Spectrum of Forward Mutants in the pyr-3 Region of Neurospora

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SUMMARY

About 200 forward mutants of Neurospora obtained as suppressors of arg were studied. All fell in the pyr region, and all appeared deficient in aspartic transcarbamylase (ATC) to various degrees: extreme deficiencies in ATC (pyr-N alleles) resulted in pyrimidine-dependence as well as suppression of arg, while partial ATC deficiencies (pyruv-arg alleles) led only to suppression. Five different complementation groups were represented among the pyr-N alleles studied. Evidence is presented which suggests that pyr-N and pyruv-arg alleles affect ATC via different mechanisms.

INTRODUCTION

The genetics and the complementation pattern of mutants at the pyr-3 (pyr) region of Neurospora was studied by Mitchell & Mitchell (1956), by Suyama, Munkres & Woodward (1959) and by Woodward (1962). Davis (1960), investigating the enzymic defects in the same mutants, showed that the pyr region is functionally heterogeneous: it comprises mutants which lack aspartic transcarbamylase (ATC) activity, as well as mutants with normal values of the same activity and presumably deficient in a previous step (Davis, 1961, 1962). The former or ATC-less mutants will be designated pyr-8N (pyr-N), and the latter pyr-8M (pyr-M). The results just outlined were obtained with mutants isolated by the usual methods for the selection of auxotrophs. A special screening method, which will be referred to as the suppressor method, is also available for the isolation of pyr mutants (Reissig, 1968). This method takes advantage of the fact that pyr mutants (pyr-N mutants, as will be seen) suppress the arginine requirement of a strain carrying the arg-2 (arg) mutant gene. Even partial decreases in the activity of the normal pyr+ allele result in suppression of arg (Reissig, 1960). Consequently the suppressor method screens for a whole spectrum of mutant alleles, ranging from the subthreshold pyruv-arg (no pyrimidine requirement) to pyr alleles allowing no trace of growth in the absence of pyrimidines. With two basically different methods available for the selection of mutants at pyr, it seemed profitable to attempt a more thorough assessment of the genetic information contained in this region.

The following abbreviations are used: arg for arg-2; pyr for pyr-3; ATC for aspartic transcarbamylase; Rgser for growth in the absence of arginine relative to growth in its presence; Rgur for growth in the absence of uridine relative to growth in its presence.

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METHODS

General methods were described in the preceding paper (Reissig, 1962). Additional details are given in this section.

Strains. Several pyr mutants obtained by auxotroph selection following treatment with u.v.-radiation, were kindly provided by K. D. Munkres (strains prefixed KS; Suyama et al. 1959) and by M. B. Mitchell; representing types N and M (Davis, 1960; and personal communication): KS36a, KS36A, 70007-45502-32723a (pyr-3d co) and 45502-1507-1A (pyr-3d T) all type N; and KS138a, KS138A and ED354-1a (a re-isolate of 87301) all type M. The St Lawrence wild types 74A and 73a were also used.

Measurement of growth responses. Growth was measured as in the usual tube method (Ryan, Beadle & Tatum, 1943); but by using 15 x 150 mm. test tubes with a notch near the open end, laid horizontally so that the notch retained the medium (5 ml.). Each strain was tested on four different media: unsupplemented, supplemented with arginine (0.1 g./l.), supplemented with uridine (0.05 g./l.), and supplemented with arginine + uridine.

Strain ED416-1a, the source of all mutants screened, is arginine-dependent, but can grow slowly on arginine-free medium. This slow growth is characterized by a long lag phase (5-6 days instead of the usual 1 or 2) followed by growth at a constant and normal rate. Therefore, linear growth rate is useless as a measure of growth response to arginine: a meaningful growth index must take into account the duration of the lag phase. The growth index used was defined as the reciprocal of the time taken by the mycelial front to travel 11 cm. from the point of inoculation. Measurements were discontinued on the 14th day, and extrapolated whenever necessary.

The growth of each mutant in the absence of arginine, relative to its growth in the presence of arginine, was called $R_{gal}$ and calculated from equation (1):

$$R_{gal} = \frac{\text{index on minimal} + \text{index on uridine}}{\text{index on arginine} + \text{index on arginine} + \text{uridine}}.$$  

Similarly, relative growth without uridine ($R_{gud}$) is given by equation (2):

$$R_{gud} = \frac{\text{index on minimal} + \text{index on arginine}}{\text{index on uridine} + \text{index on arginine} + \text{uridine}}.$$  

The measurement of response to uridine does not present complications of the type discussed in connexion with arginine response: uridine-stimulated mutants respond to this metabolite by shortening the lag phase and increasing the linear growth rate. However, for the sake of uniformity, the growth index was defined throughout as previously indicated. It should be noted that substitution of rates of linear growth for the growth indexes in equation (2), leaves $R_{gud}$ values practically unchanged. Therefore, the choice of growth index is not imposing any arbitrary distortion upon the description of the experimental data. A difference in $R_{gal}$ or in $R_{gud}$ larger than 0.2 is probably significant. However, statistical controls were not included in the experiments to be reported; therefore only the major features of these results can be discussed.

Complementation tests. Unless otherwise specified, mutants were tested in pair-
wise combinations for their ability to complement (i.e. to form a prototrophic heterokaryon) on plates of minimal medium + arginine in the manner described by de Serres (1956). Unsupplemented plates yielded similar results. Two weeks were allowed whenever possible before classing a test as negative, but positive reactions were in evidence between the 2nd and 4th day except when unrelated strains were used. Complementation tests between arg and pyr (or arg pyr) strains were made on plates supplemented with orotic acid (1 g./l.). This supplement inhibits the residual growth of arg and stimulates only slightly pyr strains.

RESULTS

The frequency distribution of $R_{g,\text{arg}}$ values among 190 mutants obtained with the suppressor method, is shown in Fig. 1. Clearly, partially arginine-dependent mutants ($R_{g,\text{arg}}$ in the 0.4-0.8 range) were also obtained. This is so with the usual scoring schedule; but by picking 2 days earlier, only fully arginine-independent mutants are recovered.

The distribution of $R_{g,\text{pyr}}$ values among the same 190 mutants is strikingly bimodal (Fig. 2). Before attempting to interpret these results we must ascertain the genetic basis of the mutations examined. This is particularly important because it is known from previous work that the screening method used yields back-mutants at arg as well as mutants at pyr. The genotype of the prototrophs was ascertained by recombination analysis, as reported in the preceding paper of this series (Reissig, 1962); the results are indicated in Fig. 2: $\text{pyr}^\text{su-arg}$ strains by vertical hatching; and back mutants by dotted areas. Pyrimidine-dependent mutants were tested for allelism by complementation criteria (see section on allelism), and all found to be allelic as indicated by horizontal hatching in Fig. 2.

Mutants with low $R_{g,\text{arg}}$ values ($< 0.85$) could not easily be tested by the recombination method, because the segregation of partial arginine-dependence interferes. Unshaded areas in Fig. 2 correspond to mutants of this type. Preliminary tests performed with some of them favour the hypothesis that they are predominantly $\text{pyr}^\text{su-arg}$.

It is clear that the distribution of mutants at pyr is bimodal. There is no reason to suspect that this may be due to an artefact of the selection method. Bimodality points, rather, to a duality in function, as will be considered in the discussion.

Complementation pattern

In a previous study (Reissig, 1959) 9 pyr mutants obtained by the suppressor method were tested for complementation in all possible pairwise combinations. This disclosed two complementation groups or cistrons: alpha, represented by 5 mutants, and beta, represented by 4 mutants. Complementation testing was now extended to 204 new mutants of independent origin, induced by u.v.-radiation, ethylmethane sulphonate or nitrous acid (Reissig, 1963). Testing in all possible combinations was not attempted; instead, 1 alpha tester and 3 beta testers were chosen at random, and their complementation reactions with all other strains were determined. The results of these and some additional tests are shown in Table 1. Four complementation groups (alpha, beta, gamma, delta) were revealed, such that any member of one group complemented with any member of another, but did not complement with members of the same group. A fifth group (non-complementing group) comprises
mutants unable to complement with representatives of any of the other 4 groups.

The failure of this fifth group to complement could be due to relationships intrinsic to the pyr region; or to extrinsic factors, such as additional mutations at loci which determine barriers to the formation of heterokaryons. To determine whether the ability to form heterokaryons was impaired in the 7 non-complementing mutants, heterokaryons were attempted between them and the arg strain

![Graph 1](image1.png)

![Graph 2](image2.png)

Fig. 1. Distribution of $R_{\text{arg}}$ values among Neurospora mutants obtained by the suppressor method. ■, Original arg strain. For definition of $R_{\text{arg}}$, see Methods.

Fig. 2. Distribution of $R_{\text{gad}}$ values among Neurospora mutants obtained by the suppressor method. ■, Original arg strain; □, back mutants (arg$^+$); ◼ not tested. Forward mutations at pyr: ■, as established by complementation; □, as established by recombination; ◼, as established by complementation and recombination. For definition of $R_{\text{gad}}$, see Methods.

from which all pyr mutants derived. Positive responses were obtained in every case, and the true heterokaryotic nature of the resulting prototrophic mycelium was confirmed by the isolation of hyphal tips, subcultivation, and plating of microconidia
on appropriately supplemented media. Therefore failure to complement was not due to incompatibility barriers, and was most likely related to the type of functional alteration at *pyr*. A clear correlation was noted between failure to complement, and failure to grow at all on minimal medium (Table 2).

Table 1. **Complementation among pyrimidine-dependent mutants of Neurospora obtained by the suppressor method**

Mutants of Neurospora were classified in 5 complementation groups. The complementation reactions of all members of each group with representative strains from the same and from other groups are indicated in the table: +, complementation; 0, no complementation. The figures in parentheses indicate the number of different pairs tested in each case.

<table>
<thead>
<tr>
<th>No. of mutants</th>
<th>Complementation group</th>
<th>Alpha</th>
<th>Beta</th>
<th>Gamma</th>
<th>Delta</th>
<th>Non-complementing</th>
</tr>
</thead>
<tbody>
<tr>
<td>155 Alpha</td>
<td>0 (444)</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>88 Beta</td>
<td>+ (207)</td>
<td>0 (41)</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>8 Gamma</td>
<td>+ (12)</td>
<td>+ (15)</td>
<td>0 (8)</td>
<td>.</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>1 Delta</td>
<td>+ (4)</td>
<td>+ (5)</td>
<td>+ (8)</td>
<td>0 (1)</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>7 Non-complementing</td>
<td>0 (28)</td>
<td>0 (28)</td>
<td>0 (21)</td>
<td>0 (7)</td>
<td>0 (1)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. **Correlation between failure to grow on pyrimidine free medium, and failure to complement**

<table>
<thead>
<tr>
<th>Growth on pyrimidine free medium*</th>
<th>No. of <em>pyr</em> mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complementing†</td>
</tr>
<tr>
<td>Yes</td>
<td>101</td>
</tr>
<tr>
<td>No</td>
<td>39</td>
</tr>
</tbody>
</table>

* Visible growth by the 14th day on arginine supplemented medium.
† Either in alpha, beta, gamma or delta complementing groups.

**Comparison with mutants obtained by auxotroph selection**

Two types of mutants are known to occur at the *pyr* region (Davis, 1960): *pyr*-N mutants lacking ATC; and *pyr*-M mutants with normal levels of ATC. In first analysis, *pyr*-N and *pyr*-M mutants can be identified with two complementation groups, which will be called, respectively, *n* and *m*. New complementation groups have appeared in more recent work (Woodward, 1962; see also Mitchell & Mitchell, 1956), but the earlier identification of *pyr*-N and *pyr*-M with two complementation groups is still useful as a preliminary criterion for comparative studies. The results just described refer to mutants obtained by auxotroph selection. It is pertinent to ask whether the same picture applies to mutants obtained by the suppressor method. The data in Table 3 show that this is not so: suppressor selection appears to yield only mutants of the *n* complementation type. But before attempting an interpretation of the data in Table 3, it was essential to find out whether the negative complementation tests might result from incompatibility factors extraneous to the *pyr* locus. Incompatibility factors were ruled out by three different criteria.

**First criterion.** Two mutants, respectively alpha and beta type, were crossed to
74A, a strain highly compatible with KS strains. Eighteen segregants (11 alpha, and 7 beta) were tested for complementation with \( m \) (KS188A, KS188a, and ED854-1a) and with \( n \) (KS86a, KS86A, pyr-8d co, pyr-8d T) strains. Although the speed and intensity of the positive reactions were much improved in most cases, all reactions reported negative in Table 3 were again negative.

### Table 8. Complementation between pyrimidine-dependent mutants of Neurospora obtained by the suppressor method and by auxotroph selection

<table>
<thead>
<tr>
<th>Suppressor mutants</th>
<th>Group m mutants</th>
<th>Group n mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Group</td>
<td>KS188a</td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>5</td>
<td>Alpha</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Beta</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Gamma</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>Delta</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Non-complementing*</td>
<td>+</td>
</tr>
</tbody>
</table>

* That is, non-complementing when tested against suppressor mutants (Table 1).

**Second criterion.** An alternative method for testing complementation involves crossing both auxotrophic strains and determining whether prototrophic pseudowild type ascospores appear. This test is more sensitive than the usual heterokaryon test (de Serres, 1960) and eliminates incompatibility barriers (Mitchell & Mitchell, 1956). A total of 10,800 viable ascospores was examined in crosses of \( n \times x \); 7700 ascospores in crosses of \( n \times \beta \); and 1030 ascospores in crosses of \( n \times \) non-complementers. In every case the results were negative. Control crosses of the same to \( m \) strains gave an average of 0-2% prototrophs.

**Third criterion.** The strain from which all suppressor mutants derive is arginine-dependent. It was a simple matter to test whether this strain (ED416-1a) is heterokaryon-compatible with KS86a, one of the strains used as \( n \) tester in the experiment of Table 3. Mixed inoculation allowed growth on orotic acid supplemented plates. Isolation of hyphal tips, cultivation on minimal medium slopes, and plating of microconidia on appropriately supplemented media, proved that a true heterokaryon had formed. Therefore, no barriers existed which prevented heterokaryon formation.

**Conclusion.** The complementation results shown in Table 3 reflect functional relations at the \( pyr \) locus, and are not distorted by extrinsic factors. Pyrimidine-dependent mutants representative of the different types obtained as suppressors of \( arg \), are all of the \( n \) complementing type, and thus presumably deficient in ATC.

**Allelism of suppressor mutants**

In this section we shall consider all mutants obtained by the suppressor method, with the exception of back mutants at \( arg \). Allelism between \( pyr^{awarg} \) and \( pyr \) was demonstrated to within \( \pm 0.1 \) map units in two instances in a previous publication (Reissig, 1960). Recently 89 new \( pyr^{awarg} \) mutations were identified by genetic tests (Reissig, 1962). These tests involved crossing \( arg \ pyr^{awarg} \times arg pyr \). If \( pyr^{awarg} \) and
pyr were not allelic, recombination would yield arg + + strains, arginine-dependent but pyrimidine-independent. Yet no arginine-dependent strain was found among 748 pyrimidine-independent segregants isolated from crosses involving the 89 new strains. Thus most if not all mutants described as pyr′′′arg′′′ are alleles of pyr, or closely linked to this locus.

Allelism of all pyrimidine-dependent mutants is most simply demonstrated by a functional criterion: they all can be classified in five complementation groups, such that one of the groups fails to complement with the other four (α, β, γ, δ). Genetic evidence for allelism between an alpha mutant and a beta mutant was presented earlier (Reissig, 1959). Five additional mutants representing the various complementation groups were crossed to a sixth pyr mutant; again no pyrimidine-independent recombinant was detected among several thousand ascospores from each cross.

Having demonstrated that the major mutations screened with the suppressor method are allelic, the possibility should be considered that concomitant mutations at modifier genes might be responsible for the minor phenotypic differences observed. This seemed unlikely, since it would require simultaneous mutation of two genes (pyr and modifier) affecting the same character, but nevertheless was tested. Four pyr mutants with \( R_{G_{ad}} = 0.6-0.7 \) (pyr\(_{0.6-0.7}\)) were crossed to a pyr strain with \( R_{G_{ad}} = 0.0 \) (pyr\(_{0.0}\)) marked with co (colonial), about 3 units away from pyr. Both parents carried also the cot (colonial at 32°) allele. When ascospores from such a cross are incubated at 32°, all growing clones make small colonies. The morphological difference between co and co + is masked at this temperature, and size differences are related to growth requirements only. Cooling then to 25° the difference between co and co + can be clearly appreciated in a few hours. Ascospores from crosses of co + arg pyr\(_{0.6-0.7}\)cot \( \times \) co arg pyr\(_{0.0}\) cot were plated on minimal medium with and without pyrimidines. On the former medium the colonies formed were of normal size, half co and half co +. On medium lacking pyrimidines, the colonies which appeared were small, and most (695 out of 702) were co +. This result shows that the factor allowing residual growth is closely linked to co.

In another experiment, ascospores from the cross co arg pyr\(_{0.4}\) cot \( \times \) co + arg pyr\(_{0.0}\) cot were plated on minimal medium. Seven days later, microcolonies could be distinguished and transferred to pyrimidine supplemented medium. On cooling, all 174 colonies were found to be co. Control platings on pyrimidine medium yielded half co and half co +. Control platings on minimal of crosses involving pyr\(_{0.0}\) \( \times \) pyr\(_{0.0}\) gave no microcolonies. Thus, linkage between the residual growth factor and co was again demonstrated. In all likelihood this means that residual growth is an attribute of the pyr alleles characteristic of each mutant, and that additional modifier mutations are not involved.

**ATC activities**

Dr R. H. Davis, at the University of Michigan, has assayed some of our suppressor mutants for ATC activity and very kindly made available his results for insertion in the present publication (Table 4). It is clear that pyr mutants belonging to the five complementing groups are all deficient in ATC. Mutants with low \( R_{G_{ad}} \) have no demonstrable ATC activity, where 5% of the normal value would have been detectable; and mutant KO493–50a, capable of appreciable growth in the absence
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Biochemical analysis

**ATC present**  **ATC absent**

Complementation analysis

Origin of mutants

Auxotroph selection

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Genotype</th>
<th>Complementation type</th>
<th>$R_{sd}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>KO498-14a</td>
<td>arg pyr</td>
<td>Alpha</td>
<td>0.18</td>
</tr>
<tr>
<td>KO498-54a</td>
<td>arg pyr</td>
<td>Beta</td>
<td>0.02</td>
</tr>
<tr>
<td>KO556-185a</td>
<td>arg pyr</td>
<td>Gamma</td>
<td>0.00</td>
</tr>
<tr>
<td>KO545-122a</td>
<td>arg pyr</td>
<td>Delta</td>
<td>0.00</td>
</tr>
<tr>
<td>KO545-81a</td>
<td>arg pyr</td>
<td>Non-complementing</td>
<td>0.00</td>
</tr>
<tr>
<td>KO498-50a</td>
<td>arg pyr</td>
<td>Alpha</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Controls:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>$R_{sd}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED416-1a</td>
<td>arg pyr+</td>
<td>1.17</td>
</tr>
<tr>
<td>KO498-56a</td>
<td>arg pyr+</td>
<td>1.01</td>
</tr>
<tr>
<td>78a</td>
<td>arg pyr+</td>
<td>ca. 1</td>
</tr>
</tbody>
</table>

Specific ATC activity in extract (μmole ureido- succinate/mg. protein/hr.)

Table 4. Aspartic transcarbamylase activities in Neurospora mutants isolated by the suppressor method, and in controls

Enzyme assays performed by Dr R. H. Davis (personal communication). Methods are described elsewhere (Davis & Woodward, 1962). Cultures in liquid minimal medium, supplemented with arginine and uridine (each 50 mg./l). All strains derived from ED416-1a by one-step mutation, except 78a.

of uridine ($R_{sd} = 0.6$), had about one-third of the activity in the controls. Mutant KO498–40a, a typical pyr<sup>mut</sup> strain, also displayed decreased ATC activity; while KO498–56a, a back-mutant of ED416–1a, had the same activity as ED416–1a or an unrelated wild strain.
Spectrum of pyr-3 mutants

Dr M. Nazario (personal communication; Instituto Nacional de Microbiología, Buenos Aires) has confirmed the absence of ATC activity in strains K0498-14, K0493-54, K0545-122 and K0545-31, by using a method capable of detecting 0.8% of the normal activity. He measured incorporation of 14C carbamyl phosphate in the acid-stable fraction, using extracts of strains cultured under conditions of de-repression, i.e. with limiting uridine.

The biochemical data reported confirm the conclusions arrived at in previous sections on the basis of genetic and complementation data alone, namely that suppressor mutants are deficient in ATC (Fig. 3); and that pyr<sup>sup-arg</sup> is an allele of pyr.

**DISCUSSION**

The mutants screened by the suppressor method fall into two groups: back mutations at arg and forward mutations at pyr. The latter involve drastic (pyr-N) or partial (pyr<sup>su-arg</sup>) deficiencies in ATC activity. Since selection is based on the suppression of the arg gene, it could be concluded that this suppression entails in every case a total or partial block in reaction (1):

\[
\text{carbamyl phosphate} + \text{aspartate} \rightarrow \text{ureidosuccinate (1)}
\]

The available evidence (Reissig, 1960; Davis, 1962) indicates that arg has a partial (cf. Fig. 1) block in the synthesis of carbamyl phosphate, a common precursor of arginine and pyrimidines. From carbamyl phosphate onwards, the arginine and pyrimidine biosynthetic pathways diverge via reactions catalysed, respectively, by ornithine transcarbamylase (OTC) and by ATC (reaction 1). Thus, OTC and ATC compete for carbamyl phosphate. Why a block in carbamyl phosphate synthesis should result in a mutant responding to arginine, but not to pyrimidines, has not been explained with certainty; but this might easily result from irreversibility of certain reactions, channelling, or prevailing regulatory systems. At any rate, suppression of arg by a second mutation creating a deficiency for ATC, is readily understood in terms of a diminished competition for carbamyl phosphate. Diminution of ATC activity to about one-third its normal value suffices for full suppression of arg (Table 4). Other mutations in the pyr region cause only partial suppression of arg (Reissig, 1960, and present paper), presumably as a result of smaller decreases in ATC activity.

There are two types of genes which control protein synthesis: regulatory and structural (Jacob & Monod, 1959). In the present study, the frequency distribution of ATC-deficient mutants is clearly bimodal when they are classified according to the extent of their pyrimidine dependence (Fig. 2), and presumably also when classified by ATC activity (Table 4). This can be most simply explained on the hypothesis that most pyrimidine-dependent types (pyr-N) are due to mutations at a structural gene, and most prototrophs (pyr<sup>sup-arg</sup>) to mutations at a regulatory gene. Of course, the evidence for the existence of both regulatory and structural genes in this system is not compelling, and is indirect. Alternative but less plausible explanations are possible. For instance, the structural relations in the ATC molecule may be such that amino acid substitution would most often lead to two discontinous activity states. Or discontinuity may follow from the peculiar effects of chromosomal sites of high mutability ('hot-spots', in the terminology of Benzer & Freese, 1958). The
latter alternative is rendered improbable by the fact that the same distribution of mutant types can be obtained with different mutagens (Reissig, 1963).

An interesting feature of the mutants discussed here is the fact that mutants defective in the same enzymic activity are able to complement in heterokaryons. It was noted (Table 2) that there is a positive correlation between residual growth of the various mutants and their ability to complement. This correlation should be kept in mind when comparing mutants obtained by the suppressor method with mutants obtained by the usual methods for the selection of auxotrophs, since the latter methods discriminate against mutants which exhibit residual growth, while the suppressor method does not. A possible theoretical basis for the correlation between residual growth and complementation is provided by the observation (Yanofsky & St Laurence, 1960; Lacy & Bonner, 1961) that tryptophan synthetase mutants which possess CRM (an immunologically related protein) complement more frequently than those which lack it. In turn, possession of CRM might be a condition for residual growth.

This work was performed during the tenure of a fellowship from the Rask Ørsted Foundation, and supported by grants from the Carlsberg Foundation and the Rockefeller Foundation. I am grateful to Professors M. Westergaard, O. Maaløe and N. H. Giles for many useful discussions; to M. B. Mitchell, K. D. Munkres and V. W. Woodward for access to their mutant strains and unpublished results; and to R. H. Davis and M. Nazario for generously facilitating their unpublished results for inclusion in this paper, and for many useful comments.

This is paper 3 in the series on forward and back mutation in the pyr-3 region of Neurospora.

REFERENCES


Structural and functional complexity in the *ad-3* region. *Genetics*, 41, 668.
Lack of complementation between different *ad-3A* mutants in heterokaryons and
pseudowild types. *Genetics*, 45, 555.
SUYAMA, Y., MUNKRES, K. D. & WOODWARD, V. W. (1959). Genetic analyses of the *pyr-3*
locus of *Neurospora crassa*: the bearing of recombination and gene conversion upon
intrallelic linearity. *Genetica*, 30, 293.
WOODWARD, V. W. (1962). Complementation and recombination among *pyr-3* hetero-