GENETICS OF ARGININE BIOSYNTHESIS IN *NEUROSPORA CRASSA*

ROWLAND H. DAVIS

Department of Molecular Biology and Biochemistry, University of California, Irvine, Irvine, California 92717

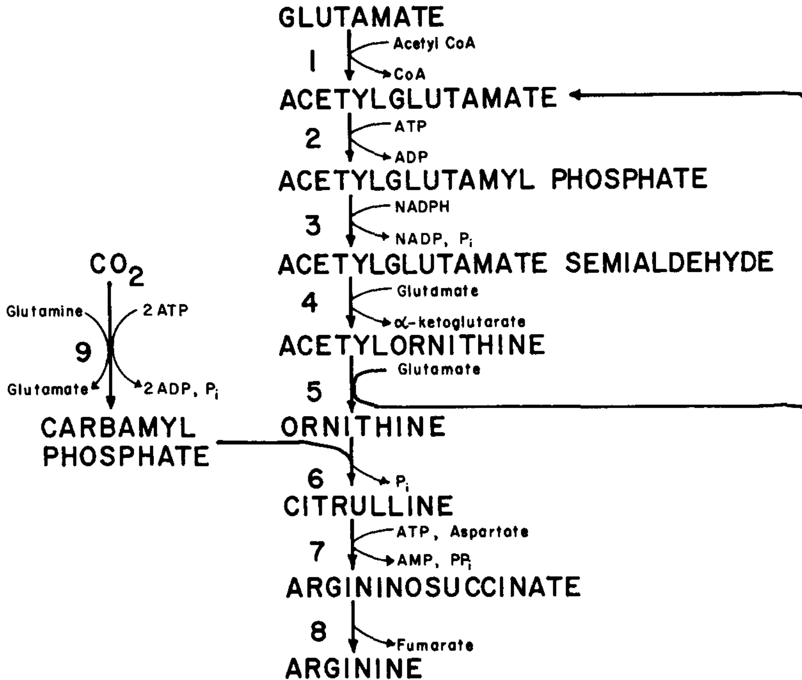
Manuscript received March 5, 1979
Revised copy received May 11, 1979

ABSTRACT

A large number of arginine-requiring mutants of Neurospora was isolated, using a strain already partially impaired in an enzyme of the pathway. Among the mutants, all previously described loci, except one, were represented, and several new loci were defined and mapped. Four groups of mutants were of particular interest. First, the large group of *arg-6* mutants, when tested for intragenic complementation, suggested a bifunctional gene, possibly controlling two steps in ornithine synthesis. This is consistent with the limited enzymic information about this locus. Second, the *arg-13* locus was represented by 14 new mutants. All five tested were quite leaky, suggesting that the function controlled by this gene can be carried out to a limited extent spontaneously or by another gene product. Third, a new locus, *arg-14*, was defined. It controls a step in ornithine synthesis. It lies in a 1 to 2 map-unit interval between *arg-2* and *pyr-3* on LG IVR, as shown by mapping in relation to translocation breakpoints. Fourth, a second new locus whose mutants render the partial mutation in starting material auxotrophic was defined and mapped near the centromere of LG VIL. These new mutants are unable to derepress enzymes of the pathway and may qualify as regulatory mutants.

THE arginine pathway of Neurospora was one of the first to be studied as an example of the one-gene, one-enzyme hypothesis (SRB and HOROWITZ 1944; SRB, FINCHAM and BONNER 1950). Despite this, gene-enzyme relationships in this pathway have never been completely determined in Neurospora. This reflects in part the difficulty of assaying the earliest enzymes of ornithine synthesis, and in part the difficulty of isolating mutants for many of the loci. For instance, only one mutant lacking ornithine transcarbamylase has even been isolated from wild-type Neurospora by the standard procedure of filtration enrichment (DAVIS and THWAITES 1963). The problem of mutant isolation reflects both the intrinsic "leakiness" of mutants at some loci, and the probable residual arginine storing or synthetic capacity of asexual spores that acquire a mutant nucleus by mutagenic treatment. The relationships of genes to enzymes known to date are shown in Figure 1.

Some years ago, the structural gene mutation, *arg-12*^{s}, was found to reduce ornithine transcarbamylase activity by over 98%, without imposing any arginine auxotrophy (DAVIS 1962a,b). When combined with leaky mutations at other loci of the pathway, however, it rendered them absolute auxotrophs (MITCHELL and MITCHELL 1952). Using *arg-12*^{s} as a starting strain, the yield of mutations



1 AcGlu synthase
2 AcGlu kinase (*arg-6*)
3 AcGluP reductase (*arg-6*)
4 AcOrn transaminase (*arg-5*)
5 AcOrn-Glu transacetylase (*arg-7*)
6 Orn transcarbamylase (*arg-12*)
7 ASA synthetase (*arg-1*)
8 ASA lyase (*arg-10*)
9 CAP synthetase A (*arg-2+3*)

FIGURE 1.—The path of carbon in the arginine biosynthetic pathway of Neurospora (heavy lines). The numbered reactions are listed, using trivial names and abbreviations, based on their substrates or their products. The gene assignments are given in parentheses. Enzyme Commission numbers for enzymes are: (1) EC 2.3.1.1.; (2) EC 2.7.2.8; (3) EC 1.2.1.38; (4) EC 2.6.1.11; (5) EC 2.3.1.35; (6) EC 2.1.3.3; (7) EC 6.3.4.5; (8) EC 4.3.2.1; (9) EC 2.7.2.9.

at many loci could be increased greatly, including further mutations at the ornithine transcarbamylase structural gene (DAVIS and THWAITES 1963). Another characteristic of *arg-12*^{s} is its ability to block the use of exogenous ornithine by mutants unable to synthesize ornithine. Double mutants of this sort will grow only on citrulline or arginine (MITCHELL and MITCHELL 1952; DAVIS and MORA 1968).

In this paper, a large series of arginine mutations selected from a strain carrying the *arg-12*^{s} mutation is described. The initial intention was to isolate mutations at the *arg-2* and *arg-3* loci, which encode the information for carbamyl phosphate synthetase A. However, mutations at all other loci were saved. These are reported as the most definitive series of mutants found to date for the pathway. Two new loci are represented among the mutants.

MATERIALS AND METHODS

The strains used were from the author's stock collection. The standard wild-type strains, *73a* and *74A*, were used, and all other strains were derived from this background in this or

other laboratories. Mutations used (singly or in various combinations) were: *pyr-3* (DFC-3), *arg-1* (UM-245), *arg-2* (MEP-7 and MEP-23), *arg-3* (MEP-6), *arg-5* (27947), *arg-6* (29997), *arg-7* (34105; locus same as "*arg-4*"), *arg-10* (B317), *arg-12* (UM-107 and *arg-12*^{s}), *arg-13* (RU-12). Strains or mutations used for mapping were *alcoy* (multiple translocation); S1229, S4342 and NM152 (insertional translocations with a break point between *arg-2* and *pyr-3*); *al-2* (15300); *trp-1*, *ylo-1* (10575, Y30539y); *cys-1*, *ylo-1* (84605, Y30539y). Strains carrying most of these mutations are available from the Fungal Genetics Stock Center, Humbolt State University, Arcata, California. Isolation numbers of all mutations isolated in the course of this work were prefixed by the letters CD.

With one exception, all operations, including stock maintenance, were done on Medium N (VOGEL 1964). Mutant selection, however, was done using the salt mixture of WESTERGAARD and MITCHELL (1947). Supplementation of media, where appropriate, was done at a level of 100 µg uridine per ml; 200 µg arginine HCl per ml; for nutritional tests, supplements were used at a concentration of 1 mM.

All genetic techniques used are described in detail in DAVIS and DE SERRES (1970). Mutant isolation was by filtration enrichment, using 0.5 mg streptomycin and 200 units penicillin per 50 ml medium to inhibit bacterial growth. Crosses were analyzed by plating activated ascospores on appropriate media and isolating colonies to tubes after 18 to 24 hr growth. Spot tests were then made on appropriate media. Complementation tests were done by adding drops of conidia to 1 ml test medium in 13 × 100 mm tubes capped with loose aluminum closures. A genetic map showing pertinent genes, including those described in this paper, is given in Figure 2.

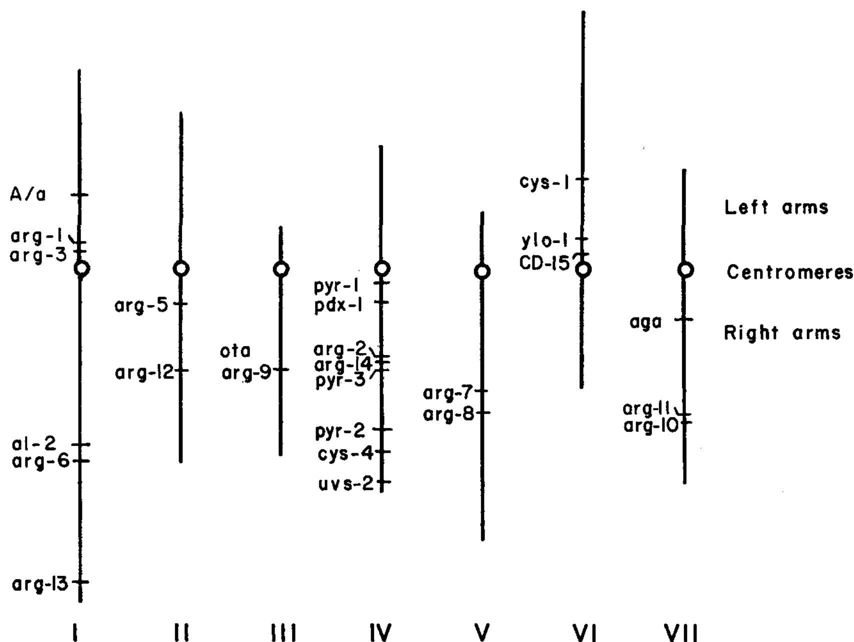


FIGURE 2.—The seven linkage groups of *Neurospora crassa* (I–VII), showing the genetic positions of loci related to the arginine pathway, and of certain other loci mentioned in this paper. The maps are based on those of DAVIS and DE SERRES (1970) and PERKINS and BARRY (1977), and are drawn without serious attention to map-distance scale. Besides arginine (*arg*) loci, others are noted: Mating type (*A/a*); albino (*al*); arginase (*aga*); cysteine (*cys*); ornithine transaminase (*ota*); pyridoxine (*pdx*); pyrimidine (*pyr*); ultraviolet-sensitive (*uvs*); and yellow (*ylo*). The order of *ota* and *arg-9* with respect to the centromere is not known. Note: *arg-7* is also known as *arg-4*; *arg-8* and *arg-9* are actually involved in proline, not arginine biosynthesis (synonyms: *pro1-3* and *pro1-4*, respectively).

Tests to distinguish *arg-12+* and *arg-12ˢ* strains (both prototrophic) required a fast assay for ornithine transcarbamylase. The one devised previously (DAVIS and THWAITES 1963) was too cumbersome. Large numbers of strains could be processed as follows. Tubes with loose aluminum caps, containing 3 ml Medium N with 200 μg L-arginine HCl per ml (and if necessary, 100 μg uridine per ml) were inoculated with conidia from strains to be tested. They were allowed to grow for two days at 32° and were shaken twice a day to submerge aerial growth. After two days, assay of whole mycelial pads was done with a permeabilization step immediately preceding the assay. First, 0.15 ml of a toluene-ethanol mixture (1:4, v/v) was added to tubes (up to 50 tubes at a time) and the tubes were shaken. The pads were lifted from the medium, squeezed against the tube, and put in a 1-ml reaction mixture at 35°. The reaction mixtures were 4.5 mM ornithine HCl, 4.5 mM dilithium carbamyl phosphate; and 100 mM Tris-HCl, pH 9.0. They were prepared and distributed just before use because of the instability of carbamyl phosphate. After 15 min (30 min for especially small pads), the pads were removed from the reaction mixtures with a spatula. To the reaction mixture, 0.5 ml of a color reagent was added. This reagent (adapted from ARCHIBALD 1944) consisted of seven parts phosphoric-sulfuric acid (3:1, v/v, concentrated) to one part 3% 2,3-butanedione-3-oxime. Reaction mixtures were mixed thoroughly, incubated at 60° for 30 min and read as positive (bright peach) or negative (blank or light pink). The test is dependable for all strains tested except those, like *arg-1* or *arg-10*, that accumulate citrulline during growth. In the latter case, reading tubes immediately yields the most dependable data.

All chemicals were of reagent grade. Nutritional supplements and enzyme reagents were obtained from Sigma Chemical Co. Dilithium carbamyl phosphate was synthesized by the method of SPECTOR, JONES and LIPMANN (1955).

RESULTS

Isolation of mutants: Attempts to isolate arginine mutants from the wild-type strain *74A* yield only about 0.5% mutants among colonies that appear on arginine-supplemented medium after filtration-enrichment in minimal Medium N. The starting strain used for this research carried the *pyr-3* and *arg-12ˢ* mutations. The former mutation blocks formation of carbamyl phosphate in the pyrimidine pathway (CAROLINE 1969) and gives more assurance of isolating carbamyl phosphate synthetase mutants (*arg-2* and *arg-3*) in the arginine pathway. Moreover, the filtration enrichment medium used was a nitrate medium, which yields less growth of leaky mutations (at least in an *arg-12+* background). From 750 colonies isolated from plates supplemented with arginine and uridine after UV mutagenesis and enrichment in uridine-containing medium, 250 were clear arginine auxotrophs. The 65-fold improvement in mutant yield can be attributed largely to the *arg-12ˢ* mutation; other experiments showed that the *pyr-3* mutation and the different medium had little effect.

Complementation tests were performed in order to group the mutants into allelic series. Members of each complementation group were then tested against mutants of known loci, previously isolated, in order to identify each group. Table 1 shows that mutants of all previously known loci except *arg-10* (argininosuccinate lyase) were found, together with two apparently new loci.

The genetic analyses of each locus were facilitated by two features of the starting material. The first is that *pyr-3* is linked to new alleles of *arg-2* and to one of the new loci, *arg-14*; *arg-12ˢ* is linked or allelic to new *arg-5* and *arg-12* mutants, and mating type is linked to new *arg-1* and *arg-3* mutants (Figure 2). Therefore

TABLE 1

Gene-enzyme relations and new mutants isolated in this study

Locus	Enzymic step	No. mutants isolated	Representative mutations Previous work	This paper	References*
arg-1	Argininosuccinate synthetase	4	36703, B369	CD-145	1, 2, 3, 5, 6
arg-2	Carbamyl phosphate synthetase A (small polypeptide)	10	33442, MEP-7	CD-4	1, 2, 3, 7, 8
arg-3	Carbamyl phosphate synthetase A (large polypeptide)	62	30300, MEP-6	CD-2 CD-77 CD-186 CD-192	1, 2, 3, 7, 8
arg-5	Acetylornithine transaminase	32	27947	CD-6	1, 2, 3, 17
arg-6	Unknown	57	29997	CD-29 CD-25 CD-63	1, 2, 3, 9
arg-7†	Acetylornithine acetyltransferase	3	21502, 34105	CD-51	1, 2, 3, 7, 16
arg-10	Argininosuccinate lyase	0	B317	—	4, 5, 6
arg-12	Ornithine carbamyltransferase	10	*arg-12^s*, UM-107	CD-3	7, 10, 11
arg-13	Unknown	14	RU-3, RU-12	CD-7	12
arg-14	Unknown	5	S1229(T)	CD-21 CD-197	13, 14, 15; This paper
Unnamed	Unknown	15	—	CD-15 CD-55	This paper
		Total = 212			

* References: (1) BEADLE and TATUM 1945; (2) SRB and HOROWITZ 1944; (3) SRB *et al.* 1950; (4) FINCHAM and BOYLEN 1957; (5) NEWMEYER 1957; (6) NEWMEYER 1962; (7) MITCHELL and MITCHELL 1952; (8) DAVIS 1967; (9) CATCHESIDE and OVERTON 1958; (10) DAVIS and THWAITES 1963; (11) HOULAHAN and MITCHELL 1947; (12) McDOUGALL and WOODWARD 1965; (13) BARRATT *et al.* 1954; (14) BARRY 1960; (15) PERKINS and BARRY 1977; (16) VOGEL and VOGEL 1963; (17) MORGAN 1965.

† Also known as *arg-4*.

many new mutants selected in the *pyr-3*, *arg-12^s A* background will immediately reveal their genetic locations in an outcross to wild type, thus confirming complementation data.

The second feature of the starting material is that no new mutant in the *pyr-3 arg-12^s* background will be able to use ornithine as a supplement (see introduction). Arginine-auxotrophic progeny from an outcross to wild type, however, will in some cases be able to use ornithine, since they are mutants blocked in ornithine synthesis in an *arg-12$^+$* background. Segregation of ornithine-utilizing and nonutilizing progeny identifies *arg-12$^+$ vs. arg-12^s* and makes the enzyme test unnecessary for such arginine auxotrophs. The nutritional behavior of strains supplements the recombination and complementation data.

Nomenclature: Strains are designated by their full genotypes, and are identified, if necessary, as in the second table, by a number following the letter R. Loci are identified by three-letter symbols (*e.g.*, *arg-1*) with a number. Locus names are used in discussions that embrace all alleles. Allele designations are numbers prefixed by CD, and these "CD" numbers are used in discussions of particular crosses and other tests where a given allele was used. The gaps in the

numerical sequence of arginine loci are accounted for as follows: *arg-4* is a synonym for *arg-7*; *arg-8* and *arg-9* are blocks in the proline path, although mutants use arginine for growth *via* a catabolic pathway (SRB, FINCHAM and BONNER 1950; DAVIS 1968); and *arg-11* mutants have a complex requirement for arginine, pyrimidine and purine, which is not presently understood (see BROADBENT and CHARLES 1965).

Characteristics of the loci

The data on each group of mutants follows in the numerical order of loci.

arg-1: Four alleles of this locus were identified by complementation tests. As expected, the original isolates (*pyr-3*, *arg-1*, *arg-12ˢ A*) failed to use citrulline as an arginine source. When the original isolate of the *arg-1* mutant, CD-145, was mated to an authentic *arg-1* mutant, the cross was sterile. (This outcome is frequently observed among allelic crosses of arginine mutants.) An outcross of *pyr-3*, CD-145, *arg-12ˢ A* to *73a* yielded progeny in the following ratios: 21 pyr^+ arg^+; 28 pyr^- arg^+; 25 pyr^+ arg^-; and 23 pyr^- arg^-. These data indicate independent assortment of *pyr-3* and CD-145 (*arg*) ($P = 0.85$, 3 *d.f.*). None of the arg^- progeny were able to use ornithine or citrulline for growth (Table 2). Of

TABLE 2

Nutritional tests of new mutants

Locus	Strain	Dry weight (mg) on MIN*	ORN	CIT	ARG
wild type	74*A* R7†	23.9	—	—	22.1
arg-1	CD-145 R1*A*	0	0	0	19.9
	CD-145 *arg-12ˢ* R2*A*	0	0	0	19.0
arg-2	*pyr-3* CD-4 R2*A*	0	0	19.7	20.9
	pyr-3 CD-4 *arg-12ˢ* R3*a*	0	0	18.6	21.6
arg-3	*pyr-3* CD-186 R1*A*	0	0	21.2	21.2
	pyr-3 CD-192 *arg-12ˢ* R1*A*	0	0	19.9	21.7
	CD-192 R2*A*	0	0	22.0	21.7
	CD-2 *arg-12ˢ* R2*A*	0	0	22.4	20.6
arg-5	CD-6 R36*a*	0	16.7	19.1	21.5
	CD-6 *arg-12ˢ* R5*a*	0	0	19.4	20.2
arg-6	CD-25 R23*A*	0	17.4	21.5	23.0
	CD-25 *arg-12ˢ* R1*A*	0	0	19.1	20.5
arg-7	CD-51 R23*a*	1.8	15.8	21.1	19.9
	CD-51 *arg-12ˢ* R6*a*	0	tr‡	18.5	18.3
arg-12	CD-3 R2	0	0	18.9	20.2
arg-13	CD-7 R32*a*	1.0	15.3	20.6	23.9
	CD-7 *arg-12ˢ* R1*A*	0	0	19.9	21.3
arg-14	*pyr-3* CD-21 R4*a*	0	17.7	20.9	20.6
	pyr-3 CD-21 *arg-12ˢ* R3*A*	0	0	20.5	19.5

Strains were grown in 10 ml medium for 48 hr at 26°.

* MIN = Vogel's minimal medium; ORN = 1 mM ornithine HCl; CIT = 1 mM citrulline; ARG = 1 mM arginine HCl. Where *pyr-3* strains are tested, all media were supplemented with 100 μg uridine per ml.

† Numbers preceded by R are ascospore reisolate numbers.

‡ tr = trace; too little to harvest.

the 48 *arg*$^-$ progeny, only five were recombinant with respect to mating type. The 10.8 map units calculated from these data for the interval is consistent with published values for recombination between *arg-1* and mating type.

arg-2: Ten alleles of this locus were identified by their failure to complement with MEP-7, the standard *arg-2* allele.

An outcross of the original strain, *pyr-3*, CD-4, *arg-12*^{s} *A* to wild-type *73a* yielded only two classes among 30 progeny: *arg*$^+$ *pyr*$^+$ and *arg*$^-$ *pyr*$^-$. Moreover, no *arg*$^-$ isolate used ornithine as an arginine source. The data are consistent with the genetic position of *arg-2* within 1 to 2 map units of *pyr-3*, and with the metabolic role of *arg-2* in carbamyl phosphate synthesis. All other original isolates were outcrossed with the same general result. The nutritional behavior of *pyr-3*, CD-4 and *pyr-3*, CD-4, *arg-12*^{s} is given in Table 2. The data show that the CD-4 mutation is not leaky and will use only citrulline or arginine for growth. It should be noted that, in the *pyr-3*$^+$ background (not shown), some leakiness of *arg-2* alleles might be expected because of a slight spillover of carbamyl phosphate from the pyrimidine pathway.

The ten new *arg-2* mutants, in the genotype *pyr-3*, *arg-2*, *arg-12*$^+$ *a*, were tested for intragenic complementation. No complementation was seen after 15 days on uridine-containing minimal medium.

arg-3: Fifty-six alleles of this locus were isolated and identified by their failure to complement with the standard *arg-3* allele, MEP-6.

An outcross of the original mutant, *pyr-3*, CD-77, *arg-12*^{s} *A* to wild-type *73a* showed independent assortment of CD-77 and *pyr-3*, but strong linkage of CD-77 to mating type (one recombinant among 26 progeny). This is consistent with the genetic position of *arg-3* (*ca.* ten units from mating type). A mating of another original mutant, *pyr-3*, CD-2, *arg-12*^{s} *A* to a standard *arg-1* mutant showed the expected strong linkage of the two arginine auxotrophs (1 prototroph among 100 isolates).

Several *arg-3* strains were tested for their nutritional behavior (Table 2), and the effect of *pyr-3* and *arg-12*^{s} mutations upon the *arg-3* phenotype. Neither *arg-12*^{s} nor *pyr-3* influences the requirement for arginine or the inability to use ornithine as an arginine source. No intragenic complementation was observed among 18 *arg-3* mutations tested in all possible pairs.

arg-5: Thirty-two alleles of this locus were identified by complementation tests. Some intragenic complementation was seen in this group; however, its members were defined by failure to complement with allele CD-6. For genetic identity, the original isolate *pyr-3*, CD-6, *arg-12*^{s} *A* was mated to an authentic *arg-5* strain. The cross was sterile. Second, the original isolate was outcrossed to wild-type *73a*. The outcome is given in Table 3, in which the segregation of all three genes is followed. The cross shows independent assortment of *pyr-3* with *arg-12*^{s} and CD-6, but the latter markers recombine only to the extent of 23%. A second cross, *arg-12*$^-$ × *pyr-3*, CD-6, *arg-12*^{s} yielded a distance of 16 map units (eight CD-6$^+$, *arg-12*^{s} among 98 isolates).

TABLE 3

*Random-spore analysis of the cross 73*a × pyr-3 *CD-6* arg-12^s A

Growth on test media: URI*	ARG	URI + ORN	URI + CIT	OTCase	No. of progeny	Genotype *pyr-3*	CD-6	*arg-12*
+	+	+	+	+	17	+	+	+
+	+	+	+	—	1	+	+	s
+	—	+	+	+	13	—	+	+
+	—	+	+	—	9	—	+	s
—	+	+	+	ND†	3	+	—	+
—	+	—	+	ND	19	+	—	s
—	—	+	+	ND	9	—	—	+
—	—	—	+	ND	25	—	—	s
					Total = 96			

Allele ratios (wild type:mutant) *pyr-3*: 40:56; CD-6: 40:56; *arg-12*: 42:54.
Recombination: *pyr-3*–CD-6: 46%; *pyr-3–arg-12*: 44%; CD-6–*arg-12*: 23%.
* URI = uridine; ARG = arginine; CIT = citrulline; OTCase = ornithine transcarbamylase.
† ND = not determined.

The progeny of the genotype CD-6, *arg-12*$^+$ were not leaky, and were able to use ornithine as an arginine source (Table 2). The data as a whole show CD-6 and, by implication, the complementation group it represents, to be *arg-5*.

arg-6: Fifty-seven alleles of this locus were identified by complementation tests, using the CD-25 allele as a tester. CD-25 failed to complement with the standard *arg-6* strain. Initial tests showed considerable intragenic complementation among original isolates (*i.e.*, in the *arg-12*^{s} background), and this phenomenon was pursued among outcross progeny in the *arg-12*$^+$ background (see below).

An outcross of the original *pyr-3*, CD-25, *arg-12*^{s}, strain to wild-type *73a* showed independent assortment of CD-25 with *pyr-3*, *arg-12*^{s} and mating type. Among arginine-requiring strains in the cross, a number were able to use ornithine as an arginine source; the remainder used only citrulline.

A cross of CD-25 (single mutant) to the standard *arg-6* strain was sterile. Crosses of CD-25 with *arg-13* (CD-7) and with *al-2* yielded 24% and 0% recombinants, respectively, among 100 isolates. These values are consistent with those expected of *arg-6* mutations.

All *arg-6* mutants were outcrossed to *73a* in order to obtain *arg-6*, *arg-12*$^+$ genotypes of one mating type (*A*) for further test of intragenic complementation. This was done in order to test the hypothesis that the locus was bifunctional. Complementation tests were run by combining drops of conidial suspension of two strains in tubes containing 1 ml of minimal medium (Davis and de Serres 1970). The tests were read each day for ten days. In most cases where complementation was observed, it was detectable on the second or third day, thus resembling nonallelic pairings. The results show there are three main types of mutants: one noncomplementing group comprising 12 mutants, and two groups of four and 27 mutants, respectively, whose members complement only with mutants of the other group. Only two mutants failed to fit this pattern. One complemented with only some of one of the complementing groups; the other

complemented only some of both complementing groups. The results, even with these complications, suggest a locus whose gene product is a bifunctional enzyme. The two-unit complementation pattern of *arg-6* mutants was first noted by CATCHESIDE and OVERTON (1958).

arg-7: Three alleles of this locus were identified among the mutants by complementation tests. The locus was difficult to identify by complementation with a standard *arg-7* strain because of the leakiness of the tester and of the new mutants. Nevertheless, the three mutants appeared to be *arg-7* on this basis.

An outcross of the original mutant, *pyr-3*, CD-51 *arg-12^s A* to *73a* showed that CD-51 assorted independently of *pyr-3*, *arg-12* and mating type. In this cross, quite leaky isolates (CD-51 *arg-12$^+$*) that grew well on ornithine were found. This is consistent with the metabolic position of *arg-7*. Nutritional data for *arg 12$^+$* and *arg 12^s* derivatives are given in Table 2. The leakiness of *arg-7* single mutants is probably due to the deacetylation of acetylornithine (enzymic or non-enzymic) even in the absence of the transacetylase reaction (HOARE, HOARE and BRAME 1967; VOGEL and VOGEL 1963).

A cross of the original mutant to *alcoy* showed strong linkage of CD-51 and *cot*. This finding is consistent with the genetic position of *arg-7* on the right arm of Linkage Group V. A cross of CD-51 (single mutant) to the standard *arg-7* strain was relatively unproductive, but yielded no prototrophs among 95 spores isolated. The data allow one to identify CD-51 and its complementation group as *arg-7*.

arg-10: No mutants for this gene were isolated.

arg-12: Ten alleles of this locus were isolated, and identified by complementation tests. The identity of one of the alleles, CD-3, was established by two lines of evidence.

First, it was expected that the mutation CD-3 took place at or near the *arg-12^s* mutation, *i.e.*, in the *arg-12* gene. Thus, in the cross of the original mutant, *pyr-3*, CD-3 (=*pyr-3$^-$*, *arg-12$^-$*) to wild type, equal numbers of four types of progeny were expected and found: 20 *pyr$^+$arg$^+$*; 25 *pyr$^-$arg$^+$*; 30 *pyr$^+$arg$^-$*; and 24 *pyr$^-$ arg$^-$* (P for independent assortment = 0.6). However, because CD-3 is a further mutation at the *arg-12* locus, no *arg-12^s* progeny were expected among the prototrophs. This expectation was borne out by direct assay of permeabilized cultures. The arginine auxotrophic class, moreover, was uniformly unable to use ornithine as an arginine source (Table 2). This is consistent with the ornithine transcarbamylase deficiency characteristic of *arg-12*.

A second cross was designed to test for the linkage expected between the mutation in the original *pyr-3*, CD-3 isolate and the standard *arg-5* mutant. In a cross between the two strains, 12 arginine-independent progeny were found among 100 isolates, suggesting 24% recombination. This is similar to the recombinant percentage (23%) in the cross of CD-6 (*arg-5*) and *arg-12* given previously. This fact, the failure to recover *arg-12^s*, and the nutritional phenotype establish CD-3 and its complementation group as *arg-12*.

arg-13: Fourteen alleles of this locus were isolated and identified by complementation tests. The leakiness of the standard strain of the *arg-13* locus (RU-12 of

McDougall and Woodward 1965) compromised the complementation test for the identity of the group. It was necessary to construct an RU-12, *arg-12*^{s} tester for the purpose, and this gave strong evidence of functional allelism of RU-12 and the new isolate, CD-7.

An outcross of the original isolate, *pyr-3*, CD-7, *arg-12*^{s} *A*, to wild-type *73a* yielded progeny among which ornithine-utilizing strains appeared. These were CD-7, *arg-12*$^+$ segregants. The ornithine-utilizing strains, moreover, were leaky on minimal medium (Table 2) and were not stimulated by glutamate (not shown). To determine whether leakiness was a general characteristic of mutations at the locus, outcrosses of four other alleles from the original series were made. Both leaky (*arg-13*, *arg-12*$^+$) and nonleaky (*arg-13*, *arg-12*^{s}) mutants were found in all of them. This suggests that all mutants so far isolated are leaky at their site of action. The present series is the first critical test of this, since the series isolated by McDougall and Woodward (1965) was selected in a regime which would yield only leaky mutants. The results suggest the existence of a second function in Neurospora able to provide to a small extent the function missing in *arg-13* mutants. It might be argued that the mutants obtained retain partial function for the locus, and that complete loss of function is lethal. However, their large number suggests that many must be complete loss-of-function mutants.

Further genetic tests were made. First, a cross of CD-7 × RU-12 yielded no wild-type progeny, although the cross was quite unproductive. Second, a cross of CD-7 × CD-25 (*arg-6*), as noted above, gave 24% recombination. The map distance is compatible with the *arg-13*—*arg-6* linkage distance established previously. Finally, CD-7 recombines to the extent of 19% ($N = 97$) with *al-2*, a marker very close to *arg-6*.

arg-14 (*new locus*): Five alleles of this locus were defined by their failure to complement with one another and by their ability to complement with representatives of all known loci.

A cross of the original isolate of one *arg-14* mutant (*ie.*, *pyr-3*, CD-21, *arg-12*^{s}, *A*) to wild-type *73a* is shown in Table 4. Two points can be drawn from the table. First, in this small progeny, there is no recombination between *pyr-3* and CD-21.

TABLE 4

*Cross of 73*a (*wild type*) × pyr-3 *CD-21* arg-12^s

Growth on test media					Genotype		
URI*	ARG	URI + ORN	URI + CIT	Number	*pyr-3*	CD-21	*arg-12*
+	+	+	+	23	+	+	+
+	+	+	+		+	+	s
—	—	+	+	8	—	—	+
—	—	—	+	9	—	—	s
Total progeny				40			

* Abbreviations as in Table 3.

The tight linkage is confirmed below. Second, half the arginine-requiring isolates, presumed to be CD-21, *arg-12*$^+$, are able to use ornithine as an arginine source. The ability to use ornithine makes *arg-14* phenotypically different from *arg-2* mutants, which also lie very close to *pyr-3* (see above). The *arg-14* strains do not use glutamate as supplement.

To exclude a chromosomal aberration in which an ornithine-requiring mutation at an unlinked locus was brought into tight linkage with *pyr-3*, three other mutants of the same complementation group (CD-197, CD-207, CD-212) were also outcrossed. All showed tight linkage with *pyr-3*. In one cross, the mutation CD-197 was resolved from *pyr-3* and isolated in a *pyr-3*$^+$ background. Thus, there is good evidence that a new locus near *arg-2* and *pyr-3* is defined by these mutants.

CD-21 and CD-197 (both *arg-14*) were mapped with respect to *pyr-3* and *arg-2*. A three-point cross designed to test recombination by selective plating was made as follows: *arg-2* × CD-21, *pyr-3*. The percentage of arginine-independent progeny was 0.48, yielding a map distance of 0.96 units between *arg-2* and CD-21. All arginine-independent progeny (*arg-2*$^+$, CD-21$^+$) were also pyrimidine-independent (*pyr-3*$^+$). This excludes *arg-2* as the medial marker, because such a gene order would require double crossovers to generate wholly prototrophic progeny. Crosses of CD-197 (*arg-14*) and *arg-2* with *pyr-3* gave distances of 1.1 and 0.9, respectively. These two-point data are not additive with respect to the *arg-2* × CD-21 distance (0.96) given previously. Nevertheless, they suggest that the original three-point cross data should be interpreted as showing CD-21 (*arg-14*) as the medial marker:

$$\frac{\textit{arg-2} \qquad\qquad + \qquad\qquad +}{+ \qquad\qquad \text{CD-21} \qquad\qquad \textit{pyr-3}}\ .$$

This order accounts for the fact that, in this cross, all *arg*$^+$ recombinants were also *pyr*$^+$.

To settle definitively the question of the location of *arg-14*, translocations were used. The motivation to do so arose when D. D. PERKINS brought to my attention that a translocation, S1229, isolated in the first years of Neurospora genetics (see BARRATT *et al.* 1954) and studied extensively by BARRY (1960), has an arginine requirement inseparable from a breakpoint between *pyr-3* and *arg-2* (PERKINS and BARRY 1977). Moreover, S1229 uses ornithine, and thus resembles the present *arg-14* mutants. When tested for complementation, it was found that S1229 was noncomplementary, and thus functionally allelic with, CD-21 and CD-197. Controls established that the lack of complementation of S1229 and *arg-14* alleles was not due to incompatibility factors: S1229 complemented vigorously with *arg-6* and *arg-3* mutants of the present series. Thus, CD-21 and CD-197 lie between *arg-2* and *pyr-3* by reference to the corresponding breakpoint of S1229.

Two other insertional translocations with breakpoints between *arg-2* and *pyr-3* are known: S4342 (T IV→III) and NM152 (T IV→I) (PERKINS and BARRY 1977; PERKINS, personal communication). These two strains, obtained from

PERKINS, were used for matings with *pyr-3*, CD-21. The rationale of the crosses is given in Figure 3, where two different outcomes discriminate whether *arg-14* is or is not on the translocated segment. The data (Table 5) show clearly that the breakpoint of NM152 is to the right of *arg-14*; that of S4342 is to the left (Figure

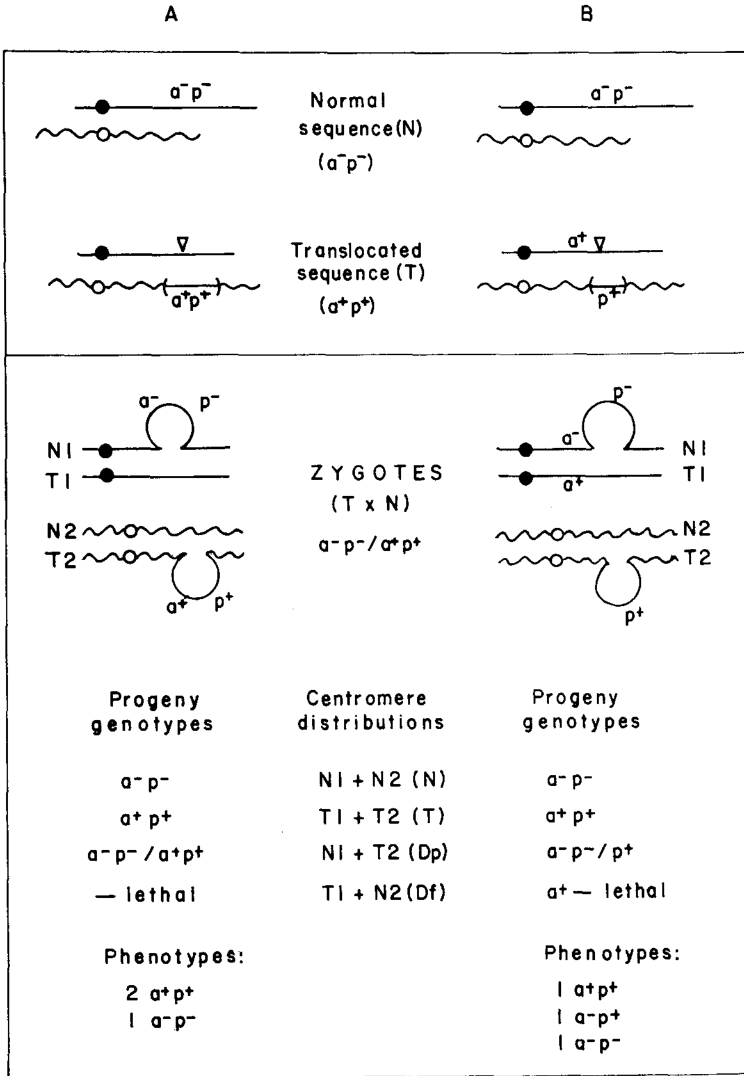


FIGURE 3.—Genetic expectations of crosses involving a normal sequence and an insertional translocation. On the left (A), both an arginine ($a+$ = *arg-14*) and a pyrimidine ($p+$ = *pyr-3*) gene have been translocated, and the mating is to a normal sequence of genotype *arg-14*, *pyr-3*. On the right (B), only the pyrimidine locus has been translocated. The expected centromere distributions at meiosis are shown, observing the restriction that homologous centromeres always segregate. Nonhomology is signified by open *vs.* closed circles (centromeres) and straight *vs.* wavy lines (chromosome arms). Progeny genotypes and phenotypes are given for each type of zygote. Abbreviations: N = normal; T = translocation; Dp = duplication; Df = deficiency.

TABLE 5

Relation of CD-21 to translocation breakpoints

Cross	Progeny *arg*$^+$ *pyr*$^+$	*arg*$^-$ *pyr*$^+$	*arg*$^+$ *pyr*$^-$	*arg*$^-$ *pyr*$^-$
pyr-3, CD-21 × NM152	74	53	2	68
pyr-3, CD-21 × S4342	121*	0	1	71

* Both barren (duplication) and fertile (translocation) progeny were found among 22 progeny tested from this category (see Figure 3).

4). By inference, the breakpoint of S1229 is within *arg-14* (Figure 4). The data as a whole show (1) that *arg-14* lies between *arg-2* and *pyr-3*; (2) that it is a new locus, aside from the S1229 occurrence; and (3) that breakpoints can occur in the *arg-2—pyr-3* interval on either side of *arg-14* without causing a pyrimidine or an arginine requirement. Other genes probably lie in the regions on either side of *arg-14*, because recombination in both regions approaches 1%.

The nutritional data on *pyr-3*, CD-21 and *pyr-3*, CD-21, *arg-12*^{s} are given in Table 2. The CD-21 strain is not leaky and responds well to ornithine, citrulline and arginine. Like other mutants blocked before ornithine, CD-21 cannot use ornithine if *arg-12*^{s} is also present. Almost identical data were obtained for CD-197, *arg-12s*.

***CD-15* et al**: Fourteen alleles of a new locus were isolated and grouped by complementation tests of the original isolates. The locus has not been named because its role in the arginine pathway is obscure. Two alleles, CD-15 and CD-55, were studied in detail. These alleles, and others tested less extensively, behave identically. Their phenotypes therefore probably represent the extreme impairment of the locus.

Outcrosses of the original isolates of these mutants yielded fewer auxotrophs than expected, as well as a category of ascospore colony which grew extremely slowly for one to three days after transfer from plate to tube (see MATERIALS AND METHODS). Upon backcrossing arginine-auxotrophic progeny to wild type, two points became clear. First, the CD-15 and CD-55 mutations impose auxotrophy only if *arg-12*^{s} is present. Second, the delayed-growth character is a reliable indicator of the presence of CD-15 or CD-55, whatever the background. Unfortu-

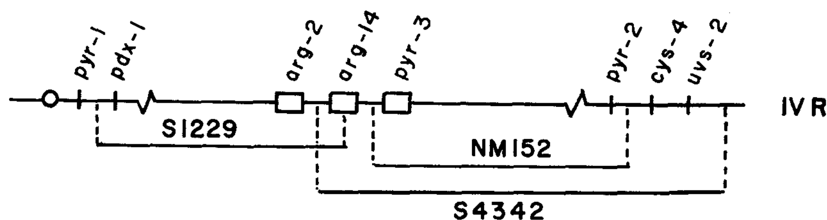


FIGURE 4.—Map of Linkage Group IVR, showing positions of breakpoints of the translocated segments in strains S1229, NM152, and S4342. The *arg-2—pyr-3* region is expanded here; the interval is 1 to 2 map units long.

TABLE 6

Data from crosses of five presumptive CD-15 strains to arg-12^s *strains*

Phenotype			Progeny in cross No.						Genotype inferred
Nutrition	Growth	OTCase	564	569	570	571	572	Sum	
prototroph	fast	high	4	4	9	11	5	33	CD-15+ *arg-12+*
prototroph	fast	low	7	6	3	5	6	27	CD-15+ *arg-12^s*
prototroph	slow	high	6	8	2	5	5	26	CD-15- *arg-12+*
auxotroph	slow	low	6	3	10	4	7	30	CD-15- *arg-12^s*

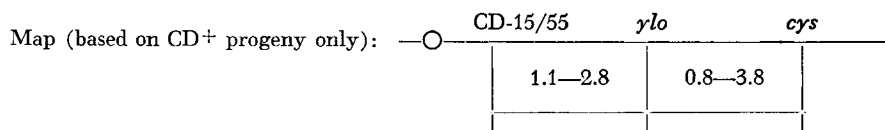
nately, the CD-15/55 phenotype was variably selected against depending upon the ascospore plating medium. The selection took the form of poor growth immediately after germination, not failure of germination itself. This tends to bias colony (or spore) isolation toward healthier colonies. Careful isolation from minimal plates, however, gave good, monogenic segregation in a cross of wild type to CD-15 (52 wild: 45 CD-15). Cysteine and tryptophan, however, severely inhibit the growth of the CD-15 class. To be certain of the identity of CD-15 isolates, five testcrosses of presumed CD-15 strains, recognized by the delayed-growth phenotype, were made to *arg-12^s* and plated on arginine-containing medium. In all cases, auxotrophic progeny appeared, which, moreover, had the low ornithine transcarbamylase character. The pooled data (Table 6) show independent assortment of CD-15 and *arg-12^s* and, in this case, no systematic selection against CD-15.

By means of crosses of CD-15 and CD-55 with the *alcoy* (PERKINS *et al.* 1969) and the *trp-1, ylo-1* strains (*cf.*, PERKINS 1964 or DAVIS and DE SERRES 1970 for summary of rationale), the new mutants were located on the left arm of linkage

TABLE 7

Crosses CD-15 × ylo-1 cys-1 *and CD-15* × ylo-1 cys-1

Progeny genotypes*		Number of progeny† CD-15 cross	CD-55 cross
CD+ *ylo*- *cys*-	(parental)	251	246
CD- *ylo*+ *cys*+	(parental)	15	28
CD+ *ylo*+ *cys*+		3	7
CD- *ylo*- *cys*-		3	3
CD+ *ylo*- *cys*+		10	2
CD- *ylo*+ *cys*-		0	0
CD+ *ylo*+ *cys*-		0	0
CD- *ylo*- *cys*+		0	0
Totals		282	286



* "CD" signifies either CD-15 or CD-55.

† The frequency of spores obviously germinated at the time of isolation was between 45 and 55% in both crosses.

TABLE 8

Nutritional tests of CD-15 strains

Strain	Hours	MIN*	ORN	CIT	ARG	GLU	PRO	TRP	CYS
		Dry weight (mg) in medium containing:							
74A	48	19.7	16.8	18.6	19.5	18.6	17.8	12.8	21.7
	72	27.1	27.9	29.3	28.7	28.3	28.1	23.6	28.6
CD-15 R71*A*	48	9.9	6.7	10.5	7.4	8.8	12.2	0	5.3
	72	21.2	19.0	16.6	21.0	36.2	23.9	1.6	18.2
CD-15 *arg-12*^{s} R3*a*	48	0	0	9.5	9.0	0	0	0	0
	72	0	0	16.6	17.2	0	0	0	0

Strains were grown in ten ml medium at 26°.
* Abbreviations in Table 2; glutamate (GLU), proline (PRO), tryptophan (TRP) and cysteine (CYS) were also tested, all at 1 mM.

group VI. Three-point crosses of CD-15 and CD-55 with *cys-1*, *ylo-1* place the new mutants proximal to *ylo-1*. The data (Table 7) show very poor segregation of the CD mutants, because cysteine was present in the plating medium. Therefore, mapping depended upon the CD-15$^+$ or CD-55$^+$ class, and only the order of loci is reasonably clear: centromere–CD-15/55–*ylo-1*–*cys-1*.

Nutritional tests of CD-15 and CD-55 showed that these mutations were not specifically in the arginine pathway. The delayed growth phenotype was indifferent to arginine, citrulline, ornithine, glutamate or proline (Table 8). The conclusion one must draw is that *arg-12*^{s} renders the double mutant specifically "arginine-less." The action of CD-15 and CD-55 is to impair or counteract the mechanism by which *arg-12*^{s} normally compensates for its defective ornithine transcarbamylase. The relationship of these mutants to *arg-12*^{s} is somewhat different from the relationship of leaky arginine mutants to *arg-12*^{s} (see introduction) because arginine can fully restore normal growth to double mutants in the latter case.

DISCUSSION

Many arginine mutants have been isolated in the course of earlier mutant hunts (Beadle and Tatum 1945; Srb and Horowitz 1944; Srb, Fincham and Bonner 1950; Newmeyer 1957; Catcheside and Overton 1958; Davis and Thwaites 1963; Woodward and Schwartz 1964; McDougall and Woodward 1965). Mapping data for the early mutants was not extensive, and the allelism or nonallelism of some of these early mutants is still obscure. Moreover, while the main outlines of the biochemical pathway are clear, certain enzymes in the synthesis of ornithine have not been identified with particular genetic loci. Because it appeared that there were more enzymes than well-characterized mutants, an exhaustive search for more mutants was undertaken here. The productivity of the filtration enrichment method was greatly enhanced (over 50-fold) by use of a strain carrying a subtle impairment of arginine metabolism, *arg-12*^{s} (*cf.*, Davis and Thwaites 1963). The yield of mutants was 33% among colonies originally isolated.

In the course of this mutant hunt, mutants of all previously known loci except *arg-10* (argininosuccinate lyase) were found. In addition, a bifunctional character of *arg-6* was suggested by tests for intragenic complementation; more representatives of the obscure *arg-13* were found; a new locus, *arg-14*, was identified; and a regulatory or organizational locus, represented by CD-15 and CD-55, was found.

The *arg-6* locus, originally identified by mutation in the BEADLE and TATUM (1945) series, has not been identified in the literature with an enzymic function. In this laboratory, CYBIS and DAVIS (unpublished results) showed that the classic isolate, 29997, lacked acetylglutamyl phosphate reductase activity. More recent studies by E. WOLF and R. L. WEISS (unpublished) show that 29997 also lacks acetylglutamate kinase activity. The same double deficiency is found in the noncomplementing mutant CD63 and in a mutant of one of the complementing groups, CD-25. A representative of the second complementing group, CD-29, has reductase activity, but lacks the kinase. These data (to be published later) clearly demonstrate the bifunctional character of the *arg-6* locus. Judging from the phenotype of CD-25, it may be that extractable kinase activity, expected in this mutant, may depend upon the integrity of the reductase. The data suggest that the kinase and reductase activities may be products of a single locus, possibly residing on a bifunctional protein. This protein would both produce and utilize the labile intermediate, acetylglutamylphosphate. The complementation pattern of *arg-6* mutants (CATCHESIDE and OVERTON 1958, and the present study) is consistent with a bifunctional organization. The pattern is highly reminiscent of the *pyr-3* locus, which controls the synthesis and utlization of another labile intermediate, pyrimidine-specific carbamyl phosphate (DAVIS and WOODWARD 1962). The *arg-6* locus organization closely resembles the situation reported for the homologous region (*arg*5–*arg*6, or *arg*B–*arg*C) of yeast (MINET *et al.* 1979). This region is reported to control both the kinase and the reductase, though the extracted activities appear to be physically separable.

A previous report (CYBIS and DAVIS 1975) suggested that the kinase was a cytosolic enzyme, while the reductase was mitochondrial. This relationship, which would be incompatible with a bifunctional protein carrying both activities, was questioned by JAUNIAUX, URRESTARAZU and WIAME (1978), who found a spurious cytosolic activity and an arginine-sensitive mitochondrial activity in assays for the kinase of yeast. Recently, WOLF and WEISS (unpublished) have shown the same is true of Neurospora: the acetylglutamate kinase of the arginine pathway lies in the mitochondrion. Thus, the kinase and reductase could be associated with the same protein.

The *arg-13* locus is represented by 14 mutants in the present series. All five of those that were tested were found to be leaky. Their metabolic position is unknown, except that it lies prior to ornithine and after glutamate, according to nutritional tests. These mutants evidently eliminate a function not wholly indispensible to ornithine synthesis. Whether they affect a previously known step, or an unknown function, remains to be determined. For the moment, *arg-13* must formally be considered a candidate for the acetylglutamate synthase structural gene, the only enzyme not now identified by mutation.

The *arg-14* locus is a second candidate for the acetylglutamate synthase structural gene. The locus is newly named, but the present results show that the complex aberration strain, S1229, carries an *arg-14* mutation coinciding with one of its breakpoints. With the aid of other translocations with breakpoints between *arg-2* and *pyr-3*, a new *arg-14* allele was definitively mapped within this *ca.* 1 map-unit interval. It is intriguing that *arg-14*, which may well specify a rate-limiting step in ornithine synthesis, and *arg-2*, which specifies the rate-limiting component of carbamyl phosphate synthetase A (CYBIS and DAVIS 1975), lie so close to one another. It suggests that some coordination of these two tributaries of the pathway might be achieved by a common control element. The interest is extended by knowledge that *pyr-3* is also in this region, inasmuch as it specifies the rate-controlling steps of pyrimidine synthesis (DAVIS 1972), one of them being a second and distinct carbamyl phosphate synthetase, specific for the pyrimidine pathway.

The translocation analysis, however, shows that the genes are almost certainly not adjacent, inasmuch as breakpoints can lie on either side of *arg-14* without causing an arginine or a pyrimidine requirement. Studies of regulation of the respective enzymes in translocation strains will nevertheless be of interest.

The locus represented by CD-15 and CD-55 is a novel one. The mutants impose no requirement for arginine, but initially they grow poorly in almost any medium. The mutants were isolated here because they apparently interfere with adaptation of *arg-12*^{s} (present in the starting material) to its low levels of ornithine transcarbamylase and thus present an auxotrophic phenotype. It might initially be thought that many types of metabolic impairment could have this effect. Some that might do so are impairments of carbon flow into or through the pathway (*e.g.*, limitation of glutamate, acetyl CoA, or NADPH) or organizational alterations of the mitochondrion, where ornithine synthesis takes place (*e.g.*, leakage of ornithine intermediates, or disaggregation of ornithine enzymes, assuming they have significant weak interaction). Yet, there is not a large number of loci with such phenotypes among the present mutants; instead, there are 15 alleles of this single gene. Thus the selective system has efficiently displayed a specific type of mutant. It is, possibly, a mutation imposing inability to derepress arginine enzymes. Depression of all arginine enzymes is characteristic of (and probably necessary for) *arg-12*^{s} growth on minimal medium, and preliminary data suggest that CD-15 and CD-55 are unable to accomplish this. Whether the phenotype extends to other pathways, as would be expected of a "cross-pathway control" mutant, is not known. Such mutants (*aas*), however, are known in yeast (WOLFNER *et al.* 1975), and it would not be surprising to find them here.

Biochemical studies to follow will clarify the metabolic effects of the mutants whose isolation and genetics is reported here.

This work was supported by research grants from the National Science Foundation (PCM 75-16245) and Public Health Service (AM 20083). I thank DEBORAH OGAWA, ELANA D. VAUGHN and KATHERINE SCHUPPERT for technical assistance; D. D. PERKINS for information and strains used in this work; the Fungal Genetics Stock Center for other strains; R. L. WEISS for advice on cell permeabilization and for unpublished data; and R. L. WEISS, D. D. PERKINS, C. L. CRAMER and L. E. VAUGHN for critical comments on the manuscript.

LITERATURE CITED

ARCHIBALD, R. M., 1944 Determination of citrulline and allantoin and demonstration of citrulline in blood plasma. J. Biol. Chem. **156**: 121–142.

BARRATT, R. W., D. NEWMEYER, D. D. PERKINS and L. GARNJOBST, 1954 Map construction in *Neurospora crassa*. Adv. Genetics **6**: 1–93.

BARRY, E. G., 1960 Genetic analysis of an insertional translocation in *Neurospora crassa*. (Abstr.) Genetics **45**: 974.

BEADLE, G. W. and E. L. TATUM, 1945 Neurospora. II. Methods of producing and detecting mutations concerned with nutritional requirements. Am. J. Bot. **32**: 678–686.

BROADBENT, J. A. and H. P. CHARLES, 1965 Some carbon-dioxide requiring mutants of *Neurospora crassa*. J. Gen. Microbiol. **39**: 63–74.

CAROLINE, D. F., 1969 Pyrimidine synthesis in *Neurospora crassa*: gene-enzyme relationships. J. Bacteriol. **100**: 1371–1377.

CATCHESIDE, D. G. and A. OVERTON, 1958 Complementation between alleles in heterocaryons. Cold Spring Harbor Symp. Quant. Biol. **23**: 137–140.

CYBIS, J. and R. H. DAVIS, 1975 Organization and control in the arginine biosynthetic pathway of *Neurospora*. J. Bacteriol. **123**: 196–202.

DAVIS, R. H., 1962a Consequences of a suppressor gene effective with pyrimidine and proline mutants of Neurospora. Genetics **47**: 351–360. ——, 1962b A mutant form of ornithine transcarbamylase found in a strain of Neurospora carrying a pyrimidine-proline suppressor gene. Arch. Biochem. Biophys. **97**: 185–191. ——, 1967 Channeling in *Neurospora* metabolism. In: *Organizational Biosynthesis*. Edited by H. J. VOGEL, J. O. LAMPEN and V. BRYSON. Academic Press, New York. ——, 1968 Utilization of exogenous and endogenous ornithine by *Neurospora crassa*. J. Bacteriol. **96**: 389–395. ——, 1972 Metabolite distribution in cells. Science **178**: 835–840.

DAVIS, R. H. and F. J. DE SERRES, 1970 Genetic and microbiological research techniques for *Neurospora crassa*. In: *Methods in Enzymology*, Vol. XVII A. Edited by H. TABOR and C. TABOR. Academic Press, New York.

DAVIS, R. H. and J. MORA, 1968 Mutants of *Neurospora crassa* deficient in ornithine-δ-transaminase. J. Bacteriol. **96**: 383–388.

DAVIS, R. H. and W. M. THWAITES, 1963 Structural gene for ornithine transcarbamylase in Neurospora. Genetics **48**: 1551–1558.

DAVIS, R. H. and V. W. WOODWARD, 1962 The relationship of gene suppression and aspartate transcarbamylase activity in *pyr-3* mutants of Neurospora. Genetics **47**: 1075–1083.

FINCHAM, J. R. S. and J. B. BOYLEN, 1957 *Neurospora crassa* mutants lacking argininosuccinase. J. Gen. Microbiol. **16**: 438–448.

HOARE, D. S., S. L. HOARE and J. BRAME, 1967 Deacetylation of N-acetyl-L-glutamic acid by *Neurospora crassa*. J. Bacteriol. **94**: 782–783.

HOULAHAN, M. B. and H. K. MITCHELL, 1947 A suppressor in Neurospora and its use as evidence for allelism. Proc. Natl. Acad. Sci. U.S. **33**: 223–229.

JAUNIAUX, J.-C., L. A. URRESTARAZU and J.-M. WIAME, 1978 Arginine metabolism in *Saccharomyces cerevisiae*: subcellular localization of the enzymes. J. Bacteriol. **133**: 1096–1107.

MCDOUGALL, K. J. and V. W. WOODWARD, 1965 Suppression of arginine and pyrimidine-requiring mutants of *Neurospora crassa*. Genetics **52**: 397–406.

MINET, M., J.-C. JAUNIAUX, P. THURIAUX, M. GRENSON and J.-M. WIAME, 1979 Organization and expression of a two-gene cluster in the arginine biosynthesis of *Saccharomyces cerevisiae*. Molec. Gen. Genet. **168**: 299–308.

MITCHELL, M. B. and H. K. MITCHELL, 1952 Observations on the behavior of suppressors in Neurospora. Proc. Natl. Acad. Sci. U.S. **38**: 205–214.

MORGAN, D. H., 1965 Acetyl-ornithine transaminase in *Neurospora.* Neurospora Newsl. **8**: 8.

NEWMEYER, D., 1957 Arginine synthesis in *Neurospora crassa*; genetic studies. J. Gen. Microbiol. **16**: 449–462. ——, 1962 Genes influencing the conversion of citrulline to argininosuccinate in *Neurospora crassa.* J. Gen. Microbiol. **28**: 215–230.

PERKINS, D. D., 1964 Multiple interchange stocks for linkage detection. Neurospora Newsl. **6**: 22.

PERKINS, D. D. and E. G. BARRY, 1977 The cytogenetics of *Neurospora. Adv. Genet.* **19**: 133–285.

PERKINS, D. D., D. NEWMEYER, C. W. TAYLOR and D. C. BENNETT, 1969 New markers and map sequences in *Neurospora crassa,* with a description of mapping by duplication coverage, and of multiple translocation stocks for testing linkage. Genetica **40**: 247–278.

SPECTOR, L., M. E. JONES and F. LIPMANN, 1957 Carbamyl phosphate. In: *Methods in Enzymology III.* Edited by S. P. COLOWICK and N. O. KAPLAN. Academic Press, New York.

SRB, A. M. and N. H. HOROWITZ, 1944 The ornithine cycle in Neurospora and its genetic control. J. Biol. Chem. **154**: 129–139.

SRB, A. M., J. R. S. FINCHAM and D. BONNER, 1950 Evidence from gene mutation in Neurospora for close metabolic relationships among ornithine, proline, and δ-amino-α-hydroxyvaleric acid. Am. J. Bot. **37**: 533–538.

VOGEL, H. J., 1964 Distribution of lysine pathways among fungi: evolutionary implications. Am. Naturalist **98**: 435–446.

VOGEL, R. H. and H. J. VOGEL, 1963 Evidence for acetylated intermediates of arginine synthesis in *Neurospora crassa* (Abstr.). Genetics **48**: 914.

WESTERGAARD, M. and H. K. MITCHELL, 1947 Neurospora V. A. synthetic medium favoring sexual reproduction. Am. J. Bot. **34**: 573–577.

WOLFNER, M., D. YEP, F. MESSENGUY and G. R. FINK, 1975 Integration of amino acid biosynthesis into the cell cycle of *Saccharomyces cerevisiae.* J. Mol. Biol. **96**: 273–290.

WOODWARD, V. W. and P. SCHWARTZ, 1964 Neurospora mutants lacking ornithine transcarbamylase. Genetics **49**: 845–853.

Corresponding editor: C. W. SLAYMAN