

# REVERSION OF ARYL SULFATASELESS MUTANTS OF NEUROSPORA

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IN previous publications from this laboratory, we have described two loci, called *cys-3* and *eth-1<sup>r</sup>*, that control a family of enzymes of sulfur anabolism in *Neurospora* (MARZLUF and METZENBERG 1968; METZENBERG 1968). These could be viewed as regulatory genes in search of a locus to control, since no proven structural mutants for any of the regulated enzymes were available. KERR and FLAVIN (1970) have noted that *eth-1<sup>r</sup>* may be the structural gene for S-adenosyl methionine synthetase, an hypothesis for which we also have some not very convincing evidence (JACOBSON, CHEN and METZENBERG, in preparation). In any event, it is not clear that *eth-1<sup>r</sup>* has a purely regulatory function.

Recently MARZLUF (1970a, 1970b) has isolated mutants which he has called *cys-13* and *cys-14*. Each of these lacks one of the two permeases which conduct inorganic sulfate into the cell, and the double mutant lacks both permeases and is an auxotroph. It is likely that these mutations correspond to the structural genes for the permeases. Likewise, we have discussed the properties of mutants that appear to be deficient only in aryl sulfatase (METZENBERG and AHLGREN 1970a). These strains, called *ars<sup>-</sup>*, are thus reasonable candidates for being structural gene mutants. We now report the isolation of revertants of two of the *ars<sup>-</sup>* alleles. We were interested in revertants on the possibility that, if *ars* is the structural locus for aryl sulfatase, some of these revertants might have an aryl sulfatase with properties distinguishable from those of the wild-type enzyme. The results reported below are negative: no qualitatively altered enzyme is apparent in any of the revertant strains. Some of these have low or very low levels of aryl sulfatase. One low-level suppressor mutation was detected. The rest of the reversion events apparently occurred at or near the *ars* locus. The failure to find altered forms of aryl sulfatase among the revertants must not be taken as proof that *ars* is *not* the structural gene for aryl sulfatase.

## MATERIALS AND METHODS

Sources of chemicals, strains, media, growth and extraction of mycelia, analytical procedures, auxanographic tests, and common procedures such as preparing crosses were as previously described (METZENBERG and AHLGREN 1970a). "Wild type" is the Oak Ridge strain, 74-OR8-1a, or its essentially isogenic counterpart of the opposite mating type, 74-OR23-1A. The strains used in this study were outcrossed to these a number of times before use. Tyrosine-O-sulfate K<sup>+</sup> salt) was prepared by the method of DODGSON *et al.* (1961).

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*Mutagenesis by ultraviolet light:* The double mutants, *ars(101); al-2* and *ars(103); al-2* were prepared by conventional crossing techniques. (The albino gene was included so that casual wild-type contaminants could subsequently be distinguished from true revertants to *ars+*). Conidia were prepared and irradiated as described by METZENBERG and AHLGREN (1970a) to 65% killing.

*Mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine (NMG):* Conidia of the above double mutants were suspended in water, filtered through glass wool to remove clumps of conidia, and adjusted to a concentration of about  $1.5 \times 10^6$ /ml. A filter-sterilized solution of NMG was added to give a concentration of 0.18 mg/ml, and the suspension was stirred for 45 min at room temperature. The conidia were washed free of the mutagen by centrifugation and resuspension in the original volume of water. The degree of "killing" could not be determined at all satisfactorily; only 0.01% of the conidia produced normal appearing, rapidly growing colonies, but many more survived to produce a spectrum of very small growth centers.

*Selection of revertants:* In principle, revertants of *ars-* mutants could be isolated by screening for colonies that turn blue in the presence of indoxyl sulfate. This procedure is simply the reverse of the one by which *ars-* mutants were obtained. In practice, the number of colonies that can be scanned by a pure screening method is quite limited. In addition, the lack of sensitivity is such that low-level revertants—potentially the most interesting class—would not be detected. One might imagine that revertants could readily be selected on the basis that unlike *ars-*, they would be able to use aryl sulfate esters as sole sulfur source. This works, but not without some limitations. First, all commercial agar samples contain some sulfur, so that it is necessary to work with liquid media. Second, *Neurospora* under conditions of sulfur limitation responds by producing enough aryl sulfatase to hydrolyze amounts of *p*-nitrophenyl sulfate that might be expected to remove the sulfur limitation and result in cross-feeding of nonrevertant spores. Thus when a "good" substrate is present in the growth medium, a small number of revertant cells can serve as breadwinner for a large retinue of unreverted cells, giving rise to a very dilute heterocaryon, from which homocaryotic revertants could be isolated only with an unacceptable amount of labor.

To minimize the problem of cross-feeding, we sought a poor substrate—one which would be cleaved only slowly by *ars+* revertants. We have found that tyrosine-O-sulfate fits this role. DODGSON, ROSE and TUDBALL (1959) reported that this compound is hydrolyzed very slowly by aryl sulfatase A and not at all by aryl sulfatases B and C of mammalian liver, though it was rapidly hydrolyzed by preparations from limpets and from some microorganisms, including one of fungal origin (*Taka-distase*). We find that, under the standard conditions for aryl sulfatase assay (pH 8.1 5 mM substrate, 37°C), tyrosine-O-sulfate is cleaved by a highly purified *Neurospora* preparation at about 0.3% of the rate for hydrolysis of *p*-nitrophenyl sulfate.

Enrichment for revertants was performed as follows. A stock medium was prepared so as to contain (after dilution): sucrose (1.5%); BC salts at half the concentration previously used (METZENBERG and AHLGREN 1970a); and tyrosine-O-sulfate, 0.05 mM as sole sulfur source. Mutagenized conidia were diluted into this medium to give a concentration of about  $1-5 \times 10^5$  total spores per ml. Aliquots (2 ml) were distributed to 200 sterile test tubes, and the mass cultures were incubated at 25° for 6 days without shaking. A drop of *p*-nitrophenyl sulfate (50 mM) was then added to each tube. Many of the cultures developed a yellow color over a period of the next day or two, showing the presence of revertants. Conidia from each of these cultures were streaked on Modified Medium P to induce colonial growth (METZENBERG and AHLGREN 1970a), and the plates were incubated overnight at 33°C. Ten colonies from each culture were picked to tubes of BC medium + cysteic acid (1 mM) and were allowed to grow for several days at room temperature. The subcultures were tested with *p*-nitrophenyl sulfate, and subcultures positive for aryl sulfatase were restreaked and the cycle of purification of the revertants was repeated until all ten subcultures were aryl sulfatase positive. Only one revertant strain from each original culture tube was kept. Finally, to assure that the revertants were homocaryotic, all of the strains were backcrossed to the original *ars-* parent [*ars(101)* or *ars(103)*]. Ascospores were germinated, and sporelings were picked to BC + cysteic acid. An aryl sulfatase positive, albino isolate was chosen as a representative of each reversion event.

*Preparation of crude extracts of revertant strains:* Cultures were grown under previously established conditions by sparging at 25°C in FRIES' minimal medium (cited by BEADLE and TATUM 1945) lacking inorganic sulfate, with 1 mM cysteic acid as the sulfur source. We have noted that the specific activity of aryl sulfatase rises steadily under these conditions and this continues after the growth rate is no longer exponential (14–15 hr). In order to obtain the higher enzyme levels needed for kinetic studies, sparging was continued for a total of 15–20 hr in these experiments. The mycelia were harvested and were extracted with Tris-acetate buffer, pH 7.5 (total Tris concentration = 0.05 M), instead of the usual pH 8.1 buffer. The pH 7.5 buffer appears to solubilize somewhat less protein than does the latter. The result of these minor modifications is that the specific activity of wild type and of many of the revertants is higher than we have previously reported.

*Michaelis constants:* These were done at 37°C, using *p*-nitrophenyl sulfate at various concentrations in Tris-acetate buffer, pH 7.5 (Tris concentration = 0.25 M), and 2-hydroxy-5-nitrophenyl sulfate ("nitrocatechol sulfate") at various concentrations in Na<sup>+</sup> acetate buffer, pH 6.0 (total acetate concentration = 0.5 M). The release of nitrocatechol was followed by stopping the reaction with ethanolic KOH in the same manner as in the standard assay and measuring the absorbancy promptly at 510 nm, before appreciable fading can occur. The Michaelis constants were determined from Lineweaver-Burk plots.

*Heat stability:* Crude extracts (pH 7.5) were heated at 70°C for 0, 5, 10, 15, 20, and 30 min and were chilled rapidly in ice water. The fraction of surviving activity of aryl sulfatase was measured.

*Arrhenius activation energy:* Activity of aryl sulfatase was measured in Tris-acetate buffer, pH 7.5 (Tris concentration = 0.25 M) at 0°C, 11°C, 21.5°C, 30°C, 36.5°C, and 44°C. The activation energy was obtained in the ordinary way, from the slope of a plot of the reciprocal of the temperature in °K against the log of the reaction rate.

*Electrophoresis on cellulose acetate strips:* This was performed at 3–5°C in diethanolamine-acetate buffer, pH 9.07 (diethanolamine concentration = 0.05 M). Sepraphore III strips, 25 mm × 170 mm, (Gelman Instrument Co.) were dampened with buffer, and were equilibrated for 30 min in the electrophoresis chamber. Extracts of the revertants and of wild type were made in the same buffer, and human hemoglobin A was added to the samples as a visible internal marker. Narrow applicator clippings of Sepraphore III (about 2 mm × 25 mm) were saturated with these preparations and were placed on the equilibrated strips at the intended origins, as recommended by Dr. K. ADACHI (personal communication). After the enzymes and hemoglobin had been allowed to diffuse into the strips for 1 hr, the applicator clippings were removed, the origin was marked, and the strips were subjected to 300 volts for 90 min. The anodic portion of the electrophoretograms was excised and the position of hemoglobin was marked. The damp strip was placed on 1% ionagar containing 5 mM K<sup>+</sup> indoxyl sulfate and 50 mM Tris-HCl, pH 8.1. The agar plates were incubated at room temperature or at 37–40°C until a blue band of indigo, indicating the position of aryl sulfatase, was obvious (typically about 1 hr).

## RESULTS

*Properties of aryl sulfatase from revertants:* The data in Table 1 do not reveal any substantial qualitative difference in Michaelis constants between wild-type and the revertant enzymes, though some of the revertant strains have reduced levels of the enzyme. In the cases of *ars(101)* revertants 11, 25, and 41, the activity is so low that kinetic measurements cannot be made with good accuracy. However, even in these cases it is clear that there are no dramatic alterations of the  $K_m$  values for either substrate. Likewise, the Arrhenius activation energy of revertant enzymes does not show any believable differences.

The heat inactivation of aryl sulfatase at 70°C usually did not show perfectly linear kinetics when log of surviving activity was plotted against time. Even with

TABLE 1

*Kinetic properties of aryl sulfatase from wild type and from revertants of ars<sup>-</sup> strains*

Strain	Mutagen	Specific Activity	$K_m$ , pN $\phi$ S (mM)	$K_m$ , NCS (mM)	Half-life at 70° (min)	Activation Energy (kcal.)
wild type (74-OR8-1 <sub>a</sub> )	-	42	0.53	0.67	20	13.7
<u>ars (101)</u>						
revertant 1		56	0.54	0.70	21	13.1
2		60	0.51	0.72	22	12.7
3		30	0.54	0.80	22	13.0
4	NMG	34	0.49	0.65	21	13.6
5		34	0.46	0.73	24	12.9
6		55	0.54	0.82	23	12.9
7		55	0.60	0.50	23	13.4
10		40	0.56	0.63	21	12.9
11	UV	0.27	0.60	0.90	—	15.0
12		63	0.50	0.65	20	13.5
14		77	0.54	0.71	26	13.6
21		52	0.54	0.70	17	13.0
22		64	0.53	0.61	17	13.1
23	NMG	50	0.52	0.70	18	13.6
24		41	0.53	0.73	21	13.6
25		3.3	0.79	0.90	28	14.4
31		36	0.68	0.64	17	13.0
32		94	0.69	0.62	19	13.0
33		65	0.63	0.68	17	13.9
34		43	0.54	0.68	23	12.9
35		43	0.50	0.70	19	12.8
36	UV	7.6	0.50	0.78	21	13.3
37		56	0.55	0.77	16	13.2
38		72	0.55	0.74	21	13.0
39		44	0.54	0.60	17	13.2
40		87	0.53	0.50	24	13.2
41		0.54	0.72	1.0	26	12.9
42		72	0.59	0.75	20	13.1
43		2.5	—	—	—	—
<u>ars (103)</u>						
revertant 1		53	0.56	0.85	23	13.0
2		68	0.54	0.78	20	13.0
3		40	0.57	0.80	21	13.2
4	NMG	55	0.57	0.94	24	13.2
5		49	0.54	0.75	21	12.9
6		84	0.70	0.91	19	13.0
7		66	0.62	0.83	19	13.0
8		41	0.56	0.76	25	13.5

Specific activity is n moles/min/mg protein at pH 8.1, 37°C (standard conditions). Abbreviations: pN $\phi$ S = *p*-nitrophenyl sulfate; NCS = nitrocatechol sulfate.

highly purified enzyme from wild type, there was a component (about 10–15%) which was more labile than the main portion of the enzyme. The decay of the remaining enzyme follows first-order kinetics. The half-lives we report are simply the time required for the activity of the enzymes to fall to 50% of their original value. The biphasic behavior is not altered by adding bovine serum albumin (10 mg/ml) to “protect” the enzyme during heating. Most of the modest amount of scatter in the half-lives of aryl sulfatase reported in Table 1 is due to variations in the amount of the more thermolabile component. It is possible that there is some microheterogeneity in aryl sulfatase. ARNOLD (1969) has reported that the heat denaturation of bulk invertase of yeast does not follow simple first-order kinetics, and that this can be explained in terms of microheterogeneity of the enzyme with respect to the amount of covalently bound carbohydrate. Whether or not a similar phenomenon occurs in aryl sulfatase can only be surmised. In any case, it is clear that there are no dramatic differences in thermostability of aryl sulfatase between wild type and any of the revertants.

Aryl sulfatases from *ars* (101) revertants 1–7 have also been compared with wild-type enzyme at pH 8.1 (results not shown in Table 1) as well as pH 7.5. The comparisons include  $K_m$  for *p*-nitrophenyl sulfate, Arrhenius activation energy, and rate of heat denaturation. In no case was any significant difference seen.

The electrophoretic mobility of aryl sulfatase of wild type was about 1.75 times that of the hemoglobin internal marker, and the mobilities of the various revertant enzymes did not differ perceptibly from this value. The activity of enzymes from *ars* (101) revertants 11, 25, 41, and 43 was too low to detect by staining with indoxyl sulfate, and hence no data are on hand concerning their electrophoretic behavior.

*Repressibility of aryl sulfatase in ars revertants:* All of the revertants were grown at 25° on Medium BC + 5 mM methionine, BC + 5 mM inorganic sulfate, and, for derepressed controls, on BC + 1 mM cysteic acid. After 2 days, the cultures were tested as usual with a drop of *p*-nitrophenyl sulfate. All of them were completely repressible by methionine and by sulfate except for *ars* (101) revertant 37. This strain showed a low level of nonrepressible activity at 25°C on both methionine and on sulfate, and it failed to grow at 40°C on any of the three media. When revertant 37 was grown at 25°C with sparging in flasks of FRIES' medium with 5 mM methionine, or 5 mM K<sub>2</sub>SO<sub>4</sub>, as sulfur sources, the aryl sulfatase levels were 2% and 4%, respectively, of that of the same strain grown on 1 mM cysteic acid.

We wished to know (1) whether the incomplete repressibility of this strain was associated with its temperature sensitivity, (2) whether the incomplete repressibility maps at the *ars* locus. To answer the first question, we crossed *ars* (101) revertant 37 to wild type and isolated 75 sporelings to BC + 5 mM inorganic sulfate and allowed them to grow at 25°C. Fifteen of the cultures were incompletely repressible, and all of these failed to grow at 40°C in subsequent tests. The remaining cultures were all temperature tolerant. Thus it appears that temperature sensitivity and incomplete repression are probably pleiotropic effects of the same mutational event. It is not clear why the proportion of the two

types of segregants is so far from the expected Mendelian ratio of 1:1; however, there were many spores that failed to germinate, and it seems likely that the discrepancy is the result of differential viability. To deal with the second question, we crossed revertant 37 to the parental *ars* (101) of the opposite mating type. Sporelings were picked to BC + 5 mM sulfate. These cultures were scored for repressibility, and were transferred to fresh tubes of the same medium to test for temperature tolerance, and to BC + 1 mM cysteic acid in order to score for ability to make aryl sulfatase under conditions of derepression. The results were clear: aryl sulfatase positive, fully repressible recombinants, and aryl sulfatase negative (*ars*<sup>-</sup>), temperature-sensitive recombinants were recovered along with the two parental types. Obviously the mutation causing partial derepression and temperature sensitivity in revertant 37 occurred independently of the reversion to the *ars*<sup>+</sup> condition. Having thus disposed of the "exceptional" revertant 37, we can state that all of the revertants are under normal repression control. Thus it is rather unlikely that the original *ars*<sup>-</sup> mutations were control mutants that were superrepressed by a variant form of a normal repressor.

All of the revertants were also tested at 40°C on Medium BC + 5 mM methionine and BC + 5 mM inorganic sulfate. All of the cultures were completely repressed by methionine. On sulfate, all of the cultures including the wild-type control were very slightly derepressed. Thus there is no evidence that any of the revertants had acquired a thermolabile repression system.

*Mapping of the reversion events:* It was important to know whether the various revertants represented mutational corrections within the *ars* locus, or whether unlinked suppressors were involved. To examine appreciable numbers of the progeny of outcrosses of the revertants to wild type, it was obviously necessary to have a method of scoring colonies for aryl sulfatase production without picking them to tubes. For this to be feasible, it was necessary to have genes for derepression of aryl sulfatase and a gene for colonial growth on both sides of the outcross. Accordingly, each of the revertants was crossed to *ars* (101), *thi-3 eth-1<sup>r</sup>*, *cys-11*; *cot-A*. Strains that were aryl sulfatase positive, thiamin independent, and colonial at 33°C and that were not repressible by either methionine or inorganic sulfate were isolated. Because *cys-11* is closely linked to the mating-type locus (MURRAY 1968), all of these were mating-type A, and because *thi-3* is closely linked to *ars*, all the derived strains will probably have the *ars* gene from the revertant parent. These derepressed revertants were crossed to *eth-1<sup>r</sup>*, *cys-5* (NM44); *cot-a*. An average of 435 heat-shocked ascospores (and never fewer than 200) from each cross were plated on Modified Medium P, allowed to grow for 3 days at 33°C, and stained for aryl sulfatase with Modified Staining Solution (METZENBERG and AHLGREN 1970a). *ars* (101) revertants 25, 36, 41, and 43, all of which had low or very low aryl sulfatase, were excluded from this experiment. *ars* (101) revertant 11 was lost after finishing the kinetic study and before genetic analysis.

It was apparent that most of the colonies on all of the plates were stained blue by the indoxyl sulfate. However, on most of the plates there were a few colonies that grew poorly and could not be classified. Therefore, the ten least-stained

colonies from each plate were picked to BC + 1 mM cysteic acid and allowed to grow 2 days at 25°C. The resulting cultures were tested with *p*-nitrophenyl sulfate, and all were found to be positive. Hence, there were no *ars*<sup>-</sup> segregants from any of these crosses, and we may rule out the possibility that the reversion events are due to unlinked or weakly linked suppressors.

*ars* (101) revertants 25, 36, 41, and 43 were crossed to wild type and germinated sporelings were picked to BC + 1 mM cysteic acid. The resulting cultures were scored for aryl sulfatase 30 min after adding *p*-nitrophenyl sulfate, and again several days later. The results in Table 2 show that revertant 41 segregates true *ars*<sup>-</sup> progeny, and is therefore really a suppressor mutation rather than a reversion at the *ars* locus. The distinction between the low-level suppressed strains and *ars*<sup>-</sup> strains was inconclusive in a few of the cultures, because during the prolonged incubation *in vivo*, some of the isolates formed some brown pigment that made it difficult to be sure whether any nitrophenol had been liberated. Therefore, all of the low sulfatase and *ars*<sup>-</sup> isolates were grown on the above medium, to which had been added 1 mM *p*-nitrophenyl sulfate labeled with <sup>35</sup>S (about 10<sup>4</sup> counts per culture tube) (METZENBERG and AHLGREN 1970b). When the cultures were grown, the mycelia were washed in the tubes with unlabeled substrate + excess inorganic sulfate, heated for 10 min at 90°C in 10% trichloroacetic acid, and the crude protein residue was counted. Wild-type controls incorporated 8–10% of the total counts, suppressed *ars*<sup>-</sup> strains incorporated 0.55–1.1% of the total counts, and *ars*<sup>-</sup> experimental and control strains incorporated 0.03–0.12%. Two cultures had been misclassified on the basis of visible color. The corrected scoring results are given in Table 2. It is also evident from Table 2 that revertant 41 is unique in the group of low aryl sulfatase strains in being the only case that cannot be explained as a reversion at the *ars* locus.

*ars* (101) revertant 25 as a temperature-conditional revertant: In the experiment dealing with the repressibility of aryl sulfatase in revertants, we noticed qualitatively that revertant 25, which makes rather low levels of aryl sulfatase under derepressing conditions at 25°C, makes much less still at 40°C. To check

TABLE 2

*Mapping of reversion events\* in low-level revertants of ars* (101)

Revertant parent	Numbers of progeny from crosses with wild type		
	High aryl sulfatase	Low aryl sulfatase	"Zero" aryl sulfatase ( <i>ars</i> <sup>-</sup> )
25	53	47	0
36	53†	47†	0
41	45	31	20
43	38	60	0

\* In each case, the revertant was outcrossed to wild type. Subsequent handling is as described in the text.

† In the case of revertant 36, there were 10 cultures that showed an intermediate level of aryl sulfatase by the visual criterion, and were assigned somewhat arbitrarily between the two classes on the basis of a "best guess". In the case of the revertants 25 and 43, there was no overlap; the distribution was completely bimodal. In the case of revertant 41, it was completely trimodal.

TABLE 3

*Aryl sulfatase levels in wild type and in ars(101) revertant 25 at various temperatures*

Strain	Temperature during growth	Time of harvest (hours)	Aryl sulfatase (specific activity)
Wild type	20°C	23	14.5
	25°C	15	21.2
	30°C	15	18.2
	35°C	15	14.7
	40°C	15	14.2
Revertant 25	20°C	23	1.3
	25°C	15	3.4
	30°C	15	2.0
	35°C	15	0.76
	40°C	15	0.28

The cultures were grown with sparging in FRIES' minimal medium lacking inorganic sulfate, with 1 mM cysteic acid as sulfur source (METZENBERG 1968).

this result, we grew the revertant and wild-type control at various temperatures and measured the specific activity of aryl sulfatase (Table 3). The results confirm that the presence of aryl sulfatase in the revertant is strongly temperature dependent. Stability *in vitro* of the revertant enzyme at 70°C, pH 7.5, appears to be normal (Table 1), and a separate comparison of the revertant with wild type at 70°C, pH 8.1 likewise revealed no differences. It seems likely that the enzyme simply fails to be synthesized by the revertant at 40°C.

#### DISCUSSION

In trying to decide whether *ars<sup>-</sup>* mutants define the structural locus for aryl sulfatase, it is necessary to consider explanations based on altered control.

Since none of the reversion events results in loss of repression of aryl sulfatase, it seems very unlikely that the absence of this enzyme in the *ars<sup>-</sup>* parents is due to some sort of locus-specific superrepression. If this were the case, we would expect that most revertants to the *ars<sup>+</sup>* condition would simply have lost all repression (WILLSON *et al.* 1964). Other types of control elements are not so easily ruled out. Two observations provide weak evidence against the hypothesis that *ars(101)* is a mutant in the promoter of the structural gene for aryl sulfatase. First, revertant 25 is temperature conditional in its synthesis of aryl sulfatase; since the promoter, defined as the binding site for RNA polymerase, is generally assumed to be DNA, it seems somewhat unlikely (but by no means impossible) that it would undergo a phase transition in the temperature range of 35–40°C. Second, "revertant" 41 is actually an external suppressor mutant. We hesitate to affirm that promoter mutants would be suppressible, though one can imagine some possible mechanisms (e.g., altered transcriptional factors). The data presented in this paper leave open the possibility that *ars<sup>-</sup>* mutants lack an activator of aryl sulfatase synthesis, an explanation we have suggested for the role of *cys-3* in the control of several genes, including aryl sulfatase. This would imply a



hierarchy of control genes with *ars* below *cys-3*. Such a model is unattractively complicated, but it cannot be ruled out.

The simplest interpretation is that *ars* is the structural locus, and that *ars* (101) and *ars* (103) are related in such a way that reversion always or usually gives an enzyme molecule with more or less normal properties, except that  $V_{\max}$  for the enzyme may be dramatically reduced in some cases. In the accompanying paper (METZENBERG and AHLGREN 1971) we show that variant forms of aryl sulfatase can be found in nature, though we have failed to obtain them in the laboratory, and that the responsible genetic factor maps at or very near the *ars* locus. Nonetheless, the view that *ars* (101) is a missense or nonsense mutant fails to explain the temperature dependence of revertant 25, and we feel that a cautious stance is indicated.

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#### SUMMARY

Revertants of aryl sulfataseless mutants have been isolated. All but one of the reversion events map at or near the locus of the original mutations. The control of enzyme synthesis by methionine and by inorganic sulfate in the revertants is normal. The levels of aryl sulfatase activity in some of the revertants was very low. No evidence was found for a qualitatively changed aryl sulfatase in any of the revertants.

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