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Chromatid Distribution at Mitosis in Cultured *Wallabia bicolor* Cells

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Abstract. An analysis of labelled centromere regions of chromosomes in metaphase cells of the Swamp Wallaby (*Wallabia bicolor*) demonstrates conclusively that chromatids do not co-segregate in sets which contain DNA template strands of identical age. Also, there is no tendency for chromatids of homologous chromosome pairs to distribute non-randomly. The data are consistent with the assumption of random distribution of chromatids at mitosis.

Introduction

In both prokaryotes and eukaryotes it is now accepted that replication and segregation of DNA is semi-conservative. In prokaryotes this is believed to involve attachment of the chromosome at a specific point on the cell membrane which is maintained throughout the replication cycle (Jacob *et al.*, 1966). Autoradiographic experiments concerning the distribution of labelled DNA in multinucleate cells of *Escherichia coli* (Lark and Bird, 1965) and elongating cell chains of *Bacillus subtilis* (Eberle and Lark, 1966) led Lark (1966) to postulate that prior to replication the bacterial chromosome is attached to the membrane by only the polynucleotide strand which had been used as a template for the previous DNA replication.

When a DNA strand is used as a template for the first time, it thus becomes permanently attached to the cell membrane. Further, Lark (1966) suggested that all strands first used as templates in a particular replication cycle would subsequently co-segregate since they would be attached to a common segregation structure.

As applied to eukaryotes, Lark's hypothesis implies that the arrangement of sub-units in a chromatid can be recognized, such that the attachment of the centromere of each chromatid to the spindle results in non-random segregation at mitotic anaphase.

The unequal distribution of autoradiographic grain counts above individual mouse and Chinese hamster cells following an ^{3}H-thymidine label led to the conclusion that sister chromatids did not segregate at random during mitosis (Lark *et al.*, 1966).

A subsequent study (Lark, 1967) showed that grain counts above the two sets of chromatids in anaphase or telophase cells of *Vicia faba* and *Triticum boeoticum* roots, at the presumed second division after replication in the presence of isotope were not equivalent and thus supported non-random chromatid segregation. These results suggest that chromatids containing the "replication-segregation" subunit synthesized in the presence of isotope co-segregate, though sister chromatid exchanges would result in the redistribution of some label between chromatid sets at second anaphase. Further support has come from similar grain distribution analyses with *Aspergillus nidulans* (Rosenberger and Kessell, 1968), cultured diploid human and rat cells (Priest and Shikes, 1970) and in part from polyploid wheat (Lark, 1969a). These results suggest that in eukaryotes, chromatids containing a strand synthesized during any particular generation tend to remain together and segregate as a group, thus providing an "accurate indexing system" (Lark, 1967) for distributing replicated chromosomes into two equivalent chromatid sets.

However, similar experiments with tetraploid rat cells (Priest and Shikes, 1970), HeLa cells (Lark, 1969b) and some wheat strains (Lark, 1969a) did not result in clear cut bimodal grain count distributions above anaphase chromosome sets. In particular Comings (1970), also using diploid Chinese hamster cells, favoured a random distribution of chromatids. The basis for such conflicting results remains unexplained.

Heddle *et al.* (1967) contended that Lark's (1967) finding of a preponderance of grains above one of the two sister ana-telophase chromatid sets in cells of *Vicia faba* was compatible with random segregation. If 12 chromosomes were segregating randomly on the basis of a binomial distribution, less than 23% of sister chromatid sets would be equally labelled. However, sister chromatid exchanges would disturb such an expectation. A possible causal factor for the conflicting results is the difficulty of accurately resolving grains above ana-telophase stages where chromatids are condensed, to some extent superimposed, and with different labelled areas exposed to the autoradiographic emulsion. Also since an activated silver halide crystal is insensitive to further interaction (Feinendegen, 1967) coincidence error would make grain counting inaccurate when labelled regions are densely apposed.

Metaphase cells at the third division after label are equivalent to second division chromatid sets at anaphase. The frequency of labelled centromeric regions in such cells would reflect whether there is a common segregation during mitosis on the basis of the age of the subunits in the centromeric regions. The centromere is the organelle specifically concerned with chromosome orientation and separation on the mitotic spindle (Nicklas, 1971). The scoring problems associated with grain counting do not arise. However, a complication in scoring centromeric labelling is the

possibility of sister chromatid exchange at the centromere occurring in previous divisions. This factor will be particularly pertinent when extensive sister chromatid exchanges occur in centromeric regions. A directed segregation of chromatids at mitosis could apply in two ways:

(i) Within the entire complement. This implies that subunits in centromeric regions are recognizable on an age basis and would result in a complete segregation of labelled versus unlabelled centromeres at the second anaphase after label.

(ii) Within individual pairs of homologues. This implies that equivalent centromeric subunits of homologous pairs of chromosomes will co-segregate. Clearly, it is not possible to test this by a study of anatelophase labelling.

An objective test is thus possible in an organism where; (a) all chromosomes are individually distinguishable and (b) a known frequency of sister chromatid exchanges occurs at the centromere. The Swamp Wallaby (*Wallabia bicolor*; $2n = 11$, XY_1Y_2) meets these requirements, allowing a stringent examination of the question of random or non-random distribution of chromatids. The results obtained conform to the proposition that chromatids segregate at random both between different chromosomes of the complement and within homologues.

Materials and Methods

Peripheral blood of *Wallabia bicolor* was collected by tail venepuncture and cultured in Falcon plastic tubes in Eagle's medium supplemented with 7.5% foetal calf serum and 2% PHA. ^{3}H-thymidine (0.25 μCi/ml; 3.0 Ci/mM) was made available from 29 to 32 hours post-initiation of culture and mitoses collected 44 hours later, after a six hour colcemid (10^{-6} M) block. Air dried preparations were coated with Kodak NTB emulsion and developed in Dektol 1:1 for two minutes after 15 days exposure. Giemsa stained metaphase spreads at the third division post label (Fig. 1) were examined for the presence or absence of label covering the centromere region of each chromosome.

Results

1. Chromatid Distribution within the Wallabia Complement

The number of labelled centromeric regions per third division post-label metaphase cell is shown in Table 1. Since the Y_1 chromosome is very small consideration was confined to the four pairs of autosomes, the X and Y_2 chromosomes.

If a directed segregation of all centromeres were operating then cells should contain either 0 or 10 labelled centromeric regions. Alternatively if the centromeres of individual chromosomes segregate randomly during mitosis, then the frequency of labelled centromeric regions should be distributed according to a binomial distribution ($p = q = 0.5$, $N = 10$), and the expected frequencies are shown in Table 1. On this hypothesis

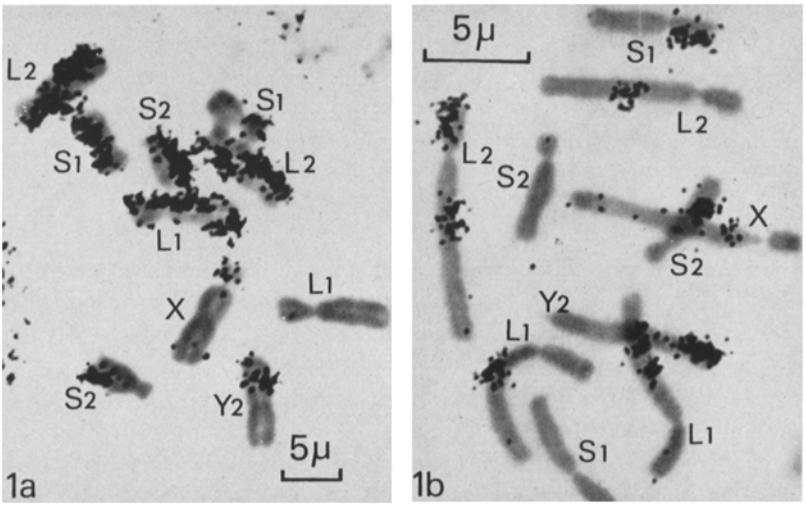

Fig. 1a and b. *Wallabia bicolor* cells at the third metaphase after ^{3}H-thymidine label; (a) cell with 6 labelled centromeres; one L1 centromere labelled, the other unlabelled; both L2 centromeres labelled; one S1 centromere labelled, the other unlabelled; one S2 centromere labelled, the other unlabelled; X centromere unlabelled; Y2 centromere labelled; (b) cell with 0 labelled centromeres. — The dot Y1 chromosome is not apparent in either cell. It should be noted that both labelled and unlabelled telomeric regions are present in both cells

Table 1. Frequency of labelled centromeres per cell in 3rd division post-label metaphases $[(p+q)^N$ where $p=q=0.5$ and $N=10]$

	Number of labelled centromere regions											
	0	1	2	3	4	5	6	7	8	9	10	Total
No. of cells	2	10	21	40	44	58	39	22	13	6	0	255
No. of cells expected assuming a binomial distribution	0.3	2.5	11.2	29.9	52.3	62.8	52.3	29.9	11.2	2.5	0.3	

the mean number of labelled centromeres per cell should be 5.0, which compares favourably with the actual mean 4.6 ± 0.12. This deficiency from the expected mean probably reflects failure to score some very lightly labelled centromeric regions. It is not due to a failure of the centromeric region of the X chromosome to be labelled, since this was labelled in 46.4% of cases.

A test of the observations against expectations gives a $\chi_7^2 = 32.355$, $P < 0.001$, indicating an unsatisfactory fit to the binomial. Whereas the results tend to follow a binomial form, cells with low numbers of labelled centromeres are over-represented. This departure from expectation is therefore in a manner which is consistent with the underscoring of lightly labelled centromeric regions and is not consistent with the expectation of a directed distribution of chromatids.

2. Chromatid Distribution within Homologous Pairs of Chromosomes

If a directed distribution mechanism was operating between equivalent centromeric subunits of homologous pairs then centromeric regions of homologues should be labelled or unlabelled. With random segregation on the otherhand, within each pair of homologues, the expected proportion of two centromeres labelled: one centromere labelled, one unlabelled: two centromeres unlabelled, is 1:2:1. Only the S_2 chromosome deviates significantly from this expectation at the 5% probability level (Table 2). Clearly a non-random distribution is not supported.

Table 2. Segregation of chromatids for each autosomal pair

	Chromosome			
	L1	L2	S1	S2
Both centromeres labelled	53	60	52	50
1 centromere labelled, 1 unlabelled	92	93	100	79
Both centromeres unlabelled	52	47	47	65
Total No. of chromosome pairs	197	200	199	194
Fit to a 1:2:1 expectation χ_2^2	0.868	2.670	0.257	8.999[a]

[a] $P < 0.05$

Discussion

The results obtained on the distribution of labelled centromeres in third division post-label metaphases of *Wallabia bicolor* clearly demonstrate that centromeres containing DNA strands synthesized during the same division cycle do not segregate as a set. The data do depart from random distribution expectations but this is almost certainly due to a failure to detect some lightly labelled centromeres. Similar distributions were found, but not tested against binomial expectations, by Heddle *et al.*, (1967) and Wolff and Heddle (1968) for both *Vicia faba* and *Potorous tridactylis* cells. A similar scoring limitation was also noted in these

cases. Cuevas-Sosa (1968) studied a small group of homologues of the human complement and found that for the three distinguishable largest chromosomes chromatid distribution was random. This agrees with the results for all but one pair of homologues in *Wallabia bicolor*. The S_2 homologues deviate significantly from random chromatid distribution since there is a significant excess of the class where two centromeres are unlabelled, and a deficiency, of the class where one centromere is labelled and the other unlabelled. This too may be attributed to underscoring of lightly labelled centromeres.

Analysis of centromeric labelling in metaphase chromosomes of third division post-label cells, avoids the possibility of mixing cells in different cell cycles, and reduces the influence of sister chromatid exchanges (SCE's). These two difficulties had been recognized in earlier studies (Lark, 1967; Heddle *et al.*, 1967). Lark (1969a) criticized Heddle *et al.*, (1967) for failing to take into account the possibility of SCE's at the centromere, which could obscure a non-random chromatid distribution. However, Heddle *et al.*, (1967) state quite clearly that label had to be distributed on both sides of the centromere to be scored. Geard (1969) has also shown that centromeric SCE's in fact comprise only 8.3% of all exchanges in the metacentric chromosomes of *Vicia faba*, which is that expected on a basis of length proportionality. This contrasts with the reported 20% centromeric SCE's in chromosome I of a Chinese hamster cell line (Martin and Prescott, 1964) and 25% in chromosomes of a *Potorous tridactylis* cell line (Gibson and Prescott, 1972), where such high levels could make scoring of centromeric label unreliable. In *Wallabia bicolor* at 44 hour post-labelling there is a significant frequency ($\simeq 18\%$) of second division post-label metaphases. However, the few chromosomes and the quality of spreads obtained allowed unequivocal decisions to be made as to whether cells were in the third division post-label. Sister chromatid exchanges occur at the centromere in *Wallabia bicolor* chromosomes but not in a disproportionate frequency. Where a sister chromatid exchange was detected at the centromere, that pair of homologues was not included in the analysis. Of 359 SCE's recorded on the L1, L2 and X chromosomes at the second division after label, 41 (11.4%) were in a 1 micron segment which included the centromere.

In eukaryotes mitotic separation of chromosomes is mediated by an interaction between the spindle system and the centromere of each chromosome (Nicklas, 1971). Since the interaction between spindle and centromere regulates chromosome orientation and subsequent movement, the centromeric region is likely to be a more sensitive indicator of chromatid segregation than an analysis of grain counts over whole sets of chromatids. In each case where the proportion of labelled centromeric regions in third division post-label metaphase have been recorded (Heddle

et al., 1967; Wolff and Heddle, 1968; Cuevas-Sosa, 1968), the results were consistent with random chromatid distribution within a set of chromosomes. As has been shown in this study, this also applies within homologous pairs of chromosomes.

In conclusion there is no sound evidence that centromeres of eukaryotes which contain DNA subunits of like age attach to a common nuclear structure in such a manner as to produce a non-random distribution of chromatids at mitosis.

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