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Action of Some Metal Ions on Yeast Chromosomes

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The effects of metal compounds on nuclear mitotic cross-over, mitotic gene conversion and reversion were studied in a diploid yeast, *Saccharomyces cerevisiae* strain D7. All metal compounds used in these experiments had cell killing effects at concentrations of 10^{-2} to 10^{-4} M. These metal compounds were classified into two groups according to their effects on strain D7. Chromium, arsenic, cadmium, cobalt and nickel compounds induced mitotic cross-over and gene conversion. Lead, mercury and zinc compounds induced mitotic gene conversion at only limited frequencies but the frequency of mitotic cross-over among the convertants was significantly higher than in the control. No increase in reversion frequencies was observed with these compounds.

Keywords——yeast recombination: metal compounds; *Saccharomyces cerevisiae*; mitotic gene conversion; mitotic cross-over

It is known that certain metal compounds present as environmental pollultants cause serious diseases of human beings or animals, and, in addition, carcinogenic effects of some metal compounds have been reported.[1] A close correlation has been found between mutagenicity and carcinogenicity for many chemicals .[2] However, the relation between mutagenicity and carcinogenicity of metal compounds is still obscure and bacterial systems are not adequate for screening metal compounds because many of them which induce cancer in experimental animals are not mutagenic to bacteria.[3]

The microbial eukaryote, *Saccharomyces cerevisiae*, is one of the best characterized eukaryotic cells and has been used for the identification of chemical mutagens.[4] We report here that the yeast strain D7, which can be used to monitor mitotic cross-over, gene conversion and reversion, is susceptible to the action of metal compounds tested.

Experimental

Metal Compounds——Metal compounds used in this study (as chlorides or oxides) are listed in Table I. They were dissolved in distilled water. Lead and arsenic compounds were dissolved in alkaline solution, since they were not soluble in distilled water.

Detection of the Effects of Metal Compounds on Yeast——*Saccharomyces cerevisiae* strain D7 was kindly provided by Prof. F.K. Zimmermann. Strain D7 is diploid and its genetic constitution is *a*/α, and *ade* 2-40/*ade* 2-119, *trp* 5-12/*trp* 5-27, *ilv* 1-92/*ilv* 1-92.[4] The occurrence of mitotic cross-over at the *ade* 2 locus results in recombinants which require adenine for growth and accumulates red or pink pigment on semisynthetic complete agar medium (Difco yeast nitrogen base medium) as recommended by Zimmermann.[4] When mitotic gene conversion occurs at the *trp* locus (or reversion at the *ilv* locus), convertants (or revertants) are able to grow on tryptophan-free (or isoleucine-free) medium. Since these characteristics were rather unstable and recombinants and mutants were accumulated upon prolonged storage, it was necessary to select a suitable clone before performing each experiment.

Five colonies were taken from a culture grown on YPD (1% yeast extract, 2% bactopeptone and 2% dextrose) 2% agar medium, inoculated separately into 1 ml of YPD liquid medium and incubated at 30°C for 18 h. The cells were washed twice and plated onto YPD agar medium and semisynthetic agar medium (lacking either tryptophan or isoleucine) to measure the number of recombinants and mutants. A clone with the least number of recombinants and mutants was selected and used for the experiments. A cell suspension made from the clone grown on a YPD plate was inoculated into 10 ml of fresh YPD liquid medium at a density of 5×10^6 cells/ml and a metal compound was added to the suspension at a concentration indi-

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cated in Table I. After incubation at 30°C for 24 h with shaking, cells were washed and suspended in water, and the cell number was counted in a hemocytometer. Cells were plated onto 3 types of semisynthetic agar media which were supplemented with a complete set of growth factors,[4] without tryptophan or without isoleucine. After incubation for 4 d at 30°C, the number of viable cells and recombinants with cross-over in the *ade* locus were counted on the complete agar medium. Frequencies of conversion and reversion were calculated from selection plates after incubation for 4 to 7 d. The experiments were done at least twice and the data presented are the averages of the results in individual experiments.

Table I. Responses of Strain D7 to Treatment with Metal Compounds for 24 h

Metal compounds	Concentration (M)	Growth[a]	Survivals		Convertants		Revertants	
			%[b]	% Crossover[c]	per 10^5 [d]	% Crossover[e]	per 10^6 [f]	% Crossover[g]
None		35.8	103.4(9569)	0.0(2)	1.3(8951)	4.5(403)	0.8(447)	0.2(1)
CrO_3	10^{-3}	9.3	77.1(1351)	0.1(2)	4.1(539)	7.8(42)	1.4(14)	(0)
	3×10^{-3}	2.4	20.4(3519)	0.4(14)	10.6(1512)	18.3(277)	3.9(47)	(0)
	10^{-2}	0.9	0.6(1011)	1.4(14)	66.5(50)	60.0(30)	(0)	(0)
As_2O_3	10^{-3}	0.8	60.3(967)	1.2(12)	31.7(3004)	3.5(106)	0.6(5)	(0)
	3×10^{-3}	0.9	29.7(1447)	1.1(16)	63.9(2438)	18.1(441)	1.8(1)	(0)
$CdCl_2$	10^{-4}	1.2	27.3(2474)	0.0(1)	6.2(489)	41.3(202)	0.8(9)	(0)
	3×10^{-4}	0.9	3.3(1870)	0.4(7)	24.6(451)	66.3(299)	3.7(4)	(0)
$CoCl_2$	10^{-3}	3·2	67.4(441)	(0)	2.1(393)	1.0(4)	0.8(9)	(0)
	10^{-2}	0.9	0.8(2873)	0.5(13)	20.7(53)	22.6(12)	(0)	(0)
$NiCl_2$	3×10^{-3}	4.7	64.6(593)	0.2(1)	4.1(233)	26.6(62)	1.1(6)	(0)
	10^{-2}	0.9	13.0(1025)	0.2(2)	10.5(288)	36.1(104)	(0)	(0)
$PbCl_2$	3×10^{-4}	25.1	76.4(381)	0.3(1)	1.8(644)	31.4(202)	0.4(15)	(0)
	10^{-3}	0.7	1.7(833)	0.1(1)	3.5(11)	63.6(7)	(0)	(0)
$HgCl_2$	10^{-4}	1.0	41.6(711)	0.6(4)	2.4(1094)	29.3(320)	0.8(41)	2.4(1)
	3×10^{-4}	1.0	25.0(1364)	0.2(3)	3.3(693)	35.5(246)	0.6(16)	(0)
$ZnCl_2$	3×10^{-3}	33.4	90.0(594)	(0)	0.9(524)	7.3(38)	0.8(9)	(0)
	3×10^{-2}	0.9	10.0(446)	(0)	3.4(69)	24.6(17)	(0)	(0)

The actual number is given in parentheses. *a*) Growth denotes the number of cells after treatment divided by the initial cell density. *b*) Number of viable cells divided by the number of cells counted in a hemocytometer. *c*) Percent of recombinants with mitotic cross-over at the *ade* locus against total number of viable cells. *d*) Number of convertants at the *trp* locus per 10^5 cells. *e*) Percent of recombinants with mitotic cross-over at the *ade* locus within the convertants. *f*) Number of revertants at the *ilv* locus per 10^6 cells. *g*) Percent of recombinants with mitotic cross-over within the revertants.

Results

The effects of metal compounds on yeast nuclear genes are summarized in Table I. For most of the metal compounds, the effects were tested at two different concentrations. All the metal compounds tested here were toxic to yeast cells. Induction of mitotic cross-over and gene conversion by the metal compounds was restricted essentially to growing cells; there was no increase in the number of recombinants or mutants among the cells treated with metal compounds in buffer solution (data not shown). In most cases, the lower the survival rate was, the higher the induction of recombination was. It appeared that these metal compounds could be divided into two classes according to their activity on yeast strain D7. The first group, to which chromium oxide, arsenic oxide, cadmium chloride, cobalt chloride and nickel chloride belonged, induced gene conversion at the *trp* locus. The frequencies of convertant formation were about 10- to 60-fold higher than the control. These metal compounds appeared to induce mitotic cross-over in convertants at the *ade* locus. In the case of 10^{-2} M chromium oxide for instance, the cross-over frequency (1.4%) was more than 50-fold higher than that of the control (about 0.02%, 2/9569). This is supported by the data showing that mitotic cross-over in convertants was significantly higher with these metal compounds (row e in Table I). They did not induce reversion at the *ilv* locus. The second group, which consisted of mercuric, lead and zinc chlorides, was less active than the first group. The ability to induce gene conver-

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sion at the *trp* locus was very low but the mitotic cross-over frequencies in convertants were 10 to 15 times higher than in the control. No increase in reversion frequencies was observed with these metal compounds.

Discussion

The results presented here show that all the metal compounds tested in these experiments were active on yeast nuclear genes. It appeared that these metal compounds could be classified into two groups; one induces both mitotic cross-over and gene conversion and the other induces mainly mitotic cross-over. Neither group induced reversion. Although the mechanism of recombination by these metal compounds is uncertain, the fact that resting cells were insensitive to these compounds suggested that growth (presumably desoxyribonucleic acid (DNA)-synthesizing steps) was necessary to induce recombinants. Sirover and Loeb reported that beryllium chloride alters the fidelity of DNA synthesis by interacting with DNA polymerase.[5] Strain D7 appears to be useful for studies on the action of metal compounds on the genome, since no single system has yet proved to be adequate for definite identification of the action of metal compounds. In a *Bacillus subtilis rec* assay system for instance, positive results were reported with arsenic, cadmium, cobalt, chlomium and mercury compounds, but negative results with nickel, lead and zinc compounds.[3c] Negative results were also reported with cadmium[6] and arsenic[7] compounds in the Ames test. It is premature, however, to conclude that the present strain is adequate for screening of the mutagenicity of metal compounds. Further work is necessary for the evaluation of strain D7 as a test strain, and such work is in progress.

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