A response to a letter from David Perkins concerning the question of whether nt mutants involve tryptophan pyrrolase.

The nt lesion must involve tryptophan pyrrolase (trp, 3-dioxygenase, EC 1.13.1.12). However, we still have no direct evidence that this is the case since tryptophan oxygenase has not been detected in N. crassa or for that matter in any other mold that we have tested: Mucor, Rhizopus, Aspergillus, Penicillium and Saccharomyces (Gaertner and Salvoni, unpublished results). We have a very sensitive fluorometric assay (1973 Arch. Biochem. Biophys. 156: 658) that works well for liver, bacterial and Drosophila tryptophan oxygenases. Our initial report of success in assaying the enzyme in Neurospora crassa slime still holds. Unfortunately, however, this only amounts to a whole cell assay and so far has not been useful for wild type N. crassa. Hence, we have only been able to assay for the presence or absence of tryptophan oxygenase in nt mutants. The following story describes what we do know about nt mutants (unpublished data).

In an attempt to isolate mutants of 3-deoxy-D-arabinoheptulosanate 7-phosphate (DAHP) synthase (trp) in N. crassa, and to test the idea that DAHP for tryptophan synthesis is channeled via the aro multienzyme system we treated conidia with nitrosoguanidine and carried out a filtration enrichment for a trp auxotroph in a medium containing high concentrations of Phe and Tyr (with the idea that the other two synthase isozymes would be inhibited). We then scored for trp auxotrophs that could grow also on quinic acid. The idea was that any trp auxotroph, that could not grow on minimal medium (in addition to no growth on minimal plus high concentrations, 400 µg/ml, of Phe and Tyr) but could grow on quinic acid, must have its lesion prior to dehydroquinic acid and should be a "channeling mutant" with a defective DAHP synthase (trp) isozyme. A long shot idea perhaps, and probably a very naive thing to try, but we were able to find such a mutant.

Its properties were the following. It was a non-leaky, it did not grow on Vogel's minimal. It was for all appearances a typical tryptophan auxotroph, responding well to the usual 20 to 40 µg/ml supplement of tryptophan. However it also grew well on Phe and on Tyr, but not at all when both Phe and Tyr were present, and it grew well on a 100 µg/ml supplement of quinic acid. Thus, we had it. A channeling mutant for the aro pathway! We then assayed for DAHP synthase (trp) expecting to find it inactive. To our surprise (and great disappointment) it had wild type levels of DAHP synthase (trp). After this, and for reasons which I no longer remember, we tried to grow the mutant on other amino acids (no response) and also tried niacin. This was all done before I knew anything of nt mutants. To my surprise, niacin permitted growth even better than tryptophan. To make a long story short, our so called channeling mutant was none other than one of the old nt mutants, now called nt 295 (FGSC #2438 A and #2439 a). It is, perhaps, the only known example of a niacin auxotroph isolated by filtration enrichment.

My hypothesis, based on the growth data and other evidence, for the rather bizarre behavior of the nt mutant goes like this. Just as there are two kynurenase-type enzymes in N. crassa, one constitutive (a hydroxykynurenase) for the synthesis of NAD and one inducible (a kynurenase) for the degradation of tryptophan (1971 J. Bact. 108: 902), there are two tryptophan oxygenases, a constitutive biosynthetic one and an inducible catabolic one. The nt mutants, then, lack the constitutive biosynthetic enzyme. Hence they have a requirement for niacin when grown on minimal medium. They show no such requirement when tryptophan is added because tryptophan acts as the inducer for the inducible tryptophan oxygenase which then takes over for the defective constitutive enzyme. The nt mutants also grow on quinic acid, not because they have a lesion prior to dehydroquinic acid, but because quinate by-passes the feedback regulatory controls and allows for an accumulation of tryptophan and a subsequent induction of the inducible tryptophan oxygenase. They also grow on Phe or Tyr because Phe or Tyr alone don't adequately block the activities of the DAHP synthase isozymes, but do apparently allow for some accumulation of chorismate and hence of tryptophan with its subsequent inductive action on tryptophan oxygenase. (Phe and Tyr together, on the other hand, do adequately block carbon flow via DAHP synthase.)

I think this hypothesis can adequately explain what we know about nt mutants. But it is still a hypothesis. A major problem in testing it is the lack of assayability of tryptophan oxygenase in the fungi. Why can't this enzyme be assayed in these organisms when it is easily assayed in all others including insects, animals and bacteria? The answer, in not known. Pete Matchett got so frustrated he suggested that the reaction might be nonenzymatic (1974 J. Bact. 118: 837). He showed very clearly the presence of N-formylkynurenine (the product of the tryptophan oxygenase reaction) in extracts of tryptophan-grown Neurospora, but despite repeated and systematic attempts to assay the enzyme in vitro was unable to detect its activity. Also, Matchett concluded in an earlier report that nt mutants were deficient in tryptophan oxygenase (1970 J. Bact. 103: 364).

P.S. In response to Dorothy Newmeyer's question about kynurenine foraminase, nt mutants carry normal levels of this constitutive activity (our unpublished data). Why niacin auxotrophs lacking foraminase have not been isolated is not known, I can't say. Perhaps there is more than one enzyme or perhaps foraminase has other functions and the mutants are lethal. It is also interesting that the foraminase of N. crassa is constitutive, whereas it is inducible in bacteria. (This research was sponsored by the Office of Health and Environmental Research, U.S Department of Energy Contract W7405-eng-26 with Union Carbide.) - - - The University of Tennessee - Oak Ridge Graduate School of Biomedical Sciences and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.