# EMERGENCE OF NOVEL FUNCTIONS IN TRANSCRIPTIONAL REGULATORS PROCEEDS THROUGH REGRESSION TO *STEM* PROTEIN TYPES

Teca Calcagno Galvão[1], Mario Mencía, and Víctor de Lorenzo
Centro Nacional de Biotecnología, CSIC, Madrid, Spain. [1]Current address: Laboratório de Genômica Funcional e Bioinformática, DBBM-IOC, FIOCRUZ; Rio de Janeiro, RJ; Brasil

Evolutionary expansion of metabolic networks entails the emergence of regulatory factors that become sensitive to new chemical species. A dedicated genetic system was developed for the soil bacterium *Pseudomonas putida* aimed at deciphering the steps involved in the gain of responsiveness of the toluene-activated prokaryotic regulator XylR to the non-natural xenobiotic chemical 2,4 dinitrotoluene (DNT). A random library of the toluene-binding module of XylR (the so-called A domain) was generated and sieved *in vivo* for those variants activated by DNT through coupling the cognate promoter *Pu* to the *P. putida* yeast URA3 homolog, *pyrF*. The resulting mutants fell in various categories according to their phenotype and their location in the protein sequence. All DNT-responsive clones maintained their sensitivity to ordinary effectors of XylR and broadened the range of inducers to unrelated aromatics. Yet, none of the altered amino acids lied in the recognizable effector-binding pocket of the polypeptide. Instead, mutations clustered in protein surfaces believed to engage in the conformational shifts that follow effector binding and modulate signal transmission to the other XylR domains. It thus seems that transcriptional factors regress into functionally multipotent forms (i.e., *stem protein* types) as a prerequisite for further definition of distinctively new functions.