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Sister Chromatid Exchanges and Chromosomal Aberrations in 5-Aminouracil-synchronized Cells

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Summary 5-AU-synchronized cells of *Allium cepa* L. meristems show increased yields of chromosomal aberrations as well as SCE's. However, although a caffeine post-treatment increases the frequency of 5-AU-induced abnormal ana-telophases, it has a negligible influence on the 5-AU-induced SCE's. These results strongly suggest that the mechanisms through which the 5-AU-induced DNA damage is transformed into chromosomal aberrations and SCE's are at least partly different.

Key words: *Allium cepa* L. – Sister chromatid exchanges – Chromosomal aberrations – 5-Aminouracil

Abbreviations

5-AU = 5-Aminouracil
FdUrd = 5-fluoro-2′-deoxyuridine
BrdUrd = 5-bromo-2′-deoxyuridine
Thd = thymidine
Urd = uridine
SCE's = sister chromatid exchanges

Introduction

5-aminouracil (5-AU) is a thymine analogue that has been extensively used in order to synchronize plant cells in the mitotic phase (Duncan and Woods 1953; Smith et al. 1963; Prensky and Smith 1965; Jakob and Trosko 1965; Mattingly 1966; Wagenaar 1966; Scheuermann and Klafke-Lobsien 1973; Diez et al. 1976; Davidson et al. 1978). The mechanisms involved in the mitotic synchronization which is usually obtained during recovery after treatment with 5-AU are still only vaguely understood. However, it has been shown that 5-AU depresses the replication rate (Prensky and Smith 1965; Jakob and Trosko 1965), and appears to inhibit in some preferential manner late replicating DNA synthesis (Scheuermann and Klafke-Lobsien 1973; Diez et al. 1976).

Several drugs that are known to interfere with DNA replication in vivo (e.g. 5-fluoro-2′-deoxyuridine, 2′-deoxyadenosine, arabinosyl adenine and hydroxyurea) often induce chromosome breakage. It has also been shown repeatedly that most compounds capable of inducing chromosomal aberrations also induce sister chromatid exchanges (SCE's) with varying degrees of efficiency (Perry and Evans 1975; Kihlman and Sturelid 1978; Popescu et al. 1979). The fact that 5-AU-treated cells usually exhibit chromosomal aberrations has been well-known since the pioneer work of Duncan and Woods (1953), but there has been no report on the possible influence of 5-AU on SCE's.

Instead, the relationships between the mechanisms involved in the formation of chromosomal aberrations and SCE's is still a controversial matter. Kato (1973, 1977) and Basler et al. (1979) suggested that both events are closely related, while several other authors postulated that chromosomal aberrations and SCE's are produced by mechanisms that are partly different if not completely different (Wolff and Bodycote 1975; Palitti and Becchetti 1977; Sasaki 1977; Kihlman and Sturelid 1978; Nakanishi and Schneider 1979; Popescu et al. 1979). In the present report we demonstrate that 5-AU-synchronized cells of *Allium cepa* L. meristems show increased yields of SCE's as well as chromosomal aberrations. We also discuss to what extent the DNA synthesis inhibition by 5-AU and the induction of chromosomal aberrations and SCE's may be related phenomena.

Material and Methods

Root meristems of *Allium cepa* L. were employed. The bulbs, 15-30 g in weight, were grown in the dark at a constant temperature of 25° C in cylindrical glass receptacles of about 80 ml capacity in tap water renewed every 24 hrs and continuously aerated by

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bubbling air at a rate of 10-20 ml/min. The bulbs were so placed that only their bases remained submerged and all treatments began when the roots reached a length of 15-20 mm. In every case after the roots has been treated with 5-bromo-2'-deoxyuridine (BrdUrd), the culture receptacles and the treated bulbs were given extra protection from light by wrapping them in aluminium foil.

BrdUrd-Treatments

BrdUrd-substitution was carried out by exposing the growing roots to a treatment solution containing 10^{-4} M BrdUrd, 10^{-9} M FdUrd and 10^{-6} M Urd throughout one cell cycle time.

5-AU-Treatments

A 0.5 mM 5-AU solution in tap water was used. The culture conditions already described were otherwise maintained throughout the treatment period, which lasted 14 hrs in every case. In those cases in which Thd incorporation has been pursued during 5-AU-treatments, the roots were exposed to a solution containing 0.5 mM 5-AU, 10^{-4} M Thd and 10^{-6} M Urd for 14 hrs. In these cases recovery occurred in the presence of 10^{-4} M Thd and 10^{-6} M Urd.

Caffeine-Post-Treatments

In those cases where after a 5-AU-treatment a caffeine post-treatment was pursued, the roots were submerged in a solution containing 2.5 mM caffeine, 10^{-4} M Thd and 10^{-6} M Urd until fixation.

Fixations

Those bulbs in which SCE's and chromosomal aberrations were going to be studied were managed as follows: 3-5 roots from each bulb were cut and fixed in ethanol-acetic acid (3:1) overnight. The remaining roots, still attached to the bulbs, after being throughly washed with tap water, were submerged in 0.05% colchicine for 3 hrs and finally all the root tips were fixed as before.

Staining Techniques

In every case where mitotic index and percentage of abnormal anatelophases were going to be studied, squashes and staining were performed according to Tjio and Levan's acetic orcein method (Tjio and Levan 1950). Root tips in which SCE's were going to be studied were squashed and stained in accordance with the FPG (fluorescent plus Giemsa) technique (Perry and Wolff 1974) as modified by Schvartzman et al. (1979).

Results

As already shown by Wagenaar (1966) and Diez et al. (1976), a wave of mitotic cells reaching mitotic index values close to 50% appears between 10 and 18 hrs after the beginning of the recovery period following the end of 5-AU-treatment in *Allium cepa* L. meristems.

Several experiments were performed in which cells were firstly allowed to replicate in the presence of BrdUrd for one cycle time and were then treated with 5-AU and Thd for 14 hrs. Recovery took place in Thd alone or in the presence of Thd and 2.5 mM caffeine, colchicine being added for the last 3 hrs. The principal aim of these schedules was to collect large numbers of 5-AU-synchronized cells in the mitotic phase showing chromosomes with their sister chromatids differentially substituted with BrdUrd. Additionally, before the colchicine treatment was started, a couple of root tips were fixed from each bulb so that chromosomal aberrations could be studied by measuring the percentage of abnormal ana-telophases.

Figure 1a shows an abnormal telophase observed in a 5-AU-synchronized meristem. A group of *Allium cepa* L. chromosomes corresponding to a 5-AU-synchronized cell with increased numbers of SCE's is shown in Figure 1b. A comparison of the yields of chromosomal aberrations and SCE's obtained in unsynchronized meristems with those obtained in 5-AU-synchronized ones, in 5-AY-synchronized meristems in which recovery after the 5-AU-treatment occurred in the presence of 2.5 mM caffeine are presented in Figure 2. It can be clearly noted that 5-AU-synchronized cells show increased yields of chromosomal aberrations, which were almost negligible in the untreated root tips. The frequency of SCE's per chromosome is also

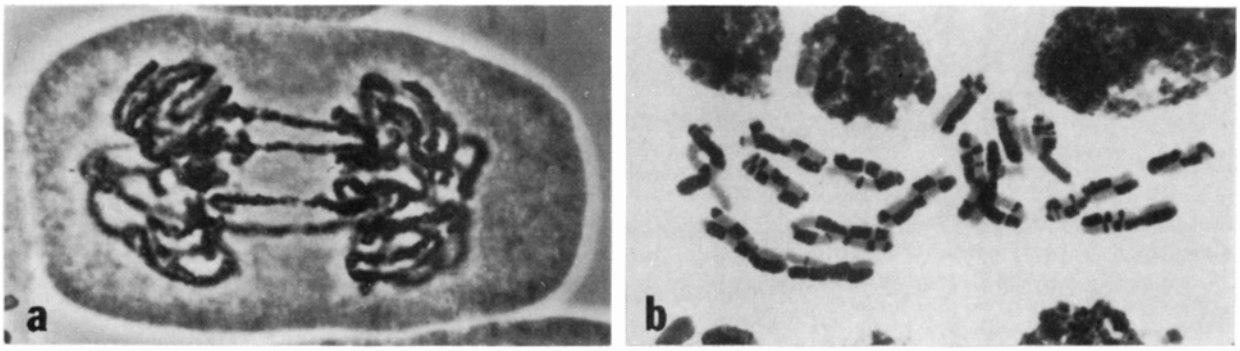

Fig. 1a and b. *Allium cepa* L. meristematic cell and chromosomes corresponding to a 5-AU-synchronized root tip. **a** Abnormal telophase showing 4 bridges **b** Chromosomes showing increased yields of SCE's

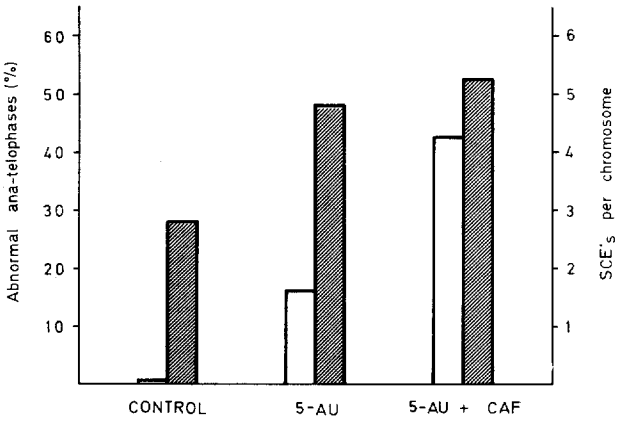

Fig. 2. Chromosomal aberrations ☐ and SCE's ▨ in controls and in 5-AU-synchronized cells with or without a 2.5 nM caffeine post-treatment. Chromosomal aberrations are expressed as the percentage of abnormal ana-telophases, while SCE's were measured on a per chromosome basis. The mean values of SCE's per chromosome were only accepted after the observed distribution fitted the expected Poisson distribution derived from each previously calculated mean value. The percentage of abnormal ana-telophases was calculated after studying 500 ana-telophases, and each value of SCE's per chromosome corresponds to the mean calculated after studying 300 chromosomes

higher in 5-AU-synchronized cells than in the untreated controls. Finally, the caffeine post-treatment almost doubles the frequency of abnormal ana-telophases, while it has no significant effect on the yield of SCE's per chromosome.

Discussion

Although the ability of serveral DNA synthesis inhibitors to provoke chromosomal aberrations have been studied (Kihlman 1977) there is no information available on the influence of these inhibitors on SCE's. According to Kihlman (1977) DNA synthesis inhibitors may be classified as a very special group of DNA damaging agents, since their effects appear to be S-dependent because they require a passage through S in order to become apparent. However, the main types of aberrations induced are gaps and open chromatid breaks, which are characteristic of S-independent agents when cells are exposed during the G_2 phase. SCE's are known to occur during DNA replication as the result of the presence of unrepaired lesions (Wolff et al. 1974; Schvartzman et al. 1979). Therefore, the fact that 5-AU induces SCE's as well as chromosomal aberrations strongly supports the S-dependent nature of 5-AU-induced DNA damage. Besides, the detection of abnormal ana-telophases showing chromosome bridges (Figure 1a) experimentally confirms Kihlman's observation that DNA damage induced by DNA synthesis inhibitors during early S may give rise to chromatid exchanges (Kihlman 1977).

The observation that a caffeine post-treatment increases the frequency of 5-AU-induced chromosomal aberrations but has no significant effect on the frequency of 5-AU-induced SCE's may be interpreted in two different ways. It may be that 5-AU induces two types of DNA lesions, one kind responsible for chromosomal aberrations and the other kind responsible for the formation of SCE's. However, this hypothesis does not appear to be shared by most workers in the field, since as already suggested by Evans (1977), different kinds of DNA lesions that might provoke the formation of chromosomal aberrations may also result in the formation of SCE's by means of a different unique mechanism operating at the time of DNA replication. The results presented in this study may be seen to agree with several previous reports, suggesting that the mechanisms responsible for the formation of chromosomal aberrations and SCE's are at least partly different, the caffeine-sensitive step being restricted to the mechanisms operating in the formation of chromosomal aberrations (Wolff and Bodycote 1975; Palitti and Becchetti 1977; Sasaki 1977; Kihlman and Sturelid 1978; Nakanishi and Schneider 1979; Popescu et al. 1979).

In short, the results presented in this report clearly show that 5-AU, like many other DNA synthesis inhibitors, while depressing the rate of DNA replication induces DNA damage. This damage appears to be responsible for the high yields of SCE's and chromosomal aberrations detected in 5-AU-synchronized cells. However, the mechanisms through which 5-AU-induced DNA damage is transformed into SCE's or chromosomal aberrations appear to be somehow different, since a caffeine post-treatment which significantly increases the frequency of 5-AU-induced chromosomal aberrations, has negligible influence on the frequency of 5-AU-induced SCE's.

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