The VirA Receptor

The original receptor was taken from the soil bacterium Agrobacterium tumefaciens, which is known as a phytopathogen causing crown gall disease in dicotyledonous species. This process is mediated by the VirA receptor, after it bound to a phenolic substance secreted by wounded plants called acetosyringone. The binding domain of VirA, the so-called linker region, is located in the cytoplasm. When binding is established, the kinase domain of VirA becomes active and catalyzes the phosphorylation of the intracellular response regulator VirG. In its active state, the transcription factor VirG recognizes a specific short DNA-sequence called virR and enhances expression of the virulence genes.

According to the literature the core structure of an inducer molecule is a phenol with at least one methoxy group in ortho position. Moreover, the binding affinity to VirA seems to be empowered by a second methoxy group in ortho position. The original receptor was modified by a second methoxy group in ortho position and the presence of a chain with high potential capacity in para position.

The VirG P

Final Construct

Characterization

To characterize our bain bacteria were grown in shake flasks and samples were drawn every hour. Depending on the reporter gene the fluorescence of mRFP or the relative light units (RLU) of luciferase were measured and normalized by the actual OD600 value. All figures shown in the following are based on multiple cultivations to gain high confidence levels of the derived values.

Bacteria with BBa_K389016 (main construct leading in mRFP) were tested, by using multiple concentrations of acetosyringone, of food visible via a light signal. The modulated system is supposed to emit light of different intensities, depending on the spiciness of a tested sample. Besides capsaicin there are other potentially detectable compounds of interest, like dopamine, adrenaline or near derivatives.

In our MARSS (Modulated Acetosyringone Receptor Sensor System) project we introduced the VirA/G two-component receptor system originating from Agrobacterium tumefaciens (CSB to Escherichia coli). The receptor from A. tumefaciens is a voltage-sensitive receptor of plants which attracts these bacteria. Binding to the receptor, acetosyringone induces an intracellular signal transduction. The receptor, the response regulator and an inducible promoter were successfully cloned into E. coli, and the signaling cascade was coupled to different reporter genes to measure the induction profile.

In a further setup we tried to alter the binding region of the VirA receptor via directed evolution in order to enable the detection of another inducer, like capsaicin. As an exemplary substance we chose capsaicin, a molecule that is responsible for the spiciness in chili, pepper and hence in a lot of food. The idea is to make the spiciness of food visible via a light signal. The modulated system is supposed to emit light of different intensities, depending on the spiciness of a tested sample. Besides capsaicin there are other potentially detectable compounds of interest, like dopamine, adrenaline or near derivatives.

In the course of our project we established a heterologous two-component system in E. coli capable of quantifying acetosyringone. Thus we proved its suitability as a biosensor. The created construct serves as a starting point for engineering novel quantification devices targeting a range of small molecules.

Even though a new receptor for capsaicin could not be achieved to date, we succeeded in developing methods for the creation and identification of modulated receptors.

Potential Candidates

When trying to detect other substances than acetosyringone, it must be considered, which chemical groups account for the activation of VirA (cmp. fig. 2). Figure 3 shows a small selection of molecules that fit the known requirements. Capsaicin is responsible for the spiciness in pepper. Dopamine is indicating misuse of doping agents and related to psychic disorders (Parkinson’s disease, schizophrenia) as its degradation product homoavamic acid is. The latter is also important for the diagnosis of tumours (pheochromocytoma, neuroblastoma) in infants.

Receptor Modulation

Using error prone PCR a library of BglII-plasmids (pSB1ATK backbone) containing randomly mutated virA genes can be created. These plasmids are brought into a pET- E. coli strain (ECL03D) keeping a second plasmid with Rha-ori, encoding the response regulator VirG and a kanamycin resistance gene under the control of the virD promoter. Selection on kanamycin, ampicillin, chloramphenicol and potential ligands then preserves cells with receptors inducible by the ligands of interest. Finally, plasmids with modified virA can be selected by electroporating both isolated plasmids into a pIa strain (TOP10), in which the second plasmid with Rha-ori cannot be replicated. Only the plasmid including the desired variant of virA remains.

Science Communication

Synthetic biology and its tool genetics are often in a negative public focus. Therefore, altering the public opinion is hard to attain. Our team embraced the opportunity of the ENAK competition to bring synthetic biology to a public discussion. We do not appreciate science hiding behind closed doors, but bringing science to a broad range of society. Only a very open contact with the media can reduce prejudices against bacteria and genetic engineering. Theresults of our work can be discussed on public relations among others, two articles in local and national magazines, a radio series in three parts broadcasted by a big federal radio station and multiple appearance on German television. Moreover, we had a public discussion at “Science Café Bielefeld” with not less than 50 guests, in order to advance the peoples opinion towards synthetic biology.

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