TEXAS WOMAN'S UNIVERSITY

DENTON • DALLAS • HOUSTON www.twu.edu

The importance of M140 and M141 protein complex in mouse Cytomegalovirus

Carla Madrid, Lisa Bolin, Laura Hanson

Biology

Abstract

Cytomegalovirus can be harmless to most but harmful to those with comprised immune systems. Between 50 to 80 percent of adults in the United States are infected by the age of 40. Once CMV is in a person's body, it stays for life. When studying the virus we work with mice cells. Since the human virus only infects people, a model of the closely related mouse virus is used. There are two viral genes which affect the severity of infection in the host, which are Protein M140 and Protein M141. In order for pM141 to avoid degradation, it must be bonded to pM140. Previous work has identified a 74 amino acid region of pM140, if it gets knocked out, it will cause the complex to bind but not protect. We are working to identify what part of this region is required for pM140 to stabilize pM141, by making smaller deletions.

Objective

The goal of this study is to identify the specific parts of m140, within the 74 amino acid region, required to protect binding partner m141 from degradation.

pM140 Sequence

MDSTLWESLPVTDDPAPYRHATRSSFLRVMLAFRDFYR
HQSNRGELASLVERRAGERLPLGIPHNWFVELTPASRYE
ELKGVDINGIVCCDEPLTVVGRIVIRNNDGYEDAGAALC
LGAWTRVFVYEIQEDAMILVAPDLDKLARFGLLHCETLY
RRPHLPQVTTTPHRLVVGLIMCQDDLDRVSDYCQENS
GRDVALYTPGFKYQPMKLLGGVRDAARYWPLDIMNPS
NLKACLDEITGRLCCFWHAFAAVGAYAPAGVFSIHHLLVI
DTFGAIYTLDMQREKFYRVADGITMLLRAGMAKAIA*F*
GARFDRPARGEGRCEMRVICPHLPDHRKSERSEVDYAN
EHEWLCRRDRFRPDMRTWDDADKLAINHAVRAMKR
***K*AGEMYTNEEEDWTDTNEWEQEDDDNRGFDGHAM**
DITGPDDGSRSCTWWPESVLTTRPDRNRDTRTLLRYM
SEKKVTFQEVATRRLLERQESHYSLSFPLRLCRP

Figure 1: Diagram of the m140 protein sequence. The 74 amino acid region required for protection of the binding partner, m141, is indicated in red. The underlining indicates where the *BmgB*I site is. Insertion of a linker at this site will result in a truncated protein.

Procedure

- Find *BmgB*I site in the protein sequence, by performing an Enzyme restriction digest.
- Running Agarose Gel and extract DNA Sequence from gel
- Add Linker to plasmid, to remove *BmgB*I site
- Ensure Linker is taken up in plasmid, via culture growth on LB Broth and LB Agarose plates containing Kanamycin Antibiotic.
- Repeat Enzyme restriction digest with same Enzyme *BmgB*I to ensure the BmgBI site no longer exists
- The linker contains a *Pac*I enzyme site. Insertion of linker will be confirmed by digestion with *PacI*.

Results

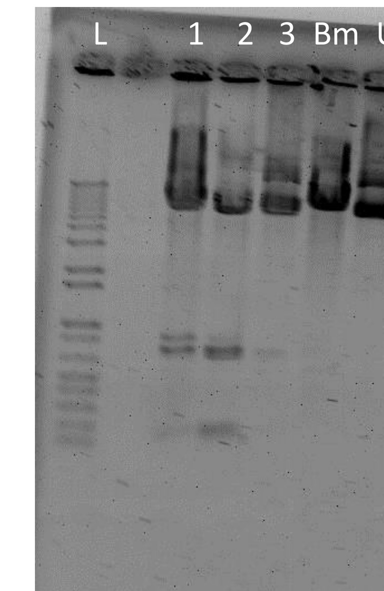

Figure 2: Testing of *BmgB*I enzyme. Three different aliquots of the *BmgBI* enzyme were tested for activity. Since the m140 plasmid is expected to have only 1 *BmgBI* site, we used a different plasmid, having 3 *BmgBI* sites, to test the enzyme. L= DNA size ladder. 1, 2, and 3 are the *BmgBI* enzymes, Bm is *BamHI*, which cuts once and U is uncut DNA.

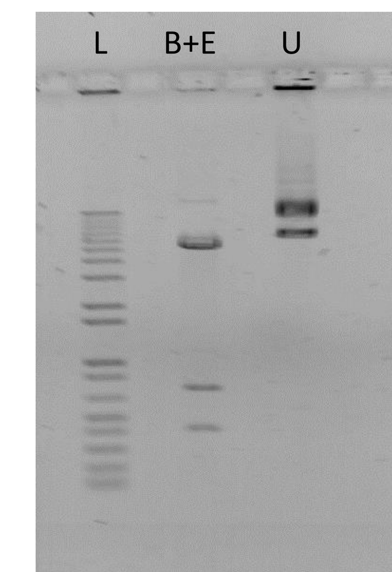

Figure 3. Confirmation of the single *BmgBI* site in m140. The m140 expression plasmid was digested with indcated enzymes, run on a 0.8% agarose gel and examined. L= DNA size ladder, B +E= double digested with *BmgBI* and *EcoRV*. U= uncut plasmid.

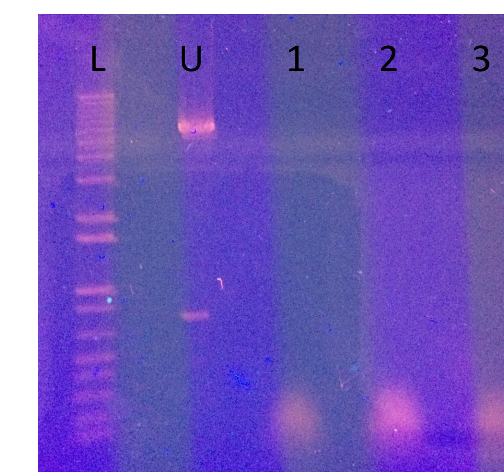

Figure 4. Testing of BmgBI linker mutants. DNA was extracted from colonies after linker insertion at the *BmgBI* site and digested with *BmgBI* + *EcoRV*. Only RNA was detected in these samples, Column 1, 2 and 3.

Conclusion

The single *BmgBI* site was confirmed in m140.

Double digestion with *EcoR*V resulted in the expected size pieces, confirming the location.

More colonies were seen on the plate which was transformed with the vector plus linker- supporting the successful insertion of linker

Initial testing of the plasmid did not give the expected results. Testing supported a problem with one of the reagents.

Further testing of the colonies is expected to confirm production of the m140 mutant which will be tested for ability to stabilize the m141 protein.

Acknowledgements

This research was supported by the Biology department at TWU.