Microbial Cell Factories

Poster Presentation

Open Access

RNA Interference mediated knockdown of genes in order to increase protein production using the baculovirus expression system

Colin Hebert*[1], Eun Jeong Kim[1], Shannon F Kramer[2], James J Valdes[3] and William E Bentley[1,4]

Address: [1]Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, MD 20742, USA, [2]Department of Biomedical Sciences, Baylor College of Dentistry, Texas A&M University System Health Science Center, 3302 Gaston Ave., Dallas, TX 75246, USA, [3]U. S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, Maryland 21010, USA and [4]Department of Chemical and Biomolecular Engineering, University of Maryland College Park, College Park, MD 20742, USA

* Corresponding author

from The 4th Recombinant Protein Production Meeting: a comparative view on host physiology
Barcelona, Spain. 21–23 September 2006

Published: 10 October 2006

Microbial Cell Factories 2006, **5**(Suppl 1):P12 doi:10.1186/1475-2859-5-S1-P12

Background

The baculovirus expression system has proven to be a robust and versatile system for recombinant protein production in insect cells. A wide range of promoters is available for the facile expression of transgenes, and yields of up to 50% of total protein have been reported [1]. However, in many cases yield is decreased as a result of proteases and host cell apoptosis [2]. Past efforts to overcome this problem include co-expressing chaperone proteins to assist with folding [3], anti-apoptotic proteins to reduce

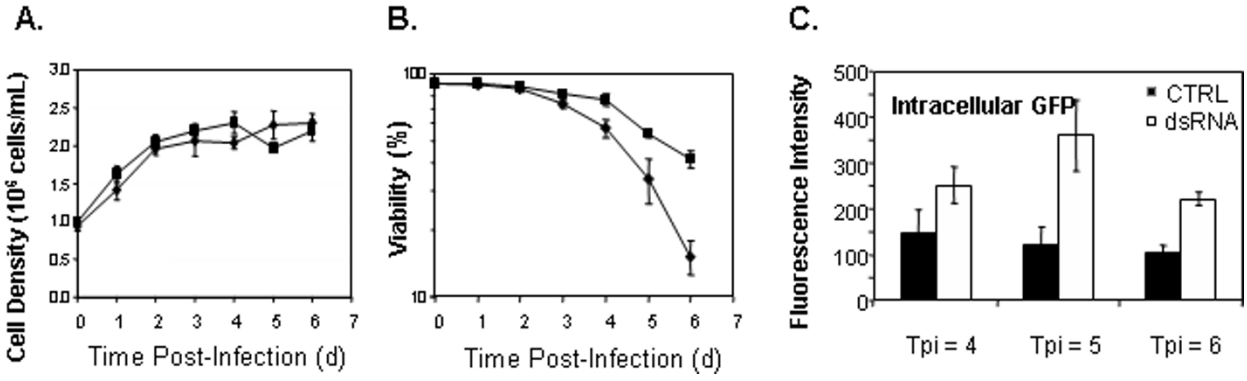

Figure 1
Effect of silencing of *v-cath* on the specific growth rate (A), the viability (B), and GFP production (C) in baculovirus infected Sf-9 cells. Cell counts were measured using a hemocytometer and viable cells were detected using trypan blue. Cell viability was defined by the ratio of the viable cell number to the total cell number. (◆) cells treated without dsRNA; (■) cells treated with dsRNA. GPF was measured quantitatively (C) using a fluorescence spectrometer. (black bar) cells without dsRNA; (white bar) cells treated with dsRNA.

cell death, or adding chemical protease inhibitors to the culture media [4]. However, these methods may have non-specific effects, prove too costly to be practical, or impose an undue metabolic burden on an already stressed cell. An alternative approach to increasing protein production is through the application of RNA interference (RNAi) to knockdown viral and host genes responsible for decreasing the yield of recombinant protein. Potential targets include proteases, cell-death proteins, and cell cycle regulators. By altering the metabolic landscape of cells prior to the introduction of the baculovirus, protein production can be improved.

Results

Initially, double stranded RNA (dsRNA) was produced *in vitro* against 19 gene targets; including acidic juvenile hormone-sensitive protein (AJHSP1) and Elongation factor 2 in *Trichoplusia ni (T. ni)* larvae, and a virus-encoded cathepsin-like protease, *v-cath*, in *Spodoptera frugiperda* (Sf-9) cell culture. The knockdown of these three genes increased the yield of Green Fluorescent Protein (GFP) produced via recombinant baculovirus. In the case of *v-cath*, the increase in infected cell culture was ~3-fold (Figure 1). Coincident with GFP yield, *v-cath* dsRNA also prolonged cell viability by over a day in infected Sf-9 cells (Figure 1). Ongoing studies are focused on identifying new host cell gene targets in both Sf-9 and *T. ni* cell culture, investigating combinations of knockdown targets, and integrating the most promising targets into expression vectors for the production of *in vivo* RNAi.

Conclusion

Overall, our results support the application of RNAi as a metabolic engineering tool, specifically for enhancing protein productivity in the baculovirus expression system.

References

1. Miller LK: **The baculoviruses.** *New York: Plenum Press*; 1997.
2. Kost TA, Condreay JP, Jarvis DL: **Baculovirus as versatile vectors for protein expression in insect and mammalian cells.** *Nat Biotechnol* 2005, **23:**567-575.
3. Ailor E, Betenbaugh MJ: **Modifying secretion and post-translational processing in insect cells.** *Curr Opin Biotechnol* 1999, **10:**142-145.
4. Kato T, Murata T, Usui T, Park EY: **Improvement of GFPuv-beta 3GnT2 fusion protein production by suppressing protease in baculovirus expression system.** *Biosci Biotechnol Biochem* 2003, **67:**2388-2395.