Growth and allelism of arg-11 and adg

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Abstract
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McDougall, K. J. and V. W. Woodward. Suppression of pyr-3 mutants of Neurospora. The mechanism by which pyr-3 mutants are suppressed has been described as follows: carbamylphosphate (CAP) is synthesized in two independent pathways; one leading to arginine synthesis and the other to pyrimidine synthesis. In the arginine pathway the enzyme carbamylphosphokinase (CPK) catalyzes the synthesis of CAP, and the arg-3 mutation is the structural gene controlling CPK synthesis. Ornithine transcarbamylase (OTC) catalyzes the coupling of CAP with ornithine to yield citrulline, and arg-12 is the structural gene for this enzyme. The enzyme catalyzing the synthesis of CAP in the pyrimidine pathway has not been described, yet certain of the pyr-3 mutants would appear to lack this enzyme activity. The enzyme aspartate transcarbamylase (ATC) couples CAP with aspartate to yield ureidoglucotic acid (US); the pyr-3 locus serves also as the structural gene for ATC. We have postulated, as has R. H. Davis, that the pyr-3 gene specifies one protein with two active sites, one site being the ATC and the other the CAP synthesizing site. The mutation arg-12 is a leaky arg-12 mutation exhibiting about 3% of wild type OTC activity. This mutant has been known for many years to suppress certain of the pyr-3 mutants. Davis and Woodward (1962 Genetics 47:1075) showed that those mutants suppressed by arg-12 have ATC activity but presumably lack the ability to synthesize CAP. We, therefore, postulate that arg-12 accumulates CAP which is then used by the pyr-3 component for pyrimidine synthesis. (Complete blocks at the arg-12 locus do not suppress pyr-3 mutants under these conditions because the required exogenous arginine inhibits or represses CPK, shutting off the remaining source of CAP.)

In a search for alleles of arg-12, four new pyr-3 suppressor mutations were found. These are designated RU-I, 3, 12 and 20. Arginine-pyrimidine double mutants were recovered after irradiating conidia of pyr-3 ATC+ mutants and plating onto minimal agar. These double mutants were crossed to wild type and tetrads yielded the suppressed strains plus the individual components. In all cases it was shown that the arginine mutants possessed wild type OTC.

The RU mutations are genetically distinct from arg-12. Woodward and Schwarz (1964 Genetics 49:846) have shown that arg-12 is located on linkage group II. RU-I is located on the right arm of linkage group V, 8 map units from arg-4, 2.8 map units from arg-7, and 14.4 map units from arg-8. The other three mutants are located on the right arm of linkage group I approximately 29 map units distal to nic-1. Since these mutants are intersterile, it is impossible to determine whether they comprise one or more loci.

In 1952 the Mitchells (Proc. Natl. Acad. Sci. U. S. 38:205) reported that arg-7 (orn-3) suppressed certain of the pyr-3 mutants. We have confirmed this and the fact that arg-6 (orn-2) does not suppress pyr-3. Collectively, these data would seem to complicate the above explanation of suppression. However, a paucity of ornithine substrate in the cell may have the same effect on pyr-3 as the accumulation of CAP. Either situation might make CAP available for pyrimidine synthesis. Studies are now under way to analyze the concentrations of the arginine intermediates in the cells of these mutants.

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Newmeyer, D. Growth and allelism of arg-11 and adg. It was subsequently mapped in linkage group VII, proximal to arg-10 (Perkins 1959 Genetics 44:1185). Mutant adg (44601), originally listed as "unknown requirement", was shown by Houlahan et al. (1949 Genetics 34:493) to segregate as a single gene unlinked to sex (21 asci). Subsequently J. Mauron (cited in G. Dubes 1953 Ph. D. Thesis, California Institute of Technology) found adg to grow suboptimally on arginine plus either adenine or a pyrimidine. This result suggested allelism with 30820, which proved to be the case (see below). However, flask assays with these strains gave unexpected results. These results have been largely superseded by the recent report of Charles and Broadbent (1964 Nature 201:1004) that 30820 will grow on minimal in the presence of 30% CO2, and that when CO2 is excluded both purine and pyrimidine become essential. However, as the relation of 30820 to the other
CO₂ mutants is still far from clear, our fragmentary results may be useful.

Flask assays: (All results are based on 3 days growth at 25°C in 20 ml of Vogel’s Neurospora minimal. ) It was found that the growth requirements of 30820 are abnormally dependent on inoculum size. With very small inocula there is negligible growth unless arginine, adenine and uridine are all present, and optimal growth when all three are added. (Alternative purines and pyrimidines were not tested.) As the inoculum size is increased, first uridine can be omitted; with a further increase in inoculum either adenine or uridine can be omitted; with still larger inocula both adenine and uridine can be omitted. (In one experiment where conidia were counted, all three compounds were essential at or below 2.2 x 10⁵ conidia per flask.) The growth of control cultures of arg-I (B369) and arg-3 (30300), which is also a CO₂ mutant, was essentially unaffected by a similar range of inoculum sizes. In preliminary tests mutant 44601 (adm) behaved much like 30820, except that 44601 appeared to be a more extreme departure from wild type. Although with small inocula the requirements for adenine and uridine are absolute, the concentrations required are very low. With 30820, essentially maximal growth was obtained with only 0.005 mg/ml each of adenine and uridine, compared with values in the literature of ca. 0.05 mg/ml for ordinary adenine mutants and ca. 0.075 mg/ml for ordinary pyrimidine mutants. The arginine requirement is also rather low (0.05 to 0.1 mg/ml). It may be significant that the concentration of pyrimidine required by 30820 is similar to that which represses or inhibits the synthesis of pyrimidine-specific CAP, according to Charles’s hypothesis (1964 J. Gen Microbial. 34:131).

Allelism tests: A cross of 30820 x 44601 gave no wild types among 268 random isolates and one true wild type out of an estimated 4,000 ascospores plated (scored by early growth on minimal sorbose, as in Lein et al. (1948 Proc. Natl. Acad. Sci. U. S. 34:435). However, the results are complicated by the fact that roughly 10 to 15% of the ascospores also failed to grow on glycerol complete medium or on supplemented minimal. When isolated to complete slants, about half of these grew up in a few days; these will be called slow, and are not uncommon. The other half either did not grow at all, or grew so slowly that it took weeks to make a macroscopically visible culture; these will be called near-lethals. Near-lethals were also found among the random isolates. Since it was possible that wild type recombinants could occur preferentially among the near-lethals, via a pair of balanced lethals, four near-lethals from this cross were revived via prolonged growth and repeated subculturing. These eventually attained a normal growth rate, and were then tested; all failed to grow on minimal. (All slow tests also failed to grow on minimal.) In addition, the balanced lethal hypothesis seems unlikely because a cross of wild type x 44601 also produced slow and near-lethals. (A cross of wild type x 30820 produced slow but no near-lethals among the small sample examined.) Allelism of 30820 and 44601 was also indicated by complementation tests. Both mutants were back-crossed until strains were obtained which appeared fully compatible, in that each gave wild type heterocaryons with an arg-I tester. These strains did not complement each other. It is therefore concluded that 30820 and 44601 are alleles.

Mr. Oliver Gillie at the University of Edinburgh has obtained much more extensive data on the variable growth of both arg-II alleles. -- -- Department of Biological Sciences, Stanford University, Stanford, California.