Project proposal for thesis CONACyT

The role of the membrane in induction of the Mlc repressor from its DNA targets.

There is an increasing number of examples where transcription factors have been found associated with membrane-bound components and where this attachment or interaction is necessary for their function (1). One of the first identified was Mlc, the repressor of the major glucose transporter in *E. coli* PtsG. Instead of being displaced from its DNA targets by interacting with a low molecular weight metabolite related to the metabolism of the repressed genes (as in the case of allolactose and the *lac* operon), growth on glucose induces the expression of the *ptsG* gene by sequestering the Mlc repressor to the membrane via an interaction with the active PtsG transporter (2). We have identified amino acids in both the transporter (3) and the Mlc repressor (4), which are necessary for this interaction. A crystal structure of the Mlc protein with the EIIB domain of the transporter has confirmed they are in close contact (5). However something in addition supplied by the membrane is required for the productive interaction leading to derepression because soluble version of the EIIB domain is non–inducing.

We have identified a homologue of Mlc from *Vibrio cholerae*, which represses *ptsG* but has a complex phenotype, which seems to involve both induction by a low molecular weight metabolite and by interaction with a membrane transporter. We propose detailed genetic studies of this protein which should shed light on how the different signals (membrane attachment and small molecule binding) lead to the changes in the DNA binding domain and release of the repressor from the DNA. Biochemical experiments will be attempted to identify the membrane component via crosslinking studies and/or pull-down experiments. Use of fluorescence labelling will also be tried but will require special care, because labels at either end of Mlc (and presumably its homologue) destroy one or other of its function, either for DNA binding or for interaction with EIIBGlc. However Mlc with a N-terminal tag (fluorescent protein or an other antibody reacting tag), should still bind the membrane and can be used to track the membrane bound form.

(5) Nam TW, Jung HI, Young JA, Park YH, Lee SH, Seok, YJ, Cha SS. (2008) Analysis of Mlc-IIIB\textsubscript{Glc} interaction and a plausible molecular mechanism of Mlc inactivation by membrane sequestration. Proc Natl Ac Sc USA 105:3751-3756

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