TM0853 AND TM0468 FORM A NOVEL TWO COMPONENT SYSTEM IN *THERMOTOGA MARITIMA*

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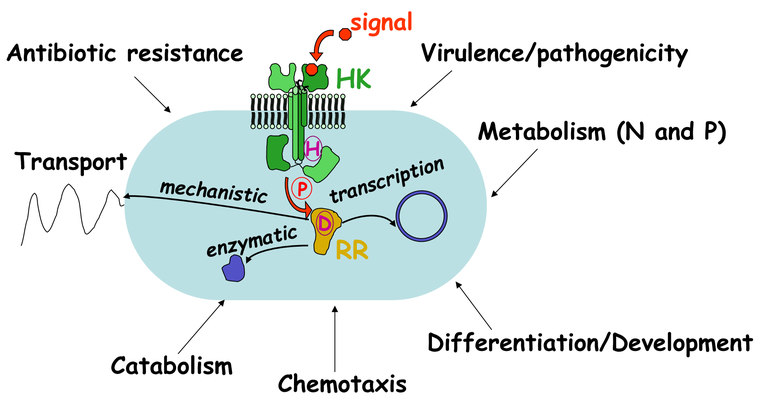
1.INTRODUCTION

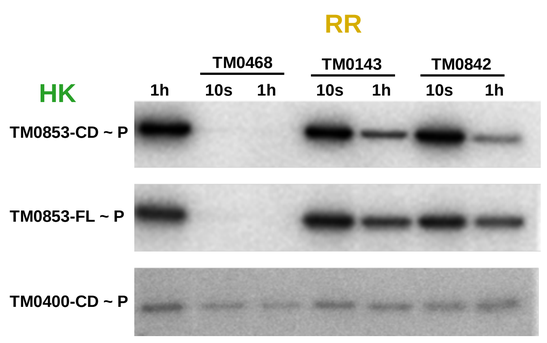
Two component systems (TSC) are the major mechanism used for signal transduction principally in prokariotes but also in some eukaryotes such as plants, fungus and yeast. A typical TSC consists of two proteins: a sensor dimeric histidine kinase (HK) serving as a signal receptor and its cognate response regulator (RR) mediating specific gene expression or enzymatic reactions required for innumerable adaptative responses as it can be seen in the figure below.



Each cognate pair of sensor/regulator works as a faithful system and they are often encoded by adjacent ORFs or within the same operon. However, some two component genes are "orphans" with no functional cognate pair encoded nearby. Two-component proteins are well characterized structurally and functionally due to the conservation of their catalytic domains, however little is known about their complex formation. We have recently solved the structure of the cytoplasmic portion of the histidine kinase TM0853, therefore we present here the studies of the complex formation with its cognate RR.

2.LOOKING FOR THE PARTNER OF THE HISTIDINE KINASE TM0853?

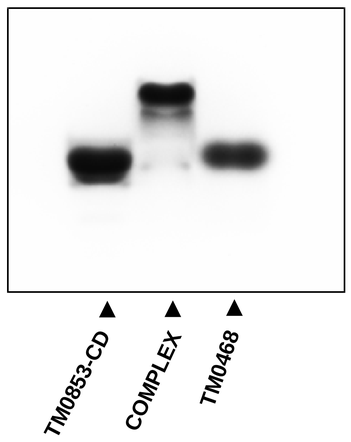
TM0853 is the only orphan HK of *Thermotoga maritima*. In order to identify with which orphan RRs of *Thermotoga maritima* (TM0143, TM0468 and TM0842) interacts "*in vivo*" we have applied a new biochemical method called *phosphotransfer profiling*.



Phosphotransfer profiling test. A HK can "*in vitro*" phosphorylate unespecifically at several RR at long times (1h) while at short times just phosphorylates specifically its "*in vivo*" partner. As it can be seen above, TM0853-full (FL) and its cytoplasmic portion (CD) transfer their phosphoryl group [γ-^{32}P] at short times (10s) to TM0468, while it is necessary long times (1h) to observe a small decrease in other RRs, TM0143 and TM0842. As a control, we have used the histidine kinase TM0400 that showed no interaction with any RR. These results indicate that TM0853 has kinetic preference for TM0468, possibly its *in vivo* target.

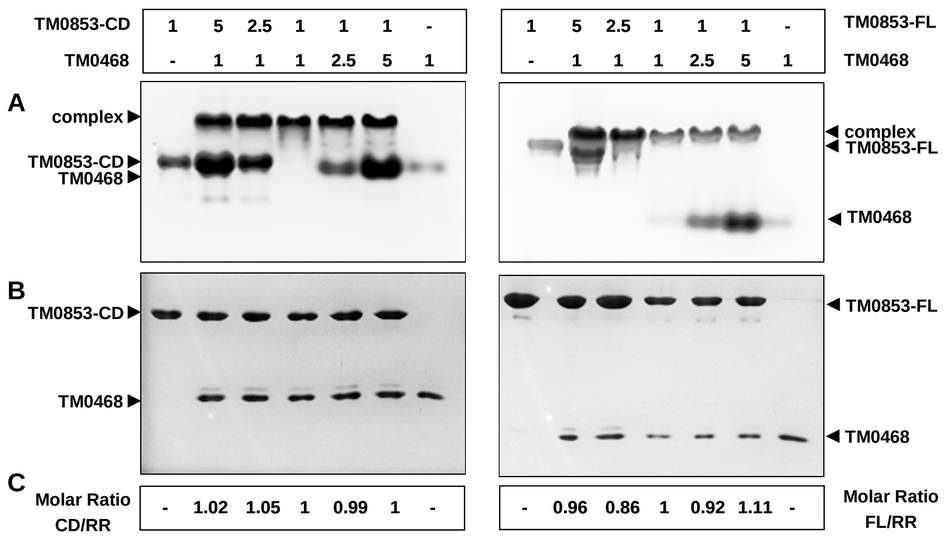
3.DOES TM0853 AND TM0468 FORM A STABLE COMPLEX?

If TM0853 and TM0468 are mixed together and a native-PAGE gel is run (as it can be seen below), a new slower band that migrates separately from the bands of the individual proteins is formed. The new band corresponds to the complex formed by both proteins and its retardation indicates that the complex is stable.



4. WHICH IS THE STOICHIOMETRY OF THIS COMPLEX?

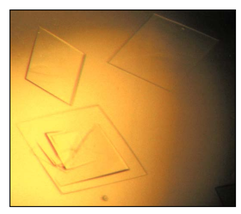
In order to calculate the stoichiometry of the complex, TM0853 and TM0468 were mixed at different molar ratios (TM0853:TM0468; 5:1, 2.5:1, 1:1, 1:2.5, 1:5) and the samples were subsequently subjected to native-PAGE (panel A). The bands corresponding to the complex were cut and subjected to SDS-PAGE (panel B) with the aim of calculating the molar ratio between both proteins within the complex. The assays were done with the pair TM0853-CD/TM0468 and TM0853-FL/TM0468, being the latter, the first analysis of this type realized using an intact TSC.



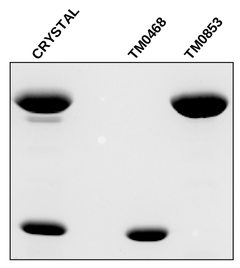
Interestingly, in the native gels (panel A) the amount of complex formed between TM0853-CD and TM0468 seems constant at varying ratios, however, the amount of complex formed between TM0853-full and TM0468 increases at increasing FL ratio.
The molar ratio (panel C) calculated between the proteins TM0853-CD and TM0468 in the complex stayed constant and equimolecular indicating that one TM0853-CD dimer binds two TM0468 molecules.
In parallel, the molar ratio calculated for TM0853-full and TM0468 showed a slightly variable ratio but close to 1 in all cases, indicating that both proteins form a 1:1 complex. Currently, ultracentrifugation assays are in progress to elucidate the results obtained.

5.CAN THE COMPLEX BETWEEN TM0853/TM0468 BE CRYSTALLIZED?

The crystals (left panel) were obtained from a protein mixture (10mg/ml TM0853-CD, 7.5mg/ml RR468, AMP-PNP-Mg) in a solution containing ammonium sulphate (1.65M), dioxane (2%) and 0.1M sodium citrate pH 5.6. Crystals grew after two days and an SDS-gel of these confirmed the presence of both proteins (right panel). Data collection is shown in the table.

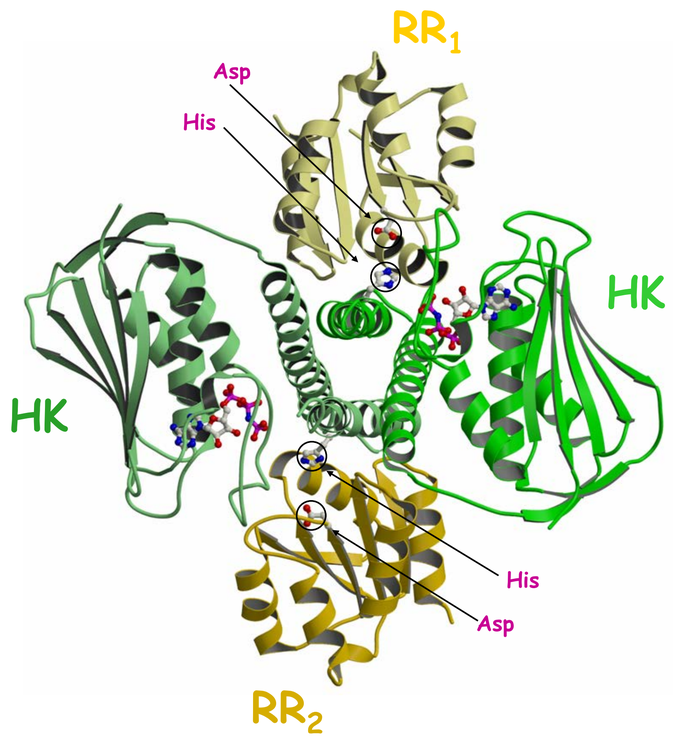


X-ray source	ESRF, Grenoble (France)
Space group	C2
Cell (Å)	a = 183.96 b = 90.67 c = 68.99 β = 108.42
Resolution (Å)	43.6-2.8
Rmerge (%)	7
I/δ(I)	15.2
Multiplicity	4.3



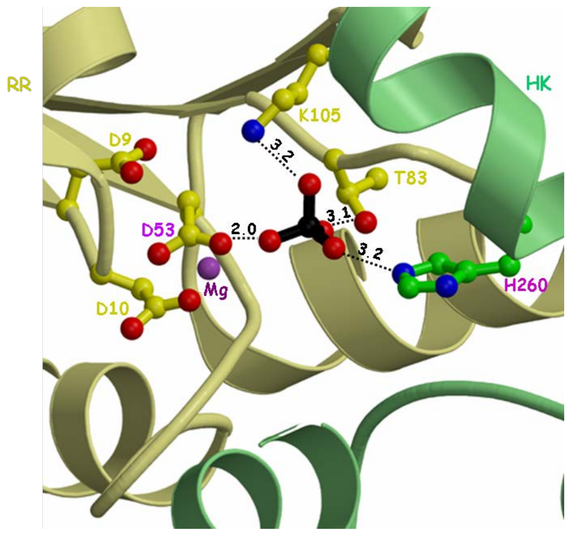
6.HOW IS THE COMPLEX STRUCTURE?

The structure solved by the MAD method is in agreement with the stoichiometry obtained in the previous assays, where the complex is formed between the dimeric HK and two molecules of the RR.



7. WHICH REACTION CAN WE OBSERVE IN THE STRUCTURE ?

Signal transduction in TSC is mediated by phosphotransfer between a conserved His and an Asp residues in the HK and in the RR, respectively. In the active site (see below) there is a sulfate ion (in black) at a bonding distance (3.2Å) of the catalytic His260 of the HK and at 2.0Å of the catalytic Asp53 of the RR. We propose that the sulphate ion is occupying the position of a phosphoryl group and that the shortest binding distance with the D53 represents the final step of the phosfotransfer reaction. Other catalytic residues involved in the phosphotransfer reaction are showed in ball and stick (D9, D10, T83, K105).



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