Chromosoma (Berl.) 69, 193–209 (1978)

CHROMOSOMA

Distribution of Heterochromatin in a Reconstructed Karyotype of *Vicia faba* as Identified by Banding- and DNA-late Replication Patterns

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Abstract. 1) The distribution pattern of heterochromatin characterized by Giemsa-banding, Quinacrine-banding and DNA-late replication has been studied in a reconstructed karyotype of *Vicia faba* with all chromosome pairs interdistinguishable. 2) By means of two Giemsa-banding methods both an interstitial and a centromeric Giemsa-banding pattern are described. The former one comprehends 14 "marker" and 18 "additional" bands of lower but characteristic visualization frequencies. The centromeric Giemsa-banding pattern consists of 7 bands, located in the centromeric and in the secondary constrictions of the metaphase chromosomes. Chromosomes with banding patterns intermediate between the interstitial and the centromeric Giemsa-banding have also been observed. 3) Quinacrine-banding revealed 10–12 brightly fluorescent bands and 1–2 regions of dim fluorescence. Most Q-bands occupy chromosomal positions also characterized by interstitial Giemsa bands. 4) The DNA-late replication pattern, analyzed both by autoradiography and by FPG-technique, revealed 9 late replicating chromosome regions; all of these correspond positionally to the sites of interstitial Giemsa bands. 5) The results are discussed with respect to (a) the relationships between the banding- and the DNA-late replication pattern; (b) banding and heterochromatin characteristics; (c) the correlations between the distribution of chromatid aberrations and special types of heterochromatin. – The patterns of heterochromatin distribution found are in basic conformity with the corresponding patterns reported for the standard karyotype of *Vicia faba*. The heterochromatin type characterized by both Giemsa-banding and late replication is characteristic of all those chromosome regions which after mutagen treatments show up as aberration hot spots. Positional correlations between interstitial Giemsa marker bands and chemically induced isochromatid breaks are indicative of preferential aberration clustering in heterochromatin/euchromatin junctions.

0009-5915/78/0069/0193/$03.40

Introduction

An unexpectedly rich longitudinal differentiation of the chromosomes of many animals and plants, mainly due to the distribution patterns of heterochromatin, has been observed by application of new cytological techniques. Among plants, *Vicia faba* is the classical and probably best studied object with respect to the features and the distribution of heterochromatin in the chromosome complement. It is also a favourable object for investigations in the field of chromosome structural changes. In several reports, regions of constitutive heterochromatin in this chromosome complement have been shown to correlate with sites of clustering of chromosome structural changes induced by chemical mutagens or irradiation (cf. Evans, 1962; Rieger and Michaelis, 1967; Kihlman, 1966; Natarajan and Raposa, 1975).

For more detailed studies as to the relationships between the distribution of heterochromatin and chromatid aberrations, the reconstructed karyotypes of *Vicia faba* with easily identifiable chromosomes, introduced by Michaelis and Rieger (1971), may be used most advantageously (Rieger et al., 1975). In the present investigation, the distribution patterns of heterochromatin characterized by Giemsa-banding, Quinacrine-banding, and DNA-late replication have been studied in one of the reconstructed karyotypes of *Vicia faba* and compared with the distribution patterns of "hot spots" for induced chromatid aberrations reported in previous publications (Rieger and Michaelis, 1970, 1972; Rieger et al., 1975).

Material and Methods

Material. The karyotype studied (ACB) differs from the normal karyotype of *Vicia faba* with respect to the morphology of chromosome pairs I, III, V and VI, which are reconstructed and made identifiable by reciprocal translocations and pericentric inversions (Michaelis and Rieger, 1971). In order to localize induced chromatid aberrations and heterochromatin regions, the karyotype has been subdivided into 28 chromosome segments as described by Döbel et al. (1973).

Banding Methods. Main root tips 3–5 cm in length have been pretreated with 0.05% colchicine for 2 h, fixed in ethanol-glacial acetic acid (3:1) for 48 h and stored in 90% ethanol at 4° C. For Giemsa-banding, they were hydrolysed for 5 min in 0.2 N HCl at 60° C. After squashing in 45% acetic acid the cover glasses were removed on dry ice and the slides air-dried.

Interstitial Giemsa bands were obtained by incubating the slides in 6 M urea at pH 7.2 for 12–20 min. Subsequently, the slides were rinsed in M/15 Sørensen phosphate buffer (pH 7.2) and treated with 0.1% trypsin at 37° C for 10–15 min. After further rinsing in Sørensen phosphate buffer, the slides were stained in a 4% Merck-Giemsa-solution in Sørensen buffer at pH 6.7 for 6–12 min.

After staining, the slides were rinsed in destilled water, airdried and mounted in Eukitt (Kindler, Freiburg i.Br.).

Centromeric Giemsa bands have been observed after pretreatment of the slides with a 3% solution of barium hydroxide at 60° C for 5–15 min prior to incubation with urea and trypsin. Before centromeric bands became visible, Giemsa-staining had to be extended up to 4 h.

In order to determine the "visualization frequency" of Giemsa bands, the percentage of the chromosomes which showed the band in question was evaluated in 250 of each of the 6 homologous chromosome pairs. The relative position of the Giemsa marker bands was determined by a method

of Scheid (1973): the contours of the chromosomes were copied from 6000× enlarged prints by engraving in sheets of transparent celluloid and the centromeric constrictions and the band centres were marked. The engravings have been projected with a further 6× enlargement and copied by hand on paper. Next, the midlines of the chromatids have been constructed. Using a chartreader, the distances were measured from the centromere to the band in question and the values obtained compared with the arm length:

$$\text{relative band position} = \frac{\text{distance of the band from centromere}}{\text{total length of the chromosome arm}}.$$

For Q-banding, a method of Kim et al. (1971) has been adapted. The fixed root tips were hydrolysed for 5 min in 0.1 N HCl at 60° C and processed into squash preparations as described for the Giemsa banding. Air-dried slides were gradually hydrated from 100% ethanol into 2% acetic acid and then stained in a solution of 0.5% atebrine in 2% acetic acid (pH 3–4) for 10–12 min. After rinsing twice in distilled water and air drying, they were mounted in distilled water and the cover glasses sealed with nail-polish. The slides were viewed and photographed with a "Fluoval"-microscope from Carl Zeiss, Jena, fitted with a HBO 200 mercury vapour lamp, using a BG 12 excitation filter and an OG 4 barrier filter. Photomicrographs were taken on ORWO (Wolfen) RS 2-films.

DNA–Late Replication. For autoradiographic determination of the DNA-late replication pattern, main roots have been incubated with ^{3}H-thymidine (5 μCi/ml) for 30 min, thoroughly washed and grown for additional 5 h in aerated tap water at 24° C. After colchicine-treatment and fixation (as described above), the roots were hydrolysed in 1 N HCl for 12 min at 60° C and Feulgen-stained. After squashing and removal of the cover glasses on dry-ice, the slides were air-dried. Subsequently, they were dipped in K6-emulsion (ORWO, Wolfen, GDR) and after exposition at 4° C for 18–21 d in the dark developed for 3 min in ORWO developer solution MH 28, diluted 1 : 4. For a quantitative estimation of the label distribution, the number of silver grains per chromosome half-segment has been counted (both in the microscope and on photographs) in 50 arbitrarily chosen labelled metaphases.

For the determination of the late replication pattern by means of the FPG-technique (Perry and Wolff, 1974), roots were incubated in the dark in BrdU (100 μM)+FdU (0.1 μM) for 4–6 h, treated with colchicine and fixed. After rinsing of the roots in 0.01 M sodium citrate buffer (pH 4.7), digestion was performed with pectinase (1% for 2 h) and cellulase (2% for 90 min) at 37° C in the same buffer. After squashing in 45% acetic acid, the cover glasses were removed on dry-ice. Subsequently, the slides were gradually hydrated from 100% ethanol to 0.5 × SSC and then treated with RNAse (0.01%) for 1 h at 37° C, dehydrated in ethanol, and air-dried. After rinsing with 0.5 × SSC, staining and UV-irradiation were performed simultaneously in 33258 Hoechst solution (0.1 ml of a 0.1% stock solution in ethanol diluted with 200 ml of 0.5 × SSC) for 45 min. Thereafter, the slides were rinsed in 0.5 × SSC, incubated in 2 × SSC at 80° C for 1 h, and finally stained in a 4% Merck-Giemsa solution, pH 6.7 for about 2 min. The preparations were made permanent by airdrying and mounting in Eukitt.

Results

1. The Giemsa Banding Patterns

In order to obtain maximal differentiation of the chromosome set of karyotype ACB by Giemsa banding, it was necessary to use two Giemsa-banding methods. By means of these methods, which differ by only one pretreatment step, two different banding patterns have been produced. In response to the urea-trypsin-Giemsa method, bands were mainly situated in interstitial chromosomal positions. In response to the bariumhydroxide-urea-trypsin-Giemsa method, most bands were found at centromeric positions. With each of these methods some metaphase plates showed chromosomes with interstitial and centromeric bands.

a) The Interstitial Giemsa Banding Pattern

The interstitial Giemsa banding pattern of karyotype ACB was characterized by 14 "marker" hands which were found in the overwhelming majority of the differentially stained chromosomes and have previously been described (Döbel et al., 1973). Due to improvements of the Giemsa banding technique 18 "additional" interstitial Giemsa bands in the chromosome set have been observed. These proved to be very faint bands visible only in a minority of the banded chromosomes.

The complete interstitial Giemsa banding pattern of ACB (Fig. 1) is more or less identical to the Giemsa banding reported for the normal karyotype of *Vicia faba* (Vosa and Marchi, 1972; Schweizer, 1973b; Burger and Scheuermann, 1974; Greilhuber, 1975; Klášterská, and Natarajan, 1975; Friebe, 1976), if the reconstruction of the chromosome set characterizing ACB is taken into consideration. By further improvements of the Giemsa banding techniques used some more additional bands may be expected to become visible.

In an attempt to quantify the differential banding of interstitial heterochromatin of ACB by the urea-trypsin-Giemsa method the "visualization frequency" of individual bands has been estimated. It was possible to classify intercalary bands into three "visualization frequency classes", viz. bands visible in approximately 10–30%, 30–60% (additional bands) and 60–100% (marker bands) of all chromosomes banded (cf. diagram in Fig. 1). Among the group of marker bands, the extreme values found are 90–100% "visualization frequency" for bands in segment 23 of chromosome V and in segments 26/27 of chromosome VI, and 50–70% for the band in segment 2 (nucleolus organizer region) of chromosome III.

In a varying proportion of chromosomes, depending on the quality of the slides, also completely unbanded chromosomes have been observed. In Figure 1b the relative positions of the interstitial marker bands on corresponding chromosome arms, as measured by the method of Scheid (1973), are given as reference points. These values proved to be remarkably constant. This is indicative of the absence of differential changes of length within the chromosomes due to the Giemsa banding procedures.

The number of discernible bands in the Giemsa-banded karyotype also depends, to some extent at least, on the state of contraction of the chromosomes. In relatively extended prometaphase chromosomes, the interstitial bands observed in segments 15, 11 and 23 (distal band) of chromosomes I, II and V respectively, have frequently been found to be subdivided into two parts (Fig. 3); in more contracted metaphase chromosomes, these bands constantly appeared in the form of a single band.

Bands in the centromeric positions of chromosomes I, II, and III have occasionally been observed in response to the urea-trypsin-Giemsa treatment (see Fig. 1, chromosome III). These bands proved to be very faint, occurred with visualization frequencies of up to 5% and coincided positionally with the centromeric bands produced by the bariumhydroxide-urea-trypsin method. The chromosomes harbouring them also showed interstitial Giemsa bands. They may represent intermediate stages between the interstitial and the centromeric banding pattern.

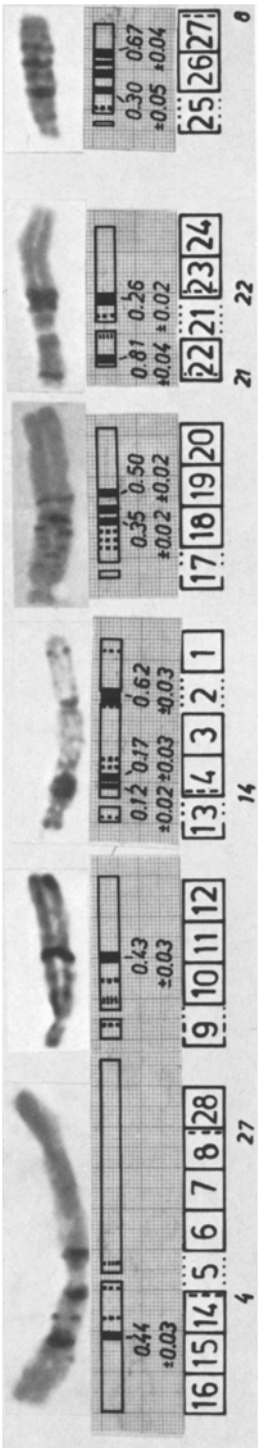

Fig. 1 a–c. a The interstitial Giemsa-banding pattern of karyotype ACB. **b** Three visualization frequency classes and the relative positions of the Giemsa marker bands. **c** The subdivision of the chromosome set of karyotype ACB into 28 chromosome segments (dotted lines: centromeres and nucleolus organizer region in segment 2).
■ = visualization frequency 60–100% = marker bands.
▒ = visualization frequency 30–60%
: = visualization frequency 10–30%
} = additional bands

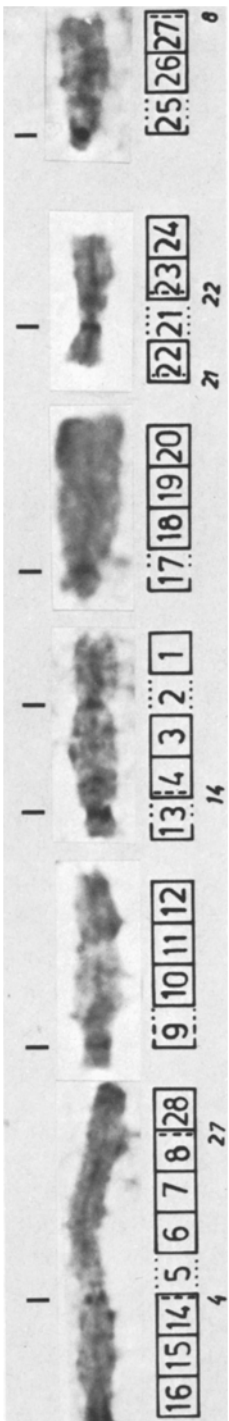

Fig. 2. The centromeric Giemsa-banding pattern (bars) of karyotype ACB

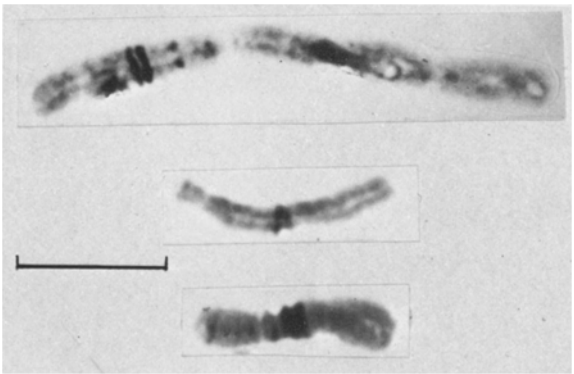

Fig. 3. Examples of relatively extended chromosomes I, II, and V (top to bottom) showing the Giemsa-bands of segments 15, 11, and 23 (distal band) subdivided into two bands. Bar = 10 μm

b) The Centromeric Giemsa Banding Pattern

An additional incubation of the slides with bariumhydroxide prior to the urea-trypsin-Giemsa treatment resulted in bands located in the centromeric constrictions of all 6 chromosome pairs as well as in the secondary constriction of chromosome III (Fig. 2).

These bands are rather prominent and completely covered the constrictions. They occurred nearly obligatory in chromosomes treated by the above-mentioned technique. Occasionally, also some interstitial Giemsa marker bands were produced by the bariumhydroxide-urea-trypsin-Giemsa method. They only slightly stood out from the chromosomal background and occurred with "visualization frequencies" up to 10% in all 6 chromosome pairs. The chromosomes harbouring them also showed centromeric and interstitial Giemsa banding, respectively (Fig. 4). It was possible to shift this intermediate pattern toward the interstitial banding pattern by shortening the bariumhydroxide-pretreatment. This resulted in a proportional increase of the number of interstitial bands at the expense of centromeric bands.

2. The Quinacrine-Banding Pattern

The Quinacrine banding pattern of karyotype ACB (Fig. 5) consists of 10–12 brightly fluorescent bands located, with 3 exceptions, on the long arm of the chromosomes near to the centromere. Only in segment 15 of chromosome I a Q-band, occasionally a double band, was found in median position. Chromosome III showed a dot-like Q-band in the short arm in segment 13 and in the long arm in segment 4 there were two, occasionally also three, Q-bands stacked one behind the other.

This pattern agrees with the Q-banding of the normal karyotype of *Vicia faba* as described by Vosa and Marchi (1972) if the structural reconstructions involved in ACB are taken into consideration. Like the Giemsa bands, the Q-bands are visible only in a limited fraction of the chromosomes. The highest

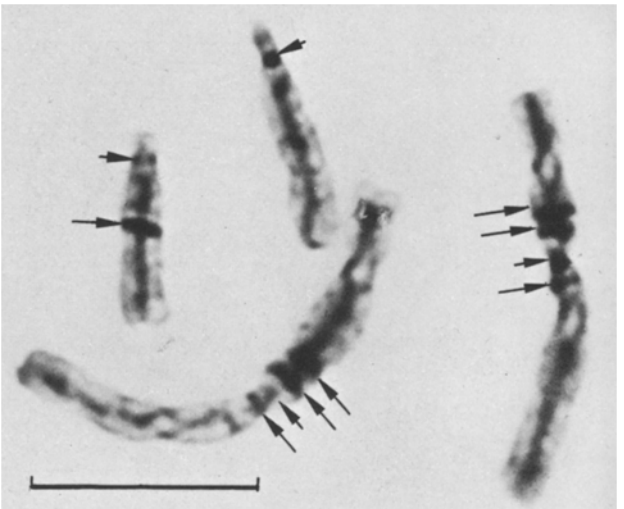

Fig. 4. Chromosomes of karyotype ACB showing intermediate stages between the centromeric (small arrows) and the interstitial (large arrows) Giemsa-banding pattern. Bar = 10 μm

"visualization frequencies" were found for the Q-bands in segment 14 and 6 of chromosome I and in segment 4 of chromosome III. These represent the most brightly fluorescent Q-bands; the Q-bands in segment 15 of chromosome I and segment 26 of chromosome VI were found, in most cases, to fluoresce relatively weakly. In segment 23 of chromosome V a band fluorescing less intensely than the rest of the chromosome has frequently been observed. A similar region of dim fluorescence has occasionally been found in segment 11 of chromosome II.

Most Q-bands appear in chromosomal positions which had been found to be also occupied by Giemsa-bands. However, only the Q-bands in segment 15 of chromosome I and segment 4 of chromosome III exactly correspond positionally to interstitial Giemsa marker bands. The Q-bands in segments 14 and 6 (chromosome I), 10 (chromosome II), 13 (chromosome III), 22 (chromosome V), and 26 (chromosome VI) correspond to additional interstitial Giemsa bands and, in most cases, were situated in paracentromeric positions, showed low "visualization frequency" and proved to be only faint. For the Q-band in segment 18 of chromosome IV a corresponding Giemsa band has not yet been found. The area of reduced fluorescence sometimes found in segment 23 of chromosome V corresponds to the interstitial Giemsa marker band.

3. The DNA-Late Replication Pattern

By autoradiography, the earliest labelled metaphases have been found $4^1/_2$–5 h after incubation of the roots in ^{3}H-thymidine. They show an inhomogeneous distribution of the label characterized by an accumulation of silver grains in certain chromosome regions (Fig. 6b). This labelling represents the pattern of chromosomal DNA replication during the late S-phase.

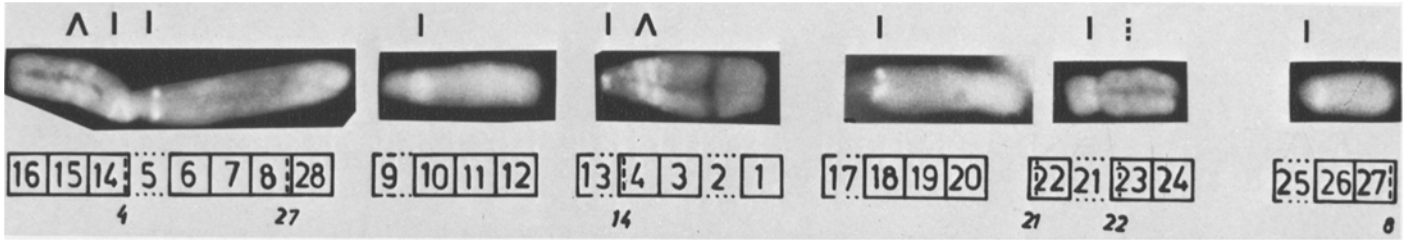

Fig. 5. The Quinacrine-banding pattern of karyotype ACB. Vertical bars indicate brightly fluorescent bands. Regions occasionally occupied by more than one band are marked by angles. Broken line = dimly fluorescent region

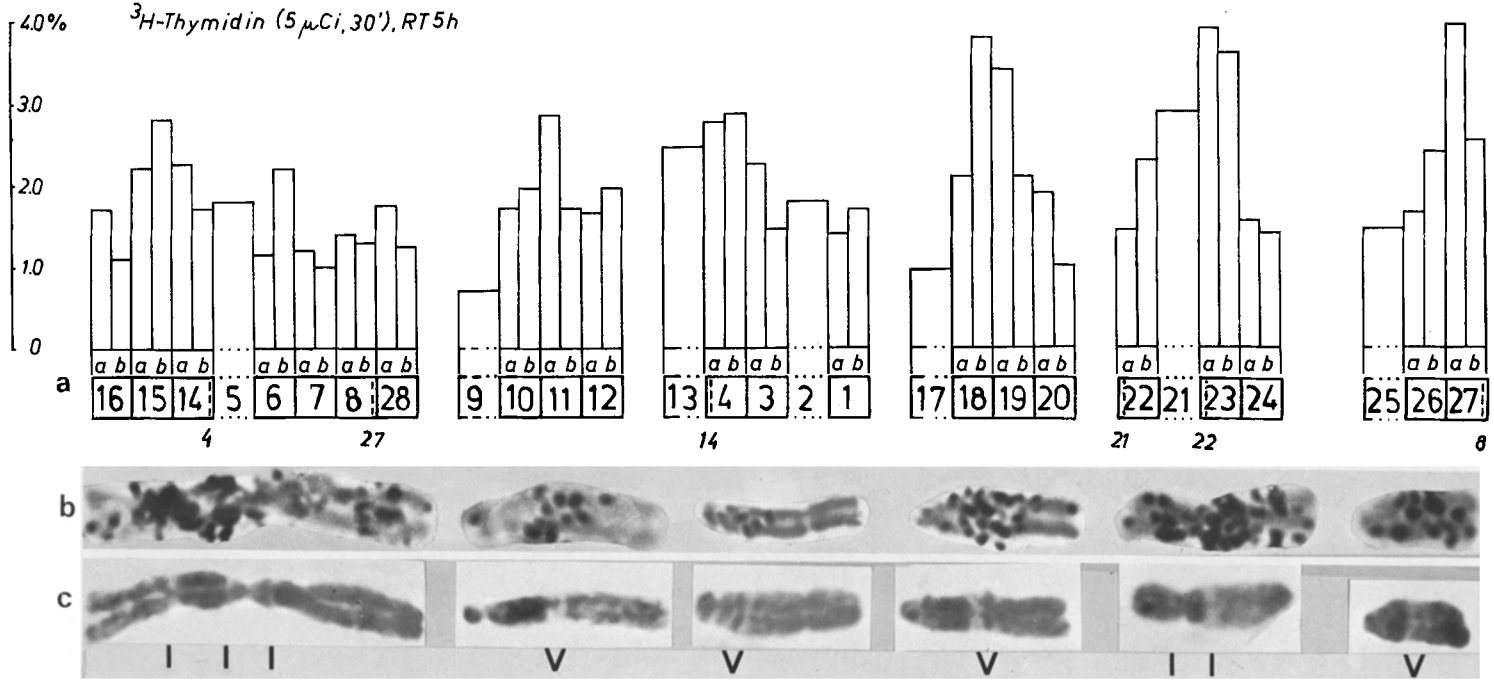

Fig. 6a–c. The DNA-late replication pattern of karyotype ACB. **a** Average percentage of silver grains per chromosome half-segment as evaluated from 50 ^{3}H-thymidine-labelled metaphases. **b** Examples of labelled chromosomes with typical distribution of silver grains. **c** Late replication pattern as demonstrated by means of the FPG-technique. The pale regions (bars or angles) are indicative of DNA-late replication

Figure 6a shows the average percentage of silver grains per chromosome half-segment, obtained by counting of 50 arbitrarily chosen labelled metaphases. Each of the 5 acrocentric chromosome pairs stands out by the heavy labelling of one chromosome region which, in most cases, was found to be located in the median part of the long arm. The metacentric chromosome I shows one distinct late replication maximum in the short arm, extending from the middle (segment 15) toward the proximal region (segment 14) and another not very distinct accumulation of silver grains in the long arm (proximal region of segment 6). This pattern is in agreement with the late replication pattern of the normal karyotype of *Vicia faba* as described by Evans (1964) and Burger and Scheuermann (1974) if the chromosomal reconstructions in karyotype ACB are taken into consideration. Though the late replicating chromosome regions are, in most of the labelled metaphases, microscopically evident by the accumulation of silver grains, the relative grain numbers found for individual chromosome segments considerably fluctuated from one cell to another. The peaks of replication intensity shown in Figure 6a are accordingly low. This is presumably caused by the wide scattering of the silver grains around the radioactive source in the autoradiographic preparations and the sporadic nature of the radioactive fission process. Possibly, cell populations with a deviating cell cycle duration, which may contribute to the blurring of the late replication maxima, also exist in the *Vicia faba* root tip meristems (Schubert and Meister, 1977).

To improve the results obtained from autoradiographic investigations a second set of experiments with the FPG-technique (Perry and Wolff, 1974) has been devised. This method rests on the substitution of 5-bromodeoxyuridine (BrdU) for thymidine during part of chromosomal DNA synthesis. Those chromosome regions which incorporated BrdU then appear as palely stained bands in response to various pretreatment steps and Giemsa staining (Latt, 1974).

It was possible by the FPG-technique to show the late replication pattern of karyotype ACB with a considerably higher degree of resolution than by autoradiography (Fig. 6c). As evident from Figure 6, the late replication patterns demonstrated by the two methods are in general agreement. The FPG-technique, however, unequivocally shows the late replicating chromosome regions to correspond to the sites of interstitial Giemsa bands. With the exception of the marker bands in segments 2 and 22 of chromosome III and V, respectively, all interstitial Giemsa marker bands are in fact sites of DNA late replication. Moreover, the interstitial additional Giemsa bands found in segments 14 and 6 of chromosome I and in segment 22 of chromosome V were also proved by the FPG-technique to be late replicating. In addition, a minority of cells has been found by application of this technique to show replication patterns different from those of the late replication pattern of DNA described above. This may be due to deviations of the cell cycle duration in these cells.

Discussion

The results of the present investigation are relevant with respect to three main topics, which deserve discussion in more detail: 1) the relationships between the three banding patterns and the DNA-late replication pattern of karyotype

ACB on the one hand and the standard karyotype of Vicia faba on the other; 2) the inferences from banding and DNA-late replication with respect to heterochromatin characteristics in karyotype ACB, and 3) the correlations between the patterns of distribution of chromatid aberrations and special types of heterochromatin in karyotype ACB.

1. Relationships of the Three Banding Patterns and the DNA–Late Replication Pattern of Karyotype ACB and of the Standard Karyotype of Vicia faba

The patterns of Giemsa banding, Q-banding and DNA-late replication in the ACB chromosome set are in basic conformity with the corresponding patterns reported for the "standard" karyotype of *Vicia faba,* if the differences in chromosome structure due to karyotype reconstructions in ACB are taken into consideration. The changed neighbourhood of some heterochromatic regions, caused by these reconstructions, remained without significant influence on the patterns studied.

Among the techniques used, the Giemsa banding methods resulted in the most clearcut euchromatin/heterochromatin differentiation in the chromosomes. Two Giemsa banding patterns have been observed after modification of the method used for Giemsa band visualization, both different with respect to the position of the bands in the chromosomes.

The interstitial Giemsa marker bands were found to coincide with the blocks of constitutive heterochromatin which are well known from earlier investigations of *Vicia faba* chromosomes by means of other techniques (Heitz, 1932; Tjio and Levan, 1950; LaCour, 1951; McLeish, 1953; Ockey, 1960; Evans and Bigger, 1961). Some of the additional Giemsa bands obviously correspond to the heterochromatin observed by these authors in the proximal part of the acrocentric chromosomes.

The centromeric Giemsa bands of *Vicia faba* have up to now been reported only by Burger and Scheuermann (1974) and by Friebe (1976). Takehisa and Utsumi (1973) report about the occasional occurrence of densely stained centromeric regions in Giemsa-banded *Vicia faba* chromosomes. No heterochromatin, however, has been detected within the centromeric regions of the *Vicia faba* chromosomes after application of any other than the Giemsa banding technique.

Apart from the chromosomal position of the centromeric bands in karyotype ACB and the method used to make them visible (which is treatment more harsh than necessary for visualization of the interstitial bands), their gradual appearance, at the expense of the interstitial bands, is in remarkable conformity with the mode of staining characteristic for C-banding in animal chromosomes. By increasing the time of the bariumhydroxide-pretreatment from 2 to about 10 min a series of intermediate stages between the interstitial and the centromeric banding can be produced in the chromosomes of karyotype ACB of *Vicia faba*. These intermediate stages correspond to the spectrum of banding patterns, ranging from G-banded to C-banded metaphases which can be induced in human (Daniel and Lam-Po-Tang, 1973) and mouse chromosomes (McKay, 1976) by prolonging the treatment time for banding.

One should be cautious, however, not prematurely to equate the two Giemsa banding patterns produced in ACB with the C- and G-banding of animal chromosomes. There is a recent tendency to equate all Giemsa banding patterns of plant chromosomes with "C-banding". "C-banding" is then understood as banding of constitutive heterochromatin, contrary to G-banding which is thought to be indicative of structural euchromatin and has been denied to be resolvable in plant chromosomes (Greilhuber, 1977). This is, in fact, not the original meaning of the term "C-banding" as defined during the Paris conference of 1971 (definition on the basis of the position of the bands and the treatment applied, but without reference to the ability of the method to differentiate heterochromatic of euchromatic regions). On the other hand, the C-bands are evidently representative of constitutive heterochromatin in most animal chromosomes. Keeping this in mind, it seems to be appropriate to avoid these terms before a definite conclusion as to the relationship between plant chromosome Giemsa banding patterns and C- and G-banding of animal chromosomes is at hand.

2. Inferences from Banding with Respect of Heterochromatin Characteristics in Karyotype ACB

The techniques used in the present investigation demonstrate four heterochromatin characteristics: differential stainability in the form of interstitial Giemsa-bands, centromeric Giemsa bands, and Q-bands as well as the presence of late replicating DNA. On this basis, the individual heterochromatic regions of karyotype ACB differ (Fig. 7) and may be classified into several distinct types. Despite extensive investigations, the mechanism of Giemsa banding is still unknown with respect to basic details. Opposite to earlier hypotheses the DNA base composition is no longer presumed to be the basic target for banding procedures but rather some protein components, presumably non-histone-proteins, which possibly become selectively extracted by the banding procedure from the chromosomes. Since proteins may recognize and interact with specific DNA-sequences or geometries, the bands produced may be viewed as indirect expressions of locally enriched specific DNA sequences (Comings et al., 1973; Löber et al., 1973, 1976). Differential chromosome contraction induced or intensified by the banding treatment has also been assumed to be involved in the production of banding patterns (McKay, 1973, 1976; Okada and Comings, 1974; Löber et al., 1976).

The presence of both brightly and dimly fluorescent Q-bands may be indicative of specificities in the DNA base composition of the corresponding chromosome regions. As found by Pachmann and Rigler (1972), Weisblum and de Haseth (1972) and recently confirmed by Schreck et al. (1977) a base composition of chromosomal DNA with preponderance of AT-or GC-pairs respectively seems to be the primary determinant of the occurrence of brightly or dimly fluorescent Q-bands. However, objections to this concept have also been raised (Bostock and Christie, 1974). Alternative interpretations put emphasis on the importance of chromosomal proteins (Comings et al., 1975; Sumner, 1977) or on differential chromosome condensation (McKay, 1976) in Q-banding. Furthermore, microen-

vironmental conditions in and around the chromosomes have been found to influence in a complex way the ability of the fluorescence stains to yield bands (Löber, 1975; Löber et al., 1976). The same conclusion has previously been drawn from the responses to other treatments (Vosa and Marchi, 1972; Takehisa, 1973; Schweizer, 1973b; Greilhuber, 1975; Vosa, 1976). According to Vosa (1976) all the bands observed in the metacentric chromosome I of the *Vicia faba* standard karyotype showed up differentially in dependence on the technique being used. This is certainly also true for most or all bands observed in the acrocentric chromosomes if the classification of Giemsa- and of Q-bands on the basis of their differential visualization frequency is taken into consideration. The differential reactivity of individual heterochromatic regions of *Vicia faba* to the techniques applied may be taken to be indicative of a certain heterogeneity of heterochromatin. As shown above, the banding methods used in the present investigation demonstrate the interstitial Giemsa marker bands and the centromeric Giemsa bands to show up rather regularly but the same methods are not well suited for the demonstration of the additional bands.

A heterochromatin type characterized by both late-replication and interstitial Giemsa bands has most frequently been found in the present study. Th heterochromatin in segments 15, 14 (proximal band) and 6 of chromosome I, in segment 4 of chromosome III, and in segment 22 (proximal) of chromosome V has been found to be additionally characterized by the presence of bright Q-bands (Fig. 7).

A corresponding coincidence between G-bands, Q-bands and late replicating chromosome regions has often been found in mammalian chromosomes (Ganner and Evans, 1971). Dutrillaux (1977) generalized for human chromosomes that G- and Q-bands represent a group of chromosomal regions which show late replication. Exceptions to this rule have, however, also been found. Based on similarities in the distribution of G- and Q-bands in both early and late replicating chromosome regions of *Dipodomys merriami* and a coincidence of Q-bands and early replicating chromosome regions in *Dipodomys ordii*, Bostock and Christie (1974) deny the existence of a general rule relating the time of replication of a chromosome segment to its ability to yield G- or Q-bands.

In mammalian chromosomes, the G-bands in most cases coincide with brightly fluorescent Q-bands. In this respect the chromosomes of *Vicia faba* deviate since most of the Giemsa marker bands do not positionally correspond to Q-bands. The very prominent Giemsa marker band observed in segment 23 of chromosome V has relatively frequently been observed to be represented by a dimly fluorescent region after Quinacrine staining and in this respect corresponds to the heterochromatic regions in *Allium-, Scilla-* and other plant chromosomes (Vosa, 1970, 1972, 1976).

3. The Patterns of Distribution of Induced Chromatid Aberrations as Related to Special Types of Heterochromatin in Karyotype ACB

It is remarkable that the heterochromatin type characterized by both Giemsa banding and late DNA replication is (except of the nucleolus organizer region)

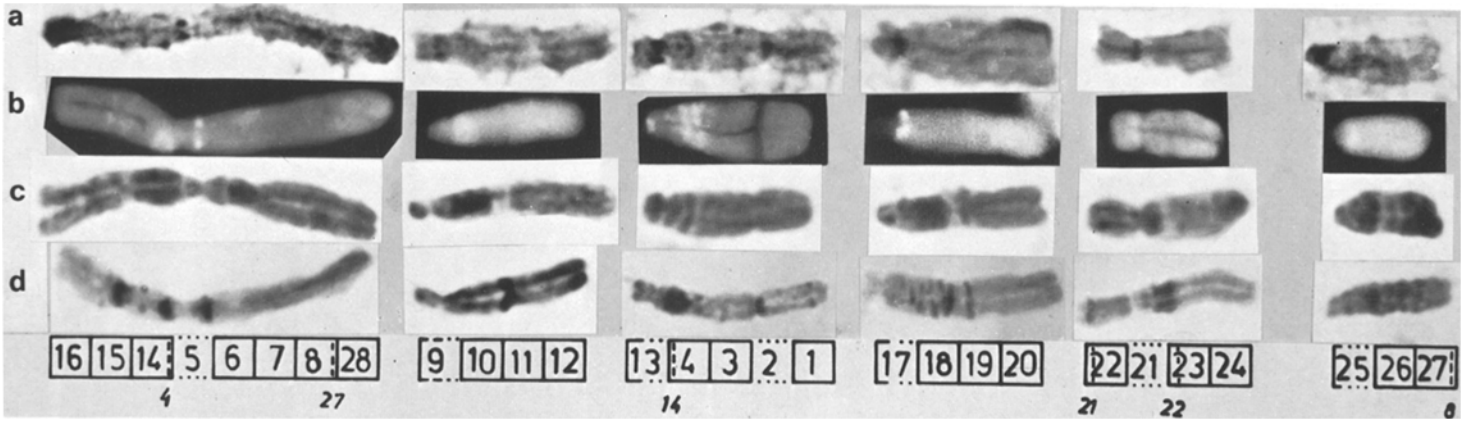

Fig. 7a–d. Survey of **a** the centromeric Giemsa-banding pattern, **b** the Q-banding pattern, **c** the FPG-late replication pattern, and **d** the interstitial Giemsa-banding pattern

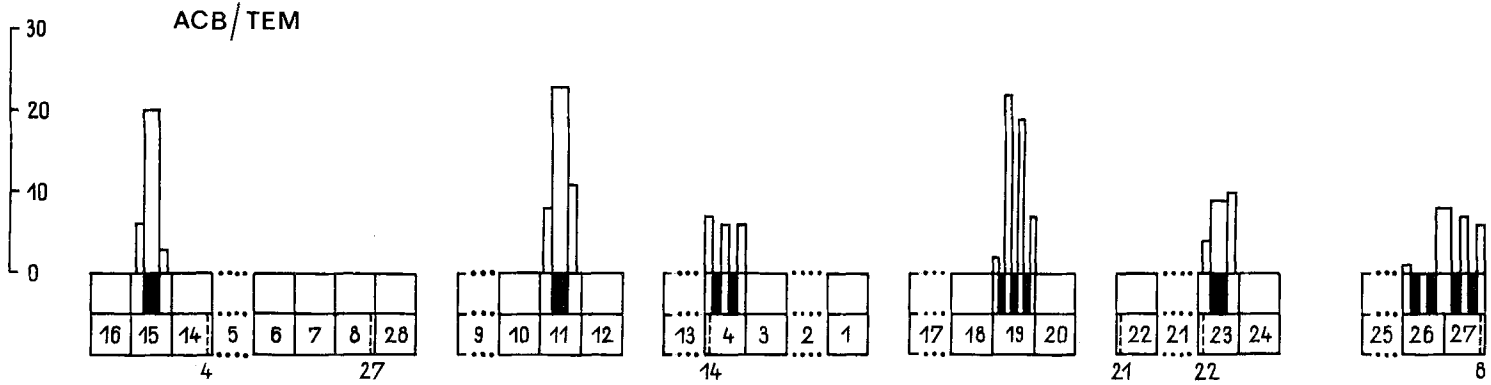

Fig. 8. Distribution of 250 isochromatid breaks, located in chromosome regions representing aberration hot spots, in relation to the Giemsa marker bands. (Mutagenic treatment: Triethylene melamine, 5×10^{-4} M, 1 h, recovery time 24 h)

localized in all those chromosome regions which after mutagen treatments show up as aberration hot spots (cf. Rieger et al., 1975). This heterochromatin type may (with the exception of segments 19 and 22) be further characterized by its tendency to respond to cold treatment by decompaction giving rise to what has been called "H-sections" (Takehisa et al., 1976). These have been found to coincide positionally with interstitial Giemsa bands or are located immediately adjacent to those. This is however a characteristic of limited value only because the relationship of cold sensitive segments and heterochromatin was found to be rather complex in *Vicia faba* (Greilhuber, 1975; Takehisa, 1976). Evidence for the presence of cold sensitive segments in chromosome regions which are not heterochromatic has in fact been reported (Schweizer, 1973a). Opposite to this, the heterochromatin type characterized both by the presence of Giemsa-banding and Q-banding but not by DNA-late replication has not been found to show clustering of chromatid aberrations (segment 10 and 13 of chromosomes II and III). With the exception of the nucleolus organizing region in segment 2 of chromosome III, the same is true for heterochromatin characterized by the presence of only Giemsa bands as found in segments 14 (distal band), 9, 11 ("additional" band), 1, 22 (marker band) of chromosomes II, I, III, and V as well as in all centromeric regions.

Due to the complexity of factors involved in chromosome banding it is difficult to use the characteristics of the heterochromatin type in chromosome regions which represent potential aberration hot spots for more than general conclusions as to the pecularities of such regions at the level of the DNA-molecule or the DNA-protein-complex. The presence of repetitive DNA sequences at these sites might favour the formation of chromosome intra- and interchanges by hybrid formation between DNA strands of different chromatids (Natarajan and Ahnström, 1974). The close correspondence between heterochromatin characterized by the presence of late replicating DNA and chromosome regions preferentially involved in chromatid aberrations calls attention to the importance of DNA-late replication in the chain of events eventually leading to the origination of chromosome structural changes.

In this context preliminary results as to the localization in karyotype ACB of the positions of the sites of clustering of chromatid aberrations in relation to the Giemsa bands may be mentioned. They indicate (Fig. 8) correlations between the pattern of Giemsa-banded heterochromatin and aberration distribution. Isochromatid breaks induced by the alkylating agents triethylene melamine and cytostasan (cf. Rieger et al., 1975) were found to show two modes of distribution. In the single and relatively thick interstitial Giemsa marker bands of segments 15, 11, and 23 they were found to mainly occupy the bands. A minor fraction of isochromatid breaks involved the immediate neighborhood of the respective bands. In the narrow bands stacked one behind the other (segments 4, 19, and 26/27) aberrations have been found to be clustered in the interband regions, indicating the junctions between heterochromatin and euchromatin to be preferentially involved in aberration clustering. Since the prominent single Giemsa bands were frequently observed to be subdivided into two bands (Fig. 2) separated by a small euchromatic zone in extended chromosomes, the same may in fact be true also for these regions.

A corresponding preferential involvement in aberrations of heterochromatin/euchromatin junctions has recently been reported for human chromosomes (Buckton, 1976; Dutrillaux et al., 1977). However, in human and animal chromosomes with many bands stacked over a relatively small distance the unequivocal correlation of breakage points with the banded regions is for optical reasons rather difficult (Savage, 1977).

Although *Vicia faba* chromosomes are clearly differentiated into specifically banded and long unbanded regions, our preliminary results with respect to aberration clustering within and/or adjacent to the Interstitial Giemsa-bands also prohibit unequivocal conclusions. The fact that euchromatic regions in no case showed up with aberration clustering however is strongly indicative of the importance of heterochromatin or heterochromatin/euchromatin junctions in aberration clustering.

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Received June 2 – August 10, 1978 / Accepted August 10, 1978 by H. Bauer
Ready for press August 15, 1978