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6. AUTHOR(S) Michael R. Lerner, M.D., Ph.D.	
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) U.T. Southwestern Medical Center 5323 Harry Hines Blvd Dallas TX 75235-9069	8. PERFORMING ORGANIZATION REPORT NUMBER N/A
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13. ABSTRACT (Maximum 200 words)

The potential to create hand held or ever smaller biosensors capable of detecting essentially any chemicals of interest remains an exciting opportunity in general and for TNT detection in particular. The basis for developing biosensors uses the concept of rapid directed G-Protein coupled receptor evolution. Mutation is accomplished by the use of the polymerase chain reaction. Selection is accomplished by the use of transfected melanophores in combination with a digital video imaging system. We have successfully integrated the pigment cells with this apparatus and can now identify the presence of a signal arising from clones present at a frequency of well under 1:100,000 in a cDNA library based on plasmid vectors. as a starting point for the mutagenesis of GPCRs in order to obtain new desired specificities, a collection of cDNAs coding for receptors from many different branches was developed. At the present time the lab has accumulated on the order of 70 such cDNAs, all of which are set up in plasmid vectors capable of being expressed in melanophores.

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FINAL REPORT

Grant#: N000149611174

PRINCIPAL INVESTIGATOR: MICHAEL R. LERNER

INSTITUTION: UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER

EMAIL: mrlerner@aol.com

GRANT TITLE: Creation of a TNT Sensor by Directed Evolution of a G-Protein Coupled Receptor

AWARD PERIOD: 15 JUL 96 through 30 JUN 99

OBJECTIVE: To develop G-protein coupled receptor (Seven Transmembrane Domain or 7TM) based biosensors using the concept of directed evolution in vitro.

APPROACH: Development of a biosensor for trinitrotoluene (TNT) is being accomplished by taking the cDNA for the human b2-adrenergic receptor (B2AR) and making selected molecular substitutions to produce new but related receptor genes. These receptors will be expressed in melanophore cells which respond to receptor activation by producing a rapid easily-read color changes that can be detected by video imaging. Receptors will be evaluated by virtue of their response to TNT. The sequences of receptors that are activated by TNT will be compared in order to identify structural features that appear to confer increased receptor specificity. These features will be selectively retained during successive rounds of mutagenesis.

ACCOMPLISHMENTS:

I Establishment of a G-Protein Coupled Receptor (GPCR) base. It is estimated that the human genome codes for approximately 1,500 GPCRs and other animals have many additional ones as well. The receptors can be grouped into classes and subclasses based on their relatedness at both the protein and cDNA levels and these relationships can be visualized as an evolutionary tree where the various branches represent the different receptors. Between them, the GPCRs from different branches are capable of detecting many thousands of small molecules. Therefore, as a starting point for the mutagenesis of GPCRs in order to obtain new desired specificities, a collection of cDNAs coding for receptors from many different branches was developed. At the present time the lab has accumulated on the order of 70 such cDNAs, all of which are set up in plasmid vectors capable of being expressed in melanophores. This large collection will enable us to swap the seven transmembrane regions, three intracellular loops and cytoplasmic tail and the three extracellular loops and amino terminal beginning between the receptors to rapidly obtain large domain mutations.

II Freezing melanophores for long term storage. To work effectively with melanophores expressing novel GPCRs it has been necessary to possess a stable base population of cells for transfections that have little genetic variability. The standard approach to this problem with other

cell types is simply to suspend them in a mixture of fetal calf serum with DMSO and to store the cells indefinitely in liquid nitrogen. Initially, this was not possible with melanophores as established freezing procedures led to their large scale cell destruction. A major difference between melanophores and other cell types is that the pigment cells possess numerous melanosomes. These pigmented organelles are responsible for the dark color and we hypothesized that their internal melanin pigment, which is a heteropolymer, might be acting as foci for ice crystal formation and subsequent cell rupture during the freezing process. Accordingly, a strategy was devised for reversibly depigmenting melanophores via the use of a copper chelator. The enzyme tyrosinase within the cells is ultimately responsible for the production of melanin and this enzyme requires a copper cofactor in order to function. It turned out it was possible to identify a concentration of the chelator phenylthiourea that could shut down the tyrosinase without disrupting other copper containing macromolecular complexes within the cells such as the cytochrome system which are required for survival. The depigmented melanophores can be frozen and thawed at will with high recoveries of viable cells.

III Setting up the 4K x 4K high resolution digital video system. The 16 mega pixel video imaging system is critical to the process of directed GPCR evolution. The sophisticated, computer controlled camera and scanning stage enables information about GPCR activity to be acquired from millions of melanophores in a matter of minutes. We have successfully integrated the pigment cells with this apparatus and can now identify the presence of a signal arising from clones present at a frequency of well under 1:100,000 in a cDNA library based on plasmid vectors.

SIGNIFICANCE: Handheld biosensors, capable of detecting different chemicals could have a broad range of uses, such as in the detection of explosives.

CONCLUSIONS: We are in a position to develop biosensors with the capability of responding to chemicals of interest pending financial support for such an endeavor.

PATENT INFORMATION: None

AWARD INFORMATION: None

PUBLICATIONS AND ABSTRACTS: None