Cell response to extracellular DNA and self-DNA inhibition

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Abstract

The discovery of DNA beyond the confinement of cell boundaries, generally defined as extracellular DNA, poses questions on its alternative roles that may differ from its being the main repository of genetic information within a cell. DNA can be extruded from cells by active or passive release, remaining in the extracellular context, that can be either the body of multicellular organisms or the natural environment, as free molecule or complexed with inorganic and organic particles, and/or organized in extracellular structures.

The presence of extracellular DNA (exDNA) outside cells, either in multicellular organisms or in the environment, has been initially discussed in terms of horizontal gene transfer, nutrient, and then as protective and/or signalling molecule.

Recently, a novel and interesting research line revealed for the exDNA additional roles depending on its nature, environmental location and structural organization. Indeed, in 2015, Mazzoleni and his colleagues demonstrated for the first time that the exposure to fragmented self-exDNA (conspecific or similar/homologous DNA) differently from the exposure to nonself-exDNA (heterologous, DNA from phylogenetically unrelated species, or, more in general, distant in sequence similarity terms), inhibits root growth and seed germination in plants in a concentration dependent manner. Later, it has been also demonstrated that it induces intracellular calcium signalling and plasma membrane depolarization and also triggers well-known markers of the immunological response, e.g. H_2O_2 production and MAPK activation. Following the results on plants, the inhibitory effect of self-exDNA on other species was also demonstrated by Mazzoleni and his group and, on the basis of these findings, they suggested the generality of the observed phenomenon which opens new perspectives in the context of self-inhibition processes.

Nevertheless, the mechanism underlying the specific recognition of either self or nonself-exDNA and subsequent responses in plants as well as in other organisms is still poorly understood.

The presented project aims at investigating the effects of the exposure of organisms from model species to nonself- and self-exDNA. In particular, one of the main objectives, is to confirm the inhibitory role of self-exDNA when compared to the exposure to nonself-exDNA, as highlighted for the first time in plants and then also in other species by Mazzoleni and colleagues in 2015. In this regard, it will be explored the effect of nonself- and self-exDNA on both terrestrial and aquatic species including representatives of unicellular and multicellular organisms from animals and plants with the purpose of confirming the generality of the phenomenon observed by Mazzoleni et al. in 2015. In particular, as representative of multicellular autotrophs, the early response to either nonself- and self-exDNA will be adressed in the model plant *Arabidopsis thaliana* through the evaluation of gene expression changes and exDNA spatial distribution at root and cellular levels. Then, to confirm the differential responses also in aquatic environments,

it will be also evaluated the effect of nonself- and self-exDNA on cell density and on the morphology of two microalgae *Nannochloropsis gaditana* and *Chlamydomonas reinhardtii*, examples of unicellular authothophs, living in marine and freshwater, respectively. Futhermore, aiming at highlighting potential differences and similarities among the responses of cells from different phyla, the effects of nonself- and self-exDNA will be evaluated on the vitality, morphological and physiological features of human cells, considered as a simplified system representing non-photosynthetic species. Finally, to further characterize the phenomenon in multicellular organisms living in marine environment, the chordate *Ciona robusta*, a model system which is considered the closest living relatives of vertebrates, will be used to investigate the effects of exDNAs on the main stages of the cordate embryos development and, thus, to infer on its potential role on vertebrates developmental processes.

The findings of this project will contribute to shed light on the differential responses of organisms of different clades to either self- or nonself-exDNA. This will have relevant ecological implications and will support the unexpected new functional roles of exDNA in species interactions at community and ecosystem levels also deserving high potentiality for the development of biotechnological and industrial applications.