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Disruption of an Amino Acid Transport Mutant of *Neurospora crassa* by KCl

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A double amino acid transport-deficient mutant (Pm$^-$NB) of *Neurospora crassa* is shown to be altered in the molecular structure of its cell wall or membrane. This alteration was revealed by a high degree of cellular disruption and cell-cell interaction following extraction by a high molar concentration of KCl.

Characterization of phenotypes associated with amino acid transport-deficient mutants of *Neurospora crassa* has been traditionally limited to a study of transport kinetics. Recently, however, biochemical studies describing the absence or reduction of protein (3) or glycoprotein (1) molecules in mutants reduced in transport of amino acids have been reported.

The conidial transport activity of the Tatum a (SY4f$_8$a) wild type and the mutants Pm$^-$N^{22}, Pm$^-$B^{37}, and Pm$^-$NB has been previously described (4). The Pm$^-$N^{22} mutant is reduced in transport of neutral-aromatic but not in the basic amino acids. The mutant Pm$^-$B^{37} is reduced in transport of the basic but not the neutral-aromatic amino acids. Pm$^-$NB is a double transport-deficient mutant obtained from a cross of Pm$^-$N^{22} and Pm$^-$B^{37} phenotypes. This double mutant is presumed to retain only a general transport activity for neutral and basic amino acids.

The two loci, *Pm N* and *Pm B*, have been shown to be associated with the chromatographic (affinity column chromatography) disappearance of KCl (4.82 M) extractable "glycoproteins" (1). The absence of 260-nm absorbance material on the affinity column could be due to structural modification of the glycoprotein, i.e., loss of "affinity" for bound ligand (arginine), or complete loss of biosynthetic capabilities for that glycoprotein. In either case, the association of genetically altered molecules (or even their complete absence) with the cell wall or membrane of *N. crassa* conidia should tend to weaken its structural integrity.

Conidia were obtained from 7-day-old vegetative cultures grown on Vogel minimal medium N (2). The conidia were harvested into either glass-distilled water or 4.82 M KCl (saturated solution at 25 C), shaken for 1 to 2 min, filtered through glass wool, and then centrifuged to sediment the extracted cells. The saturated KCl solution was decanted off, and 1 ml of 4% gluteraldehyde in Millonig's phosphate buffer (*p*H 7.4) was added to the cells and allowed to fix for 1 hr at 4 C. The cells were then rinsed twice with phosphate buffer (*p*H 7.4) to affect a phosphate buffer wash (S. Sines, *personal communication*).

A 1-ml amount of 1% osmium tetraoxide in phosphate buffer (*p*H 7.4) was added to the cells and allowed to fix for 1 to 1.5 hr at 23 ± 1 C. The cells were then centrifuged and the liquid was decanted. Dehydration was accomplished by passage of cells through 10% gradations of ethyl alcohol (50 to 100% in 20-min intervals). After the last dehydration in 100% EtOH, the cells were filtered onto nitrocellulose filters (type AA, 0.8-μm pore size; Millipore Corp.).

The filter was then mounted onto standard SEM stubs, coated with gold palladium (20 to 25 ± 2.5 nm) by using a Denton DV 502 vacuum evaporator, and scanned with a scanning electron microscope (Cambridge Stereoscan Mark 2A).

Scanning electron micrographs of conidia of wild type or any of the transport mutants revealed no visible differences in external morphology after extraction with sterile glass-distilled water.

Figure 1 presents micrographs of conidia of wild type, Pm$^-$N^{22}, Pm$^-$B^{37}, and Pm$^-$NB extracted with 4.82 M KCl. The wild type, Pm$^-$N^{22} (neutral), and Pm$^-$B^{37} (basic) strains remain intact and resemble their water-extracted counterparts. The Pm$^-$NB double mutant, however, appears to be extensively disrupted by KCl extraction (Fig. 1D). Because of the pronounced tendency of the Pm$^-$NB conidia to aggregate after KCl extraction, a

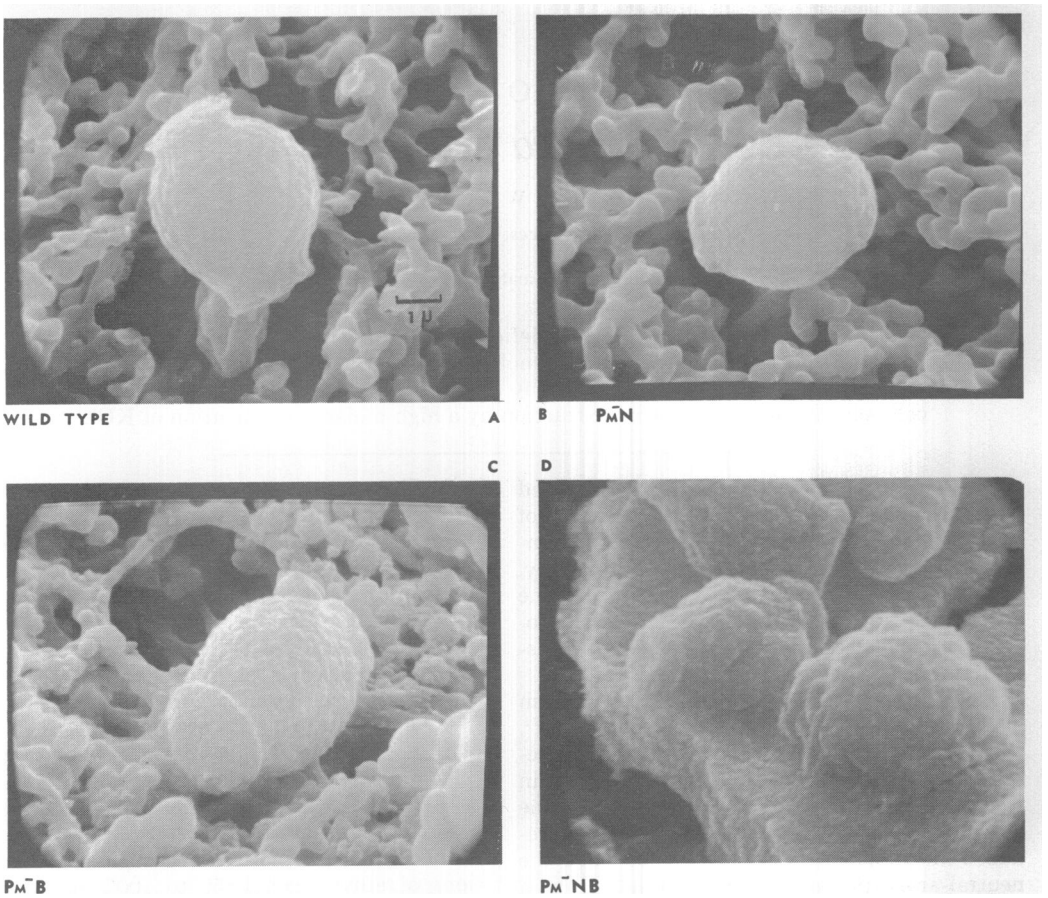

FIG. 1. *Scanning electron micrographs of wild type (A), Pm⁻N²² (B), Pm⁻B³⁷ (C), and Pm⁻NB (D) conidia extracted with 4.82 M KCl. Background material supporting the conidia is the nitrocellulose filter.*

determination of viability of the extracted cells has been difficult. It should be noted here that the disruption of Pm⁻NB conidia by KCl was first noted as a visible abnormality of the conidial suspension and may serve as a convenient means to screen large numbers of mutant strains.

The observed sensitivity of Pm⁻NB conidia to 4.82 M KCl could be due to disruption of ionic bonding between structural components of the cell wall or the membrane. Alternatively, the high molar concentrations of KCl used could constitute an osmotic shock to the cells. Either case might be expected to cause release of cellular materials which would appear as a homogeneous matrix in the electron micrograph.

Regardless of the mechanism(s) of disruption by KCl, it may be noted that, in neither case where the loci appear singularly does the sensitivity to KCl appear. The structural modifications produced by the altered *Pm N* and *Pm B* loci appear to be additive, showing the KCl sensitivity only in the Pm⁻NB double mutant. Further, the involvement of these two loci in permeation of metabolites across the cell membrane and wall suggests that the structural modifications proposed might occur in either (or both) structures. The sensitivity of Pm⁻NB conidia to KCl might therefore be attributable to the integration of more than one structurally altered component into the cell wall or membrane.

We are presently unable to ascertain whether the material released by the Pm⁻NB strain after KCl extraction is cytoplasmic or cell surface material. A detailed biochemical analysis of the material released and the dis-

ruptive mechanism(s) of KCl are in progress.

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LITERATURE CITED

1. Stuart, W. D., and A. Gib DeBusk. 1971. Molecular transport. II. *In vitro* studies of isolated glycoprotein subunits of the amino acid transport system of *Neurospora crassa* conidia. Arch. Biochem. Biophys. **144**:512–518.
2. Vogel, H. J. 1964. Distribution of lysine pathways among fungi: evolutionary implications. Amer. Nat. **97**:435–446.
3. Wiley, W. R. 1970. Tryptophan transport in *Neurospora crassa*: a tryptophan-binding protein released by cold osmotic shock. J. Bacteriol. **103**:656–662.
4. Wolfinbarger, L., Jr., and A. Gib DeBusk. 1971. Molecular transport. I. *In vivo* studies of transport mutants of *Neurospora crassa* with altered amino acid competition patterns. Arch. Biochem. Biophys. **144**:503–511.