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Why Plant Chromosomes Do Not Show G-bands

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Summary. Giemsa techniques have refused to reveal G-banding patterns in plant chromosomes. Whatever has been differentially stained so far in plant chromosomes by various techniques represents constitutive heterochromatin (redefined in this paper). Patterns of this type must not be confused with the G-banding patterns of higher vertebrates which reveal an additional chromosome segmentation beyond that due to constitutive heterochromatin. The absence of G-bands in plants is explained as follows: 1) Plant chromosomes in metaphase contain much more DNA than G-banding vertebrate chromosomes of comparable length. At such a high degree of contraction vertebrate chromosomes too would not show G-bands, simply for optical reasons. 2) The striking correspondence of pachytene chromomeres and mitotic G-bands in higher vertebrates suggests that pachytene chromomeres are G-band equivalents, and that this may also be the case in plants. G-banded vertebrate chromosomes are on the average only 2.3 times shorter in mitosis than in pachytene; the chromomeric pattern therefore still can be shown. In contrast, plant chromosomes are approximately 10 times shorter at mitotic metaphase; their pachytene-like arrangement of chromomeres is therefore no longer demonstrable.

Key words: Plant chromosomes - G-bands - Chromomeres - Heterochromatin

Ever since Giemsa banding techniques have been applied in plant cytology it was irritating that nothing could be demonstrated which was comparable with the G-bands in higher vertebrates. In fact, any of the bands stained in plant chromosomes hitherto can be identified as constitutive heterochromatin (specific banding: C-banding), if we define it correctly as inherited, unconvertable chromosome segments which do not decondense during telophase, so forming chromocenters during interphase, including all such very small bodies (heterochromomeres), which are consubstancial with chromocenters as revealed by staining behaviour or cytochemical tests.[1] Whenever cytologists have claimed to have demonstrated G-bands in plants, this was due to a misunderstanding of what G-banding really means: Visualization of chromosome segmentation in contracted, dividing chromosomes along their whole length, and consequently also in other regions than those occupied by constitutive heterochromatin. The sources of this terminological vagueness have already been outlined (Greilhuber 1975) - the use of banding terminology according to the treatments applied, or according to the band position, i.e. centromeric or non-centromeric.

The inability to demonstrate G-bands in plants was explained either by a consequence of the different mode of chromosome preparation in plants or by a genuine absence of G-banding chromatin in the plant kingdom (Greilhuber 1975), or, in terms of molecular biology, by the high ratio of moderate repetitive DNA in plants (Nagl 1976). It was not realized that the impossibility to demonstrate G-bands in plant chromosomes might simply be due to their high degree of contraction. It is evident from Fig. 1 that human chromosomes would not show G-bands simply for reasons of optical resolution, if they were contracted to the same extent as bean or rye chromosomes. The objection can be made that G-bands in plants, if they exist, might be larger than those in man, and on principle therefore demonstrable by appropriate treatments. However, the above explanation is consistent with another consideration, which is outlined in the following. It has been demon-

[1] This definition closely refers to the classical understanding of heterochromatin (Heitz 1932), but omits the dubious precocious prophase condensation as a criterion, and clarifies the distinction of eu- and heterochromomeres. Widely used characteristics of constitutive heterochromatin, like late replication, content of satellite DNA, highly repetitive DNA, should be avoided in definitions of heterochromatin, since exceptions are known in each case. Furthermore, euchromatin can also have some of these characters, e.g. late replication, and highly repetitive DNA.

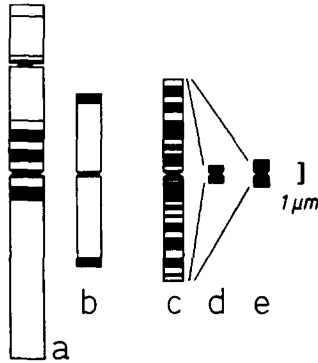

Fig. 1. a-c the usual size relations in cytological preparations of the C-banded M-chromosome of *Vicia faba* (a; Greilhuber 1975), the C-banded chromosome no. 1 of *Secale cereale* (b; Weimarck 1975), and the G-banded human chromosome no. 1 (c; Luciani et al. 1975). d, e the size of the human chromosome no. 1, if contracted as strongly as bean (d) or rye (e) chromosomes; under such conditions no G-bands would be demonstrable

strated in Chinese hamster (Okada and Comings 1974) and man (Luciani et al. 1975) that G-bands in prometaphase and chromomeres in pachytene show close correspondence. We may therefore look for corresponding structures in plants: these are obviously the long known pachytene chromomeres. The comparison of relevant features in man, insects, and plants (Table 1) makes the following evident: human chromosomes are the least contracted chromosomes at mitosis, have the largest chromomere distance at pachytene and the smallest ratio of pachytene/mitotic chromosome length. This results in a distance of chromomeres (=G-bands) of approximately 0.6 µm at prometaphase of mitosis. Plants show a considerable size difference of pachytene and mitotic chromosomes. This reduces a hypothetical chromomere distance (from center to center) at mitotic metaphase to 0.1 µm or less, which is, of course, not resolvable at the light microscopical level. If we accept the complementarity of pachytene chromomeres and G-bands, it becomes obvious that we cannot see G-bands at metaphase in plants, again simply for reasons of chromosome condensation.

Then are G-bands in plant chromosomes visible, if we look at them with the electron microscope? Supposedly not. Firstly, any arrangement other than in a straight line (coiling, folding of the chromatid) would make the pattern indistinct. Secondly, it was recently argued that G-bands in man are no longer demonstrable when chromosomes pass through true metaphase (Sumner 1976). This process of genuine (not simply optical!) fusion of G-bands obviously is brought about already by only a twofold contraction from prometaphase to metaphase. It could be expected, therefore, that G-bands in plants would be visualized at best in mitotic prophase, if at al.

It has been demonstrated in man that many bands are present in early mitotic prophase; by progressive and orderly fusion they compose the typical G-banding pattern of prometaphase (Yunis 1976). It is likely that a similar fusion also occurs in plants from interphase to prophase of mitosis and meiosis. If we accept the concept of a genuine band fusion, the crucial questions are at which moment such a fusion occurs, and at which moment the bands are no longer demonstrable by special treatments.

It is of some significance that chromomeric chromosome organization has been described in meiotic prophase II and mitotic prophase of several plants, and that the proportionality of chromomere number and stage dependent chromosome length has been shown (Lima-de-Faria 1975). However, "chromomeres" of this type are much less distinct than pachytene chromomeres, and obviously open to different subjective interpretations, e.g. as chromonematic coils. Formally, such structures can be interpreted as fused pachytene chromomeres. Respecting these results, it should be recalled that the parallelism of G-banding patterns and variations in DNA base composition is well established in higher vertebrates by base specific fluorochromy (Schweizer 1976). In consequence of the structural correspondence between G-bands and chromomeres, the pachytene chromomeres must be considered as morphological structures determined from the molecular level. With regard to a definite solution of the problem of G-banding in plants, it is therefore of utmost interest to find out whether pachytene chromomeres and interchromomeres in plants also show differences in base composition or not. So far, no fluorochrome banding has been found in the euchromatic parts of mitotic plant chromosomes.

The present explanation for the failure of G-banding in plants is also applicable to those animal groups where G-banding has not been found or has been questioned, i.e. especially in Urodeles (Amphibia), and

Table 1. Dependence of G-banding from chromomere distance at pachytene and length of mitotic relative to pachytene chromosomes

	Homo sapiens	*Achaeta domestica*	*Vicia faba*	*Agapanthus umbellatus*	*Secale cereale*	*Ornithogalum virens*	*Antirrhinum majus*	*Salvia viridis*
C-value (pg)	2.53	2.00	13.33	13.28	8.29	5.28	1.60	-
pg DNA per μm chromatid length in mitotic (pro-)metaphase	0.02	0.06	0.24	0.15	0.15	0.33	0.07	-
mitotic chromosome contraction relative to human	1 ×	3 ×	12 ×	7.5 ×	7.5 ×	16.5 ×	3.5 ×	-
average chromosome size in mitotic (pro-)metaphase (μm)	4.7	2.7	9.1	6.1	7.8	5.3	2.8	3.0
pachytene length (male) relative to mitotic length	2.3 ×	6.9 ×	-	14.8 ×	10.8 ×	18.3 ×	8.0 ×	9.6 ×
chromomere distance in male pachytene (μm)	1.43	1.10	-	0.84	0.89	1.07	0.50	1.06
hypothetical chromomere distance in mitotic (pro-)metaphase (μm)	0.62	0.16	-	0.06	0.06	0.06	0.07	0.11
G-banding expected	yes	hardly	no	no	no	no	no	no

Relevant parameters are compared in man, an insect, and plants. If the correspondence of G-bands and pachytene chromomeres is accepted, there is no theoretical possibility to demonstrate metaphase G-banding in plants. Data compiled, calculated, or estimated from Nagl 1976, Luciani et al. 1975, Schnedl 1971 (on human chromosomes), Lima-de-Faria et al. 1973a, b (on *Achaeta domestica*), Bennett and Smith 1976 (on DNA contents in plants), Schweizer 1973 (on *Vicia faba*), Lima-de-Faria 1954, Sharma and Mukhopadhyay 1963 (on *Agapanthus umbellatus*), Lima-de-Faria 1952, Heneen 1962 (on *Secale cereale*), Lima-de-Faria et al. 1959 (on *Ornithogalum virens*), Ernst 1939, 1940 (on *Antirrhinum majus*), Lima-de-Faria and Sarvella 1962, Afzal-Rafii 1975 (on *Salvia viridis*).

Orthoptera (Insecta). There is only one remarkable case of metaphase G-banding in a locust, *Chortoicetes terminifera* (Webb 1976). However, it is quite possible that this "G-banding" actually represents a case of inverse C-banding, because structural euchromatin appears homogeneously darkly stained after a trypsin treatment, while only constitutive heterochromatin shows dark and pale segments, revealing a heterogeneity of heterochromatin rather than G-banding.

It should be emphasized that the present interpretation of G-bands in plants - even if it appears obvious - is still speculative, and requires experimental evidence. The crucial test would be to demonstrate banding within structural euchromatin. In this respect we evidently have not succeeded yet.

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