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The Localisation of Radiation Induced Chromosome Aberrations in Relation to the Distribution of Heterochromatin in *Secale cereale*

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Abstract. The distribution of two break chromosome exchanges (dicentrics and centric rings) following gamma or neutron irradiation of resting seeds of rye, *Secale cereale*, has been investigated. The localisation of heterochromatin in the terminal ends of the chromosomes of rye facilitates distinguishing aberrations involving heterochromatin from others. Dicentrics found in or near heterochromatic regions were about 5 times more frequent after gamma irradiation and about 2.5 times more after neutron irradiation, than expected on a random distribution. The implications of these findings in relation to aberration formation are discussed.

Introduction

The possible role of the content and distribution of heterochromatin in the manifestation of radiation and chemically induced chromosomal aberrations was discussed by us in an earlier paper (Natarajan and Ahnström, 1969). It was shown that in *Nigella damascena* (a species which seems to lack heterochromatin), a lower frequency of chromosome aberrations was induced for a given dose of gamma radiation or mitomycin C, when compared to *Vicia faba* (a species possessing several heterochromatic regions). It was also indicated that the differential response of organisms with regard to chromosome aberrations induced by sparsely ionizing and densely ionizing radiations may vary according to their content and distribution of heterochromatin, thus affecting the RBE (Relative Biological Effectiveness) for the production of chromosome aberrations.

In order to gain further information on this point, investigations on radiation induced chromosome aberrations were conducted using diploid rye, *Secale cereale*. This material has a low chromosome number ($2n = 14$), and in addition well defined heterochromatic regions at the terminal ends of the chromosomes, which facilitates differentiation between chromosome aberrations occurring in heterochromatin and euchromatin, respectively.

Materials and Methods

Resting seeds of *Secale cereale* were irradiated with γ-rays from a Co^{60} source (3,000 Ci) or with fast neutrons from a reactor (Ahnström and Ehrenberg, 1961).

The gamma irradiation and the soaking of the seeds was performed under conditions giving either no oxygen effect (irradiation of seeds of 13% water content, soaking in nitrogen bubbled water) or giving a sizable oxygen effect (irradiation of seeds containing 6% water, soaking in oxygen saturated water). The seeds were germinated in dark and the root tips were fixed in acetic alcohol (1:3), following a pretreatment of 0.1% of colchicine solution for 3 hours to collect the first cell division. The slides were prepared as Feulgen squashes and made permanent by the dry ice method.

Autoradiographs to detect late replicating regions were prepared following a 15 minutes pulse labelling with tritiated thymidine (5 μCi/ml, specific activity 5 Ci/mmole). The slides were prepared as Feulgen squashes, dipped in Ilford K4 emulsion, and stored cold in the dark for a week, before developing in Kodak 19b developer.

The measurements of the chromosomes and nuclei were made from squash preparations by the use of camera lucida drawings.

Results

Karyotype of Secale cereale

The diploid complement has 14 chromosomes, 3 pairs with nearly median centromeres and 4 pairs with submedian centromeres. One pair of the nearly median chromosomes is satellited. The chromosomes measure about 105 μ in total length. However, the relative length determinations between the chromosomes and the arms do not agree with the pachytene measurements (Lima-de-Faria 1952). This may be due to differential contraction of various regions in pachytene chromosomes.

The Localisation and Estimation of the Quantity of Heterochromatin in Secale cereale

Lima-de-Faria (1959) has shown that rye chromosomes have late replicating regions in the terminal ends as well as near the centromeres thus indicating the presence of heterochromatin in those parts. However, in a more detailed study, Darlington and Haque (1965) demonstrated that only terminal heterochromatin exists in rye. Our results confirm this latter finding. Pulse labelling experiments indicate that there are 18 to 20 terminal segments (out of 28 terminal ends) which are late replicating and hence probably heterochromatic. This number is in agreement with the number of knobs, in the pachytene nuclei, estimated earlier (Lima-de-Faria, 1952).

The number of chromocentres per nucleus was counted from 100 interphase nuclei. The range varied from 7 to 18 with an average of 12 per nucleus. This indicates that two or more heterochromatic regions remain fused to form a chromocentre in the interphase, since one should expect about 18 to 20 chromocentres in each nucleus if there is no association between the chromocentres.

An estimate of the total length of the heterochromatic regions was made by measuring the lengths of late labelled regions of the chromosomes and compared to the total length. This indicates that heterochromatic regions comprise $10 \pm 2\%$ of the total length. We have also

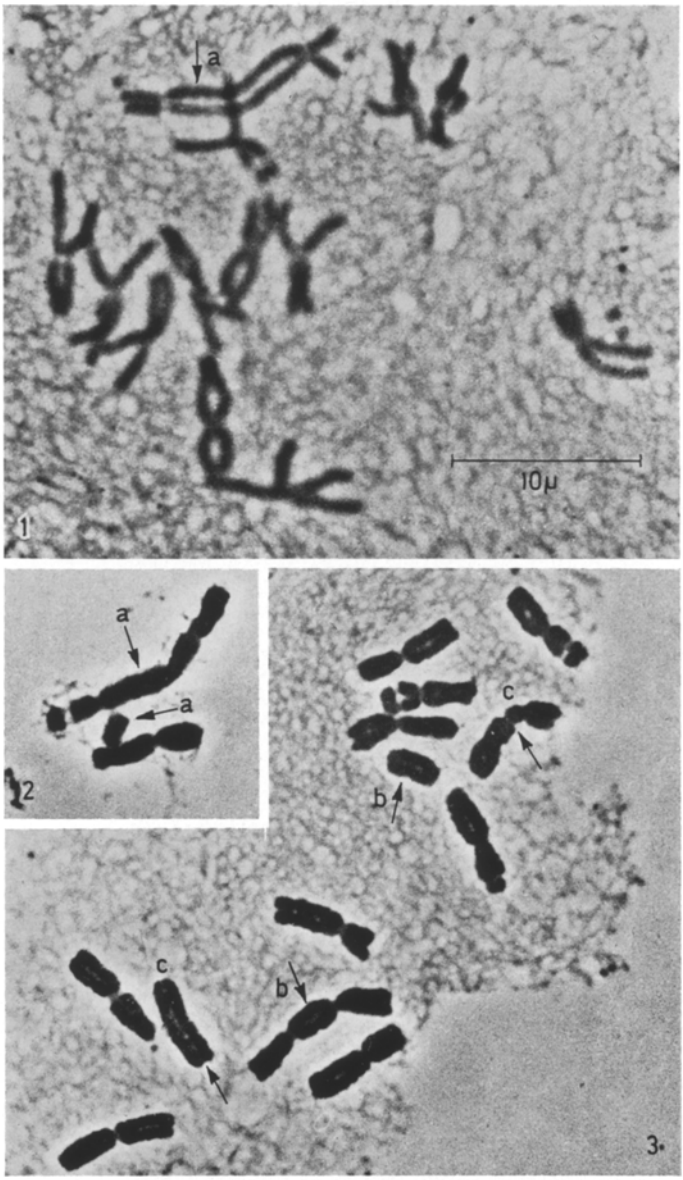

Fig. 1. An "a" type of dicentric without a fragment (arrow). × 2,500

Fig. 2. An "a" type of dicentric with a small fragment (arrows). × 2,500

Fig. 3. "b" and "c" types of dicentrics and their respective acentric fragments (arrows). × 2,500

Table. *Chromosome aberrations/100 cells*

	Dicentrics			Rings	Ratio dicentrics: rings
	a	b	c		
γ-rays					
10 krads	18	23	3	17	2.7
20 krads (no oxygen effect)	51	41	20	27	4.0
Percentage of various types of dicentrics	45	41	14		
γ-rays					
4 krads (oxygen effect)	50	25	10	15	5.7
Percentage of various types of dicentrics	59	29.5	11.5		
Neutrons					
200 rads	4.5	9	2	1	15.5
400 rads	8	15	3	2	13.0
800 rads	13	32	8	2	25.5
Percentage of various types of dicentrics	27	59	14		

compared the volume of the condensed heterochromatic regions (chromocentres) in G_2 with the total nuclear volume of a G_1 cell where all the nuclear material is in condensed state. This gives a value for heterochromatin per nucleus of 12%, in agreement with the metaphase measurements.

Study of Chromosome Aberrations

Since resting seeds were irradiated and studied in the first mitosis on germination, only chromosome aberrations were encountered. Detailed analyses of the rings and dicentrics were made. Attention was paid to the type of dicentrics induced and the accompanying acentric fragment, in order to make an estimate of dicentrics involving heterochromatic regions. These dicentrics will be long, with a long intercalary segments, (almost the size of two arms) with or without a small acentric fragment

(type a, Figs. 1 and 2). Dicentrics involving the regions near the centromeres, will be small with a small intercalary segment and accompanied by a long acentric fragment (type c, Fig. 3). Those involving the middle regions of the arms will form dicentrics with varying lengths of intercalary segment as well as accompanying acentrics (type b, Fig. 3).

Similarily, rings involving heterochromatic regions, will be large (equivalent to a whole chromosome) with or without a small fragment. Both small and large rings were encountered, as well as rings unaccompanied by fragments.

The data for both γ-irradiation and fast neutrons are presented in the Table (p. 253). Minutes were only found in the high gamma dose treatment, while acentric fragments unaccompanied by dicentrics or rings were common in the treatment involving oxygen.

Discussion

Relative frequencies of the three types of dicentrics are presented in the Table. From a random distribution of aberrations, one should expect about 10% of the dicentrics to involve heterochromatic regions. It can be seen that especially after γ-irradiation, a high proportion of the dicentrics is localised at or near the terminal regions — 5 times more than expected. Even after neutron irradiation, more dicentrics are found to involve terminal regions than expected — about 2.5 times.

The assumption that these aberrations really occur in the heterochromatin is based on the fact that terminal dicentrics are rarely found in other materials. In barley, for example, which has a comparable karyotype, the predominant type of dicentrics are those occurring near the centromere (Kumar and Natarajan, 1966).

Role of Heterochromatin in Formation of Chromosome Aberration

The reasons for high localisation of chromatid aberrations (induced in G_2) were discussed in an earlier paper (Natarajan and Ahnström, 1969). However, very little is known about the organization of the chromosomes (and heterochromatin) in the nucleus in G_1 or G_0 stage of the interphase. The present results offer some elucidation of the above question.

For the formation of dicentrics and rings, close interaction between different chromosomes and/or arms of the same chromosome is necessary. The high radioresistance of resting seeds when irradiated with sparsely ionizing radiations indicates that interactions between chromosomes are probably limited during the first hours of water uptake during the germination process. When the seeds are soaked before irradiation there is a gradual increase in radiosensitivity during the first 10—12 hours. After this period a constant level is reached where the seeds are about 50 times more sensitive than in the dry state. The repair processes start to

operate soon after soaking of the seeds in water and seem to be completed within the first 5 hours (Ahnström and Natarajan, 1970) and therefore most of the lesions induced by gamma rays are repaired before the nucleus enters a stage where closer interaction between lesions can occur as judged by the above mentioned increase in radiosensitivity upon soaking. One could then visualise the localisation in heterochromatin, either as pairing of heterochromatin in the dry state (G_0) or the repair process being delayed in the heterochromatic region — in correspondence with the delayed normal DNA-synthesis in these regions — and therefore permit interaction between the lesions later on in the G_1 where the chromosomes are in closer contact with each other. The limitations of interaction between chromosomal lesions during early hours of water uptake by the seeds, may be due to the fact that during this period the chromosomes are rigidly held in the nuclear matrix. That the mobility is low is also supported by the fact that radiation-induced oxygen sensitive centres are stable for hours especially when the seeds are soaked at low temperatures (Ahnström and Mikaelsen, 1968; Ahnström, 1968; Conger *et al.*, 1969).

Chromatid aberrations induced by chemicals as well as radiation have been found to be preferentially localised in the heterochromatin. This has been attributed to be due to the simultaneous DNA synthesis in these regions (Evans and Scott, 1969), as well as the tendency of these regions to associate with each other (Michaelis and Rieger, 1968; Natarajan and Ahnström, 1969). Since chromosome aberrations are formed prior to the normal DNA synthesis in the seeds (Ahnström and Natarajan, 1970) the role of DNA synthesis itself seems to be of little importance. The close association or even an intimate pairing in these regions should play a greater role. The fact that we observe always a lower number of chromocentres than the number of heterochromatic regions indicates that the majority of these regions are associated. It seems that the structural heterochromatin is not just a condensed state only during the interphase, but is also differentiated in other stages, e.g. pachytene stages in meiosis or even in somatic metaphases (Caspersson *et al.*, 1969). Hence heterochromatic regions are probably able to influence aberration formation at all stages[1].

1 *Note Added in Proof.* Since this paper was sent to the press it has been shown that structural heterochromatin in mouse cells mainly consists of so-called satellite DNA (Yasmineh and Yunis, 1970 and quoted papers). Satellite DNA has a base composition little different from the main DNA has been found to be easily renaturable indicating the occurrence of repetitive base sequences. If this is a common property of structural heterochromatin in all species it could explain chromocentre formation as well as localised chromosome exchanges, as the occurrence of repetitive base sequences would offer maximum possibilities for hybrid formation between DNA-strands in homologous or nonhomologous chromosomes.

Differential Response between γ-Rays and Neutrons

The frequency of dicentrics involving heterochromatin following neutron irradiation is less than after gamma irradiation. This may mainly be due to the fact that neutron induced lesions are not repaired to the same extent as the gamma ray induced lesions as shown by the fact that caffeine which enhances the damage after gamma radiation has no effect in neutron irradiated seeds (Ahnström and Natarajan, 1970). This could indicate that neutron induced lesions are repaired less efficiently and therefore persist to a later stage when interaction is possible thus being less dependent on (a) pairing of heterochromatic regions during G_0 or (b) delayed repair in the heterochromatic regions.

It can be seen from the table, that the proportion of dicentrics to rings is much low in gamma treated material than the neutron treated material. To a lesser extent a similar relationship between these two types of radiations has been found earlier in *Hordeum vulgare* (Kumar and Natarajan, 1966), *Nigella damascena* (unpublished results), species lacking heterochromatin, as judged by various criteria (Natarajan and Ahnström, 1969). In *Tradescantia* microspores, it has been shown that environmental changes such as temperature, carbon monoxide etc., can modify the ratio between dicentrics and rings, which has been attributed due to differential movement of chromosomes and chromosome arms (Steffensen, 1959).

Since the quantity and the distribution of heterochromatin affect differentially the frequency of chromosome aberrations produced by neutrons and gamma rays, it is expected that the RBE values determined for different species should vary according to their heterochromatin content. This point was discussed previously comparing *Nigella damascena* and *Vicia faba* (Natarajan and Ahnström, 1969). Diploid barley and diploid rye can also be compared in a similar way. Barley lacks any well defined heterochromatin in contrast to rye. The RBE calculated for rye is between 25 to 30 (Table) and for barley it is between 70 to 80 (calculated from the data of Kumar and Natarajan, 1967), for the production of dicentrics and rings. These findings support the concept that the RBE for chromosome aberrations is, under identical conditions, affected by the presence of heterochromatin.

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