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Mutagen specificity among reversions of ultraviolet-induced *adenine-1* mutants of *Schizosaccharomyces pombe*

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1. INTRODUCTION

Current theories concerning the chemical nature of induced mutations have stimulated many experiments in which mutants produced by a given mutagen were tested for the ability to revert spontaneously and under the influence of different mutagens (Freese, 1959, 1963; Kirchner, 1960; Eisenstark & Rosner, 1964; Margolin & Mukai, 1961; Weinberg & Boyer, 1965; Barnett & de Serres, 1962; Tessman, Poddar & Kumar, 1964; Drake, 1964; Kreig, 1963). Often the results of such tests have confirmed the chemical hypotheses which had provoked them. This has been so especially in experiments with bacteriophage, while similar tests on bacteria and fungi have given less consistent results. In these latter organisms what appears to be a specific response of a particular auxotrophic allele to a given mutagen may be due to a variety of effects: true reversion (including intragenic suppressors that change the reading frame (Drake, 1963)), suppressor mutations which may be specific for particular alleles, or effects of the mutagen on one or more of the many cellular processes concerned in the genetic translation and phenotypic expression of the restored prototrophic function.

In the present study a number of *adenine-1* mutants of the fission yeast *Schizosaccharomyces pombe* (Leupold, 1955, 1957), all induced by ultra-violet irradiation, and most of them located in the same cistron, have been tested for reverse mutability after treatment with ultra-violet light or nitrous acid, or without treatment (spontaneous reversions). In agreement with all previous tests of this nature, in bacteriophage and bacteria, differences in response were found to exist between the alleles. Samples of the reversions obtained were classified into two classes: true reversions, including intragenic or very closely linked suppressors, and reversions due to unlinked or loosely linked suppressors. This revealed a further striking type of specificity, namely a difference in the proportions of suppressor mutations among revertants depending on the particular allele and mutagen chosen.

2. MATERIALS AND METHODS

The ten U.V.-induced mutations at the *adenine-1* locus of *S. pombe* are located at ten different sites within the locus (Ramirez, Friis & Leupold, 1963; Leupold,

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personal communication; Clarke, unpublished results). The isolation numbers of these mutations are 3, 25, 40, 51, 107, 153, 169, 199, 233 and 249. Mutant 40 is located towards one end of the locus, mutants 3 and 233 are representatives of a cluster of mutants located in the centre of the locus, and mutants 25, 51, 107, 169, 199 and 249 all occur in a close cluster at the other end of the locus. Mutant 233 will complement mutants 25, 51, 107, 169 and 199. Mutants 169 and 199 are temperature-sensitive auxotrophs.

Details of media, procedures for nitrous acid and U.V. reverse mutation experiments, and methods for crosses have been published earlier (Leupold, 1955, 1957; Clarke, 1962, 1963, 1965).

Abbreviations used: adn^- = adenine-dependent, adn^+ = adenine-independent, MMA = minimal medium agar, YEA = yeast extract agar.

3. RESULTS

(*i*) *Reverse mutation*

A comparison was made between the frequencies of spontaneous and induced reverse mutation of ten *adenine*-1 mutants. The mutagens used were nitrous acid and U.V. radiation. Table 1 gives a summary of the data for those mutants that responded to at least one of the two mutagens. Table 2 presents a qualitative 'response pattern' for all ten mutants; it includes two which do not figure in Table 1 because they did not respond to either mutagen.

Quantitative estimates for spontaneous mutation frequencies are difficult to obtain, since spontaneous revertants include pre-existing as well as newly arisen ones. Qualitatively however, differences between the mutants in spontaneous reversion frequency were obvious. Mutants 25, 169, 199, 3 and 40 reverted with much higher frequencies than mutants 249, 51, 107, and 244. No spontaneous revertants at all were obtained from mutant 153, even when some 10^9 cells from twenty independent YEA cultures were plated on MMA. Mutant 153 was also recalcitrant to the action of the two mutagens. In addition, two mutants (3 and 249) failed to respond to nitrous acid. In the other seven mutants, nitrous acid induced clear increases in mutation frequencies over the controls, measured either as increases in absolute colony number or as increases in the frequencies per 10^7 surviving cells. U.V. produced reversions in only two mutants, both of them responsive also to nitrous acid. Apart from this, there appears to be no correlation between the frequencies of reversions occurring spontaneously or induced by HNO_2 or U.V. light.

(*ii*) *Genetic analysis of adenine reversions for the presence of suppressor mutations*

The adenine-independent revertants were back-crossed to wild-type and 200–500 random ascospore progeny from each cross were tested for the presence of adenine-requiring recombinants indicative of a suppressor-type revertant. Table 3 summarizes the results obtained for all the *adenine*-1 revertants so far tested. For

Table 1. *Summary of reverse mutation data for* U.V.*-induced* adenine-1 *mutants**

			Untreated controls		Treated series			
Mutant	Treatment	No. of experiments†	Absolute no. of *adn*†	Spontaneous revertants/10^7 viable cells	Mean Survival %	Absolute no. of *adn*†	Revertants/ 10^7 survivors	Induced *adn*† ‡
25	HNO_2	18	122	0·79	67·8	2290	15·9	15·1
40	HNO_2	22	267	2·35	76·3	2117	15·5	13·1
51	HNO_2	12	3	ca. 0·03	53	1678	25·1	25·1
107	HNO_2	2	1	ca. 0·06	77·9	97	1·4	1·3
169	HNO_2	4	8	0·27	82	75	1·05	0·8
199	HNO_2	3	21	0·99	69·9	1123	13·8	12·8
233	HNO_2	3	0	0	63·3	73	1·27	1·3
40	UV	5	234	4·18	71·2	948	24·5	20·3
199	UV	4	12	0·86	69	883	12·2	11·3

* All ten *adenine-1* mutants were tested for reversion with both HNO_2 and UV. Only the positive results are shown in this table.

† In all experiments treatment was with 0·1 M $NaNO_2$, or at a constant distance from the U.V. source, for varied periods of time so that 5 or 6 different doses were given in each experiment. Survival usually ranged from 100 down to 10%. Mean survivals have been calculated from the 5 or 6 treated series in each experiment. (Clarke, 1963).

‡ Revertants/10^7 survivors *minus* spontaneous revertants/10^7 viable cells.

Table 2. *Reverse mutation response pattern of ten U.V.-induced* adenine-1 *mutants*

Mutant	Reversions: Spontaneous (per 10^7 cells plated)	Reversions: HNO_2	Reversions: UV
153	< 0·02	‡	‡
249	< 0·2	‡	‡
51	< 0·2	*	‡
107	< 0·2	†	‡
233	< 0·2	†	‡
25	> 0·2	*	‡
169	> 0·2	†	‡
199	> 0·2	*	*
3	> 2	‡	‡
40	> 2	*	*

* Ten induced $adn^+/10^7$ survivors within the survival range of 50–80%.
† Approx. one induced $adn^+/10^7$ survivors in the survival range of 50–80%.
‡ No increase over spontaneous level was detectable.

purposes of comparison the results for revertants of other adenine mutants, both at the *adenine-1* and other adenine loci, are shown in the lower half of Table 3. In all cases where a suppressor mutation was detected 20–30% of progeny ascospores were adn^-, indicating that the suppressor was unlinked or only very loosely linked to the adenine locus. As an example, Table 4 presents detailed results of a genetical analysis of fifteen U.V.-induced revertants of allele 199; in every case, the suppressor appears to be unlinked to the *adenine-1* locus. While the revertants of 199 that are not due to detectable suppressors grow like wild-type, many of the suppressor revertants form small colonies after seven days at 30° on MMA. In addition, there is one type of adenine suppressor which allows only extremely slow growth, resulting in minute colonies after two weeks' incubation at 30°. This minute suppressor type has not been included in the present data.

Table 3 shows that the proportion of suppressor revertants among all revertants depends both on the allele tested and on the mutagen used. Particular attention may be drawn to *adenine-1* mutants 25, 40, 199 and H774 and to *adenine*-7 mutant 84. About 80% of spontaneous revertants of mutant 25 are due to distant (unlinked or very loosely linked) suppressor mutations, whereas only one of nineteen revertants of mutant 25 isolated following HNO_2 contains a detectable suppressor. Similarly, about 80% of spontaneous revertants of mutant 40 are due to distant suppressors, but it appears that both nitrous acid and U.V. irradiation induce mainly or solely revertants containing no distant suppressors. Mutant 199 gives spontaneous and HNO_2-induced revertants of a type containing no detectable suppressor, whereas many U.V.-induced revertans of 199 are of a distant suppressor type.

Mutant H774, in the four cases tested, gave suppressor-type spontaneous revertants, whereas none of nine HNO_2-induced revertants contained a detectable

Table 3. *Tests for the presence of suppressor mutations in* adn$^+$ *revertants**

	Revertants					
	Spontaneous		Isolated following HNO_2		Isolated following UV	
Mutant	No. tested	Sup. type	No. tested	Sup. type	No. tested	Sup. type
Adenine-1, 3	27	25				
25	21	16	19	1		
40	54	41	29	0	10	1
51	9	1	15	0		
107	3	1	8	0		
169	30	5	9	0		
199	37	0	15	0	38	26
233	1	1	10	0		
249	2	0				
Adenine-1,H 12	1	0	1	0		
H264	2	0	7	0		
H268			3	0		
H293			4	2		
H330	2	0	8	0		
H538	3	1	10	3		
H563			5	0		
H652	1	0	9	0		
H774	4	4	9	0		
Adenine-2,SP5 (spontaneous)			14	0		
Adenine-6,D2	1	0	19	0		
Adenine-7,84	9	8	19	1		
407			25	0	14	0
486	4	2				
541	2	2				
Adenine-8,106	1	0	12	0		
177	2	0	28	0		
313	5	0	19	1		
321			15	0		
333	1	0	14	0		

Sup. type = suppressor mutation present. In all cases 20–30% *adn*$^-$ recombinants were found among the progeny spores upon back-crossing the revertant to wild-type. Results are based on an analysis of 200–500 progeny per cross.

H = isolated by Dr H. Heslot.

All mutants are UV-induced except *adn*-2, SP5 (spontaneous) and *adenine*-1 mutants H330, H538, H563, H562 and H774 (italicized in the table) which are of di-ethyl sulphate origin.

* Of the ten UV-induced *adenine*-1 mutants, 153 gave no spontaneous, HNO_2-, or UV-induced revertants.

suppressor mutation. Finally, *adenine-7* mutant 84 gave mainly (8 cases out of 9) suppressor-type spontaneous revertants, but with nitrous acid the revertants were (18 cases out of 19) of a non-suppressor type.

These examples represent obvious and drastic cases of mutagen specificity for different types of reversion from adenine-requirement to adenine-independence

Table 4. *Testing of* 15 *U V-induced revertants of mutant* adenine-1,199 *for the presence of distant suppressor mutations*

Revertant no.	Total progeny tested	No. of *adn*⁻ recombinants	% *adn*⁻
1	187	0	0
2	191	0	0
3	250	0	0
4	371	132	36
5	150	51	33
6	214	66	31
7	371	91	25
8	408	115	28
9	374	81	21
10	270	90	33
11	288	85	30
12	242	58	24
13	363	126	35
14	257	68	26
15	378	96	25

(*adn*⁻→*adn*⁺). Some mutational processes favour distant suppressor-type revertants, others favour the production of revertants due to events at or very close to the original site of mutation. This specificity depends not only on the mutagen but also upon the particular mutant involved.

The results given in Table 3 indicate also that HNO_2-induced revertants of U.V.-induced adenine mutants are predominantly not of a distant-suppressor type, whereas spontaneous revertants of such mutants are fairly often due to such suppressor mutations.

Only one distant suppressor revertant was found among the ninety-eight spontaneous and HNO_2-induced revertants from five different U.V.-induced *adenine-8* mutants (Table 3). This is in contrast to the results with revertants of *adenine-1* and *adenine-7* mutants.

In only three cases (mutants *adenine-1,* 40, *adenine-1,* 199 and *adenine-7,* 407) has a comparison been made between HNO_2- and U.V.-induced revertants. In two cases, 40 and 407, both HNO_2- and U.V.-induced revertants were predominantly of a non-suppressor type. In the other case, that of the temperature-sensitive mutant 199, nitrous acid-induced revertants were of a non-suppressor type whereas most of the U.V.-induced revertants were due to distant suppressors.

4. DISCUSSION

The results obtained in this study indicate that the ten U.V.-induced mutants at the *adenine-1* locus of *S. pombe* differ not only in their sites of mutation but also in their spontaneous, nitrous acid-, and U.V.-induced frequencies of reversion to adenine-independence.

Seven (numbers 25, 40, 51, 107, 169, 199 and 233) of the ten mutants responded to HNO_2 mutagenesis, but only two of the ten U.V.-induced mutants reverted with U.V. Both of the mutants (40 and 199) which revert with U.V. also do so with HNO_2. Indeed, only mutant 40 gives reversions with U.V. which do not contain

detectable suppressor mutations. The other mutant to respond to U.V. is the temperature-sensitive mutant 199, and this reverts with U.V. mainly via suppressor mutations at a locus or loci distant from *adenine-1* (Table 4).

These results suggested that most mutants induced by U.V. irradiation tend to be revertible by nitrous acid treatment. Tests of twenty-three additional U.V.-induced mutants located at adenine loci other than *adenine-1* and at loci unrelated to purine biosynthesis confirm this observation. Fourteen gave a definite reversion response, four a weak response, and five no detectable response to HNO_2 treatment. These results are in quite good agreement with those of Drake (1963, 1964), using a different system (rII mutants of bacteriophage T4), who found that about half the U.V.-induced mutants could be induced to revert with base analogues, while of the rest many were proflavin-revertible. This would suggest that U.V. can induce not only transition type mutations (Freese, 1959) but also non-transition mutations (Drake, 1964).

Genetic analysis of adenine-independent revertants revealed a second type of mutagen specificity distinct from that of a particular mutagen for different mutant sites. This second type of mutagen specificity concerned different classes of reversions given by one and the same mutant allele with different mutagens. Schwartz (1963) had already shown that there were two types of revertants which he called 'feeders' and 'non-feeders', in a $lac^- \rightarrow lac^+$ system in *Escherichia coli*. The former approximated to reversions back to wild-type while the latter were due to suppressor mutations. The ratio of these two types of lac^+ revertants depended on how the original mutant had been induced (ethylmethane sulphonate or U.V. light) and also upon the mutagen causing reversion (ethylmethane sulphonate, U.V. light, or 2-aminopurine).

Loprieno and Clarke, 1965, studying reversions of the methionine auxotroph *met-4, D19* in *S. pombe* showed that different mutagens induced reversions due to linked suppressors, unlinked suppressors, and only rarely to events in the vicinity of the original mutation. The ratios of these three types of revertants differed between mutagens.

In the present work, individual mutants differed in the proportion of their spontaneous revertants due to distant suppressors (Table 3). Some, like mutants 3, 25, and 40, frequently gave suppressor-type spontaneous revertants. Others, like mutants 169 and 199, only rarely did so. Spontaneous revertants due to suppressors were common among *adenine-1* and *adenine-7* mutants but not among the *adenine-8* mutants tested. More extensive work will have to be done on *adenine-8* revertants before any significance can be attached to this observation. Nitrous acid was shown, in a whole series of adenine auxotrophs, to induce mainly or perhaps solely reversions containing no detectable suppressors (Table 3). The few revertants isolated after HNO_2 treatment which did contain suppressors could probably all have been spontaneous since survival levels above 10% were used.

Particularly striking were cases in which one and the same mutant reverted mainly via distant suppressor mutations under one set of conditions, e.g. spontaneously, but mainly by events probably within the *adenine-1* locus under another

set of conditions, e.g. after treatment with nitrous acid. Mutants 25, 40, 199, H774 and 84 in Table 3 represent examples of this phenomenon.

Why distant suppressors rarely occur among HNO_2- induced adenine reversions is unknown. Possibly only those events involving, for example, base pair deletions or additions, or replacement of thymine by another base at the suppressor locus can lead to a phenotypic suppression of adenine-requirement. Nitrous acid, being unable to bring about these types of mutational change would thus not be expected to induce suppressor revertants. Conversely only HNO_2, and not the spontaneous mechanism of mutation, might be able to bring about those types of base change at the *adenine* locus able to restore near-wild type-function.

Alternatively HNO_2 treatment may actually induce both distant supperssor and what may be tentatively called non-suppressor revertants, but in a cell which has been treated with HNO_2 the metabolic conditions may be such that only the non-suppressor type of revertant can be phenotypically expressed. The example found by Witkin and Theil (1960) in which one type of U.V.-induced mutation in *Escherichia coli* required post-irradiation protein synthesis for its expression ($lac^- \rightarrow lac^+$) whereas a second type of mutation ($str^s \rightarrow str^r$ or $str^d \rightarrow str^i$) did not do so, may be pertinent in this context. Experiments are planned in which a U.V. revertible mutant will first be treated with U.V. and then with HNO_2 to test this possibility.

Until more is known of the number, mode of action, and locus- or site-specificity of the adenine suppressors it is probably foolhardy to speculate further at what level of the mutation process the mutagen specificities found in the present work actually reside. Attempts to determine the number of suppressor loci involved in *adenine-1* reversions have met so far with difficulties due to recombinant classes able to grow slowly on minimal medium.

SUMMARY

Ten U.V.-induced mutants at the *adenine*-1 locus of *Schizosaccharomyces pombe* differ not only in their intragenic location but also in their spontaneous, HNO_2- and U.V.-induced frequencies of reversion to adenine-independence. Seven mutants reverted with HNO_2 and of these only two reverted also with U.V.; the other three did not revert with either HNO_2 or U.V.

Genetic analysis of revertants showed that they could be due to suppressor mutations at a locus or loci far from the *adenine-1* locus, or to events extremely close to, if not identical with, the original site of mutation. Many distant suppressors were found among spontaneous revertants of some *adenine-1* and *adenine-7* allles, but such suppressors appear to be less frequent among the five *adenine-8* mutants tested. There are large differences between different sites at the same locus in the proportion of their spontaneous revertants due to distant suppressor mutations. Nitrous acid-induced revertants of *adenine1-*, *adenine-7*, and *adenine-8* mutants were almost all of a type containing no detectable suppressor mutation. In the three cases studied, U.V.-induced revertants could be either predominantly of a suppressor or non-suppressor type, depending on the particular mutant examined.

Striking examples of mutagen specificity were found in which a particular mutant gave mostly suppressor-type revertants with one mutagen, while with another mutagen revertants were mainly of a type containing no detectable suppressor mutation.

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