Allele Specific and Locus Non-specific Suppressors in *Aspergillus nidulans*

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Using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, ultraviolet irradiation, ethyl methanesulphonate or 4-nitroquinoline-1-oxide mutagenesis and an enrichment method for the isolation of auxotrophs, 25 mutants with defects in the *ada* locus were obtained after screening 41 376 colonies. One of these, *adaA24*, did not complement with any of the other *ada* mutants, had a very high reversion rate and had some other properties which usually characterize strains carrying nonsense mutations. All revertants of *adaA24* carried dominant suppressor mutations. A group of *adaA24* suppressors was tested for allele and locus specificity. They were found to suppress only some *ada* alleles, and at the same time, some mutations in the *methG*, *methH*, *argB* and *proA* loci. It is proposed that the allele specific and locus non-specific adenine suppressors are suppressors of nonsense mutations.

INTRODUCTION

Among Eukaryota, suppressors of nonsense mutations have been identified in yeast (Hawthorne & Mortimer, 1963; Barben, 1966), *Neurospora crassa* (Seale, 1968) and probably in *Podospora anserina* (Picard, 1973) and *Drosophila melanogaster* (Lindsley & Grell, 1968). In the case of yeast (Sherman *et al.*, 1970; Stewart & Sherman, 1972; Stewart *et al.*, 1972; Hawthorne & Leupold, 1974; Randerath *et al.*, 1975; Piper *et al.*, 1976), and to a lesser extent *N. crassa* (Seale *et al.*, 1977), genetical studies on these suppressors were followed by biochemical analysis involving studies on tRNA structure, which made it possible to distinguish between amber, ochre and opal suppressors. This paper describes the selection and genetical analysis of nonsense suppressors in another eukaryotic organism, *Aspergillus nidulans*. Preliminary data on these suppressors have been presented in a short communication (Bal *et al.*, 1978).

In bacteria and viruses, several different approaches for the direct identification of nonsense suppressors are available (Benzer & Champe, 1962; Kennedy & Crowlesmith, 1975; Mindich *et al.*, 1976). In Eukaryota, no such approach exists. The first nonsense suppressors were found in yeast by Hawthorne & Mortimer (1963) who studied the frequency of simultaneous reversion of several mutations. They demonstrated co-suppression of mutations in different genes by a single suppressor gene.

To increase the chances of isolating nonsense suppressors a method similar to that used by Seale (1968) in *N. crassa* was applied. This method involved two steps. Firstly, a number of mutants defective in a particular gene were isolated and from them those showing properties expected for nonsense mutations were chosen. Secondly, suppressors obtained for presumed nonsense mutations were characterized.

We decided to look for nonsense mutations in mutants defective at the *ada* locus. The enzyme specified by the *ada* gene is well characterized and strains carrying *ada* mutations are unable to convert adenylosuccinate (AMPS) to adenosine 5'-monophosphate (AMP)

(Foley *et al.*, 1965). They are thus blocked in the last step of AMP biosynthesis (Fig. 1) and metabolic suppressors of *adA* mutants are unlikely to be obtained. Furthermore, it is known that certain *adA* mutations show intragenic complementation (Foley *et al.*, 1965). This helps to select potential nonsense mutations which are unlikely to complement other mutations within the same locus. There are good methods for the selection of mutants blocked in the last stages of adenine synthesis and for distinguishing *adA* mutations from mutations in other adenine loci (Foley *et al.*, 1965; Bal & Pieniżek, 1979).

METHODS

Strains. The following strains were obtained from the Fungal Genetics Stock Center: *sulA1*, *galA1*, *pyroA4*, *facA303*, *lacA1*, *choA1*, *chaA1* (FGSC 465); *biA1*, *adH23*, *acrA1*, *wA3*, *nicB8* (FGSC 239); *adG14*, *proA1*, *pabaA1*, *yA2*, *wA3* (FGSC 72); *adF17*, *yA2*, *pabaA1*, *fpaD11* (FGSC 273); *yA2*, *wA2*, *thiA4*, *cnxE16*, *add3*, *biA1* (FGSC 268); *adB1*, *veA1* (FGSC 217); *riboA1*, *yA2*, *adE20*, *methG1*, *suCadE20*, *palC4*, *pabaB22*, *pyroA4* (FGSC 517); *sulA1adE20*, *pyroA4*, *adE20* (FGSC 47). Strains *lysB5*, *nicB8*, *veA1*, *wA3*, *acrA1*, *adA3*; *lysB5*, *nicB8*, *veA1*, *wA3*, *acrA1*, *adA55*; *biA1*, *yA2*, *acrA1*, *riboB2*, *veA1*, *adA3* and *biA1*, *yA2*, *acrA1*, *riboB2*, *veA1*, *adA55* were obtained from Dr C. F. Roberts via Dr H. N. Arst. The *adA3* and *adA55* mutations were described previously as AM3 and AM55 (Foley *et al.*, 1965). Strains *methA17*, *yA2*, *proA2* and *methD10*, *yA2*, *pyroA4* were obtained from the Department of Genetics, Polish Academy of Sciences, Warsaw, Poland. Other strains used in this work originated from the collection of the Department of Genetics, Warsaw University. Mutation terminology was as in Clutterbuck (1973).

Chemicals. 4-Nitroquinoline-1-oxide (NQO) was purchased from ICN (Cleveland, Ohio 44128, U.S.A.), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and ethyl methanesulphonate (EMS) from Koch-Light and adenylosuccinate (AMPS) from Sigma. *N*-Glycosyl-polifungin was obtained from the Department of Drug Technology and Biochemistry of the Gdańsk Institute of Technology, Poland.

Genetic analysis. The minimal medium (MM) and methods of genetic analysis were standard (Pontecorvo *et al.*, 1953). The frequency of reversion was examined as described by Bal *et al.* (1977). Haploidization was performed as described earlier (Bal *et al.*, 1975), with *pabaA1*, *acrA1*, *phenA2*, *lysB5*, *nicB8*, *pyroA4*, *sB3*, *riboB2*, *chaA1* (FGSC 146) being used as a 'master strain'.

Mutagenesis. The mutations were induced by ultraviolet (u.v.) irradiation (Pontecorvo *et al.*, 1953), or by treatment with EMS (Fink, 1970), NQO (Bal *et al.*, 1977) or MNNG (Chattoo & Sinha, 1974; Mallig & De Serres, 1970). The polifungin method (Bal *et al.*, 1974) was used to enrich for adenine auxotrophs.

Biochemical analysis. Adenylosuccinate lyase (EC 4.3.2.2; adenylosuccinate AMP-lyase) activity was assayed by a modification of the method described by Foley *et al.* (1965). The assay mixture contained, in a final volume of 1 ml: 0.05 M-Tris/HCl buffer pH 8.5, 0.08 M-AMPS, 30 to 250 µg protein and 15% (v/v) glycerol. In the control, AMPS was omitted. Assays were done at 37 °C with a Beckman model 25 spectrophotometer. One unit of activity of the enzyme was defined as $10^4 \times \Delta A_{280} \text{ min}^{-1} (\text{mg protein})^{-1}$. Protein was estimated by the method of Lowry. The accumulation of adenine biosynthetic pathway metabolites was determined as described by Bal & Pieniżek (1979).

RESULTS

Selection of adA mutations

Mutations in the *adA* gene were obtained after EMS, MNNG or NQO treatment. They were induced in strains with different genetic backgrounds in order to facilitate further genetic analysis. The polifungin method (Bal *et al.*, 1974) for the enrichment of auxotrophs after mutagenesis was applied on hypoxanthine-supplemented medium. Only *adA* and *adB* mutants cannot grow on this medium (see Fig. 1) and hence should be selectively isolated. A total of 559 such mutants were isolated after screening of 41 376 colonies (Table 1) and were examined for adenylosuccinate lyase activity and 5-amino-4-imidazole(*N*-succinyl-carboximide)ribotide (SAICAR) accumulation. It was previously observed (Giles *et al.*, 1957; Foley *et al.*, 1965) that only *adA* mutants accumulate this metabolite, while *adB* mutants accumulate hypoxanthine. On the basis of the above tests 25 mutants were assigned as *adA*. Of these some were obtained from the same polifungin selection and therefore could represent clones of a single original mutant (Bal *et al.*, 1974). Only one mutant from each polifungin selection was used in further tests.

Fig. 1. Pathway of adenine biosynthesis in *Aspergillus nidulans* (Foley *et al.*, 1965). Positions of metabolic blocks in *adA* and *adB* mutants are shown. PRPP, 5-Phosphoribosyl-1-pyrophosphate; PRA, phosphoribosylamine; GAR, phosphoribosyl-glycinamide; FGAR, phosphoribosyl-formylglycinamide; FGAM, phosphoribosyl-formylglycinamidine; AIR, phosphoribosyl-aminoimidazole; CAIR, phosphoribosyl-aminoimidazole carboxylate; SAICAR, 5-amino-4-imidazole-(*N*-succinyl-carboximide)ribotide; AICAR, phosphoribosyl-aminoimidazole carboxamide; FAICAR, phosphoribosyl-formamido-imidazole carboxamide; IMP, inosine 5'-monophosphate; AMPS, adenylosuccinate; AMP, adenosine 5'-monophosphate.

Table 1. Isolation of mutations in the *adA* gene following treatment with four mutagens

In all cases the polyifungin method for enrichment of auxotrophs was used.

Mutagen*	No. of colonies tested	No. of experiments	Hypoxanthine non-utilizing mutants		<i>adA</i> mutants		Symbols of <i>adA</i> mutants
			No.	%	No.	%	
U.v.	17397	19	117	0.67	4	0.023	24, 25, 101, 116
NQO	9113	14	378	4.1	12	0.13	1, 11, 12, 15, 36, 45, 48, 51, 54, 115, 117, 120
MNNG	9946	11	56	0.56	9	0.090	202, 204, 210, 213, 224, 226, 264, 280, 289
EMS	4920	9	8	0.16	—	—	—
Total	41376	53	559	1.35	25	0.06	

* U.v., Ultraviolet irradiation; NQO, 4-nitroquinoline-1-oxide; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; EMS, ethyl methanesulphonate.

Analysis of *adA* mutations

'Classical' nonsense mutations usually have several properties. They are non-leaky, do not complement with other mutations within the same locus, have a high rate of reversion and their phenotype is not reversed under different growth conditions. The results of tests performed in order to select the *adA* mutants which best fulfilled the criteria of strains carrying nonsense mutations are given in Tables 2 and 3 and in Fig. 2. The *adA24* mutant met all the requirements: it did not complement with any other *adA* mutant tested (Fig. 2), it reverted with a frequency higher than that of the other *adA* mutants (Table 3), and, in all analysed cases, reversion was due to a suppressor mutation (Table 4). The properties of some other *adA* mutants show that they could carry nonsense mutations too. However, most of the subsequent experiments were performed with the *adA24* mutant and its suppressors.

Table 2. Growth of *adA* mutants on minimal medium with or without supplements* at two different temperatures

Mutant symbol	25 °C					37 °C				
	MM	MM + ad	MM + hypo	MM + KCl	MM + KCl + ad	MM	MM + ad	MM + hypo	MM + KCl	MM + KCl + ad
Wild-type	+	+	+	+	+	+	+	+	+	+
<i>adA1, 11, 12, 15, 24</i> <i>36, 45, 48, 51, 54,</i> <i>101, 120, 202, 213</i>	-	+	-	-	+	-	+	-	-	+
<i>adA25</i>	+	+	+	+	+	-	+	-	-	+

* MM, minimal medium; ad, adenine (100 $\mu\text{g ml}^{-1}$); hypo, hypoxanthine (75 $\mu\text{g ml}^{-1}$); KCl (150 mg ml^{-1}).

Fig. 2. Results of complementation tests between mutations in the *adA* locus: ■, complementation; □, lack of complementation.

Properties and genetic analysis of suppressor mutations

As mentioned above, reversion of the *adA24* mutant was due to suppressor mutations. These suppressors were tested for dominance, and the suppressed strains were tested for their growth properties and adenylosuccinate lyase activity (Table 5). Growth tests with heterokaryons produced between particular suppressed strains and an *adA24 su*⁺ strain showed that all suppressors are dominant. Some of the suppressors were also tested for dominance in diploids and the results of these tests were in full agreement with those obtained in heterokaryons. All suppressed strains grew more slowly than the wild-type on the minimal medium while on adenine-supplemented medium these differences were not observed. This suggests that the slow growth of suppressed strains on minimal medium is due to low efficiency of suppression and is not a property of the suppressor mutation itself. The same conclusion can be drawn from the fact that a strain carrying the *su-23* mutation alone (i.e. *adA*⁺*su-23*) grew at the same rate as the wild-type on minimal medium. On the other hand, there was no correlation between the rate of growth on adenine-less medium and the activity of adenylosuccinate lyase of suppressed strains (Table 5). Generally, in suppressed strains the level of adenylosuccinate lyase did not exceed 30% of wild-type activity. In 14 suppressed strains no activity was detected, probably due to low sensitivity of the assay.

Table 3. Frequency of reversion of *adA* mutations

Mutant symbol	Frequency of reversion*		
	Spontaneous	NQO-induced	MNNG-induced
<i>adA1</i>	$< 10^{-12}$	$< 10^{-9}$	$< 10^{-8}$
<i>adA11</i>	$< 10^{-12}$	4.0×10^{-9}	4.5×10^{-9}
<i>adA12</i>	1.0×10^{-9}		
<i>adA15</i>	2.6×10^{-9}		
<i>adA24</i>	2.8×10^{-6}	6.4×10^{-5}	9.0×10^{-6}
<i>adA25</i>	3.6×10^{-9}		
<i>adA36</i>	$< 10^{-12}$		
<i>adA45</i>	2.4×10^{-9}		
<i>adA48</i>	4.9×10^{-11}	9.0×10^{-7}	5.3×10^{-7}
<i>adA51</i>	3.7×10^{-9}		
<i>adA54</i>	8.8×10^{-11}	2.5×10^{-6}	3.5×10^{-7}
<i>adA101</i>	$< 10^{-12}$	$< 10^{-9}$	$< 10^{-8}$
<i>adA120</i>	1.6×10^{-12}	3.2×10^{-7}	2.9×10^{-8}
<i>adA202</i>	4.0×10^{-7}		
<i>adA213</i>	$< 10^{-13}$		

* About 10^{10} to 10^{12} viable conidia were tested in each experiment.

Table 4. Frequency of adenine-requiring colonies in crosses between revertants of the *adA24* mutant and the wild-type

Suppressor symbol*	Origin of revertant	No. of colonies tested	Adenine-requiring colonies	
			No.	%
<i>su-22</i>	Spontaneous	126	29	23.0
<i>su-23</i>	Spontaneous	259	66	25.4
<i>su-24</i>	Spontaneous	130	9	6.9†
<i>su-26</i>	Spontaneous	70	14	20.0
<i>su-27</i>	Spontaneous	148	46	31.1
<i>su-28</i>	Spontaneous	129	31	24.0
<i>su-29</i>	Spontaneous	78	23	29.5
<i>su-34</i>	Spontaneous	428	65	15.2†
<i>su-36</i>	Spontaneous	143	34	23.7
<i>su-39</i>	Spontaneous	127	42	33.1
<i>su-44</i>	Spontaneous	693	100	14.4†
<i>su-51</i>	MNNG-induced	90	26	28.8
<i>su-52</i>	MNNG-induced	104	17	16.4†
<i>su-53</i>	MNNG-induced	127	23	18.1
<i>su-54</i>	Spontaneous	130	34	26.1
<i>su-55</i>	Spontaneous	52	3	5.8†
<i>su-56</i>	Spontaneous	128	20	15.6†
<i>su-57</i>	NQO-induced	174	55	31.6
<i>su-58</i>	NQO-induced	130	38	29.2
<i>su-59</i>	MNNG-induced	128	22	17.2†
<i>su-60</i>	NQO-induced	104	35	33.6
<i>su-61</i>	NQO-induced	103	17	16.5†
<i>su-62</i>	MNNG-induced	129	21	16.3†
<i>su-63</i>	NQO-induced	233	55	23.6
<i>su-64</i>	Spontaneous	129	38	29.4

* In all cases reversion was due to a suppressor mutation.

† Suppressor mutation locus is linked to the *adA* locus.

Table 5. *Properties of adA24 su strains*

Strain	Rate of growth on MM at 37 °C*	Growth on MM with or without supplements					Relative adenylo-succinate lyase activity†
		37 °C					
		MM + ad	MM + KCl (2 M)	MM + ad + KCl (2 M)	42 °C		
					MM	MM + ad	
Wild-type	++++	+	+	+	+	+	100
<i>adA24</i>	—	+	—	+	—	+	< 3·0‡
<i>adA24 su-22</i>	++	+	—	+	+	+	5·0
<i>adA24 su-23</i>	++	+	—	+	+	+	< 3·0‡
<i>adA24 su-24</i>	+++	+	+	+	+	+	12·0
<i>adA24 su-26</i>	+++	+	—	+	+	+	26·0
<i>adA24 su-27</i>	+	+	+	+	+	+	5·0
<i>adA24 su-28</i>	+++	+	+	+	+	+	< 3·0‡
<i>adA24 su-29</i>	+	+	—	+	—	+	< 3·0‡
<i>adA24 su-34</i>	++	+	—	+	+	+	< 3·0‡
<i>adA24 su-36</i>	+++	+	—	+	+	+	< 3·0‡
<i>adA24 su-39</i>	+	+	—	+	+	+	25·0
<i>adA24 su-44</i>	++	+	+	+	+	+	< 3·0‡
<i>adA24 su-51</i>	++	+	—	+	+	+	28·0
<i>adA24 su-52</i>	++	+	+	+	+	+	< 3·0‡
<i>adA24 su-53</i>	++	+	+	+	+	+	< 3·0‡
<i>adA24 su-54</i>	++	+	—	+	+	+	< 3·0‡
<i>adA24 su-55</i>	++	+	—	+	+	+	10·0
<i>adA24 su-56</i>	+++	+	+	+	+	+	5·0
<i>adA24 su-57</i>	+++	+	—	+	+	+	< 3·0‡
<i>adA24 su-58</i>	++	+	—	+	+	+	< 3·0‡
<i>adA24 su-59</i>	+++	+	+	+	+	+	< 3·0‡
<i>adA24 su-60</i>	+	+	+	+	+	+	24·0
<i>adA24 su-61</i>	++	+	—	+	+	+	12·0
<i>adA24 su-62</i>	+++	+	+	+	+	+	26·0
<i>adA24 su-63</i>	++	+	+	+	+	+	< 3·0‡
<i>adA24 su-64</i>	+++	+	+	+	+	+	12·0

* + + + +, Distinct growth after 24 h; + + +, growth after 24 h but retarded relative to wild-type; + +, distinct growth after 48 h; +, distinct growth after 72 h.

† The wild-type strain specific activity ($8\cdot1 \pm 1\cdot8$ units) was taken as 100%.

‡ Below sensitivity of assay.

Table 6. *Linkage of su-51 to phenA in the cross adA24 su-51 × phenA2*

Phenotypes of progeny	Distribution of phenotypes		
	Expected*	Observed	
		No.	%
Ad Phen ⁺	12·5	8	8·0
Ad ⁺ Phen	37·5	34	34·0
Ad ⁺ Phen ⁺	37·5	42	42·0
Ad Phen	12·5	16	16·0

* Assuming *su-51* and *phenA* are unlinked: linkage would produce a deficiency of Ad Phen⁺ and Ad⁺Phen phenotypes and an excess of Ad⁺Phen⁺ and Ad Phen phenotypes.

Table 7. Linkage of *su-63* to *lysB* in the cross *adA24 su-63* × *lysB5*

Phenotypes of progeny	Distribution of phenotypes		
	Expected*	Observed	
		No.	%
Ad Lys ⁺	12.5	9	6.7
Ad ⁺ Lys	37.5	42	32.3
Ad ⁺ Lys ⁺	37.5	57	43.8
Ad Lys	12.5	22	17.5

* Assuming *su-63* and *lysB* are unlinked: linkage would produce a deficiency of Ad Lys⁺ and Ad⁺Lys phenotypes and an excess of Ad⁺Lys⁺ and Ad Lys phenotypes.

Table 8. Specificity of the *su-23* mutation for different *adA* alleles

Strains carrying different *adA* alleles were crossed to a strain of genotype *adA⁺su-23*.

<i>adA</i> allele	No. of colonies tested	Adenine-requiring colonies	
		No.	%
<i>adA11</i>	468	234	50.0
<i>adA12</i>	468	134	28.6*
<i>adA15</i>	282	81	28.7*
<i>adA25</i>	156	35	23.2*
<i>adA36</i>	144	66	44.3
<i>adA48</i>	312	133	42.6
<i>adA51</i>	234	73	31.2*
<i>adA101</i>	130	26	20.0*
<i>adA116</i>	154	33	21.4*
<i>adA120</i>	234	50	21.4*
<i>adA202</i>	720	351	48.7
<i>adA213</i>	175	86	49.0
<i>adA3</i>	289	76	26.4*
<i>adA55</i>	468	230	49.1

* Mutations whose phenotype is suppressed. The χ^2 test indicates that the observed results are in agreement with a 3 Ad⁺:1 Ad phenotypic distribution; 20 to 30% of the adenine prototrophs in the progeny of these crosses showed weak growth and presumably represented the *adA su* class.

Only one suppressed strain, *adA24 su-29*, was temperature-sensitive. The temperature sensitivity is not the property of the *su-29* mutation alone, as the *adA24 su-29* strain grew normally at 42 °C on adenine-supplemented medium (Table 5). The growth of 13 suppressed strains was inhibited by 2 M-KCl. Again, this inhibition was only observed on adenine-less medium and not on adenine-supplemented medium (Table 5).

As all suppressor mutations obtained were dominant, it was not possible to establish the number of suppressor loci by complementation tests. Direct recombination analysis was impossible as in no case did we succeed in crossing two suppressor-carrying strains. In this situation the number of suppressor loci was tentatively established by analysis of the linkage of particular suppressor mutations with other loci. Crosses of the type *adA su* × *adA⁺su⁺* had shown that in several cases the number of adenine-requiring progeny was less than 25% (Table 4), indicating linkage between *adA* and *su* loci. Suppressors linked to the *adA* locus were tentatively assigned to two loci, one 34 map units (*su-34*, 44, 52, 56, 59, 61, 62) and the other 12 map units (*su-24*, 55) from the *adA* locus. Other crosses showed that *su-51* is linked to the *phenA* locus (chromosome III) by 32 map units (Table 6) and that *su-63* is linked to the *lysB* locus (chromosome V) by 27 map units (Table 7). By haploidization (about 100 haploids were tested in each experiment) three suppressor mutations (*su-36*, 37 and 64)

Table 9. *Specificity of adA24 suppressors for mutations in other genes*

Strains carrying different *adA24* suppressors were crossed to strains with tester mutations in unrelated loci and suppression of the tester mutations was examined.

Suppressor strain	Mutation examined	No. of colonies tested	Colonies unable to grow on the test medium	
			No.	%
<i>adA24⁺su-23</i>	<i>lu-2*</i>	105	41	39.0
	<i>lu-3*</i>	128	53	41.4
	<i>lu-4*</i>	129	55	42.6
	<i>lu-6*</i>	129	70	53.8
	<i>lu-7*</i>	104	41	39.4
	<i>lu-8*</i>	130	58	44.6
	<i>pabaA1</i>	249	121	48.6
	<i>pabaA2</i>	103	45	43.7
	<i>pabaA18</i>	146	76	52.0
	<i>pabaA9</i>	311	160	51.4
	<i>pabaA23</i>	128	69	53.9
	<i>pabaB22</i>	283	135	47.4
	<i>paba-101*</i>	128	55	43.0
	<i>paba-104*</i>	130	72	55.4
	<i>paba-105*</i>	129	51	51.4
	<i>paba-106*</i>	127	54	42.5
	<i>argB2</i>	169	31	18.3†
	<i>proA1</i>	144	68	47.2
	<i>proA6</i>	362	165	45.6
	<i>proA7</i>	122	51	41.8
	<i>lysB5</i>	257	113	44.0
	<i>lysF51</i>	104	45	39.4
	<i>nicA2</i>	526	244	46.1
	<i>nicB8</i>	171	85	49.7
	<i>pyroA4</i>	849	374	44.0
	<i>pyro-2*</i>	128	57	44.5
	<i>methE6</i>	182	80	43.9
	<i>methG1</i>	690	300	43.5
	<i>methG2*</i>	155	78	50.3
	<i>methG4*</i>	71	35	49.3
	<i>methG55</i>	303	86	28.1†
	<i>methA11*</i>	182	84	46.1
	<i>methA17</i>	194	91	46.9
	<i>methD10</i>	155	65	41.9
	<i>methH2</i>	169	71	42.0
	<i>methH3*</i>	147	32	21.7†
	<i>methB41</i>	130	55	42.3
	<i>methB49</i>	129	57	44.1
	<i>biA1</i>	517	249	48.2
	<i>ino-1*</i>	180	84	46.6
	<i>panto-1*</i>	181	103	56.9
	<i>panto-2*</i>	182	93	51.1
	<i>acrA1</i>	1562	742	47.5
	<i>fpaD11</i>	249	103	41.4
	<i>phenA2</i>	499	256	51.3
	<i>riboD5</i>	867	439	50.6
	<i>puA2</i>	131	62	47.3
	<i>facA303</i>	104	45	43.3
	<i>galA1</i>	102	52	51.0
	<i>lacA1</i>	102	42	41.2
	<i>adB142*</i>	125	55	44.0
	<i>adB1</i>	128	57	44.5
	<i>adD3</i>	130	62	47.7
	<i>adE20</i>	119	54	45.4
	<i>adF9</i>	122	53	43.4
	<i>adF17</i>	150	80	53.3

Table 9 (cont.)

Suppressor strain	Mutation examined	No. of colonies tested	Colonies unable to grow on the test medium	
			No.	%
	<i>adG14</i>	156	63	40.4
	<i>adI50</i>	130	57	43.8
	<i>ad-101*</i>	147	64	43.5
	<i>ad-102*</i>	154	81	52.6
<i>adA24 su-23</i>	<i>adH23</i>	171	116	67.8‡
<i>adA24 su-1 </i>	<i>proA10*</i>	389	93	23.9†
	<i>lysB5</i>	200	103	51.5
<i>adA24 su-22</i>	<i>phenA2</i>	126	57	45.2
	<i>lysB5</i>	126	67	53.2
	<i>riboD5</i>	126	60	47.6
<i>adA24 su-27</i>	<i>adF9</i>	148	102	68.9‡
<i>adA24 su-29</i>	<i>methG4*</i>	177	80	54.2
<i>adA24 su-34</i>	<i>riboD5</i>	141	58	41.4
	<i>lysB5</i>	405	165	40.7
	<i>nicA2</i>	139	59	42.4
<i>adA24 su-36</i>	<i>lu-3*</i>	143	63	44.0
	<i>lysB5</i>	143	55	38.4
	<i>phenA2</i>	143	58	40.5
	<i>riboD5</i>	143	58	40.5
<i>adA24 su-39</i>	<i>methG4*</i>	148	44	29.7†
	<i>acrA1</i>	148	65	43.9
	<i>phenA2</i>	127	67	52.7
	<i>lysB5</i>	127	49	38.6
<i>adA24 su-44</i>	<i>adG14</i>	104	53	50.9‡§
	<i>methG4*</i>	204	56	27.4†
	<i>methG2*</i>	129	72	55.8
	<i>phenA2</i>	228	112	49.1
	<i>lysB5</i>	278	131	47.1
	<i>paba-106*</i>	278	125	45.0
	<i>riboD5</i>	282	115	40.8
	<i>methD10</i>	128	64	50.0
<i>adA24 su-51</i>	<i>lysB5</i>	100	57	57.0
	<i>phenA2</i>	100	48	48.0
	<i>riboD5</i>	100	50	50.0
<i>adA24 su-52</i>	<i>lysB5</i>	102	64	62.7
	<i>phenA2</i>	102	62	60.8
	<i>riboD5</i>	102	42	41.2
<i>adA24 su-54</i>	<i>lysB5</i>	127	58	45.7
	<i>phenA2</i>	127	54	42.5
	<i>riboD5</i>	127	54	42.5
<i>adA24 su-56</i>	<i>lysB5</i>	128	57	44.5
	<i>phenA2</i>	128	55	43.0
	<i>riboD5</i>	128	50	39.0
<i>adA24 su-57</i>	<i>pabaA1</i>	122	68	55.8
	<i>riboD5</i>	122	60	49.2
	<i>trypB403</i>	148	82	55.4
<i>adA24 su-58</i>	<i>lysB5</i>	129	58	45.0
	<i>phenA2</i>	129	65	50.4
	<i>riboD5</i>	129	71	55.0
<i>adA24 su-59</i>	<i>lysB5</i>	128	71	55.5
	<i>phenA2</i>	128	70	54.7

Table 9 (*cont.*)

Suppressor strain	Mutation examined	No. of colonies tested	Colonies unable to grow on the test medium	
			No.	%
<i>adA24 su-60</i>	<i>lysB5</i>	103	57	55.3
	<i>phenA2</i>	103	48	46.6
	<i>riboD5</i>	103	50	48.5
<i>adA24 su-61</i>	<i>lysB5</i>	104	44	42.3
<i>adA24 su-62</i>	<i>lysB5</i>	129	79	61.2
	<i>phenA2</i>	129	62	48.1
	<i>riboD5</i>	129	56	43.4
<i>adA24 su-63</i>	<i>puA2</i>	103	49	47.6
	<i>lysB5</i>	258	130	50.4
	<i>lu-14*</i>	94	50	53.2
	<i>phenA2</i>	104	49	47.1
	<i>riboD5</i>	104	46	44.2
<i>adA24 su-64</i>	<i>lysB5</i>	129	67	52.0
	<i>phenA2</i>	129	57	44.2
	<i>riboD5</i>	129	54	42.0

* Isolated in the Department of Genetics, Warsaw University.

† Mutations whose phenotype is suppressed by the particular suppressor involved. The chi² test indicates that the observed results are in agreement with a 3 M⁺:1 M phenotypic distribution. Approximately 30% of the prototrophic progeny in these crosses showed weak growth, and presumably represented the *m su* class (where *m/M* denotes the mutation/mutant phenotype under test).

‡ Theoretically, 37.5% of adenine-requiring progeny would be expected if the suppressor was effective and 62.5% if it was not.

§ The *su-44* mutation is linked to the *adA* locus (see Table 4) so the number of adenine-requiring colonies is less than when the mutations tested are unlinked.

|| Further data on the *su-1* mutation are given by Bal *et al.* (1978).

were assigned to linkage group VI. The remaining suppressor mutations do not belong to any of the loci mentioned above. We can therefore assume that the minimum number of *adA24* suppressor loci is six.

Determination of suppressor specificity

The main property of suppressors of nonsense mutations is that they are allele specific and locus non-specific. The specificity of adenine suppressors was studied either by testing the suppressor effect on several different mutations or by selection of simultaneous revertants of two different mutations (one being invariably *adA24*). The results obtained from the second approach have been presented elsewhere (Bal *et al.*, 1977). Here we present the results obtained by the first approach for the specificity of several *adA24* suppressors. Most experiments were carried out with the *su-23* mutation.

Specificity of su-23 for adA mutations. The effects of *su-23* on 14 different *adA* mutations were examined. The tests were done in crosses of the type *adA*⁺*su-23* × *adA su*⁺. As *su-23* is unlinked to *adA*, 50% of adenine-requiring progeny is expected when *su-23* is not effective against a given mutation, while only 25% is expected when the suppressor is effective. On this basis *su-23* suppresses 8 out of 14 *adA* mutations tested (Table 8).

Specificity of adenine suppressors for mutations in loci other than adA. The specificity of the adenine suppressors for mutations in several different loci was examined. Again, the method used was to analyse progeny of crosses between *adA*⁺*su* strains and strains carrying the mutations under test. Assuming that the loci of the suppressor and of the mutation tested are not linked, 25% of progeny showing the mutant phenotype is expected when the suppressor is effective against the test mutation and 50% when the suppressor is not effective. On

this basis, 5 out of 62 mutations tested were suppressed by adenine suppressors (Table 9). The adenine suppressors *su-1*, 23, 39, 44 are clearly locus non-specific. Moreover, the results of some crosses strengthen the notion that the suppressors are allele specific. Thus *su-23* suppresses *methH3* and *methG55* but not *methG4*, *methG2*, *methG1* and *methH2*; similarly, *su-44* suppresses *methG4* but not *methG2*.

Specificity of *suAadE20* and *suCadE20* suppressors

Two adenine suppressor mutations (*suAadE20* and *suCadE20*) were described by Pritchard (1955). We tested the effect of these suppressors on the *adA24* mutation by examining the number of colonies which could not grow on hypoxanthine-supplemented medium among progeny of crosses between *suAadE20 adE⁺* or *suCadE20 adE20* strains and the *adA24* strain. Of 123 and 534 colonies examined, 44.9% and 55.3%, respectively, failed to grow on the test medium. This means, that the *adA24* mutation is not suppressed by the *suA* and *suC* mutations.

DISCUSSION

Aspergillus nidulans serves as a model organism for many kinds of genetical and biochemical studies. Nonsense mutations and their suppressors would be of great use in these studies but have not so far been identified in this organism, although some attempts have been made (H. N. Arst, personal communication). In this paper we describe mutants and suppressors which by genetical criteria can be considered as nonsense mutations and nonsense suppressors. This conclusion is based mainly on the analysis of suppressors of the *adA24* mutation. Strains carrying this mutation have all the properties which usually characterize nonsense mutations: non-leakiness, lack of phenotypic reversion under different growth conditions and a high rate of reversion. All revertants of *adA24* strains appeared to carry dominant suppressor mutations, which is expected if we assume, in accordance with the classical scheme of nonsense suppression, that suppressor mutations result in the appearance of a new class of tRNA molecules. That the adenine suppressors are mutations in tRNA genes is further indicated by the mapping of the *adA24* suppressors to at least six loci. It is known that tRNA genes in Eukaryota exist in many copies (Schweizer *et al.*, 1969; Dutta & Ray, 1973; Maleszka, personal communication) and therefore a large number of nonsense suppressor loci is to be expected. Nineteen such loci have been found in *Saccharomyces cerevisiae* (Hawthorne & Leupold, 1974) and seven are known both in *Schizosaccharomyces pombe* (Barben, 1966) and *N. crassa* (Seale, 1972). Thirteen of 25 adenine suppressors examined were sensitive to KCl. This sensitivity may arise from an effect of high osmotic strength (the same results were obtained when KCl was replaced by glycerol) on the tertiary structure of the mutant tRNA molecule, leading to a reduced efficiency of suppression. Many nonsense suppressors in *S. cerevisiae* produce osmotic-sensitive growth but this sensitivity is a property of the suppressor mutations *per se* and is not dependent on the presence of a suppressible mutation, as with the present suppressors (Singh, 1977).

The most important property of nonsense suppressors is, however, their allele specificity and locus non-specificity. The adenine suppressors described here have this property as well. Suppressors selected for the *adA24* mutation were effective against mutations in four other loci – *methH*, *methG*, *proA* and *argB*. On the other hand, only some alleles in *adA*, *methG* and *methH* were suppressed while others were not (Table 9).

Suppressors of the *adA24* mutation differ in the extent of growth in the absence of adenine and in the level of adenylosuccinate lyase activity which they restore. However, the activity of the enzyme never exceeded 30% of the activity in the wild-type. In several cases no adenylosuccinate lyase activity was detected in suppressed strains but we believe that this is due to the insensitivity of the assay procedure. We were not able to detect activities lower than 3% of that in the wild-type, while it is known that leaky mutants of *N. crassa*, which

have only 2% of the wild-type activity of this same enzyme, are still able to grow on adenine-less medium (Giles *et al.*, 1957). The mutation designated *su-29* differs from the other suppressor mutations in that it is not effective at 42 °C. This is not unusual for nonsense suppressors. Similar cases were reported in studies on nonsense suppressors in yeast (Rasse-Messenguy & Fink, 1973) and *Escherichia coli* (Gallucci *et al.*, 1970; Oescher & Woods, 1976). Moreover, in *E. coli* it was demonstrated that temperature sensitivity was the property of a mutant tRNA molecule (Abelson *et al.*, 1970; Smith *et al.*, 1970). On the other hand, it is possible that in the *adA24 su-29* strain adenylosuccinate lyase itself becomes temperature-sensitive due to a particular amino acid substitution.

The four locus non-specific suppressors differ in the particular tester mutations they suppress (Table 9). This difference in specificity probably reflects the existence of the three types of nonsense mutations within the sample of mutations tested. The data on suppressor specificity are, however, insufficient to permit speculation on which mutants are amber, ochre or opal type.

Can all mutations suppressed by nonsense suppressors be classified as nonsense mutations? From studies on bacteria and yeasts it is known that from 15 to 30% of all point mutations in a given gene are nonsense mutations (Singh & Sherman, 1975), although some exceptions exist (Denis-Duphil & Lacroute, 1971; Korch & Snow, 1973). In the *adA* locus, most thoroughly studied in this work, 8 out of 14 alleles were suppressed by *su-23*. This apparent high frequency of nonsense mutations is probably due to the method of isolating this series of mutants. All leaky mutants, among which nonsense mutants are not expected, would have been eliminated during polifungin selection. On the other hand, not all mutations which are suppressed by nonsense suppressors can be automatically classified as nonsense mutations. It is known that missense (Newcombe & Griffiths, 1973; Simarov *et al.*, 1971; Maisurian & Pozdniakov, 1971; Picard, 1973) and frameshift (Atkins & Ryce, 1974) mutations can be suppressed by nonsense suppressors. We believe that a similar mechanism of suppression is responsible for the effect of *su-23* on the *adA101* and *adA120* mutants. These two mutants complement with some other mutants with defects in the *adA* locus and show a very low reversion rate compared to *adA24*; thus it would be unlikely that these mutants carry nonsense mutations. The same is true for the *methH3* mutation; strains carrying this mutation have a much lower reversion rate than those carrying other mutations in the *methH* locus (Gajewski & Litwińska, 1968).

The exceptions mentioned above do not rule out the thesis that the remaining mutations? suppressed by the suppressors of *adA24* are nonsense mutations. However, all evidence for this thesis comes from genetical data and should be supported by experiments at the molecular level. Some of these are now in progress.

Locus non-specific suppressors have also been obtained in *A. nidulans* by Roberts *et al.* (1978). We have not, however, had an opportunity to test them and to compare them with the suppressors described in this paper.

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