## The levels of mRNA expressed by gene *palF* of *A. nidulans* do not appear to be pH regulated

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Although *pal* genes are putative members of a signaling cascade involved in ambient pH sensing and in the consecutive activation of PacC protein, recent findings show that most of them (*pal*A, B, C, H and I) do not respond to ambient pH at the transcriptional level. Here, we show that mRNA levels of the remainding *palF* gene are also constant at various growth pH values.

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In *Aspergillus nidulans* ambient pH-regulated gene expression is under the control of a complex genetic circuit comprising at least seven genes that have been cloned and sequenced (Negrete-Urtasun et al. 1999. Mol. Microbiol. 33: 994-1003). The gene *pac*C codes for a transcription factor that undergoes proteolysis at alkaline growth pH, yielding a functional protein responsible for the induction of genes expressing enzymes with optimal activity at alkaline pH (e.g. alkaline phosphatase) and repression of those with optimal activity at acidic pH (e.g. acid phosphatase). Transcription of *pac*C is itself pH regulated, being induced under alkaline growth conditions. The genes *pal*A, B, C, F, H, and I are putative members of a signalling cascade involved in ambient pH sensing and the consecutive activation of PacC protein. Although the role of *pac*C in gene regulation has been well defined in *A. nidulans*, no function has yet been assigned to any of

the *pal* genes. The *pal*B gene codes for a calpain protease that is not involved directly in PacC processing, whereas the other genes have revealed, solely by sequence prediction analysis, a few features concerning cell localization signals and putative protein structural motifs. Thus, much effort is needed to elucidate the role of *pal* genes in the transduction of the ambient pH signal to PacC. An interesting finding however is that the transcription of the genes *pal*A, B, C, H, and I is not pH regulated like *pac*C. In the present study, we completed the transcriptional analysis by studying the influence of ambient pH on the transcription of the uncharacterized *palF* gene.

Strain *paba*A1 ($10^9$ spores) was grown with shaking in 100 ml of liquid minimal medium (Cove, 1966. Biochim. Biophys. . 113: 51-56) buffered at pH 5.0 and pH 6.3 with 50 mM sodium citrate, and at pH 8.0 with 50 mM Tris-HCl for 12 h at 37°C. Mycelia were harvested and ground in liquid $N_2$ and total RNA was extracted with the Trizol reagent (Gibco BRL) according to manufacturer's instructions. Northern blot was carried out as follows: total RNA (10 µg) was subjected to electrophoresis in a 1% agarose/formaldehyde gel, stained with ethidium bromide, photographed, and blotted onto a nylon membrane as previously described (Maccheroni et al. 1997. Gene 194: 163-167). A 3.3-kb DNA fragment corresponding to a *palF* cDNA (100 ng) was labeled by random priming with [$\alpha$-$^{32}$P]dCTP (>3000 Ci/mmol; 10 mCi/ml) to a specific activity of $10^9$ CPM/µg. The membrane was hybridized overnight at 65°C in a solution containing 250 mM $NaH_2PO_4$, 7% SDS and washed once at 37°C (40 mM $NaH_2PO_4$, 5% SDS) for 1 h and once at 55°C (40 mM $NaH_2PO_4$, 1% SDS) for 30 min. Hybridization signals were detected after 5-days exposure to X-ray film.

As shown in Figure 1, a single transcript (3.5-kb) is observed at similar abundance under all growth conditions, indicating that *palF* transcription is also unchanged in response to ambient pH. This raises the possibility of post-transcriptional processing exerted by *pal* genes as the mechanism of ambient pH signal transduction or even that other *pal* genes are still waiting to be identified.

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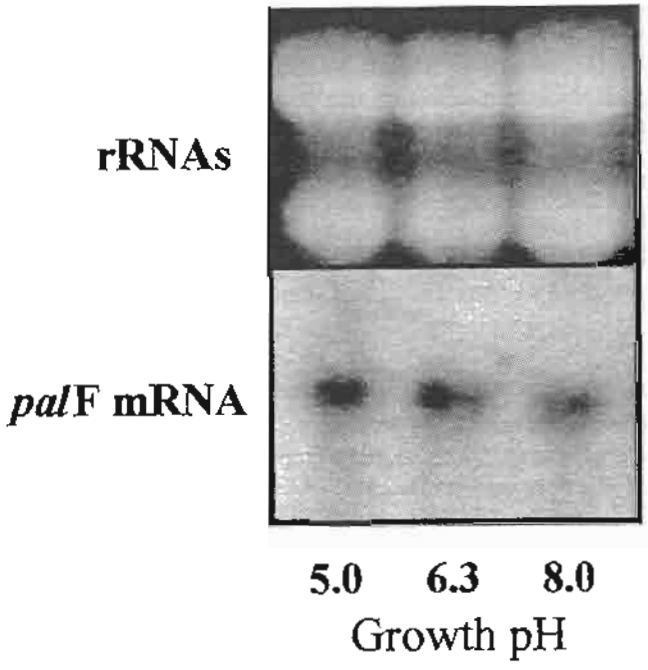


Figure 1. Influence of ambient pH on *palF* gene transcription. Ribosomal RNAs were used as control to indicate that equal loads of total mRNA, obtained at different growth pHs, were used in each lane.