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Abstract

No mutation affecting the structure or expression of the housekeeping (low affinity) phosphate permease of Neurospora crassa has been reported. The null double mutant lacking both high affinity repressible permeases cannot grow at low phosphate concentrations and high pH. A presumptive mutant affecting the housekeeping permease has been isolated as a suppressor of the double mutant's inability to grow under the restrictive condition.

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N. crassa is capable of making three phosphate permeases. In the presence of abundant phosphate and at the typical growth pH of 5.8, a "housekeeping" permease with a Km of about 1 mM is the active transporter (Lowendorf *et al.* 1974 Biochim. Biophys. Acta 373:369-382), though its Km seems itself to be a function of the phosphate concentration during growth (Burns and Beever 1977 J. Bacteriol. 132:511-519). The gene encoding this housekeeping phosphate permease is unmapped, since no mutations affecting it have previously been reported. Under conditions of very low external phosphate concentration, a regulatory cascade acts to turn on the synthesis of two structurally and functionally distinct repressible phosphate permeases encoded by the pho-4 and pho-5 genes (Versaw 1995 Gene 153:135-139; Versaw and Metzenberg 1995 Proc. Nat. Acad. Sci. USA 92:3884-3887). The PHO-4 and PHO-5 transporters have much lower Km values (2.56 mM and 37.4 mM, respectively) than does the housekeeping phosphate permease. The Km of the housekeeping permease rises sharply under alkaline conditions, so that only very high phosphate concentrations allow appreciable transport by this permease. Thus at a pH above neutrality and a phosphate concentration of 50 mM, phosphate uptake and growth require the function of at least one of the repressible, low Km permease genes, plus the ability of the regulatory cascade to turn it on. The double mutant pho-4RIP; pho-5RIP cannot grow under these restrictive conditions.

In one experiment, about 10^6 conidia of the a mating type of this strain (FGSC#8348) were inoculated into a number of flasks of a medium modified from Fries 1.5% sucrose. In this modified medium, the KH2PO4 was lowered to 50 mM, the molar deficit of potassium was replaced by KCl, and the pH was held at 7.75 with 100 mM Na HEPES buffer. No visible mycelium appeared in any of the flasks for a week, but then three of the flasks rather abruptly showed vigorous growth proceeding in each case from a single focus, apparently as a result of spontaneous suppressor mutations. The suppressed strains were backcrossed to the opposite mating type of the parental double pho-4RIP; pho-5RIP (FGSC #8347) in order to isolate homocaryons, and ascospore cultures were tested for their ability to grow at 50 mM phosphate and pH 7.75. Two of the strains failed to give rise to any such progeny, but the third yielded a single isolate of the desired kind among 24, suggesting that the original strain was a dilute heterocaryon of mutant nuclei in a mostly parental mycelium. The homocaryotic strain, in turn, passed readily through a second backcross, as judged by scoring progeny on sorbose medium under the restrictive conditions.

On ordinary Vogel's (high phosphate) medium, the original homocaryotic triple mutant and its reisolates from a cross accumulate a brownish pigment. They grow and conidiate considerably less well than does the parental pho-4RIP; pho-5RIP, which is visually indistinguishable from wild type. Its ability to grow under the restrictive condition is presumably due to a change in the houskeeping permease, which might have an elevated Vmax (either an enzyme with a higher intrinsic activity per molecule or wild-type enzyme made in greatly increased amounts) or a mutant enzyme with a lower Km than that of wild type. In either case, the availability of a suppressor mutant, and potentially of many mutants, makes this enzyme accessible to study. The present data are consistent with the possibility that the mutation is the structural gene for the houskeeping phosphate permease, but do not rule out a dominant regulatory mutation. I suggest that the gene be called hkpp, and have put the suppressed triple mutant, hkppsu; pho-4RIP; pho-5RIP, into the collection as FGSC strains #8349 and 8350, of the A and a mating types, respectively.

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