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Negative interallelic complementation of ad-6 temperature dependent mutants of Schizosaccharomyces pombe

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Genetica. — *Negative interallelic complementation of* ad–6 *temperature dependent mutants of* Schizosaccharomyces pombe (*). Nota (**) di NICOLA LOPRIENO, presentata dal Corrisp. F. D'AMATO.

RIASSUNTO. — Sono state utilizzate due coppie di mutanti *temperatura-dipendenti* nel locus *ad–6* di *Schizosaccharomyces pombe* per un'analisi di complementazione interallelica negativa. Le due coppie di mutanti sono collocate nelle sue parti distali del segmento genetico ad una distanza di $543{,}7 \times 10^{-6}$ (ricombinazione intragenica); per ogni coppia i due mutanti distano tra di loro di $1\text{–}2 \times 10^{-6}$ (ricombinazione intragenica).

La capacità di complementazione interalellica di questi quattro mutanti è stata valutata sia con gli alleli selvatici che con una serie di alleli costituiti da mutazione di tipo *mis–sense*, alle due diverse temperature di 30° e 35° C.

È stata accertata una complementazione interallelica negativa soltanto per alcune combinazioni rappresentate da mutazioni temperatura–dipendenti, per cui si deve ammettere una interazione di una singola o di un numero molto piccolo di subunità della proteina codificata dal locus *ad–6*.

Il secondo risultato di un certo interesse è rappresentato da una bassa attività di complementazione dei diploidi *td*/+ alla temperatura di 35° C, il che fa pensare all'esistenza di un certo grado di dominanza dell'allele mutato sull'allele selvatico.

I presenti risultati, inoltre, confermano quanto già osservato in via preliminare in altri microorganismi: nella presente analisi, la capacità di complementazione interallelica è stata messa in relazione anche alla mappa di ricombinazione intragenica del locus *ad–6*.

It has been shown that particular types of mutant alleles (remedial mutants) are unable to complement in diploid heterozygotic combinations even in conditions where haploids have an unmutated phenotype: such a phenomenon has been called « negative complementation » [1].

Moreover, particular types of mutant alleles at given loci in *Aspergillus nidulans* [2], bacteriophage T_4 [3], *Neurospora crassa* [4], and *Saccharomyces cerevisiae* [5, 6] were shown to display a certain degree of dominance over wild-type alleles: it has been suggested that such a behaviour could be due to an interaction of a mutant polypeptide with a normally active wild-type polypeptide.

In the present paper, four *ad–6* temperature dependent heteroallelic mutants (*ad*-6, *td*) of *Schizosaccharomyces pombe* have been tested for their interallelic complementation ability in heterozygotic diploids i) with a group

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of mis-sense complementing mutants; ii) with wild type alleles; iii) in all their reciprocal combinations at different temperature conditions.

The four *ad–6 , td* mutants have been located in the *ad–6* locus map, in order to assess possible relationships between negative complementation and intragenic position.

Experimental.

Strains: the four temperature dependent *ad–6* mutants chosen for such analysis, were EMS–240 and EMS–201 (induced by ethylmethanesulfonate), HA–180 (induced by hydroxylamine) and MMS–678 (induced by methylmethanesulfonate) [7, 8]. These mutants, when incubated at 35° C on minimal agar medium (MMA), did not grow, whereas, at 30° C, they showed a background of growth all over the surface of the medium.

For interallelic complementation analyses a set of *ad–6* known complementing mutants was employed [9, 10]: they were UV–566, UV–445, UV–670, UV–271, UV–256, UV–442, UV–588, induced by UV light [11]. Wild type strains *972 , h^-* and *975 , h^+* of opposite mating type were also employed.

For intragenic recombination analyses, four non-complementing *ad–6* mutants were used, namely UV–250, UV–428, UV–463, UV–430 [11].

Intragenic recombination: analyses were done with random ascospores collected from crosses: the ascospores, were plated at 35° C, when the crosses involved one or both of temperature-sensitive mutants. At least two experiments for each reciprocal cross were analyzed.

Interallelic complementation: the material to be analyzed was plated on MMA plates and incubated at either 30° or 35° C, and the ability of diploid combinations to grow on minimal medium was evaluated. The following combinations were evaluated:

a) temperature-dependent mutants *x* wild type; (*ad–6 , td*/*ad*$^+$);
b) temperature-dependent mutants *x* temperature dependents mutants (*ad–6 , td_x*/*ad–6 , td_y*);
c) temperature dependent mutants *x* mis-sense mutants (*ad–6, td*/*ad–6*);
d) wild type *x* wild type (*ad*$^+$/*ad*$^+$).

In all cases diploid colonies grown on MMA plates were classified as complementing when they showed a positive response to the iodine reaction [9].

Results.

Table I reports data on the intragenic recombination analyses for eight mutants (36 combinations): the data for each combination is the total of at least two reciprocal crosses; the relative position of the eight alleles within the map of the *ad–6* locus is shown in fig. 1.

TABLE I.

Result of the intragenic recombination analysis of eight mutants at the ad–6 *locus of* Schizosaccharomyces pombe.

CROSSES	N° ascospores ($\times 10^6$)	Total N° recombinants	Recombination frequency ($\times 10^{-6}$)
UV –250×UV –428	20.93	2758	131.77
UV –250×UV –463	13.53	5829	430.82
UV –250×UV –430	13.43	15491	1153.46
UV –428×UV –463	10.34	2435	235.49
UV –428×UV –430	14.77	15227	1030.94
UV –463×UV –430	10.49	6802	648.42
UV –250×UV –250	6.46	0	—
UV –428×UV –428	14.10	0	—
UV –463×UV –463	2.06	0	—
UV –430×UV –430	19.81	0	—
EMS–201×EMS–240	26.61	3260	122.51
EMS–201×MMS–678	63.72	150	2.35
EMS–201×HA –180	9.29	5051	543.70
EMS–240×MMS–678	12.10	1775	146.69
EMS–240×HA –180	47.88	56	1.16
MMS–678×HA –180	8.11	3615	445.74
EMS–201×EMS–201	22.18	0	—
EMS–240×EMS–240	23.70	0	—
MMS–678×MMS–678	22.47	0	—
HA –180×HA –180	19.64	0	—
EMS–201×UV –250	6.57	3726	567.12
EMS–201×UV –428	12.76	7884	617.86
EMS–201×UV –463	3.20	1006	314.37
EMS–201×UV –430	4.94	1151	232.99
EMS–240×UV –250	2.53	64	25.29
EMS–240×UV –428	4.63	88	19.00
EMS–240×UV –463	2.09	589	281.81
EMS–240×UV –430	2.70	1087	402.59
MMS–678×UV –250	3.98	3772	947.73
MMS–678×UV –428	5.10	4383	859.41
MMS–678×UV –463	9.67	2584	267.21
MMS–678×UV –430	20.34	2263	111.25
HA –180×UV –250	5.83	828	142.02
HA –180×UV –428	15.36	586	38.15
HA –180×UV –463	37.56	2682	71.40
HA –180×UV –430	33.58	7376	219.65

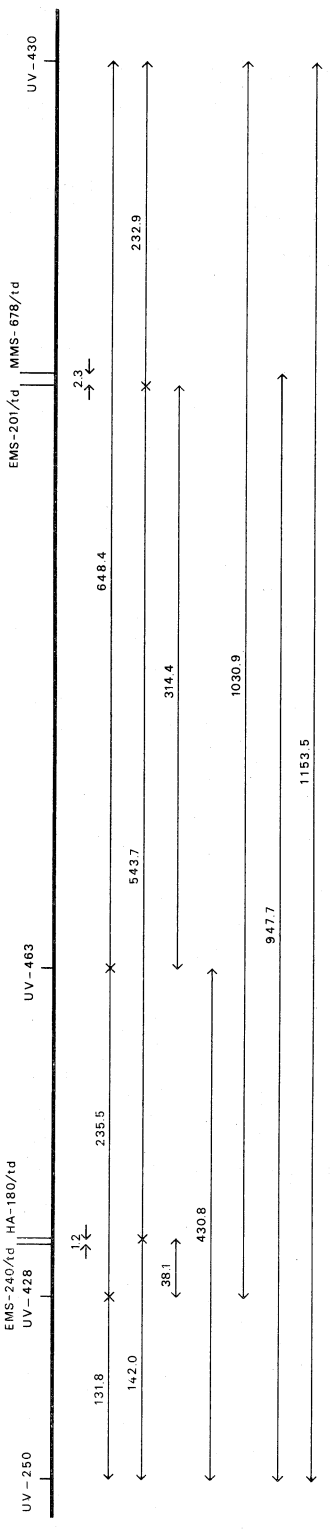

Fig. 1. – Linkage relationships of EMS–240, EMS–201, HA–180, and MMS–678 *ad–6* mutants (adenine temperature dependent alleles) and UV–250, UV–428, UV–463, and UV–430 *ad–6* tester mutants of *Schizosaccharomyces pombe*. Intragenic map distances are presented in terms of prototrophs per 10^6 viable ascospores plated.

TABLE II.

Interallelic complementation patterns of ad–6 *temperature dependent alleles of* Schizosaccharomyces pombe.

(+, Complementation; —, No complementation).

MUTANT TESTED	EMS–240		HA–180		EMS-201		MMS–678	
	30°	35°	30°	35°	30°	35°	30°	3° 5
EMS–240	—	—	—	—	+	—	+	—
HA–180			—	—	+	—	+	—
EMS–201					—	—	—	—
EMS–678							—	—

In Table II and III, the results of the interallelic complementation analyses for the heterozygotic combinations *ad–6* , $td_x/ad–6$, td_y and *ad–6*, *td*/*ad–6* for two different temperature conditions (30° and 35° C) are reported. In Table IV, the results of the complementation behaviour of the combinations *ad–6* , *td*/ad^+ are gathered.

TABLE III.

Interallelic complementation patterns of ad–6 *temperature dependent alleles of* Schizosaccharomyce pombe *in diploid combinations with* ad–6 *tester mutant strains.*

(+, Complementatíon; —, No complementation).

MUTANT TESTED	UV–566		UV–445		UV–670		UV–271		UV–256		UV–442		UV–588	
	30°	35°	30°	35°	30°	35°	30°	35°	30°	35°	30°	35°	30°	35°
EMS–240	—	—	—	—	+	—	+	—	+	—	+	+	+	+
HA–180	—	—	—	—	—	—	+	—	+	—	+	—	+	+
EMS–201	+	+	+	+	+	+	+	—	+	—	—	—	+	+
MMS–678	+	+	+	—	+	—	+	—	—	—	—	—	+	—

An attempt at a quantitative analysis of the complementation ability of the diploids *ad–6* , *td*/ad^+, showed that the number of complementing diploids at 30° C was 10 to 100 times higher than that at 35° C, whereas there was a ratio of 1 : 1 between the diploids ad^+/ad^+, formed at both temperatures.

TABLE IV.

Diploid allelic complementation of ad–6 *temperature dependent alleles and wild type alleles of* Schizosaccharomyces pombe.

(+++, 5% diploids; +, 0.5 — 0.05% diploids).

MUTANT TESTED	*wt*–975, h^+		*wt*–972, h^-	
	30°	35°	30°	35°
EMS–240	+++	+	+++	+
HA–180	+++	+	+++	+
EMS–201	+++	+	+++	+
MMS–678	+++	+	+++	+
wt–972, h^-	+++	+++		
wt–975, h^+			+++	+++

DISCUSSION.

Negative complementation has been suggested by Finchman [1] to occur when « a pair of mutants might interact to form an enzyme less active or more abnormal in properties than the enzyme which would have been formed by one of the alleles by itself ".

In the present analysis, four temperature-dependent *ad–6* alleles of *S. pombe* have been tested for their interallelic complementing ability in heterozygotic diploid combinations within themselves ($ad–6\ ,\ td_x/ad–6\ ,\ td_y$) or with normally complementing alleles ($ad–6\ ,\ td/ad–6$) at two particular conditions of growth temperature (30° and 35° C): some heterozygotic diploid combinations showed negative complementation at 30° C; at 35° C, the interallelic complementation ability of the four temperature-dependent alleles was greatly reduced.

The first interesting aspect that has emerged from this analysis is the relationship between intragenic location of *td* alleles and their negative complementation pattern when in heterozygotic combinations of either type td_x/td_y or *td*. It has been demonstrated that interallelic complementation is the result of hybridization between different mutant polypeptides [4]: the activity of such a hybrid protein will depend on the type of interaction displayed by the two components. Since not all combinations involving *td* alleles show negative complementation, it seems reasonable to assume that the above interaction is operative at a level of single, or of a very small number of, subunits of the protein.

The second aspect is represented by the efficiency of complementation in heterozygotic combinations of the type *ad-6 , td/ad*$^+$: at the higher temperature (35° C), the allelic complementation is very low compared to that shown at 30° C. Since at 35° C the aploid strains carrying the *ad-6 , td* alleles are strongly mutant, a certain degree of dominance of the *td* alleles over the corresponding wild type alleles might be assumed. This is in agreement with other results obtained on employing different microbial systems [2, 3, 4]. The hypothesis put forward to explain this phenomenon assumes the presence of a multimeric protein due to the random association of polypeptides: reduction in complementation, would, therefore indicate the formation, in the majority of cases, of hybrid protein molecules, resulting from mutant and wild type polypeptides.

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