FINAL REPORT

GRANT #: N00014-98-C-0358

PRINCIPAL INVESTIGATOR: Dr. Alexey G. Zdanovsky

INSTITUTION: Promega Corporation

GRANT TITLE: A Rapid Sensitive Universal Detection System for Biological Agents of Mass-destruction

AWARD PERIOD: January 26, 1999 - June 2000

OBJECTIVE: To identify and isolate the specific regulatory elements that are activated in response to interaction of a eukaryotic cell with specific biologically active agents or groups of biologically active agents.

APPROACH: The human genomic library coupled with the sequence encoding the reporter proteins was introduced into mammalian cell; cells not producing the reporter protein would be isolated by the cell sorting technique. Then the isolated cells would be challenged with the biologically active agent of interest and sorted one more time according to their ability to produce reporter protein. Cells producing reporter protein would contain plasmids with cloned regulatory elements activated in response to interaction between the cell and the biologically active agent. By repeating the same procedure with different biologically active agents, we planned to create library of responsive elements. In this library, each element would be able to recognize only one biologically active agent but taken together these elements would be able to detect and identify a broad spectrum of biologically active agents.

ACCOMPLISHMENTS: We have developed a set of interlinked protocols that allowed efficient manipulation with plasmid DNAs in both mammalian and bacterial cells. Particularly, by systematic testing of multiple commercially available transfection reagents and adjusting conditions of transfection we have identified protocol that could be used for efficient introduction (80% of the population) of plasmid DNAs with the average size of 16 k.b. into Cos cells on a massive scale (10^9 cells per experiment). We have found that even after five days since transfection DNA introduced into Cos cells remained intact and could be isolated in the form that than could be introduced into *E. coli* for amplification. We have developed protocols for plasmid DNA purification from mammalian cells and subsequent electroporation into *E. coli* cells that have allowed us to recover and than amplify plasmid DNA practically from each single transfected Cos cell. Also, we have generated a set of derivatives of pseudomonas exotoxin A that could be used for localization of exotoxin A areas that are capable of inducing specific responsive elements of mammalian cells. These proteins as well as mutant form of adenovirus were originally planned to be used as model biological agents in the screening experiments. All these agents were produced in quantities sufficient for treatment of Cos cell population transfected with the whole human genome library. Also we have identified concentrations of adenovirus and recombinant proteins that

DTIC QUALITY INSPECTED 4

DISTRIBUTION STATEMENT A
Approved for Public Release
Distribution Unlimited

20001013 024

should be used for treatment of Cos cells during screening of the library.

We have constructed plasmid vector that is capable of replicating in *E. coli* cells and contains reporter cassette. The cassette is flanked on both sites by terminators of transcription and contains multiple cloning sites that are followed by the Internal Ribosome Entry Site from encephalomyocarditis virus and sequence encoding Green Fluorescent protein (GFP). This vector was used to construct human genomic library composed of 10^8 of independent clones. This library was introduced into Cos cells and transfected cells were used to isolate from the original library those human DNA containing plasmids that under normal conditions do not direct production of green fluorescent protein. We were not able to continue screening procedures further because of multiple malfunctions of the cell-sorter.

Finally, we have constructed hybrid gene that encode GFP fused at the N-terminus with ubiquitine. This protein was designed so that upon deubiquitination it would generate a new form of GFP with a very short half live time. As a result fluorescence of cells expressing such unstable protein would change rapidly upon changes in the level of protein synthesis. Thus cells expressing such hybrid GFP can be used as general detectors of toxic agents.

CONCLUSIONS: All developed protocols in combination with our model biologically active agents represent the set of tools required. These are sufficient for identification and isolation of specific regulatory elements that are activated in response to interaction of a eukaryotic cell with specific biologically active agents. Nevertheless, the inabilities of vendor of the flow cytometer to correct multiple defects associated project cell sorter have interfered with the progress of the project. Because of nearly a year's delay, the decision to was made to end the project.

SIGNIFICANCE: Our work have provided a set of interlinked techniques and reagents that can be used for the search of specific regulatory elements that are activated in response to interaction of a eukaryotic cell with specific biologically active agents.

PATENT INFORMATION: None

PUBLICATIONS AND ABSTRACTS: None

FINAL REPORT

GRANT #: N00014-98-C-0358

PRINCIPAL INVESTIGATOR: Dr. Alexey G. Zdanovsky

INSTITUTION: Promega Corporation

GRANT TITLE: A Rapid Sensitive Universal Detection System for Biological Agents of Mass-destruction

AWARD PERIOD: January 26, 1999 - June 2000

OBJECTIVE: To identify and isolate the specific regulatory elements that are activated in response to interaction of a eukaryotic cell with specific biologically active agents or groups of biologically active agents.

APPROACH: The human genomic library coupled with the sequence encoding the reporter proteins was introduced into mammalian cell; cells not producing the reporter protein would be isolated by the cell sorting technique. Then the isolated cells would be challenged with the biologically active agent of interest and sorted one more time according to their ability to produce reporter protein. Cells producing reporter protein would contain plasmids with cloned regulatory elements activated in response to interaction between the cell and the biologically active agent. By repeating the same procedure with different biologically active agents, we planned to create library of responsive elements. In this library, each element would be able to recognize only one biologically active agent but taken together these elements would be able to detect and identify a broad spectrum of biologically active agents.

ACCOMPLISHMENTS: We have developed a set of interlinked protocols that allowed efficient manipulation with plasmid DNAs in both mammalian and bacterial cells. Particularly, by systematic testing of multiple commercially available transfection reagents and adjusting conditions of transfection we have identified protocol that could be used for efficient introduction (80% of the population) of plasmid DNAs with the average size of 16 k.b. into Cos cells on a massive scale (10^9 cells per experiment). We have found that even after five days since transfection DNA introduced into Cos cells remained intact and could be isolated in the form that than could be introduced into *E. coli* for amplification. We have developed protocols for plasmid DNA purification from mammalian cells and subsequent electroporation into *E. coli* cells that have allowed us to recover and than amplify plasmid DNA practically from each single transfected Cos cell. Also, we have generated a set of derivatives of pseudomonas exotoxin A that could be used for localization of exotoxin A areas that are capable of inducing specific responsive elements of mammalian cells. These proteins as well as mutant form of adenovirus were originally planned to be used as model biological agents in the screening experiments. All these agents were produced in quantities sufficient for treatment of Cos cell population transfected with the whole human genome library. Also we have identified concentrations of adenovirus and recombinant proteins that

should be used for treatment of Cos cells during screening of the library.

We have constructed plasmid vector that is capable of replicating in *E. coli* cells and contains reporter cassette. The cassette is flanked on both sites by terminators of transcription and contains multiple cloning sites that are followed by the Internal Ribosome Entry Site from encephalomyocarditis virus and sequence encoding Green Fluorescent protein (GFP). This vector was used to construct human genomic library composed of 10^8 of independent clones. This library was introduced into Cos cells and transfected cells were used to isolate from the original library those human DNA containing plasmids that under normal conditions do not direct production of green fluorescent protein. We were not able to continue screening procedures further because of multiple malfunctions of the cell-sorter.

Finally, we have constructed hybrid gene that encode GFP fused at the N-terminus with ubiquitine. This protein was designed so that upon deubiquitination it would generate a new form of GFP with a very short half live time. As a result fluorescence of cells expressing such unstable protein would change rapidly upon changes in the level of protein synthesis. Thus cells expressing such hybrid GFP can be used as general detectors of toxic agents.

CONCLUSIONS: All developed protocols in combination with our model biologically active agents represent the set of tools required. These are sufficient for identification and isolation of specific regulatory elements that are activated in response to interaction of a eukaryotic cell with specific biologically active agents. Nevertheless, the inabilities of vendor of the flow cytometer to correct multiple defects associated project cell sorter have interfered with the progress of the project. Because of nearly a year's delay, the decision to was made to end the project.

SIGNIFICANCE: Our work have provided a set of interlinked techniques and reagents that can be used for the search of specific regulatory elements that are activated in response to interaction of a eukaryotic cell with specific biologically active agents.

PATENT INFORMATION: None

PUBLICATIONS AND ABSTRACTS: None