCE possa risultare utile per identificare composti che inducono tale effetto.*

Distribution of sister chromatid exchanges

Sister chromatid exchanges (SCEs) detect S-dependent DNA damaging agents and accordingly are routinely used in genotoxicity bioassays [1-3].

In this paper we discuss data on the mechanism of SCE induction.

The distribution of spontaneous SCEs in V79-AP4 Chinese hamster cells is reported in Fig. 1. The mean and the variance of the numbers of SCEs per metaphase were comparable, following a Poisson distribution. Similar results were obtained when increasing concentrations of bromodeoxyuridine (BUdR) were used (Table 1). As previously shown [4, 5], BUdR induced an enhancement of the number of SCEs per cell and the ratios between the variance and the mean number of SCEs per metaphase at the various concentrations were comparable and consistent with a Poisson distribution.

Analogously these ratios did not change when the cells were treated with direct DNA damaging agents. Table 2 shows the results obtained by treating V79-AP4 cells with ethylmethansulphonate (EMS), a direct alkylating DNA damaging agent; the distribution of SCEs was unchanged although the absolute number was increased.

Different results were obtained when the replication of V79-AP4 cells was inhibited by different metabolic inhibitors [6]. For example, we report the results obtained with aphidicoline (APC), whose primary site of action is DNA polimerase α. In the presence of appropriate concentrations of the drug, DNA synthesis of V79-AP4 cells was inhibited. After 1 h of APC treatment, the distribution of SCEs was altered; in addition to a subpopulation showing the pattern of untreated cells, a second subpopulation arose showing a higher number of SCEs (Fig. 2). Quantitation of this fraction revealed that about 30% of the treated cells exhibited a level of SCEs above 17, which represents the furthermost limit of the spontaneous SCE distribution (Table 3). The observation that the fraction of cells with an elevated level of SCEs increases at longer treatment times (Table 4) supports the position that cells with a higher level of SCEs were indeed blocked at a particular stage of the cell cycle.

The general conclusion made from these and other similar experiments was that the diverse distribution of

Table 1. - *SCEs in V79-AP4 Chinese hamster cells cultured at different concentrations of BUdR*

BUdR μM	Number of metaphases	Mean	Variance	Variance/mean
10	115	8.01	9.83	1.23
80	90	12.27	12.96	1.01
160	50	18.38	20.60	1.12

Table 2. - *SCEs in V79-AP4 Chinese hamster cells treated with EMS*

EMS mM	Number of metaphases	Mean	Variance	Variance/mean
0	260	7.52	8.51	1.17
1	40	11.38	9.18	0.81
10	112	36.79	40.01	1.09

Table 3. - *Metaphases with normal and high levels of SCEs in V79-AP4 Chinese hamster cells treated with APC*

APC	Normal level of SCEs				High level of SCEs	
μg/ml	No. metaphases	Mean	Variance	Variance/mean	No. metaphases	%
0	64	8.09	8.27	1.02	0	0
5	49	8.71	9.02	1.04	15	23.43
10	43	8.95	11.11	1.24	21	32.81
20	45	7.87	8.03	1.02	19	29.68

Table 4. - *Metaphases with normal and high levels of SCEs found in V79-AP4 Chinese hamster cells treated with 1 μg/ml APC for 8 and 24 h*

Treatment	Normal level SCEs		High level SCEs	
h	No. metaphases	%	No. metaphases	%
8	20	37.73	33	62.26
24	24	28.23	61	71.76

SCEs could distinguish between agents that damage DNA from agents that interfere with the replication machinery. In the first case, any cell might be damaged, so that the development of SCEs during the traversing of S phase would depend on the number of unrepaired or persistent lesions present in the cells. This would explain the progressive shift of SCE distribution observed by increasing the dose of the agent (Fig. 3). In the second case, the biological target is represented by cells in S phase; while a fraction of cells will maintain levels of SCEs within the normal range (Fig. 4a), only cells blocked in DNA replication will show induction of SCEs (Fig. 4b). In the latter instance a longer treatment time will cause an increase in the number of cells affected by higher numbers of SCEs, rather than a general increase of SCEs spread over the entire cell population.

An implication of these conclusions is that the induced SCEs should be present until the causes of their induction persist. In EMS-treated V79-AP4 cells (Fig. 5) we found that SCEs were induced not only imme-

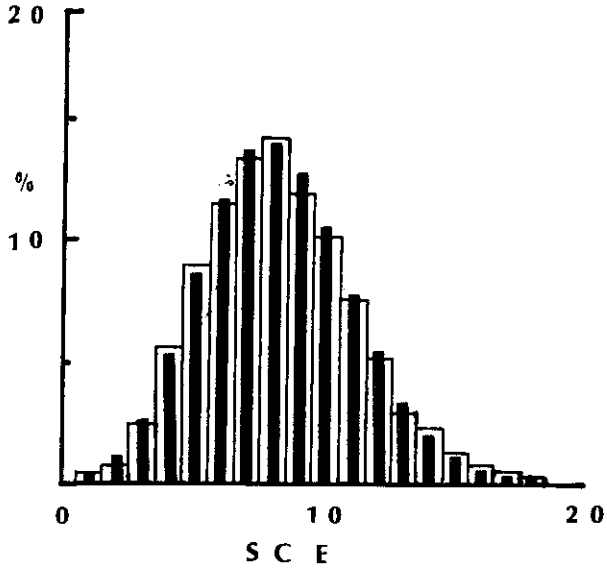


Fig. 1. - Distribution of spontaneous SCEs (open bars) compared with the equal mean Poisson distribution (shaded bars). n = 895; mean = 8.21; variance = 8.70; variance/mean = 1.05.

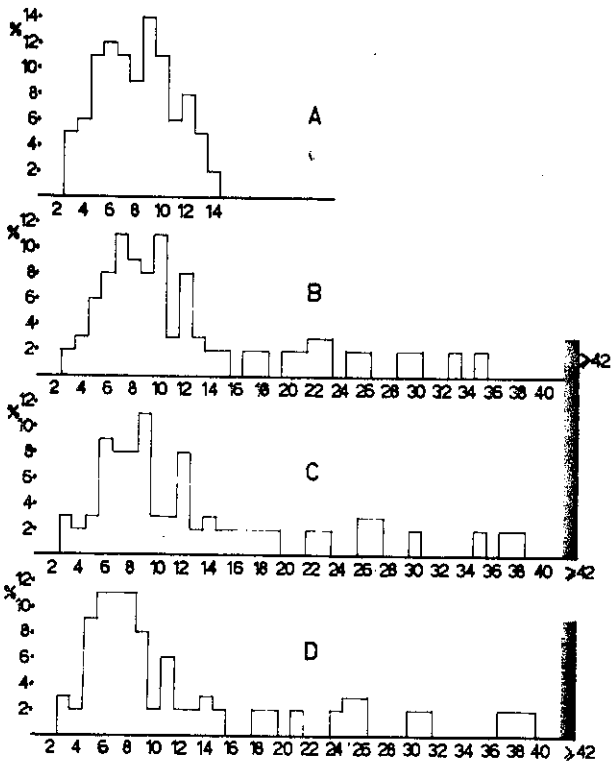


Fig. 2. - Distribution of SCEs induced by aphidicoline. A: untreated cells; B: 5 µg/ml; C: 10 µg/ml; D: 20 µg/ml. Abscissa, SCE per metaphase; ordinate, percentage of scored metaphases. For each dose n = 64 (from [6]).

diately after the end of the treatment but also 24 h (about 2 cell divisions), or 48 h (about 4 cell divisions) afterwards. By contrast, this prolonged effect was not observed in APC treated cells. When BUdR was added in

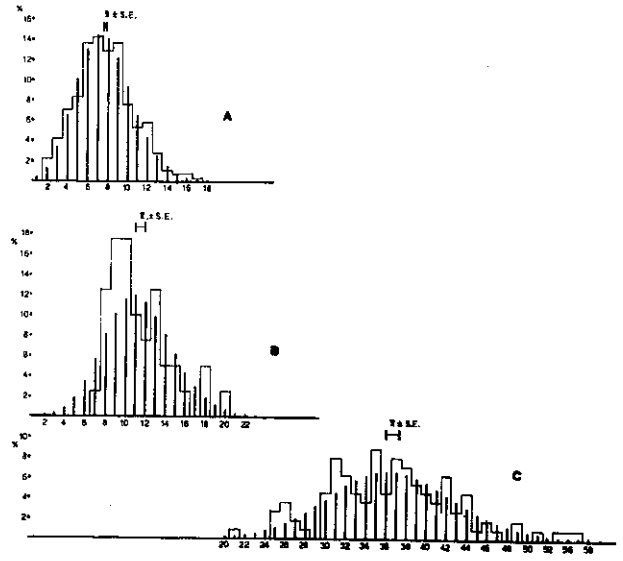
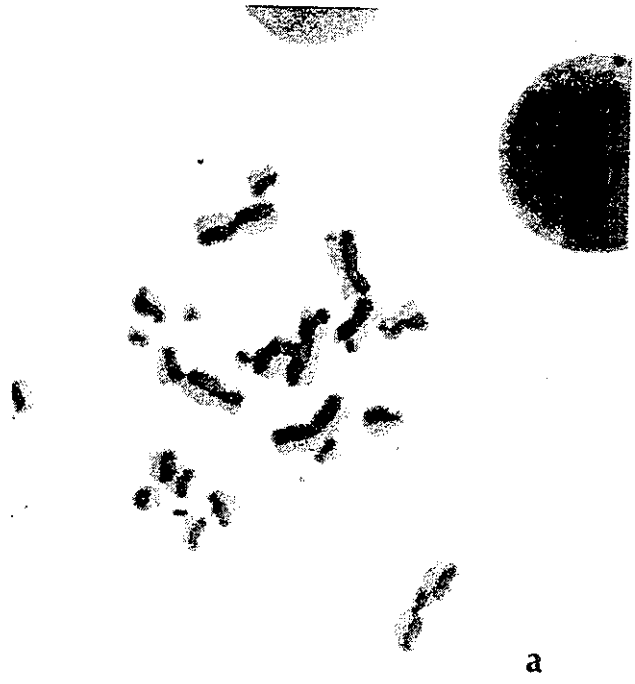


Fig. 3. - Distribution of SCEs induced by EMS compared with the equal mean Poisson distribution (vertical bars). A: untreated cells; B: 1 mM EMS; C: 10 mM EMS. Abscissa, SCEs per metaphases; ordinate, percentage of metaphases (from [6]).

the culture medium 6 or 12 h after the removal of the APC blockage, the number of SCEs dropped to the baseline level (Table 5). This result suggests that SCEs induced by inhibition of DNA synthesis are generated until the target cells had traversed the S phase, while those induced by DNA damaging agents are produced until unremoved damage is present in dividing cells.

As a consequence the kinetics of SCE disappearance could provide a means to distinguish between compounds that damage DNA and agents that interfere with DNA replication. This conclusion may have important practical consequences in genotoxicity studies, in that it could avoid the erroneous definition of a compound that inhibits DNA replication as a DNA-damaging agent (*i.e.*, false positives).

An example of the usefulness of the SCE distribution analysis can be seen in the results we obtained with potassium dichromate [7]. This compound is known to inhibit DNA synthesis very efficiently [8] as well as to damage DNA both at the chromosomal and molecular levels [9, 10]. On these bases and according to our interpretation, both a modification of SCE distribution owing to chromium inhibition of DNA synthesis, and a dose-dependent induction of SCEs due to its interaction with DNA, are expected to occur. In fact we found that the number of SCEs was enhanced by treatment with potassium dichromate and the ratios of the variance/mean were different from 1 (Table 6). These data suggest that the distribution of SCEs might represent a useful tool to identify compounds that have various biological targets. A similar example is represented by UV irradiation which, in addition to increasing the number of SCEs in a dose-dependent manner, also generates a spread of the data around the mean values (data not shown).



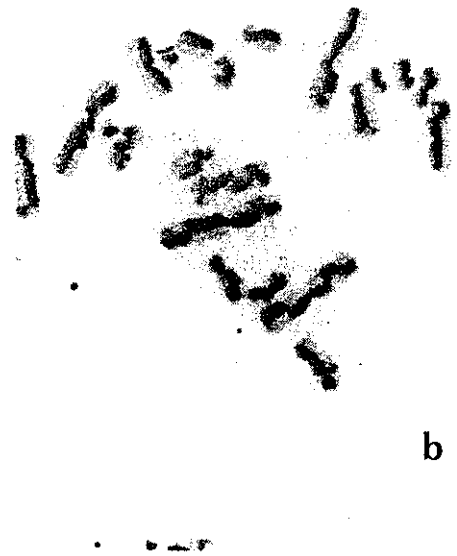


Fig. 4. - Examples of the two cell populations present in V79-AP4 cells after the removal of the blockage of DNA synthesis. a: metaphase with a SCE frequency not differing from that of untreated cells; b: metaphase with elevated SCE frequency that is characteristic for S-blocked cells.

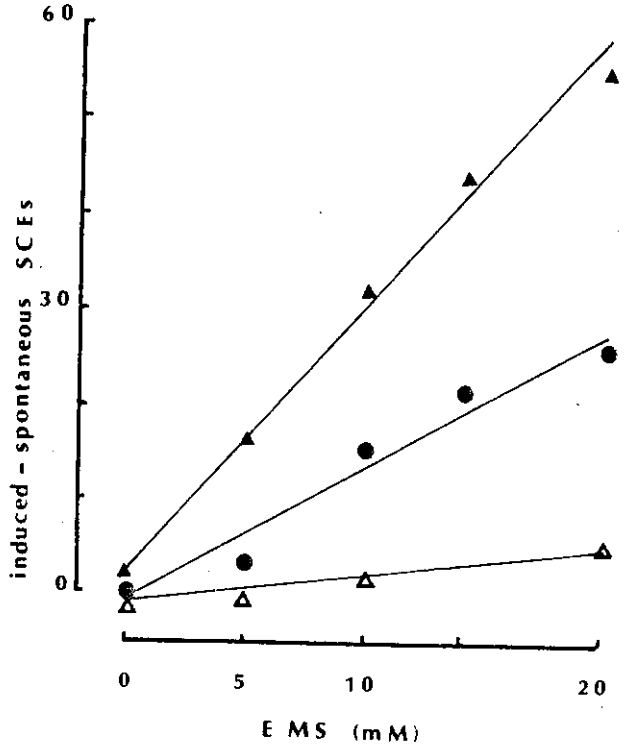


Fig. 5. - V79-AP4 Chinese hamster cells were seeded 16 h before treatment to produce an exponentially growing cell population. The treatment was performed by exposing the cultures for 1 h to EMS dissolved in Dulbecco's modified medium supplemented with 5% of foetal calf serum. After treatment, the cells were seeded in BUdR-containing medium at different periods after EMS treatment. (▲) 0-24 h; (●) 24-48 h; (△) 48-72 h.

Table 5. - *SCEs in V79-AP4 Chinese hamster cells treated with 1 μg/ml APC for 8 h*

BUdR-labeling	Number methaphases	Mean	Variance	Variance/mean
0-24	64	33.4	513.02	15.35
6-30	100	8.08	10.64	1.31
12-36	100	6.77	6.34	0.93

Table 6. - *SCEs in V79-AP4 Chinese hamster cells treated with potassium dichromate*

Potassium dichromate μg/ml	Number metaphases	Mean	Variance	Variance/mean
0	66	8.6	9.2	1.1
16	75	25.9	78.8	3.0
32	96	30.0	121.9	4.0
64	36	39.8	126.2	3.2

Aberrant induction of sister chromatid exchanges

In previous studies we observed that following a block of DNA synthesis either for short or long periods, about 30% (Table 3) and 70% (Table 4) of the cell population respectively, presented an higher level of SCEs, as compared with the remaining cells in the population. The position of the block along the cell cycle of V79-AP4 cells treated with various DNA synthesis inhibitors was determined by the premature chromosome-condensation technique and microspectrophotometric measurements of nuclear DNA. A reduction of the fraction of cells in G1 and in G2+M and an increase of the fraction of cells in S phase at the end of the block were observed. A detailed analysis showed that cells in S phase with extended, highly decondensed chromatin regions were more frequent in treated than in untreated samples (Fig. 6). To determine the stage of DNA synthesis corresponding to the level of decondensation, we measured the DNA content per cell in the treated cell population. An increase in the number of cells with nuclear DNA content slightly higher than the 2C value was found (Fig. 7). Thus, it appears that the exposure of the cells to DNA synthesis inhibitors causes the cells to accumulate in the early S phase of the cell cycle.

Fig. 6. - Mitotic CHO cell fused with V79-AP4 cell. The fusion product shows sister chromatid differentiation of CHO chromosomes and pulverization of V79-AP4 chromosomes. The degree of pulverization indicates that V79-AP4 cell was at the early S phase of the cell cycle (from [11]).

To investigate whether the accumulation in early S phase and the induction of SCEs were associated, synchronyzed cells were exposed to the inhibitory drug at various times after seeding. A progressive decrease in the proportion of cells with numerous SCEs was observed as a function of increasing time intervals between the seeding of the mitotic cells and the addition of the drug. Thus, the progression of the cells through the S phase before treatment determined the fraction of cells with high number of SCEs (Fig. 8); these data show

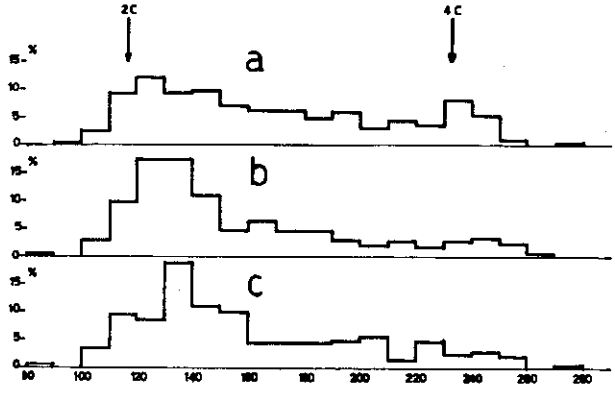


Fig. 7. - Percentage of V79-AP4 cells with a given DNA content in arbitrary units. a: control; b: cytosine arabinoside (araC) 1 x 10^{-6} M; c: deoxythymidine (TdR) 1 x 10^{-3} M (from [11]).

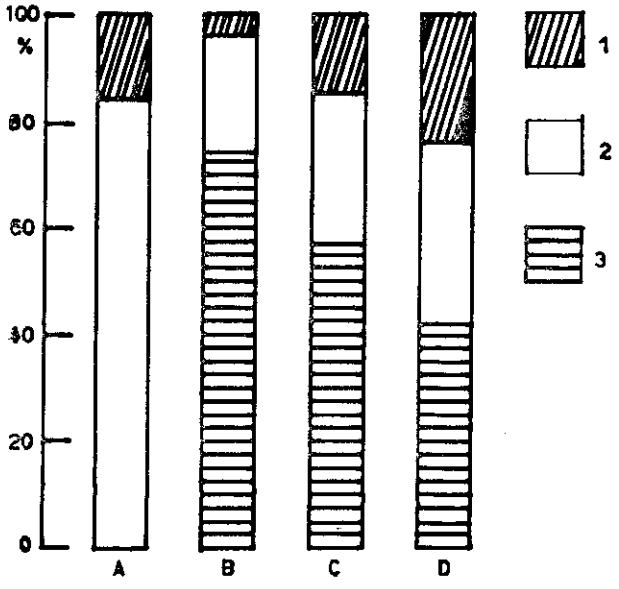


Fig. 8. - Mitotic cells were detached from the culture dishes and reseeded in the absence (A) or presence of araC 1 x 10^{-6} M added to the medium at 1 h (B), 3 h (C), and 5 h (D) after seeding. After 24 h the drug was removed and the cells were allowed to replicate twice in medium containing BUdR (3 µg/ml). After 3 h of block with Colcemid the cells were collected and the metaphases were scored as: (1) metaphases showing non-reciprocal sister chromatid differentiation (SCD); (2) metaphases showing reciprocal sister chromatid differentiation and 0-17 SCE per metaphase; (3) metaphases showing reciprocal sister chromatid differentiation and more than 17 SCE per metaphase (from [11]).

that cells blocked in early S phase of cell cycle are those most prone to form SCEs. These results are consistent with the model of Painter [10] for SCE formation since, by blocking or slowing down of DNA chain elongation, adjacent unreplicated and replicated regions might have the opportunity to recombine old and new DNA strands; the longer the blockage, the higher is the probability that SCE will be initiated.

Another possible interpretation of our data is that the number of SCEs might depend on the number of replicative origins employed in DNA replication. This number has been demonstrated to change in the same cell by varying the culture conditions [12], suggesting that many potential replicative origins can be utilized when a modification of the replicative schedule is required or induced. Thus, we hypothesize that the block of the chain elongation by inhibitors of DNA synthesis could represent a signal for the cells to activate additional replicative origins. After the removal of the block, the original timing of DNA replication of the cells at the early S phase cannot be resumed. The consequent desynchronization of the replication fork movements would therefore enhance the probability for the old and new strands to generate SCEs by joining each other. Thus, according to this model, the activation of potential replicative origins might be responsible for the aberrant induction of SCEs in cells at the early S phase of the cell cycle.

An unscheduled firing of replication origins has also been proposed to explain the occurrence of another important biological event: gene amplification [13, 14]. Therefore, if an unscheduled firing represents the basis for SCE induction as well as for gene amplification, the agents that induce aberrant formation of SCEs would also induce gene amplification. We investigated this possibility by studying cells with a high level of SCEs (presumably due to unscheduled firing of origins) and cells with a normal level of SCEs (presumably due to scheduled firing of origins of replication) for their ability to amplify selected genes. The outcome of this scenario would be gene amplification, measured as N-phosphonacetyl L-aspartate (PALA) resistance, paralleled by the appearance of dividing cells with high levels of induced SCEs, suggesting that the two events may occur in the same subset of dividing cells.

The mechanism by which the two phenomena are associated is a matter of speculation. Aside from the model of aberrant DNA replication [13] as a basis for the generation of gene amplification, other mechanisms such as an asimmetric segregation [16] or unequal exchanges [17-18] have been proposed to explain the occurrence of gene amplification.

We propose that the aberrant induction of SCEs might give rise to unequal SCEs in some cells. As a consequence, a balanced and unbalanced distribution of gene copy numbers should occur and gene-dosage effects should be present among the survivors. A further important aspect derives from this model: genes that were masked in parental cells would possibly be expressed. A matter of investigation in the future will concern not only the role of aberrant induction of SCE on the generation of gene amplification but also whether proto-oncogenes dosage-effects could be induced.

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