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Review Articles

Sister Chromatid Exchanges and Heterochromatin

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Summary. The inter- and intrachromosomal distribution patterns of SCEs obtained with or without mutagen treatment are reviewed and compared, with each other as to their relation to heterochromatin and with the distribution patterns of chromatid aberrations that occurred either "spontaneously" in chromosomes of repair-defective human syndromes or after treatment with the mutagens (BrdU, ethylalcohol, DMBA, TMBA, maleic hydrazide, MMS, MMC). The conclusions are: No general rule is detectable for nonrandom involvement of heterochromatin in spontaneous SCEs. Mutagen-induced SCEs show the same or very similar distribution patterns as the spontaneous ones and are in no case as preferentially located as chromatid aberrations (which involve mainly the junctions between eu- and heterochromatin or other special regions). Therefore, a specific mutagen sensitivity of heterochromatin-containing chromosome regions as observed for chromatid aberrations does not exist (or is less pronounced) for SCEs. This supports the inference that different mechanisms underlie the origins of the two phenomena.

1. Introduction

Since Taylor et al. (1957) discovered sister chromatid exchanges (SCEs) in chromosomes of *Vicia faba* and *Bellevalia romana* by means of autoradiography, and especially since methods were developed for demonstrating differential substitution in chromosomal DNA of thymidine by bromodeoxyuridine (BrdU) (Zakharov and Egolina 1972; Latt 1973; Perry and Wolff 1974; Korenberg and Freedlender 1974), several authors have reported on the intra- and interchromosomal distribution of "spontaneous" or "mutagen-induced" SCEs in different species. (Though the fraction of truly spontaneous SCEs is unknown since BrdU is able to induce them, the term spontaneous will be used in this report for SCEs appearing without, and induced, for those showing up after additional mutagen treatment.) Data concerning distribution of spontaneous SCEs along the metaphase chromosome complement are available for 19 species (Tables 1 and 2). The distribution of SCEs induced by different mutagens [methyl-methane sulfonate (MMS), dimethylbenz(a)-anthrazene (DMBA), trimethylbenz(a)anthrazene (TMBA), mitomycin C (MMC), maleic hydrazide (MH), ethyl alcohol (EA), and BrdU itself] has been studied in one plant species *(Vicia faba)* and six mammalian species (man, mouse, rat, Chinese hamster, cactus mouse, and Indian muntjak). In this paper, new results obtained in *Vicia faba* (broad bean) will be reported and compared with data published for other species.

The data presently available will be considered from the following points of view:

1) Are SCEs distributed randomly (i.e., in proportion to chromosome length)?
2) If there are deviations from random distribution, which chromosome regions are concerned?
3) Are there correlations between heterochromatin and clustering of SCEs?
4) Are there correlations between the distribution patterns of spontaneous and induced SCEs or between SCEs and chromatid aberrations induced by the same mutagens?

The answers to these questions should not only reveal which features of SCE distribution are generally true for the species tested and whether nonrandom involvement is a typical characteristic of heterochromatin, but they should also permit conclusions as to the relationship between the mechanisms giving rise to SCEs and/or chromatid aberrations.

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2. The Inter- and Intrachromosomal Distribution of Spontaneous SCEs

Of the 19 species investigated in this respect (Tables 1 and 2) 6 are plants *(Vicia faba, Hordeum vulgare, Allium cepa, Secale cereale, Bellevalia romana,* and *Haplopappus gracilis).* Except *Drosophila melanogaster,* all animals studied are mammals: man as the only primate, eight rodents *(Cricetulus griseus, C. triton, Mus musculus, Rattus norvegicus, Peromyscus eremicus, Microtus agrestis, M. montanus, Dipodomys ordii),* one member of Artiodactyla *(muntiacus muntjak),* and two marsupials *(Macropus parma* and *Potorous tridactylis).*

Vicia faba (Broad Bean)

Kihlman and Kronborg (1975) described a random distribution of SCEs among the metacentric and the five acrocentric pairs of chromosomes after BrdU incorporation into one of the four DNA strands of the metaphase chromosomes. Vosa (1976) looked at the intrachromosomal distribution of SCEs after trifilar substitution with BrdU in the metacentric chromosome pair. He subdivided the satellite into two segments and the short (satellite-bearing) and the long arms each into four. The

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centromere and the nucleolus organizing secondary constriction were excluded from this evaluation. Although the involvement of both arms in SCEs was proportional to their lengths, Vosa found nonrandom SCE distribution within the long arm. Both middle segments were more often, and the telomeric regions and the segments adjacent to the centromere, less frequently involved in SCEs than expected. These results cannot be correlated with the heterochromatin content of the segments as revealed by Giemsa staining techniques or by labeling of late-replicating DNA (Döbel et al. 1973, 1978; Schweizer 1973a; Takehisa and Utsumi 1973; Burger and Scheuermann 1974; Greilhuber 1975; Friebe 1976).

Schubert et al. (1979a) used two different reconstructed karyotypes of *Vicia faba* (denoted EF and ACB) with six clearly identifiable chromosome pairs (due to the presence of homozygous translocations and inversions) to study intrachromosomal SCE distribution. The 28 segments into which each of the two karyotypes had been subdivided were found to be randomly involved (1185 and 986 SCEs, 1% level of significance). The only exception to this rule was the nucleus organizing secondary constriction (NOR), which is an SCE hot spot with more than double the frequency of SCEs expected for random distribution at the upper limit of confidence, in both karyotypes. Some smaller additional deviations from random distribution of SCEs in both directions (significant at the 5% level), which occurred in other segments in some experiments, were nonreproducible.

Schweizer (1973b) also observed an SCE hot spot in the NOR after differential incorporation (into one of the four DNA strands) of ^{3}H-thymidine. Furthermore he described an SCE hot spot in the centromere of the metacentric chromosome and additional nonrandom SCE distribution (at the 5% level of significance) in cold-inducible heteropycnotic regions of the *Vicia faba* chromosomes. While Schweizer (1973b) found ~17% of all SCEs of chromosome 1 in its centromere, Geard (1969) observed (in autoradiographs) 8.3% of all SCEs of the same chromosome in the centromeric region.

The results obtained in *Vicia faba* may be summarized as follows: The NOR of this species is an SCE hot spot. SCE clustering in the centromere region of the metacentric chromosome probably is due to twisting of sister chromatids in this region rather than to preferential involvement in real SCEs (without evaluation of the third or fourth mitosis after ^{3}H-thymidine incorporation this is a rather common misinterpretation of autoradiographs). Other deviations from random SCE distribution are less pronounced or appear only occasionally, and such results have not been reproducible by various investigators. Segments containing constitutive heterochromatin (besides the NOR) do not deviate in either direction from random SCEs involvement.

Hordeum vulgare (Barley)

A reconstructed karyotype (MK 14/18) of barley with seven individually recognizable chromosome pairs (due to two homozygous translocations between chromosomes 2 and 7 and 3 and 4) was subdivided into 44 segments and investigated for intrachromosomal SCE distribution after BrdU was incorporated into one of the four DNA strands of the metaphase chromosomes (Schubert et al. 1980). At the 1% level of significance, 1030 SCEs were distributed randomly among the 44 segments; only one segment (the satellite of chromosome 6) showed fewer SCEs than expected. Since this segment has no heterochromatin (Giemsa technique) and other segments without bands were found to be randomly involved in SCEs, this observation is presently without a satisfying explanation. Contrary to the NORs of *Vicia faba,* those of barley showed no SCE clustering.

Allium cepa (Onion)

Schvartzman and Cortes (1977) reported a nonrandom distribution of 2273 SCEs in *Allium cepa* chromosomes after BrdU treatment during the last-but-one S phase. The telomeric heterochromatin (containing Giemsa bands and late-replicating DNA) and the pericentromeric heterochromatin (harboring late-replicating DNA only) of each chromosome showed significantly fewer SCEs than expected for random involvement; the remaining chromatin showed correspondingly more SCEs.

Secale cereale (Rye)

Studies of SCE involvement of the satellite chromosomes (three of the four DNA strands labeled with BrdU) by Friebe (1978) led to the conclusion that in this species the lowest SCE frequency was in the telomeric heterochromatin and the highest, at the junctions between eu- and heterochromatin.

Haplopappus gracilis and Bellevalia romana

For both these species Sparvoli and Gay (1973a and b) described SCE clustering in the centromeric regions (labeling with ^{3}H-thymidine and autoradiography). Sometimes slight clustering occurred in the midarm regions of the *Bellevalia* chromosomes 1 and 2.

Drosophila melanogaster

This is the only invertebrate animal in which SCEs have been studied extensively. Dolfini (1978) observed more SCEs in males than in females, notwithstanding the fact that the X chromosome showed more SCEs per unit length than the autosomes. According to Dolfini (1978) the pericentromeric heterochromatin and the heterochromatic Y chromosomes showed no SCEs. Contrary to this, Gatti et al. (1979) reported more SCEs per cell in the female karyotype (with a random interchromosomal distribution of SCEs) as compared with the male, in spite of the fact that the Y chromosome showed three times more SCEs than expected on the basis of its length. A third contradiction between the results of Dolfini (1978) and Gatti et al. (1979) is that the latter found more SCEs than expected in the heterochromatic regions. The only data that agree from the two papers is that SCEs occur frequently at the euchromatin-heterochromatin junctions. Contrary to the above authors, Tsuji and Tobari (1979) could not find any difference in SCE frequency between the two sexes. The different proportions of SCEs in heterochromatic regions, including that of the Y chromosome, of *Drosophila* may be explained by the fact that sister chromatids in these regions often lie so tightly together that it is difficult or sometimes even impossible to decide whether or not an SCE occurred (Dolfini 1978; Wienberg 1977).

Homo sapiens

The distribution of SCEs along the metaphase chromosomes in mammals has been most extensively investigated in man (see

Table 1. Selected chromosome regions of the human karyotype showing nonrandom involvement in spontaneous SCEs[a]

Centromere	Facultative heterochromatic X	C bands	G^+ or Q^+ bands	G^- or Q^- bands	Band junctions	Methods for SCE detection	References
+ (~40%)						AR[b]	Herreros and Gianelli 1967[c]
+ (~25%)		+				Modified Giemsa technique, Hoechst 33258	Kim 1974
				+	+	Q-banding, Hoechst 33258	Latt 1974
(5%)		–				FPG	Galloway and Evans 1975
					+	Modified Giemsa technique	Pathak et al. 1975
+						FPG	Sperling et al. 1975
+ (18%)						Acridine orange	Tice et al. 1975[d]
	+					Hoechst 33258, late replication (^{3}H-thymidine)	Schnedl et al. 1976
No clustering		No clustering	(+)			AR[b]	Smyth and Evans 1976
					+	See Korenberg and Freedlender 1974	Crossen et al. 1977[e]
					+	Q- and R-banding, see Korenberg and Freedlender 1974	Dutrillaux et al. 1977[f]
			–			G bands, see Korenberg and Freedlender 1974	Morgan and Crossen 1977
–		–				FPG	Shiraishi and Sandberg 1977[g]
+ (20%)			– (13%)	+ (76%)	(11%)	Quinacrine mustard	Haglund and Zech 1979

[a] +, SCE clustering; (+), SCE clustering inferred indirectly; –, less SCEs than expected
[b] AR, autoradiography
[c] Endoreduplicated chromosomes
[d] After three cycles in BrdU
[e] C-band junctions also
[f] Mentioned as unpublished results
[g] Bloom syndrome cells used

Table 1). Differential ^{3}H-thymidine incorporation (Smyth and Evans 1976) and differential labeling with BrdU (Latt 1974a; Galloway and Evans 1975; Schroeder 1975; Sperling et al. 1975; Crossen et al. 1977; Morgan and Crossen 1977; Shiraishi and Sandberg 1977; Stoll et al. 1977; Haglund and Zech 1979) have shown that the involvement of individual chromosomes in SCEs increases with metaphase length of the chromosomes in question. However, even more SCEs have been found in the large chromosomes (groups A and B), and fewer, in the small chromosomes (group E, F, G) than expected on the basis of their metaphase length (Latt 1974a; Galloway and Evans 1975; Schroeder 1975; Sperling et al. 1975; Crossen et al. 1977; Shiarishi and Sandberg 1977; Haglund and Zech 1979). Smyth and Evans (1976) observed (autoradiographic investigations) no clustering of SCEs, neither in centromeres nor in C-band regions of chromosomes 1, 9, and 16, but chromosomes with many tightly stacked G^+ bands showed a high frequency of SCEs per unit length. Galloway and Evans (1975), using the fluorescence-plus-Giemsa (FPG) technique, found that fewer SCEs than expected were in C-band regions and more in midarm regions ($P < 0.05$) and, that 5% of the SCEs were located in centromeres. Crossen et al. (1977) described SCE clusters at the junctions between C bands and euchromatin of chromosomes 1, 2, 4, and 5. Sperling et al. (1975) observed (FPG-stained slides) a high frequency of SCEs in centromeres, however, the number of SCEs (246) evaluated was very low. The results of Tice et al. (1975) point in the same direction. They localized 18% of all SCEs in the centromeric regions after three cell cycles in BrdU medium; this means that in this case twisting of the sister chromatids could not have been responsible for the high incidence of SCEs in centromeres. Kim (1974) reported 25% of 2700 SCEs to be located in centromeres and pericentromeric regions. These regions and the constitutive heterochromatin, e.g., region 1qh, were more frequently involved in SCEs than the telomeres (but these were not defined quantitatively). According to Herreros and Gianelli (1967) even 40% of the SCEs occur in the centromeres of human chromosomes after ^{3}H-thymidine incorporation; however Shiraishi and Sandberg (1977) found only a few SCEs in centromeric regions.

After sequential staining with quinacrine and 33258 Hoechst, Latt (1974a) observed that for chromosome 1 most SCEs occurred in faint Q bands and between these and Q^+ bands (especially if the latter are very pronounced).

With similar techniques Haglund and Zech (1979) found 20% of SCEs in the centromeres. They described the following SCE distribution pattern: 76% in faint Q bands, 13% in Q^+ bands, and 11% at the borders between the two types of bands. Dutrillaux et al. (1977) mentioned unpublished results, according to which most SCEs occurred in so-called "interbands" between Q^+ and R^+ bands (though Q^+ and R^+ bands are generally accepted to be complementary to each other, most SCEs occurred in faint bands in both cases when sequential staining was done). Pathak et al. (1975) scored most SCEs in human and Chinese hamster chromosomes in regions between G^+ and G^- bands (however the simultaneous microscopic resolution of SCEs and G-banding is rather poor).

A similar result was obtained by Morgan and Crossen (1977): None of 161 SCEs were found within G^+ bands. Schnedl

et al. (1976) scored nearly twice as many (0.63) SCEs per chromosome in the heterochromatic X chromosome as in the euchromatic X chromosome (0.26). Per X chromosome, 0.33 SCEs would have been expected according to the chromosome's metaphase length. In this case, the inactive X was labeled by ^{3}H-thymidine incorporation during late S, and control experiments excluded the possibility that β-rays of ^{3}H-thymidine were responsible for the increased SCE frequency in the inactive X.

The inter- and intrachromosomal distribution patterns of SCEs in lymphocytes of patients with the Bloom syndrome (Schroeder 1975; Shiraishi and Sandberg 1977), Fanconi's anemia (Sperling et al. 1975, who reported the evaluation of only 88 SCEs, i.e., less than 2 per chromosome!), or ataxia telangiectasia (Galloway and Evans 1975) showed no significant differences as compared with the controls, despite a nearly twelve-fold increase in the spontaneous SCE frequency in Bloom syndrome cells (Shiraishi and Sandberg 1977).

The comparison of the rather different and in part contradictory results from 14 papers reporting intrachromosomal distribution of spontaneous SCEs in man reveals some methodological weak points:

1) The numbers of SCEs scored are often so small that the results cannot be reproduced (e.g., Sperling et al. 1975).

2) Occasionally the sizes of the chromosome regions being compared are insufficiently defined (e.g., "paracentromeric regions," "telomeric region," Kim 1974).

3) The results have not or only insufficiently been treated statistically (Kim 1974; Latt 1974a; Pathak et al. 1975; Sperling et al. 1975; Haglund and Zech 1979).

4) Microscopic resolution is rather poor when SCEs and Q- or G-banding patterns are demonstrated simultaneously or sequentially, and so some doubts remain as to the exact localization of SCEs in the respective $^+$bands, $^-$bands, or the regions between them (Latt 1974a; Kim 1974; Pathak et al. 1975; Morgan and Crossen 1977; Haglund and Zech 1979).

5) Some authors mentioned large deviations in SCE distribution between different individuals and stressed that sometimes the results were difficult to reproduce (Crossen et al. 1977).

A special contradiction awaiting elucidation is the occurrence of SCEs in centromeres. Tice et al. (1975) found no difficulty in distinguishing between real SCEs and mere twisting of sister chromatids; they evaluated cells after three DNA replication cycles in BrdU and found 18% of SCEs in centromeric regions.

The results reported by various authors differ substantially. Conformity is more or less confined to the observation of SCE clustering in weakly fluorescent or Giemsa-stained bands and their borders, but only Morgan and Crossen (1977) tried to prove this observation statistically.

Therefore the question of whether definite regions of the human karyotype that unequivocally and reproducibly deviate from random involvement in spontaneous SCEs really exist still awaits a final answer.

Cricetulus griseus (Chinese Hamster)

Data concerning intrachromosomal distribution of SCEs in this species are in part contradictory. Marin and Prescott (1964), using the cell line CHEF 125, found that in the third metaphase after a ^{3}H-thymidine pulse, 20% of the SCEs observed in chromosome 1 were located in the centromere. Stone et al. (1972) used the same technique plus an additional twofold synchronization of ovary cells (first at the beginning and then at the end of ^{3}H-thymidine incubation): They found that in the third and fourth mitosis after incubation, one-third of all SCEs occurred in centromeric regions, i.e., five times more than expected per unit length.

Rommeleare et al. (1973) subdivided chromosome 1 of cell line V 79 into five segments of equal length and reported that SCEs were distributed "relatively uniformly along the chromosome, although their frequency is slightly lower in the terminal segments; this agrees with the observations of Marin and Prescott (1964) showing that the centromeric region is the most affected." After BrdU incorporation into the chromosomes of the Don-cell line and differential staining of sister chromatids according to Korenberg and Freedlender (1974), Hsu and Pathak (1976) observed fewer SCEs than expected in chromosomes with a high proportion of C bands when compared with chromosomes of the same length but with less C-band material. For two marker chromosomes of cell line C 14 (derived from ovary cells) Ockey (1980) found a nonrandom distribution of SCEs, independent of the time of fixation (FPG technique). However, there was no clear-cut correlation to G- or C-banding, nor to the DNA late-replication pattern. SCE clusters appeared in the NOR and in 19 of 28 G-band borders. Strong involvement in SCEs was observed for 87% of the G^+ bands, 68% of their borders, and 59% of G^- bands. Centromeres had been excluded from evaluation in this study.

Mus musculus

Holmquist and Comings (1975) studied chromosomes of L cells by the FPG technique and found fewer SCEs than expected in the heterochromatin. The individual centromeres showed different SCE frequencies; some of them represented SCE hot spots. Just the opposite was reported by Lin and Alfi (1976) for RAG cells after differential staining with DAPI (4'-6-diamino-2-phenylindole). Inspecting cells fixed immediately after one round of replication in BrdU-containing medium, the authors found 1.82 SCEs per unit length in the asymmetric bands covering the pericentromeric heterochromatin (which makes up about 11% of the mouse genome). Only 0.27 SCEs per unit length were found in the euchromatic chromosome arms (89% of the genome) after two cell cycles in BrdU.

Rattus norvegicus

Popescu and DiPaolo (1977) used embryo cultures to investigate intrachromosomal SCE distribution in a sample of two hundred chromosomes 2. Of 156 SCEs 80.1% were located in four light G bands, which represent less than half of the metaphase chromosome length. By comparison with photographs of G-banded chromosomes it was further shown that 23.1% of the 156 SCEs involved region 2q24, a light G band like the other three SCE hot spots. Like these, it is free of heterochromatin (no C band material, no late-replicating DNA) and of NORs.

Microtus agrestis (European Field Vole)

The X chromosomes (77% of their chromatin is heterochromatin) of this species showed (FPG-stained slides) twice as many SCEs per unit length as the autosomes (Natarajan and Klašterska 1975). Data from in vivo experiments by Pera and Mattias (1976) showed a clearly higher SCE frequency per unit length not only in heterochromatic regions of X (1.1) and Y

chromosomes (0.75) but also in euchromatic regions of the X chromosome (1.2) when compared with the mainly euchromatic autosomes (0.1). However this statement is based on only 76 SCEs.

Microtus montanus

Hsu and Pathak (1976) used a modified Giemsa technique to study chromosomes of this species. They found only half the number of SCEs per unit length in the heterochromatic Y as in the X chromosome, with only a small heterochromatin content.

Peromyscus eremicus

The C-band-positive heterochromatin is preferentially localized in all short arms of the chromosomes of the cactus mouse (Schneider et al. 1980). After application of the FPG technique, 29.7% of 487 SCEs were found to involve the heterochromatic short arms (36.2% of the genome). Therefore the mainly euchromatic long arms (63.8% of the genome) showed a slight excess (70.3%) of all SCEs observed.

Muntiacus muntjak

For this species with only six chromosomes in the female and seven in the male (the chromosomes being easily distinguished from one another) Carrano and Wolff (1975) obtained the following results (FPG-stained slides): (1) The interchromosomal SCE distribution corresponded to the DNA content of each chromosome, and (2) in euchromatic chromosome regions the SCE frequency paralleled the DNA content; heterochromatic regions (especially the morphologically clearly recognizable heterochromatic neck of the X chromosome) showed significantly fewer, and their junctions to the euchromatin, significantly more SCEs than expected. It is of interest that morphologically these junctions belong to the heterochromatin fraction and that the frequency of SCEs for the large central part and the small peripheral parts of the heterochromatic neck together fit well the value of expectation (Carrano and Wolff 1975); this shows that the peripheral regions of heterochromatin contain many more SCEs than the central parts.

Cricetulus triton and Macropus parma

Kato (1979) found a pattern of intrachromosomal SCE distribution similar to that observed in the Indian muntjak for *Cricetulus triton* (663 SCEs localized) and for the marsupial *Macropus parma* (742 SCEs localized) when he compared photographs of C-banded and FPG-stained chromosomes.

Dipodomys ordii

In this species Bostock and Christie (1976) observed similar SCE frequencies in C-banded regions and in euchromatin; however two-thirds of the SCEs involving heterochromatin were located in the peripheral parts of heterochromatin, which makes up less than one-third of total heterochromatin.

Potorous tridactylis

According to autoradiographic investigations performed by Gibson and Prescott (1972), the centromeric regions showed 25% of all SCEs, and the remaining chromatin was randomly involved in SCEs.

From a comparison of all data concerning distribution patterns of spontaneous SCEs the conclusion is that no general pattern of intrachromosomal distribution is recognizable. This is true for the data from different species as well as for the results obtained by different researchers from a given species (see especially the data for man, Drosophila, Chinese hamster, and mouse, Tables 1 and 2).

Sometimes centromeres were found to react as SCE hot spots. However, reliable data concerning SCE clustering in centromeres can be obtained only from the third and fourth mitoses after incubation in ^{3}H-thymidine or BrdU.

NORs may, though seldom, show SCE clustering (*Vicia faba,* Chinese hamster).

Different approaches have been used to look for correlations between SCE frequency and certain heterochromatin fractions or banding patterns:

1) Comparisons between chromosomes containing large amounts of heterochromatin and those showing small or no detectable heterochromatin portions have been made (e.g., Hsu and Pathak 1976). But such comparisons are not very exact, and the interferences should be viewed with caution. Usually, it remains an open question whether the whole chromosome or only certain of its regions show nonrandom involvement in SCEs [compare data for sex chromosomes of *Microtus agrestis* from Natarajan and Klašterska (1975) and from Pera and Mattias (1976)]. When SCE clustering occurs, the important question as to whether it involves the heterochromatin itself or its neighboring regions remains unsolved. When SCE clustering in one region of the chromosome is compensated for by a lack of SCEs in another (Carrano and Wolff 1975), both phenomena will be left undetected.

2) Some results were obtained by simultaneous or sequential demonstration of SCEs and certain banding patterns. However, the exact localization of SCEs in light or dark bands or their borders is not without problems, since microscopic resolution of most banding patterns is inadequate for this purpose.

3) Most frequently, the position of SCE sites in relation to the banding patterns was determined from photographs of appropriately stained chromosomes (Bostock and Christie 1976; Kato 1979; Ockey 1980) or on the basis of a subdivision of the chromosomes into segments, allowing SCEs and heterochromatin fractions to be localized in definite chromosome regions (Schweizer 1973b; Galloway and Evans 1975; Crossen et al. 1977; Schvartzman and Cortes 1977; Friebe 1978; Schubert et al. 1979a, 1980). But even the photographic standardization of chromosome length does not completely exclude some uncertainties, since the degree of contraction between individual chromosomes or different chromatin regions is not necessarily the same.

4) The frequency of SCEs in heterochromatin may be measured in asymmetric bands immediately after one DNA replication in BrdU (Lin and Alfi 1976) and compared with the overall SCE frequency after another cell cycle with or without BrdU (provided the heterochromatin AT content in the two DNA strands is uneven).

5) However the most impressive results came from subjects with heterochromatin that could be recognized morphologically with the FPG technique [the neck of the X chromosome of Indian muntjak (see Carrano and Wolff 1975); the short arms of cactus mouse chromosomes (see Schneider et al. 1980)].

The results concerning intrachromosomal distribution of spontaneous SCEs, which (the relative proportion of SCEs in

Table 2. Selected chromosome regions of animal (for man see Table 1) and plant species showing nonrandom involvement in spontaneous SCEs[a]

Species	Centro-mere	NOR	Constitutive hetero-chromatin[b]	Eu hetero-chromatin junctions	G^+ bands	G^- bands	Method of SCE detection[d]	References
Vicia faba	+Chr.1	+					AR	Schweizer 1973b[c]
Vicia faba							FPG	Vosa 1976[c]
Vicia faba		+					FPG	Schubert et al. 1979a
Allium cepa			−				FPG	Schvartzman and Cortes 1977[c]
Secale cereale			−	+			FPG	Friebe 1978[c]
Hordeum vulgare							FPG	Schubert et al. 1980[c]
Haplopappus gracilis	+						AR	Sparvoli and Gay 1973a
Bellevalia romana	+						AR	Sparvoli and Gay 1973b[c]
Drosophila melanogaster			−	+			FPG	Dolfini 1978[c]
Drosophila melanogaster			+	+			FPG	Gatti et al. 1979[c]
Cricetulus griseus (CHEF 125)	+						AR	Marin and Prescott 1964
Cricetulus griseus (CHO)	+						AR	Stone et al. 1972
Cricetulus griseus (V79)	(+)						AR	Rommeleare et al. 1973[c]
Cricetulus griseus (Don)			−				See Korenberg and Freedlender 1974	Hsu and Pathak 1976
Cricetulus griseus (C14)		+		(+)	(+)	(+)	FPG	Ockey 1980[c]
Mus musculus (L-cells)	(+)		−				FPG	Holmquist and Comings 1975[c]
Mus musculus (RAG-cells)			+				DAPI	Lin and Alfi 1976[c]
Rattus norvegicus						+	See Korenberg and Freedlender 1974	Popescu and DiPaolo 1977
Microtus agrestis			+				FPG	Natarajan and Klašterska 1977
Microtus agrestis (in vivo)			+				FPG	Pera and Mattias 1976[c]
Microtus montanus			−				Modified Giemsa staining	Hsu and Pathak 1976[c]
Peromyscus eremicus			−				FPG	Schneider et al. 1980
Dipodomys ordii			−	+			FPG	Bostock and Christie 1976
Muntiacus muntjak			−	+			FPG	Carrano and Wolff 1975
Macropus parma			−	+			FPG	Kato 1979
Cricetulus triton			−	+			FPG	Kato 1979
Potorous tridactilis	+						AR	Gibson and Prescott 1972

[a] +, SCE clustering; (+), SCE clustering weakly expressed or true only for certain conditions; −, less SCEs than expected
[b] C-band regions and late replicating regions
[c] For additional remarks see text
[d] AR, autoradiography; FPG, fluorescence-plus-Giemsa technique

centromeres excluded) describe relationships between nonrandom SCE distribution and heterochromatin may be divided into two groups: One group of results shows, despite occasional nonrandom SCE distribution, no general correlation between SCEs and constitutive heterochromatin (Vosa 1976; Schubert et al. 1979a, 1980; Ockey 1980). The majority of results of the other group describe a diminished frequency of SCEs in heterochromatin (or at least in its central parts; Carrano and Wolff 1975; Galloway and Evans 1975; Holmquist and Comings 1975; Bostock and Christie 1976; Crossen et al. 1977; Schvartzman and Cortes 1977; Shiraishi and Sandberg 1977; Dolfini 1978; Friebe 1978; Kato 1979; Schneider et al. 1980) and often an additional clustering of SCEs at the junctions between eu- and heterochromatin (Carrano and Wolff 1975; Bostock and Christie 1976; Crossen et al. 1977; Dolfini 1978; Friebe 1978; Gatti et al. 1979; Kato 1979).

For the rat (Popescu and Di Paolo (1977) and in some papers on human chromosomes, SCE clustering was reported to occur in faint Q or G bands and at their borders (Latt 1974a; Pathak et al. 1975; Morgan and Crossen 1977; Haglund and Zech 1979). Very seldom were the C-band regions themselves reported to represent SCE hot spots (Kim 1974; Lin and Alfi 1976; Gatti et al. 1979).

3. Relationships Between the Distribution Patterns of Mutagen-Induced SCEs, Mutagen-Induced Chromatid Aberrations, and Heterochromatin

As a rule, mutagen-induced chromatid aberrations are not randomly distributed along the metaphase chromosome com-

plements. Up to now this is true for all subjects tested for aberration distribution patterns after the application of various mutagenic agents.

Especially mutagen treatment with delayed effects (due to the S-phase dependency of their clastogenic action) results in very pronounced aberration clustering in certain chromosome regions (sometimes specific for a given mutagen) (Schubert and Rieger 1977).

In *Vicia faba,* potential aberration hot spots are those chromosome segments in which the positions of Giemsa marker bands (C bands, according to Greilhuber 1977) and of late-replicating DNA coincide (Döbel et al. 1978; Rieger and Michaelis 1972; Rieger et al. 1975, 1977; Schubert and Rieger 1976, 1977; Kaina et al. 1979). For the large heterochromatin blocks characterized by these features the majority of aberration breakpoints were located inside the Giemsa marker bands. In regions containing tightly stacked bands, the majority of aberration breakpoints occurred at the junctions between $^{+}$bands and $^{-}$bands (Döbel et al. 1978).

The expression of aberration clustering in *Vicia faba* may be influenced, at least in part, both by the mutagen being used (mutagen specificity) and/or by the position in the genome of the potential hot spot in question (karyotype specificity) (Rieger et al. 1975, 1977; Kaina et al. 1979; Schubert et al. 1979b; Michaelis et al., in press).

From these observations of aberration distribution the following questions arise as to the chromosomal distribution of mutagen-induced SCEs: Are mutagen-induced SCEs distributed like spontaneous SCEs (i.e., nearly randomly in the case of *Vicia faba*) or preferentially as observed for induced chromatid aberrations? In other words: Do heterochromatin-containing potential aberration hot-spot segments also represent SCE hot-spot segments? Do the distribution patterns of mutagen-induced SCEs show a mutagen specificity similar to that of the distribution patterns of chromatid aberrations? Can karyotype reconstruction influence SCE distribution patterns?

The answers to these questions should make clear whether the outstanding "mutagen sensitivity" of certain heterochromatin-containing chromosome segments and the factors influencing their reaction hold true for both mutagen-induced SCEs and chromattid aberrations. Additionally, similar distribution patterns, if existent, might be taken as a hint that the two phenomena have common origins, while significant differences would point in the opposite direction.

3.1. The Intrachromosomal Distribution of Mutagen-Induced SCEs in Vicia faba

Treatment with EA, MH, MMC, or long-wave UV light ($\lambda = 320–380$ nm) about one cell cycle before fixation was found to increase by more than 100% the frequency of SCEs in *Vicia faba* chromosomes with unifilar BrdU substitution (Kihlman et al. 1977; Schubert et al. 1979a). While EA, MH, and MMC resulted in highly significant clustering of chromatid aberrations in heterochromatin-containing chromosome segments with a clearly mutagen-specific expression of individual aberration hot spots and with position-dependent expression in one case (after MMC treatment), the SCEs induced by the same mutagens were distributed like those observed without additional mutagen treatment (Schubert et al. 1979a), i.e., randomly distributed except in the NOR. The only difference from the distribution of spontaneous SCEs was a decrease of the hot spot character of the NOR. The reason for this is that since the NOR is so small, maximally two SCEs are resolvable, but ten or even more are resolvable in the other chromosome segments. Therefore the SCE hot spot character of the NOR decreases relatively with increasing SCE frequency per cell.

Long-wave UV light, which has no clastogenic effect in non-BrdU-substituted chromosomes, strongly increased SCE frequency in BrdU-substituted *Vicia faba* chromosomes when given during S phase. An analogous increase of the frequency of chromatid aberrations was observed after irradiation with long-wave UV light during S or G_2 (Kihlman et al. 1977, 1978). Contrary to chromatid aberrations induced by long-wave UV light, which were clustered in heterochromatin-containing segments, the SCEs observed after long-wave UV irradiation (Schubert, unpublished) were distributed like SCEs induced by EA, MH, or MMC, i.e., randomly, in proportion to length, again with the exception of the NOR.

From these results obtained in *Vicia faba* it may be concluded that unlike mutagen-induced chromatid aberrations, mutagen-induced SCEs are distributed like spontaneous SCEs, i.e., randomly (except for the NORs, which show up as SCE hot spots in all cases), for the individual chromosome segments into which the *V. faba* karyotype had been subdivided. There was no preferential involvement in SCEs of heterochromatin-containing segments, no mutagen-specific and no karyotype-specific SCE clustering. Differential exertion of influence by various factors (e.g., heterochromatin content of segments, type of mutagen used, effects of segment transposition) on intrachromosomal distribution patterns of mutagen-induced chromatid aberrations and on SCEs may be used as an argument against a common mechanism of origination of chromatid aberrations and SCEs (Schubert et al. 1979a).

The detuction that the initial lesions which lead to SCEs and chromatid aberrations might also be different (Wolff et al. 1977; Wolff and Carrano 1979) and responsible for the different distribution patterns is rather improbable (at least in the case of *Vicia faba*), since the mode of action of long-wave UV light on BrdU-substituted DNA is rather well known (Hutchinson 1973): The photolytic debromination eventually results in one type of lesion, the single-strand break. This lesion is apparently responsible for the induction of SCEs during S and of chromatid aberrations during S and G_2.

To overcome the objection that (though SCE distribution no doubt is less specific than that of chromatid aberrations) a different involvement of *V. faba* eu- and heterochromatin in SCEs might exist but remains undetectable due to the "rough" subdivision of the karyotype into 28 segments (most of which are many times larger than, for example, the Giemsa marker bands), we performed a more detailed analysis of SCE distribution in chromosome 3 of the karyotype ACB. The long arm of this reconstructed chromosome 3 consists mainly of the satellite arm of the metacentric chromosome 1 of the standard karyotype (reciprocal translocation).

This chromosome is of special interest since the proximal half of the region between the centromere and NOR harbors two Giemsa marker bands, two late-replicating regions, two Q^{+} bands (Döbel et al. 1978), and two asymmetric bands (the latter indicating uneven distribution of adenine and thymidine between the DNA strands when stained by the FPG technique immediately after incubation for one cell cycle in BrdU medium; see Schubert and Rieger 1979). After treatment with MMC this region reacts as a very pronounced hot spot for chromatid aberrations. Furthermore, this chromosome contains the NOR, which after MMC treatment also shows aber-

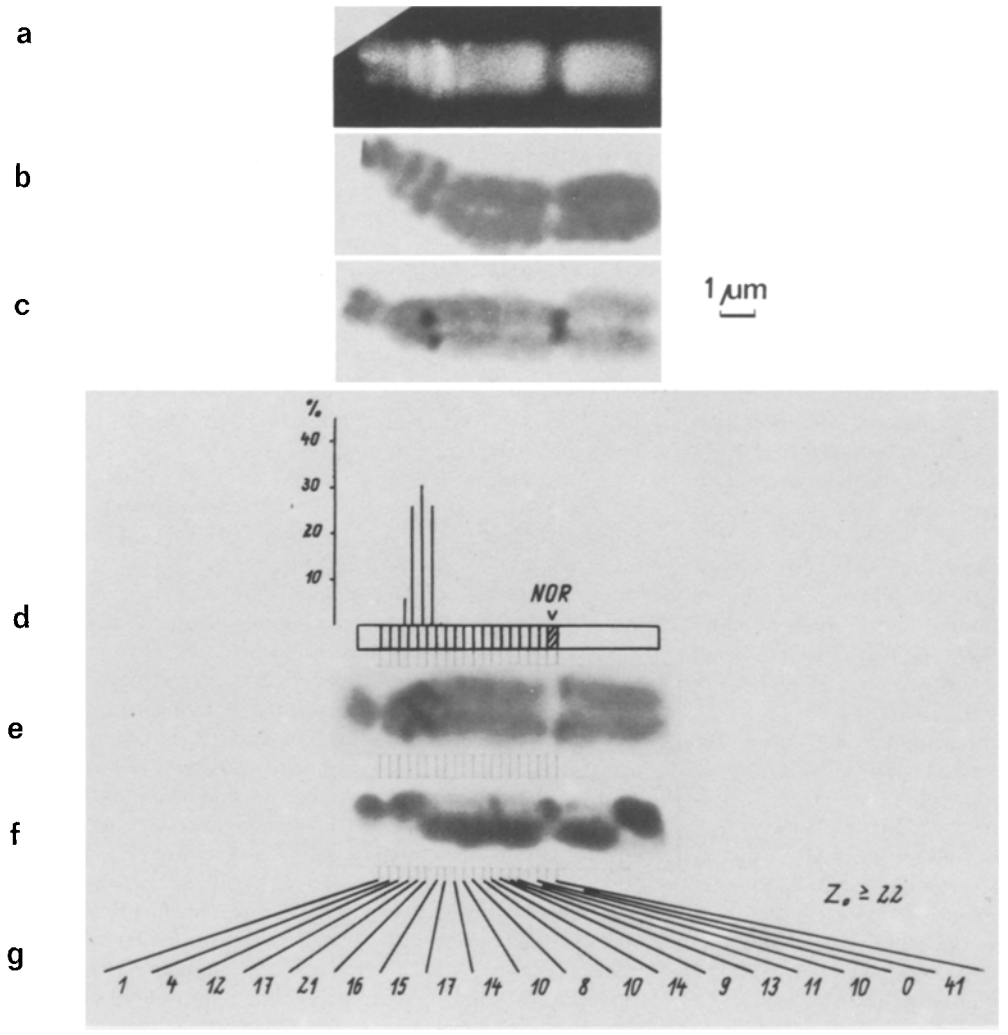

Fig. 1. **a** Chromosome 3 of the reconstructed *Vicia faba* karyotype ACB with Q-banding (method of Döbel et al. 1978); **b** Late replication indicated by two light bands after pulse labeling with BrdU at late S and FPG staining (method of Döbel et al. 1978); **c** Two asymmetric bands showing uneven A-T content of DNA strands (after about one round of replication in BrdU medium and FPG staining); the symmetric band in the NOR is indicative of early DNA replication in this region (method of Schubert and Rieger 1979); **d** Schematic representation of subdivision into 19 segments of chromosome 3 starting from centromere and terminating with the secondary constriction (NOR). The columns show participation of segments in the proximal Giemsa-marker bands as determined from a sample of 35 photographically standardized chromosomes; **e** Two Giemsa-marker bands in the proximal half of the long chromosome arm and one band in the distal part of the NOR (method of Döbel et al. 1978); **f** Chromosome 3 with SCEs (method of Schubert et al. 1979a); **g** Involvement of the 19 segments in 243 MMC-induced SCEs (from 65 photographically standardized chromosomes). Z_0, upper confidence limit (1% level) of random SCE distribition (for statistics see Kaina et al. 1979)

ration clustering and represents the only hot spot of spontaneous as well as mutagen-induced SCEs in *Vicia faba.*

Starting from the centromere and ending with the NOR, we subdivided this part of chromosome 3 into 19 segments of equal lengths, each representing about 0.5% (roughly the size of a Giemsa marker band) of the total metaphase genome length (Fig. 1). Then the relative share of individual segments in the two proximal Giemsa marker bands was determined by means of standardized photographs from 35 Giemsa-banded chromosomes 3 (for banding procedure see Döbel et al. 1978).

From 65 MMC-treated and FPG-stained chromosomes (for methods see Schubert et al. 1979a) the involvement of the 19 segments in 243 MMC-induced SCEs was measured, again by means of standardized photographs (see Fig. 1). Using a formula described by Kaina et al. (1979), we found that at the 1% level of significance, no segment except the NOR surpassed the upper confidence limit (22 SCEs per segment) for random participation in SCEs.

This result clearly shows that

1) In *Vicia faba,* there is no correlation between a nonrandom segment involvement in SCEs and the presence of Giemsa marker bands (or other banding structures characterizing heterochromatin, see Fig. 1).
2) MMC-induced SCEs are, contrary to MMC-induced chromatid aberrations, not clustered in heterochromatin-containing chromosome regions.
3) The NOR is a significant SCE hot spot. It seems improbable that the heterochromatin fractions surrounding the NOR are responsible for SCE clustering in the secondary constriction, since no such correlation has been detected in any other region of the karyotype. Up to now, no convincing explanation for this observation is at hand.

3.2. *Distribution Patterns of Mutagen-Induced SCEs and Chromatid Aberrations. Comparison of the Results Obtained in Vicia faba with Data Available from Other Species*

BrdU. Schneider et al. (1980) compared the intrachromosomal distribution patterns of BrdU-induced chromatid aberrations with SCE distribution in chromosomes of the cactus mouse *Peromyscus eremicus:* 2.55% of 2470 cells contained a total of 63 aberrations (7 chromatid translocations, 14 chromatid breaks, and 42 gaps). Of the aberration breakpoints, 56 were located in an extremely BrdU-sensitive region of the long arm of a submetacentric chromosome. This region showed neither characteristics of heterochromatin nor SCE clustering.

DMBA, TMBA. Popescu and DiPaolo (1977) compared the distribution patterns of DMBA- and TMBA-induced SCEs and chromatid aberrations with the intrachromosomal distribution of spontaneous SCEs in chromosomes 1 and 2 of cultivated rat cells. Of 156 SCEs in two hundred chromosomes 2 from a control experiment, 80.1% occurred in four light G bands; 23.1%, in one of these bands (2q24). The same four G bands showed 82.4% of the 216 DMBA-induced SCEs; 30.1% of these were located in region 2q24. Of 82 DMBA-induced chromatid aberrations (mostly gaps), 96.3% were localized in the above-mentioned four G^- bands; however, 59.8% of these involved region 2q24. This region was also found to be an aberration hot spot when other mutagens were applied. Similar results have been obtained with TMBA.

Ueda et al. (1976) studied the distribution pattern of chromatid aberrations (~84% gaps and ~16% breaks) in vivo and of SCEs in tissue cultures of the rat using the same mutagens as Popescu and DiPaolo (1977). In these experiments, two aberration hot spots were observed in chromosome 2 after DMBA-treatment; each of these contained more than 30% of the aberrations scored. The same regions (and an additional one) also showed SCE clustering (in this case, however, each of these contained only about 10% of a total of 34 SCEs).

DMBA treatment in these experiments started as late as 6 h before fixation, the frequency of SCEs per cell (11.5) was very low, and control experiments without mutagen were not performed. Therefore some doubts remain as to whether the low number of SCEs observed was really induced by the mutagen. (Popescu and DiPaolo found a spontaneous SCE frequency of 13.6 per cell!)

MMS. Ockey (1980) compared the distribution patterns of MMS-induced and spontaneous SCEs in two marker chromosomes of synchronized cells of the Chinese hamster cell line C 14. In spite of a nearly threefold increase of SCE frequency after MMS treatment, the distribution patterns were fairly similar. Only regions that were just replicating during the MMS pulse showed increased involvement in SCEs (exception: the late-replicating C-band region of marker chromosome 2).

Haglund and Zech (1979) observed very similar distribution patterns of spontaneous and MMS-induced SCEs in chromosomes of human lymphocytes.

MMC. Lin and Alfi (1976) studied the distribution of spontaneous and MMC-induced SCEs and chromatid aberrations in mouse chromosomes (RAG cells). After one cell cycle in BrdU they observed 1.82 SCEs per unit length in the asymmetric bands of pericentromeric heterochromatin and, after two rounds of replication in BrdU, 0.27 SCEs per unit length in euchromatic arms. Additional treatment with MMC increased SCE frequency in asymmetric bands to 7.27 and in the remaining chromatin to 6 SCEs per unit length. Simultaneously, chromosome breaks were found in 10% of the metaphases and localized mainly in the centromeric regions.

The intrachromosomal distribution of MMC-induced SCEs was investigated in chromosomes of the Indian muntjak *(Muntiacus muntjak)* by Carrano and Johnston (1977). As in control experiments without MMC (see Carrano and Wolff 1975), the participation of euchromatic regions after MMC treatment corresponded to their DNA content. At low doses of MMC, the X-chromosomal heterochromatin showed fewer SCEs than expected; at higher doses the number observed was close to the expected. At the same time, however, SCE frequency in the short arm of the X chromosome decreased when compared with the controls. As in these controls, the junctions of X-chromosomal heterochromatin were more frequently involved in SCEs than the central parts ("value of saturation": 0.5 SCEs per junction at higher doses). Huttner and Ruddle (1976) localized the breakpoints of 59 MMC-induced chromatid translocations (37 of these involved identical sites in homologous chromosomes) on chromosomes of the Indian muntjak. Of 118 translocation breakpoints 85 were located in constitutive heterochromatin, and 63 (53.4%!) at the q-junction of the X-chromosomal heterochromatic neck. The same authors observed that most of the translocation breakpoints outside constitutive heterochromatin occurred in G^--band regions.

Latt (1974b) and Shiraishi and Sandberg (1978) examined the intrachromosomal distribution pattern of MMC-induced SCEs in human lymphocyte chromosomes. In spite of a nearly sixfold increase of SCE frequency, Latt (1974b) found that the SCE distribution corresponded exactly to that of the controls (SCE clustering in faint Q bands and at the junctions between bright and dull fluorescing regions; see Latt 1974a). MMC-induced chromatid breaks were similarly distributed, but breakpoints of chromatid translocations were distributed much more preferentially, occurring mainly in the pericentromeric regions of chromosomes 1, 9, and 16 (Latt 1974b).

Shiraishi and Sandberg (1978) reported a distribution pattern of MMC-induced SCEs similar to the pattern of spontaneous SCEs in cells of patients with the Bloom syndrome. In good agreement with the data of Latt (1974b) their data showed MMC-induced chromatid breaks to be similarly distributed, while breakpoints of chromatid translocations were located preferentially in chromosomes 1, 9, and 16.

Comparing these data on distribution patterns of mutagen-induced SCEs (see Table 3), one arrives at the following conclusions:

1) Independent of the species-specific differences in distribution of spontaneous SCEs and independent of the mutagen being used, the intrachromosomal distribution patterns of mutagen-induced SCEs are very similar to those of spontaneous SCEs in a given species.

2) Chromatid aberrations obtained after treatment with the same mutagens are much more preferentially distributed (mostly at the borders between eu- and heterochromatin) than SCEs (this is true especially for the breakpoints of chromatid translocations). Even in cases where SCE and aberration clusters occupy identical regions of the chromosomes (Ueda et al. 1976; Lin and Alfi 1976; Popescu and DiPaolo 1977), the expression of aberration hot spots is much more pronounced.

3) Heterochromatin-containing regions are less preferred sites for SCEs than for chromatid aberrations.

Table 3. Comparison of involvement in spontaneous SCEs, mutagen-induced SCEs, and mutagen-induced chromatid aberrations of selected chromosome regions from different animals and *Vicia faba*[a]

Species	Phenomenon	Centro-mere	NOR	Constitutive hetero-chromatin[b]	G^+ or Q^+ bands	G^- or Q^- bands	Junc-tions	Other regions	References
Vicia faba	SCE spontaneous		+						Schubert et al. 1979a[c]
	SCE EA MH MMC Long-wave UV light		+						
	Chromatid aberrations (EA, MH, MMC, long-wave UV[j])		+	+++					
Peromyscus eremicus	SCE spontaneous			–					Schneider et al. 1980[d]
	Chromatid aberrations (BrdU induced)							+++	
Rattus norvegicus	SCE spontaneous					+			Popescu and Di Paolo 1977[e]
	SCE DMBA TMBA					+			
	Chromatid aberrations DMBA TMBA					+++			
	SCE DMBA TMBA					(+)			Ueda et al. 1976[f]
	Chromatid aberrations DMBA TMBA					(++)			
Cricetulus griseus (C^{14} cells)	SCE spontaneous		+		(+)	(+)	(+)		Ockey 1980[g]
	SCE MMS		+		(+)	(+)	(+)		
Homo sapiens	SCE spontaneous	+(20%)			–(13%)	+(76%)	(11%)		Haglund and Zech 1979
	SCE MMS	+			–	+			
	SCE spontaneous					+	+		Latt 1974a
	SCE MMC					+	+		
	Chromatid breaks MMC					+	+		Latt 1974b
	Chromatid translocations MMC			+++					
	SCE Bloom syndrome	–		–					Shiraishi and Sandberg 1977
	SCE MMC	–		–					Shiraishi and Sandberg 1978
	Chromatid translocations MMC			+++					
Mus musculus	SCE spontaneous			+					Lin and Alfi 1976[h]
	SCE MMC			+					
	Chromatid aberrations MMC			++					
Muntiacus muntjak	SCE spontaneous			–			+		Carrano and Wolff 1975
	SCE MMC			–			+		Carrano and Johnston 1977
	Chromatid aberrations MMC					(+)	+++		Huttner and Ruddle 1976[i]

[a] +, Clustering of SCEs; –, less SCEs than expected; +++, very pronounced clustering of chromatid aberrations; (+), clustering of SCEs or aberrations weakly expressed or true only under certain conditions
[b] C bands and late-replicating regions.
[c] Aberration clustering in chromosome segments containing constitutive heterochromatin.
[d] Aberration clustering in an especially BrdU-sensitive region without other special features.
[e] Aberration clustering especially in one of the G^- bands, which is also sensitive to other mutagens.
[f] Aberration and SCE clustering not attributed to special banding structures (whether or not the SCEs are mutagen-induced is ambiguous).
[g] G^+, G^- bands and junctions between them may contain SCE clusters.
[h] SCE frequency after one cycle in BrdU in asymmetric bands as compared with SCE frequency of the total genome after two cycles in BrdU.
[i] Aberration clustering especially at the q end of the heterochromatic neck of the X chromosome.
[j] Schubert unpublished

4) The same is true for the distribution patterns of spontaneous SCEs and spontaneous chromatid aberrations in lymphocyte chromosomes of Bloom syndrome patients (Schroeder 1975; Shiraishi and Sandberg 1977) and probably also in those of patients with Fanconi's anemia. Though in Fanconi's anemia 29% of the aberration breakpoints coincided with positions of SCEs, aberrations were found to be much more preferentially distributed than SCEs (Dutrillaux et al. 1977). According to Sperling et al. (1975), the latter are distributed like spontaneous SCEs in normal lymphocytes.

5) The fundamental differences between the distribution of mutagen-induced SCEs and that of mutagen-induced chromatid aberrations support the inference, based on the experiments of SCE and aberration distribution in *Vicia faba* and on other types of investigation, that in all probability SCEs and chromatid aberrations originate by different molecular mechanisms (Schubert et al. 1979a). However, a small proportion of chromatid aberrations may arise via "incomplete" SCEs (Schubert and Meister 1979).

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Note Added in Proof

Since this paper was submitted we became aware of the following data:

1. J. J. Hoo and M. I. Parslow ("Relation between the SCE points and the DNA replication bands." Chromosoma 73:67–74, 1979) found in human lymphocyte chromosomes 191 of 285 SCEs in replicating G bands and only 94 in late replicating G^+ bands; 4 of 14 SCEs were localized in the facultative heterochromatic X chromosome.

A. Sono and K. Sakaguchi ("The distribution of sister chromatid exchanges and chromosomal aberrations induced by 5-fluorodeoxyuridine and ethylmethanesulfonate in the euchromatin and heterochromatin of Chinese hamster cells." Cell Struct Funct 5:175–182, 1980) found fewer spontaneous and induced SCEs than expected in constitutive heterochromatin while the same regions were hot spots for chromatid aberrations.

3. Clustering of spontaneous SCEs was observed at euchromatin-heterochromatin junctions of *Allium cepa* (F. Cortes "Occurrence on sister chromatid exchanges at euchromatin-C-band junctions in *Allium cepa* chromosomes." Experientia 36:1290–1291, 1980) and *Ornithogalum* (P. Ambros and D. Schweizer: personal communication).