CYTODUCTION AS A NEW TOOL IN STUDYING THE CYTOPLASMIC HEREDITY IN YEAST

I. A. ZAKHAROV and B. Ph. YAROVOY

Leningrad Nuclear Physics Institute, Academy of Sciences of the USSR, Gatchina, Leningrad district, 188350, USSR

Summary

When crossing the haploid cells of genetically marked yeast strains we observed the appearance of both normal diploid zygotes and haploid nuclear cytoplasmic hybrids. The latter had the nuclear markers of one and the cytoplasmic marker (rho^+) of the other parent. The autonomous cytoplasmic factor transfer was termed as cytoduction. Cytoduction is supposed to be the abortive form of yeast cell mating. Only about 1% of cytoductants is usually observed.

Cytoduction can be used as a simple test on cytoplasmic determination of some characters. We observed the transfer into cytoductant cells of not only rho^+ marker but of resistance factors to antibiotics (erythromycin, neomycin) and killer factor as well. Cytoduction can be applied towards constructing strains having the identical nucleus genotype with mitochondria and other cytoplasmic factors of different origin.

In crossing strains with doubly marked mitochondria recombination of mitochondrial markers in cytoductant haploid cells was observed, the pattern of which was similar to that of mitochondrial recombination in normal zygotes.

Introduction

It is usually supposed that when two yeast cells of opposite mating type mate, plasmogamy is just followed by karyogamy. But in 1957 WRIGHT and LEDERBERG[1] observed that young zygotes could form haploid buds obviously before karyogamy. In the progeny of such haploid buds they revealed nuclear cytoplasmic recombinants, i.e. cells with nuclear genotype from one parent and cytoplasmic (mitochondrial) marker from the other. Some years ago we[2] discovered a similar but not identical phenomenon, namely the occurrence of nuclear cytoplasmic haploid hybrids without formation of diploid zygotic nuclei. We called this phenomenon "cytoduction". In this paper we shall describe some peculiarities of cytoduction, as well as transfer, segregation and recombination of cytoplasmic markers during cytoduction.

Materials and Methods

Strains The strains of our breeding stocks descend from Saccharomyces cerevisiae race XII and are highly homozygous as they have originated from one haploid 15v-P4 after selecting *a* to α mutation[3] and marking by different nuclear and mitochondrial mutations. All petites (rho^-) used in this work are of spontaneous origin. Furthermore, two strains from USA breeding stocks were obtained from Dr. R. MORTIMER and used with genotypes α ade2 and a leu2. The spontaneous rho^- mutant was selected from the first of these strains. In some of the experiments we used homothallic strain 437 *Sacch. cerevisiae* that was a 'killer'[4].

Nomenclature As far as possible, we have followed the yeast genetic nomenclature recommendations[5]. The symbols used for strains of our breeding stocks are the following:

a/α – mating type;
ade1, ade2 – adenine requirement, red colony;
his8, hisZ – histidine requirement;

rad2 – UV sensitivity (uvs1 in our previous publications[2,6];
rgh4 – rough colony[7];
pet – nuclear petite, respiration deficiency (alc4 in our previous publications[2,8];
canR – canavanine resistance;
eryR – erythromycin resistance;
neoR – neomycin resistance;
ery0, neo0 – absence of resistance loci in genome of rho$^-$ strains;
rho$^-$ – respiration deficiency, cytoplasmic petite;
k – cytoplasmic killer factor;
[ ] – cytoplasmic genetic factors.

Determination of phenotypes and genotypes
The procedure for determination of auxotrophy, mating types and UV sensitivity was standard, and the details are described in our previous publications[6,9]. Tests on respiration deficiency or competence were made on glucose-free, 2% ethanol medium [see Media,[8]]. The eryR and neoR phenotypes were determined on ethanol media containing antibiotics. The cells of each colony tested were suspended and plated by steel 5×5 point replicator[10] on selective and non-selective media.

The persistence of eryR and neoR loci in the genomes of petite mutants isolated from proper resistant strains was proved by crossing these mutants with an antibiotic sensitive rho$^+$ strain and testing hybrid diploids thus obtained.

Media
Our basal medium contained K_2HPO_4 2 g/l, $MgSO_4$ 1 g/l, $(NH_4)_2SO_4$ 1 g/l, agar 30 g/l. The complete medium included the basal one supplemented with 20 g/l glucose and 10 ml/l yeast autolysate. The selective medium for antibiotic resistance mutants was the complete ethanol medium supplemented with either 2 g/l erythromycin or 0.7 g/l neomycin. The selective medium for respiration deficient mutants was the complete medium with glucose replaced by 20 ml/l ethanol.

Cytoduction was followed on two media. One was for respiration deficient parents and consisted of the basal medium supplemented with glucose 1 g/l, ethanol 20 ml/l, yeast autolysate 10 ml/l. The other medium was for one respiration deficient parent and respiration sufficient but auxotrophic other. It consisted of basal media supplemented with thyamine 200 μg/l, ethanol 20 ml/l, biotine 2 μg/l, glucose 1 g/l, adenine 5 mg/l.

Genetic crosses
Crosses for studying cytoduction were made as follows: Two parents, one rho$^-$, and the other rho$^+$ but nuclear petite or auxotroph were taken from 2–5 day agar cultures and suspended in sterile water and simultaneously plated on selective agar media for cytoduction (see Media) where they mated. We plated 10^6 cells per petri dish of rho$^-$ parent and 1000 cells per dish of rho$^+$ parent. The plates were incubated at 30° for about two weeks and the colonies analysed and retested when necessary. Usually about 500 zygotic colonies were obtained per each plate.

Results and Discussion

The phenomenon of cytoduction
In the first of our experiments we made crosses of the type [rho$^-$]α ade2×[rho$^+$] a pet and plated the parent cells on the selective medium where they mated. In these crosses we observed not only white respiration sufficient prototrophic colonies but mixed white/red colonies and completely red respiration sufficient colonies as well.

The results of several experiments are shown in Table 1. The mosaic white/red colonies may have been the result of the process described by WRIGHT and LEDERBERG[1], i.e. haploid bud formation by the transient dikaryon. As to the complete red rho$^+$ colonies, tests on the mating

Table 1. Cytoduction frequency in progenies of [rho$^+$] a pet crossed to different petites

Expt. No.	Genotype of petite	Number of colonies scored			
		white	mosaic white-red	complete red	
				number	%
3	[rho$^-$]α ade2	1573	11	9	0.56
5	[rho$^-$]α ade2 rad2	2513	12	6	0.24
6	[rho$^-$]α ade2 rad2	1275	10	7	0.54
9	[rho$^-$]α ade1 rgh4	1665	39	18	1.05
10	[rho$^-$]α ade1 rgh4	1390	14	8	0.57
11	[rho$^-$]α ade1 rgh4	583	21	7	1.15

type of their cells have shown all to be of α type, which proves the autonomous (without transfer of nuclear material) transfer of rho^+ factor during yeast mating from the donor (rho^+) strain to the recipient (rho^-) one. This abortive form of sexual process was termed as cytoduction. The haploid nature of the cytoductants was evident from the results of crossing [rho^-]α ade1 rgh4 × [rho^+] a pet and [rho^-]α ade2 rad2 × [rho^+] a pet. In the first of these all (33 colonies) red cytoductants were rough, in the second all (7 colonies) red cytoductants were not only α mating type but also UV sensitive.

The ability to form cytoductants was not the peculiarity of only our yeast strain. Crossing two strains from Dr. R. MORTIMER's breeding stocks, [rho^-]α ade2 × [rho^+] a leu2, gave similar results with a cytoduction frequency of 3.7%.

Therefore, it can be concluded that cytoduction occurs during mating of different yeast strains, in the crosses of rho^- strains with nuclear petite strains, as well as with respiration sufficient auxotrophic strains, no matter what mating type the donor and recipient parents have.

In another publication we have described the rho^+ (donor) strain (named Hfcd-strain) as having a high frequency of cytoductants (up to 45%)[2]. Thus the frequency of cytoduction is under genetic control and a possibility exists for isolating mutants with a high frequency of cytoduction, which are the mutants that are partly deficient in karyogamy.

Cytoduction of different cytoplasmic factors

The experiments described above demonstrated the transfer of normal mitochondria (rho^+ factor) through cytoduction. If in such crosses two parents differed in other cytoplasmic factors we would obtain either the transfer of this cytoplasmic markers or their segregation[11].

Table 2 summarizes the results of some of our experiments. We observed the transfer of the mitochondrial antibiotic-resistance markers eryR and neoR as well as non-mitochondrial killer factor to all of the cytoductants investigated. In a similar experiment AIGLE and LACROUT[12] found that the cytoplasmic URE 3 marker could be transfered to all cytoductants in the absence of the nuclear ure2 mutation. The lack of segregation of eryR and neoR

Table 2. Cytoduction of different cytoplasmic factors

Genotype of petite (recipient)	Genotype of donor	Cytoproduction frequency	No. of cytoductants scored	Genotype of cytoductants
[rho^-]α ade2	[rho^+eryR] a hisZ	0.3	66	all [rho^+eryR] α ade2
[rho^-]α ade2	[rho^+eryR] a hisZ adel	0.3	36	all [rho^+eryR] α ade2
[rho^-]α ade2	[rho^+neoR] a hisZ	0.7	29	all [rho^+neoR] α ade2
[rho^-]α adel canR	[rho^+K] ade^+ canS*	-	3	all [rho^+k] α adel canR

* spores of homothallic prototrophic strain killer - K

characters in the crosses outlined shows that our rho^- strains were ery0 and neo0 respectively. Thus cytoduction is a unique way of constructing yeast strains having identical nuclear genotype but different cytoplasms. Such strains appear to be very useful in studying nuclear cytoplasmic interactions.

In our laboratory, KALDMA investigated the genotypic effects on mitochondrial recombination[13]. He prepared a series of yeast strains by cytoduction that were isogenic but anisomitochondrial and found that the mitochondrial origin essentially influences both the parent and recombinant class distribution and the susceptibility of the transmission to the effect of the mating type locus. Mitochondria of different sexual types and those with different sensitivity to the mating type locus effect have been found to exist[13].

Mitochondrial recombination during cytoduction

It is known that the parental mating type[14] and the pattern of zygotic bud formation[15] have effects on mitochondrial marker transmission. Thus it was interesting to investigate the influence of such abnormal zygot formation as cytoduction on mitochondrial marker transmission and recombination. In our experiments we had mixed populations of clones, zygotic clones and cytoductants. For that reason we could not use the standard mass mating technique[16] that was employed in our laboratory[13,17]. In this case, we studied mitochondrial transmission and recombination by subcloning individual primary colonies, the products of mating.

For this purpose, the zygotic and cytoductant primary colonies grown on plates were harvested, suspended and in portions plated on appropriate selective media to score the

Table 3. Analysis of diploid and haploid progeny of cross αade1 [rho⁻eryS neoR] x [rho⁺eryR neoS] a his8

Phenotype of colonies	Zygotes					Cytoductants				
	No. colonies scored	Number of subclones and their phenotypes				No. colonies scored	Number of subclones and their phenotypes			
		eryS neoR	eryR neoS	eryR neoR	eryS neoS		eryS neoR	eryR neoS	eryR neoR	eryS neoS
eryS neoS	10	-	-	-	-	12	-	-	-	-
eryS neoR	7	94	0	0	46	4	80	0	0	0
eryR neoS	82	0	196	0	4	73	0	193	0	7
eryR neoR	21	122	225	41	12	11	119	71	30	0

phenotype of the primary colony. One part of each suspension was restreaked for single colonies on a complete nonselective medium. The colonies arising after subcloning (about 20 per 1 primary colony) were individually suspended and retested.

We performed three types of crosses: αade1[rho⁻ eryR neoS]×[rho⁺ eryS neoR] a his8; αade1[rho⁻ eryS neoR]×[rho⁺ eryR neoS] a his8; αade1 [rho⁻ eryR neoR]×[rho⁺ eryS neoS] a his8. The transmission and recombination of our markers eryR and neoR in the normal (rho⁺ by rho⁺) crosses by standard techniques were studied by KALDMA[17]. The frequency of recombination between eryR and neoR was found to be about 16%.

The results of our experiments are shown in Tables 3, 4, 5. It can be seen that the patterns of marker segregations in the progenies of zygotes and cytoductants are very similar if not identical. Thus we can conclude that the deficiencies in normal zygote formation leading to cytoduction have no influence on mitochondrial transmission and that mitochondrial recombination takes place both in haploid and diploic cells.

We can conclude that cytoduction is the abortive form of yeast mating process and is characterized by cell fusion and plasmogamy without karyogamy. We thus suggest the existence of a real physiological (and sometimes genetical) block of karyogamy. As an abortive sexual process, cytoduction may be compared with some sexual phenomena in higher organisms, androgenesis and gynogenesis[18]. It is especially similar to cybrid formation' that was recently described for mammalian cells[19]. Since cytoduction is an easy and unique way of combining any nuclear genotype with cytoplasmic hereditary factors, it is a very promising tool for studying yeast cytoplasmic inheritance.

Table 4. Analysis of diploid and haploid progeny of cross αade1 [rho⁻eryR neoS] x [rho⁺eryS neoR] a his8

Phenotype of colonies	Zygotes					Cytoductants				
	No. colonies scored	Number of subclones and their phenotypes				No. colonies scored	Number of subclones and their phenotypes			
		eryS neoR	eryR neoS	eryR neoR	eryS neoS		eryS neoR	eryR neoS	eryR neoR	eryS neoS
eryS neoS	1	-	-	-	-	2	-	-	-	-
eryS neoR	8	151	0	0	9	7	116	0	0	2
eryR neoS	17	0	151	2	45	25	2	224	1	26
eryR neoR	74	254	188	84	66	58	162	253	93	70

Table 5. Analysis of diploid and haploid progeny of cross αade1 [rho⁻eryR neoR] x [rho⁺eryS neoS] a his8

Phenotype of colonies	Zygotes					Cytoductants				
	No. colonies scored	Number of subclones and their phenotypes				No. colonies scored	Number of subclones and their phenotypes			
		eryS neoR	eryR neoS	eryR neoR	eryS neoS		eryS neoR	eryR neoS	eryR neoR	eryS neoS
eryS neoS	33	-	-	-	-	26	-	-	-	-
eryS neoR	6	46	0	21	43	1	14	0	0	6
eryR neoS	35	10	46	0	108	16	0	141	1	98
eryR neoR	14	49	13	0	158	15	59	51	48	119

References

1. Wright, R. E., Lederberg, J., 1957. Proc. Nat. Acad. Sci. USA, 43, 919–925.
2. Zakharov, I. A., Yurchenko, L. V., Yarovoy, B. Ph., 1969. Genetika, 9, 136–141.
3. Zakharov, I. A., Simarov, B. V., 1966. Genetika, 3, 118–122.
4. Naumov, G. I., Naumova, T. I., 1973. Genetika, 11, 140–145.
5. Sherman, F., Lawrence, C. W., 1974. Microbial Genetics Bulletin, 37, 11–12.
6. Zakharov, I. A., Kozina, T. N., 1967. Proc. (Doklady) Acad. Sci. USSR, 176, 1417–1420.
7. Zakharov, I. A., Kozina, T. N., Kuznetsov, V. V., 1968. Genetika, 4, 78–82.
8. Yarovoy, B. Ph., Yurchenko, L. V., Zakharov, I. A., 1970. Genetika, 6, 138–143.
9. Zakharov, I. A., Kozina, T. N., 1967. Genetika, 11, 110–115.
10. Kovalitzova, S. V., Levitin, M. M., 1968. Genetika, 5, 148–151.
11. Yarovoy, B. Ph., 1973. Proc. (Doklady) Acad. Sci. USSR, 208, 1461–1463.
12. Aigle, M., Lacroute, F., 1975. Molec. Gen. Genet., 136, 327–335.
13. Kaldma, I. A., 1975. Genetika, 8, 88–95.
14. Callen, D., 1974. Mol. Gen. Genet., 128, 331–339.
15. Wilkie, D., Thomas, D. Y., 1973. Genetics, 73, 367–377.
16. Coen, D., Deutsch, J., Netter, P., Petrochilo, E. and Slonimski, P. P., 1970. Symposia of the society for experimental biology, Cambridge, XXIV, 449–496.
17. Kaldma, I. A., 1975. Genetika, 3, 111–121.
18. Rieger, R., Michaelis, A., 1958. Genetisches und cytogenetisches Wörterbuch. Springer-Verlag. Berlin–Göttingen–Heldeberg.
19. Bunn, C. L., Wallace, D. C., Eisenstadt, J. M., 1974. Proc. Nat. Acad. Sci. USA, 5, 1681–1692.