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 recognition 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-fragment called virR-box and enhances transcription of the virulence genes.

Abstract

In our MARSS (Modulated Acetosyringone Receptor Sensing System) project we introduced the VirA/G two-component receptor system originating from Agrobacterium tumefaciens CS8 to E. coli. The receptor from A. tumefaciens recognizes acetosyringone, a secondary metabolite 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 other compounds than the native inducer acetosyringone. As an exemplary substance we chose capsaicin, a molecule that is responsible for the spiciness in chilli, 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.

When trying to detect other substances than acetosyringone, it must be considered, which chemical groups account for the activation of VirA (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 psychiatric disorders (parkinson disease, schizophrenia) as its degradation product homovanillic acid is. The latter is also important for the diagnosis of tumours (pheochromocytoma, neuroblastoma) in infants.

Potential Candidates

When trying to detect other substances than acetosyringone, it must be considered, which chemical groups account for the activation of VirA (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 psychiatric disorders (parkinson disease, schizophrenia) as its degradation product homovanillic 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 Bodriblock-plasmids (pSB1AT3 backbone) containing randomly mutagenized VirA genes can be created. These plasmids are brought into a pir - E. coli strain (EColiDD) keeping a second plasmid with RMA-ori, encoding the response regulator VirG, and a kanamycin resistance gene under the control of the virP promoter. Selection on kanamycin, ampicillin, chloramphenicol and potential ligands then preserves cells with receptors inducible by the ligands of interest. Finally, plasmids with modulated virA can be validated by electrophorising both isolated plasmids into a pir - strain (TOPO10), in which the second plasmid with RMA-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 IKEM 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.

Results of our strong emphasis on public relations are among others, two articles in local and national magazines, a radio series in three parts broadcasted by a big federal radio station and multiple appearances on German television. However, we had a public discussion at “Science Café Bielefeld” with not less than 100 guests, in order to advance the people's opinion towards synthetic biology.

Acknowledgment

We would like to say thank you to Prof. Dr. Alfred Pühler, Prof. Dr. Karsten Niehaus, Dr. Hannes Franke, Dr. Christian Rückert, Dr. Jörn Kalinowski, Dr. Hans-Peter Kayser, Prof. Dr. Jörgen Kuhlman, all members of the Center for Biotechnology (CeBiTec Bielefeld) for the excellent and helpful working atmosphere we enjoyed.

Moreover, we want to express our thanks to our sponsors