

Crystal structure of the quorum sensing protein TraM and its interaction with the transcriptional regulator TraR, Alessandro Vannini, Cinzia Volpari and Stefania Di Marco*, *IRBM, Italy*. E-mail: stefania_dimarco@merck.com

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Quorum sensing is a term that reflects the ability of bacteria to control the expression of specific operones in a cell density-dependent manner and is based on the production, release and "sensing" of small signal molecules called autoinducers, which accumulate in the environment as a function of cell density. Obeying to quorum sensing is the soil bacterium *A. tumefaciens*, responsible for substantial loss of perennial crops worldwide [1]. Pathogenesis involves transfer of the Tumor-inducing (Ti) plasmid from the bacterium to the host cell nucleus and the subsequent transcription of opines and phytohormones, which cause large tumors to form on stems. The conjugal transfer of the Ti plasmid is strictly controlled by a LuxRI-type quorum sensing circuit composed of the activator TraR (homologous to LuxR), a *cis*-acting DNA inverted repeat called *trabox*, and the autoinducer, N-(3-oxo-octanoyl)-L-homoserine lactone. Recently, the crystal structure of TraR bound to its autoinducer and to DNA has been reported [2]. Additional regulatory proteins modulate the activity of LuxR-type proteins, such as defective LuxR homologues that form inactive heterodimers and other regulators that form inhibitory complexes [3]. Among these, the Ti plasmid encoded protein TraM from *A. tumefaciens*, modulates TraR-dependent transcriptional activation by direct protein-protein interaction. TraM acts as an anti-activator of TraR by binding to its C-terminal domain and therefore prevents TraR from binding DNA [3]. This inhibition is required for the normal operation of the quorum-sensing pathway and, as a consequence, null mutations in TraM result in constitutive conjugation even at low population density. TraM acts either to prevent TraR from initiating transcription of the *tra* regulon under non-inducing condition, or to shut down it efficiently when the opine signal is no longer available during infection. Thus, TraM plays a key role in determining the threshold level of the bacterial population required for initiating the Ti plasmid conjugal transfer [3]. We have determined the crystal structure of the recombinant TraM protein. The structure reveals two molecules per asymmetric unit, arranged as a dimer. In order to understand the molecular basis of TraR-TraM interaction, we have reconstituted and characterized, *in vitro*, the complex. Dimeric TraM binds tightly dimeric TraR with an equimolar ratio, forming a stable oligomeric complex of ~150 KDa. A model of the TraR/TraM complex is proposed.

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