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The preg^{C} strain of N. crassa has abnormal vesicles when grown on both low- and high-P_i media

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Abstract

The genetic and molecular mechanisms controlling the synthesis of de-repressible phosphatases in Neurospora crassa include four regulatory genes, nuc-2, preg, pgov, and nuc-1, involved in a hierarchical relationship (Metzenberg, 1979. Microbiol. Rev. 43: 361-383).

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The *preg*^{c} strain of *Neurospora crassa* has abnormal vesicles when grown on both low- and high-P_i-containing media

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The genetic and molecular mechanisms controlling the synthesis of de-repressible phosphatases in *Neurospora crassa* include four regulatory genes, *nuc*-2, *preg*, *pgov*, and *nuc*-1, involved in a hierarchical relationship (Metzenberg, 1979. Microbiol. Rev. 43: 361-383). The action of the transcriptional activator nuc-1, required for the expression of phosphorous-specific genes such as *pho*-2 (which encodes a P_i-repressible alkaline phosphatase), is antagonised by the putative pgov-preg complex, which is antagonised by nuc-2, which in turn is antagonised by P_i or its derivatives (Peleg et al.1996. Fungal Genet. Biol. 20:185-191). Thus, nuc-1 is relieved from the negative effect of the pgov-preg complex in strains growing under derepressing conditions or in *preg*^{c} mutant, selected for its ability to synthesise P_i-repressible alkaline phosphatase and secrete acid phosphatase constitutively. Actually, *preg*^{c} strains still respond to variations in extracellular P_i levels. Strains 74A and *preg*^{c} show not only distinct patterns of P_i-repressible alkaline phosphatase secretion, but also distinct properties for the enzyme, such as heat stability and kinetic behaviour for the hydrolysis of the substrate, as a function of variations in the exogenous P_i concentration. Furthermore, the *preg*^{c} strain promptly starts to secrete the *pho*-2$^+$-encoded alkaline phosphatase at pH 7.8, whereas strain 74A does so with a lag of at least 24 h (Thedei Jr. and Rossi, 1994. Plant Cell Physiol. 35: 837-840), an effect probably due to alterations in cell structure. Thus, electron micrographs of sectioned hyphae were taken to investigate further this response. For this, mycelia of strains 74A and *preg*^{c}, grown for 72 h at 30°C, pH 5.4, and collected by centrifugation at full speed in a microtube, were incubated overnight at 4°C in a fixative solution containing 3.0% (v/v) glutaraldehyde and 0.1 M phosphate buffer, pH 7.4. After washing with phosphate buffer, mycelia were post-fixed for 2 h with 0.1% (w/v) OsO_4 in 0.1 M phosphate buffer, pH 7.4. After washing again with phosphate buffer, samples were dehydrated and then embedded in epoxy resin. Ultrathin sections of hyphae were cut, stained with uranila acetate and Pb-subacetate (0.5% w/v) and transmission electron micrographs (TEM) were taken. As shown in Figure 1, many vesicles were located close to the plasma membrane or dispersed in the cytosol when strain 74A was grown in low- or high-P_i media, respectively, whereas a small number of large vesicles is observed when strain *preg*^{c}A was grown in both low- and high-P_i media.

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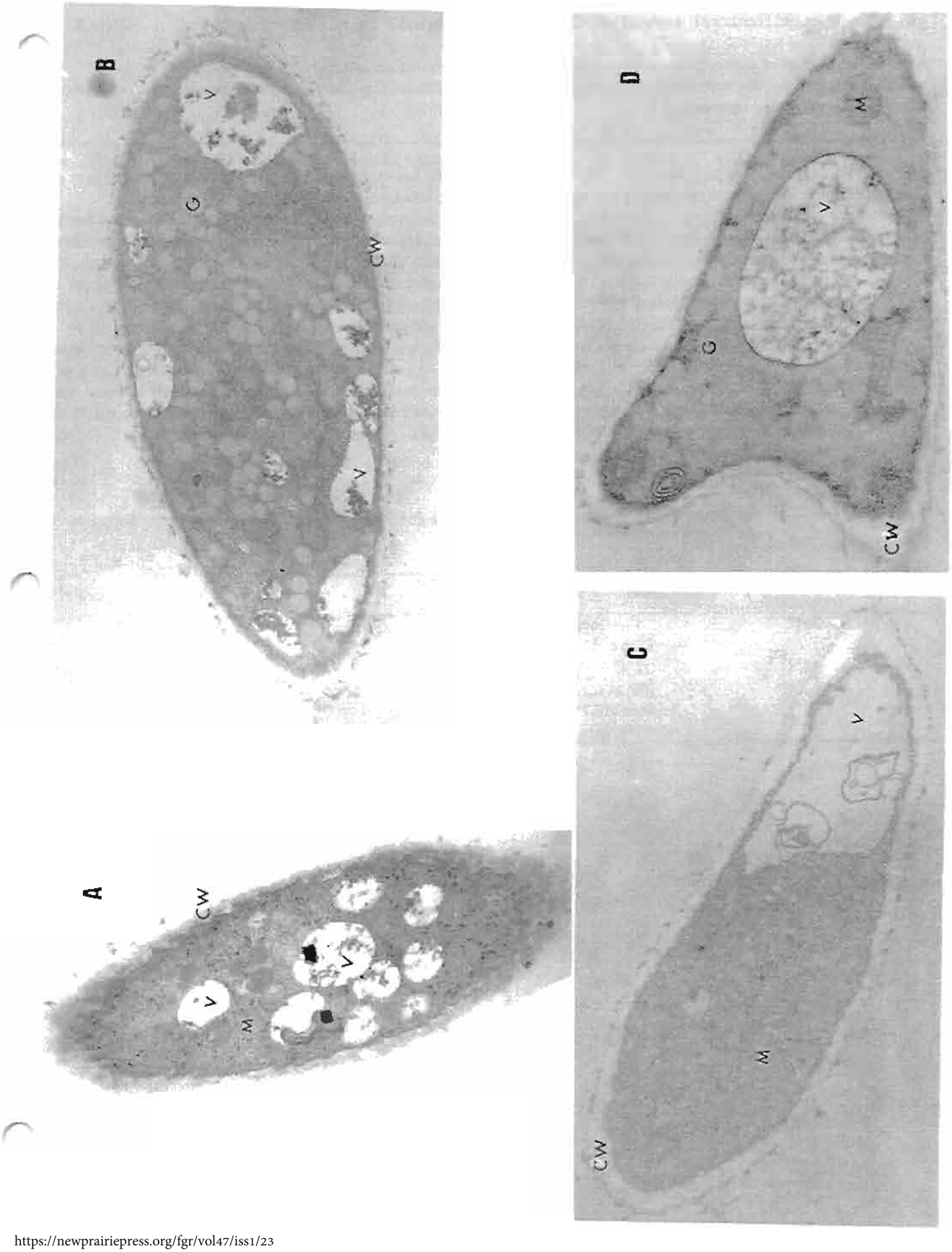
Figure 1. (Following page) Transmission electron micrographs of sectioned hyphae of *N. crassa*. A, B represent sectioned hyphae of strain 74A grown at pH 5.4 in 10 mM Pi and 50 µM Pi, respectively. C, D represent sectioned hyphae of strain *preg*^{c} grown at pH 5.4 in 10 mM Pi and 50 µM Pi, respectively. CW, M, V and G indicate cell wall, mitochondrion, vacuole and granule, respectively.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