POSITIVE REGULATION IN A EUKARYOTE, A STUDY OF THE *ua*Y GENE OF *ASPERGILLUS NIDULANS*: I. CHARACTERIZATION OF ALLELES, DOMINANCE AND COMPLEMENTATION STUDIES, AND A FINE STRUCTURE MAP OF THE *ua*Y − *oxp*A CLUSTER

CLAUDIO SCAZZOCCHIO, NICHOLAS SDRIN[1] AND GLORIA ONG[2]

Department of Biology, University of Essex, Wivenhoe Park, Colchester, CO4 3SQ, Essex, England

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ABSTRACT

In this paper we characterize genetically a positive eukaryotic regulatory gene: the *ua*Y gene of the ascomycete *Aspergillus nidulans*. Several steps in the uptake and degradation of purines are under the control of the *ua*Y gene (summarized in SCAZZOCCHIO and GORTON 1977). In the present paper 12 *ua*Y⁻ mutations are characterized with respect to their inducibility for adenine deaminase, xanthine dehydrogenase (purine hydroxylase I) and urate oxidase and by the absence of the uric acid-xanthine permease scored *in vivo* by resistance to 2-thiouric acid. While 10 mutations are uniformly unleaky, two others are almost wild type for the induction of urate oxidase. A fine structure map of the *ua*Y gene shows that the two "leaky" mutations are not clustered. The fine structure mapping unambiguously positions six *ua*Y alleles and provides preliminary but interesting trends regarding the pattern of gene conversion in the *ua*Y gene. The enzyme levels in all *ua*Y⁻/*ua*Y+ heterozygous diploids are intermediate between the corresponding *ua*Y⁻/*ua*Y⁻ and *ua*Y+/*ua*Y+ homozygous diploids, suggesting that one functional copy of the *ua*Y gene is able to mediate the complete induction of only one set of structural genes. No complementation was found between any two *ua*Y⁻ alleles. This establishes that the mutations showing either of the phenotypes are alleles in the same gene; it fails to provide evidence for intracistronic complementation. A mutation, *oxp*A5, causes resistance to the xanthine analogue oxypurinol (4, 6-dihydroxypyrazolo-(3, 4-d)-pyrimidine) and partial constitutivity of adenine deaminase, xanthine dehydrogenase (purine hydroxylase I) and urate oxidase. The constitutive phenotype is suppressed by mutations blocking the synthesis of intracellular inducers. The mutation is recessive and complements fully with the 11 *ua*Y⁻ mutations tested. It maps to the left of all 12 *ua*Y mutations to which it has been crossed. The data indicate that both the resistance and constitutivity arise from one mutational event in a gene, *oxp*A, different from *ua*Y and possibly adjacent to it. We propose that the *oxp*A gene codes for a protein involved in limiting the flow of inducers into the cell nucleus. Thus *oxp*A and *ua*Y constitute a regulatory gene cluster, indicating that *ua*Y is the regulatory gene.

[1] Present address: Pastra's High School, Pastra, Cephalonia, Greece.
[2] Present address: Imperial Cancer Research Fund, Burtonhole Lane, Mill Hill, London N.W.7.

SINCE the seminal work of JACOB and MONOD (1961), the establishment of regulatory circuits has depended on the identification of regulatory mutations and the study of their interactions. Regulatory genes can be defined when pleiotropic mutations resulting in noninducibility or constitutivity can be identified. However, genetic evidence is generally insufficient to establish the level at which the product of a regulatory gene acts. Rather than directly affecting transcription or translation, the product of a putative regulatory gene could be involved in a step that catalyzes the synthesis of the true co-inducer (or co-repressor). The insufficiency of the purely genetic approach is especially manifest when a putative regulatory mutation results in apparent noninducibility. This phenotype could also arise from mutations in genes coding for common subunits, enzymes involved in a common cofactor synthesis or in post-translational modification common to several proteins. Identifying a regulatory mutation becomes an almost intractable problem at the formal genetic level when the putative regulatory gene affects the induction or repression of only one activity.

The ability to obtain mutations with opposite phenotypes (noninducible and constitutive, or derepressed and nonderepressible) is strong circumstantial evidence for a true regulatory gene. Noninducible and constitutive mutations have been described for some regulatory genes in simple eukaryotes (for example *nir*A, PATEMAN and COVE 1967; *apl*A, SCAZZOCCHIO, HOLL and FOGUELMAN 1973; SCAZZOCCHIO, *et al.* 1978; *qa*-a VALONE, CASE and GILES 1971). In only one case has a fine structure map proved conclusively that the constitutive mutations map within the putative regulatory gene as defined by the noninducible mutations (*gal-4*, MATSUMOTO *et al.* 1980).

On the other hand the inability to obtain mutations of opposite phenotype does not *per se* preclude a regulatory role. In some cases, one of the phenotypes might be impossible (SCAZZOCCHIO, unpublished).

In this paper and elsewhere (PHILIPPIDES and SCAZZOCCHIO 1981) a positive regulatory gene in the ascomycete, *Aspergillus nidulans* is defined and described. This is the *ua*Y gene necessary for the induction of several of the enzymes of purine degradation and at least one permease (SCAZZOCCHIO and DARLINGTON 1967, 1968; SCAZZOCCHIO, HOLL and FOGUELMAN 1973; SCAZZOCCHIO and GORTON 1977; SCAZZOCCHIO and ARST 1978). While a regulatory role was postulated as far back at 1965, strong evidence of the direct regulatory role of *ua*Y has only recently been obtained (SCAZZOCCHIO and ARST 1978).

In strains carrying *ua*Y$^-$ mutations, xanthine dehydrogenase (purine hydroxylase I), urate oxidase and the uric acid-xanthine permease are noninducible (*loc. cit.*). These activities are induced in the wild type by uric acid and some of its thio-analogues (SCAZZOCCHIO and DARLINGTON 1968; SCAZZOCCHIO 1973; SEALY-LEWIS, SCAZZOCCHIO and LEE 1978, ARST and SCAZZOCCHIO 1975). Allantoinase and allantoicase are inducible in the wild type by both uric acid and allantoin; *ua*Y$^-$ mutations are noninducible by uric acid but inducible by allantoin (SCAZZOCCHIO and DARLINGTON 1968). Recently it was shown that adenine deaminase responds to the same inducers as the enzymes under *ua*Y

control and we presented indirect evidence that the xanthine alternative pathway is under *ua*Y control. It has been seen that only uric acid and its 2- and 8-thio analogues are effective inducers of the activities under *ua*Y control (SEALY-LEWIS, SCAZZOCCHIO and LEE 1978) Figure 1 shows the purine degradation pathway and Table 1 lists all the characterized genes presumed to be under *ua*Y control.

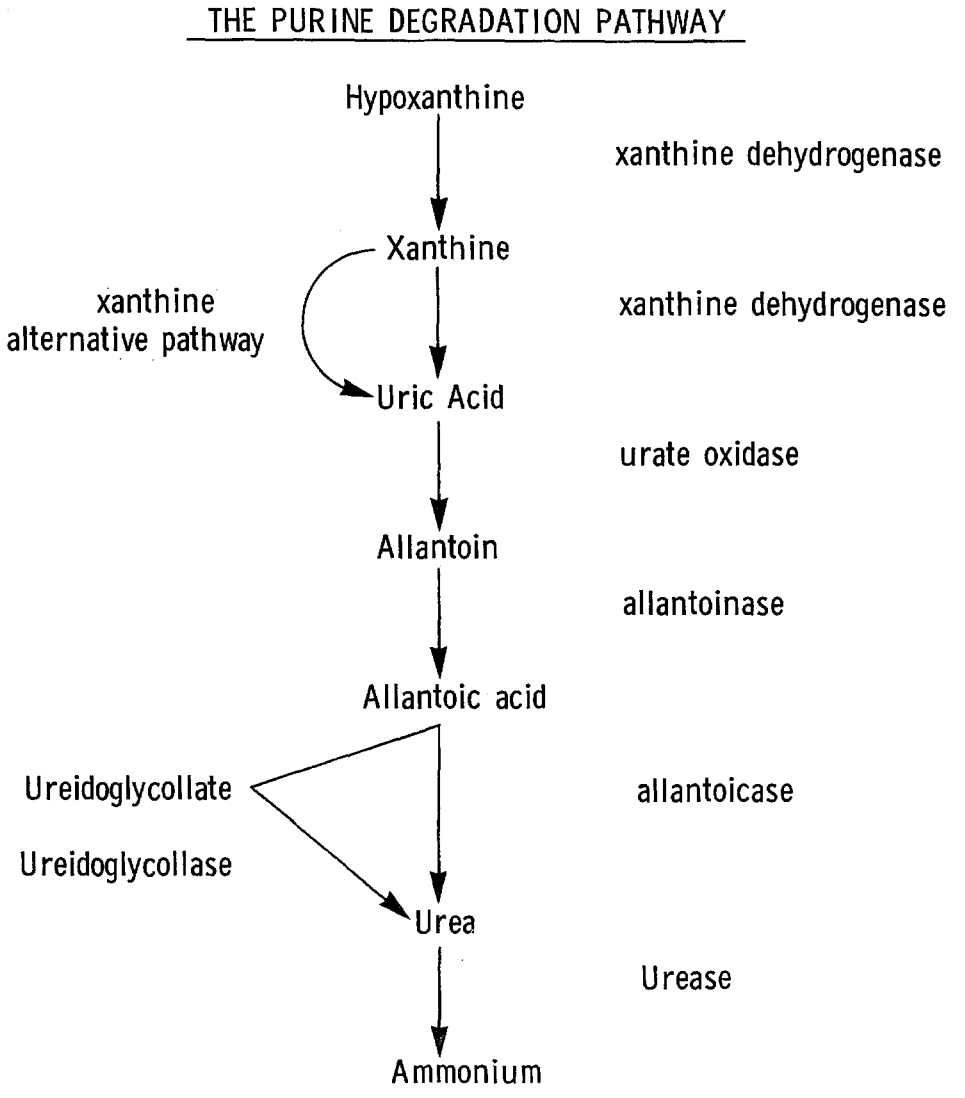

FIGURE 1.—In the center (connected by arrows) are the metabolites involved in the purine degradation pathway. Adjacent to the arrows are the names of the enzymes that catalyze each step. The last two enzymes of the pathway, ureidoglycollase and urease seem to be constitutive and are not under *ua*Y control (SCAZZOCCHIO and DARLINGTON, 1968).

TABLE 1

Genes under ua*Y control*

Gene	Function	Linkage group	Nearest gene(s) and distance in centimorgans	References
*nad*A	Putative structural gene for adenine deaminase	I	No linkage detected	VANCE and SCAZZOCCHIO unpublished
*hx*A	Structural gene for xanthine dehydrogenase I	V	*fac*A, (13 cm) and *acu*G (7 cM)	SCAZZCCCHIO and DARLINGTON 1968; SCAZZOCCHIO *et al.*, 1973; ARMITT, MCCULLOUGH and ROBERTS 1975; SEALY-LEWIS and SCAZZOCCHIO 1978.
*hx*B	Codes for a protein necessary for both xanthine dehydrogenase (purine hydroxylase) I and purine hydroxylase II activities.	VII	No linkage detected	SCAZZOCCHIO and DARLINGTON 1968; SCAZZOCCHIO 1973; SCAZZOCCHIO *et al.* 1973.
*xan*A	Putative structural gene for (a step on) the xanthine alternative pathway.	VIII	No linkage detected	SEALY-LEWIS, SCAZZOCCHIO and LEE 1978.
*ua*Z†	Structural gene for urate oxidase	I	*ribo*A (9 cM)	SCAZZOCCHIO 1966; SCAZZOCCHIO and DARLINGTON 1968; COVE unpublished, SEALY-LEWIS and SCAZZOCCHIO unpublished.

TABLE 1—Continued

Gene	Function	Linkage group	Nearest gene(s) and distance in centimorgans	References
*al*X	Putative structural gene for allantoinase	III	*fmd*S (2 cM) and *sor*B (32 cM)	DARLINGTON 1966; SCAZZOCCHIO and DARLINGTON, 1968; HYNES, personal communication, ELORZA and ARST, 1971.
*aa*X	Putative structural gene for allantoicase	VI	No linkage detected	DARLINGTON 1966; SCAZZOCCHIO and DARLINGTON 1978.
*uap*A	Putative structural gene for a uric acid-xanthine permease	I	No linkage detected	DARLINGTON and SCAZZOCCHIO 1967; ARST and SCAZZOCCHIO 1975; SCAZZOCCHIO and ARST 1978.

* This table shows which genes are presumably under *ua*Y control. In each case the enzymes lacking in mutations mapping in each gene are also noninducible in strains carrying *ua*Y⁻ mutations. In *hx*A, *hx*B, *ua*Z, and *uap*A it is possible to show by complementation tests that they are directly under *ua*Y control (text and SCAZZOCCHIO and DARLINGTON 1967; SCAZZOCCHIO, HOLL and FOGUELMAN 1973; SCAZZOCCHIO and ARST 1978); *nad*A, *xan*A, *uap*A, *al*X and *aa*X have been called "putative structural genes" as this is the more likely interpretation of their phenotypes. The *hx*A is the structural gene for the xanthine dehydrogenase I as electrophoretic variants and mutants with altered substrate specificity, cold sensitive, and nonconditional alleles, map at this locus (SCAZZOCCHIO and SEALY-LEWIS 1978). Mutations at the *hx*B gene result in loss of activities of both xanthine dehydrogenase and another enzyme, not under *ua*Y control, purine hydroxylase II (SCAZZOCCHIO, HOLL and FOGUELMAN 1973). It is not clear whether it codes for a common subunit or a post translational modification common to both enzymes. The variable levels of cross-reacting material present in a number of unleaky *ua*Z mutations are only consistent with *ua*Z being the structural gene for urate oxidase (SEALY-LEWIS and SCAZZOCCHIO unpublished).

† *ua*Z was previously located in chromosome VIII (ref. cited and SCAZZOCCHIO and GORTON 1977). The reason for this was that the only allele available at the time, *ua*Z11 carries a I–VIII translocation associated with the *ua*Z mutation and placing *ua*Z and presumably other markers on chromosome I between the *s*D and *orn*B markers on chromosome VIII (SEALY-LEWIS, HODGE and SCAZZOCCHIO, unpublished).

We characterize 12 *ua*Y⁻ alleles by phenotype, dominance, complementation relationships, and position in a fine structure map. We also describe an apparently constitutive mutation, *oxp*A5, closely linked to the *ua*Y gene, and present evidence that the *oxp*A gene specifies a mechanism that restricts the accessibility of the product of the *ua*Y gene by intracellular inducers.

The data presented here are compatible with *ua*Y acting as a positive regulatory gene. Evidence of this conclusion will be presented elsewhere (SCAZZOCCHIO, unpublished), and the isolation of a protein likely to be coded by the *ua*Y gene has been published (PHILIPPIDES and SCAZZOCCHIO 1981).

MATERIALS AND METHODS

Strains: All the strains were isolated in a *bi*A-1 background (auxotrophic for biotin); *ua*Y2 was isolated after UV mutagenesis while *ua*Y4, 5, 6, 7, 8, 9 and 12 were isolated by diethyl sulphate mutagenesis. The latter originally were called UA-2, 4, 5, 6, 7, 8, 9, and 12 (DARLINGTON, SCAZZOCCHIO and PATEMAN 1965); HARTLEY (1969) isolated *ua*Y205 and *ua*Y207 by the selection procedure of ALDERSON and SCAZZOCCHIO (1967) after diepoxybutane mutagenesis. Both strains were reported not to revert (HARTLEY 1969), but while this was confirmed for *ua*Y207 (PHILIPPIDES and SCAZZOCCHIO 1981), *ua*Y205 was reverted by *N*-methyl-*N'*-nitro-N-nitrosoguanidine treatment (SCAZZOCCHIO, unpublished). These strains were called respectively DU5 and DU7 in HARTLEY's thesis (1969); *ua*Y109 and *ua*Y110 are mutations isolated by HARTLEY (1969) after nitrous acid mutagenesis and originally called AH9 and AH10. In contrast to other *ua*Y mutations, these result in only slight impairment of the utilization of uric acid as sole nitrogen source (see RESULTS) and map inside the *ua*Y gene. The *oxp*A5 was selected in a *bi*A1 (auxotrophic for biotin) strain after diethyl sulphate mutagenesis (ALDERSON and SCAZZOCCHIO 1967) on a minimal medium (COVE 1966) containing uric acid as nitrogen source in the presence of 250 μg/ml oxypurinol (4,6 dihydroxypyrazolo (3, 4-d)- pyrimidine). Uric acid was used as nitrogen source to eliminate *ua*Y⁻ mutants, which themselves are somewhat resistant to oxypurinol due to the noninducibility of the *uap*A permease (SCAZZOCCHIO and ARST 1978). Sodium deoxycholate was included in the medium to induce compact growth (MACKINTOSH and PRITCHARD 1963). On this medium, wild-type strains produce very small nonconidiating colonies. Resistant mutants can be easily distinguished as strongly growing, fully conidiating colonies. Of approximately 40,000 colonies, 22 resistant strains were isolated. One, carrying a mutation *oxp*A5 (previously named *oxp*ʳ-5; COVE 1970, SCAZZOCCHIO, HOLL and FOGUELMAN 1973, SCAZZOCCHIO and GORTON 1977), was constitutive for xanthine dehydrogenase (purine hydroxylase I, LEWIS, *et al.* 1978) and urate oxidase and was selected for further work.

Growth tests: These were done on Aspergillus minimal medium supplemented with adenine, hypoxanthine, xanthine, uric acid, urea or ammonium d-tartrate as nitrogen sources, as indicated by ARST and COVE (1973). Purines were added at a final concentration of 0.1 mg/ml. The presence of an active uric acid-xanthine permease was investigated by the effect of 2-thioxanthine and 2-thiouric acid on conidial pigmentation. Resistance to 2-thiouric acid results from a block in the uric acid-xanthine permease. Resistance to 2-thioxanthine results from a block in either the permease or xanthine dehydrogenase as 2-thioxanthine is taken up by the same permease as 2-thiouric acid (DARLINGTON and SCAZZOCCHIO 1967) but has to be oxidized to 2-thiouric acid *via* xanthine dehydrogenase to be effective (ALDERSON and SCAZZOCCHIO 1967). This was tested by DARLINGTON and SCAZZOCCHIO (1967) and ALDERSON and SCAZZOCCHIO (1967).

In situ *staining of* A. nidulans *colonies*: Colonies were stained after 24 hr growth on minimal medium for xanthine dehydrogenase and urate oxidase activities. The colonies were made permeable to the reagents in the reaction mixture and the intracellular substrates leaked out by flooding the petri dish with toluene and washing out the toluene after a few seconds with the appropriate buffer. After incubating in buffer for 30 min, sufficient reaction mixture to cover the colonies was poured onto the petri dish. Xanthine dehydrogenase was detected by the tetra-

zolium stain described by SCAZZOCCHIO, HOLL and FOGUELMAN (1973) and urate oxidase by the histochemical technique of GRAHAM and KARNOVSKY (1965). Strong staining for either enzyme was obtained with the wild type only when inducers (SEALY-LEWIS, SCAZZOCCHIO and LEE 1978) were included in the medium. On medium without inducers, strains carrying *oxp*A5 stained strongly for both xanthine dehydrogenase and urate oxidase. The clearest results were obtained using L-arginine as sole nitrogen source, and in the case of xanthine dehydrogenase, inducing compact growth with sodium deoxycholate.

Construction of diploids: Nonleaky complementing markers were used to force heterokaryons. The markers used were *bi*A1, *pyro*A4, *panto*B100, *paba*A1, *pu*A2, *ribo*C5, (auxotrophies resulting in biotin, pyridoxine, pantothenic acid, p-aminobenzoic acid, putrescine and riboflavine respectively). The conidial color markers *y*A2 (yellow), *w*A4 (white) and *fw*A1 (fawn) were also used. Diploids were selected following the usual procedures employed in Aspergillus (CLUTTERBUCK 1974).

Fine structure mapping: This was done by conventional crosses between either *oxp*A5 and a given *ua*Y mutation or between two given *ua*Y mutants. Alleles were ordered using the external markers *cbx*C34 (carboxin resistance, GUNATILLEKE, ARST and SCAZZOCCHIO 1975) and *fpa*D43 (*p*-fluorophenylalanine resistance, SINHA 1969) mapping respectively to the left and the right of the *oxp*A5-*ua*Y cluster in the Aspergillus conventional map of linkage group VIII.

Determination of enzyme activities: The growth of mycelia in liquid culture, preparation of cell-free extracts and enzyme assays have been described in detail (SCAZZOCCHIO, HOLL and FOGUELMAN 1973; SEALY-LEWIS, SCAZZOCCHIO and LEE 1978). A 50 mM, pH 7.4, sodium phosphate extraction buffer, 100 μM in Na4 EDTA and 100 μM in dithiothreitol was used when adenine deaminase was assayed. The determination of xanthine dehydrogenase cross-reacting material was as described by SEALY-LEWIS, SCAZZOCCHIO and LEE (1978).

RESULTS

Characterization of the mutations—growth tests: Growth tests subdivide the *ua*Y alleles into two groups. Mutations in the group comprising *ua*Y2, 4, 6, 7, 8, 9, 12, 205 and 207 result in equally poor growth on adenine, hypoxanthine, xanthine or uric acid as nitrogen source. This growth is equivalent to the residual growth of the wild type on medium without addition of a usable nitrogen source. The second group, comprising *ua*Y109 and *ua*Y110, grows slightly better than any member of the previous group on adenine, hypoxanthine and xanthine and only slightly less well than the wild type on uric acid. All strains are equally resistant to 2-thioxanthine and 2-thiouric acid, which indicates a similar impairment of the uric acid-xanthine permease. The slight impairment of growth on uric acid as nitrogen source resulting from the *ua*Y109 and *ua*Y110 mutations is comparable to the impairment found in strains that carry mutations in the *uap*A gene specifying a uric acid-xanthine permease under *ua*Y control (ARST and SCAZZOCCHIO 1975; SCAZZOCCHIO and ARST 1978).

Characterization of the Mutations—enzyme levels: Table 2 shows the xanthine dehydrogenase (purine hydroxylase I) activity, xanthine dehydrogenase cross-reacting material and urate oxidase activity levels that result from a selection of *ua*Y mutations when noninduced or induced with the physiological inducer uric acid or the gratuitous inducer 2-thiouric acid. Table 3 shows another series of experiments in which all 12 mutations are compared for their adenine deaminase, xanthine dehydrogenase and urate oxidase activities when noninduced or induced with 2-thiouric acid. While all mutations in the first group are uniformly noninducible for all activities tested, mutations in the second

TABLE 2

Xanthine dehydrogenase, xanthine dehydrogenase-cross reacting material and urate oxidase activities of ua*Y alleles**

Strains	Xanthine dehydrogenase			Xanthine dehydrogenase-cross reacting material			Urate oxidase		
	Noninduced	Induced with uric acid	Induced with 2-thiouric acid	Noninduced	Induced with uric acid	Induced with 2-thiouric acid	Noninduced	Induced with uric acid	Induced with 2-thiouric acid
Wild type	12	100	131	11	100	122	8	100	106
*ua*Y5	14	13	9	11	9	7	6	4	6
*ua*Y7	12	13	14	10	9	11	2	2	2
*ua*Y9	13	11	10	12	9	11	6	2	5
*ua*Y109	16	31	26	22	27	36	5	78	82
*ua*Y207	12	15	14	11	11	11	2	2	3

* All strains carry the *bi*A1 marker resulting in biotin auxotrophy. The origin of the different *ua*Y mutations is described in MATERIALS AND METHODS. "Noninduced" grown for 20 hr on 5 mM urea, "induced with uric acid" grown on 5 mM urea and induced at 15 hr with 300 μM uric acid, "induced with 2-thiouric acid" grown on 5 mM urea and induced at 15 hr with 2.7 μM 2-thiouric acid. The specific activities are expressed in percent of the wild type induced with uric acid. This arbitrary value of 100 for induced wild type mycelia grown and assayed in parallel corresponds for the activities reported in this and the following tables to 43 $\pm$ 5.4 nanomoles of ammonia produced/min/mg of protein (adenine deaminase,) 30.1 $\pm$ 4.4 nanomoles of cytochrome c reduced/min/mg of protein (xanthine dehydrogenase) and 14.0 $\pm$ 1.3 nanomoles of uric acid oxidised/min/mg of protein (urate oxidase) (SEALY-LEWIS, SCAZZOCCHIO and LEE 1980).

TABLE 3

Enzyme activities of ua*Y alleles**

Mutation	Adenine deaminase		Xanthine dehydrogenase		Urate oxidase	
	Noninduced	Induced	Noninduced	Induced	Noninduced	Induced
*ua*Y+	4	100	12	100	3	100
*ua*Y2	7	2	10	7	1	1
*ua*Y4	2	6	14	16	1	1
*ua*Y5	5	4	14	10	2	1
*ua*Y6	3	6	8	8	2	1
*ua*Y7	2	7	12	14	3	4
*ua*Y8	7	3	12	16	4	4
*ua*Y12	7	18	3	2	2	2
*ua*Y205	3	2	12	11	1	2
*ua*Y207	5	1	9	12	2	1
*ua*Y109	3	29	9	27	2	63
*ua*Y110	5	47	6	39	1	70

* All procedures are as in Table 2 except that the results are expressed in percent of the wild type induced with 2.7 μM 2-thiouric acid, grown, extracted and assayed in parallel. "Induced" refers to induction with 2.7 μM 2-thiouric acid.

group (*ua*Y109 and *ua*Y110) are clearly less affected for the induction of urate oxidase than for adenine deaminase or xanthine dehydrogenase and its cross-reacting material.

Dominance: Heterozygous diploids carrying each of the 12 *ua*Y$^-$ mutations and the *ua*Y$^+$ allele were constructed. All the *ua*Y$^-$ mutations appeared recessive in growth tests and for 2-thioxanthine and 2-thiouric acid resistance; all heterozygous diploids can utilize adenine, hypoxanthine, xanthine, or uric acid as nitrogen source, and are sensitive to 2-thioxanthine and 2-thiouric acid. In each case the relevant *ua*Y$^-$/*ua*Y$^-$ homozygous diploids and a *ua*Y$^+$/*ua*Y$^+$ diploid were included as controls in the same plate as each *ua*Y$^-$/*ua*Y$^+$ heterozygous diploid.

Table 4 shows the adenine deaminase, xanthine dehydrogenase, and urate oxidase activities of all 12 heterozygous diploids noninduced or induced with 2-thiouric acid. Most of the *ua*Y$^-$/*ua*Y$^-$ homozygous diploids are also included as controls in Table 4. For all three enzymes the heterozygous diploids show levels intermediate between the *ua*Y$^-$/*ua*Y$^-$ and *ua*Y$^+$/*ua*Y$^+$ homozygous diploids; there seems to be strict dependence on the dosage of the *ua*Y product.

Complementation: Preliminary crosses showed that all the remaining 11 *ua*Y$^-$ mutations map at less than 0.2 centimorgans from *ua*Y9. *ua*Y9 was mapped in linkage group VIII between *cbxC* (carboxin resistance, GUNATILLEKE, ARST and SCAZZOCCHIO 1975) and *pfa*D (*p*-fluorophenylalanine resistance, SINHA 1969).

We have stated that *ua*Y9, while complementing in diploids with mutations in three genes under putative *ua*Y control, *hx*A1, *hx*B13 and *ua*Z11, fails to complement in heterokaryons (SCAZZOCCHIO and DARLINGTON 1967). Complementation with these three mutations has been investigated for *ua*Y5 and *ua*Y7. These *ua*Y alleles behave exactly as *ua*Y9. This pattern seems to be a general

TABLE 4

Enzyme activities of ua*Y*+/ua*Y*⁻ *diploids**

Diploid	Adenine deaminase		Xanthine dehydrogenase		Urate oxidase	
	Noninduced	Induced	Noninduced	Induced	Noninduced	Induced
*ua*Y+/*ua*Y+	2	100	12	100	3	100
*ua*Y2/*ua*Y2	2	3	8	10	3	4
*ua*Y2/*ua*Y+	1	37	9	38	4	44
*ua*Y4/*ua*Y+	2	33	6	40	12	66
*ua*Y5/*ua*Y5	2	5	11	12	3	2
*ua*Y5/*ua*Y+	2	19	2	40	8	53
*ua*Y6/*ua*Y6	3	3	12	13	3	2
*ua*Y6/*ua*Y+	3	26	16	67	3	35
*ua*Y7/*ua*Y7	1	2	5	6	2	2
*ua*Y7/*ua*Y+	2	33	14	27	2	36
*ua*Y8/*ua*Y8	1	1	6	7	1	1
*ua*Y8/*ua*Y+	2	38	14	37	3	40
*ua*Y9/*ua*Y9	4	4	11	12	3	5
*ua*Y9/*ua*Y+	2	38	6	32	2	36
*ua*Y12/*ua*Y12	2	2	15	10	3	2
*ua*Y12/*ua*Y+	2	27	11	36	3	36
*ua*Y205/*ua*Y+	5	56	9	52	3	31
*ua*Y207/*ua*Y207	1	3	8	9	2	2
*ua*Y207/*ua*Y+	4	65	8	65	3	67
*ua*Y109/*ua*Y109	4	24	13	32	3	54
*ua*Y109/*ua*Y+	1	52	9	59	4	87
*ua*Y110/*ua*Y+	2	29	6	29	2	82

* The enzyme specific activities are expressed in percent of a *ua*Y+ homozygous diploid induced with 2.7 μM 2-thiouric acid. "Noninduced" mycelia grown for 20 hr on 5 mM urea, "induced" grown of 5 mM urea for 20 hr and induced after 15 hr growth with 2.7 μM 2-thiouric acid. Some of the diploids were also tested after induction with the natural inducer (300 μM uric acid) with substantially the same results.

characteristic of *ua*Y mutations and could be interpreted either as a genuine nuclear restriction or as a result of the dose effect seen in Table 4 (SCAZZOCCHIO and DARLINGTON 1967; SCAZZOCCHIO, HOLL and FOGUELMAN 1973). This apparent nuclear restriction makes it necessary to investigate the complementation of mutations that show the *ua*Y phenotype in diploids rather than heterokaryons.

A complete diploid complementation grid among the 12 *ua*Y mutations in this work would involve obtaining 66 heterozygous diploids. We failed to obtain *ua*Y205/*ua*Y4, *ua*Y205/*ua*Y207, *ua*Y109/*ua*Y205, *ua*Y110/*ua*Y205, and *ua*Y109/*ua*Y207. The remaining 61 heterozygous diploids were tested for growth on adenine, hypoxanthine, xanthine and uric acid as sole nitrogen source, and for sensitivity to 2-thioxanthine and 2-thiouric acid. Each *ua*Y⁻/*ua*Y⁻ heterozygous diploid was tested on the same plates with both relevant *ua*Y⁻/*ua*Y⁻ homozygous diploids, both relevant *ua*Y⁻/*ua*Y+ heterozygous diploids and a *ua*Y+/*ua*Y+ homozygous diploid. All *ua*Y⁻/*ua*Y⁻ heterozygous pairs that comprise *ua*Y2, *ua*Y4, *ua*Y5, *ua*Y6, *ua*Y7, *ua*Y8, *ua*Y9, *ua*Y12, *ua*Y205 and *ua*Y207 failed to grow on any of the nitrogen sources or to show 2-thioxanthine or 2-thiouric acid sensitivity. As mentioned, *ua*Y109 and *ua*Y110 only grow marginally better

than the nonleaky mutations on adenine, hypoxanthine, or xanthine; this allows complementation to be scored in diploids carrying these alleles. Pairs comprising *ua*Y109 or *ua*Y110 and any of the other nonleaky *ua*Y mutations grew somewhat less than *ua*Y109/*ua*Y109 or *ua*Y110/*ua*Y110 homozygous diploids and were resistant to 2-thioxanthine and 2-thiouric acid. The heterozygous diploid *ua*Y109/*ua*Y110 grew as leakily on uric acid as the corresponding homozygous diploids and failed to show complementation on any other medium. All diploids grew normally on urea and ammonium d-tartrate as nitrogen sources.

Thus, no complementation is apparent between any two mutations that show either the complete or the partial (*ua*Y109 and *ua*Y110) *ua*Y$^-$ phenotype.

Constitutivity of oxp*A5*: Table 5 shows that the mutation *oxp*A5 results in partial constitutivity of adenine deaminase, xanthine dehydrogenase and its cognate cross-reacting material, and urate oxidase.

Further induction is afforded by both the natural inducer uric acid and its analogue 2-thiouric acid. The introduction of mutations resulting in loss of xanthine dehydrogenase activity, *hx*A1 mapping in the structural gene (SCAZZOCCHIO and SEALY-LEWIS 1978) and *hx*B13 (SCAZZOCCHIO 1973) result in loss of the constitutive phenotype for adenine deaminase and urate oxidase. The *hx*B13 mutation maps in a gene necessary for xanthine dehydrogenase activity,

TABLE 5

The constitutivity of oxp*A5*

	Wild type	*oxp*A5	*hx*B13	*oxp*A5*hx*B13
Adenine deaminase				
Noninduced	6	90	15	20
Induced with uric acid	100	128	109	113
Induced with 2-thiouric acid	127	156	135	148
Xanthine dehydrogenase				
Noninduced	13	61	0	0
Induced with uric acid	100	95	0	0
Induced with 2-thiouric acid	134	152	0	0
Xanthine dehydrogenase cross-reacting material				
Noninduced	15	54	6	10
Induced with uric acid	100	87	84	68
Induced with 2-thiouric acid	117	140	116	102
Urate Oxidase				
Noninduced	5	54	4	7
Induced with uric acid	100	82	93	98
Induced with 2-thiouric acid	146	150	127	117

* All strains carry the *bi*A1 marker resulting in biotin auxtrophy. Specific activities are expressed in per cent of the wild type induced with uric acid assayed, grown, extracted and assayed in parallel with each set of determinations. Each value is an average of at least three determinations. Xanthine dehydrogenase cross-reacting material determined as in Table 2. "Noninduced" were grown for 20 hr on 5 mM urea; "induced with uric acid," grown on 5 mM urea and induced at 15 hr with 300 μM uric acid; "induced with 2-thiouric acid," grown on 5 mM urea and induced at 15 hr with 2.7 μM 2-thiouric acid. Analogous results to that for *bi*A1 *hx*B13 *oxp*A5 were obtained for a number of *hx*B13 *oxp*A5 double mutants arising from the same cross and for *hx*A1 *oxp*A double mutants (only urate oxidase assayed, results not shown).

but does not affect the presence of the xanthine dehydrogenase protein (SCAZZOCCHIO 1973, 1980). This allows us to establish that the constitutivity of this protein also depends on the presence of xanthine dehydrogenase activity.

The double mutants *hx*A1 *oxp*A5 and *hx*B13 *oxp*A5 conserve the resistance to oxypurinol toxicity, which indicates that the constitutivity of the enzymes of the purine degradation pathway is not a prerequisite for the expression of the resistance. This is also true in the cases of *hx*B20 *oxp*A5 double mutants and strains carrying *oxp*A5 plus any of the following cofactor mutations leading to loss of xanthine dehydrogenase activity (PATEMAN *et al.* 1964): *cnx*A9, *cnx*C5, *cnx*F8, *cnx*G4, and *cnx*H3.

Cosegregation of the constitutive phenotype with the oxypurinol resistance: A strain carrying *oxp*A5 was crossed to a wild type and 455 progeny were scored for oxypurinol resistance and the constitutivity of xanthine dehydrogenase and urate oxidase by the *in situ* staining technique. No exception to the cosegregation of the three phenotypes was observed.

While these results do not exclude the possibility that the resistance and the constitutivity result from tightly linked mutations rather than from the same mutational event, another experiment makes this interpretation unlikely. Using the *in situ* staining for xanthine dehydrogenase, three colonies showing the constitutive phenotype were isolated from approximately 28,000 colonies screened after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The three strains are oxypurinol resistant and one of them, when crossed to *oxp*A5, gave no wild-type recombinants in the 350 progeny tested. It is thus probable that the same mutational event results in constitutivity and in oxypurinol resistance.

Recessivity of oxp*A5 and complementation of* oxp*A5 and* ua*Y mutations*: As *oxp*A5 was shown to be very tightly linked to *ua*Y alleles, complementation between *oxp*A5 and *ua*Y2, *ua*Y4, *ua*Y5, *ua*Y6, *ua*Y7, *ua*Y9, *ua*Y12, *ua*Y109, *ua*Y110, *ua*Y205 and *ua*Y207 was tested. The *oxp*A5 *ua*Y$^+$/*oxp*A$^+$ *ua*Y$^-$ diploids were constructed and tested on adenine, hypoxanthine, xanthine and uric acid as sole nitrogen sources, for 2-thioxanthine and 2-thiouric acid resistance and for resistance to oxypurinol. They were compared in each case with the corresponding *ua*Y$^-$ *oxp*A$^+$/*uaY*$^-$ *oxp*A$^+$; *ua*Y$^+$ *oxp*A5/*ua*Y$^+$ *oxp*A5; *ua*Y$^+$ *oxp*A$^+$/*ua*Y$^+$ *oxp*A$^+$ homozygous diploids and *ua*Y$^-$ *oxp*A$^+$/*ua*Y$^+$ *oxp*A$^+$ and *ua*Y$^+$ *oxp*A5/*ua*Y$^+$ *oxp*A$^+$ heterozygous diploids. These growth tests showed that the *oxp*A5 mutation was completely recessive (*i.e.*, a *uaY*$^+$ *oxp* A5/*ua*Y$^+$ *oxp*A$^+$ diploid is as sensitive to oxypurinol as the *ua*Y$^+$ *oxp*A$^+$/*ua*Y$^+$ *oxp*A$^+$ wild-type homozygous diploid), and that all the *ua*Y$^-$ mutations complement with *oxp*A5. All heterozygous diploids grow on adenine, hypoxanthine, xanthine and uric acid as nitrogen sources and are sensitive to 2-thioxanthine, 2-thiouric acid and oxypurinol.

Table 6 shows that the constitutivity resulting from the *oxp*A5 mutation is completely recessive to the wild type and that in no case is *oxp*A5 dominant to a *ua*Y$^-$ mutation, *i.e.*, in no case a *ua*Y$^+$ *oxp*A5/*ua*Y$^-$ *oxp*A$^+$ heterozygous diploid is constitutive. In most cases, *ua*Y$^-$ *oxp*A$^+$/*ua*Y$^+$ *oxp*A5 heterozygous diploids show a dose effect *vis-à-vis* inducibility of the three enzymes tested; the fully-induced level is lower than a *ua*Y$^+$ *oxp*A5/*ua*Y$^+$ *oxp*A$^+$ control.

TABLE 6

Complementation of oxp*A5 and* ua*Y⁻ mutations**

Diploid	Adenine deaminase Noninduced	Adenine deaminase Induced	Xanthine dehydrogenase Noninduced	Xanthine dehydrogenase Induced	Urate oxidase Noninduced	Urate oxidase Induced
*ua*Y+*oxp*A+/*ua*Y+*oxp*A+	2	10	14	100	3	100
*ua*Y+*oxp*A5/*ua*Y+*oxp*A5	70	150	73	158	41	130
*ua*Y+*oxp*A5/*ua*Y+*oxp*A+	2	70	13	81	3	78
*ua*Y+*oxp*A5/*ua*Y2*oxp*A+	1	23	6	54	2	34
*ua*Y+*oxp*A5/*ua*Y4*oxp*A+	2	31	14	38	2	36
*ua*Y+*oxp*A5/*ua*Y5*oxp*A+	2	89	15	70	3	48
*ua*Y+*oxp*A5/*ua*Y6*oxp*A+	1	29	6	67	2	48
*ua*Y+*oxp*A5/*ua*Y7*oxp*A+	2	32	16	72	4	43
*ua*Y+*oxp*A5/*ua*Y9*oxp*A+	2	78	10	81	4	40
*ua*Y+*oxp*A5/*ua*Y12*oxp*A+	6	89	9	90	4	51
*ua*Y+*oxp*A5/*ua*Y205*oxp*A+	2	62	17	75	3	26
*ua*Y+*oxp*A5/*ua*Y207*oxp*A+	4	43	8	41	3	31
*ua*Y+*oxp*A5/*ua*Y109*oxp*A+	1	75	7	78	5	84
*ua*Y+*oxp*A5/*ua*Y110*oxp*A+	1	54	6	61	6	71

* All procedures are as in Table 2 except that the results are expressed in percent of the wild-type diploid induced with 2.7 μM 2-thiouric acid, grown and assayed in parallel. "Induced" refers to induction with 2.7 μM 2-thiouric acid. Only the relevant genotype of the diploids is included, forcing markers have been omitted.

Fine structure map indicating that oxp*A5 maps to the left of 6* ua*Y⁻ mutations*: Preliminary crosses showed the order of markers in the region of linkage group VIII comprising *ua*Y to be *cnx*B, *cbx*C, *ua*Y, *fpa*D. The distance between *cbx*C and *ua*Y was approximately 0.5 cM and the distance between *ua*Y and *fpa*D was approximately 6 cM. In preliminary crosses, *oxp*A5 mapped approximately 0.1 cM from *ua*Y9, and between *cbx*C and *fpa*D (*cbx*C carboxin resistance, GUNATILLEKE, ARST and SCAZZOCCHIO 1975; *fpa*D *p*-fluorophenylalanine resistance, SINHA 1969). To establish the relative order of *oxp*A5 and *ua*Y2, *ua*Y5, *ua*Y8, *ua*Y109 and *ua*Y110 the following strategy was used. The *cbx*C34 *oxp*A5 and *fpa*D43 *ua*Y⁻ double mutants were constructed by crossing. Recombinants in the *cbx*C34-*pfa*D43 interval were selected by plating ascospores from crossed fruiting bodies on minimal medium containing urea as nitrogen source and 50 μg/ml carboxin and 10 μg-ml *p*-fluorophenylalanine. Only events that result from an odd number of crossovers in this interval are detected by this procedure.

Let *ua*Yi be any one *ua*Y⁻ mutation. If the order is *cbx*C34, *oxp*A5 *ua*Yi *fpa*D43 single crossovers could yield *oxp*A⁺ *ua*Y⁻, *oxp*A⁻ *ua*Y⁻ and *oxp*A⁻ *ua*Y⁺ progeny but a triple crossover would be necessary to obtain *oxp*A⁺ *ua*Y⁺ progeny. Conversely, if the order is *cbx*C34 *ua*Yi *oxp*A5 *fpa*D43 the triple crossover class would be *ua*Y⁻ *oxp*A⁻. We did not know *a priori* whether a *ua*Y⁻ *oxp*A⁻ recombinant could be distinguished phenotypically from *ua*Y⁻ *oxp*A⁺, but the presence or absence of *ua*Y⁺ *oxp*A⁺ wild-type recombinants would have sufficed to distinguish between the two orders. However, *ua*Y⁻ *oxp*A⁻ recombinants can be recognized, making the results unequivocal. While *ua*Y⁻ mutations show a slight resistance to oxypurinol, strains with the *ua*Y⁻ phenotype, but also showing high resistance to oxypurinol characteristic of *oxp*A5 strains, appeared in

every cross and were easily distinguished from *ua*Y$^-$ *oxp*A$^+$ strains. In several cases the genotype of the double mutants was confirmed by outcrossing and recovering *ua*Y$^+$ *oxp*A$^-$ strains. The strategy for the mapping of *oxp*A is illustrated in Figure 2.

Table 7 gives the results of the six crosses. These crosses establish for each allele the order *cbx*C34 *oxp*A5 *ua*Yi *fpa*D43. Taking 4.8 cM as the unweighted mean distance between *cbx*C34 and *fpa*D43 the recombination frequency between *cbx*C34 and *oxp*A5 would be 0.3 cM and the mean recombination frequency between *oxp*A5 and *ua*Y 0.5 cm.

Fine structure mapping, the order of the ua*Y$^-$ alleles*: The *ua*Y alleles were placed in a linear order by crosses involving external markers in a manner analogous to that used by PRITCHARD (1955) in his classical mapping of the *ad*E locus of *A. nidulans*. We isolated strains carrying *ua*Y2, *ua*Y5, *ua*Y109 and *ua*Y110 in coupling with *cbx*C34, *oxp*A5 and *fpa*D43 (*cbx*C carboxin resistance, *oxp*A oxypurinol resistance, *fpa*D *p*-fluorophenylalanine resistance, above). These were crossed to *cbx*C$^+$ *oxp*A$^+$ *fpa*D$^+$ strains carrying a different *ua*Y mutation. The *ua*Y$^+$ recombinants were selected on minimal medium with the appropriate supplements and hypoxanthine as sole nitrogen source on which *ua*Y$^-$ mutants do not grow. The order of the genes in this region of linkage group VIII is *cbx*C *oxp*A *ua*Y *fpa*D (see above). Let *ua*Yi be a mutation in coupling with *cbx*C34, *oxp*A5, and *fpa*D43 and *ua*Yi, a different *ua*Y mutation in repulsion. If the order is *cbx*C *oxp*A *ua*Yi *ua*Yj *fpa*D wild-type recombinants resulting from single crossover events will be *cbx*C$^+$ *oxp*A$^+$ *ua*Y$^+$ *fpa*D43; if the order is *cbx*C *oxp*A *ua*Yj *ua*Yi *fpa*D single crossover events in the same interval would result in strains of genotype *cbx*C34 *oxp*A5 *ua*Y$^+$ *fpa*D$^+$. In each case,

TABLE 7

Location of oxp*A5 relative to* ua*Y alleles*

*ua*Y allele	Recombination frequency between *cbx*C and *fpa*D	Total recombinants scored	Classes of recombinants Number recovered in each class			
			*ua*Y$^+$*oxp*A$^+$	*ua*Y$^+$*oxp*A5	*ua*Y$^-$*oxp*A$^+$	*ua*Y$^-$*oxp*A5
*ua*Y2	4.0	171	0	147	11	13
*ua*Y5	6.6	73	0	68	1	4
*ua*Y8	1.0	176	0	164	5	7
*ua*Y9	3.0	168	0	159	4	5
*ua*Y109	not tested	168	0	156	4	8
*ua*Y110	not tested	168	0	159	1	8

In each of the crosses the relevant *ua*Y allele was in coupling with *fpa*D43 [*para*-fluorephenilalanine resistance (SINHA, 1969)] and *oxp*A5 in coupling with *cbx*C34 [carboxin resistance (GUNATILLEKE, ARST and SCAZZOCCHIO 1975)]. Recombinants in the *cbx*C-*fpa*D interval were selected on minimal medium plates containing 10 μg/ml *para*-flurophenylalanine and 50 μg/ml carboxin. The recombinants were scored for *ua*Y$^+$ or *ua*Y$^-$ by their growth on hypoxanthine and uric acid as sole nitrogen sources and for *oxp*A$^+$ or *oxp*A5 by their resistance to 250 μg/ml oxypurinol on 10 mM nitrate as nitrogen source. The recombinants were also scored for the segregation of the markers used to establish the cross (results not shown). In four of the crosses the total number of progeny and thus the frequency of recombination in the *cbx*C-*fpa*D interval was determined by plating in parallel ascospores on selective medium and dilutions on minimal nonselective medium, supplemented for all relevant auxotrophies.

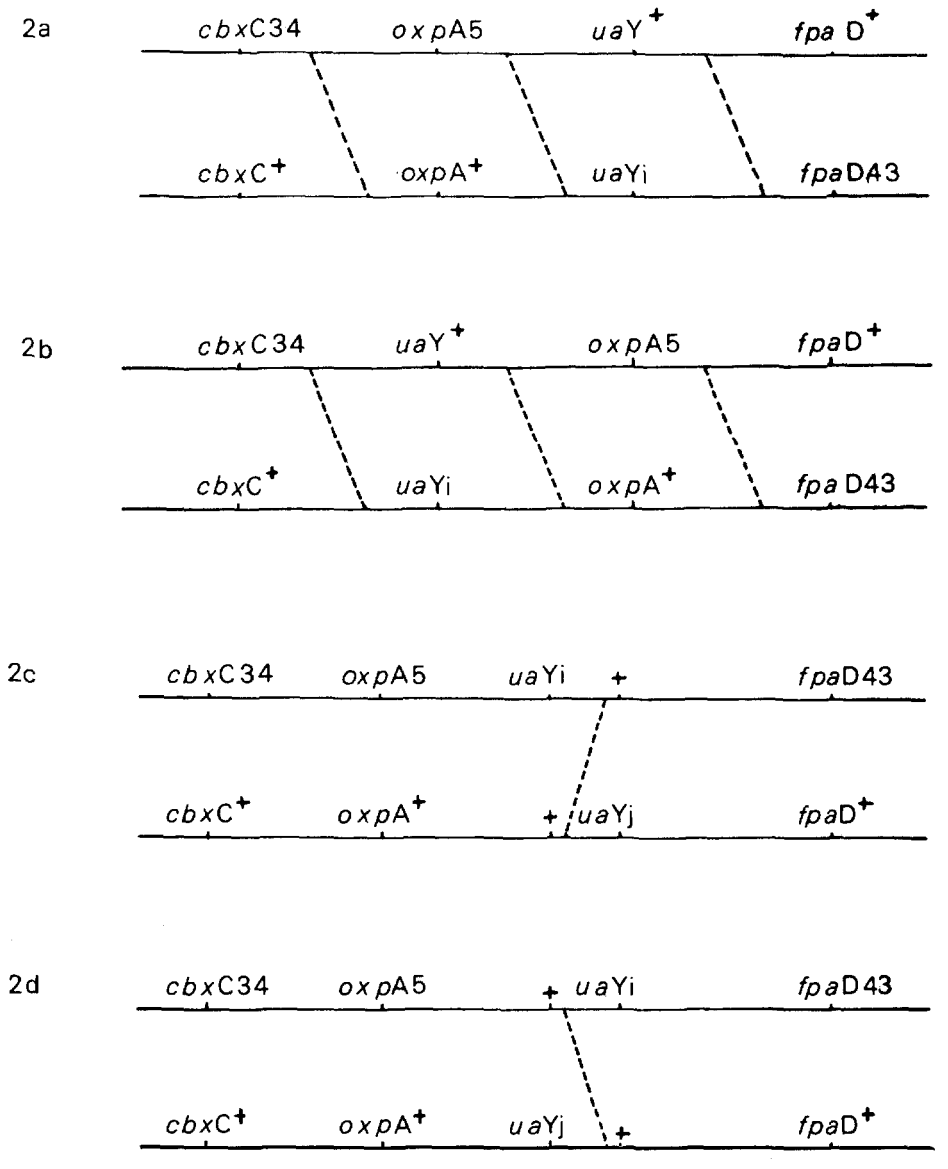

FIGURE 2.—*Strategy for the fine structure mapping.* Schemes 2a and 2b indicate the genotypes that arise from single crossovers in the *cbx*C34–*pfa*D43 intervals, selecting for the double resistant phenotype if a *ua*Yi allele maps to the left (2a) or to the right (2b) of *oxp*A5. Schemes 2c and 2d indicate the genotypes that arise from single crossover events between two *ua*Y alleles *ua*Yi and *ua*Yj when selecting for a *ua*Y+ recombinant when *ua*Yj maps to the left (2c) or to the right of *ua*Yi(2d); ua*Y*i is the allele in coupling with *cbx*C34 *oxp*A5 and *pfa*D43. In schemes 2a and 2b all markers have been placed arbitrarily at equal distances; in schemes 2c and 2d external markers have been placed arbitrarily at equal distances and the *ua*Y alleles arbitrarily closer together.

the reciprocal class would necessitate a triple cross-over event. This strategy is illustrated in Figure 2.

Gene conversion (and double crossover) events would result in strains with parental configuration of flanking markers (*cbx*C34 *oxp*A5 *ua*Y$^+$ *fpa*D43 or *cbx*C$^+$ *oxp*A$^+$ *ua*Y$^+$ *fpa*D$^+$). Only single crossover events are used to establish the order of any two markers. Possible reversion events (or wild-type contaminants) would contribute only to the gene conversion class and would not affect the establishment of an order. Nevertheless, to gain information on the pattern of gene conversion, we attempted to prevent contamination and monitored the reversion rate. No *ua*Y$^-$ allele tested to date reverts spontaneously with a frequency higher than 1×10^{-7}. The reversion rate was also investigated in crosses homozygous for *ua*Y2, *ua*Y5, *ua*Y109 and *ua*Y110 and heterozygous for the external markers. Between 620,000 and 1,755,000 progeny were plated with no wild-type revertants recovered. For clarity, we assumed in the above argument that *oxp*A5 maps to the left of every *ua*Y$^-$ allele. This has been rigorously established previously only for *ua*Y2, *ua*Y5, *ua*Y8, *ua*Y9, *ua*Y109 and *ua*Y110. If in any cross the order is *ua*Yj *oxp*A5 *ua*Yi two types of *ua*Y$^+$ recombinants could be obtained, with, respectively, a parental and a recombinant configuration of *cbx*C and *oxp*A alleles. In all the crossover progeny scored in all the crosses performed, *oxp*A and *cbx*C alleles were in a parental configuration, placing *oxp*A5 to the left of all *ua*Y alleles tested (*vide infra*).

Thirty-four crosses were performed in which a given *ua*Y allele in coupling with *cbx*C$^+$, *oxp*A$^+$ and *fpa*D$^+$ was crossed with either *ua*Y2, *ua*Y5, *ua*109 or *ua*Y110, these alleles in coupling with *cbx*C34, *oxp*A5 and *fpa*D43. These crosses allowed us to place any *ua*Y allele in one of the five intervals defined by the four "test" mutations. A sample of these crosses is shown in Table 8. The single crossover classes generate a consistent unambiguous order of the *ua*Y alleles, as shown in Figure 3. Six alleles, *ua*Y110, *ua*Y5, *ua*Y7, *ua*Y2, *ua*Y109 and *ua*Y12 have been placed in a linear order. Three alleles, *ua*Y8, *ua*Y205 and *ua*Y207 map to the left of *ua*Y110, *ua*Y6 and *ua*Y9 map in between *ua*Y5 and *ua*Y109 while *ua*Y4 is anywhere to the left of *ua*Y2. The following points can be established:

1. It is possible to locate unambiguously, by conventional crosses with flanking markers on both sides, every *ua*Y allele. The map derived from the procedure detailed above (MATERIALS AND METHODS) is without exception self-consistent. In each cross only one class of *ua*Y$^+$ recombinants showing recombination of external markers has been recovered. This was true even when a considerable number of recombinants were scored (*ua*Y2 $\times$ *ua*Y207, *ua*Y5 $\times$ *ua*Y205, *ua*Y109 $\times$ *ua*Y2). This and the self-consistency of the map allowed us to order two alleles (*ua*Y5 and *ua*Y7) on the basis of only one recombinant obtained in crosses with *ua*Y110 and *ua*Y5 respectively.

2. Recombination frequencies are not a useful parameter for fine structure mapping in this system. While some agreement between the map in Figure 3 and a qualitative map derived from recombination frequencies was found (for example, in each set of crosses *ua*Y12 would be the more distal allele to the right), there were some inconsistencies between the allele order determined

TABLE 8

Recombination data from heteroallelic ua*Y*⁻ *crosses**

Allele	"Tester" allele	Total progeny analyzed	Total no. *ua*Y+ recombinants	Single crossover events *cbx*C34 *oxp*A5 *fpa*D+	Single crossover events *cbx*C+ *oxp*A+ *fpa*D43	Gene conversion events Of "tester" allele *cbx*C34 *oxp*A5 *fpa*D43	Gene conversion events Of "tested" allele *cbx*C+ *oxp*A+ *fpa*D+	Relative order
*ua*Y7	*ua*Y2	149,000	4	2	0	0	2	*ua*Y7 — *ua*Y2
*ua*Y12	*ua*Y2	23,000	16	0	14	0	2	*ua*Y2 — *ua*Y12
*ua*Y205	*ua*Y2	56,000	14	7	0	0	7	*ua*Y205 — *ua*Y2
*ua*Y207	*ua*Y2	313,000	32	25	0	7	0	*ua*Y207 — *ua*Y2
*ua*Y9	*ua*Y5	28,000	4	0	3	1	0	*ua*Y5 — *ua*Y9
*ua*Y205	*ua*Y5	281,000	23	14	0	0	9	*ua*Y205 — *ua*Y5
*ua*Y207	*ua*Y5	174,000	5	5	0	0	0	*ua*Y207 — *ua*Y5
*ua*Y2	*ua*Y109	175,000	25	12	0	6	7	*ua*Y2 — *ua*Y109
*ua*Y8	*ua*Y109	9,400	7	3	0	4	0	*ua*Y8 — *ua*Y109
*ua*Y207	*ua*Y109	160,000	14	9	0	5	0	*ua*Y207 — *ua*Y109
*ua*Y8	*ua*Y110	334,000	5	2	0	3	0	*ua*Y8 — *ua*Y110
*ua*Y12	*ua*Y110	11,400	10	0	7	1	2	*ua*Y110 — *ua*Y12

* This table presents some of the 34 crosses analyzed between different *ua*Y⁻ alleles. "Allele" indicates the one in repulsion with the *cbx*C34 *oxp*A5 *fpa*D43 markers while "tester allele" indicates the allele in coupling with the external mutant markers thus defining five mapping intervals.

A "tester strain" *cbx*C34 *oxp*A5 *ua*Yi *fpa*D43 was crossed with another strain carrying any *ua*Y allele; *ua*Yi was either *ua*Y2, *ua*Y5, *ua*Y109 or *ua*Y110. Suitable nutritional markers to force the cross were included in both parents. The complete results can be obtained from the senior author and are on file with the editor of GENETICS.

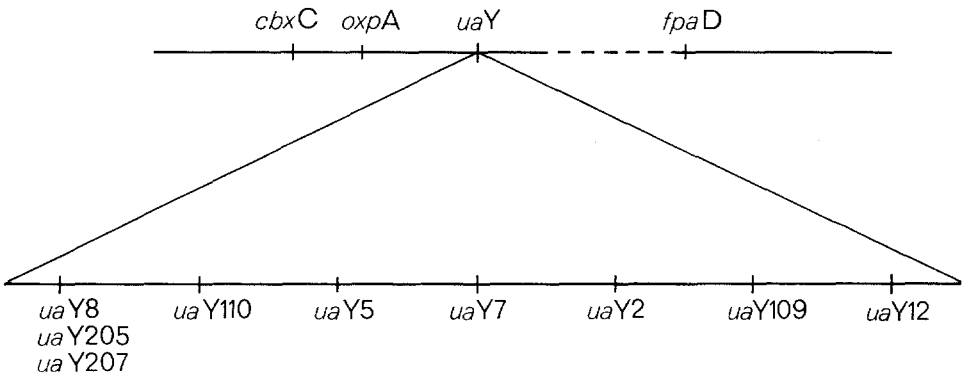

FIGURE 3.—*Fine structure map of the* ua*Y gene.* The *ua*Y alleles have been placed in the order derived from 34 crosses described in the text and in the legend to Table 8. The alleles have been placed equidistantly, ignoring recombination frequencies (see text). The *ua*Y8, *ua*Y205 and *ua*Y207 map to the left of *ua*Y110 (Table 4) and have not been mapped in relation to each other; *ua*Y6 and *ua*Y9 have not been mapped in relation to *ua*Y2 and have thus been positioned between *ua*Y5 and *ua*Y109. The *ua*Y4 has only been mapped in relation to *ua*Y2 and *ua*Y109 and can map anywhere to the left of *ua*Y2. The left of the drawing indicates arbitrarily the *cbx*C *oxp*A side (centromere proximal) and the right the *fpa*D side (centromere distal).

using the reassortment of external markers and recombination frequencies. Thus we ignored recombination frequencies and derived qualitatively the map order shown in Figure 3 exclusively from the class of progeny showing recombination of external markers in each cross.

3. As anticipated, all *ua*Y$^-$ alleles map to the right of *oxp*A5. We have shown that *ua*Y8 maps to the right of *oxp*A5. The only alleles that could map to the left of *ua*Y8 are *ua*Y4, *ua*Y205 and *ua*Y207. Thus, only these three alleles could map to the left of *oxp*A5. *ua*Y4 was crossed to *ua*Y2 and *ua*Y109; a total of nine *ua*Y$^+$ recombinants showing reassortment of external markers was obtained. In all of these the *cbx*C and *oxp*A markers remained in the parental configuration. Strains carrying *ua*Y205 and *ua*Y207 have been crossed to the four mapping strains and a total of 28 and 41 *ua*Y$^+$ strains showing recombination of external markers were obtained, respectively. In all cases the *cbx*C and *oxp*A markers remained in the parental configuration, providing evidence that these alleles map also to the right of *oxp*A5.

4. *ua*Y207 is a nonrevertible allele (HARTLEY 1969); it recombines with *ua*Y110 and maps to the left of it. Assuming that the mutant alleles represent a random population along the gene, if *ua*Y207 is a deletion it is probably a relatively short one.

5. The two mutations that show a partial *ua*Y phenotype, *ua*Y109 and *ua*Y110 are clearly within the *ua*Y gene. They do not seem to be clustered as they are separated by at least five different sites able to mutate to the more stringent *ua*Y phenotype.

6. In most crosses, besides *uaY*$^+$ recombinants showing reassortment of external markers, a class of *ua*Y$^+$ strains nonrecombinant for external markers was found. We defined such recombinants operationally as belonging to a "gene

conversion" class, without prejudgement as to the mechanism underlying their appearance.

DISCUSSION

Twelve *ua*Y alleles have been characterized for their adenine deaminase, xanthine dehydrogenase and urate oxidase activities. The ten nonleaky alleles *ua*Y2, 4, 6, 7, 8, 9, 12, 205 and 207 show uniform noninducibility of the three enzymes. In contrast *ua*Y109 and *ua*Y110 are somewhat inducible for adenine deaminase and xanthine dehydrogenase, but highly inducible for urate oxidase. Preliminary results of PERMAUL and SCAZZOCCHIO (unpublished) suggested that adenine deaminase was noninducible in *ua*Y$^-$ mutations. This has now been confirmed for all 12 *ua*Y alleles. All mutations result in similar impairment in uptake, as judged qualitatively by resistance to 2-thiouric acid.

The "leaky" alleles *ua*Y109 and *ua*Y110 are particularly interesting. Fine structure mapping established that *ua*Y109 and *ua*Y110 map within the *ua*Y gene and are not clustered, as they are separated by *ua*Y5, *ua*Y7, *ua*Y2, *ua*Y6 and *ua*Y9. An interpretation of the properties of these alleles would be that they are affected in the DNA (or RNA) binding site of the *ua*Y protein, the specific differences in inducibility being related to differences in the *cis*-acting regions adjacent to the structural genes.

The intermediate levels of enzyme activity in *ua*Y$^-$/*ua*Y$^+$ diploids are indicative of a dose effect and provide no evidence of the mode of control. In fact, when coupled with a dose effect, either a positive or a negative mode of control could give the results in Tables 4 and 5. A similar dose effect was found by COVE (1969) for the *nir*A gene and by SCAZZOCCHIO, HOLL and FOGUELMAN (1973) for the *apl*A gene of *Aspergillus nidulans*.

The phenotype of deletions in the *ua*Y gene, the frequency of mutations to the *ua*Y$^-$ phenotype and the reversion pattern of *ua*Y point mutants show that the *ua*Y$^-$ phenotype arises from loss of function, i.e. that *uaY*$^+$ acts by eliciting rather than preventing the expression of structural genes (SCAZZOCCHIO unpublished). The dominance data for our 12 alleles are thus consistent with a positive mode of control in which the concentration of *ua*Y regulatory protein in heterozygous diploids is lower than needed to elicit the full expression of two sets of structural genes.

In previous work (DARLINGTON, SCAZZOCCHIO and PATEMAN 1965; ALDERSON and SCAZZOCCHIO 1967; ALDERSON and HARTLEY 1969) mutations were assigned to the *ua*Y gene on the basis of a double stab heterokaryon complementation test. This is invalid as the action of *ua*Y is limited to the nucleus (SCAZZOCCHIO and DARLINGTON 1967; SCAZZOCCHIO and ARST 1978). In this publication we have shown that the 12 alleles tested belong to the same complementation group. Our complementation grid included two alleles (*ua*Y109 and *ua*Y110) separable by recombination that necessarily have a partially functional *ua*Y product. Intracistronic complementation could be expected if the *ua*Y product were present as an oligomer as is the case for the *lac* (RIGGS and BOURGEOIS 1968) and lambda and phage 434 repressors (PIROTTA, CHADWICK and PTASHNE 1970). Our

complementation grid provides strong evidence that mutations with either the "unleaky" or "leaky" *ua*Y phenotypes represent events that affect the same gene; the absence of intracistronic complementation is not significant in a sample of this size.

The nature of the pseudoconstitutive mutation *oxp*A5 merits some discussion.

Xanthine dehydrogenase catalyzes the oxidation of hypoxanthine to xanthine and of xanthine to uric acid. The necessity for an active xanthine dehydrogenase (purine hydroxylase I, LEWIS, *et al.* 1978) for the constitutive phenotype of *oxp*A5 to be expressed could be rationalized by proposing that a pleiotropic effect of this mutation was to make the control system respond to low concentrations of uric acid produced through the noninduced levels of xanthine dehydrogenase (approximately 10% of the wild type induced level; see for example Tables 3 and 6). As uric acid induces xanthine dehydrogenase a shift upward in the sensitivity to uric acid induction would result in production of more uric acid until a new steady state is achieved through the induction of urate oxidase. An equivalent proposal would be that *oxp*A5 modifies the control system making it sensitive to low concentrations of xanthine also produced *via* xanthine dehydrogenase catalysis.

An alternative possibility is that the same kind of autogenous regulation (GOLDBERGER 1974; COVE 1974) exists by which the xanthine dehydrogenase protein is involved in its own induction and that of the other enzymes of the pathway. A large number of xanthine dehydrogenase structural gene mutants are normally inducible for the other enzymes of the pathway (see for example DARLINGTON, SCAZZOCCHIO and PATEMAN 1965 and SCAZZOCCHIO and DARLINGTON 1968). The mutation used in this study, *hx*B13, is a totally unleaky, revertible, complementing, mutation mapping in the *hx*B gene (SEALY-LEWIS, LEE and SCAZZOCCHIO, unpublished). The *hx*B gene codes either for a small subunit common to both xanthine dehydrogenase and purine hydroxylase II (SCAZZOCCHIO, HOLL and FOGUELMAN 1973) or for a common post-translational modification (SCAZZOCCHIO 1980). The xanthine dehydrogenase present in *hx*B13 strains is immunologically identical to the wild-type protein, has the same molecular weight and conserves the ancillary NADH dehydrogenase activity. Even the less leaky *hx*B mutations seem to affect only the ability to hydroxylate the specific substrates (SCAZZOCCHIO 1980, SEALY-LEWIS, LEE and SCAZZOCCHIO, unpublished). Thus all the evidence points to the role of xanthine dehydrogenase in the expression of the constitutivity that results from the *oxp*A5 mutation as being entirely metabolic.

An attractive early hypothesis (discussed in COVE 1970) was that *oxp*A5 represents a mutation in *ua*Y resulting in tighter binding of uric acid (or xanthine). The tight linkage between *oxp*A5 and *ua*Y$^-$ mutations was thought to be consistent with this hypothesis. This hypothesis is difficult to reconcile with the data presented here. The mapping of *oxp*A5 to one side of every *ua*Y$^-$ allele tested is necessary but not sufficient evidence to place *oxp*A5 in a gene different from *ua*Y. The uniform complementation of *oxp*A5 with all the *ua*Y$^-$ alleles tested, provides compelling evidence for the existence of a new locus. If *oxp*A5 were a new kind of *ua*Y allele leading to altered function rather than to loss of

function it should be dominant to the wild type and at least to some *ua*Y alleles, noticeably to the putative deletion *ua*Y207, a mutation that results in the absence of any effector binding protein (PHILIPPIDES and SCAZZOCCHIO 1981).

We propose that *oxp*A5 defines a gene different from *ua*Y and possibly adjacent to it and that constitutivity and oxypurinol resistance are the pleiotropic expression of the same mutational event. The resistance to oxypurinol is not a result of the constitutivity of any enzyme or permease under *ua*Y control as even *oxp*A5 *ua*Y$^-$ double mutants are fully resistant. The fact that they are distinguishable from *oxp*A$^+$ *ua*Y$^-$ strains indicates that *oxp*A$^+$ is functional in *ua*Y$^-$ strains, in other words *oxp*A is not stringently under the control of *ua*Y. This result also argues against *oxp*A5 being a new kind of allele in the *ua*Y locus (*vide supra*).

The frequency of mutation of the *oxp*A$^-$ phenotype (whether selected by oxypurinol resistance or by constitutivity) and its complete recessivity to *oxp*A$^+$ indicate that both oxypurinol resistance and the constitutive phenotype are the result of loss rather than modification of function.

It can be proposed that the *oxp*A gene specifies a function resulting in a reduction of the local concentration of inducer in a cellular compartment to which the *ua*Y product is restricted. Nuclear limitation of the *ua*Y product has been proposed on the basis of entirely different evidence (SCAZZOCCHIO and DARLINGTON 1967; SCAZZOCCHIO and ARST 1978); and both uric acid and xanthine are toxic to the cell when allowed to accumulate (DARLINGTON and SCAZZOCCHIO 1967; LEE and SCAZZOCCHIO unpublished). If this hypothesis is correct, we need to explain why *oxp*A5 results in increased sensitivity to induction by endogenous uric acid (or xanthine) while resulting in efficient exclusion of the xanthine analogue oxypurinol. It could be argued that the target of oxypurinol toxicity lies outside the compartment containing the target of induction, *e.g.*, that induction occurs in the nucleus, while oxypurinol toxicity occurs in the cytoplasm. Alternatively, it could be proposed that a permeation mechanism acting in both directions, according to the concentration gradient, is absent in *oxp*A5, and that this results in accumulation of inducer(s) and exclusion of oxypurinol from the same cellular compartment. Previous work has established that uric acid but not xanthine is an efficient inducer of activities under *ua*Y control (SEALY-LEWIS, SCAZZOCCHIO and LEE 1978). Our *in vivo* experiments did not establish whether xanthine is an inefficient inducer because it is not a ligand of the *ua*Y protein or because it is efficiently excluded from the nucleus, while uric acid is not. The recent isolation of a protein likely to be coded by *ua*Y (PHILIPPIDES and SCAZZOCCHIO 1981) and the construction of *oxp*A5 strains completely blocked in xanthine breakdown (SEALY-LEWIS, SCAZZOCCHIO and LEE 1978) will allow a direct answer to this question.

The fine structure mapping of the *uaY* alleles show some preliminary trends bearing on the pattern of gene conversion within the *ua*Y gene. While in at least one case (*ua*Y109 × *ua*Y2) both alleles seem to convert with similar frequencies, in others strong bias is observed. The three "leftward" alleles—*ua*Y8, *ua*Y205 and *ua*Y207—tested with all the four "tester strains," could serve to illustrate this asymmetry. In crosses involving *ua*Y8, with the possible exception of

*ua*Y5 × *ua*Y8 where only one convertant was recovered, the other *ua*Y⁻ allele is converted to *ua*Y⁺ preferentially. When conversion progeny are recovered from a cross involving *ua*Y205 it is always *ua*Y205 that is found converted. Finally, *ua*Y207, a nonrevertible allele, is never converted, but all conversion events involve the other *ua*Y⁻ allele in the cross. It would be tempting to speculate that this lack of conversion is due to *ua*Y207 being a deletion, inversion, or some other gross alteration of the DNA sequence (*vide supra*). However, conversion of deletions in *Saccharomyces cerevisiae* has been observed in the *his4* and the *cyc1* loci (FINK and STYLES 1974; LAWRENCE *et al.* 1975).

The relative position of the alleles does not seem to bear a clear relation with the asymmetry of conversion. In some cases the bias favors the leftward (centromere proximal) allele and in some others the rightward (centromere distal) allele. This contrasts with the situation observed by PUTRAMENT, ROZBICKA and WOJCIECHOWSKA (1971) in the *meth*A gene of *A. nidulans* where the centromere distal allele converts predominantly.

The work presented here constitutes an attempt to completely characterize a eukaryotic regulatory gene. The isolation of effector binding protein likely to be coded by the *ua*Y gene is being published separately (PHILIPPIDES and SCAZZOCCHIO 1981).

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