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Nonrandom Distribution of Sister Chromatid Exchanges in the Chromosomes of Three Mammalian Species

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ABSTRACT—The frequency and distribution of mitomycin C (MMC)-induced sister chromatid exchanges (SCEs) were investigated in the fibroblast chromosomes of three mammalian species, *Microtus montebelli, Apodemus argenteus* and *Chimarrogale himalayica*, by the fluorescence-plus-Giemsa (FPG) and C-band staining methods, paying special attention to the large C-band area (C-block)-carrying and/or nucleolus organizer region (NOR)-carrying chromosomes. The junctions of heterochromatin and euchromatin (HE-junctions) and NORs were found to be "hot spots" of SCEs in all the species examined: their SCE frequencies were 35.3% and 24.2% in the HE-junction of the X chromosomes of *M. montebelli* and *A. argenteus*, and 16.7% and 17.8% in the NORs of the No. 1 chromosomes of *M. montebelli* and *C. himalayica*, respectively. In *M. montebelli* and *A. argenteus* the SCE frequency was apparently lower in the C-block region than in the euchromatic one, when compared with each other based on equal length, while in *C. himalayica* no such marked difference in the SCE frequency was found between these two regions of the chromosome. These findings may indicate that occurrence of SCEs is significantly suppressed in the C-block region of *M. montebelli* and *A. argenteus*, but not in that of *C. himalayica*. In addition, the C-blocks of the No. 1 homologue of *C. himalayica* showed a highly varied individual-to-individual heteromorphism in length. The biological implication of SCEs was discussed in connection with the generation of heteromorphism.

INTRODUCTION

Sister chromatid exchange (SCE) is a cytogenetic phenomenon reflecting misrepair in the process of cutting and subsequent rejoining of DNA strands packed in chromosomes [12]. Thus, SCE is regarded as a product caused by intrachromosomal chromatid exchange. In this sense, SCE is essentially distinguished from so-called crossing-over in meiosis, i.e., chromatid exchange between homologous chromosomes. If an SCE takes place at a homologous locus of a given chromosome, the result is genetically neutral. However, if an unequal SCE occurs between sister chromatids, the resultant daughter cells should sustain partial gain or partial loss of DNA involved in the SCE. Large C-band areas (C-blocks) and nucleolus organizer regions (NORs) generally consist of highly repeated sequences and rRNA gene clusters, respectively [8, 34]. Because of this unique nature of base sequences, these chromosomal regions could be particularly susceptible to the production of heritable variation by SCE formation or by unequal crossing-over.

Several reports of nonrandom intrachromosomal SCE distribution suggest the uneven distribution is related to the chromosomal G- and C-bands and also to particular sites such as HE-junctions and NORs. Both spontaneous and MMC-induced SCEs occur more frequently at the HE-junctions than at the non-junction regions in Indian muntjac chromosomes [3]. Similar findings have been obtained in several species of mammals including human [2, 6, 13, 14, 21]. In some species the SCE frequency is lower in the heterochromatic regions than in the euchromatic regions [2, 3, 13, 24], whereas in other species the heterochromatic regions show a higher frequency of SCEs [15, 18]. As yet, no convincing rule has been obtained for the nonrandom involvement of C-blocks in both spontaneous and chemical-induced SCEs. More information from a variety of species carrying typical C-blocks is needed.

In the present study, the frequencies of MMC-induced SCEs in the heterochromatic and euchromatic regions, the HE-junctions and NORs are investigated by the FPG method in the fibroblasts of three mammalian species of Japan: grass voles, long-tailed field mice and water shrews, all of which are known to carry unusually large C-blocks on either the X chromosome or the No. 1 homologue [19, 20, 31, 33]. The aims of this study are not only to determine whether or not the HE-junctions and NORs are SCE hot spots, but also to inquire into the biological significance of SCE in the C-blocks of these species of mammals.

MATERIALS AND METHODS

Animals

The grass voles (*Microtus montebelli*), the long-tailed small field mice (*Apodemus argenteus*) and the water shrews (*Chimarrogale himalayica*, formerly *C. platycephala*) were used in the present study. The former two belong to the family Muridae (Rodentia) and the latter one to the family Soricidae (Insectivora). Except for five laboratory-reared albino grass voles, all animals used were caught in orchard and mountain areas of Aomori Prefecture, Japan. Collecting sites and the number of individuals examined are as follows; *M. montebelli*: 3 males and 2 females from Hirosaki, *A. argenteus*: 2

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males and 4 females from Hirosaki, and *C. himalayica*: 2 males and 2 females from the Nakano river, Kuroishi.

Cell culture and chromosome preparation

Fibroblast cultures were performed with the lung tissue of each specimen. Cells were grown for 1 to 2 weeks in TC199 medium (containing 100 units/ml of penicillin and 100 μg/ml of streptomycin) supplemented with 20% fetal calf serum at 37°C under humidified 5% CO_2-95% air, and harvested at the primary culture or first subculture stage. To visualize SCEs, cells were grown in the dark to prevent photolysis of BrdU for two or three cell cycles (60–66 hr) in the presence of both 0.1 μg/ml BrdU and 1.0×10^{-7}M MMC at final concentration (see RESULTS). After colchicine block (0.5 μg/ml, final 1 hr of culture), cells were harvested by trypsin (0.25%) digestion for 5 min at 37°C. Chromosome preparations were made with hypotonic treatment (0.075 M KCl, for 20 min), fixation with Carnoy's fixative (methanol : acetic acid=3:1) and conventional air-drying.

Differential staining of sister chromatids

Chromosomes were differentially stained according to the FPG method of Wolff and Perry [29] with modifications in the mounting solution and light condition after mounting. The staining procedure is as follows: the chromosome preparations were immersed in the Hoechst 33258 solution (50 μg/ml) for 15 min, rinsed briefly in distilled water and air-dried. Each preparation was then temporarily mounted with a cover slip in PBS (pH 6.8), and exposed to black light blue fluorescent light (Toshiba FL20SBLB). Approximately 30 min later, the cover slips were removed and the preparations were incubated for 30 min in 2×SSC at 60°C, briefly rinsed and stained with 4% Giemsa in Sörensen's phosphate buffer (pH 7.0) for 10–12 min.

G-, C- and NOR-banding and fluorescent staining

After inspecting FPG-stained metaphases under microscope and photographing well-spread metaphases, the Giemsa stain was removed with Carnoy's solution, and followed with C-band staining. This sequential staining technique facilitated identification of the target chromosomes in the diploid complements and exact recognition of the HE-junctions of chromosomes. For production of G- and C-bands and NORs, the ASG method of Sumner *et al.* [27], the BSG method of Sumner [26] and the one step silver method of Howell and Black [9] respectively were adopted. If necessary, quinacrine mustard dihydrochloride (QM) or chromomycin A_3 (CMA) staining was applied. The former method (AT-base specific fluorescent staining) was done according to Caspersson *et al.* [4], and the latter (GC-base specific fluorescent staining) according to Amemiya and Gold [1].

RESULTS

BrdU-labelling time

BrdU-substituted metaphases were classified as 1st, 2nd and 3rd divisions according to the criteria of Crossen and Morgan [5]. The most efficient time of treatment with BrdU to obtain 2nd division metaphase cells was determined to be approximately 60 hr in *M. montebelli* by the differential labelling method [28]: by 60 hr after addition of BrdU, the 2nd division cells amounted to more than 50% of metaphases without MMC. Similarly, treatment times for *A. argenteus* and *C. himalayica* were determined to be 60 and 66 hr, respectively.

Effects of MMC on SCEs

In all species examined, less than 10 SCEs per cell occurred in the presence of 0.1 μg/ml BrdU at final concentration. These SCEs were regarded as "spontaneous" [12]. Under these conditions the frequency of SCEs occurring in the target chromosomes, i.e., the X chromosomes of *M. montebelli* and *A. argenteus* and the No. 1 chromosomes of *M. montebelli* and *C. himalayica*, was too low to get sufficient number of SCEs. In order to increase the frequency of SCEs, the SCE-inducing antibiotic MMC was added to the culture at a final concentration of 1.0×10^{-7}M together with BrdU according to Yamagata *et al.* [30]. With this treatment, the SCE-frequency per cell was increased more than 3 times over that of the control. A concentration of 1.0×10^{-7}M MMC lowered the mitotic index but still assured a reasonable number of mitotic cells necessary for effective SCE counting.

Karyological characteristics of three mammalian species

Microtus montebelli: The specimens examined had the diploid number of 30, in agreement with earlier reports [31]. Most of C-heterochromatin was localized on the sex chromosomes, X and Y (Fig. 1, inset). The X chromosome was a

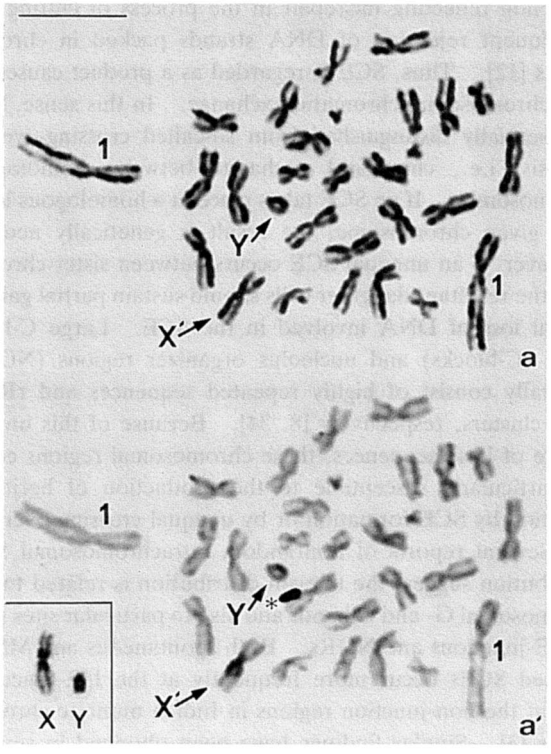

FIG. 1. FPG-stained (a) and C-banded (a') profiles of the same metaphase in the second division from an albino individual of *M. montebelli*. The bar represents 10 μm. X, X chromosome; X', variant X chromosome; Y, Y chromosome; 1, No. 1 chromosome; *, debris (not C-band). Inset: X and Y chromosomes from a wild male with a normal C-banding pattern.

metacentric with large C-blocks of almost equal size on either side of its centromere, thus having two HE-junctions. The Y chromosome was the only acrocentric, and appeared totally heterochromatic (Fig. 1, inset). The laboratory-reared albino grass voles had a variant X caused probably by inversion, which carried C-blocks of unequal size on either side of its centromere (Fig. 1a'). Identification of the X chromosome was difficult in the FPG-stained metaphases, because of its morphological similarity to the metacentric autosomes. Sequential FPG and C-band staining was necessary for identification of the X chromosome in this species. A vestigial secondary constriction (SC) was detectable, in well-spread metaphases, on the proximal region of the long arm of the largest submetacentric autosome. This chromosome carried two NORs: a deeply-stained telomeric NOR and a vestigial interstitial NOR which corresponded to the indistinct SC. The SC region could not be visualized as a conspicuous constriction by either conventional Giemsa or NOR-staining, but was markedly enhanced by the FPG staining (Fig. 2a, b and d).

Apodemus argenteus: The diploid chromosome number was 46, consistent with the previous reports [17, 32, 33]. All chromosomes carried large amounts of centromeric C-heterochromatin (Fig. 3a' and b'). The largest subtelocentric X chromosome had an unusually large C-block (Fig. 3a' and b'): the entire short arm and the proximal region of the long arm were totally heterochromatic. Thus, the X chromosome of this species has only one HE-junction in its long arm. The X chromosome could be easily identified by size and morphology, by conventional Giemsa staining, and

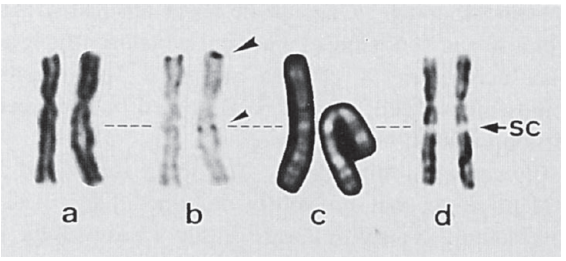

FIG. 2. Giemsa-stained (a), Ag-NOR-stained (b), QM-stained (c) and FPG-stained (d) No. 1 homologues of *M. montebelli*. a and b are sequentially stained same homologues. a, b and c are from bone marrow metaphases, and d is from a fibroblast metaphase in the third division. Large and small arrowheads indicate active and vestigial NORs, respectively. SC, secondary constriction.

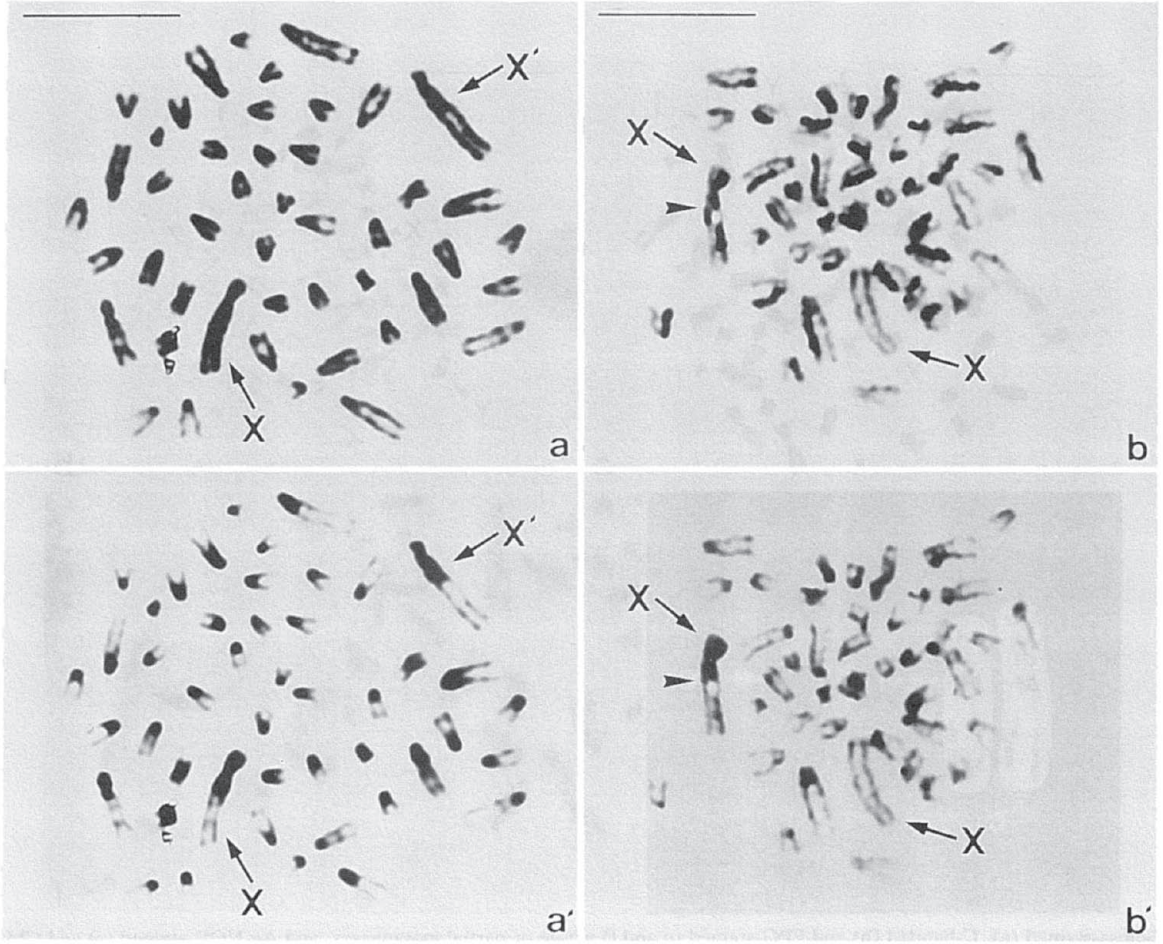

FIG. 3. FPG-stained (a, b) and C-banded (a', b') profiles of the same metaphases of *A. argenteus*. The bars represent 10 μm. a and a', first division; b and b', third division. Arrowheads indicate either the SCE site (b) or corresponding HE-junction (b').

by C-band staining. One female specimen had a heteromorphic X pair consisting of a normal subtelocentric X and a variant acrocentric X (Fig. 3a and a'). This variant X chromosome seemed to have been formed by a pericentric inversion within C-block.

Chimarrogale himalayica: The water shrew had 2n= 52, identical to that previously described [20]. The sex chromosomes, X and Y, were easily identified by their morphology: the X is a medium sized submetacentric and the Y is the only acrocentric in the complement (Fig. 4a). The No. 1 homologue, the largest element, could be easily identified by the conventional Giemsa staining. Further, the entire short arm was totally heterochromatic, excluding the SC region (Fig. 4b). The No. 1 chromosome carried a typical SC on the proximal region of its short arm, which was regarded as an active NOR site on the basis of the Ag-NOR staining (Fig. 4c). For our purposes, the major short arm from the distal boundary of the SC to the telomeric end was defined as C-block. The C-block of the No. 1 homologue showed a highly variable individual-to-individual heteromorphism (Fig. 4b), as pointed out by Obara and Tada [20].

SCE analysis in the target chromosomes

The frequency and distribution of SCEs were scored using as target chromosomes the X chromosomes of *M. montebelli* and *A. argenteus* and the No. 1 chromosomes of *M. montebelli* and *C. himalayica*. Profiles of these chromosomes are schematically depicted as idiograms (Fig. 5) based upon the morphological characteristics summarized in Table 1. Taking their relative lengths into acount, the FPG stained target chromosomes were evenly divided on photoprints into small fractions of equal size along their entire length, since the simultaneous analysis of G-banding pattern and SCEs was hardly made in our system. In addition to these fractions the HE-junctions and/or NOR were also considered as special fractions. The figures objectively show the distribution patterns of SCEs in the target chromosomes. In each of the target chromosomes, a fraction on either side of the centromere was excluded from the SCE counting. This was done since pericentromeric regions contain kineto- chores and the organization of these regions is distinguished from ordinary chromatin structure [23]. The SCEs were counted in practice in 25 and 49 fractions in the X and No. 1 chromosomes of *M. montebelli*, respectively (Table 2). Similarly, 29 fractions in the X chromosome of *A. argenteus* and 25 fractions in the No. 1 chromosome of *C. himalayica* were set up for the SCE counting (Table 2).

Microtus montebelli: The frequency and distribution

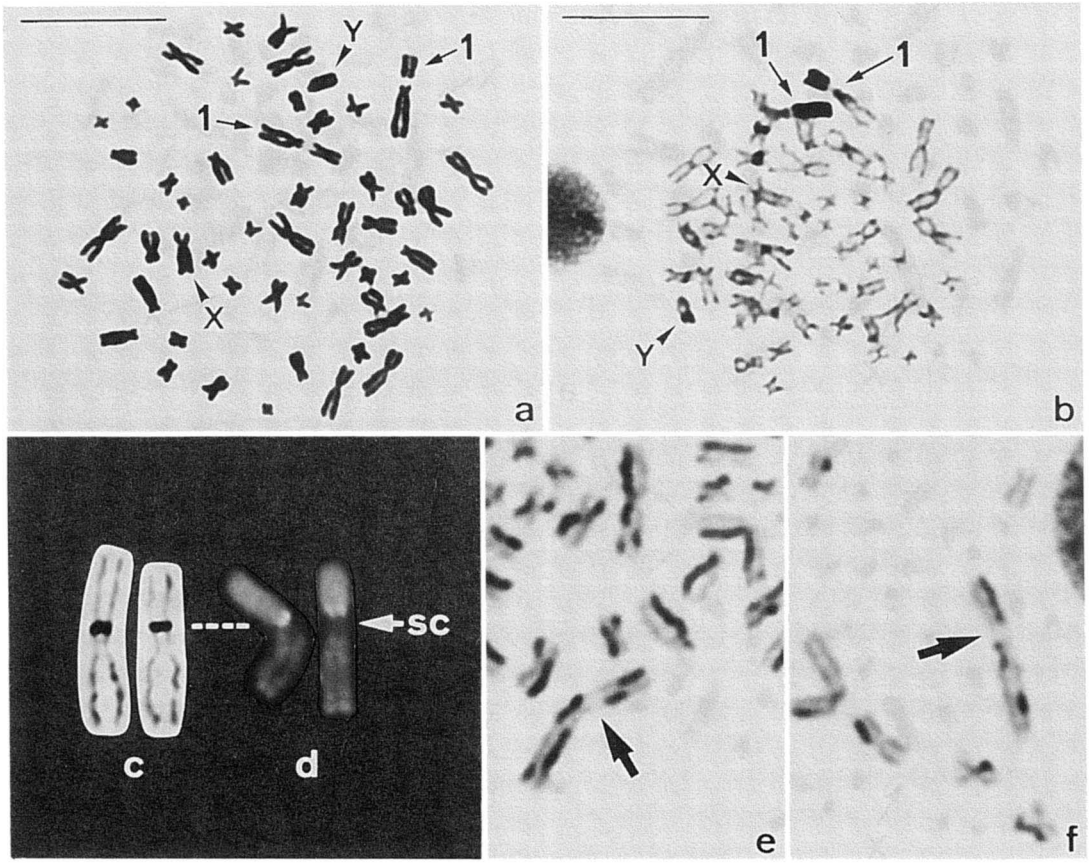

FIG. 4. Giemsa-stained (a), C-banded (b) and FPG-stained (e and f) whole or partial metaphases, and Ag-NOR-stained (c) and CMA-stained (d) No. 1 homologues of *C. himalayica*. The bars represent 10 μm. a-d, bone marrow chromosomes; e and f, fibroblast chromosomes showing SCEs on the No. 1 chromosomes in the second (e) and third (f) divisions. Thick arrows indicate SCEs at the NOR.

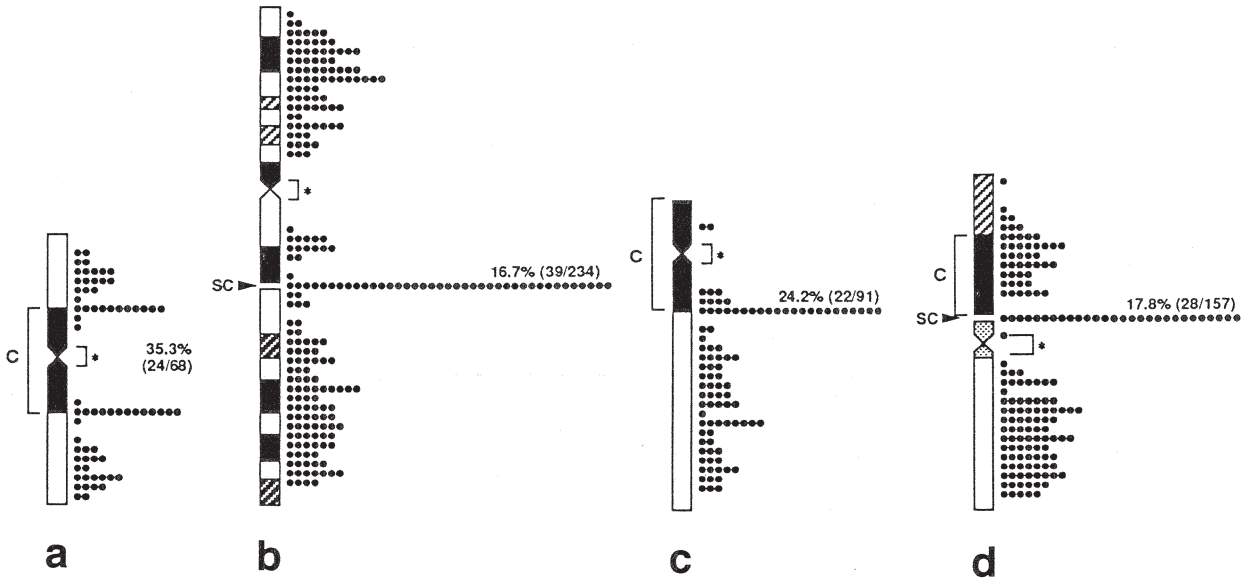

FIG. 5. C-banded (a, c and d) and G-banded (b) idiograms showing the SCE distribution on the X (a and c) and No. 1 (b and d) chromosomes of *M. montebelli* (a and b), *A. argenteus* (c) and *C. himalayica* (d). C, C-block; *, fractions free from the SCE counting; SC, secondary constriction; parenthesis, actual SCE numbers on the HE-junctions or SC/total SCE count on the entire chromosome length. The shaded and dotted areas (d) show the range of C-band heteromorphism and rather slightly stained C-heterochromatin, respectively.

TABLE 1. Morphological characteristics of the target chromosomes

Species	Chromosome	Relative length[a]	arm ratio	C-block (%)
M. montebelli	X	1.0	1.17	41.0
	No. 1	1.85	1.71	—
A. argenteus	X	1.15	4.75	36.5
C. himalayica	No. 1[b]	1.03	1.51	28.2

[a] The X chromosome of *M. montebelli* is considered to be 1.0.
[b] Carries the smallest C-block among varying sizes of heteromorphic C-blocks.

pattern of SCEs in the X chromosome were determined using a solid dot-piling system (Fig. 5a), in which a total of 49 metaphases were analysed for SCEs. Each of the solid dots indicates one SCE observed in the corresponding fraction. In total, 68 SCEs were plotted. The SCEs were the most frequent at the HE-junctions (Fig. 5a), where the SCEs comprised 35.3% of the total SCEs counted. The frequency of SCEs per arbitrary unit length is apparently lower in the C-block region than in the euchromatic region. In the No. 1 chromosomes, for which 234 SCEs from 100 metaphases were counted, the SC fraction constituted 16.7% of the total SCEs (Fig. 5b). Application of the ASG method on well-spread metaphases established the G-banding pattern of the No. 1 chromosome, however, no general correlation between clustering of SCEs and G-banding patterns was obtained in this chromosome.

Apodemus argenteus: A total of 91 SCEs from 42 metaphases were plotted in the X chromosome (Fig. 5c). As in *M. montebelli*, SCEs were the most frequent at the HE-junction, where the SCEs amounted to 24.2% of the total. The frequency of SCEs per arbitrary unit length was extremely low in the C-block region, as compared with that in the euchromatic region.

Chimarrogale himalayica: Eighty metaphases were

TABLE 2. Numbers of fractions divided for SCE-counting along the long axis of chromosomes

Species	Chromosome	C-block	Euchromatin	HE-junction or NOR	Total
M. montebelli	X	9(7)	16	2	27(25)
	No. 1	—	50(48)	1	51(49)
A. argenteus	X	10(8)	20	1	31(29)
C. himalayica	No. 1	8*	16	1	25

Parentheses indicate the numbers after subtraction of 2 fractions of either side of the centromere.
* The terminal region showing the short arm heteromorphism was excluded from the evaluation of the number of fractions.

TABLE 3. Frequency of SCEs on the C-blocks of three mammalian species examined

Species	Chromosome	C-block			obs./exp. ×100 (%)
		Number of SCEs observed	Number of SCEs expected*	χ^2	
M. montebelli	X	3	13	7.64 ($P<0.01$)	23.1
A. argenteus	X	9	20	5.28 ($P<0.05$)	45.0
C. himalayica	No. 1	39	43	0.29 (n.s.)	90.7

* Calculated by dividing the total number of SCEs observed with the total number of fractions and multiplying the quotient in proportion to the number of fractions within the C-block. In this evaluation, the counts at the HE-junctions and 2 fractions in the centromeric region were excluded. In the No. 1 homologue of *C. himalayica*, the terminal region showing the short arm heteromorphism was also excluded from the evaluation of SCE counting. χ^2, chi-square test; n.s., not significant.

analyzed in the No. 1 chromosomes (Fig. 5d). The SCEs occurred with a high frequency in the NOR fraction adjoining to the C-block (17.8% of the total 157 SCEs). In contrast to *A. argenteus* and *M. montebelli*, no significant difference in the frequency of SCEs was found between the arbitrary unit lengths of the C-block and euchromatic regions. The shaded part of the short arm was excluded from calculation of the SCE frequency, because it was involved in the heteromorphism.

Interspecific variation of SCE-sensitivity in C-blocks

In order to quantitatively show the SCE-sensitivity in the C-block region, both actual and expected counts of SCEs were obtained and compared among these three species (Table 3). Assuming SCEs are proportional to the chromosome length, expected counts of SCEs in C-blocks can be calculated by a proportional allotment (Table 3). The SCE-sensitivity of the C-blocks in these three species was different: *C. himalayica* showed the highest sensitivity, which was almost equal to that of the euchromatic region (90.7% of the expected counts), *M. montebelli* the lowest sensitivity, which was almost one-fourth of the expected value (23.1%), and *A. argenteus* an intermediate value (45.0%).

DISCUSSION

Recently, Rachel *et al.* [22], using Indian muntjac chromosomes, found the BrdU plus MMC-induced SCEs occur more frequently in R-bands than in G-bands; but after BrdU incorporation alone SCEs occur, on the contrary, more frequently in G-bands than in R-bands. These findings suggest that SCE formation may not be simply restricted by chromatin structure *per se* as defined by euchromatin or heterochromatin. In this study the formation of the BrdU plus MMC-induced SCEs was investigated with special attention to the frequency distribution of SCEs in the C-blocks and NORs. Furthermore, the activity of the C-block-carrying X was not taken into consideration here, as X chromosome inactivation has no effect on SCE formation [10, 35].

SCEs at the HE-junctions and NORs

In general, the SCE frequency is proportional to the length of chromosomes, excluding specific chromosome regions such as HE-junctions [12]. In several mammalian species including human both NORs and HE-junctions are the preferred sites for the occurrence of SCEs [25]. A similar frequency distribution of SCEs was found in murid and soricid species examined here (Fig. 5).

The marked preference for SCEs at the HE-junctions of two mammalian species (Fig. 5a and c) is more persuasive in that the target chromosomes carry unusually large C-blocks, making it possible to accurately identify the HE-junctions by application of sequential FPG and C-band staining. The cytologic and molecular mechanisms accounting for a high incidence of SCEs at the HE-junctions remain unsolved, but the observations suggest that HE-junctions may be the so-called switch of DNA replication and a point of conversion of chromatin structure. Euchromatin tends to decondense into loosely arranged chromatin fibers, thus replicating DNA in the early S phase, while heterochromatin condenses into tightly packed chromatin fibers, thus replicating DNA in the late S phase. Such a contrast in chromatin organization may cause the strand breaks during DNA replication at HE-junctions, and hence the high occurrence of SCEs.

Clustering of SCEs of the NORs has been described in Chinese hamster and Indian muntjac cells [11, 21]. In the present study the SC regions of the No. 1 chromosomes of both *M. montebelli* and *C. himalayica* were apparently hot spots for the occurrence of SCEs (Fig. 5b and d). The SC of *C. himalayica* appeared as a typical achromatic structure, regarded as an active NOR (Fig. 4c), while that of *M. montebelli* as an atypical vestigial one, showing low activity of the NOR (Fig. 2b), as Yamakage *et al.* [31] have pointed out. The conspicuous SC of *C. himalayica* is bounded by heterochromatin on either side, and the SCEs seemed to occur preferentially at the site with a high frequency. On the other

hand, the vestigial SC of *M. montebelli* is in contact with euchromatin on either side, and this SC region also showed a high SCE frequency, as in *C. himalayica*. These findings suggest that the high susceptibility to SCEs in the SC (NOR) region has nothing to do with the condition of chromatin surrounding NORs. Instead, specific DNA sequences within NORs consisting of highly repeated rRNA gene clusters may take part in SCE formation. The presence of a large numbers of replication units may cause breakage of DNA strands leading to the induction of break point misrepair. Evidence for chromosomal replicons as units for SCEs has been presented by Lugo *et al.* [16]. Current hypotheses of the SCE formation postulate that the SCE site is associated with an active replicon or replicon cluster.

It should be noted that the vestigial SC of the No. 1 chromosome of *M. montebelli* was remarkably enhanced by the FPG staining in almost all of the 2nd and 3rd division cells (Fig. 2d). Since the SC region fluoresces brightly with Q-band staining (Fig. 2c), some AT-rich DNA may remain in this vestigial SC. Therefore, the FPG-induced large achromatic SC region might have resulted from undercondensation being caused by preferential substitution of BrdU with thymidine of AT-rich DNA comprising the SC.

SCEs in the C-blocks

Schubert and Rieger [25] reviewed the SCE current literature for plant and animal species, and found that the SCE frequency in C-block regions was lower than expected in some systems, but higher than expected in other systems. They concluded that the nonrandom involvement of heterochromatin in spontaneous SCEs does not hold as a general rule. In the X chromosomes of *M. montebelli* and *A. argenteus*, the SCE frequencies in C-block regions were significantly low, as compared with that in their euchromatic regions (Fig. 5a and c). The marked differences between the expected and observed SCE frequencies strongly suggest preferential suppression of SCEs in the C-block regions. On the other hand, the C-block region of the No. 1 chromosome of *C. himalayica* showed a number of SCEs nearly equal in frequency to those found in the euchromatic region of the same length (Fig. 5d). Statistical analysis revealed no significant difference between the observed and expected SCE counts in the C-block region (Table 3). This species-specific tendency of SCE distribution seems to suggest that there is no direct relation of SCE formation with chromatin condensation. The fluorescence analysis with a GC-specific fluorochrome chromomycin A_3 indicated that the C-block of *C. himalayica* consists of two kinds of GC-rich DNA: one is a major part of the C-block, fluorescing brightly along its entire length, and the other a small-sized C-heterochromatin organizing the junction of the C-block and NOR, which is observed as the brightest spot (Fig. 4d). The antibiotic MMC is a relatively GC-specific drug, which reacts preferentially with GC-rich regions [22]. Therefore, it may be reasonable to consider that the greater part of the SCEs on the MMC-treated BrdU-incorporated C-block could be caused by the greater number of lesions induced by MMC.

The C-block of the No. 1 homologue of *C. himalayica* shows a remarkable heteromorphism in size (Fig. 4b). Our previous work [19] revealed that the extent of the C-block heteromorphism (the balance of relative lengths between heteromorphic C-blocks) ranged from 6.4 to 36.4%. Unequal crossing-over in meiosis may also be a plausible mechanism for the occurrence of this heteromorphism. On the other hand, "unequal SCEs" could also be proposed as a mechanism for somatic recombination. The most probable factor for induction of unequal SCEs may be the presence of highly repeated DNA sequences on the basis of their intrinsic nature of base pairing. The larger such a domain becomes, the more frequently unequal SCEs may occur. Thus, comparatively large C-blocks free from suppression of SCEs, e.g., those of the No. 1 homologue of *C. himalayica*, may be more likely to induce unequal SCEs. Holden *et al.* [7] were the first to present, by means of the differential sister chromatid staining technique, clear-cut figures of unequal SCEs at the homogeneously staining region (HSR) in four different chromosomes of a human melanoma cell line MeVo. They found that the frequency of SCEs within the HSR was increased over other chromosomal regions, and proposed based on the prominent looping out of the longer chromatids a model of unequal SCE for the amplification of DNA sequences in these HSRs. It is tempting to opine that such unequal SCEs may occur in the C-blocks of gonial cells of *C. himalayica*. Chromosome analysis in the second meiotic division provides direct evidence for unequal crossing-over. Structural analysis of the synaptonemal complex with special attention to the recombination nodules might also provide useful information to understand this phenomenon. These studies are now in progress in our laboratory.

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