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 ureidosuccinic 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^{s} 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^{s} have ATC activity but presumably lack the ability to synthesize CAP. We, therefore, postulate that arg-12^{s} 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^{s}, four new pyr-3 suppressor mutations were found. These are designated RU-1, 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-1 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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