Chromosoma (Berl.) 59, 63–72 (1976)

CHROMOSOMA

Univalent Behaviour at Anaphase I in Desynaptic Rye

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Abstract. The Anaphase-I behaviour of univalents produced by desynapsis has been studied in four inbred lines of rye. — The analyses show that the distribution of numbers of equationally dividing univalents per cell at anaphase-I does not conform to the statistical expectation of randomness. The pattern of this distribution leads us to the assumption that two types of univalent pairs are present at metaphase I: in type I both members of the pair can divide equationally or reductionally, independent of one another. In type II both members of the pair always divide reductionally. Under this assumption a theoretical model was made which fits the observed distribution of the number of equationally dividing univalents per cell. — It is suggested that the difference of anaphase-I behaviour between these two types of univalent pairs is determined by the moment of metaphase I at which they are formed.

Introduction

The simultaneous orientation of the two centromere chromatid subunits to the same pole of the cell is called syntelic, and is normally restricted to first meiotic division. At second meiotic division as at mitosis, the two centromere chromatid subunits orient to oposite poles (amphitelic orientation).

Centromere behaviour at first meiotic division is responsible for the reduction of chromosome number during the meiotic process.

Syntelic orientation of bivalents is unvariable. However when univalents are present at metaphase I their centromeres can reorient amphitelically and then divide equationally at first anaphase (Bauer et al., 1961).

From this difference between bivalent and univalent behaviour it can be deduced that the maintenance of chromosome pairing during metaphase I can influence centromere orientation and therefore the subsequent behaviour in anaphase.

In this work, anaphase behaviour of univalents produced by desynapsis is

studied. As was shown by Sybenga (1958) in inbred rye, desynapsis leads to gradual univalent formation during late diakinesis and metaphase I. Desynapsis phenomenon allows us to study the possible relationships between the moment in which univalents are formed and their subsequent behaviour in anaphase.

Material and Methods

Four inbred lines of rye, *Secale cereale* (*V*, *P*, *M* and *A*) formed the material for this study. All lines were obtained by self pollination during 14 to 20 generations (*V*, I_{20}; *P*, I_{14}; *M*, I_{15}; *A*, I_{14}) at the experimental station of Aula Dei (C.S.I.C. Zaragoza, Spain).

All observations were made in Feulgen stained squash preparations of pollen mother cells, following fixation in acetic-alcohol 1:3.

Preparations were made permanent by inclusion in sandeural.

Results

Metaphase I

A minimum of four plants per line (100 to 700 cells per plant) were analysed at metaphase I. In all cases univalents were observed at this stage. Table 1a shows the univalent per cell frequencies at metaphase I in the four lines studied (see also Fig. 1). In order to ascertain that the presence of univalents was produced by desynapsis, 100 diakinesis cells per line were observed (see Table 1b). The difference between the mean univalents per cell at diakinesis and metaphase I indicates that univalents are produced by desynapsis.

Table 1. (a) Distribution of numbers of univalents per cell in the four lines at metaphase I. The number of univalents varies from 0 to 8 per cell

Class (univalents per cell)	Line *V*	Line *P*	Line *M*	Line *A*
0	482	352	213	1022
2	107	207	141	1059
4	11	33	41	402
6	–	8	4	103
8	–	–	1	14
Number of cells	600	600	400	2600
Mean univalents per cell	0.43	0.99	1.19	1.71

(b) Mean univalents per cell at diakinesis in the four lines studied (100 P.M.C. per line)

	Line *V*	Line *P*	Line *M*	Line *A*
Mean univalents per cell	0.02	0.08	0.10	0.18

Anaphase I

Equationally dividing chromosomes at this stage always move to the poles later than reductionally dividing chromosomes (Fig. 2). In the other hand, in telophase I scored cells per line no lagging univalents were seen in 200 cells scored for each line. Therefore chromosomes that remained at the equator at anaphase I (Fig. 2c) were condidered as equationally dividing chromosomes that had not begun its division in the moment at what the cell was fixed. These equatorial univalents represented about 25% of the chromosomes classified as equationally dividing univalents.

Only three anaphase I cells of the total analysed (Tables 2 and 4) showed misdivision, and were not included in these results.

Thus, only two classes of chromosomes were considered, those dividing equationally and those dividing reductionally.

Anaphase I cells were classified according to the number of equationally dividing chromosomes which they contained.

Equationally dividing chromosomes at anaphase I undoubtely proceed from chromosomes forming univalents at metaphase I. Reductionally dividing chromosomes may proceed either from chromosomes forming bivalents or from chro-

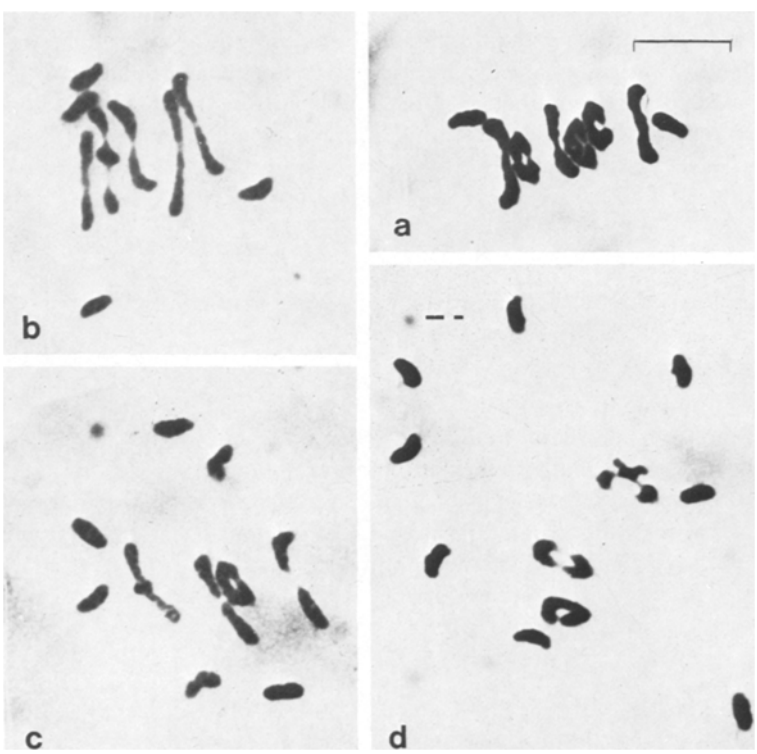

Fig. 1a–d. Metaphase I. **a** Two univalents. **b** Four univalents. **c** Six univalents. Bivalents with terminalised chiasmata are clearly distinguishable from univalents. **d** Eight univalents

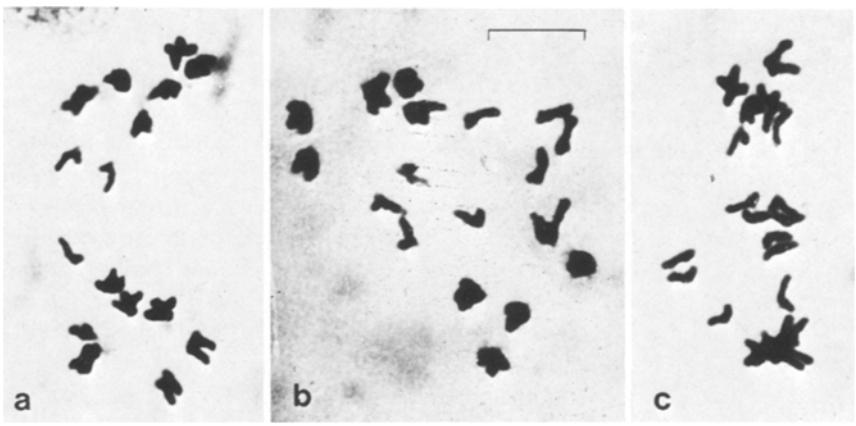

Fig. 2a–c. Anaphase I cells. **a** Two equationally dividing univalents. **b** Four equationally dividing univalents. **c** Six equationally dividing univalents. Note that equational division of univalents begin when the rest of chromosomes are near the poles

mosomes forming univalents at metaphase I. Therefore the number of reductionally dividing univalents cannot be known by direct observations of anaphase I cells. However an estimation of this fraction is possible taking into account both the data from metaphase I and anaphase I cells. For a given line, in a population of M cells at metaphase I the total number of univalents is U. For the same line, in a population of A cells at anaphase I the total number of equationally dividing univalents is E. Therefore the average probability for a univalent to divide equationally in this line is:

$$e=(E\times M)/(A\times U).$$

And therefore the probability for a univalent to divide reductionally is:

$$r=1-e.$$

Now, if all the univalents present in a given cell can divide equationally or reductionally at random, and the probability of dividing equationally is the same for all univalents, we can calculate the probabilities for an anaphase I cell to have 0, 1, 2, ... x equationally dividing univalents on the basis of a binomial distribution. An anaphase I cell proceeding from a cell having i univalents at metaphase I will show x equationally dividing univalents with a probability of:

$$\binom{i}{x} e^x r^{i-x}.$$

In a population of M cells at metaphase I N_i contain i univalents ($i=0, 2, 4,$ ... 14). Therefore the probability for an anaphase I cell to proceed from a metaphase I cell having i univalents is:

$$P_i=N_i/M.$$

Table 2. Distribution of numbers of equationally dividing univalents per cell at anaphase I in the four lines compared to the expected distributions calculated with formula (1). (See text)

Class (equationally dividing univalents per cell)	Line *V*		Line *P*		Line *M*		Line *A*	
	Frequency		Frequency		Frequency		Frequency	
	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.
0	396	393.1	447	412.2	264	240.7	979	790.2
1	1	6.6	4	71.6	6	48.0	31	359.6
2	3	0.2	47	14.1	27	9.9	322	182.6
3	–	–	1	1.4	–	1.1	6	49.7
4	–	–	1	0.2	3	0.1	59	14.4
5	–	–	–	–	–	–	–	2.8
6	–	–	–	–	–	–	2	0.5
7	–	–	–	–	–	–	–	0.05
8	–	–	–	–	–	–	1	0.004
No. of cells	400		500		300		1400	
			$\chi^2 = 76.766$ signif.		$\chi^2 = 64.638$ signif.		$\chi^2 = 626.519$ signif.	
			2 d.f.		2 d.f.		4 d.f.	

Thus, the probability for an anaphase I cell to have x equationally dividing univalents is:

$$E_x = \sum_{i=0}^{14} P_i \binom{i}{x} e^x r^{i-x}, \tag{1}$$

where $i = 0, 2, 4, \ldots 14$ and, of course $x \leqq i$.

Table 2 presents the distributions of the numbers of equationally dividing univalents per cell in the four lines studied as compared to expected distributions calculated with formula (1). In all lines except *V* a highly significant difference between the observed and expected values is found. In all cases the number of cells having an even number of equationally dividing univalents is higher than expected, and the number of cells having an odd number of equationally dividing univalents is less than expected. This fact could be explained assuming that the behaviour of a univalent at anaphase I is not independent of the behaviour of its homolog. So one could suppose that two types of univalent pairs are present in metaphase I cells:

Type I: Pairs of univalent of this type behave at anaphase I as "true univalents" i.e. each member of the pair can divide equationally with a probability a and reductionally with a probability s, independently of one another.

Type II: Pairs of univalent of this type behave at anaphase I as the two members of a bivalent, i.e. both members of a pair always divide reductionally.

Let us assume now that the probabilities of belonging to type I (q_{I}) and to type II (q_{II}) are the same for all univalent pairs, and that these two types of uni-

valent pairs can exist in the same cell at random. Then, we can calculate the probabilities for an anaphase I cell to have 0, 1, 2, 3, ... 14 equationally dividing univalents as follows: Let P_i be the probability for an anaphase I cell to proceed from a metaphase I cell having i univalent pairs. Then, the probability for an anaphase I cell to proceed from a metaphase I cell having h type I univalent pairs and $i-h$ type II univalent pairs is:

$$P_i \binom{i}{h} q_{\mathrm{II}}^{i-h} q_{\mathrm{I}}^{h}.$$

This cell will have k equationally dividing univalents with a probability of:

$$\binom{2h}{k} a^k s^{2h-k}.$$

Now, the probability for an anaphase I cell to have k equationally dividing univalents is:

$$E_k = \sum_{i=0}^{7} \sum_{h=0}^{i} P_i \binom{i}{h} q_{\mathrm{II}}^{i-h} q_{\mathrm{I}}^{h} \binom{2h}{k} a^k s^{2h-k}, \tag{2}$$

where $i=0, 1, 2, \ldots 7$; $k \leqq 2h$ and $h \leqq i$.

P_i values can be calculated directly from metaphase I cells as:

$$P_i = N_i/M,$$

where N_i is the number of cells having i univalent pairs in a population of M metaphase I cells.

q_{I}, q_{II}, a and s cannot be calculated with accuracy because there are no morphological differences between type I and type II univalents at metaphase I. However, an estimate of these values can be made as follows:

Anaphase I cells having $2x$ and $2x+1$ equationally dividing univalents indicates that they contain respectively at least x and $x+1$ type I univalent pairs. If $A_0, A_1, \ldots A_i$ are the numbers of cells having respectively 0, 1, ... i equationally dividing univalents in A anaphase I cells, the minimum number of type I univalent pairs present in these A anaphase I cells will be:

$$U_{\mathrm{I}}' = (A_1 + A_2) + 2(A_3 + A_4) + 3(A_5 + A_6) + \cdots.$$

Taking U_{I}' as the actual number of type I univalent pairs, q_{I} can be estimated as:

$$q_{\mathrm{I}} = (U_{\mathrm{I}}' \times M)/(U_p \times A),$$

where U_p is the total number of univalent pairs present in M metaphase I cells.

Then:

$$q_{\mathrm{II}} = 1 - q_{\mathrm{I}}.$$

Now, the total number of equationally dividing univalents at anaphase I is:

$$E = A_1 + 2A_2 + 3A_3 + 4A_4 + \cdots.$$

And the total number of univalents belonging to type I pairs is:

$$2U_{\mathrm{I}}'.$$

Table 3. Estimated values of q_I, q_{II}, a and s in the four lines

	Line *V*	Line *P*	Line *M*	Line *A*
q_I	0.046 ± 0.03	0.222 ± 0.07	0.217 ± 0.08	0.411 ± 0.13
q_{II}	0.935 ± 0.03	0.778 ± 0.07	0.782 ± 0.08	0.589 ± 0.13
a	0.875 ± 0.23	0.954 ± 0.02	0.923 ± 0.03	0.962 ± 0.03
s	0.125 ± 0.23	0.045 ± 0.02	0.077 ± 0.03	0.038 ± 0.03

Table 4. Distribution of numbers of equationally dividing univalents per cell in the four lines compared to the expected distributions calculated with formula (2). (See text)

Class (equationally dividing univalents per cell)	Line *V*		Line *P*		Line *M*		Line *A*	
	Frequency		Frequency		Frequency		Frequency	
	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.
0	396	396.03	447	447.08	264	263.25	979	973.94
1	1	0.87	4	4.38	6	4.97	31	26.41
2	3	3.03	47	46.01	27	29.86	322	339.03
3	–	0.07	1	0.33	–	0.46	6	7.56
4	–		1	1.76	3	1.39	59	47.99
5	–		–	0.44	–	0.07	–	0.93
6	–		–		–		2	4.0
7	–		–		–		–	0.04
8	–		–		–		1	0.14
No. of cells	400		500		300		1400	
			$\chi^2 = 0.1411$ not signif. 2 d.f.		$\chi^2 = 0.9222$ not signif. 2 d.f.		$\chi^2 = 5.2684$ not signif. 4 d.f.	

Then, the probability for a univalent belonging to type I to divide equationally is:

$$a = E/2\,U_I'$$

and therefore:

$$s = 1 - a.$$

Table 3 shows the values of q_I, q_{II}, a and s estimated for each line.

Table 4 presents the distribution of the numbers of equationally dividing univalents per cell in the four lines studied as compared with the expected distributions calculated after formula (2). As can be seen there is a good fit in all cases. No significant difference is found in the three lines in which a χ^2 test was made.

Discussion

From the anaphase I and telophase I observations it can probably be deduced that, excluding the few cases of mis-division, only two alternatives for univalent anaphase behaviour exist in our material i.e. equational or reductional division.

This study demonstrates the existence of two types of univalent pairs produced by desynapsis. Type I univalent pairs behave at anaphase I as "true univalents" i.e. both members of a pair can divide equationally or reductionally, independently of one another. Type II univalent pairs behave at anaphase I as the two members of a bivalent i.e. both members of a pair always divide reductionally.

The distribution of the number of equationally dividing univalents per cell fits a theoretical model in which all pairs of univalents have the same probability of belonging to type I. Therefore, the possibility that only particular chromosomes of the genome form type I univalent pairs can probably be excluded.

Anaphase behaviour of a univalent depends on the orientation of its two centromeres at metaphase I. When amphitelic orientation is produced (bipolar orientation for univalents), equational division at anaphase I follows. Reductional division is produced when syntelic orientation is maintained (unipolar orientation for univalents) (Bauer et al., 1961; Luykx, 1970).

The instability of unipolar orientations during metaphase I has been demonstrated in several cases, both in bivalents (Bauer et al., 1961; Nicklas, 1967; Henderson and Koch, 1970) and in univalents (Bauer et al., 1961). However, in our material type II univalent pairs seem to maintain unipolar orientation since both members of these pairs always divide reductionally.

The micromanipulation studies of Nicklas and Staehly (1967) show direct evidence of a firmer attachment of chromosomes to spindle fibers in anaphase than in metaphase. This fact leads to the possibility that the later the metaphase the firmer the attachment of chromosomes to spindle fibers. In late metaphase these strong connections would make reorientations of univalent half centromeres impossible. Direct evidence for this was given by Bauer et al. (1961) in living spermatocytes of *Tipula oleracea*, in which both types of univalent orientations (syntelic and amphitelic) became stable a short time before the onset of anaphase I.

Now, if gradual univalent formation observed by Sybenga (1958) in inbred rye is produced in our material, the moment at which a univalent pair is formed can determine its subsequent anaphase I behaviour.

Supposing that at the beginning of metaphase I two chromosomes form a bivalent with their kinetochores syntelicaly oriented, if this bivalent gives rise to two univalents at early metaphase I (when the attachment of chromosomes to spindle fibers is weak) then a change from syntelic to amphitelic orientation of kinetochores is possible. These early-formed univalent pairs belong to type I.

If the same bivalent gives rise to two univalents at late metaphase I, the attachment of chromosomes to spindle fibers is strong, and kinetochore reorientations are not possible. The two formed univalents divide reductionally at anaphase I. These late-formed univalent pairs belong to type II (Fig. 3).

If this is true we can suppose that, for a given line, the earlier the moment at which univalents begin to form, the higher the value of q_1, and the higher the total number of univalents present at metaphase I for this line.

Figure 4 shows the relationship between the values of q_1 and the mean number of univalents per cell at metaphase I for each line. As expected, the regression of q_1 values on mean univalents per cell is positive and significant ($b = 0.277$; $t = 7.863$; $p < 0.02$). It can then be concluded that in desynapsis the frequency of univalents

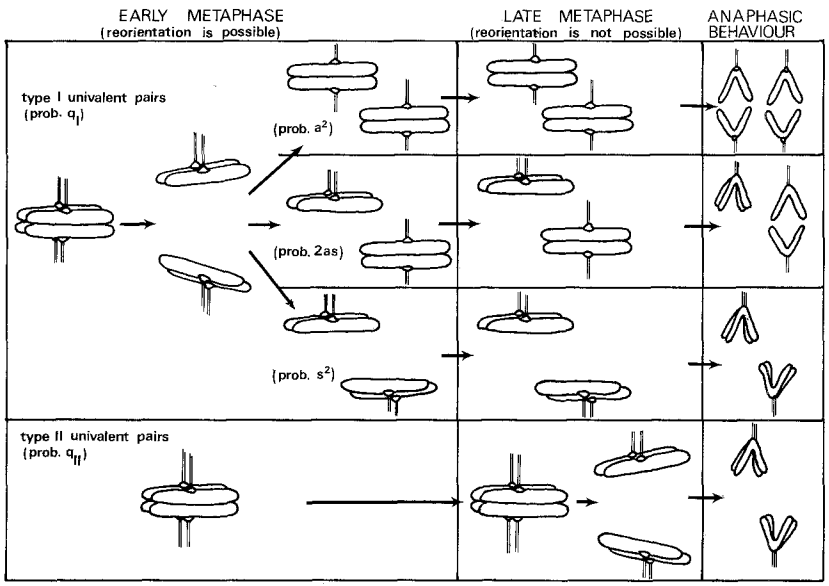

Fig. 3. Univalent behaviour at anaphase I depending on the time at which they are formed during metaphase I. The early-formed univalents (type I univalent pairs) can divide equationally or reductionally. The late-formed univalents (type II univalent pairs) always divide reductionally

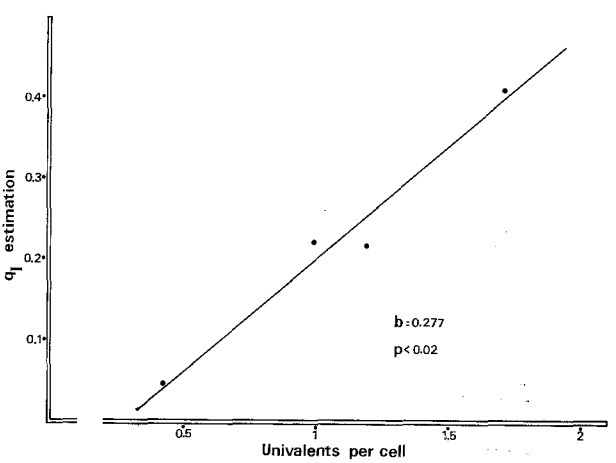

Fig. 4. The estimation of the frequency of the early-formed univalents (q_I) plotted against the mean univalents per cell in each line

at metaphase I depends on the moment at what they form, i.e. the higher the univalent frequency the earlier they have been formed.

That pairing and distribution are related events is a well-known fact. There is a considerable list of findings suggesting that pairing plays the role of maintaining correct kinetochore orientation until the beginning of anaphase I movement.

On the basis of univalent behaviour described in this paper it is proposed that

during metaphase I there is a transference of this role from pairing to spindle attachment forces. Therefore at late metaphase I pairing neither is necessary for maintaining syntelic orientation nor normal anaphase I distribution.

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Received June 10–July 21, 1976 / Accepted August 24 by H. Bauer
Ready for press August 26, 1976