Activation of the twin-arginine translocation pathway of *Streptomyces coelicolor* under phosphate limited conditions

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**Introduction**

Phosphate is an essential nutrient for the growth of all microorganisms. For this reason a limitation of phosphate in the medium results in a specific response. In *Streptomyces* coelicolor the expression of several secreted proteins is activated directly by the two component system PhoR-PhoP under phosphate limited conditions (1, 3). Many of these proteins are secreted via the twin-arginine translocation pathway (Tat), which is a major route of secretion in *S. coelicolor* (4).

To determine whether the expression of the Tat system is also controlled by phosphate, we amplified the promoter of the twin arginine translocase gene A (*tatA*). We studied the existence of PHO boxes (PhoP binding sites) and the binding of PhoP to the promoter region of *tatA* by electrophoretic mobility shift assays (EMSA) as well as its activity, coupling it to the luciferase reporter genes in the integrative plasmid pLUXAr(+). The expression was studied under conditions of high and low phosphate concentrations in the strains *S. coelicolor* M145 (wt) and INB101 (ΔphoP).

**Possible PHO boxes in the *tatA* promoter**

Two possible PHO-box sequences (framed) with similarity to the conserved PHO consensus sequence G(7)TCAYYYR(G) (3) were localized in the promoter region of *tatA*. One in the complementary strand, 274 bp upstream of the start codon (GTCAGACCGGATTTCCAGGG) and one in the coding strand, 96 bp upstream of the start codon (ATGACACCGGATTTCCAGGG). To test whether PhoP binds to one of these sequences, the *tatA* promoter was amplified and gel retardation experiments (EMSA, electrophoretic mobility shift assay) were performed using PhoP[13] (the DNA-binding domain of PhoP) as described by Sola-Landa et al. (2). Employing 20 pmol of PhoP[13], the highest concentration used in all previous trials conducted in our laboratory with other promoters, no promoter shift could be observed. This result supports the importance of the conservation of the first 6-7 nucleotides in at least two of the direct repeats forming the PhoP binding site (3).

**Construction of plasmid pLUX-tatA**

To measure its expression, the *tatA* promoter was amplified by PCR and cloned into plasmid pLUXAr(+) according to the strategy depicted above. An Nde restriction site was introduced so that the ATG of the *tatA* gene coincided with the start codon of luxA.

The resulting plasmid (pLUX-tatA) is conjugative and integrative, permitting its construction in *Escherichia coli*. Its introduction into *S. coelicolor* by conjugation, and its integration into the genome. The *luxAB* genes and d-luxA constitute only a part of the luciferase operon and thus, to measure the promoter expression by luminescence, r-decarboxylase has to be added as a substrate.

**Expression of the *tatA* promoter in *S. coelicolor***

M145 and INB101

The strains *S. coelicolor* M145 and INB101 (ΔphoP), containing the plasmid pLUX-tatA, were grown in MG medium with 15 or 2.5 mM phosphate. With starting concentrations of 15 mM phosphate is not a limiting factor whereas at concentrations of 2.5 mM it is depleted after 50 h. Nevertheless, growth remains stable in the wt strain and is only affected in the ΔphoP mutant strain INB101 at low phosphate concentrations as previously described (1).

The expression of the *tatA* promoter is identical in all conditions up to 48 h. After this time phosphate is exhausted under limited phosphate conditions and the *tatA* expression is strongly increased in *S. coelicolor* M145, reaching values of more than twice its normal expression. However, this increase is not observed in INB101. The expression of *tatA* in the ΔphoP mutant strain is independent of the phosphate concentration and matches in all time points with the expression measured in the wt strain with 15 mM of initial phosphate. This indicates that *tatA* is activated by PhoP under conditions of phosphate starvation. The activation takes place in parallel to the activation of the rest of the PHO regulon genes (1), including catalytic proteins which were shown previously to be secreted via the Tat pathway (4).

**Conclusions**

*tatA* expression increases up to 2-3 times in conditions of phosphate limitation. This increase takes place after phosphate depletion and does not occur in the ΔphoP mutant strain INB101, indicating together with the results of gel retardation experiments, an indirect activation by PhoP. Thus *tatA* activation involves a hitherto unknown regulator, which could be among the transcription factors whose expression has been shown to be controlled by PhoP (1).

As the genes *tatA* and *tatC* form a possible operon, this suggests an indirect control of *PhoP* on the twin arginine translocation pathway. An activation of this system in phosphate shortage makes sense, since many genes activated by PhoP are secreted by this route, as demonstrated by Widdick et al. (4).

**References**