Adenovirus Terminal Protein Localization

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Introduction

The Life Sciences Summer Institute (LSSI) connects high school students to San Diego's Life Sciences Industry since 2005. Students complete a one-week pre-internship "boot cam p" training followed by 7-weeks of paid research work experience.

Christina is a rising senior at Harvard Westlake School, Class of 2014. The LSSI boot camp gave her a great introduction to laboratory science. During her internship she performed SDS-PAGE, Western blot, immunofluorescence, and general mammalian tissue culture techniques.

- Adenovirus is a linear double strand DNA virus that replicates its DNA in the nucleus of human cells.
- The components of the cellular DNA damage response pathway can detect, bind to, and block viral DNA replication.
- Adenovirus proteins target and neutralize DNA damage response proteins to facilitate its replication.
- Adenovirus Terminal Protein (TP) is covalently attached to the ends of adenovirus genomes.
- We hypothesize that adenovirus TP localizes adenovirus DNA to distinct biochemical compartments within the nucleus that are advantageous to adenovirus gene expression and replication.
- These compartments may allow adenovirus DNA escape detection by the DNA damage response proteins.

Approach

The purpose of this project is to determine if TP localizes to a distinct biochemical fraction within the nucleus of human cells.

Approach:

1. Overexpress TP or other viral proteins in human cells
2. Perform *in vitro* fractionation of these cells
3. Quantify the association of these viral proteins to each distinct biochemical fraction.

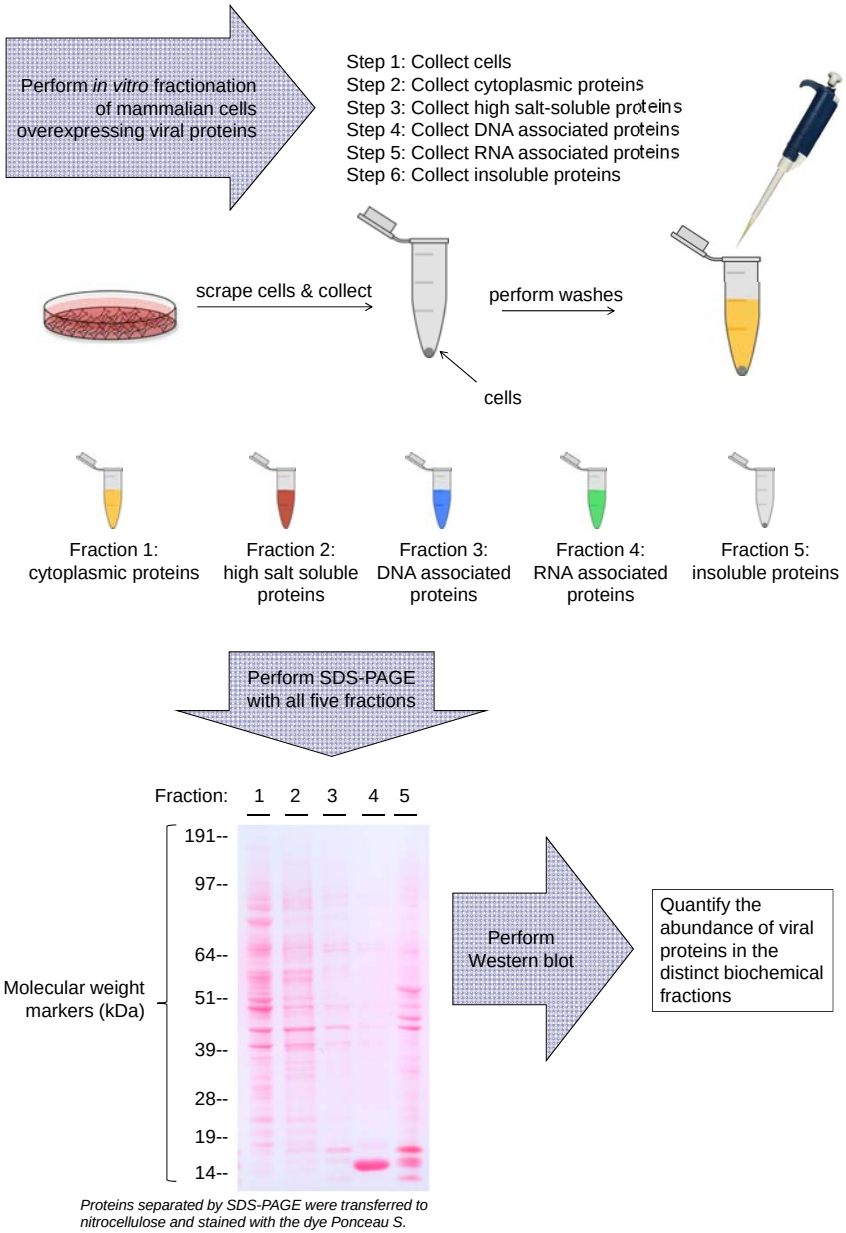
Techniques:

in vitro fractionation = separate proteins based on biochemical properties

SDS-PAGE = separate proteins based on size

Western blot = detect and quantify proteins using anitbodies

Methods and Results



Proteins separated by SDS-PAGE were transferred to nitrocellulose and stained with the dye Ponceau S.

Conclusion

- Ponceau S staining detects all of the proteins collected in each fraction. This technique shows us that distinct proteins are being extracted into each fraction and shows the quality of our technique.
- A Western blot would demonstrate how much TP or other proteins-of-interest are being extracted in each fractionation step.
- The localization of TP to a distinct biochemical compartment suggests that virus DNA is directed to distinct region of the nucleus that is advantageous to viral replication.
- Our initial interest is to study the association of DNA damage response proteins to viral DNA. Thus, probing for DNA damage response proteins by Western blot from these fractions would demonstrate if TP localizes to the same biochemical compartment as these cellular proteins.
- We can also use this technique to determine if other cellular proteins that are advantageous to viral gene expression or replication localize to these biochemical compartments.
- In future experiments, we can overexpress TP or other viral proteins that localize to the biochemical compartment that is most advantageous to viral replication and block the putative binding sites for adenovirus DNA within the nucleus.

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