Occurrence of sister chromatid exchanges at euchromatin-C band junctions in *Allium cepa* chromosomes

F. Cortés

Departamento de Citología, C-XV, Universidad Autónoma de Madrid, Madrid-34 (Spain), 11 February 1980

Summary. Distribution of SCE in C band and non-C band regions of *Allium cepa* chromosomes.

The incidence of sister chromatid exchanges (SCE) after treatment with 5-bromodeoxyuridine (BrdU) has proved to be different in euchromatic and heterochromatic regions of metaphase chromosomes. Nevertheless, the results obtained by different authors have been contradictory. Thus, while the results obtained from human chromosomes[1] and chromosomes of *Microtus agrestis*[2] suggested that SCE occur preferentially in heterochromatic regions, a lower frequency than expected has been reported for heterochromatic chromosomal regions in the Indian muntjac[3], the kangaroo rat *(Dipodomys ordii)*[4], Chinese hamster[5], *Microtus montanus*[5], and *Allium cepa*[6]. In addition an unexpectedly high occurrence of SCE at the heterochromatin-euchromatin junctions in metaphase chromosomes of animal cells has been reported[3,4,7]. Such findings are very interesting and important for increasing our knowledge of chromatin structure and function.

In the present report, an analysis of SCE along the metaphase chromosomes of *Allium cepa* L. meristematic cells has been performed in order to study the distribution of SCE in euchromatin and C-band heterochromatin and, especially, in the junctions between both chromosomal regions.

Material and methods. Meristem roots of *Allium cepa* L. bulbs were grown in the dark at a constant temperature of 25±0.5 °C in tap water renewed every 24 h and aerated continuously (by bubbling air) at a rate of 10–20 ml/min. The bulbs were placed in such a manner that only their bases remained submerged in the water. The experiments began when the roots reached 15–20 mm. In order to analyze the occurrence of SCE, bulbs were treated with 10^{-4} M BrdU and 10^{-7} M fluordeoxyuridine (FdU) for a total time of 25 h (renewing the treatment solution after the first 12 h) to ensure BrdU incorporation into DNA throughout 2 consecutive rounds of replication. During the BrdU-treatment, the culture receptacles and the bulbs were protected from light by wrapping them with aluminum foil. Finally, roots still attached to the bulbs were treated with 0.05% colchicine for 3 h and root tips were fixed overnight in ethanol-acetic acid (3:1) at 4 °C.

After fixation, roots were treated with pectinase (Sigma, from *Aspergillus niger*) dissolved in citrate buffer adjusted to pH 4.2, at 37 °C for 1 h and then squashed. Coverslips were removed by the dry ice method and preparations were hydrated by passing them through ethanol solutions (absolute, 96%, 70%, 50%, 30%) and distilled water. Then, the preparations were incubated with RNase (Sigma, ribonuclease-A from bovine pancreas) at 25 °C for 2 h and slides were washed with 0.5 × SSC (sodium saline citrate). Afterwards, slides were treated with Hoechst 33258 at room temperature for 30 min and, subsequently, exposed to a fluorescent sun lamp radiating in the 280–380 nm band (Philips HP 3202) at a distance of 10 cm. Such exposure was performed in a moist chamber with 0.5 × SSC for 1 h. Slides were treated at 55 °C in the same solution again for 1 h and finally, stained during 9 min with 3% Giemsa in phosphate buffer adjusted to pH 6.8, briefly washed in the same buffer, air dried and mounted with Euparal.

Chromosomal region	Relative length	Number of SCEs* Expected	Observed	χ^2-test
Total	1.00	2082	2082	
Euchromatin	0.82	1707.2	1676	χ^2: 0.5 df: 1 $p<0.005$
C-bands	0.18	374.7	312	χ^2: 10.5 df: 1 $p<0.005$
Euchromatin-C band junctions	-	-	94	

* SCEs in 500 chromosomes were studied.

Results and discussion. C bands are located in *Allium cepa* L. at telomeric levels in metaphase chromosomes, as has been reported by several authors[9,10]. Since it can be considered, in general, that the incidence of sister chromatid exchanges is roughly proportional to the lengths of the metaphase chromosomes, one can expect a certain number of exchanges to occur randomly at euchromatin and C bands. Nevertheless, as can be seen in the table, SCE seem not to occur randomly, but preferentially in chromosome regions corresponding to euchromatin. In addition, a remarkable fact was the unexpectedly high occurrence of SCE at the junctions between euchromatin and C bands. This section is considered to be minimal in comparison with the other chromosomal regions studied.

This last result agrees with the one previously reported for animal cells[3,4,7] and so represents a confirmation in a plant species. Due to the fact that BrdU incorporation per se induces SCE, the percentage of SCE detected by the fluorescent plus Giemsa (FPG) technique that is spontaneous, and the percentage induced by BrdU[10] has not been well established up to now, although the existence of the former has been demonstrated in vivo[11].

With respect to the localization of SCE along chromosomes; despite the frequent observation of a suppressed occurrence, of SCE in C bands, an explanation for this fact remains to be found. In this sense, Kato[7] pointed out that 'the suppressed occurrence of SCE within C bands seems to be caused by their tertiary structure rather than their nucleotide sequence'. According to this reasoning, the occurrence of SCE might be determined not only by the BrdU incorporation into DNA but also by the degree of coiling of nucleoproteins. If we admit such a hypothesis, the euchromatin-C band junctions would represent especially uncoiled transitional regions, highly sensitive to the occurrence of SCE.

Since proteins associated with DNA determine the degree of coiling, a longitudinal differentiation along chromosomes according to their SCE sensitivity could be established. This sensitivity might be related to the type and relative proportion of proteins associated with the DNA at each region. One of the current concepts considers histone H1 in the role of inducer and stabilizer of the supercoil of nucleosomes and intranucleosomal DNA and its packing into the chromatin fibre. With this in mind, we can postulate that the relative proportion of histone H1 could be responsible for the differences in sensitivity of SCE observed among different chromosome regions.

1 W. Schnedl, W. Pumberger, R. Czaker, P. Wagenbichler and H.G. Schwarzacher, Hum. Genet. *32*, 199 (1976).
2 A.T. Natarajan and I. Klásterská, Hereditas, Lund *79*, 150 (1975).
3 A.V. Carrano and S. Wolff, Chromosoma *53*, 361 (1975).

4 C.J. Bostock and S. Christie, Chromosoma *56*, 275 (1976).
5 T.C. Hsu and S. Pathak, Chromosoma *58*, 269 (1976).
6 J.B. Schvartzman and F. Cortés, Chromosoma *62*, 119 (1977).
7 H. Kato, Chromosoma *74*, 307 (1979).
8 S.M. Stack and C.R. Clarke, Cytologia *39*, 553 (1974).
9 J.B. Schvartzman and J.L. Díez, Cytobiologie *14*, 310 (1977).
10 R. Tice, J. Chaillet and E.L. Schneider, Nature *256*, 642 (1975).
11 R. Tice, J. Chaillet and E.L. Schneider, Cell Res. *102*, 426 (1976).

Release of Ca^{2+} from intracellular pools stops cytoplasmic streaming in *Tradescantia* staminal hairs

M. Dorée and A. Picard

Station biologique, F-29211 Roscoff (France), 7 February 1980

Summary. Ionophore A 23187 reversibly stops cytoplasmic streaming in *Tradescantia* staminal hairs. Cessation of streaming occurs even in the absence of free Ca^{2+} ions in the medium.

Cytoplasmic streaming occurs in many plant and protozoan cells[1,2]. The welldocumented suppression of streaming by cytochalasin B, which appears to weaken the actin filament structure[3–6], suggests that bundles of microfilaments are involved in the mechanism of streaming. The presence of such bundles has actually been reported in plant cells which demonstrate cytoplasmic streaming[4,7]. In non-muscle cells Ca^{2+} seems to be involved in the control of the formation of bundles of actin filaments[8] and actin-containing gels[9] as well as in the interaction of actin with myosin[10]. On the other hand it has long been known in *Charophyta* that streaming halts transiently when an action potential passes along a cell[11,12]. Barry found that replacement of Ca^{2+} in the medium with Mg^{2+} left *Nitella* giant cells excitable but abolished the blockage of streaming that normally accompanies excitation[12]. Although this finding supports the view that the action potential is normally accompanied by a net influx of Ca^{2+} which has an inhibitory effect on streaming, this explanation lacks clear experimental support, since Barry failed in his attempts to inhibit streaming by driving Ca^{2+} directly into the cytoplasm either ionophoretically or by microinjecting it. This led us to test the effect of the rather Ca^{2+}-specific ionophore A 23187[13] on cytoplasmic streaming in *Tradescantia* staminal hairs, a very favorable material for quantitative studies of streaming rates[14].

Material and methods. Staminal hairs were used on the day following opening of the flowers. They were mounted on a slide in a 100-µl chamber filled either with pure water, 1 mM $CaCl_2$ or 10 mM EDTA, with bubbling air for at least 1 h before starting the experiments. A Wild microscope equipped with a ×40 phase contrast objective under ordinary light was used, heating of the slide being avoided by the interposition of a water cell. Readings were made of the motion of the smallest visible particles (figure 1) in transvacuolar strands of a single cell, using a calibrated ocular micrometer and a stop watch. A 200-µM ionophore solution in methanol was used as stock solution: 1 µl was added directly into the 100-µl chamber. Controls with 1% methanol were run in parallel to the inhibitor experiments: no change in the rate of streaming was observed. Ionophore A 23187 was a gift of Eli Lilly.

Results. Figure 2, a, shows that, in our experimental conditions, streaming-rate remains constant for at least 1.5 h in aerated pure water. It is not altered when water is changed for 1% methanol. 10 mM EDTA or 50 mM $CaCl_2$ (nor when 50 mM $MgCl_2$ is substituted for 50 mM $CaCl_2$). Figure 2, b and c, show that 1 min after addition of 2 µM ionophore A 23187 a decrease of streaming rate is already noticeable. Within 5 min streaming completely stops. No recovery occurs within at least 30 min in the presence of ionophore. However, a rapid and complete restoration occurs after the chamber containing the staminal hair has been washed thoroughly several times either with 10 mM $CaCl_2$ (figure 2, b) or with 10 mM EDTA (figure 2, c). This result suggests that despite its hydrophobic nature, ionophore can be washed out relatively easily from the membrane, as already reported by others[15,16]. A 2nd ionophore application again suppresses cytoplasmic streaming. Comparison of figures 2, b, and 2, c, makes clear that the presence of Ca^{2+} in the medium is not required for ionophore A 23187 inhibition of cytoplasmic streaming. Even after standing 30 min in 10 mM EDTA, with several changes of medium, staminal hairs respond to ionophore as well as they do in the presence of Ca^{2+}. Inhibition is neither reversed nor enhanced when Ca^{2+} concentration is raised to 50 mM.

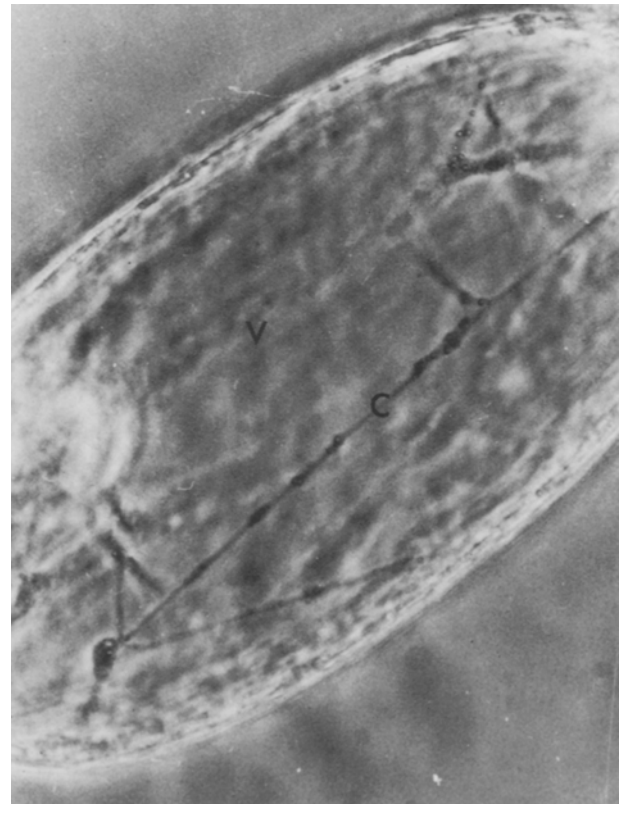

Fig. 1. *Tradescantia* staminal hairs. Arrows point to particles whose rate of rectilinear movement was measured in transvacuolar strands. v, vacuole; c, cytoplasm. ×700.