

GENETIC CONTROL OF PHOSPHORUS ASSIMILATION IN
NEUROSPORA CRASSA: DOSE-DEPENDENT DOMINANCE AND
RECESSIVENESS IN CONSTITUTIVE MUTANTS

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ABSTRACT

Mutants called *nuc-1^c*, constitutive for alkaline phosphatase synthesis, were isolated and mapped very close to *nuc-1* mutants in which this enzyme is not expressed. *nuc-1* is epistatic to *nuc-1^c*. *nuc-1^c* acts only if it is *cis* to normal *nuc-1* function. The preparation of partial diploids heterozygous for various *nuc-1* alleles is described; *nuc-1^c* is dominant to *nuc-1⁺*, which in turn is dominant to *nuc-1*. In heterocaryons with *nuc-1⁺*, *nuc-1^c* is dominant when it is present in high proportion, but essentially recessive if it is present in low proportions. In heterocaryons with *nuc-1*, *nuc-1^c* is again dominant when present in high proportions, but in low proportions it "complements" to give essentially normal repressibility. A model of regulation consistent with these findings is presented.

WHEN wild-type *Neurospora* is limited in its growth by the supply of phosphorus in the medium, it produces a number of enzymes that are made in much smaller, or undetectable, amounts during growth in phosphorus-adequate medium. These enzymes, including repressible alkaline and acid phosphatases, a special high-affinity phosphate permease, a phosphoethanolamine permease and a number of nucleases, all function in obvious ways to make more inorganic phosphate available to the cell (NYC, KADNER and CROCKEN 1966; KADNER, NYC and BROWN 1968; BURTON and METZENBERG 1974; LOWENDORF and SLAYMAN 1975; HASUNUMA, TOH-E and ISHIKAWA 1976; HASUNUMA and ISHIKAWA 1977). All of the known members of this "phosphorus family" of enzymes are placed in such a way that they directly encounter the external environment, either by being in the cell membrane, as permeases, or between the membrane and the wall, as is the bulk of the repressible alkaline phosphatase, or by being actually secreted into the medium, as in the case of the repressible acid phosphatase and the nucleases.

Elsewhere, we have discussed two of the structural genes, *pho-2⁺* and *pho-3⁺*, which code for the repressible alkaline and acid phosphatases, respectively. These are under the control of three regulatory genes, *nuc-1⁺*, *preg⁺* and *nuc-2⁺* (LEHMAN and METZENBERG 1976; NELSON, LEHMAN and METZENBERG 1976). A

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model based on results from dominance and epistasis tests has been developed (LITTLEWOOD, CHIA and METZENBERG 1975) in which the product of the *nuc-1*⁺ gene is required for expression of the structural genes. The synthesis of the *nuc-1*⁺ gene product, or its action, is opposed, in some unspecified way, by the *preg*⁺ gene product. Similarly, the action of the *preg*⁺ gene, or its product, is opposed by the *nuc-2*⁺ gene product. This, in turn, is opposed by inorganic phosphate or a co-repressor derived from it. The result is a regulatory hierarchy, in which the product of the *nuc-1*⁺ gene has the last word about whether or not alkaline phosphatase and its cognate enzymes will be synthesized (see Figure 1 and the explanation in its legend).

An immediate problem posed by such a model is: how does *preg*⁺ exert its effect? Does it repress the synthesis of *nuc-1*⁺ product, perhaps by binding to some operator next to the *nuc-1* structural gene, or does it titrate out, or otherwise inactivate, existing *nuc-1*⁺ product? The identical question can, of course, be asked as to how *nuc-2*⁺ works against the *preg*⁺ gene or its product.

Properties of constitutive mutants of the *nuc-1* gene have afforded some insight. *nuc-1* null mutants, which are unable to express the structural genes of the phosphorus family, have long been known (TOH-E and ISHIKAWA 1971; LEHMAN *et al.*, 1973) and were interpreted as lacking a positive regulator or "expressor" necessary for activation of the structural genes of the family (LITTLEWOOD, CHIA and METZENBERG 1975). CHIA (1976) isolated mutants called *nuc-1*^c, which were in, or closely linked to, the region specified by the *nuc-1* mutations. These *nuc-1*^c mutants were constitutive for alkaline phosphatase synthesis. These mutants allowed the question of *preg*⁺ antagonism to *nuc-1*⁺ gene product to be restated in the form: why does *preg*⁺ fail against *nuc-1*^c? One can imagine sev-

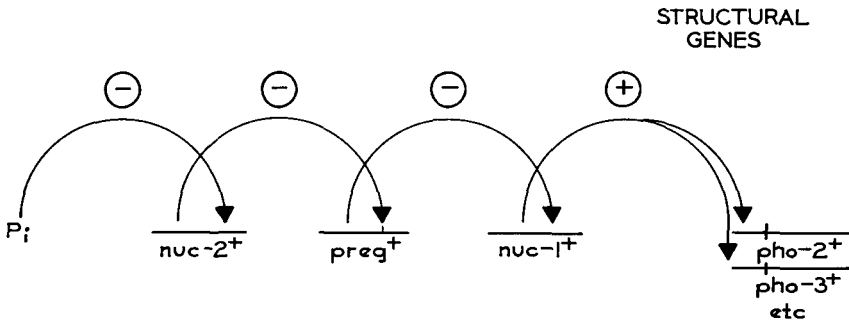


FIGURE 1.—Model for the control of synthesis of the phosphorus family of enzymes. *pho-2*, *pho-3*, etc., are the structural genes, which are under positive control by the product of the *nuc-1*⁺ gene. This product is inactivated by the *preg*⁺ product, which in turn is inactivated by the *nuc-2*⁺ product, which in turn is inactivated or repressed by phosphate or something derived from it.

The phenotypes of various strains carrying one or more mutations can be "calculated" by multiplying the positive or negative signs of the regulatory products. Only those regulatory products that are connected to the structural genes by a sequence unbroken by mutation can be included in the calculation. For example, in *nuc-2* strains, only the final two regulatory steps are operative ("−" times "+") and such strains are "−", i.e., the enzymes are not made.

eral possibilities; for example, (1) *preg*⁺ normally binds to an operator next to *nuc-1*⁺, but *nuc-1*^c mutants are mutant in the operator and are therefore blind to *preg*⁺ product. (2) *preg*⁺ product normally binds to the *nuc-1*⁺ product, inactivating it essentially by titrating it out; *nuc-1*^c product is structurally altered so that *preg*⁺ product cannot bind to it. (3) *preg*⁺ product normally binds to the *nuc-1*⁺ product, inactivating it essentially by titrating it out; *nuc-1*^c is an over-producer of qualitatively normal *nuc-1*⁽⁺⁾ product, such that the amount of *preg*⁺ in the cell is less than stoichiometric with it, and can never titrate it out.

A finding that helps in choosing between these possibilities came from the properties of certain strains that were partial diploids for the *nuc-2*—*preg* region. While the euploid strain *nuc-1*^c; *nuc-2*⁺ *preg*⁺ is constitutive, the partial diploids, *nuc-1*^c; *nuc-2*⁺ *preg*⁺/*nuc-2*⁺ *preg*⁺ and *nuc-1*^c; *nuc-2* *preg*⁺/*nuc-2*⁺ *preg*⁺ are essentially normally repressible. If either of the two *preg*⁺ alleles is replaced by *preg*^c, the strain becomes strongly constitutive, and if both alleles of *preg* are *preg*^c, it is still more strongly constitutive (METZENBERG and NELSON 1977). In other words, *nuc-1*^c is constitutive in a background of one *preg*⁺, but is essentially repressible in a background of two *preg*⁺ genes; and *preg*^c, which is normally fully recessive to *preg*⁺ when it is in a *nuc-1*⁺ background (METZENBERG, GLEASON and LITTLEWOOD 1974) becomes dominant to it in a *nuc-1*^c background.

We argued that these paradoxes are hard to reconcile with any hypothesis except one in which *nuc-1*^c is an overproducer of *nuc-1* product, and we suggested the following simple dosage-titration model. Suppose that *nuc-1*⁺ normally makes 100 arbitrary units of its product under all conditions, and *preg*⁺ makes, say, 150 units of its product, which are capable of titrating it out stoichiometrically, and that *nuc-2*⁺ makes, say, 500 units of its product, capable of titrating out *preg*⁺ product, and finally that the activity or effectiveness of *nuc-2*⁺ product is somehow controlled by phosphate or its derived co-repressor. If *nuc-1*^c were a mutation that raised the level of *nuc-1* product from 100 to 250 units, one dose of *preg*⁺, making 150 units, could never titrate it out, but two doses, making 300 units, could restore normal repressibility. In other words, the necessary rank order for repressibility, *nuc-2*⁺ > *preg*⁺ > *nuc-1*⁽⁺⁾, has been restored.

The dosage titration model makes some nontrivial predictions about the properties of strains heterocaryotic for *nuc-1*^c and *nuc-1*⁺, and especially those heterocaryotic for *nuc-1*^c and *nuc-1*. It also suggests that the control of alkaline phosphatase in these heterocaryons and in the corresponding partial diploids heterozygous at the *nuc-1* locus should be different in specific ways. In the case of heterocaryons of *nuc-1*^c + *nuc-1*⁺, let us assume that each of the two kinds of nuclei makes *nuc-1* product at its characteristic, autonomous rate, and that the two kinds discharge their products into a perfectly-mixed cytoplasm. Take the case, for the moment, of a heterocaryon, in which each nuclear type is present as 50% of the total. Using the rather arbitrary numbers we have picked, the relative concentration of *nuc-1* product will be $(0.5 \times 250) + (0.5 \times 100) = 175$. If both of the nuclear types are also *preg*⁺, the relative concentration of *preg* product will be 150 and untitrated *nuc-1* product will be present ($175 - 150 = 25$), that is, the strain will be weakly constitutive. Consider, however, a hetero-

caryon having 30% *nuc-1^c* and 70% *nuc-1⁺* nuclei. The level of product will be $(0.3 \times 250) + (0.7 \times 100) = 145$. Since this is less than the 150 units that can be titrated out by *preg⁺* product, the strain should be repressible. In the general case, we would expect that, at low proportions of *nuc-1^c* nuclei, heterocaryons would be repressible, and that on high phosphate, even the production of alkaline phosphatase per *nuc-1^c* nucleus would be very low. Above some threshold proportion of these nuclei, the heterocaryons would become constitutive, and the production of alkaline phosphatase in high phosphate per *nuc-1^c* nucleus would rise sharply. If the nuclei are not randomly distributed in the cytoplasm, or cytoplasmic products are otherwise incompletely mixed, the cutoff may not be sharp; but the nature of the prediction is not changed. A partial diploid like *nuc-1^c/nuc-1⁺; preg⁺*, on the other hand, should have a level of *nuc-1* product of $250 + 100 = 350$, of which *preg⁺* could titrate out only 150. Such a strain should, therefore, be strongly constitutive.

The case of *nuc-1^c + nuc-1* heterocaryons is another test of the model. If we assume that *nuc-1* makes no active product, a heterocaryon in which the nuclear proportions were, say, 50% of each kind would have a level of 125 units, and would be repressible. In that case, such heterocaryons should show something not seen in either homocaryon—normal repressibility. This repressibility should turn to constitutivity above some threshold proportion of *nuc-1^c* nuclei. Once again, the partial diploid *nuc-1^c/nuc-1; preg⁺* should be constitutive.

In this paper, we describe in detail the isolation and mapping of *nuc-1^c* mutants. We show that they are hypostatic to *nuc-1* in intragenic double mutants, and that *nuc-1^c* is dominant to both *nuc-1* and *nuc-1⁺* in partial diploids. Heterocaryons of varying nuclear proportions do show the predicted behavior: those with a high proportion of *nuc-1^c* nuclei are constitutive for alkaline phosphatase synthesis, and those with a high proportion of *nuc-1⁺* or *nuc-1* nuclei are essentially repressible.

MATERIALS AND METHODS

Strains: "Wild-type" *Neurospora crassa* used in this study 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 to get stocks that were fully heterocaryon-compatible. *nuc-1*, except where otherwise specified, is allele T28-M1. An apparently nonrevertible allele, *nuc-1* (JFL-611) was selected as described in the text for alleles RHB-13 and RHB-141. *nuc-1^c*, except where otherwise specified, is allele BC-152. The auxotrophic marker strains were obtained from the FGSC, Humboldt State University Foundation, Arcata, California, as were some of the translocation strains. Translocations with nutritional, color, and antibiotic resistance markers were kindly furnished by DAVID PERKINS. The following abbreviations are used in describing strains: *ad*, *al*, *arg*, *cyh^r*, *cys*, *his*, *inl*, *lys*, *met*, *nic*, *thi*, and *un* are adenine, albino, arginine, cycloheximide-resistant, cysteine-or-methionine, histidine, inositol, lysine, methionine, nicotinamide, thiamine, and "unknown" (temperature-sensitive); NS = Normal Sequence, T = translocation, LG = Linkage Group. The terminology of partial diploids is given in the legend of Table 2.

Media: Fries medium with 1.5% sucrose was described by BEADLE and TATUM (1945). It is 7.35 mM in inorganic phosphate, and is called "high P_i." Fries "low P_i" has the KH₂PO₄ lowered to 50 μM, with the deficit of potassium ions being made up with equimolar KCl. High pH, low P_i medium is the same, but with the pH adjusted to 7.3 with 100 mM Na⁺ MOPS

(morpholinopropane sulfonic acid); *nuc* mutants were scored by their failure to grow on this medium. Plating media were solidified with 1.5% Difco agar, with sucrose being substituted by 1% sorbose, 0.05% glucose, and 0.05% fructose to induce colonial growth (BROCKMAN and DESERRES 1963); this will be referred to subsequently as BdeS sugars. Crosses were made on the synthetic medium of WESTERGAARD and MITCHELL (1947), supplemented as needed. All amino acids were used at 1 mM, inositol was used at 50 μ g per ml, and all other vitamins at 2 μ g per ml.

Scoring: Replica plating was done by the method of LITTLEWOOD and MUNKRES (1972), modified as follows. To detect (for example) arginine auxotrophs, colonies growing on arg BdeS medium were replicated not to minimal, but to 5mM lysine BdeS to prevent uptake of arginine carried over during replication. Similarly, in detecting histidine auxotrophs, colonies were replicated from histidine to 5 mM arginine + 5 mM methionine to prevent uptake of histidine. Mating type was scored by spotting loopfuls of conidial suspensions onto lawns of the standard fluffy testers, and the Barren trait used to score for partial diploids was observed on the same plates (TURNER *et al.* 1969). Repressible alkaline phosphatase was scored by staining colonies on high and low P_i plates as described by TOH-E and ISHIKAWA (1971). Alkaline phosphatase was assayed quantitatively as described by LEHMAN *et al.* 1973).

Preparation and analysis of heterocaryons: Strains were constructed in which the *nuc-1^c* component carried one auxotrophic forcing marker and the *nuc-1⁺* or *nuc-1* strain carried another. In some cases, the *nuc-1^c* component was also marked with the recessive albino color marker, *al-2*. The heterocaryons were formed by inoculating approximately equal amounts of conidial suspension of each auxotroph into a small tube of liquid minimal medium. The prototrophic heterocaryons were allowed to grow to conidiation, and a small transfer was made to second tube. Several sequential transfers were made to allow the nuclear proportions to approach steady state. To estimate the proportions of the two kinds of nuclei in a heterocaryon, conidia were suspended in water, diluted quantitatively, and plated to BdeS Fries minimal, single supplement of one kind, single supplement of the other kind, and double supplement. The proportion of the two kinds of nuclei in each heterocaryon was calculated from differential plate counts as described by ATWOOD and MUKAI (1955). The presence of *al-2* in the *nuc-1^c* component in some of the heterocaryons allowed homocaryotic colonies of that nuclear type to be distinguished from heterocaryotic prototrophs growing on the same (supplemented) plate. This reduced the error due to sampling and pipetting.

*Isolation of *nuc-1^c* mutants:* The complete failure of *nuc-2* strains to grow on high pH, low P_i medium allows strong selection of revertants which are able to grow under these conditions. In a typical experiment, conidia of *arg-12 nuc-2* (MKG-139) were UV-irradiated to about 75% killing and plated to the selective medium with arginine at 33°. Out of 1.5×10^6 survivors plated, 156 gave rise to colonies. These revertants were picked, conidial suspensions were spotted to high P_i plates with arginine, incubated at 33° for three days, and stained to detect alkaline phosphatase. Eleven of the revertants were seen to be highly constitutive. (A considerably larger proportion of the revertant colonies from such experiments were actually heterocaryons between a constitutive revertant and the unmutated parental strain.) The 11 candidates were outcrossed to obtain assured homocaryotic cultures. Eight of the 11 (and a roughly similar proportion from later selections) proved to be due to mutations of *preg⁺* to *preg^c*, recessive, constitutive mutants that are linked to *nuc-2* and epistatic to it (LITTLEWOOD, CHIA and METZENBERG 1975). In outcrosses of these eight to *nuc-2⁺ preg⁺*, about 2% of the progeny were *nuc-2⁺ preg⁺*, showing that *nuc-2* was still present. Partial diploids of the putative *nuc-2⁺ preg^c* strains made with the previously-characterized translocation strain, T(II \rightarrow I) NM177 *nuc-2⁺ preg^{c-1}* were all constitutive, showing that the new mutations were functionally *preg^c* mutants (LITTLEWOOD, CHIA and METZENBERG 1975). Finally, several of the new constitutive mutations were tested; they remained constitutive when extracted into a *nuc-2⁺* background (B. LITTLEWOOD, unpublished data).

The remaining three reversion events segregated about 20% *nuc-2* progeny from outcrosses, not far from the 25% expected of unlinked suppressor mutations. Each of the unlinked suppressor-reversion events was found to be linked to mating type at a distance of roughly 10 centi-

morgans—consistent with the idea that they constituted a new class of mutant at the *nuc-1* locus. Since the work to be described below shows that this is indeed the case, we will refer to them prospectively as *nuc-1^c* (BC-30), -(BC-73) and -(BC-152). *nuc-1^c* isolates free of *nuc-2* and of *arg-12* were obtained from outcrosses of the original isolates; as expected, these were constitutive in a *nuc-2⁺* background as well.

RESULTS

Genetic analysis

Order of markers to the right of the centromere in Linkage Group I: The gene order *A/a* (centromere) *his-2 lys-4 nic-2* has been established by conventional crossover mapping (DESERRES 1969). The order of the rest of the genes on the right arm of LG I that were used in this study has been proven most rigorously by duplication mapping (PERKINS and BARRY 1977a, p. 211) and, taken with DESERRES' (1969) data, is: *un-2 his-2 lys-4 nic-2 thi-1 met-6 ad-9 cyh-1 al-2 al-1*.

nuc-1 was located between *his-2* and *lys-4* on the basis of the following crosses. A strain *a* (centromere) *his-2 nic-2* was crossed to *A* (centromere) *nuc-1*. Ascospores were plated to high pH, low P_i medium with nicotinamide, but without histidine. Among 10,220 spores plated, there were ten *his-2⁺ nuc-1⁺* recombinants, of which eight were *A* and two were *a*. A cross of *a* (centromere) *lys-4 al-2* to *A nuc-1* was made, and ascospores were plated to high pH, low P_i minimal medium. Among 6750 spores, there were 24 *nuc-1⁺ lys-4⁺* recombinants, and all of them were mating type *a*. Eight of them were *al-2* and 16 were *al-2⁺*. Support for this gene order comes from crosses in which *his-2 nuc-1* strains were crossed to *nuc-1^c lys-4* strains carrying various *nuc-1^c* alleles, and the progeny ascospores were plated to low pH, low P_i medium, which is permissive for growth of both *nuc-1^c* and *nuc-1*. The rare *his⁺ lys⁺* recombinant colonies were replicated to medium with ordinary (high) phosphate, and the masters and replicas were stained to score the segregants as constitutive, null or repressible. If the gene order were (centromere) *nuc-1 his-2 lys-4*, we would expect all the prototrophs to carry the form of *nuc-1* that entered the cross in coupling with *his-2*. This was not the case. The prototrophs were divided between *nuc-1* and *nuc-1^c* in about the proportions expected if the *nuc-1* gene were about one-fifth of the way between *his-2* and *lys-4*. On a number of occasions, however, we have selected recombinants between *his-2* and *nuc-1*, and have seen that such crosses often show "high negative interference," or failure of flanking markers to recombine. Thus, the majority, but not all, of our crossover data agree with the gene order *his-2 nuc-1 lys-4*.

There is an independent way of corroborating this order through the use of an insertional translocation, *T(I→VII; I; V; VII)AR173*, in which a small piece of LG I carrying *un-2⁺* and *his-2⁺* is moved to LG VII; (PERKINS and BARRY 1977a, p. 249). To test whether *nuc-1⁺* is translocated from I to VII, NS *a nuc-1 met-6 al-1^y* was crossed to the prototrophic translocation strain. Sixty-three isolates carrying the near-distal marker *met-6* (of which 49 also carried the far-distal marker, *al-1^y*) were spotted in the usual way to fluffy mating-type testers, and the crosses were later scored for Barren (= partial diploidy) as well. Of the 63

isolates, 25 were partial diploids, and of these, 23 were mating type *a*. All the cultures were spotted to methionine-supplemented high pH, low P_i and low pH, low P_i plates. The former were scored for growth, and the latter were stained and scored for color. There were no discrepancies between these two methods of scoring for *nuc-1*. All of the Barren duplications failed to grow on the high pH plates and grew but failed to stain on the low pH plates. Five of the fertile, euploid strains were *nuc-1*⁺, apparently the products of crossing over between *nuc-1* and *met-6*. Elsewhere in this paper we show that *nuc-1*⁺ is dominant to *nuc-1* in partial diploids, as well as in heterocaryons, so that the *nuc-1* phenotype of the AR173 partial diploids indicates that they do not contain *nuc-1*⁺. Therefore *nuc-1* cannot lie between *un-2*⁺ and *his-2*⁺. Since it clearly does lie to the right of *un-2*, it must also be to the right of *his-2*.

Close linkage of nuc-1^c with nuc-1: Preliminary experiments indicated that crosses between the new constitutives and the *nuc-1* type-strain did not yield repressible, *nuc-1*⁺ recombinants among modest numbers (e.g., 100) of random isolates. Therefore, crosses were set up in which obligate recombinants in the region of *nuc-1* were selected by the use of nutritional forcing markers, and the recombinants were examined for the presence of *nuc-1*⁺ types. This was done in the following way (illustrated with *nuc-1^c* (BC-152), which was later taken as the type-strain).

The strain *A* (centromere) *his-2*⁺ *nuc-1^c* *lys-4* *nic-2*⁺ *al-2* was crossed to *a* (centromere) *his-2* *nuc-1* *lys-4*⁺ *nic-2* *al-1^y*. Ascospores were suspended in 0.02% agar, and the titer estimated by plating aliquots onto fully supplemented medium, and by direct count of spores under the microscope. (These were in satisfactory agreement). About 24,000 viable ascospores were plated, at a density of 500–1000 per plate, to high pH, low P_i medium containing nicotinamide. With the exception of possible disomics for LG I (not encountered in this work), the only progeny expected to grow on this medium are those that are recombinant in the short region between *his-2* and *lys-4*, and are also either *nuc-1^c* or *nuc-1*⁺. A total of 160 colonies appeared on the combined plates. These were replicated to ordinary, high P_i , low pH medium containing nicotinamide, and after the usual growth period, the high P_i replicas were stained to detect alkaline phosphatase. The results (see Table 1, Cross 3) were that all of the 160 colonies were constitutive. Because it was equally possible that the placement of the forcing markers was such as to select against production of *nuc-1*⁺ by ordinary crossing over rather than to favor it, a similar cross was made with the forcing markers reversed; that is, *a* (centromere) *his-2* *nuc-1^c* *lys-4*⁺ *nic-2* *al-1^y* was crossed to *A* (centromere) *his-2*⁺ *nuc-1* *lys-4* *nic-2*⁺ *al-2*. Of 33,500 viable spores plated, 49 gave rise to colonies; of these, 48 were constitutive and one was repressible. (See Table 1, cross 6). The sole repressible segregant was found to be *A nuc-1*⁺ *nic-2 al-1^y*. If it is assumed that this arose from a conventional crossover, its genotype suggests the map order *A/a* (centromere) *his-2 nuc-1^c* (BC-152) *nuc-1 lys-4 nic-2*, with the *nuc-1^c* to *nuc-1* distance being much less, even, than the roughly 1 centimorgan between *his-2* and *lys-4*, perhaps of the order of magnitude of 0.006 centimorgans. Since the *nuc-1^c* alleles (BC-30 and BC-73) gave no *nuc-1*⁺ segregants in such crosses, nothing at all can

TABLE 1

Rarity of recombination between nuc-1^c and nuc-1

Cross	<i>nuc-1^c</i> allele used	Viable spores plated	<i>his-2⁺ nuc-1^c lys-4⁺</i> progeny	<i>his-2⁺ nuc-1⁺ lys-4⁺</i> progeny
1	(BC-30)	70,000	297	0
2	(BC-73)	35,400	182	0
3	(BC-152)	33,500	160	0
4	(BC-30)	57,200	88	0
5	(BC-73)	28,400	68	0
6	(BC-152)	24,000	48	1

Crosses 1, 2 and 3 employ different alleles of *nuc-1^c* in matings between *his-2⁺ nuc-1^c lys-4* and *his-2 nuc-1 lys-4⁺*. Crosses 4, 5 and 6 were between *his-2 nuc-1^c lys-4⁺* and *his-2⁺ nuc-1 lys-4*. Ascospores were plated to minimal medium and the prototrophs were classified for *nuc* phenotype. For further details of the crosses and classification, see the text.

be said about their map position with respect to *nuc-1*, except that they are very closely linked to it.

When the data in Table 1 are pooled, it can be seen that crosses 4, 5 and 6 gave 204 non-*nuc-1*, *his⁺* recombinants among 109,600 spores. Assuming that the reciprocal recombinants were equally frequent, this corresponds to a *his-2—nuc-1* distance of 0.37 centimorgans. Crosses 1, 2 and 3 yield a *nuc-1—lys-4* distance of 0.92 centimorgans. In a separate experiment (not shown) we found 15 *lys-4⁺ nic-2⁺* recombinants among 767 *lys-4⁺* spores, which gives a distance of 2.0 centimorgans between these markers.

Hypostasis of nuc-1^c to nuc-1: Ideally, one would like to prepare the double mutant, *nuc-1^c nuc-1*, by crossing the two single mutants and ask which phenotype prevails. Given the extremely close linkage between the two, however, this seemed out of the question. Fortunately, it was possible to arrive at an answer in two ways, one of them inferential and the other direct.

When *nuc-1* is mutagenized with UV and plated to high pH, low P_i medium, reversions are obtained at a rate of about 2×10^{-5} . About one-third of these are normally repressible, and the rest are capable of growing on the restrictive medium, but do not produce enough alkaline phosphatase to detect by staining. If *nuc-1^c* were epistatic to *nuc-1*, it should be possible to induce constitutive mutations onto a *nuc-1* background exactly as they can be induced onto a *nuc-1⁺* background. To test this, *nuc-1*; *nuc-2*; *inl-a* conidia were irradiated as usual, and 2.3×10^7 surviving conidia were plated to inositol-containing, high pH, low P_i plates. The 285 colonies that arose were picked to inositol tubes, and conidial suspensions were spotted to minimal and to inositol high P_i and low P_i plates. None grew on minimal medium, showing that contaminants were absent from the collection. Neither did any of the cultures stain on low or high phosphate, whereas *nuc-1^c*; *nuc-2* would have done so on both media. On the basis of the frequency of *nuc-1^c*; *nuc-2* types arising from reversion of *nuc-1⁺*; *nuc-2*, we would have expected, conservatively, 13 such colonies in the sample. The simplest interpretation is that *nuc-1^c nuc-1*; *nuc-2* mutants did arise during mutagenesis, but that the phenotype of *nuc-1^c nuc-1* is null, not constitutive.

The converse approach was to isolate new *nuc-1* mutants on a *nuc-1^c* background. *cys-11 nuc-1^c*; *arg-5 nuc-2*; *inl-a* was mutagenized, and about 10^5 survivors were spread on each plate. The medium was Fries salts without P_i , with 2 mM filter-sterilized phosphoryl ethanolamine as sole phosphorus source, BdeS sugars, methionine, arginine, inositol and 0.2 mM sodium arsenate. The plates were sealed in bags and incubated at 33° for three weeks. (Under these conditions, strains with the *nuc* phenotype, which lack the repressible, high-affinity phosphate permease, are arsenate-resistant and grow very slowly, whereas repressible or constitutive strains do not grow at all. *cys-11* seems to improve this discrimination, for reasons not understood). Two-hundred resistant colonies were picked, and conidia were streaked to the same medium to isolate probable homocaryons. Three were discarded as being arsenate-sensitive. The remaining cultures were tested for growth on appropriately supplemented high pH, low P_i medium, and on low pH, low P_i medium. Of the 197, 187 had the *nuc* null phenotype. These putative *nuc-1* mutants were spotted to grids of *nuc-1*; *fl*, and progeny ascospores were collected in inverted glass caps. A few hundred spores were heat-shocked and spread to restrictive medium to check for recombination. All of the strains that failed to grow on high pH medium and failed to stain on low pH medium also failed to show facile recombination with standard *nuc-1*. Presumably most or all of them are the double mutant, *nuc-1^c nuc-1*.

To see if *nuc-1^c* was really present, two of these new *nuc-1* mutants, alleles RHB-13 and RHB-141, were picked at random for a reversion study. Conidia were irradiated as usual, and about 5×10^5 survivors per plate were spread onto appropriately supplemented high pH, low P_i plates. This gave about 20 colonies per plate. Thirty-six colonies from each of the strains were picked and were tested for constitutivity on high P_i medium. Allele RHB-13 gave 16 recognizably constitutive revertants among 36, and RHB-141 gave 11 among 36. Both showed a range of degree of constitutivity among the revertants. Ordinary *nuc-1*, induced on a wild-type background, gives no constitutives at all among its revertants. Therefore, it is clear that the *nuc-1* mutants RHB-13 and RHB-141 do harbor a *nuc-1^c* allele and are epistatic to it.

Preparation of very unstable partial diploids of the genotype nuc-1/nuc-1⁺: The insertional translocation *T(I→V)AR190* moves all known markers in the right arm of LG I, except *un-2*, to the left arm of LG V (PERKINS and BARRY 1977a, p. 253). Since *his-2⁺* is among those moved, *nuc-1⁺* should be moved as well. *nuc-1 met-6 al-1^u-A* was crossed to *T(I→V)AR190 nic-2 ad-9 cyh-1^r al-2^p*. Unordered tetrads were collected as described by PERKINS (1966). When tetrads include four black spores and four white spores, these spores are expected to represent partial diploids and lethal deficiencies, respectively. Examination of five such tetrads showed that this was the case. All of these prototrophs, with the exception of two from one ascus, were mating-type A. None showed any sign of being barren, however, as is usually seen with partial diploids. The fertility of partial diploids of AR190 with Normal Sequence has been described before (PERKINS and BARRY 1977a) and is evidently due to a rapid loss during vegetative growth of the terminal segment of the translocated arm, so that the strain returns rapidly to

the euploid, fertile condition. In the present case, we would expect that loss of the piece bearing *nuc-1*⁺, *met-6*⁺, and *al-2*^p would give back the parental type, *nuc-1 met-6 al-1*^v with its associated requirement for methionine. If the newly isolated partial diploid is kept on minimal medium at all times, we would expect that, at most, some proportion of the nuclei would become euploid, but that selective pressure would maintain at least a minority of partial diploid nuclei in heterocaryosis with the euploid ones. This is because any mycelial region that completely lost *met-6*⁺ would stop growing.

Conidia from two of the putative partial diploids, chosen at random, were suspended in water and 400 to 500 were spread to ordinary Fries BdeS plates supplemented with methionine, nicotinamide, and adenosine. The plates were incubated two days at 33° and then replicated to minimal low pH, low P_i plates and to low pH, high P_i methionine plates. The master plates, containing fully permissive medium, supported strong growth of all colonies. The print of these onto minimal medium showed that all colonies did transfer, but they barely grew. Their slight growth was presumably supported by traces of nutrients carried over during replication. Twelve of 430 colonies from one strain and six of 452 of the other grew strongly on minimal. Staining of the minimal plates showed that these colonies had alkaline phosphatase, though in much less than wild-type amounts. All of the colonies grew vigorously on methionine plates, indicating that those which ceased growth on minimal were methionine auxotrophs. In agreement with this, plating of an ordinary conidial suspension of the partial diploids to minimal and methionine plates shows about 20 to 50 times more colonies on the methionine plates than on minimal. Cultures or colonies growing on minimal medium are, in fact, yellowish-orange, and are clearly intermediate between the very pale yellow color of *al-1*^v and the salmon-orange color of wild type. Colonies, or cultures grown to maturity on methionine become the color of *al-1*^v as they become auxotrophs. No colonies showing the pinkish color of *al-2*^p have ever been seen in platings of these or similar partial diploids.

All of the partial diploids grew readily on minimal high pH, low P_i medium and stained weakly on minimal low pH, low P_i medium. Seventeen separate isolates of the partial diploid were examined quantitatively for their ability make alkaline phosphatase. Conidia were inoculated into Fries minimal low P_i medium and grown for two days at 25°. The specific activity of alkaline phosphatase of these cultures ranged from 14.7 to 75.5 nmoles per minute per mg protein, with a mean of 26.2 and medium of 26.0. The *nuc-1* and wild-type controls were 0.89 and 794, respectively. Thus the alkaline phosphatase levels are about 3% that of wild type, which is still much higher than that of *nuc-1*. This is especially true since it is known that even the small amounts of alkaline phosphatase in *nuc-1* are virtually entirely the "housekeeping" alkaline phosphatase, not the repressible enzyme coded by the *pho-2*⁺ gene (LEHMAN and METZENBERG 1976). It is noteworthy that the ability of the dilute partial diploid to make alkaline phosphatase is in very rough agreement with the proportion of nuclei containing a *nuc-1*⁺ allele, as if the latter did not make a large excess of product.

Preparation of semi-stable partial diploids of the genotypes $nuc-1/nuc-1^+$, and $nuc-1^c/nuc-1^+$: While the data from the very unstable partial diploid were sufficient to show that *nuc-1* is recessive to *nuc-1⁺*, the parallel question of whether *nuc-1^c* is recessive or dominant to *nuc-1⁺* could not be asked properly in this system. The finding that an unstable partial diploid is constitutive could not be interpreted because the large majority of the nuclei would be expected to be haploid *nuc-1^c*. Unfortunately, no other insertional translocation than AR190 has been found with the breakpoint in the short region between the centromere and *nuc-1*.

Nevertheless, it was possible to make partial diploids of greater stability by a different approach. We postulated that, in the unstable diploids containing LG I of Normal Sequence and LG V of AR190, the latter breaks spontaneously at some appreciable frequency, exactly at the original attachment point of the translocated material, so that a euploid nucleus results. This euploid grows somewhat faster than the partial diploid, so that it increases in proportion to the diploid until its high rate of division is counterbalanced by a deficit of a nutrient—in this case, methionine. If one arranged things so that breakage at the “fragile point” in LG V would give rise to a strain lacking not merely the wild-type allele of *met-6*, but a large bloc of genes, any nucleus in which this breakage occurred might be dead, or at a severe disadvantage. Such a diploid can be constructed by crossing two insertional translocations, *T(I→V)*AR190 and *T(I→V)*-ALS182. The latter has a piece of the right arm of LG I moved to the left arm of LG V; the breakpoint in LG I is well to the right of the breakpoint in AR190 (PERKINS and BARRY 1977a, p. 253; 1977b). The principle involved in stabilization of such a diploid can be seen in Figure 2. Any diploid that loses the trans-

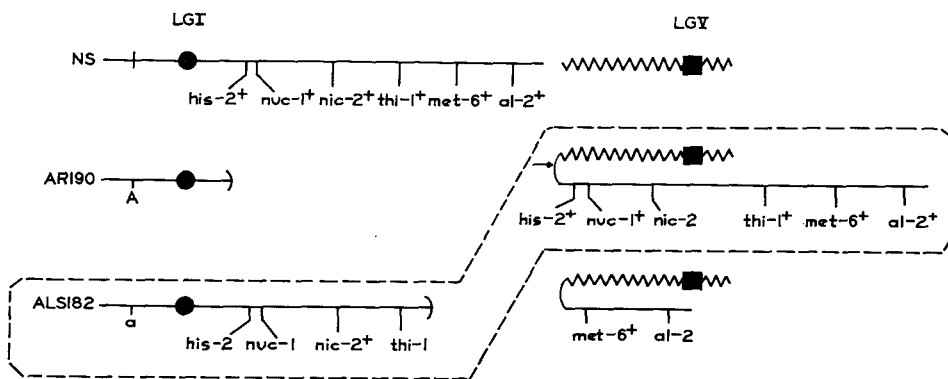


FIGURE 2.—Preparation of a partial diploid by crossing two insertional translocations, *T(I→V)*AR190 and *T(I→V)* ALS182. [The Normal Sequence (NS) strain is shown at the top for comparison]. Centromeres of Linkage Group (LG) I are shown as solid circles, those of LG V as solid squares. Random assortment gives the combination enclosed by the dashed line as the only viable prototroph. The arrow indicates the junction between the left arm of LG V and the translocated piece; simple breakage at this apparent “fragile point” will produce not only haploidy for the *nuc-1-nic-2-thi-1* region, but deficiency for all the distal genes not present on LG I of this strain.

located material on the left arm of LG V from AR190 will be deficient in all the genes that normally reside in the right arm of LG I distal to the LG I break-point of ALS182. Such a partial diploid can generate a viable euploid only by crossingover followed by breakage, not by breakage alone.

In the actual preparation of diploids heterozygous for various alleles at *nuc-1*, the alleles were put into the *T(I→V)*ALS182 background by conventional crosses. In addition, proximal and distal forcing markers *his-2* and *thi-1* were built into the ALS182 parents. The AR190 parents always carried *nic-2*, a forcing marker between *nuc-1* and *thi-1*. Thus, when spores from such a cross are plated to minimal medium, the only prototrophs should be partial diploids. About 150 ascospores per plate were spread onto BdeS high P_1 and low P_1 media. The plates were incubated for 2½ to three days at 33° and were then stained. The putative *nuc-1/nuc-1*⁺ colonies failed to stain on the high P_1 plates, but stained strongly on the low P_1 plates—*i.e.*, they were repressible. The putative *nuc-1*^c/*nuc-1*⁺ partial diploids stained strongly on both types of plates.

Colonies from stained plates, or young prototrophic sporelings from unstained plates were picked to small (1 ml) tubes of liquid minimal medium and grown to conidiation. When conidia of these prototrophs were spotted to fluffy lawns to test for both mating type and the Barren/fertile phenotype, most of them proved to be the mating type of the ALS182 parent, as expected. In our experiments, anywhere from about 50% to more than 90% of the progeny were nearly Barren, though even these putative partial diploids shot considerably more spores than we have seen in comparable tests with other partial diploids. The rest of the prototrophs were fertile, and apparently euploid.

When the Barren, or nearly Barren, cultures were grown on a somewhat larger scale, or transferred once or twice, they become fertile euploids. Plating of conidia from these cultures to supplemented plates and replication to minimal showed that the diploid prototrophs had segregated vegetatively and become heterocaryotic, with prototrophic nuclei being in the minority. The auxotrophic segregants arising from a single partial diploid ascospore often proved to be of more than one sort. They include every combination of histidine, nicotinamide and thiamine requirement except nicotinamide + thiamine. The auxotrophic segregants were tested for their *nuc* phenotype. It was found that they had the phenotype of the *nuc-1* allele expected if the original *nuc-1* and *his-2* associations had been preserved. The strains had obviously become haploid at the *nuc-1* locus, as well as for the nutritional markers. It is clear that even these “stabilized” partial diploids for the *nuc-1* region do break down rather rapidly, probably by mitotic crossingover between homologous segments followed by breakage at the “fragile point” of the AR190 rearrangement. As in the much more unstable partial diploids made by crossing AR190 by Normal Sequence, the end result is a heterocaryon containing euploid, auxotrophic nuclei, with a minority of partial diploid prototrophic nuclei maintained selectively on minimal medium. In the present case, however, the partial diploids are stable enough to allow them to be grown as colonies of 3 to 5 mm. diameter on plates, and to be classified as null, repressible, or constitutive before they undergo severe mitotic segregation. The

TABLE 2

Alkaline phosphatase phenotypes in prototrophic partial diploids for the nuc-1 region

Partial diploid	Phenotype
<i>nuc-1/nuc-1⁺</i>	repressible
<i>nuc-1⁺/nuc-1^c</i>	constitutive
<i>nuc-1^c/nuc-1⁺</i>	constitutive
<i>nuc-1^c/nuc-1^c</i>	constitutive
<i>nuc-1/nuc-1^c</i>	constitutive
<i>nuc-1^c/nuc-1</i>	constitutive

Phenotypes of partial diploids with several combinations of *nuc-1^c*, *nuc-1⁺* and *nuc-1*. Example of terminology: "*nuc-1⁺/nuc-1^c*" means that *nuc-1⁺* entered the cross in the ALS182 parent and remains linked to the centromere of LG I; the constitutive allele, *nuc-1^c*, entered the cross in the AR190 parent and is linked to the centromere of LG V. The classification "constitutive" means that the prototrophic colonies stained strongly on both low phosphate and high phosphate plates; "repressible" means that the colonies stained strongly on low phosphate but did not stain detectably on high phosphate plates. No "null" partial diploids that failed to stain on low phosphate were found. No cross gave equivocal results; from any given cross, about 35 to 50 prototrophs were grown on low and on high phosphate BdeS plates at 33° for 2.5 days and stained. All colonies on any given plate showed a single phenotype.

phenotypes of a number of such partial diploids is given in Table 2. Larger cultures of these strains, grown under conditions used for quantitative assays of alkaline phosphatase, gave wildly erratic results. Tests of such cultures showed that they had become euploid.

Are nuc-1 and nuc-1^c part of the same cistron or transcription unit? The extremely close linkage between *nuc-1* and *nuc-1^c* does not, of course, prove that they are part of a genetic unit of function; only a *cis-trans* test can establish that. Since *nuc-1^c* is dominant in partial diploids (see below), it is not possible to do an ordinary complementation test. There are ways of circumventing this, however. Let us provisionally call *nuc-1^c* "*nuc-x^c*," which may or may not be an allele of *nuc-1*. We know that the double mutant, *nuc-x^c nuc-1* has the phenotype of *nuc-1*, i.e., it is a null mutant. It is demonstrated below that the partial diploid, *nuc-1/nuc-1^c* (that is, *nuc-x⁺ nuc-1/nuc-x^c nuc-1⁺*) is constitutive. We can then ask whether *nuc-x^c* acts as a constitutive if it is *trans* to a normal *nuc-1⁺*; that is, is *nuc-x^c nuc-1/nuc-x⁺ nuc-1⁺* also constitutive? To test this, we prepared *T(I→V)ALS182 cys-11 nuc-x^c nuc-1 (RHB-141) thi-1; inl* and crossed it to *T(I→V)AR190 nuc-x⁺ nuc-1⁺ ad-9 cyh-1^r al-2^p*. A partial diploid, which was *cys-11* and did not require inositol, thiamin or adenine, was isolated. It grew well on high pH, low P_i medium and presumably was heterozygous for the *nuc-1*, *thi-1* and *ad-9* regions, and was Barren. From the few spores produced by a cross of this strain to Normal Sequence, *nuc-1* segregants could be isolated, proving that this allele was indeed present. When the alkaline phosphatase of this strain was examined on high and low phosphate, it was found to be completely normally repressible. Evidently *nuc-x^c* functions as a constitutive only if it is *cis* to *nuc-1⁺*. We will resume calling it *nuc-1^c*.

Isolation of his-2 nuc-1^c in the insertional translocation background T(I→V)-AR190: As noted before, this translocation moves *his-2*, *nuc-1* and all known IR

markers except *un-2* to *VL* (PERKINS and BARRY 1977a, pp. 253–254; 1977b; and data in the present paper). The breakpoint in IR is therefore proximal to *his-2* and extremely close to it. Attempts to isolate the desired recombinant by screening a manageable number of progeny from crosses of Normal Sequence strains to AR190 derivatives failed. Selection from the following cross was successful (See Figure 3).

$T(I \rightarrow V)$ AR190 *nic-2A* was crossed to Normal Sequence (NS) *un-2 his-2 nuc-1^c-a*. Very roughly, 2.8×10^5 ascospores were heat-shocked and suspended in 100 ml of ordinary Fries minimal medium containing only 0.5% sucrose, so as to give less interference with the action of sorbose used in subsequent steps. The suspension was shaken at 25° for 48 hours, and was filtered through cheesecloth at intervals to remove growing hyphae. The resulting suspension, from which most *his-2*⁺ sporelings had been removed, was distributed to 70 plates containing histidine BdeS agar. These plates were then incubated for three days at 38° to select against *un-2*. Of the 104 colonies that arose, 54 were found to be *un-2*⁺ *his-2 nuc-1^c-A* and nine were *un-2*⁺ *his-2 nuc-1^c-a*. Many of the rest proved to be heterocaryotic for mating type, and were probably so for other genes as well; these cultures were discarded. There were no unequivocal *un-2*⁺ *his-2 nuc-1*⁺ homocaryons. One of the *A* cultures was arbitrarily chosen as the type-strain.

To establish that *his-2* and *nuc-1^c* were no longer on LG I, we crossed the putative $T(I \rightarrow V)$ AR190 *his-2 nuc-1^c-A* to authentic $T(I \rightarrow V)$ AR190 *nic-2-a*. Among 70 viable *his-2* progeny picked from a minimal plate, 32 proved to be the parental mating type, *A*, and 38 were *a*; thus *his-2* has become unlinked to mating type. About 8,000 to 16,000 spores from the same cross were plated to minimal BdeS medium, yielding 101 prototrophs. If *his-2* were still in a NS strain, about one-fourth of the total spores, or one-third of the viable spores would have been heterozygous partial diploids for both *his-2* and *nic-2*, and would have been detected as thousands of prototrophs. All 101 of the prototrophs proved to be *nuc-1*⁺. Because the *nuc-1* gene is much more closely linked to *his-2* than to *nic-2*, only a minority of *nuc-1^c* segregants would have been expected, but their complete absence among the progeny suggests that crossingover in the *his-2*—*nuc-1* region is reduced in the translocation strain even below its normal, low level.

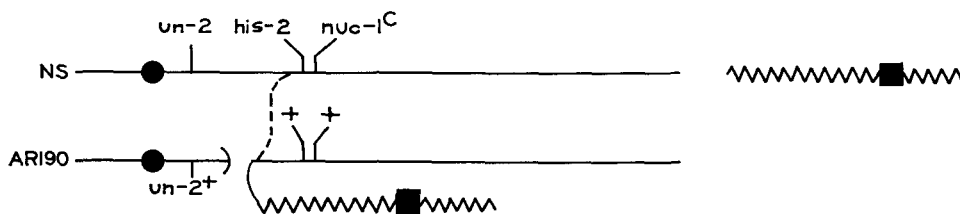


FIGURE 3.—Selection of rare crossovers between the right arm of Normal Sequence (NS) and the translocated segment of $T(I \rightarrow V)$ AR190. The desired crossover is shown by the dashed line. Histidine-independent progeny, including AR190 parentals and partial diploids heterozygous for *his-2*/*his-2*⁺ were removed by filtration enrichment. Nongrowing germings were plated to histidine at 40° to select for *un-2*⁺.

Preparation of T(I→V)AR190 nuc-1^c nic-2: *T(I→V)AR190 his-2 nuc-1^c-A* was crossed to *NS a un-2 nuc-1^c nic-2*. A few thousand spores were plated to BdeS medium containing nicotinamide and incubated at 38° to 40° to select against *un-2*, as well as against *his-2*. Two large vigorous colonies were seen, as well as a much larger number of spindly, poorly growing ones. The two large ones proved to be of the desired genotype. The poorly growing ones have not been characterized. Quite possibly, they are heterocaryons of mixed mating type, since these are known to grow very poorly.

Preparation of T(I→V)AR190 nuc-1 nic-2: This strain was made in an analogous manner from a cross involving *NS un-2 nuc-1 nic-2* and *T(I→V)AR190 his-2 nuc-1^c*.

Preparation of stable partial diploids of the genotypes nuc-1/nuc-1^c, nuc-1^c/nuc-1, nuc-1⁺/nuc-1^c, and nuc-1^c/nuc-1⁺: In these strains, the *nuc-1* allele entering with the *T(I→V)AR190* parent was not *nuc-1⁺*; this parent was prepared as described in the previous section. The partial diploids were then made, as before, by crossing to the appropriately marked *T(I→V)ALS182* derivative. As before, *his-2*, *nic-2* and *thi-1* were used as forcing markers.

The phenotypes of the partial diploids are shown in Table 2. It is apparent that *nuc-1^c* is dominant to both *nuc-1⁺* and to *nuc-1*, and that *nuc-1⁺* is dominant to *nuc-1*.

Heterocaryons between nuc-1^c and nuc-1⁺: Conidia of heterocaryons were inoculated into Fries minimal high and low P_i media (20 ml per flask). The cultures were incubated at 35° for 42 hours. Then the mycelial mats were harvested, ground and assayed for repressible alkaline phosphatase and for protein. Another aliquot of the original inoculum was quantitatively diluted and plated to estimate the proportions of the two nuclear types. The results listed in Table 3 are from a series of heterocaryons showing various proportions.

TABLE 3

Alkaline phosphatase in heterocaryons of nuc-1^c + nuc-1⁺

Strain	<i>nuc-1^c</i> marker(s)	<i>nuc-1⁺</i> marker	% <i>nuc-1^c</i> nuclei	Sp. act. on low P _i , raw data	Sp. act. on high P _i , raw data	% Constitu- tivity*	Sp. act. on high P _i normalized to 100% <i>nuc-1^c</i> nuclei
wild type	—	—	(0%)	1037	0.57	0.055%	"0/0"
Het 39	<i>nic-1</i>	<i>arg-12</i>	31.7%	985	5.77	0.58%	18.2
Het 40	<i>met-6</i>	<i>arg-12</i>	36.6%	1345	7.97	0.59%	21.8
Het 37	<i>inl; al-2</i>	<i>arg-12</i>	47.1%	1124	52.9	4.70%	112
Het 52	<i>nic-1</i>	<i>his-2</i>	71.6%	809	105	13.0%	147
Het 22	<i>lys-4 al-2</i>	<i>met-6</i>	74.6%	1119	69.6	6.22%	93.3
Het 23	<i>lys-4 al-2</i>	<i>his-3</i>	81.2%	1284	83.7	6.51%	103
<i>nuc-1^c</i>	—	—	(100%)	1138	241	21.2%	241

The construction and analysis of the heterocaryons is described in the text.

* % constitutivity is defined as $100 \times$ (specific activity on high phosphate/specific activity on low phosphate).

From inspection of the raw specific activities, it is clear that the specific activity of alkaline phosphatase is quite low in the heterocaryons with a low proportion of *nuc-1^c* nuclei and rises rather abruptly as this proportion increases. This is seen more easily when the specific activity is normalized to 100% *nuc-1^c* nuclei (this cannot, of course, be done in the case of the wild-type control). In the column called % constitutivity, (defined as 100 times the ratio of specific activity of a high phosphate culture divided by specific activity of a low phosphate culture), it is apparent that heterocaryons 39 and 40 are essentially repressible, while heterocaryons 37, 52, 22 and 23 are, to varying degrees, constitutive.

Heterocaryons between nuc-1^c and nuc-1: These were grown and analyzed as above. The results (Table 4) show that heterocaryons with a low proportion of *nuc-1^c* nuclei show "complementation" with *nuc-1* to give, essentially, a repressible phenotype. Cultures having a high proportion of *nuc-1^c* nuclei are constitutive. One of the heterocaryons shown here, heterocaryon 20, employed a different allele of *nuc-1*, namely, *nuc-1* (JFL-611). This allele was included in the series because, unlike our standard allele, it appears to be nonrevertible (J. F. LEHMAN, unpublished data), and might be a deletion mutant. Hence, its "complementation" with *nuc-1^c* is not likely to be due to interaction between *nuc-1* (JFL-611) and *nuc-1^c* polypeptides.

DISCUSSION

The results from the *Neurospora phosphorus* system are most easily explained on the "dosage-titration" model presented in the introduction of this paper and outlined previously by METZENBERG and NELSON (1977). We suggest that *nuc-1^c* is constitutive because it is an over-producer of qualitatively normal *nuc-1* product, so that a normal amount of *preg⁺* product is unable to titrate it out. *nuc-1^c* may be brought to an essentially repressible condition by increasing the dosage of

TABLE 4
Alkaline phosphatase in heterocaryons of nuc-1^c + nuc-1

Strain	<i>nuc-1^c</i> marker(s)	<i>nuc-1</i> marker	% <i>nuc-1^c</i> nuclei	Sp. act. on low P _i , raw data	Sp. act. on high P _i , raw data	% Constitu- tivity*	Sp. act. on high P _i normalized to 100% <i>nuc-1^c</i> nuclei
wild type	—	—	(0%)	1037	0.57	0.055%	"0/0"
Het 17	<i>lys-4 al-2</i>	<i>nic-1</i>	13.8%	231	1.74	0.75%	12.6
Het 16	<i>met-6</i>	<i>nic-1</i>	14.3%	300	2.51	0.83%	17.6
Het 20†	<i>lys-4 al-2</i>	<i>nic-1</i>	29.4%	381	6.10	1.60%	20.7
Het 18	<i>his-2</i>	<i>arg-12</i>	33.8%	484	6.72	1.39%	19.9
Het 26	<i>met-6</i>	<i>nic-1</i>	34.9%	554	7.12	1.29%	20.4
Het 3	<i>nic-1</i>	<i>inl</i>	53.2%	1000	97.0	9.70%	182
Het 1	<i>inl; al-2</i>	<i>nic-1</i>	64.6%	1155	146	12.6%	226
<i>nuc-1^c</i>	—	—	(100%)	1138	241	21.2%	241

* % constitutivity is defined as in the legend of Table 3.

† In Het 20, the *nuc-1* component is allele JFL 611, as described in the text.

preg⁺ as in a partial diploid for this gene, or by diluting out the excess *nuc-1* product in the cytoplasm of a heterocaryon with *nuc-1*⁺ or *nuc-1*.

This interpretation goes against the mindset of many workers in the field of regulation. Where there is a cascade or hierarchy of genetic regulatory elements, most would probably have guessed that the product of one regulatory gene should repress the synthesis of the product of another regulatory gene, rather than impairing its activity directly. Also, there was a body of evidence from a very well-characterized system that at least did not contradict the idea. This system, galactose catabolism in yeast, amounts to a cascade or hierarchy of two regulatory products: that of the *GAL4* gene, whose activity is needed for the expression of several enzymes of galactose metabolism, and that of the *GAL80* gene, which acts to oppose the positive effect of the *GAL4* gene unless the *GAL80* product is inactivated by galactose. Mutations mapping very close to the *GAL4* gene and acting *cis* to it could be isolated; they cause constitutive expression of the structural genes of galactose catabolism. Such mutations were interpreted as operator mutations that prevented the *GAL4* gene from being repressed by the *GAL80* product (at that time, called the *i*-gene product (DOUGLAS and HAWTHORNE 1966)). This model for the galactose system continued to fit the known facts for another ten years. Recently, however, two very different kinds of evidence have supported a model in which the *GAL4* gene makes its product all the time. Using kinetic evidence obtained with temperature-sensitive mutants, MATSUMOTO, TOH-E and OSHIMA (1978) argued that at the permissive temperature, product of the *GAL4* gene must have been present even under circumstances in which its actions were not seen. Very strong evidence for this has come from the work of PERLMAN and HOPPER (1979). They showed that galactose could induce the synthesis of mRNAs for the enzymes of galactose metabolism even when protein synthesis had been blocked by cycloheximide. Therefore, any protein (*viz.*, the *GAL4* product) necessary for the transcription of the structural genes or processing of the transcripts into active form must already have been present at the time protein synthesis was blocked. This, in turn, means that *GAL80* product in the absence of galactose probably impairs the activity of *GAL4* product, rather than repressing its synthesis. These authors also adduced another kind of evidence that *GAL4* product itself is somehow opposed by the *GAL80* product, or at least by the mutant *GAL80*^s product. When *GAL80 GAL4 gal1* cells are mated synchronously to *GAL80*^s *gal4 GAL1* cells, the presence of *GAL80*^s product in the zygote somehow prevents *GAL4* product contributed by the other conjugant from causing the expression of the *GAL1* gene.

Comparing the galactose system of yeast to the phosphorus system of Neurospora, we see a formal resemblance between the *nuc-1*⁺ gene and the *GAL4* gene; each produces something necessary for expression of the structural genes. There is also an obvious formal parallel between the *nuc-1*^c mutants and the *cis*-acting dominant constitutive mutants linked to *GAL4*, now called *GAL81* mutants. The *preg*⁺ gene product, or the *nuc-2*⁺ and *preg*⁺ gene products taken sequentially, controls the activity of the *nuc-1*⁺ product as the *GAL80* product controls that of the *GAL4* product; here the parallelism becomes indistinct because of the greater

complexity of the phosphorus hierarchy and the fact that the galactose enzymes are inducible, while the phosphorus enzymes are repressible. The analogy can be pushed a bit more, though. NOGI *et al.* (1977) found that combining a dominant *GAL81* constitutive allele with a certain dominant *GAL80^s* "superrepressor," uninducible mutant gave a double mutant with a surprising phenotype: it was normally repressible. Admitting that it is risky to extend our interpretations to a different system and organism, we would suggest that *GAL80^s* may really be just an overproducer of normal *GAL80* product that can titrate out the excessive *GAL4* product made by *GAL81*, just as two doses of *preg⁺* product acting on *nuc-1^c* can give almost normal repressibility. A mutation in the phosphorus system truly analogous to *GAL80^s* would be a dominant "null" mutation in the *preg* gene. No such mutant has yet been found.

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