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Src function in a unicellular relative of metazoans



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RESUMEN

Motivación: Tyrosine kinases play important roles in different cell signaling cascades in metazoans (1). The non-receptor Src family has been found to be involved in the regulation of cell-cell interaction, cell adhesion and migration (2). For this reason, its malfunctioning is implicated in diseases, such as cancer. Tyrosine kinases were thought to be specific to Metazoa, but recent comparative genomic analyses showed that they are also present in some of the closest unicellular relatives of metazoans (3). The role played by these kinases in these unicellular organisms remains unknown. The goal of this project is to decipher the function of Src in unicellular organisms.

Métodos: The *src* gene was predicted in the genome of *Creolimax fragrantissima*. The gene was cloned from cDNA by PCR. A dominant negative (DN) version of the gene was designed introducing an amino acid change in the two conserved Tyr that are important for the regulation of the protein, blocking the downstream cascade (4). Both the wild-type version and DN were cloned in a vector under the beta-tubulin promoter (b-tub) with constitutive expression, and fused to the mCherry fluorescent marker. Transformation was done by electroporation using the Neon® transfection system by Invitrogen (1 µg each vector). The experiment consisted on transforming *C. fragrantissima* with either the normal Src or the DN constructs plus b-tub::venus and b-tub::H2B-mCherry as controls for the transformation. Negative control had the two last constructs but none of the *src* constructs. The cells were examined using a Nikon fluorescence microscope, and time-lapse was performed when needed.

Resultados: We know that the constitutive expression of *src* affects cell-cell interaction in cell culture experiments. *C. fragrantissima* has a colonial stage during its cell cycle that “explodes” giving rise to free-living amoebas (see <http://www.youtube.com/watch?v=7Gvrg1I8jBA>). We assume that cell-cell interaction may have some relevance during this stage. For this reason we expected to see a resulting phenotype either during the colony formation or during its development. The preliminary results showed some different phenotypes although they are not significant enough. Experiments on transformation and time-lapse need to be repeated, in order to be sure that differences are found between the overexpressed and the DN *src* transformants, in the colonies or amoebas.

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