REGULATION OF PHOSPHATE METABOLISM IN *NEUROSPORA CRASSA*. CHARACTERIZATION OF REGULATORY MUTANTS

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ABSTRACT

A mutant of *Neurospora crassa*, called *UW-6*, differs from wild type in being partially constitutive for synthesis of a species of alkaline phosphatase, and also for a species of phosphate permease that has a high affinity for phosphate at high pH. *UW-6* is possibly allelic with a mutant called *nuc-2* that was previously isolated by ISHIKAWA. *nuc-2* has the converse phenotype, in that it cannot be derepressed for either of these two activities. *UW-6* is co-dominant with its wild-type allele in heterokaryons and in partial diploids. An unlinked mutant, *nuc-1*, is like *nuc-2* in that it fails to make the alkaline phosphatase or the permease referred to above. *nuc-1* is epistatic to *UW-6* in the double mutant. The control of phosphorus metabolism is discussed, and is compared with some other control systems in filamentous fungi.

MUCH is known about the control of protein synthesis in bacteria. Advances in this area have depended heavily on the availability of mutants that display altered regulation of a particular enzyme or family of metabolically related enzymes. By contrast, we know very little about control of specific enzyme synthesis in eukaryotes, and there is still a dearth of systems that are amenable to genetic dissection.

We therefore decided to examine the control of alkaline phosphatase in Neurospora. The corresponding enzyme is highly repressible in various bacteria, and there is a considerable body of knowledge about its regulation (see, for example, GAREN and ECHOLS 1962a, 1962b; WILKINS 1972; PRATT and GALLANT 1973; GLENN and MANDELSTAM 1971). The enzyme is an attractive one to study because it is easily purified from most organisms and the assay is unusually simple. Studies of control of this enzyme in *Aspergillus nidulans* have resulted in a very complicated picture, involving many genes (DORN and RIVERA 1966; DORN 1968). The control of alkaline phosphatase has been examined in the basidiomycete *Coprinus lagopus* (NORTH and LEWIS 1971). Again, the results are quite hard to interpret by any simple model. In *Neurospora crassa*, a constitutive alkaline phosphatase can be demonstrated under all growth conditions (KUO and

BLUMENTHAL 1961; DAVIS and LEES 1969), but during growth on limiting phosphate, a separate, repressible enzyme is made in much larger amounts (NYC, KADNER and CROCKEN 1966; KADNER and NYC 1969). An analogous situation exists for the acid phosphatases (NYC 1967).

TOH-E and ISHIKAWA (1971) recently reported that two unlinked mutants of Neurospora, *nuc-1*, and *nuc-2*, that are unable to grow on nucleic acids as a phosphorus source are also unable to *derepress* the synthesis of alkaline and acid phosphatase in response to phosphate starvation. In the present paper we describe the isolation of a mutation, *UW-6*, that is unable to fully *repress* the synthesis of alkaline phosphatase on high phosphate medium though the specific activities are never as high as under conditions of phosphate starvation. This mutant is closely linked to *nuc-2*, and is hypostatic to *nuc-1*. *UW-6* is roughly co-dominant in heterokaryons. As we will develop in the discussion, there are intriguing parallels between the control of repressible alkaline phosphatase and the control of the family of sulfur-repressible enzymes.

MATERIALS AND METHODS

Strains: "Wild-type" *Neurospora crassa* used in these studies is the Oak Ridge stock, 74-OR8–1a, Fungal Genetics Stock Center (FGSC)# 988. All other strains were inbred to this or to an essentially isogenic strain of the opposite mating type, 74-OR23–1A (FGSC #986) for several generations so as to get stocks that were fully heterokaryon-compatible. *nuc-1* is allele T28-M1 (FGSC# 1994); *nuc-2* is allele T28-M2 (FGSC# 1998). The visible markers used are *cot-1* (a temperature-conditional colonial mutant), *al-2* (albino), *pe* (peach-colored conidia). *ad-3A* and *ad-3B* are closely-linked adenine-requiring mutants that are not allelic (FGSC# 662 and 677, respectively). *UW-6* was selected from an inbred strain of *cot-1*. For the identity of other strains used in this study, see METZENBERG and AHLGREN (1970).

Media and growth conditions: Crosses were made on the medium of WESTERGAARD and MITCHELL (1947). Amino acid and purine auxotrophs were furnished with the required supplement at a concentration of 1mM. Inositol was furnished to *inos* strains at 50 μg/ml. All repression studies were carried out in Fries minimal medium (cited in BEADLE and TATUM 1945), modified as described in the protocols of individual experiments. "Repression medium" contains 7.35 mM phosphate, and is unmodified Fries medium. "Derepression medium" contains KH_2PO_4 at only 0.05 mM, the deficit of potassium being made up by an equivalent amount of KCl. Cultures were inoculated and sparged with sterile air as described previously (METZENBERG 1968) for 15 hours at 25° except where otherwise indicated.

For scoring *UW-6 vs. UW-6+* (the wild-type allele), plates of Fries minimal medium with the sugar mixture of BROCKMAN and DE SERRES (1963) were spotted with conidial suspensions to be tested. After 2–3 days at room temperature, the plates were stained for alkaline phosphatase (TOH-E and ISHIKAWA 1971). *UW-6* growth centers started showing a purplish brown color in a few minutes, whereas *UW-6+* took a number of hours to give a comparable intensity of color.

nuc-1 and *nuc-2* were scored by spotting onto Fries 1.5% sucrose plates in which the phosphate was replaced by 100 μg./ml of *Torula* yeast RNA (TOH-E and ISHIKAWA 1971), or on plates of low phosphate (0.05 mM) Fries sucrose adjusted to pH 7 with 0.1M Na-MOPS (morpholinopropane sulfonic acid) buffer. Both mutants failed to grow on these media.

Extraction and assay of enzymes: Except where aryl sulfatase was to be assayed (see Table 4) harvested mycelia were always homogenized with alumina with a motor-driven pestle for 1 min. at 0°, the extraction medium being Na-acetate buffer, pH 5.0 (0.05 M). This pH destroys most of the constitutive alkaline phosphatase, which was not measured in these experiments (NYC, KADNER and CROCKEN 1966). Repressible alkaline phosphatase was assayed as described by the same authors, with p-nitrophenyl phosphate (5 mM), Na-glycine buffer, pH 9.0 (30 mM) and

ethylenediamine tetra-acetate (1 mM). The latter inhibits any residual constitutive alkaline phosphatase that survives the pH 5 extraction. The assay mixtures were incubated at 37° for 5–120 minutes and were ordinarily deproteinized with half the volume of 1 M KOH in 90% ethanol. When phosphatase assays were to be run on strains bearing *arom-1*, the protocatechuic acid accumulated by the latter turned brown in strong alkali and interfered with the determination of free nitrophenol. Therefore, these incubation mixtures were stopped and deproteinized with a mildly alkaline mixture of 6.5% (v/v) ethanolamine and 3% (v/v) sirupy phosphoric acid (85%) in 50% ethanol. The phosphate at this concentration inhibits the enzyme completely.

Protein in extracts was assayed as described by LOWRY *et al.* (1951), using bovine serum albumin as a standard. In experiments in which uptake of ^{32}P-phosphate was to be measured, an aliquot of washed young mycelia was centrifuged at 700 × g for 5 minutes and the pellet was taken up in the NaOH-Na_2CO_3 reagent of LOWRY *et al.* without copper tartrate. The suspension was left at room temperature for 16 hours to extract the protein. The residue was removed by centrifugation, and protein was assayed in an aliquot of the supernatant solution.

EXPERIMENTAL RESULTS

Isolation of UW-6: The parent strain was the temperature-conditional colonial mutant, *cot-1 a*. A suspension of conidia in water was irradiated with a UV germicidal lamp to about 99% killing and plated so as to give about 200–300 colonies per plate. The medium for plating contained sorbose (1%), and glucose and fructose (each 0.05%) (BROCKMAN and DESERRES 1963) and the salts base of Fries (cited in BEADLE and TATUM 1945) modified by reducing the concentration of KH_2PO_4 to 2 mM and substituting KCl for the remainder of the phosphate on an equimolar basis. In subsequent experiments, a medium containing unmodified Fries salts was used (KH_2PO_4 = 7.35 mM), as it gave essentially identical results. The plates were incubated for 2–3 days at the restrictive temperature (33°) and the plates, containing a total of about 5000 colonies, were irrigated with 2.5 ml. of a mixture of *p*-nitrophenyl phosphate (5 mM), Na-glycine buffer pH = 9.0 (300 mM) and ethylene diamine tetra-acetic acid (EDTA, 1 mM). After about 15 minutes, one bright yellow colony was seen. This was picked, purified by restreaking, and outcrossed to wild-type *A*. All of the work described in this paper was done with the *UW-6* mutation reisolated from this outcross.

Mapping of UW-6: UW-6 a was crossed to an *A* mating type strain that was marked on all seven linkage groups (*al-2 tryp-3 tyr-1 pdx-1 inos chol-2 thi-3 ars-101*). Analysis of random spore progeny showed that *UW-6* was weakly linked (about 30 centimorgans) to *tryp-3*, and therefore was on linkage group II. In a cross of UW-6 to *arg-12*, about 5% recombination was observed. A three-point cross (UW-6 *arg-12 a* × *pe A*) was made, and random spores were isolated and the resulting cultures were classified for the visible marker, *pe* (peach-colored conidia) and were scored for the other two markers. The results, given in Table 1, show that *UW-6* is about 3.8 centimorgans to the left of *pe*, which, in turn is just to the left of *arg-12*, in agreement wtih the results of GILES *et al.* (1967). In another three-point cross, *UW-6 arom-1 A* was mated to *arg-5 a*. Forty-five arg⁻ sporelings were picked and the cultures were scored. Thirty-eight cultures were *arg-5* parentals, five were *arg-5 UW-6 arom-1*, and two were *arg-5 arom-1*. None was *arg-5 UW-6*. The order of the genes deduced from these crosses is: *centromere, arg-5, UW-6, pe, arg-12, arom-1* (GILES *et al.* 1967).

TABLE 1

Mapping of UW-6 *by a three-point cross*

Class of segregant	Genotypes	Number
Parentals	*UW-6*+, *pe*⁻ , *arg-12*+	116
	UW-6 , *pe*+, *arg-12*⁻	135
Crossovers, Reg. 1	*UW-6*+, *pe*+, *arg-12*⁻	5
	UW-6 , *pe*⁻ , *arg-12*+	5
Crossovers, Reg. 2	*UW-6* , *pe*+, *arg-12*+	1
	UW-6+, *pe*⁻ , *arg-12*⁻	1
Double crossovers	*UW-6*+, *pe*+, *arg-12*+	0
	UW-6 , *pe*⁻ , *arg-12*⁻	0

The cross was *UW-6, arg-12-a* × *pe-A* as described in the text. Region 1 is the interval *UW-6-pe,* and Region 2 is the interval *pe–arg-12.*

Possible allelism of UW-6 *with* nuc-2: *UW-6 a* was crossed to *nuc-2 arom-1 A.* Of 208 random sporelings isolated, all were either *UW-6* or *nuc-2.* There were 7 *UW-6 arom-1* recombinants and 7 *nuc-2* recombinants. HASUNUMA and ISHIKAWA (1972) have located *nuc-2* between *arg-5* and *arom-1*, and TOH-E and ISHIKAWA (1971) have shown that *nuc-2* cannot be derepressed for alkaline phosphatase synthesis, whereas *UW-6* cannot be fully repressed. The results in Table 2 show the specific activities of alkaline and acid phosphatase of wild type, *UW-6, nuc-1*, and *nuc-2* grown on low and on high levels of phosphate at 25° and at 37°. It is apparent that *UW-6* synthesizes substantial amounts of alkaline phosphatase

TABLE 2

Specific activities of repressible alkaline and acid phosphatases in mycelia of various strains of Neurospora

Strain	Phosphate in growth medium	25° Repressible alkaline phosphatase	25° Repressible acid phosphatase	37° Repressible alkaline phosphatase	37° Repressible acid phosphatase
Wild type *a*	low	874	418	518	278
	high	0.68	1.3	0.88	3.9
UW-6-A	low	1290	538	625	340
	high	75	1.7	112	30.7
nuc-1-a	low	1.8	1.0	1.8	10.1
	high	3.8	7.1	1.2	23.7
nuc-2-a	low	1.1	12.2	1.1	10.8
	high	1.0	7.4	1.1	24.7

All the cultures were grown on Fries medium with high (7.35 mM) or low (0.05 mM) phosphate by sparging with sterile air for 15 hours at 25° or at 37°. Details of media, extraction of enzymes, and assay methods are described in the text.

at either temperature even at high phosphate concentrations, but that it is, at most, slightly derepressed for acid phosphatase, and only at 37°. *nuc-1* and *nuc-2* show no appreciable derepression of alkaline phosphatase on low phosphate at either temperature, in confirmation of the results of TOH-E and ISHIKAWA (1971), and no derepression of acid phosphatase. The slightly elevated levels of acid phosphatase seen on high phosphate in *nuc-1* and *nuc-2* under certain conditions are probably not significant.

Failure of nuc-1 *and* nuc-2 *to grow at high pH on low concentrations of inorganic phosphate:* The basal medium contained per liter): ammonium tartrate, 5 g; KCl, 0.55 g; NH_4NO_3, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; NaCl, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; biotin, 5 μg; trace elements as described by BEADLE and TATUM (1945); sucrose, 15 g; and Na-MOPS buffer (pH 6.94) to 100 mM. Potassium phosphate buffer (pH 7.0) was added to concentrations ranging from 0.05 mM to 10 mM. The final pH was 6.88. Test tubes (18 × 150 mm) were charged with 5 ml of buffered media (the use of Erlenmeyer flasks was avoided so as to minimize acidification by atmospheric CO_2). The tubes were inoculated with a drop of conidial suspension of wild type, *nuc-1*, or *nuc-2* and incubated one week at room temperature. The mycelial pads were dried at 110° and weighed.

The results in Figure 1 show that wild type grew reasonably well even at 0.05 mM phosphate, whereas both *nuc-1* and *nuc-2* required much higher levels of phosphate before any appreciable growth occurred. In a separate experiment at

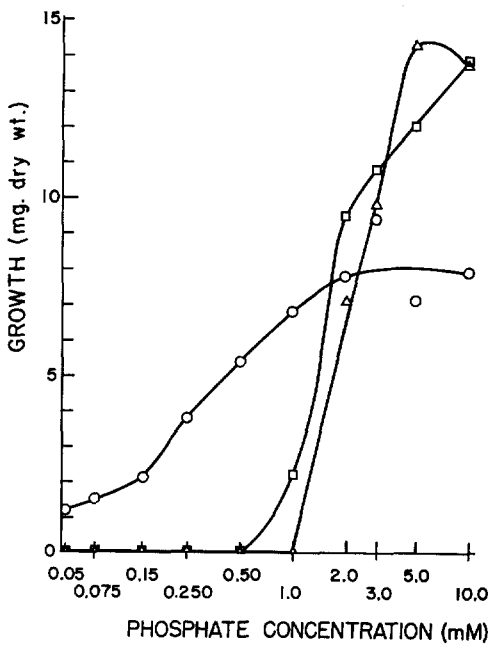

FIGURE 1.—Growth of wild type, *nuc-1*, and *nuc-2* at pH 6.88 as a function of phosphate concentration. The experiment is described in the text. ○, wild type; △, *nuc-1*; □, *nuc-2*. Note that the scale along the abscissa is logarithmic.

pH 7.35 (Tris-HCl buffer) the differences were even more dramatic; wild type grew in a manner almost identical with that shown at pH 6.88, but both *nuc-1* and *nuc-2* grew only at the highest phosphate concentration (10 mM). In an experiment performed with a simple Fries-based medium (pH 5.6) with varying amounts of phosphate, all three strains grew even at the lowest concentration of phosphate (0.05 mM), though *nuc-1* and *nuc-2* grew less well (results not shown).

Transport behavior and enzyme levels in UW-6, nuc-1 *and* nuc-2: LOWENDORF and SLAYMAN (1970) and LOWENDORF (1972) have studied transport in *Neurospora crassa* as a function of phosphate concentration present during growth. They found that phosphate starvation of wild type causes a several-fold derepression of the transport system. A more striking observation, however, was that the apparent K_m for phosphate transport is low under all conditions at low pH, but at high pH, the K_m is high for phosphate-sufficient cultures, but low for phosphate-starved cultures. That is, cultures that are "derepressed" by phosphate starvation can more effectively make use of low concentrations of phosphate at high pH than can cultures that have not been thus "derepressed". LOWENDORF (1972) considered two hypotheses: (1) that there is one permease which is constitutive and does not function well at high pH, and a second permease which is repressible, and is responsible for transport at high pH; (2) that there is a single permease which can be modified under conditions of phosphate starvation so as to decrease the K_m at high pH. On the basis of his kinetic data, he favored the latter explanation.

The failure of *nuc-1* and *nuc-2* to grow on low phosphate at high pH suggested that phosphate fails to be transported efficiently in these strains at high pH, though in no way does it lend weight to one or the other of LOWENDORF's hypotheses. To test the possibility that phosphate transport is altered in *nuc-1*, *nuc-2*, and *UW-6*, conidia were germinated in medium containing low (0.05 mM) or high (7.35 mM) phosphate and examined for uptake of ^{32}P-phosphate at high pH, as described in the legend of Table 3. Phosphate concentrations during the uptake experiment were 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, and 2.0 mM. The amount of phosphate transported was small compared to the amount present, so that the uptake is a measure of initial velocity. Lineweaver-Burk plots (1/v *vs.* 1/s) did not give straight lines, particularly in the case of wild type grown on high phosphate and *nuc-1* and *nuc-2* grown under either condition, so that K_m and V_{max} cannot be obtained from these data. In Table 3 we have therefore recorded only the specific activity of each culture at the extremes of phosphate concentration (0.1 mM and 2.0 mM). The ratio of these two activities, shown in the last column, is a very rough measure of the affinity of the uptake systems. As found by LOWENDORF, wild type grown on low phosphate has a "high affinity" transport system. This system is not repressible by high phosphate in *UW-6*, and is not made on high or low phosphate in *nuc-1* and *nuc-2*.

Co-dominance of UW-6 *in heterokaryons:* Twelve nutritionally-forced heterokaryons were prepared, each containing one *UW-6* component and one *UW-6*$^+$ component. A wide variety of forcing markers were used, including *inos*, *inv*,

TABLE 3

Uptake of inorganic phosphate at high pH (7.5) in mycelia of various strains of Neurospora

Strain	Phosphate in growth medium	Phosphate uptake, sp. activity		Ratio: uptake at 0.1 mM
		At 0.10 mM phos.	At 2.0 mM phos.	uptake at 2.0 mM
Wild type *a*	low	2.91	5.76	0.505
	high	0.083	0.875	0.095
UW-6-A	low	4.06	7.85	0.516
	high	2.21	3.75	0.590
nuc-1-a	low	0.291	3.38	0.086
	high	0.205	3.19	0.064
nuc-2-a	low	0.376	4.17	0.090
	high	0.262	2.37	0.111

Conidia were shaken in siliconized flasks charged with Fries medium with low (0.05 mM) or high (7.35 mM) phosphate for 10 hours at 25°. The young mycelia were harvested by centrifugation and washed twice with ice-cold KCl (20 mM) and then resuspended in half the original volume of 20 mM KCl. Aliquots (5 ml) of these suspensions were added to flasks along with KCl and Na-HEPES (N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid), pH 7.5 to give 20 mM each. The suspension was shaken at 25° for 20 minutes and 0.2 ml of ^{32}P sodium phosphate buffer at the same pH was then added to give final concentrations ranging from 0.1 mM to 2.0 mM (see text). Shaking was continued for 4 minutes. Then the contents of each flask were quickly poured onto a Millipore filter (pore size = 1.2μ). The mycelia were quickly washed with water at 0°, and the filter plus mycelia were counted in the scintillation fluid of BRAY (1960). The specific activities above are n moles taken up/min/mg protein, and only the values at 0.1 mM and 2.0 mM are recorded. The right-hand column shows the ratio of specific activities at these two phosphate concentrations.

tryp-3, *hist-6*, *hist-2*, *cys-11*, *cys-3*, *arg-12*, *ad-3A*, and *ad-3B* in various combinations. All of the heterokaryons were grown in Fries minimal (high phosphate) medium in the usual manner and assayed for repressible alkaline phosphatase. None of the heterokaryons showed a specific activity in the range of wild type grown on high phosphate (0.6–1.2), the lowest value being 14.7 and the highest being 93. The mean specific activity was 42, or roughly half that of the *UW-6* homokaryon (see Table 2).

One problem in the interpretation of results from heterokaryons is the possibility that there might be a tendency for nuclei to practice "residential segregation". That is, nuclei of two genotypes may be nonrandomly distributed in a heterokaryon so that a given nucleus tends to have neighbors of its own genotype, even in a nutritionally forced heterokaryon (for discussion see BURTON and METZENBERG 1972). An obvious way to see whether any appreciable fraction of the *UW-6* nuclei are out of biochemical earshot of *UW-6*$^+$ nuclei would be to put *UW-6* in coupling with known recessive genes that govern a second, unrelated system of repressible enzymes. If this latter system is not fully repressible, "residential segregation" would be indicated. One of the twelve heterokaryons mentioned above was constructed with the intent of testing this point. Synthesis of aryl sulfatase is normally repressible by either inorganic sulfate or by methionine. In *cys-11*, the enzyme cannot be repressed by sulfate because this mutant lacks ATP sulfurylase and cannot convert sulfate to the corepressor (RAGLAND 1959; METZENBERG and PARSON 1966; MARZLUF 1970). On the other

TABLE 4

Co-dominance of UW-6 *in a heterokaryon*

Homokaryotic or heterokaryotic strain	Sulfur source cystic acid plus	Specific activity: Alkaline phosphatase	Specific activity: Aryl sulfatase	Nuclear proportions
(*UW-6; cys-11, eth-1^r,*	sulfate	22.5	0.22	31% *UW-6*
hist-2) + (*UW-6+; tryp-3;*	methionine	23.8	0.07	35% *UW-6*
al-2) heterokaryon	———	28.7	15.5	29% *UW-6*
UW-6+ wild-type	sulfate	0.71	0.09	
homokaryon	methionine	0.78	0.03	(0% *UW-6*)
	———	1.4	8.1	
UW-6 prototrophic	sulfate	95.4	0.08	
homokaryon	methionine	74.0	0.04	(100% *UW-6*)
	———	90.0	17.6	
cys-11, eth-1^r;	sulfate	1.1	18.5	
UW-6+ homokaryon	methionine	1.1	25.0	(0% *UW-6*)
	———	1.4	17.1	

All the strains were grown on high phosphate (7.35 mM) media as described in the text by sparging 15.5 hours at 33°, which allows maximum derepression of *eth-1^r* on methionine.
The extracts were prepared in Tris-HCl buffer, pH 8.1 (0.05M) instead of acetate buffer at pH 5.0 since both aryl sulfatase and alkaline phosphatase are stable at the higher pH.

hand, *eth-1^r* is not repressible by methionine (METZENBERG 1968), apparently because it has reduced levels of S-adenosyl methionine synthetase (KERR and FLAVIN 1970; JACOBSON 1973). A multiply-marked strain (*UW-6 cys11 eth-1^r his-2*) was therefore prepared, and a heterokaryon of the latter was made with *UW-6+ tryp-3 al-2*. This heterokaryon was grown on Fries medium without sulfate, with ordinary (high) phosphate, but in which 1 mM cysteic acid (a non-repressing sulfur source) was present in all flasks. In some, this was the sole sulfur source; in others, K_2SO_4 or methionine was added to 5 mM. The specific activities of alkaline phosphatase and of aryl sulfatase are given in Table 4. It is apparent that the latter enzyme is virtually fully repressed in the heterokaryon, whereas the former is not. The two kinds of nuclei are therefore in good communication with respect to corepressor of sulfur metabolism, but *UW-6+* nuclei fail to repress *UW-6* nuclei with respect to phosphorus metabolism.

To estimate the proportion of the two nuclear types in the heterokaryon, samples of mycelial suspension were transferred to minimal agar just prior to harvesting. After these cultures had conidiated, suspensions of conidia were plated to selective media, and colony counts made as described by ATWOOD and MUKAI (1955). The recessive albino gene, *al-2*, in the *UW-6+* component was a gratuitous aid in confirming the proportion of homokaryotic conidia of this genotype. The nuclear proportions recorded in Table 4 are in rough agreement with the fractional derepression of alkaline phosphatase.

Epistasis of nuc-1 *to* UW-6: *UW-6 inos A* was crossed to *nuc-1 a*. Ordered tetrads were dissected, the ascospores were germinated, and the resulting cultures were classified with respect to ability to grow on low phosphate plus inositol at elevated pH to score for *nuc-1*; ability to make alkaline phosphatase on high phosphate plus inositol to score for *UW-6*; and for mating type, which is quite closely linked to *nuc-1*. A nonparental ditype ascus, containing four *nuc-1*$^+$ *UW-6*$^+$ *A* spores was examined further. (The ascus was tetratype with respect to inositol requirement). The other four spores gave cultures that failed to grow on low phosphate plus inositol at high pH, and were presumed to be *nuc-1 UW-6*. One of the cultures (*inos nuc-1*; putative *UW-6*) was outcrossed to wild type *A* and sixteen random progeny were examined. Five of them were *UW-6*, showing that the genotype of the strain in question was as expected. This strain showed very low levels of repressible alkaline phosphatase on media containing either high phosphate or low phosphate (specific activities of 0.96 and 0.53, respectively). Numerous other *nuc-1 UW-6* double mutants have been prepared and have always been found to be indistinguishable from *nuc-1*.

DISCUSSION

The *UW-6* mutation causes incomplete repressibility of alkaline phosphatase and of a permease activity that is responsible for the transport of phosphate with high affinity at high pH. A possible allele, *nuc-2*, and an unlinked mutation, *nuc-1*, are unable to be derepressed for the synthesis of alkaline (and acid) phosphatase (Toh-E and Ishikawa 1971), and it is shown above that they are unable to transport phosphate normally at high pH. *UW-6* is roughly co-dominant with its wild-type allele in heterokaryons, and it is hypostatic to *nuc-1*.

According to the lactose operon model, a constitutive mutant like *UW-6* might be expected to be lacking the diffusible repressor, or altered in an operator region so that the latter does not recognize repressor. The *UW-6* mutant is not readily explained by the hypothesis that a cytoplasmic repressor is not made, because the mutant is co-dominant in heterokaryons with wild type. The hypothesis that the two kinds of nuclei are widely separated in the heterokaryons ("residential segregation") seems improbable because they are still in good communication with respect to repression of aryl sulfatase. Still, it could be argued that a low molecular weight corepressor might be more rapidly diffusible than a macromolecule such as a repressor. It is likely, however, that protoplasmic streaming plays a major role in intra-mycelial mixing, and this process, unlike simple diffusion, would not be expected to discriminate between molecules of various sizes. Hence, "residential segregation" seems unlikely to be the correct explanation of the co-dominance of *UW-6*; it cannot be completely ruled out, however.

The possibility remains that *UW-6* is the operator region for a group of structural genes concerned with phosphorus metabolism. Since none of the structural genes has unequivocally been identified, it remains possible that these genes will turn out to be closely linked to *UW-6*. However, one of us (M.K.G.) has isolated two apparently allelic mutants that produce no detectable alkaline phosphatase of the repressible sort. One of these has been studied in some detail. It grows

normally on low concentrations of phosphate at high pH, and it appears to take up phosphate normally. It also produces acid phosphatase under conditions of derepression. We suspect that the mutation, which is unlinked to *UW-6* and to *nuc-1*, represents the structural gene for alkaline phosphatase. If this is correct, *UW-6* cannot be the operator region for alkaline phosphatase. Likewise, TOH-E and ISHIKAWA (1971) have isolated a mutant, called *pho-1*, which has sharply reduced levels of the repressible alkaline phosphatase. *pho-1* is not linked to *nuc-1*, and it is weakly linked or unlinked to *nuc-2*, and therefore to *UW-6*.

Little can be said about the number of enzymes controlled by *UW-6*. HASUNUMA and ISHIKAWA (1972) have suggested, as a working hypothesis, that *nuc-1* is the structural gene for one of the nucleases, and that the *nuc-2* locus codes for an inhibitor of this nuclease. *nuc-2* locus codes for an inhibitor of this nuclease. *nuc-2* mutants presumably make a more efficient inhibitor, thus explaining the failure of these mutants to grow on nucleic acids as a sole phosphorus source. The hypothesis is less successful in explaining why both these mutants are incapable of being derepressed for the synthesis of alkaline and acid phosphatases (TOH-E and ISHIKAWA 1971). We wish to advance an alternate hypothesis. The failure of these mutants to grow on nucleic acids as a phosphorus source may be attributed to failure to liberate inorganic phosphate from nucleotides at a normal rate, and failure to transport the free phosphate efficiently at low concentrations. Nonetheless, there is evidence that nuclease(s) themselves may be repressible by phosphate (ISHIKAWA, personal communication). Thus, *nuc-1* and *nuc-2* could be deficient in as many as three successive steps that are needed for the assimilation of phosphate from nucleic acids.

It is mildly surprising that *UW-6* retains normal repression control over acid phosphatase, at least at 25°, and therefore is not the pure converse of *nuc-2*. At 37°, *UW-6* is somewhat derepressed compared with wild type, but *nuc-1* and *nuc-2* also have appreciably elevated levels of acid phosphatase at that temperature (Table 2). The meaning of these results is not clear. However, TOH-E and ISHIKAWA (1971) have studied revertants of *nuc-1* and *nuc-2*. While all *nuc-1* revertants were completely repressible, a few of the *nuc-2* revertants were incompletely repressible for alkaline phosphatase but showed normal repression of acid phosphatase. A reasonable explanation is that acid phosphatase production is more sensitive to repression than is alkaline phosphatase or repressible phosphate permease production, and that whenever derepression is less than complete, acid phosphatase remains fully repressed.

Several other control systems is filamentous fungi bear comparison to the one controlling phosphorus metabolism in Neurospora. In the control of aryl sulfatase and its family of sulfur-metabolizing enzymes, the wild-type allele of *cys-3* locus makes a product which is needed for the synthesis of all the enzymes of the family (MARZLUF and METZENBERG 1968; METZENBERG and AHLGREN 1971). A mutant called *scon*^{c} makes all of these enzymes constitutively. *scon*^{c} and *cys-3* are unlinked to the three identified structural genes of the family, and to one another (METZENBERG and AHLGREN 1970, 1971; MARZLUF 1970; BURTON and METZENBERG 1972). *cys-3* is epistatic to *scon*^{c} (DIETRICH and METZENBERG 1973). *scon*^{c}

is roughly co-dominant in heterokaryons with its wild-type allele, and by use of electrophoretic variants of aryl sulfatase, it was possible to show that most, and perhaps all, of the aryl sulfatase that was produced under conditions of sulfur adequacy was of the form coded by the *scon*^{c} nucleus (BURTON and METZENBERG 1972; DIETERICH and METZENBERG 1973). No such test is yet possible with the *UW-6* mutant, since electrophoretic variants of alkaline phosphatase have not yet been obtained. It seems possible, however, that *UW-6* is analogous to *scon*^{c}, and that *nuc-1* is analogous to *cys-3*.

Other constitutive mutants are known in Neurospora, most notably the *qa-1*^{c} mutants, which cause constitutive synthesis of the enzymes for quinic acid degradation (VALONE, CASE and GILES 1971). Like *UW-6* and *scon*^{c}, these are semi-constitutive in heterokaryons. Unlike *scon*^{c}, *qa-1* is closely linked to the structural genes it controls, so that the possibility that it is an operator locus cannot be discarded. In Aspergillus, constitutive mutants for the synthesis of enzymes of nitrate reduction have been found (COVE and PATEMAN 1969; COVE 1969). These mutants, called *nir*^{c}, are not recessive, and are *not* closely linked to the structural genes they control. Like *UW-6*, they are approximately co-dominant with their wild-type alleles. It will be interesting to compare and contrast these systems as more becomes known about them.

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