G AND/OR C-BANDS IN PLANT CHROMOSOMES?

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SUMMARY

Similarities and differences become evident from comparisons of centromeric and non-centromeric banding patterns in plant and animal chromosomes.

Similar to C and G-banding in animals (at least most of the reptiles, birds and mammals), centromeric and nucleolus-organizing region bands as well as interstitially and/or terminally located non-centromeric bands may occur in plants, depending on the kind and strength of pretreatment procedures. The last group of bands may sometimes be subdivided into broad regularly occurring 'marker' bands and thinner bands of more variable appearance.

Non-centromeric bands in plants often correspond to blocks of constitutive heterochromatin that are rich in simple sequence DNA and sometimes show polymorphism; they thus resemble C-bands. However, most of these bands contain late-replicating DNA. Also they are sometimes rich in A-T base-pairs, closely adjacent to each other and positionally identical to Feulgen* and Q* bands, thus being comparable to mammalian G-bands.

Although banding that is reverse to the non-centromeric bands after Giemsa staining is still uncertain in plants, reverse banding patterns can be obtained with Feulgen or with pairs of A-T versus G-C-specific fluorochromes.

It is therefore concluded that not all of the plant Giemsa banding patterns correspond to C-banding of mammalian chromosomes. Before the degree of homology between different Giemsa banding patterns in plants and G and/or C-bands in mammals is finally elucidated, the use of the neutral term 'Giemsa band', specified by position (e.g. centromeric, proximal, interstitial, terminal), is suggested to avoid confusion.

INTRODUCTION

By means of various treatment procedures before Giemsa staining a variety of banding patterns has been demonstrated in the chromosomes of many species, thus allowing their lengthwise differentiation. The two fundamental patterns are the so-called C and G-banding patterns originally established for mammalian chromosomes by Pardue & Gall (1970), Arrighi & Hsu (1971) and Sumner, Evans & Buckland (1971), respectively. (The techniques were later improved, modified, and adapted to a variety of species by many investigators.) On the basis of different reactions to various banding techniques, Comings (1978) subdivided the chromatin of mammals into three types: (1) constitutive heterochromatin resulting in C-bands; (2) interstitial (A-T-rich and late-replicating) heterochromatin* resulting in G-bands (positionally

*This type of heterochromatin is in accordance with Nagl’s (1976) ‘functional’ heterochromatin but was called ‘ontogenetic’ (non-transcribed) euchromatin by Goldman et al. (1984).
correlated with Q-bands, see below); and (3) euchromatin resulting in R-bands (reverse to the G-banding pattern). While G-banding results in closely adjacent bands along the chromosomes, which in man and Chinese hamster correspond to the chromomere pattern in pachytene chromosomes (Luciani, Morrazzani & Stahl, 1975; Okada & Comings, 1974), C-banding reveals blocks of constitutive heterochromatin located mainly around the centromeres of mammalian chromosomes (ISCN, 1978).

For more than a decade, a large number of studies have been done to differentiate plant chromosomes, by the application of techniques identical or similar to those used for animal chromosomes. Most authors called the banding patterns obtained in plant chromosomes C-banding.

The main reasons for the widespread use of this term for chromosome banding in plants are: (1) the morphological similarity of these banding patterns, in many cases at least, to C-banding patterns of animal chromosomes (large dark bands, but in most cases not at the centromeric position, and large chromosome regions without bands); (2) the similarity of the procedures (often Ba(OH)₂ pretreatment) resulting in this type of banding patterns; (3) the argument of Greilhuber (1977) that the more dense compaction of plant chromosomes during mitosis, as compared to mammalian chromosomes, prevents microscopic resolution of G-bands in plant chromosomes.

While the first two arguments are not without exceptions (see below), the third one became less convincing since Anderson, Stack & Mitchell (1982) found, under comparable conditions, that the ratios of DNA to volume and DNA to length are similar in both plant and mammalian chromosomes (see also Bennett, Heslop-Harrison, Smith & Ward, 1983). Also, Takayama (1976) reported that the G-banding procedure results, parallel to the contraction of chromatin in G⁺-band regions, in an extension of chromatin in G⁻-band regions.

In this paper we report on the similarities and differences of the Giemsa banding patterns observed in mammalian and plant chromosomes that allow us to reach preliminary conclusions and recommendations with respect to the nomenclature of the various types of chromosome bands in plants.

SIMILARITIES OF CENTROMERIC AND NON-CENTROMERIC GIEMSA BANDS IN PLANTS AND C- AND G-BANDS IN MAMMALS

(1) As in mammals, two main types of Giemsa banding patterns may occur in the same plant. One, similar to C-banding in mammals, involves heterochromatin adjacent to centromeres and secondary constrictions only. It has been observed in *Vicia faba* (Döbel, Schubert & Rieger, 1978), *Allium cepa*, *Rhoeo discolor*, *Ornithogalum virens*, *Tradescantia edwardsiana* (Stack, 1974), *Avena vistii* (Yen & Filion, 1977), *Tulipa gesneriana* (Filion & Blakey, 1979) and *Nigella damascena* (Klašterska & Natarajan, 1975). In *N. damascena*, the three terminal or subterminal Giemsa bands, which occur in addition to the centromeric bands, indicate nucleolus-organizing region (NOR) positions as demonstrated by AgNO₃ staining (Hizume, Tanaka & Shigematsu, 1982; Schubert, unpublished).

A second pattern, consisting of interstitial and/or terminal bands, appears in
G and/or C-bands in plant chromosomes

V. faba and A. cepa following a procedure published by Schwarzacher, Ambros & Schweizer (1980) or after using a urea/trypsin technique (Döbel et al. 1978; Schubert, Ohle & Hanelt, 1983). In Avena aestivii and T. gesneriana, the same type of pattern occurred after shortening of HCl-hydrolysis time before Ba(OH)₂ treatment (Yen & Filion, 1977; Filion & Blakey, 1979).

(2) C and G-banding do not exclude each other; intermediate forms may in fact be observed. For mammalian chromosomes, Daniel & Lam-Po-Tang (1973) demonstrated the stepwise transition of one banding pattern into the other. Comings, Avelino, Okada & Wyandt (1973) wrote: ‘relatively mild conditions allow staining of both C- and G-bands. To obtain only C-bands the chromosomes must be treated more harshly to disrupt or destroy the G-bands’. Similar observations have been made in V. faba. The harsher Ba(OH)₂ pretreatment resulted in a C-like pattern. Shortening of the Ba(OH)₂ treatment time allowed the stepwise appearance of single interstitial bands, additional to the centromeric bands. On the other hand, the procedure usually giving rise to interstitial bands sometimes also results in centromeric bands. In Avena aestivii and T. gesneriana, a transition between both types of Giemsa bands has also been observed (Yen & Filion, 1977; Filion & Blakey, 1979) after slight changes of the pretreatment conditions.

(3) A urea pretreatment technique, originally developed for G-banding in human cells by Berger (1971) and Shiraishi & Yoshida (1972), resulted in interstitial bands when applied to V. faba (Döbel, Rieger & Michaelis, 1973); and in at least two chromosomes, these bands were close to each other thus resembling mammalian G-banding patterns (Döbel et al. 1978).

A pattern with many closely adjacent Giemsa bands was also reported for Leopoldia comosa by Bentzer & Landström (1975), for Pinus resinosa by Drewry (1982) and for Zea mays by T. C. Hsu & Shi Liming (personal communication).

(4) Comparable to mammalian G-bands, some of the interstitial Giemsa bands of V. faba may (depending on the degree of chromosome contraction) appear as a large block (in rather contracted chromosomes of late colchicine metaphases) or subdivided into two bands in more extended chromosomes of early metaphase cells (Döbel et al. 1978).

(5) Like mammalian G-bands (Grzeschik, Kim & Johannsmann, 1975; Dutrillaux, Couturier, Richer & Viegas-Pequignot, 1976; Holmquist, Grey, Porter & Jordan, 1982) most interstitial Giemsa bands of V. faba contain late-replicating DNA as demonstrated after incorporation of [³H]thymidine or bromodeoxyuridine during late S-phase (Döbel et al. 1978). The only exception is the NOR-associated heterochromatin. It shows up as a dark band after pretreatment, resulting in either interstitial or centromeric bands, respectively, and as a single dark band after N-banding* (see Funaki, Matsui & Sasaki, 1975; D’Amato, Bianchi, Capineri & Marchi, 1979; Schubert, 1984), which was found to be the earliest replicating region of the V. faba genome (Schubert & Rieger, 1979).

* In conformity with the nomenclature for mammalian chromosome banding, the term used for description of a banding pattern should follow the phenomenon specified rather than the procedure used. Thus, N-banding should be reserved for the exclusive staining by Giemsa of NORs or NOR-associated heterochromatin.
(6) In human and mouse chromosomes, dark bands (F+ bands) appear after prolon-
ged hydrolysis with HCl before Feulgen staining. These are positionally coincident
with G+ bands (Rodman, 1974; Rodman & Tahiliani, 1973). In V. faba, prolonged
hydrolysis with HCl and subsequent Feulgen staining revealed dark bands (Tem-
pelaar, de Both & Versteegh, 1982; Schubert, unpublished), which – notwithstanding
the different staining intensities of some of these bands – correspond positionally to
the interstitial Giemsa bands (except for the secondary constriction, which never
shows up as a dark band after Feulgen staining). Also in other plants, Anemone
blanda, Fritillaria lanceolata (Marks, 1983), Buglossoides purpurocaerulea
(D’Amato, Capineri & Marchi, 1981b)), rather striking homologies between inter-
stitial Giemsa bands and F+ bands have been reported.

(7) In mammals, the position of G-bands is similar to that of the so-called Q-bands
obtained after treatment with quinacrine dihydrochloride or quinacrine mustard,
respectively (Evans, Buckton & Sumner, 1971; ISCN, 1978); these regions are
thought to be rich in A-T base-pairs (Holmquist et al. 1982). In V. faba all the
brightly fluorescing chromosome regions after staining with Hoechst 33258 – a
fluorochrome with specific affinity for A-T base-pairs (Latt & Wohlleb, 1975;
Schweizer, 1981) – corresponded positionally to the sites of interstitial Giemsa bands
(Döbel & Schubert, 1981). A similar fluorescence pattern was obtained by Caspersson
et al. (1969) using quinacrine mustard. After treatment of Vicia chromosomes with
quinacrine dihydrochloride, either enhancement or depression of fluorescence
occurred in regions characterized by interstitial Giemsa bands (Döbel et al. 1978;
Döbel & Schubert, 1981). In Vicia melanops (D’Amato, Bianchi, Capineri & Marchi,
1980) and Allium carinatum (Vosa & Marchi, 1972) terminal Giemsa bands coincide
with Q+ -bands, in A. cepa (Vosa, 1976) with Hoechst 33258+ and Q− bands, and in
some other cases, e.g. Tulbaghia leucantha (Vosa & Marchi, 1972), Allium fistulosum
(Kamizyo & Tanaka, 1978), Buglossoides purpurocaerulea (D’Amato et al. 1981b),
Scilla siberica (Deumling & Greilhuber, 1982), also with Q−-bands.

DIFFERENCES BETWEEN GIEMSA BANDING PATTERNS IN PLANTS AND
MAMMALS

(1) Non-centromeric Giemsa bands (those located neither at nor adjacent to the
centromere) in plants occur, in many cases, in the form of blocks, leaving large parts
of the chromosome free of bands; multiple closely adjacent bands, like mammalian
G-bands, are less frequently observed.

(2) In some plants, the interstitial bands may be subdivided into a group of regularly
occurring large bands (called ‘marker bands’ by Döbel et al. 1973, 1978) and a group
of thinner bands, which either occur after special modifications of the pretreatment
procedures, as chromosomal polymorphisms in inbred lines (e.g. see Lelley, Josifek
& Kaltsikes, 1978), or even in different metaphases of the same slide. Such additional
interstitial bands occur in V. faba (Greilhuber, 1975; Döbel et al. 1978), P. resinosa
(Drewey, 1982), Secale cereale (Lelley et al. 1978; Schlegel & Kynast, personal
communication), in some Fritillaria species (La Cour, 1978) and (probably) in
A. cepa (Schubert et al. 1983). In the last case, additional interstitial chromosome regions other than those normally showing up as Giemsa bands are late-replicating (Cortes, Gonzalez-Gil & Lopez-Saez, 1980). In some plant inbred lines, e.g. Zea mays (Hadlaczky & Kalman, 1975) and Hordeum vulgare (Linde-Laursen, 1978), pronounced Giemsa bands, which show polymorphism – similar to C-bands but unlike G-bands in mammals (see Schweizer, 1980) – have also been observed.

(3) Contrary to the situation in mammals, no complete R(reverse)-banding has so far been described for plants after Giemsa staining. Schweizer (1973) showed that occasionally reverse Giemsa banding patterns may occur in chromosomes of F. lanceolata and F. recurva.

However F⁻⁻-banding reverse to Giemsa banding was described by Marks (1983) for F. lanceolata after short hydrolysis with HCl before Feulgen staining; prolongation of HCl hydrolysis led to F⁺ bands at sites positionally identical to those of Giemsa bands. Indications of a similar phenomenon in Ornithogalum montanum were reported by D'Amato, Bianchi, Capineri & Marchi (1981a). In V. faba, reverse banding (F⁻⁻-bands) obtained by modified HCl hydrolysis conditions before Feulgen staining was restricted to the metacentric satellite chromosome (Takehisa, 1970; D'Amato et al. 1981a).

In spite of the absence of unambiguous reverse Giemsa banding patterns, reverse banding patterns comparable to the situation observed in mammals (ISCN, 1978), have also been obtained after application of different fluorochromes in some plant species. A fluorescence pattern the reverse of that of the A·T-specific fluorochrome 4′-6-diamino-2-phenylindole (DAPI) has been detected in the metacentric satellite chromosome of V. faba after counterstaining with the G·C-specific combination of methylgreen plus fluorochrome chromomycin A₅ (Schweizer, 1976). The same is true for the whole karyotype of V. faba when the Hoechst 33258 fluorescence pattern (Döbel & Schubert, 1981) is compared to the counterstaining pattern obtained after chromomycin + distamycin + DAPI (Schweizer, personal communication). Any of the positive fluorescent regions after DAPI or Hoechst staining corresponds to a negatively fluorescent region after counterstaining. Only the G·C-rich NOR, the single chromosomal site exhibiting negative fluorescence after Hoechst 33258 or DAPI, shows intensive fluorescence after counterstaining. Pairs of reverse fluorescence patterns have also been found in A. cepa (Vosa, 1976), S. siberica, O. caudatum, P. mugo and Anemone blanda (Schweizer, 1976, 1980).

(4) Eiberg (1974) reported on Cd (centromeric dot) bands in human chromosomes. In spite of the morphological similarity between some centromeric Giemsa bands in plants and Cd-bands, they do not correspond to each other, since Cd-banding never includes nucleolus-organizing secondary constrictions and is obtained after quite another pretreatment procedure than used for obtaining most of the centromeric Giemsa banding patterns described in plants.

(5) Unlike G-bands, but similar to C-bands in mammals, interstitial and terminal Giemsa bands in plants frequently contain a large amount of simple sequence DNA. This is true for some species of Secale (Bedbrook et al. 1980) and Scilla (Deumling & Greilhuber, 1982), as well as for Luzula purpurea (Ray & Venketeswaran, 1979),
barley, wheat (Dennis, Gerlach & Peacock, 1980) and maize (Peacock, Dennis, Rhoades & Pryor, 1981). In V. faba many highly repetitive DNA sequences, even when cloned in the plasmid pBR322, gave random distribution of silver grains along the whole chromosome complement (Kaina, Bäumlein, Wobus & Schubert, 1979; Rowland, 1980). However, Bassi, Cremonini & Cionini (1982) found preferential in situ hybridization of a repetitive palindromic sequence in chromosome regions known to be sites of intercalary Giemsa bands.

**Conclusions**

From the observations summarized above the following conclusions may be drawn.

1. As in animals, there are different types of heterochromatin in plants (e.g. see Vosa, 1970; Stack, Clarke, Cary & Muffly, 1974; Schweizer & Nagl, 1976; La Cour, 1978; Sato, Kuroki & Ohto, 1979; Sato, 1980; Dobel & Schubert, 1981).

2. Giemsa banding patterns of plant and mammalian chromosomes show a number of similarities as well as differences.

3. Centromeric Giemsa bands of plants correspond to C-bands of mammals, rather than to Cd-bands of human chromosomes, since Cd-banding does not include the nucleolus-organizing secondary constrictions. The formal similarity to the Cd-bands is probably due to the fact that centromeric heterochromatin in plants is often quantitatively less represented than in many animal chromosomes.

4. Whether the existence of two main types of Giemsa banding patterns in plants — a mainly centromeric one obtained after harsh pretreatment procedures, and interstitial/terminal patterns obtained after application of milder conditions — are the rule or the exception deserves further study. However, the fact that such patterns were found in at least four unrelated genera makes it rather probable that thorough future investigations will reveal additional positive examples. The same may be true for reverse banding patterns.

5. Provided the above-mentioned similarities between interstitial/terminal Giemsa bands in plant chromosomes and mammalian G-bands on the one hand, and centromeric Giemsa bands in plants and animal C-bands on the other are genuine and the differences not fundamental in nature, then one should very carefully consider which banding pattern in plant chromosomes is to be called G or C-banding, respectively (see Drewry, 1982).

6. If, however, differences (e.g. in the content of simple sequence DNA and the degree of polymorphism) between Giemsa banding patterns in plants and G or C-banding in mammals are due to fundamental differences between the various heterochromatin fractions in mammals and plants, respectively (as suggested by Nagl, 1979), the use of the term C-banding should be avoided in connection with Giemsa bands in plant chromosomes.

7. The different Giemsa banding patterns obtained in various plants are evidently not always identical to C-banding in mammals. Therefore, one should be aware of the fact that the term 'C-banding' used (often uncritically) for plant chromosomes does not necessarily describe the same phenomenon referred to as C-banding in mammals.
Before a final solution with respect to the homology of the different types of Giemsa banding patterns in mammals and plants is at hand, the use of the neutral term 'Giemsa banding', specified by a description of the band positions, e.g. centromeric, proximal, intercalary/terminal (and possibly the main pretreatment procedure used) is suggested for plants. This will avoid confusion.

We thank Dr G. E. Marks (John Innes Institute, Norwich, England), Dr G. P. Holmquist (Baylor College of Medicine, Houston, Texas, U.S.A.) and Professor Dr D. Schweizer (Institute of Botany, University of Vienna, Austria) for their critical review of the manuscript and helpful suggestions.

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(Received 9 April 1984 – Accepted 14 May 1984)