MOLECULAR AND CELLULAR BIOLOGY, Nov. 1982, p. 1299–1303
0270-7306/82/111299-05$02.00/0

Vol. 2, No. 11

Propranolol, Atenolol, and Trifluoperazine Reduce the Spontaneous Occurrence of Meiotic Diploid Products in *Saccharomyces cerevisiae*

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Received 16 April 1982/Accepted 26 July 1982

The effect of atenolol, propranolol, trifluoperazine, and caffeine on the occurrence of meiotic diploid and disomic products in *Saccharomyces cerevisiae* was investigated. We demonstrated that atenolol, propranolol, and trifluoperazine reduce the occurrence of meiotic diploid products and that propranolol also slightly decreases the spontaneous frequency of disomics. On the other hand, caffeine appears to be a powerful inducer of diploid meiotic products, but also shows a lesser effect on disomic induction. Since spontaneous or caffeine-induced diploids arise from a failure of the second meiotic division, it appears that the target of these drugs is at the beginning of the second meiotic division. The only common effect of trifluoperazine and propranolol, mainly investigated in mammals, was an inhibition of calmodulin activity via direct interaction. We tend, therefore, to believe that calmodulin activity must be a crucial point for the second meiotic division to begin. The increased induction of diploids, due to caffeine, may be interpreted as a consequence of an increased cyclic AMP level.

The study of spontaneous and induced "nondisjunction," using the *Saccharomyces cerevisiae* strain DIS13 (8a), provided evidence of the occurrence of two kinds of anomalous meiotic products: aneuploids ($n+1$) and diploids. Aneuploids spontaneously arose during both meiotic divisions, whereas diploids appeared to originate from a failure of the second meiotic division. Very often, chemical treatments during meiosis result in an increase of meiotic diploid progeny. For example, bleomycin and mitomycin C induce, almost exclusively, diploids, which originate from a failure of the second meiotic division (S. Sora, M. Crippa, and G. Lucchini, Mutat. Res., in press).

The spontaneous or induced failure to enter into the second meiotic division is similar to the effects of some *spo* and *cdc* mutants on meiosis (5, 8). It is possible that compounds able to induce the failure of meiosis II are able to interfere with structures or signals responsible for the coordination of the sequence of the meiotic process.

In this study, we report the effect of some drugs on the induction of diploid meiotic products. The tested compounds are known to interact with tubulinic structures and with the regulation of the level of cyclic AMP (cAMP). Trifluoperazine is known to interact with calmodulin, which is involved in the regulation of several cell activities. Of interest is its role in the modulation of cAMP and in the disassembly of tubulins (3). The β-adrenergic receptor system is involved in adenylate cyclase regulation and is located on microtubulinic structures (7). For this reason, we assayed the two β-adrenergic receptor antagonists, propranolol and atenolol. Caffeine was also investigated because of its action on the cAMP level (6).

Our findings show caffeine to have an action opposite to that of atenolol, propranolol, or trifluoperazine. The majority of effects were on diploid induction. Caffeine increased the total amount of meiotic diploid progeny, whereas all three of the other compounds reduced their frequency of occurrence.

MATERIALS AND METHODS

Strains. 6122/3a α *lys2* and 6122/12b a *lys2* were used as haploid testers in all of the crosses. The genotype of the diploid strain DIS13 is given in Fig. 1.

Experimental procedure. As reported elsewhere (Sora et al., in press), our methodology consisted of the following steps. (i) Strain DIS13 was treated during sporulation with increasing doses of the compounds to be investigated. (ii) The extent of the inhibition of sporulation was controlled. (iii) The lysis of the ascus walls and seeding of the spore suspensions were performed on different media that allowed: selection of clones heterozygous for the six markers on the right arm of chromosome V, estimation of the frequency of recombination between two of the markers on chro-

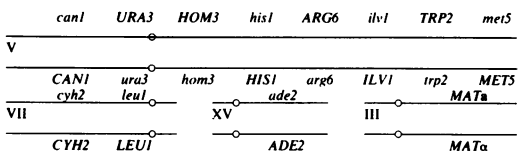

FIG. 1. Genotype of diploid strain DIS13. The chromosomes are indicated by Roman numerals.

mosome V (*hom3* and *his1*), and survival frequency. (iv) The phenotypic analysis of the selected clones took into account two markers located on other chromosomes (*ade2* and *leu1*) and the different combinations of the mating type alleles. This allows the attribution of each clone either to the $n+1$ or the $2n$ class.

RESULTS

Spontaneous events. The reported values of the spontaneous frequencies of diploids, disomics, and recombination per viable spore, $1.05 \pm 0.478 \times 10^{-4}$, $0.68 \pm 0.309 \times 10^{-4}$, and $1.70 \pm 0.467 \times 10^{-2}$, respectively, are the means of 24 independent experiments. The values obtained after treatment with the different chemicals are compared with the spontaneous frequencies, given that the internal control is within the confidence limits.

Treatment with chemicals. The following tables report the data obtained from cultures treated during meiosis with trifluoperazine (Table 1), propranolol (Table 2), and atenolol (Table 3). These three compounds acted in the same direction with respect to the induction of meiotic diploid products. All of the three drugs reduced the spontaneous frequency of diploids, up to 10-fold for propranolol and trifluoperazine, and up to 5-fold for atenolol. Propranolol was the most active chemical in relation to the reduction of disomic formation, reducing the frequency of disomics up to threefold at the maximal dose used. It is evident that all of these effects were observed at the lowest doses used, i.e., doses that have little influence on the sporulation frequency. Although sporulation was inhibited by all of the drugs, only the propranolol treatment at doses higher than those reported may influence spore viability (6×10^{-3} surviving spores at 16 μM concentration).

TABLE 1. Frequencies of the analyzed meiotic events after treatment with trifluoperazine

Dose (μM)	Sporulation inhibition (%)	Recombination HOM3-HIS1/10^2 viable spores	Diploid clones/10^5 viable spores	Disomic clones/10^5 viable spores
0	0	1.70 ± 0.47	10.5 ± 4.8	6.8 ± 3.1
7.5	18	1.50	1.52	7.98
10.0	12	1.60	1.10	6.01
12.5	29	1.74	0.93	4.76
15.0	31	1.80	0.84	4.32
17.5	34	1.74	0.70	3.57
20.0	38	0.96	0.66	3.34
25.0	62	1.58	0.92	4.69

TABLE 2. Frequencies of the analyzed meiotic events after treatment with propranolol

Dose (μM)	Sporulation inhibition (%)	Recombination HOM3-HIS1/10^2 viable spores	Diploid clones/10^5 viable spores	Disomic clones/10^5 viable spores
0	0	1.70 ± 0.47	10.5 ± 4.8	6.8 ± 3.1
1	9	1.46	1.57	3.23
2	12	1.00	2.09	4.31
4	18	1.24	1.21	2.49
8	28	1.07	0.85	1.75

TABLE 3. Frequencies of the analyzed meiotic events after treatment with atenolol

Dose (μM)	Sporulation inhibition (%)	Recombination HOM3-HIS1/10^2 viable spores	Diploid clones/10^5 viable spores	Disomic clones/10^5 viable spores
0	0	1.70 ± 0.47	10.5 ± 4.8	6.8 ± 3.1
200	4	1.69	4.00	8.20
400	7	1.24	1.96	4.04
1,000	12	1.11	2.09	4.31
2,000	12	1.60	3.04	6.26
4,000	15	1.33	2.38	4.92

TABLE 4. Frequencies of the analyzed meiotic events after treatment with caffeine

Dose (μM)	Sporulation inhibition (%)	Recombination HOM3-HIS1/10^2 viable spores	Diploid clones/ 10^5 viable spores	Disomic clones/10^5 viable spores
0	0	1.70 ± 0.47	10.5 ± 4.8	6.8 ± 3.1
1,250	0	2.32	35.4	10.5
2,500	0	2.83	69.8	31.7
5,000	0	2.51	159.3	41.0
6,250	0	3.15	437.0	28.9
7,500	14.7	3.18	730.2	ND[a]
8,750	34.2	ND	2,500.0	ND
10,000	46.1	ND	4,015.0	ND
12,500	74.8	ND	5,220.0	ND

[a] ND, Not determined because the large number of diploid clones masked the recombinant and disomic clones.

Caffeine showed (Table 4) a very different effect. We found caffeine to be the most efficient compound, among those so far tested, in inducing the occurrence of diploid meiotic products. The frequency of diploids at the maximal tested dose was around 5%; this corresponded to an increase over the spontaneous frequency of more than 500-fold. Considering an estimated loss of 90% of the diploids due to the selection system (Sora et al., in press), the real overall frequency of induced diploids must reach 50% of the entire spore population. The data resulting from the segregational pattern of markers *ade2* and *leu1* indicate that all of the induced diploids originate from a failure of the second meiotic division. An effect was also found on disomic induction but with different potency. No effects on the recombination frequency were observed. At the highest doses (from 7,500 to 12,500 μM) caffeine caused a significant death (up to 60%) of ascospores.

Direct isolation of diploid meiotic products. In our previous work, we did not have any direct evidence of the origin of diploid clones from spores. All of the information was based on the segregation of the markers *ade2* and *leu1*. The high increase of diploid frequency induced by caffeine allowed us to obtain such direct evidence by means of spore isolation with a micromanipulator.

TABLE 5. Phenotypes of some clones derived from two-spored asci from caffeine treatment[a]

Ascospore	Supplemented minimal medium			Supplemented minimal medium omitting:									Cross	
	Full	+ *cyh*	+ *can*	*ade*	*leu*	*ura*	*hom*	*his*	*arg*	*ilv*	*trp*	*met*	× a	× α
1a	+	+	−	−	−	+	−	+	−	+	−	+	+	−
1b	+	−	−	+	+	−	+	−	+	−	+	−	−	+
4a	+	+	+	+	+	−	+	−	+	+	+	−	−	+
4b	+	+	+	−	+	−	−	+	−	+	−	+	+	−
5a	+	−	−	+	−	−	+	−	−	−	+	−	−	+
5b	+	−	−	−	+	−	−	+	−	+	−	+	−	+
8a	+	−c	−	+	+	+	−	+	−	+	+	+	−	−
8b	+	−c	−c	+	−	−	+	−	+	−	+	+	−	−
10a	+	−	−c	+	+	−	+	+	+	+	+	−	−	+
10b	+	−c	−c	+	−	+	+	+	−	+	−	+	+	−
12a	+	−	−	+	−	−	−	+	+	+	+	+	+	−
12b	+	−	−	−	−	+	−	+	−	+	−	+	−	+
18a	+	+	−c	−	−	−	+	+	+	+	+	−	+	−
18b	+	−	−c	+	+	+	+	+	+	+	−	+	−	−
19a	+	+	−c	+	+	+	+	+	+	+	+	+	+	−
19b	+	−	−c	−	+	+	+	+	+	+	+	+	−	+
20a	+	+	+	+	+	+	+	−	+	+	−	+	+	−
20b	+	+	−	+	+	+	−	+	−	+	−	−	−	+
21a	+	−	+	+	+	+	+	+	+	+	+	+	−	−
21b	+	+	−	−	−	−	+	+	+	+	+	+	−	−
23a	+	−c	−	−	−	−	+	+	+	+	+	+	+	−
23b	+	+	−	+	+	+	+	−	+	−	+	−	−	+

[a] Symbols: +, growth; −, nongrowth; c, few colonies on negative spots.

One of the apparent effects of treatment with caffeine was an increased frequency of two-spored asci with a dose-response relationship (from 17% in the controls to 81% at 12,500 μM). Two-spored asci were isolated and dissected both from the control cultures and from the cultures treated with 12,500 μM caffeine. A phenotypic analysis was carried out on the clones derived from asci with two surviving spores (69 out of 81 asci from controls, 21 out of 119 from caffeine treated).

A rather important criterion for identifying diploid clones was the appearance of resistant colonies on the negative replicas on media containing cycloheximide or canavanine. This indicates a sensitive phenotype and somatic segregation of resistance, i.e., heterozygosity for the resistance marker. In addition, the segregation of the markers of chromosome V and of the mating type alleles allowed the identification of every diploid among the unselected clones.

Among 138 ascospores from the control asci, none turned out to be diploid. Among the clones derived from the spores of the 21 asci treated with caffeine, some showed anomalous segregations, all of which are reported in Table 5, along with segregations of normal spores. Clones from asci 1, 4, 5, 12, and 20 appeared to have normal segregations. Clones from asci 10, 19, 23, 8, 18, and 21 showed the appearance of colonies on media containing cycloheximide or canavanine, suggesting the presence of two chromosome VIIs or Vs or both.

Clones from asci 10, 19, 23, and clone 18a were able to mate. They were, therefore, crossed with the appropriate haploid partner, together with the normal clones from asci 1, 4, 5, 12, and 20. The survival of spores from asci from such crosses is reported in Table 6. It turned out quite clearly that zygotes formed by clones from asci 1, 4, 5, 12, and 20 are diploid, whereas those derived from clones 10a, 10b, 18a, 19a, 19b, and 23a showed the typical survival pattern of a triploid. We can, therefore, conclude that these asci contained diploid mater spores. Clones 8a, 8b, 18b, 21a, and 21b were able to sporulate and showed the typical survival segregation of diploids.

It was, therefore, found that out of 21 two-spored asci analyzed, 5 carried two diploid spores, and 1 carried a diploid and a haploid spore. The case of spore 18a can be interpreted as a diploid **a**α with a loss of the chromosome III carrying the **a** allele. Even taking into account the small sample analyzed, the data obtained confirm our previous assumptions. We expected a loss of 90% of the diploid clones during the selection procedure. Our expectation was, therefore, to find 50% of diploid spores among the caffeine-treated samples; we found 26%. Considering also the fact that we analyzed only the progeny of asci with two surviving spores, this result seems acceptable. We expected a 1:1 ratio for **a**α/(**aa** + αα); the observed ratio was 6:5. It is apparent that in 5 out of 6 cases, caffeine induced the failure of both chromosomal groups to separate. In the remaining case (ascus 23), there originated a diploid and a haploid.

TABLE 6. Survival of spores from tetrads of sporulating clones and from progeny of crosses involving mater clones

Clone N	Tetrads: Isolated total	Tetrads: With surviving spores per ascus 0	1	2	3	4	Mating type and ploidy of the clone	
8a	8			1	2	5	**a**α	*2n*
8b	8			2	1	5	**a**α	*2n*
18b	10				1	9	**a**α	*2n*
21a	7				2	5	**a**α	*2n*
21b	11				4	7	**a**α	*2n*
1a	11			1	3	7	α	*1n*
1b	7				2	5	**a**	*1n*
4a	8			4	4		**a**	*1n*
4b	7				3	4	α	*1n*
5a	9				5	4	**a**	*1n*
5b	7			1	2	4	**a**	*1n*
12a	8			2	1	5	α	*1n*
12b	7			1		6	**a**	*1n*
20a	6			1	1	4	α	*1n*
20b	5					5	**a**	*1n*
10a	7	6	1				**aa**	*2n*
10b	10	9	1				αα	*2n*
18a	10	10					α?	*2n-1* (III)?
19a	11	9	2				αα	*2n*
19b	8	7	1				**aa**	*2n*
23a	9	7	2				αα	*2n*
23b	8				3	5	**a**	*1n*

DISCUSSION

The data presented here give direct evidence that diploid clones selected with our procedure are derived from spores. Whether the diploids arose spontaneously or by treatment with caffeine, they always originated from a failure of the second meiotic division. This is also true for other drugs which increase the frequency of meiotic diploid products (Sora et al., in press). The meiotic events, particularly recombination and segregation during the first meiotic division, proceeded without error until meiosis II. In yeasts, similar diploid meiotic products have resulted due to the interruption of sporulation (9) and as a consequence of *cdc5* and *cdc14* (8) and of *spo2* (5).

The drugs tested in this study may be capable of interfering with the start signal of the second meiotic division. The two β-receptor antagonists reduced the spontaneous frequency of meiotic

diploid products, as did trifluoperazine. Caffeine increased the frequency of diploids considerably.

The data in the literature on the action of these drugs in mammals and in yeasts may be some help in a preliminary interpretation of our data. The β-blockers (propranolol, in particular, was used) are able to lower the intracellular cAMP level by acting on the regulatory adenylate cyclase subunit (2). Data have also been reported on the ability of propranolol to antagonize calmodulin and the calmodulin-dependent activity of cyclic nucleotide phosphodiesterase (10). In yeasts, propranolol displaces cAMP from the binding site of form-II yeast glyceraldehyde-3-phosphate dehydrogenase (1). Trifluoperazine inhibits calmodulin activity in mammalian cells (3).

The finding that atenolol, propranolol, and trifluoperazine affect diploid induction in the same way seems to indicate that the common target is calmodulin. Calmodulin modulates enzymatic activities that are Ca^{2+} dependent. An interplay exists in the metabolism and function of cyclic nucleotides and also in those of Ca^{2+}. The disassembly of tubulinic structures is correlated with Ca^{2+}-dependent functions. One may hypothesize that a decrease in disassembly may stabilize or favor spindle formation in the second meiotic division with the consequent reduction of diploid formation.

Caffeine is able to inhibit phosphodiesterase, and as a consequence, there is an increase in the level of intracellular cAMP (6). Caffeine also interferes with DNA repair, but it seems unlikely that repair may be involved in diploid spore formation, particularly during the second meiotic division. At the point at which meiosis II is to take place, all of the phenomena of DNA duplication and repair must already be accomplished. It also seems unlikely that the removal of catabolite repression would occur at this stage due to the fact that it must occur before DNA synthesis begins. The most probable way for caffeine to influence the formation of diploid meiotic products seems to be in its ability to influence the intracellular level of cAMP via phosphodiesterase inhibition. It is possible that high levels of cAMP stimulate the calmodulin activity via a mechanism which is opposite to that of the β-receptor antagonists. The findings reported by Jameson and Caplow (4) may make possible another interpretation of diploid formation. High levels of cAMP, via microtubule-associated proteins, influence the quality and the extent of microtubule fibers. If this phenomenon occurs also in yeasts, anomalous spindles may originate and be the cause of diploid formation and possibly nondisjunction (Tables 2 and 4).

A clearcut demonstration of the mechanism is not possible from our work due to the intricate network of the functions dependent on cAMP and Ca^{2+}. Nevertheless, cAMP may have a fundamental role in the induction of the second meiotic division in yeasts.

ACKNOWLEDGMENTS

We are indebted to G. E. Magni for his helpful criticisms and to M. Presta, who stimulated us to consider β-blockers.

This investigation was supported by a grant from the Consiglio Nazionale delle Ricerche (Roma), Progetto Finalizzato "Promozione della Qualità dell'Ambiente," and by grant ENV/345/1 of the European Community.

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