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Genetica. — *Reversion studies with ad_7 "hot spot" mutants of* Schizosaccharomyces pombe (*). Nota di ENRICA MICHEL, MARIA L. MELEN e NICOLA LOPRIENO, presentata (**) dal Corrisp. F. D'AMATO.

RIASSUNTO. — È stato analizzato, per mezzo di analisi di reversione alla adenina-indipendenza effettuata con i raggi UV e l'acido nitroso, il comportamento di 8 mutanti adenina-dipendenti indotti dai raggi UV nel *locus* ad_7 di *Schizosaccharomyces pombe*. Questi mutanti, che non presentano ricombinazione tra di loro, sono ritenuti corrispondenti allo stesso *site* mutazionale e rappresentano ripetizioni indipendenti dello stesso evento: il *site* occupato è il 407 (« hot spot ») del *locus* ad_7. A seguito della presente analisi è stata tuttavia dimostrata la non omogeneità degli 8 mutanti appartenenti all'« hot spot » in esame: ciò indica che in *S. pombe* l'analisi genetica di ricombinazione non permette di distinguere due o più *sites* vicini tra di loro, o che gli otto mutanti presentano una o più alterazioni genetiche di tipo molecolare in corrispondenza del *site* in questione. I risultati ottenuti in *S. pombe* corrispondono a quelli riportati per altri organismi, in relazione allo stesso fenomeno.

Extensive studies by Leupold [1], [2], Gutz [3], [4], Leupold and Gutz [5] have shown the occurrence in the ad_6 and ad_7 loci of *Schizosaccharomyces pombe* of " hot spot " mutants after chemical and physical mutagenic treatments. The classification of these mutants is based on the absence of wild type recombinants from reciprocal crosses. Mutants located at the same site have been found in other organisms [7], [6]: in phage T_4 and bacteria reversion analyses by means of different chemical treatments have provided evidence that " hot spots " may be resolved [8], [9].

In order to ascertain this possibility in *S. pombe* also, reversion studies with UV light and nitrous acid have been carried out with eight out of 25 mutants induced by UV light, occurring at the 407 site in the ad_7 locus. The results of this analysis are reported here.

EXPERIMENTAL.

The following mutant strain, $ad_7\, h^-$, of *S. pombe* (kindly provided by Prof. U. Leupold) have been employed:

407/1 s, 433/1 s, 435/1 s, 439/1 s, 506/1 s, 516/1 s, 520/1 s, 529/1 s.

Conditions for mutagenic treatments, plating media for scoring reversion and genetic analyses have been those previously reported [10].

(*) Work done at the Istituto di Genetica – Università di Pisa.
(**) Nella seduta del 22 giugno 1966.

Results.

Several independent cultures on YEA of the mutants have been analyzed for their spontaneous reversion frequency to adenine independence: Table I shows that, for all mutants tested, the reversion frequency is of the order of 10^{-8} per viable cells.

Table I.

Spontaneous reversions to adenine independence in 8 ad_7^- *hot spot mutants of* Schizosaccharomyces pombe.

Mutant no.	No. of independent cultures tested	No. of viable cells evaluated $\times 10^9$	No. of ad^+ scored	ad^+ per 10^8 survivors
407	49	10.42	60	0.57
433	43	8.89	78	0.87
529	71	15.67	170	1.08
435	70	24.18	68	0.28
439	39	10.48	497	4.74
516	60	18.61	76	0.40
520	119	18.37	30	0.16
506	52	9.37	137	1.45

For UV-light and nitrous acid treatments, the reversion to adenine independence has been evaluated in several independent experiments, by varying both the UV doses and the times of treatment with nitrous acid. The results of this analysis are reported in Tables II and III; for each mutant only one dose is reported, and a comparable survival level is considered. UV light induces reversions in only three out of the eight mutants, whereas nitrous acid reverts all but the ad_7^- h^-, 506/1 s.

Revertant strains spontaneously occurring in all the eight mutants (each one from independent cultures), UV-induced revertants obtained from treated 407, 433, and 529 mutants, and nitrous acid-induced revertants obtained from the seven sensitive strains have been crossed back to the wild type 975 h^+ strain for an analysis of the suppressor mutations; in all cases, more than 1,000 random ascospores have been evaluated (Table IV). Only 16 revertant strains appear to be due to a mutation of disinct suppressor loci: for these strains, the genetic analysis has been repeated several times and the percentage of the ad^- purple recombinants is given in Table V. The percentages of recombination vary from 0.16 to 4.44, thus indicating that several closely linked suppressor loci are involved.

TABLE II.

Reversion to adenine independence induced by UV *light in* 8 ad_7^- *hot spot mutents of* Schizosaccharomyces pombe.

(3 min. of treatment; dose rate = 48 erg/mm²/sec.).

Mutant no.	TREATED SERIES				CONTROL SERIES		
	Survival %	No. viable cells evaluated ($\times 10^9$)	No. of *ad*+ scored	*ad*+ per 10^8 survivors	No. viable cells evaluated ($\times 10^9$)	No. of *ad*+ scored	*ad*+ per 10^8 survivors
407	56.47	1.75	43	2.45	4.44	4	0.09
433	60.53	0.53	25	4.71	1.16	2	0.17
529	46.79	0.72	47	6.52	1.63	1	0.06
435	65.25	0.48	9	1.87	0.66	0	< 0.01
439	82.81	0.59	5	0.85	0.71	0	< 0.01
516	64.90	0.76	7	0.92	1.16	0	< 0.11
520	56.32	0.67	6	0.89	1.21	0	< 0.008
506	73.40	1.47	10	0.68	2.25	3	0.13

TABLE III.

Reversion to adenine independence induced by nitrous acid (12 min. of treatment) *in* 8 ad_7^- *hot spot mutants of* Schizosaccharomyces pombe.

Mutant no.	TREATED SERIES				CONTROL SERIES		
	Survival (%)	No. viable cells evaluated ($\times 10^9$)	No. of *ad*+ scored	*ad*+ per 10^8 survivors	No. viable cells evaluated ($\times 10^9$)	No. of *ad*+ scored	*ad*+ per 10^8 survivors
407	29.68	0.90	38	4.22	4.09	4	0.09
433	59.33	0.44	976	221.81	0.75	1	0.13
529	42.97	0.51	274	53.72	0.97	4	0.41
435	42.51	0.22	39	18.05	0.51	1	0.19
439	56.09	1.02	57	5.59	1.79	12	0.67
516	45.17	0.45	40	8.88	1.51	1	0.06
520	52.83	0.28	30	10.86	0.52	1	0.19
506	49.51	0.23	1	0.43	0.43	3	0.69

Discussion.

The reversion pattern induced by UV light and nitrous acid in eight UV–induced ad_7 mutants of *S. pombe*, occurring at the site 407 of ad_7 cistron shows that the " hot spot " analyzed is not homogenous: at this site, one finds UV and nitrous acid revertable strains (407, 433, 529), nitrous acid revertable strains (435, 439, 520, 516), and only one spontaneously reverting strain (506). These findings are in agreement with available data on UV–induced or spontaneous " hot spot " *rII* mutants of phage T_4 [11], [12] and on chemically induced " hot spot " mutants of *Salmonella typhimurium* [9].

Table IV.

Genetic analysis of the induced and spontaneous revertants of the ad_7^- *hot spot mutants of* Schizosaccharomyces pombe.

Mutants no.	Revertants induced by agent	No. of strains evaluated	Suppressor-revertants	
			No.	%
407	None (Spontaneous)	31	1	3.2
	UV	51	0	0.0
	NA	75	4	5.3
433	None (Spontaneous)	23	0	0.0
	UV	89	3	3.3
	NA	59	0	0.0
529	None (Spontaneous)	36	2	5.5
	UV	52	0	0.0
	NA	60	0	0.0
435	None (Spontaneous)	29	0	0.0
	NA	77	0	0.0
439	None (Spontaneous)	31	0	0.0
	NA	67	2	2.9
520	None (Spontaneous)	15	1	6.6
	NA	70	0	0.0
516	None (Spontaneous)	39	1	2.5
	NA	66	0	0.0
506	None (Spontaneous)	27	2	7.4

TABLE V.

Ad^- recombinant frequencies obtained from crosses suppressor revertants X wild type (975 h^+).

Revertant no.	ORIGIN (mutant no.-treatment)	ad^-/tot.	Recombination (%)
1292	407–SP	58/3734	1.55
1089	407–NA	110/4650	2.36
1105	407–NA	88/5042	1.74
1081	407–NA	19/3816	0.49
1106	407–NA	145/4070	3.56
1707	433–UV	37/2228	1.66
1751	433–UV	47/3391	1.39
2001	433–UV	9/4524	0.16
1243	529–SP	263/5919	4.44
1391	529–SP	439/11309	3.88
1614	516–SP	18/3818	0.47
1061	439–NA	191/7453	2.56
1037	439–NA	215/6267	3.43
1480	520–SP	71/4462	1.59
1430	506–SP	62/21193	0.29
1382	506–SP	71/14932	0.47

In *S. pombe*, the reversion pattern found in the UV–induced "hot spot" 407 is similar to the reversion pattern of UV–induced mutants in other loci in the same organism. For instance, among ten mutants induced by UV light in different sites of the ad_1 locus, seven reverted with NA and, of these, only two reverted with UV also [14].

The non-homogeneity of "hot spot" mutants may indicate that either genetic analysis in *S. pombe* does not permit a resolution of two (or more) closely linked sites, or more than one kind of change can occur at a given "hot spot" site. Other "hot spots" in the ad_7 locus of *S. pombe* have been resolved by either suppressor studies (Leupold, personal communication), or reversion analyses [13].

The 407–site mutants offer a suitable material for reversion analyses, because reversion to adenine independence occurs mainly by back-mutation

(see Table IV): only 16 out of 927 genetically analyzed revertants have been found due to a mutation of suppressor loci, situated in a region proximal to the ad_7 locus, as shown by the recombination frequency of adenine-dependent ascospores (Table V).

If the reversion pattern observed represents a specific response of a given nucleotide base in the eight mutants, the mutants 407, 433, 529 might have a cytosine base as a target, sensitive to both UV and nitrous acid [15], [16], whereas the mutants 435, 439, 520, 516 might have an adenine base as a target., sensitive to nitrous acid only [15]; the mutant 506 may represent a non-transition mutant, according to the indications provided by the UV–induced *rII* mutants of T_4 [12].

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