Lipid driven genetically engineered machine

Team Debrecen Hungary
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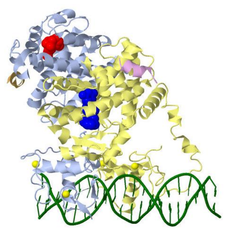
Background

Lipids constitute a broad group of naturally occurring molecules including fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, etc. The main biological functions of lipids include energy storage, structural components of cell membranes, and important signaling molecules. These lipids have specific regulatory effects on gene regulation mostly through nuclear receptors.

Sponsors

1. Nuclear receptors

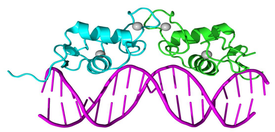
Nuclear hormone receptors belong to a well conserved family of proteins present in all metazoans. They may be viewed as *lipid activated transcription factors*. Each nuclear receptor has a conserved DNA-binding domain which contains two Zn-fingers. They can directly bind to the DNA and regulate gene expression.



PPARγ (yellow) and RXRα (grey) heterodimer on DR1 response element in complex with specific activators, Rosiglitazone (blue) for PPARγ and 9-cis retinoic acid (red) for RXRα (1.).

2. Zn-fingers

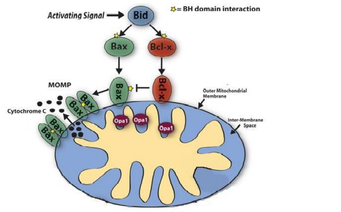
A great deal of progress in the development of modular protein domains that recognize specific triplets of DNA sequence has been made. The domains can be fused together to create proteins that can bind to a chosen DNA sequence. Combining DNA-binding domains with effector domains yields brand new transcriptional activators or repressors, enabling us to have transcriptional control of virtually any gene of interest.

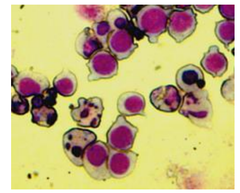


Crystallographic structure of the human progesterone receptor DNA-binding domain dimer (cyan and green) complexed with double strained DNA (magenta). Zinc atoms of are depicted as grey spheres.

3. Apoptosis

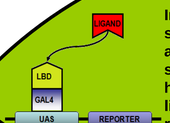
The process of programmed cell death (PCD), is an elaborate cellular homeostasis mechanism that ensures correct development and function of multicellular organisms. Biochemical events lead to characteristic changes (morphology) and the death of cells.
We would like to find a way how could we terminate our process, thats why we try to induce apoptosis of the so called genetically engineered cell.
In our system we used Bax pro-apoptotic protein to induce programmed cell death which is affecting the mitochondrial pathway leading to mitochondrial outer membrane permeabilization.



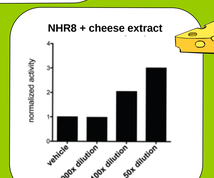


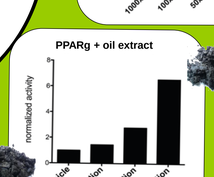
Upon receiving an activating signal, a direct activator BH3-only protein such as Bid will transiently interact with Bax and Bcl-xL, causing their translocation to the outer mitochondrial membrane. Once inserted into the outer mitochondrial membrane, Bax can form homo-oligomers through BH3 domain interactions, leading to pore formation and MOMP. Bcl-xL can bind to Bax via its BH3 domain and inhibit homo-oligomerization of Bax, thereby inhibiting MOMP and apoptosis.

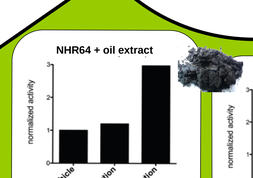
1. Receivers

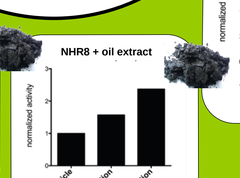


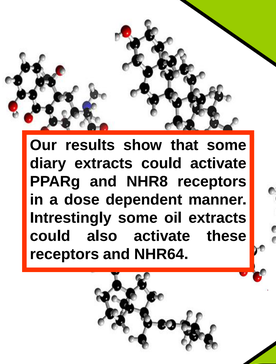
In order to find specialized lipid sensors, we carried out luciferase assays. These experiments are a special form of the mammalian two hybrid system. In our system, if the ligand can bind to the Gal4 fused receptor ligand binding domain and activate it, there will be a detectable increase in the luciferase activity.







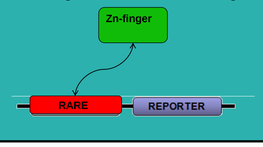




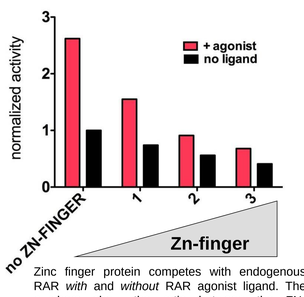
Our results show that some diary extracts could activate PPARg and NHR8 receptors in a dose dependent manner. Intrestingly some oil extracts could also activate these receptors and NHR64.

2. Zn-fingers

Ligand binding domains respond only to predefined lipids and selectively activate predetermined gene expression. The tool for regulating directly the gene expression of the DNA binding domains of nuclear hormone receptors are tiny zinc finger motifs. These elements bind directly to the DNA and can act as direct gene expression regulators so they can operate as specific effectors. We designed Zn-fingers to a well known RAR (Retinoic Acid Receptor) response elements. We performed another special type of luciferase assay which is very good to detect protein-DNA interactions. Intrestingly one of our fully synthetic biological designed Zn-finger could compete with the endogenous RAR for binding sites.

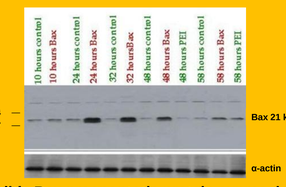


DNA binding capacity of the designed ZN-FINGER motif



Zinc finger protein competes with endogenous RAR *with* and *without* RAR agonist ligand. The numbers show the ratio between the ZN-FINGERS and the buffer plasmids:
1.ZN-FINGERS:buffer plasmids-1:8;
2.ZN-FINGERS:buffer plasmids-1:2;
3.Only ZN-FINGERS

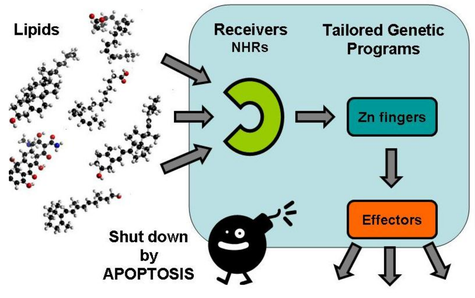
3. Apoptosis



Fully Synthetic Biology compatible Bax proapoptotic protein expressing vector (BBa_K626000) was assessed from pSB1A3. Detection of the protein level of Bax by Western blot. The level of Bax is the highest 24 hours after transfection.

The nuclear fragmentation was visible by Hoechst staining, which is a known marker of apoptosis. The arrows show apoptotic cells.

Lipid Driven Genetically Engineered Machines



Summary

We set up a complex system which is driven by lipids found in everyday life. We proved that several nuclear hormone receptors can be specifically activated by lipids from oil and diary products extracts (**1**). We also designed and tested Zn-fingers to regulate gene expression directly (**2**). Furthermore we found harmless way to switch off our machine by using the apoptosis (**3**).
We have supported 3 important mechanisms to build up a fully synthetic biological engineered cell, which is able to receive the specific signal and translocates it to the nucleus, where the tailored genetic programme could start. We also have the possibility to shutting down the program by apoptosis.