LETHAL SECTORING AND THE DELAYED INDUCTION OF ANEUPLOIDY IN YEAST[1]

A. P. JAMES, ELIZABETH R. INHABER AND GABRIELLE J. PRÉFONTAINE

Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6

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ABSTRACT

Persistent lethal sectoring in a homothallic strain of yeast has been ascribed to tetrasomy for chromosome I. Such aneuploids can appear many generations after irradiation. The data thus indicate that an induced predisposition towards aneuploidy can be prolonged through successive post-irradiation cell divisions. Sporadic cell death in tetrasomics for chromosome I was found to result from a metabolic imbalance and not from a genetic instability conseqent to aneuploidy. This imbalance may be due to a dosage effect involving cistrons for ribosomal RNA since many of these are known to be located on chromosome I. Tetrasomy is not the only cause of persistent lethal sectoring; the phenomenon has been initiated through genetic recombination involving normal diploids. It has also been concluded that, in trisomics, equational division of the supernumerary chromosome sometimes occurs at the first meiotic division.

IN yeast lethal sectoring, the sporadic production of dead cells, is of two types—transient and persistent. Transient lethal sectoring is one of the immediate and frequent consequences of irradiation (JAMES and WERNER 1966). Persistent lethal sectoring, on the other hand, is usually found in clones derived from cells in which transient sectoring has been detected (JAMES *et al.* 1968; JAMES and SAUNDERS 1968). Persistent lethal sectoring is more amenable to investigation by classical genetic techniques and the studies described here were undertaken with the expectation that conclusions about this kind of instability would lead to a better understanding of transient lethal sectoring.

Attention was concentrated on one mutant, the colonies of which are morphologically distinctive. Previous studies had shown that the genetic lesion responsible for lethal sectoring in this mutant is recessive or nearly so (JAMES 1972). A study of meiotic reversion had shown that reversion occurs during the first meiotic division, suggesting that aneuploidy is involved (JAMES 1973). On the other hand, many meiotic revertants are only partial, a fact which is seemingly in conflict with this interpretation. The present investigation has shown that aneuploidy is indeed a cause of persistent lethal sectoring. It is concluded that radiation-induced transient lethal sectoring results from a prolonged disturbance of normal centromere behavior or spindle formation which can, in turn, lead to the production of aneuploids in post-irradiation mitotic generations.

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MATERIALS AND METHODS

The unstable strain was isolated as a segregant from the UV-induced unstable line of the homothallic yeast *Saccharomyces cerevisiae* (var. *ellipsoideus*) termed B67 in this laboratory. The mutant was termed *uns1* in a previous publication (JAMES 1972). The phenotype, referred to as s1 in this report, is distinctive; colonies are ragged in appearance when viewed microscopically at 24 hours. This morphology is due to a high frequency of large, abnormally shaped cells many of which are dead.

B67 contains few genetic markers. Consequently, for linkage studies it was necessary to cross the mutant to well-marked heterothallic strains. These strains, X1012-1D, X1687-16C, X2928-7D, X2944-14A, X2950-5C, and X4002-2B, were obtained from R. K. MORTIMER, University of California, Berkeley. Unfortunately, the spores produced by such hybrids germinated very poorly and the growth of spore colonies was so inadequate that frequently the unstable phenotype could not be identified with any degree of certainty. This problem was solved by inserting genetic markers individually into B67 in a series of backcrosses. Backcrossing was continued until the growth of segregants was normal or near-normal. At this point these genetically marked homothallic strains were crossed to the unstable strain.

Media and methods were routine and have been described previously (JAMES and WERNER 1966). Matings were either spore to spore, cell to spore, or were carried out by a mass mating technique involving genetically marked strains. Phloxine B, to a concentration of .025 mg/ml, was added to the solid medium to enhance the distinguishing characteristics of the unstable phenotype. The dead cells in the mutant colonies are stained brightly with this dye. Medium containing phloxine B becomes toxic on exposure to visible light. For this reason a red filter was attached to the microscope.

The pattern of lethal sectoring was determined by pedigree analysis. This involved the isolation of an individual vegetative cell in logarithmic growth phase to a slab of agar medium. Subsequently, bud separations were carried out through four generations to produce a total of sixteen cells. These were incubated and inspected microscopically at 24 hours. A lethal sector was considered to have been initiated in any one of these cells when all its vegetative progeny produced abortive clones.

RESULTS

Tetrad analyses:

Data from initial crosses involving the unstable mutant and the markers *trp1* and *ade2* indicated that the mutation responsible for lethal sectoring, *s1*, was either centromere-linked or involved a whole chromosome. The relevant data are summarized in the first row of Table 1. *Trp1* is known to be centromere-linked, whereas *ade2* is independent. The near absence of tetratype segregations for *s1* and *trp1* indicated that *s1* tended to segregate at the first meiotic division.

With this information a systematic survey was initiated to determine the location of the lesion responsible for lethal sectoring. Centromere markers were used when available. Results from the first ten chromosomes that were assayed are summarized in Table 1. These data confirmed the association of the mutant with a centromere. They also provided presumptive evidence that the lesion existed as a discrete locus separable from its centromere. This evidence, which later proved to be completely misleading, came from those instances in which the lesion segregated at the second meiotic division. Such events can be detected with a low probability of error when the mutant under test is associated with two or more centromere-linked genes. Thus, the data of Table 1 indicate that a second-division segregation occurred in at least 8 of 444 tetrads (1.8%). It was

TABLE 1

Tetrad ratios for crosses involving s1 *and markers on ten different chromosomes*

Test chromosomes				Cross	Back-cross	No. asci	No. discarded*		Tetrads PD	NPD	T	2nd div. seg. Locus	No.
XV	*ade2*	IV	*trp1*	*s1 ade2* + / + + *trp1*	5	134	6	*s1* – *ade2*	10	20	98		
								s1 – *trp1*	58	69	1		
								ade2 – *trp1*	12	18	98		
III	*leu2*	IV	*trp1*	*s1* + *trp1* / + *leu2* +	5	72	16	*s1* – *leu2*	23	24	9	*leu2*	9
								s1 – *trp1*	25	31	0		
								leu2 – *trp1*	23	24	9		
V	*ura3*	IV	*trp1*	*s1* + *trp1* / + *leu2* +	4	76	21	*s1* – *ura3*	29	22	4	*ura3*	4
								s1 – *trp1*	28	25	2	*trp1*	2
								ura3 – *trp1*	21	28	6		
VI	*his2*	IV	*trp1*	*s1* + *trp1* / + *his2* +	4	70	14	*s1* – *his2*	12	11	33	*s1*	1
								s1 – *trp1*	28	25	3	*his2*	30
								his2 – *trp1*	11	13	32	Any 2	2
VII	*leu1*	IV	*trp1*	*s1* + *trp1* / + *leu1* +	3	15	0	*s1* – *leu1*	7	8	0		
								s1 – *trp1*	8	7	0		
								leu1 – *trp1*	10	5	0		
VII	*leu1*, *trp5*	XV	*ade2*	*s1* + + *ade2* / + *leu1* *trp5* +	1	88	27	*s1* – *leu1*	28	25	18		
								leu1 – *trp5*	43	0	18		
								s1 – *trp5*	14	23	24		
								s1 – *ade2*	13	5	43		
X	*ilv3*	IV	*trp1*	*s1* + *trp1* / + *ilv3* +	3	89	33	*s1* – *ilv3*	21	26	9	*s1*	1
								s1 – *trp1*	25	25	6	*ilv3*	5
								ilv3 – *trp1*	25	21	10	*trp1*	2
XI	*met14*	IV	*trp1*	*s1* + *trp1* / + *met14* +	3	90	17	*s1* –*met14*	31	40	2	Any 2	3
								s1 – *trp1*	32	38	3	*s1*	2
								met14 – *trp1*	36	36	1	*trp1*	1
XIII	*lys7*	IV	*trp1*	*s1* + *trp1* / + *lys7* +	4	64	8	*s1* – *lys7*	11	16	29	*s1*	2
								s1 – *trp1*	30	22	4	*trp1*	2
								lys7 – *trp1*	10	17	29	*lys7*	27
XIV	*lys9*	IV	*trp1*	*s1* + *trp1* / + *lys9* +	3	144	67	*s1* – *lys9*	15	11	51	*s1*	2
								s1 – *trp1*	30	45	2	*lys9*	49
								lys9 – *trp1*	15	13	49		

* Data were discarded when fewer than three spores germinated (7%), when small colony size precluded identification of *s1* (16%), or when segregation for markers was irregular (2%).

evident that a second division segregation for *s1* would be incorrectly scored as a crossover in those instances in which a crossover for each of the other two markers occurred in the same cell. However, it seemed unlikely that all instances of second division segregation for *s1* had such an origin: the frequency of these was even higher than those for *trp1* alone within the same set of data (7/444).

The mutation was found to involve chromosome I, the chromosome on which *ade1* is located. Summarized data are presented in Table 2. Two aspects of these data are of particular interest. Firstly, although the mutant segregated normally (2:2) in each of 201 instances, the segregations for *ade1* were anomalous: the great majority (158/201) were 4:0 (+:−) while a very few (2/201) were 3:1. Secondly, *s1* and *ade1* showed close linkage; only parental ditypes appeared among 41 segregations.

Two explanations of the anomalous segregations for adenine requirement were considered. (1) The 4:0 segregations for adenine were attributable to the presence of revertants (*ADE1 ADE1*) among the presporulation cells, and the rare 3:1 segregations were attributable to gene conversion. (2) The hybrids were trisomic for chromosome I, being of the genetic constitution *ADE1 ADE1/ade1*. If so, it would follow that the lethal sectoring displayed by the mutant homozygote was a consequence of tetrasomy for that chromosome.

Positive support for the first explanation was provided by the evidence, considered above, of recombination between *s1* and its centromere. Further support was provided by the fact that the frequencies (158, 41, 2) of 4:0, 2:2 and 3:1 segregations differed very significantly ($P < .01$) from the frequencies 127, 60, and 14, expected of a trisomic as calculated from the formulae $1/3\ (2-x)$, $1/3\ (1-x)$, and $2/3\ x$, where x is the frequency of second-division segregation (.10) expected of *ade1*. The frequency of 3:1 segregations was fewer than expected by a factor of seven. Furthermore, the frequency of 4:0 segregations was greater than the frequency of 2:2 segregations by a factor of nearly four rather than by a factor of about two, as would be expected if the low frequency of crossovers involving *ade1* was a result of an overestimate of the distance between that locus and its centromere.

A test for the second explanation, trisomy of the hybrids and tetrasomy as a cause of lethal sectoring, was available using the segregations which provided the data of Table 2. If those spores that germinated to produce lethal sectoring

TABLE 2

The phenotypes of segregants of crosses s1 ADE1 trp1 × S ade1 TRP1
(complete tetrads only)

Strain no.	*S:s1* 2:2	*Ade⁺:Ade⁻* 4:0	2:2	3:1	*s1-Ade* PD	NPD	T	*s1-Trp* PD	NPD	T	*Ade-Trp* PD	NPD	T
S 221	117	94	21	2	21	0	0	57	60	0	12	9	0
S 197	33	25	8	0	8	0	0	9	23	1	1	7	0
S 230	13	12	1	0	1	0	0	6	7	0	0	1	0
S 231	38	27	11	0	11	0	0	15	22	1	5	6	0
Total	201	158	41	2	41	0	0	87	112	2	18	23	0

TABLE 3

Tetrad analysis of spore colonies produced by three asci derived from the cross s1 ADE1 trp1 × S ade1 TRP1

Spore Col.	A				B			C			D		
Phenotype	*s1 Ade+*				*s1 Ade+*			*S Ade+*			*S Ade+*		
	Ade+:Ade−				*Ade+:Ade−*			*Ade+:Ade−*			*Ade+:Ade−*		
	4:0	2:2	3:1	0:4	4:0	2:2	3:1	4:0	2:2	3:1	4:0	2:2	3:1
Ascus													
1	5	2	0	0	4	0	2	7	0	0	8	0	0
2	12	0	1	0	6	1	2	7	0	0	6	0	0
3	50	15	14	2	3	4	0	14*	0	0	6	0	0
Total	67	17	15	2	13	5	4	28	0	0	20	0	0

* Colony morphology was aberrant in a majority of the 56 spore colonies.

colonies (*s1*) were indeed disomic, then among those segregations that were 4:0 for adenine, all *s1* segregants should be heterozygous for adenine. Since the strain was homothallic, this could be tested directly by sporulating these segregants.

Positive evidence of aneuploidy was obtained from the segregations of the 12 spore colonies from three asci (Table 3). Each of the six unstable spore colonies, when resporulated, produced one or more adenine-requiring segregants. In contrast, the six stable spore colonies produced none. The accumulated frequencies of 4:0, 2:2, and 3:1 segregations from the six unstable spore colonies were 80:22:19. This ratio was in general accord with that expected of a tetrasomic of genotype *ADE1 ADE1 ade1 ade1*, 73:33:15, as calculated from the formulae provided by ROMAN, PHILLIPS and SANDS (1955) for bivalent pairing.

Confirmation of tetrasomy as a cause of lethal sectoring was provided by the segregations from three exceptional zygotic cultures (*s1 Ade+ Trp− × S Ade− Trp+*). These cultures segregated 4S:0*s1*, an indication that reversion to stability had occurred before mating. In each instance (Table 4) adenine, as well as tryptophan, segregated normally. It is clear that reversion of *s1* to wild type was accompanied by a resumption of normal 2:2 segregations for adenine and that, despite other evidence to the contrary, persistent lethal sectoring in *s1* was caused by aneuploidy. The existence (Table 1) of second-division segregation of *s1* must be interpreted not as a consequence of recombination between *s1* and a centromere, but as a consequence of equational division of the supernumerary chromo-

TABLE 4

The normal segregation of the adenine locus (ade1) *in crosses* s1 Ade+ Trp− × S Ade− Trp+ *in which s1 reverted to wild type at or prior to mating*

	S:s1			*Ade+:Ade−*				*Ade-Trp*		
	4:0	3:1	2:2	4:0	3:1	2:2	1:3	PD	NPD	T
S 294	7	0	0	0	1	6	0	4	2	0
S 295	7	0	0	0	0	6	1	1	3	2
S 296	8	0	0	0	0	8	0	1	6	1
Total	22	0	0	0	1	20	1	6	11	3

some in the trisomic at the first meiotic division rather than at the second. Such behavior has been noted in plants (BURNHAM 1962).

Two asci in the data of Table 3 were exceptional in producing only adenine-requiring spore colonies despite the fact that the premeiotic cells were tetrasomic for chromosome I and presumably of the genotype *ADE1 ade1/ADE1 ade1*. Sporulation of these eight segregants produced only adenine-requiring spore colonies, and crosses to wild type (*S ADE1*) produced strains (*ade1 ade1/ADE1*) which segregated 2:2 for *s1* and for adenine. These segregants, when of *s1* phenotype, in turn segregated as expected of tetrasomics *ADE1 ade1/ADE1 ade1*. It was thus demonstrated that the original exceptional segregants had indeed been of the genotype *ade1 ade1/ade1 ade1*. In the absence of a better explanation, the complete "loss" of two *ADE1* loci was attributed to mitotic crossing over in both pairs of chromosomes prior to the meioses in which the exceptional segregants appeared.

Metabolic imbalance and lethal sectoring:

The effect of culture conditions on lethal sectoring in the tetrasomic strain was studied by transferring individual cells in logarithmic phase from YEPD medium to minimal medium. The cells were then subjected to pedigree analysis through four subsequent mitotic generations.

The results (Table 5) showed that the stability of *s1* is probably a consequence of metabolic imbalance rather than of genetic instability. The frequency with which lethal sectors were produced was reduced by a factor of about 14 during four mitotic generations on a synthetic medium. Furthermore, it was apparent that cells which were moribund on YEPD were reactivated in the presence of unfortified medium. Thus, 15 of 40 cells were abortive when transferred to YEPD, whereas only one of 40 was abortive when cells from the same culture were transferred to synthetic medium.

Lethal sectoring as an indicator of aneuploidy:

Five unstable lines of independent origin have now been isolated from the progeny of irradiated cells of B67. All five have demonstrated the "centromere linkage" expected of tetrasomics, and preliminary tests have indicated that at least two different chromosomes are involved. However, the correspondence

TABLE 5

The influence of growth medium on lethal sectoring in a strain of yeast tetrasomic for chromosome I

Strain	Medium	No. isol.	Abort.	Lethal sectoring			
				Gen. 1	Gen. 2	Gen. 3	Gen. 4
Tetrasomic	YEPD	40	15/40=.38	11/25=.44	17/36=.47	22/52=.42	33/78=.42
	Synthetic	40	1/40=.03	2/39=.05	3/75=.04	6/146=.04	3/271=.01
Diploid	YEPD	10	0/10= 0	0/10= 0	0/20= 0	0/40= 0	0/80= 0
	Synthetic	10	0/10= 0	0/10= 0	0/20= 0	1/40=.03	0/78= 0

TABLE 6

The appearance of a locus for lethal sectoring through genetic recombination involving a homothallic (B67) and a heterothallic (1687–16C) strain

	Diploid	Phenotype	Germ.	Segregants Unstable	Tetrads (stable:unstable) 0:4	1:3	2:2
S1	(1687–16C × B67)	stable	51/64 = .80	28/51 = .55	0	3	5
S2	(S1–15B × B67)	stable	56/68 = .82	40/56 = .71	2	4	4
S68	(S2–9C × B67)	stable	158/172 = .92	88/158 = .56	0	2	34
S72	(S68–17B × B67)	stable	189/192 = .98	95/189 = .50	0	1	44

between lethal sectoring and aneuploidy is far from complete: lethal sectoring can exist in the absence of aneuploidy and aneuploidy can exist in the absence of lethal sectoring.

The existence of lethal sectoring in the absence of aneuploidy was demonstrated in the "synthesis" of a mutant locus through recombination between the homothallic and a heterothallic strain, both of which were free of lethal sectoring. The initial hybrid (Table 6) was stable. Many of its segregants displayed poor colony forming ability but some produced typical unstable colonies. A series of backcrosses of unstable segregants to B67 resulted in a strain, S72, which produced a clear 2:2 segregation for stability. An unstable "mutant" from this strain produced a stable diploid when crossed to *s1*. This diploid when sporulated produced a segregation pattern in which the double mutant was identified through its production of abortive colonies. The frequencies of tetrad types, PD:NPD:T, was 2:2:10, an indication that the mutant locus under test was not centromere-linked.

Tetrasomy in the absence of lethal sectoring was demonstrated in an isolate of B67 which had been exposed to ultraviolet irradiation. This line was evidently tetrasomic for chromosome IX: in crosses involving *his5* and *lys1* the frequencies of 4:0, 2:2 and 3:1 segregations were 12, 1, 11 for *his5*, and 15, 3, 5 for *lys1*. Markers on four other chromosomes segregated normally.

DISCUSSION

These studies have shown that the persistent lethal sectoring that appears in the progeny of irradiated yeast is frequently a result of aneuploidy. Earlier studies have indicated that this persistent lethal sectoring can be initiated many vegetative cell generations after irradiation (JAMES and SAUNDERS 1968). The data imply, then, that the induction of aneuploidy need not be confined to the immediate post-irradiation generation. It seems that irradiation can induce a heritable predisposition towards aneuploidy. Comparable phenomena have been reported for plants and for animals. VAN DER MEY (1973) has noted the delayed induction of chromosome aberrations after irradiation of tomato plants, and ZARTMAN, FECHHEIMER and BAKER (1969) have made similar observations using cultured leukocytes.

Since it is among yeast cells engaged in so-called transient lethal sectoring that aneuploids have been detected, the data further imply that it is in these particular cells that the predisposition exists. It is thus of some interest to review what is now known about this initial damage: (1) The damage is dominant, since it is expressed with high frequency in the immediate vegetative progeny of irradiated diploids. (2) The most prominent expression is the sporadic occurrence of lethal sectors among the vegetative progeny of an irradiated cell. These tend to originate in daughter cells (JAMES and WERNER 1966). (3) The propensity for lethal sectoring is accompanied by a reduced rate of cell division and a tendency towards cell enlargement (JAMES *et al.* 1968). (4) Repair of the damage occurs spontaneously and with such high frequency that affected cells tend to be rare among older clones. Nevertheless, the damage is transient only at the population level; affected cells can persist through many generations, as demonstrated by a procedure involving serial transfer of selected clones (JAMES and SAUNDERS 1968). (5) Spontaneous recovery is a multistep process. Thus, the progeny of a single irradiated cell are heterogeneous in regard to propensity for producing lethal sectors, some demonstrating complete recovery, others only a partial recovery (JAMES and WERNER 1969a). (6) There is some reason to believe that the damage responsible for lethal sectoring is qualitatively similar to a fraction of that which produces outright death, but differs from it in the number of "sites" which have been affected within an irradiated cell (JAMES and WERNER 1967). (7) Recovery of affected cells is promoted by post-irradiation treatment with β-mercaptoethanol, evidently through the action of this substance as a mitotic inhibitor. Recovery is promoted by treatment in the interval between the first and second post-irradiation division as well as in the interval prior to the first post-irradiation division (JAMES and WERNER 1969b).

To these characteristics may now be added a predisposition toward aneuploidy, a state which suggests that the initial damage, characterized as transient lethal sectoring, involves a tendency toward abnormal centromere behavior which, though subject to attenuation, can persist through repeated mitotic generations.

The fact that tetrasomy for chromosome I induces lethal sectoring is of particular interest since recent data have indicated that cistrons for ribosomal RNA are located on this chromosome (FINKELSTEIN, BLAMIRE and MARMUR 1972; ØYEN 1973). Few other genes have as yet been located on this chromosome, a fact which invites the speculation that lethal sectoring is attributable to an excess of ribosomal RNA. This possibility is in accord with the evidence that the lethal sectoring in the tetrasomic is a consequence of metabolic imbalance rather than of genetic instability. It should be noted that the trisomic is phenotypically normal except for a slight reduction in rate of cell division; the data thus indicate a threshold for this dosage effect.

Aneuploidy involving chromosome I has been reported by HAWTHORNE and MORTIMER (1960), who detected the state through irregular segregation of a genetic marker, and by COX and BEVAN (1962), who detected the state through the occurrence of genetic instability. The present study has uncovered a third method of detecting aneuploidy for this same chromosome: the state is accomp-

anied by a distinct colonial morphology which involves lethal sectoring. Thus, in yeast as in Aspergillus (KÄFER 1961), aneuploids may display distinctive phenotypes. The fact that the authors referred to above did not note the presence of lethal sectoring is not surprising since, as the present investigation has shown, the abnormal phenotype is largely absent under some conditions of culture. The phenotype is also influenced by genetic background: although the phenotype of the trisomic is near-normal within the homothallic strain, it is similar to that of the tetrasomic in the hybrid resulting from a cross of the homothallic to a heterothallic strain.

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Corresponding editor: G. R. FINK