JAMES RENNIE BEQUEST

REPORT ON EXPEDITION/PROJECT/CONFERENCE

Expedition/Project/ Conference Title: **"Interactions between motor protein-dynein and HIV.**

Travel Dates: 2nd June 2010 – 1st September 2010

Location: Westmead Millenium Institute, Sydney, Australia

Group member(s): Piotr Janas

Aims: Verify theory claiming that HIV corrupts microtubules of host cell to gain access to nucleus.

OUTCOME (not less than 300 words):-

"Interactions between motor protein-dynein and HIV.

Three months summer undergraduate experience took place at Westmead Millenium Institute for Medical Research, University of Sydney. The goal of my project was to verify whether HIV corrupts dynein of host cells to move along microtubules. Project involved several different research techniques with direct focus on Yeast Two-Hybrid assay.

Dynein

Cellular transport along microtubule can be distinguish by the direction in which molecules are transported. Transport from nucleus towards cell membrane is called anterograde and depends from kinesin. Movement towards nucleus is called retrograde transport and requires dynein, this particular model was of our interest.

Dynein is a motor protein (also called molecular motor or motor molecule) in cells which converts the chemical energy contained in ATP into the mechanical energy of movement. Dynein transports various cellular cargo by "walking" along cytoskeletal microtubules towards the minus-end of the microtubule, which is usually oriented towards the cell center. Thus, they are called "minus-end directed motors," while kinesins, motor proteins that move toward the microtubules' plus end, are called plus-end directed motors.

HIV is believed to bind to dynein and by hijacking cellular transport molecules can move towards nucleaus and multiply. Two subunits of dynein have been investigated: RP3 and LC8. Both molecules belong to the light chain of dynein, responsible for binding to cargo molecules.

HIV

Human immunodepressant virus is a main cause of death worldwide. It is a retrovirus and uses reverse polymerase to transcript DNA to RNA. HIV infects primarily vital cells in the human immune system such as helper T cells (CD4+ T cells), macrophages, and dendritic cells. HIV infection leads to low levels of CD4+ T cells through three main mechanisms: First, direct viral killing of infected cells; second, increased rates of apoptosis in infected cells; and third, killing of infected CD4+ T cells by CD8 cytotoxic lymphocytes that recognize infected cells. When CD4+ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections. Most untreated people infected with HIV-1 eventually develop AIDS. These individuals mostly die from opportunistic infections or malignancies associated with the progressive failure of the immune system. HIV progresses to AIDS at a variable rate affected by viral, host, and environmental factors; most will progress to AIDS within 10 years of HIV infection: some will have progressed much sooner, and some will take much longer.

Four proteins of HIV have been investigated using Y2H assay: capsid (CA), integrase (IN), matrix (MA) and Vpr. All four of them are encoded within Gag gene and synthesized at the same time as a polyprotein. Due to the action of enzymes those inserts are cut and separated, therefore able to perform separate distinguish functions.

Yeast two-hybrid (Y2H) assay

Two-hybrid screening (also known as yeast two-hybrid system or Y2H) is a molecular biology technique used to discover protein-protein interactions and protein-DNA interactions by testing for physical interactions (such as binding) between two proteins or a single protein and a DNA molecule, respectively. The premise behind the test is the activation of downstream reporter gene(s) by the binding of a transcription factor onto an upstream activating sequence (UAS). For two-hybrid screening, the transcription factor is split into two separate fragments, called the binding domain (BD) and activating domain (AD).

System GAL4 (called Gold) has been provided by Clonetech and involves application of two different strains of S. cerevisiae. Strain Y187 acts as a bait, while Gold strain acts as a target. Two different vectors are used to carry cloned inserts. Vector pGADT7 is incorporated into Gold yeast, while pGBKT7 into Y187 yeast. First vector contains binding domain (BD), while latter activation domain (AD). If the bait and prey proteins interact (i.e. bind), then the AD and BD of the transcription factor are indirectly connected, bringing the AD in proximity to the transcription start site and transcription of reporter gene(s) can occur. If the two proteins do not interact, there is no transcription of the reporter gene. In this way, a successful interaction between the fused proteins is linked to a change in the cell phenotype (blue colonies can be observed).

Results

To optimize outcome of the experiment all inserts (DNA sequences of protein of interest) were cloned into both binding (BD) and activation (AD) domains. Subunit RP3 of dynein indicates very strong autoactivation in this system, therefore its interactions cannot be confirmed. There were no observed interactions between investigated subunits of dynein and HIV proteins in GAL4 yeast two-hybrid assay. Interestingly integrase (IN) of HIV has showed strong interaction with another molecule of integrase. This phenomenon has not been previously observed.

Undergraduate Summer Placement at WMI has given me new insight into fascinating world of research. My handling skills have improved drastically over this period. I had a great time at the Westmead Millenium Institute which helped me explore culture of Australia and people of Sydney. This project assured my intentions of pursuing career in Science. Many thanks to James Rennie Bequest, Prof. Russell Diefenbach and all members of HSV lab.

Acknowledgment
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