

CONCEPTS AND COMMENTS:

Are the structural genes of Pi-repressible phosphatases regulated by multiple circuits in the filamentous mold *Neurospora crassa*?

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The filamentous mold *Neurospora crassa*, and probably all living organisms, synthesize a number of phosphatases which are necessary to scavenge phosphate from medium containing DNA or RNA as the sole phosphorus source, i.e., these enzymes are synthesized in response to signals of the absence of inorganic phosphate (Pi) in the medium. This response has suggested the existence of repressible phosphatases whose structural gene transcription may be regulated in *N. crassa* by at least three genes, *nuc-2*⁺, *preg*⁺ and *nuc-1*⁺, involved in a hierarchical relationship (*nuc-2* and *nuc-1* mutants do not grow on DNA or RNA as the sole phosphorus source, and the *preg*^c mutant should synthesize and excrete these phosphatases constitutively). This regulatory model elaborated from genetic studies postulates that the positive action of *nuc-1*⁺, required for the expression of the structural genes, is antagonized by *preg*⁺, which is antagonized by *nuc-2*⁺, which in turn is antagonized by Pi or by a co-repressor derived from it (Metzenberg, 1979; Furukawa *et al.*, 1987; Kang and Metzenberg, 1993; Kang, 1993). A regulatory hierarchy of this type implies that the *preg*^c mutant should synthesize and secrete on high-Pi medium the same enzymes synthesized by the wild-type strain grown on low-Pi medium, i.e., the *preg*^c mutant

should not sense signals of Pi starvation. Indeed, the regulation of this adaptive response has proved to be even more complex, not only because of the identification of a large number of genes involved in the signalling of Pi starvation, but also because the synthesis of these enzymes may be under the action of nitrogen, carbon and pH regulatory circuits (Grove and Marzluf, 1980; Nahas *et al.*, 1982; Espeso *et al.*, 1993). [The pH regulatory mechanism ensures that extracellular enzymes are secreted only at pH values at which they can function effectively, i.e., the mold *N. crassa* secretes for example acid and alkaline phosphatases at acidic and alkaline pH, respectively]. However, there is increasing evidence indicating that *N. crassa* synthesizes these phosphatases regardless of the exogenous levels of Pi and that genes *nuc-2*, *preg* and *nuc-1* are involved in a regulatory hierarchy responsible for the excretion process by causing the enzymatic form to be excreted, i.e., they may participate in the regulation of structural signalling of the enzymes for a subsequent step in the excretion pathway (Han and Rossi, 1989; Han *et al.*, 1994; Thedei and Rossi, 1994). Thus, if the gene *preg* is functioning (high Pi medium), a signal would occur for the processing of the structural modifications needed for the excretion of high Km (low-affinity) enzyme forms. If the gene *preg* is not functioning (low-Pi medium), the gene *nuc-1* would signal for structural modifications leading to the excretion of low Km (high affinity) enzyme forms, a fact that would permit the utilization of nucleic acids as the sole Pi source (Han *et al.*, 1994). This implies that nitrogen, carbon and pH regulatory circuits should also play a role in the excretion pathway of these enzymes.

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If we consider the transcription control of gene *pho-3*, which seems to be the structural gene for the Pi-repressible acid phosphatase synthesized by the mold *N. crassa* (Han *et al.*, 1994; Nelson *et al.*, 1976), the nitrogen regulation of phosphatases in this organism displays an unexpected picture because: a) growth is supported by adenine, AMP and RNA but not by either single or double stranded DNA when these compounds are the only available nitrogen source; b) no rise in phosphatase activity levels is detected when AMP is the sole phosphorus or nitrogen source; c) only low levels of acid phosphatase activity is detected in mycelial extracts when RNA is the sole nitrogen source (Grove and Marzluf, 1980). Furthermore, this enzyme should account alone for the complete hydrolysis of RNA. These results are also difficult to reconcile with a possible control of transcription of structural genes of the phosphatases excreted by *N. crassa* since the utilization of RNA as a nitrogen source occurs in high-Pi medium, a growth condition under which gene *preg* is functional. Under these growth conditions, the low affinity forms of the phosphorus family enzymes should be synthesized. This may indeed be occurring because the acid phosphatase activity detected was increased approximately 8-fold during nitrogen limitation as opposed to 60-to-100-fold in cells grown with limited phosphorus (Metzenberg, 1979; Grove and Marzluf, 1980). It is possible that the structural modifications necessary for transport into the cell membrane of permeases and probably also of some enzymes involved in the utilization of different nitrogen sources (Pombeiro-Sponchiado *et al.*, 1993) are shared by all phosphorus family enzymes through a diversion pathway triggered by nitrogen limitation. The existence of other pathways involved in phosphatase processing may also explain the detection of different levels of elevation of enzyme activity as a function of the utilization of sucrose or acetate as a carbon source by *N. crassa* (Thedei *et al.*, 1994; Han *et al.*, 1987). In addition, the synthesis and/or secretion of only Pi-repressible alkaline phosphatase is affected by mutations in *acu-1*, and *acu-5* and *acu-7* genes which also indicate distinct pathways for the secretion of acid and alkaline phosphatases in *N. crassa* (Han *et al.*, 1987). However, these structural modifications may not be adequate for obtaining high-affinity forms of these enzymes (with the consequent lack of detection of high levels of enzyme activity) or permitting the fungus to grow on DNA as a nitrogen source. Furthermore, the excretion of low affinity forms of the phosphatases (Han *et al.*, 1994) may permit the utilization of AMP as a sole phosphorus or nitrogen source without the activation of the signals of deprivation of these nutrients.

In conclusion, it appears that the action of the nitrogen regulatory circuit does not occur at the level of regulation of transcription of the *pho-3* gene of the mold *N. crassa* but rather, like the phosphorus, carbon and pH regulatory circuits, at the level of the control of phosphatase excretion, by causing the enzymatic form to be excreted into the culture medium. Thus, all the data concerning the synthesis or secretion of phosphatases by the mold *N. crassa* of which we are aware fits a single model, i.e., the hypothesis of structural signaling (Han *et al.*, 1994) formulated to explain the regulation of phosphatase excretion in the mold *N. crassa*.

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