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Transcriptional Control for Protein Engineering and Regulation of DNA Replication by miRNA

Our laboratory mainly focuses on the gene regulation studies in insect and mammalian systems and also applies these basic research results to various useful applications. One of the major molecular biological tools used in our laboratory is baculovirus. Baculovirus is a rod-shaped insect-specific virus, which contains a dsDNA genome of around 80-180 kb. It is a non-human infectious virus and thus safe and easy for manipulation. This virus has long been used for the expression of engineered proteins in insect cells for medical studies and biotechnological applications. It can also be used as a safe and efficient tool for gene delivery in mammalian cells and organisms. In our laboratory, we are not only manipulating baculovirus for the expression of engineered proteins, but also applying it as a versatile tool for molecular biological studies. We have found that a baculovirus gene product, IE2, which can recruit G-actin and RNA polymerase II to form a novel micro-machine, visible under light microscopy, for the high level transcription of target genes in mammalian cells. We have also found that cellular miRNAs can target and regulate DNA replication. This is an important and novel discovery with profound significant to future molecular biological studies of cellular functions. Our research work and various findings are described as follows:

I. The enhancement of foreign gene expression in mammalian cells using baculovirus vector (Fig. 1).

We have identified baculovirus IE2 as a strong activator to stimulate CMV promoter expression in mammalian cells. This finding is not only for further assisting baculovirus as an effective gene delivery tool in mammalian cells, but also useful for developing baculovirus as a new tool for humanized protein production.

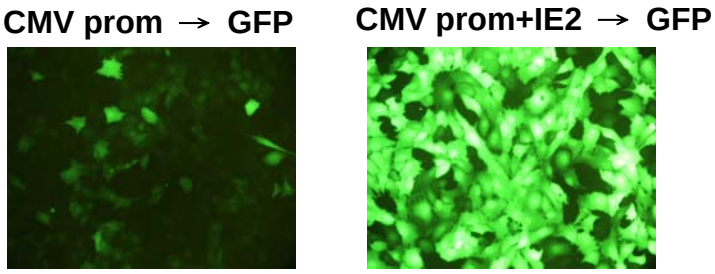

Fig. 1. IE2 strongly activates CMV promoter in mammalian cells for foreign protein expression.

II. A novel, efficient micro-machine in the cell nucleus for high level mRNA expression (Fig. 2).

The mechanism by which IE2 activates CMV promoter expression in mammalian cells was further studied. We found that IE2 can form a unique cage-like structure, which encloses G-actin and further recruits activated RNA polymerase II. This structure, we name it as transcriptsome, which can serve as a very strong micro-machine to generate large amounts of mRNA for gene expression. Since the NC is likely to be the first machinery for gene expression visible at the light microscopic level, the mechanism by which IE2 and G-actin work in concert with RNA polymerase II could be a notable emerging subject in the molecular biological studies.

A.

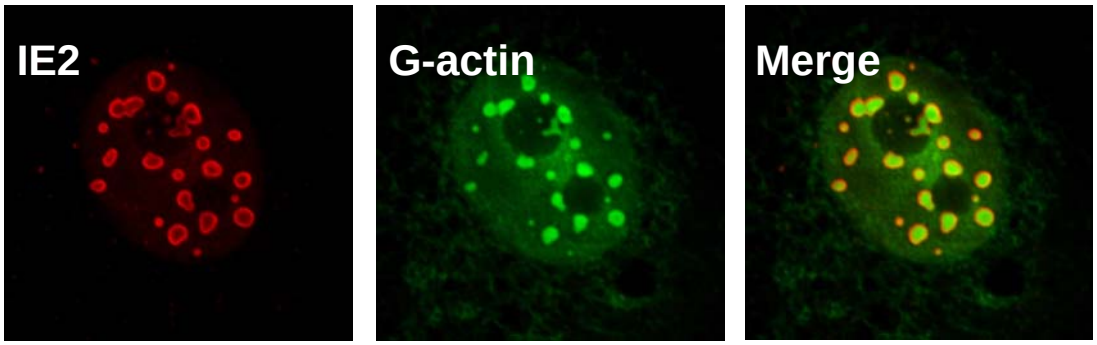

Fig. 2A. In the nucleus of a single cell, IE2 forms numerous transcriptsomes (red hallow balls) which strongly recruit G-actin (green) to the center of the individual cage-like transcriptsomes.

B.

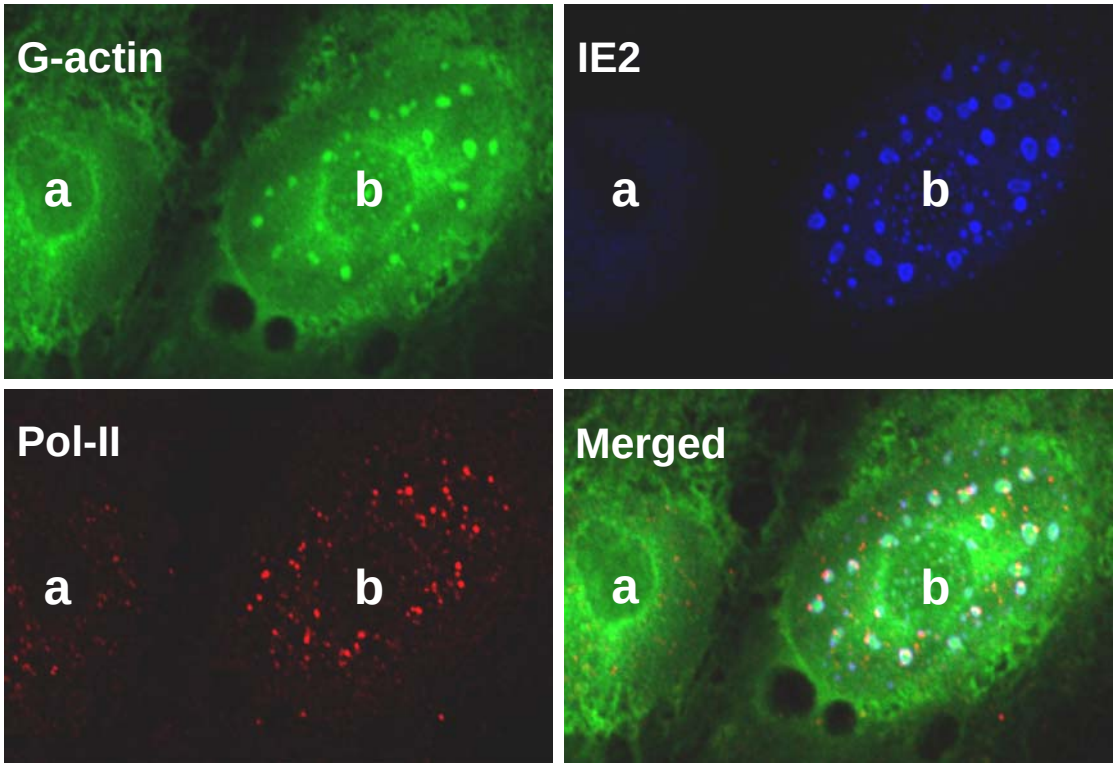

Fig. 2B. The same two cells in four sub-pennals showing interactions of IE2, G-actin, and activated RNA polymerase II. The expression of IE2 (blue) induces the gathering of activated RNA polymerase II (red), which associates with IE2 transcriptsomes as enlarged red granules (compare **a** and **b** cells) to make NC as micro-machines (around 0.5 μ) visible under light microscopy for strong mRNA transcription.

C. **D.**

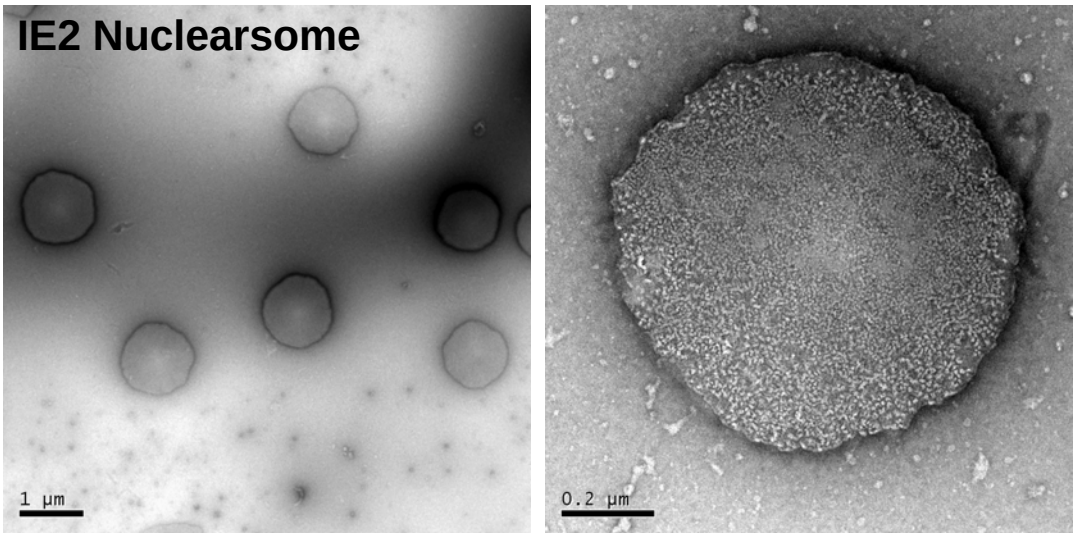

Fig. 2C. Purified transcriptsomes.

Fig. 2D. Blow up of a transcriptsome. This is a newly discovered powerful nature micro-machine for strong mRNA transcription.

III. Regulation of DNA replication by miRNA.

MicroRNAs are a class of small RNAs so far known to function as post-transcriptional regulators in cellular processes such as mRNA degradation, translational repression, and deadenylation. Recently, we have identified a group of miRNAs that can recognize and bind directly to the replication origin of DNA and repress DNA replication, thus uncovering a new function for miRNA. Since miRNA is very likely to regulate DNA replications in many viral and cellular DNA replication origins, this novel discovery is very important for the future study of DNA replication as controlled or mediated by miRNA. Now, the important question is to identify what is the mechanism mediate miRNA to recognize the origin of DNA replication, what are proteins involve in this novel mechanism. The results will be very fruitful, and extend to many replication origins of cells and viruses in the future.

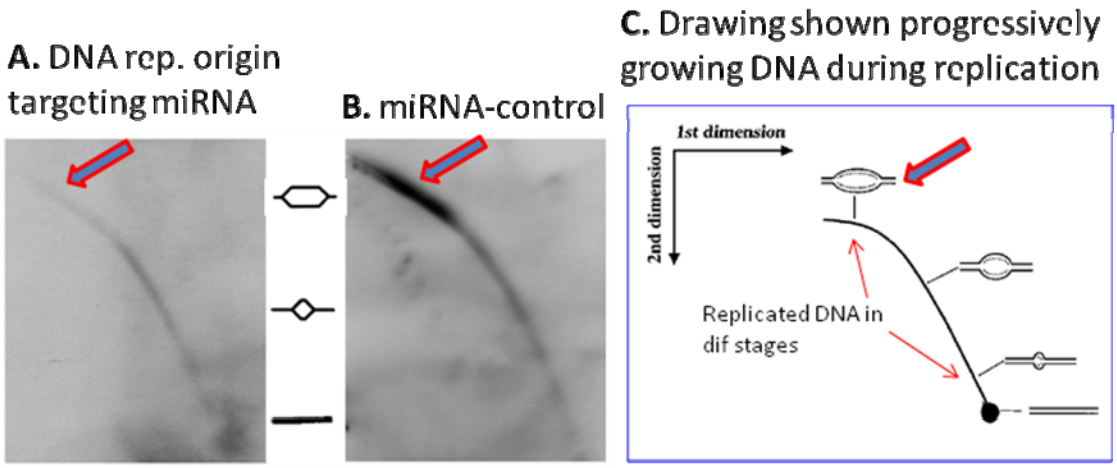

Fig. 3. 2-D DNA gel electrophoresis shows that a group of cellular miRNAs can control the replication of plasmid DNA. (A) and (B): Arrows showed that cellular miRNA can suppress DNA replication. (C) Cartoon shows the migration of DNA in the gel and their correlation with different stages of DNA replication.

IV. Protein aggregation in the nucleus for easy isolation of engineered proteins.

Harvesting engineered proteins from diluted media or cells requires considerable effort. We have developed measures to concentrate the proteins as granules in the nucleus or cytosol. These particles can then be harvested easily by simple lysis of the cells followed by mild centrifugation. These proteins, although aggregated as granules, obviously maintain their proper structure well, as the green fluorescent and red fluorescent proteins still emit strong fluorescence (Fig. 4). This is a novel and cost effective technology useful to harvest engineered proteins for biotechnology industries.

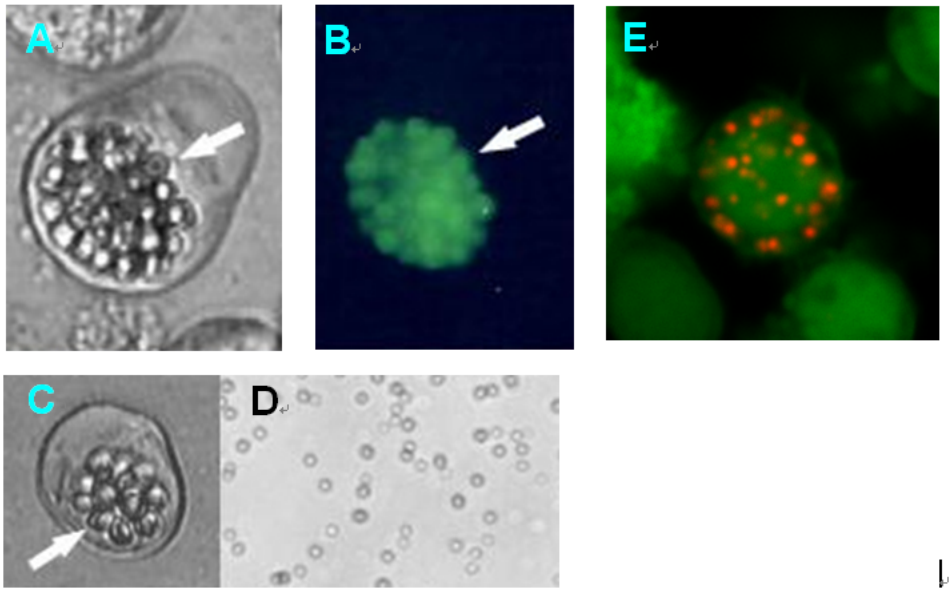

Fig. 4. Protein aggregation in the nucleus or cytosol for easy purification.

Selected recent publications:

1. Chao, Y. C*., S. L. Chen, and C. F. Li. 1996. Pest control by fluorescence. Nature 380, 396-397.

2. Liu, C. Y. Y., C. H. Wang, W. K. Hsiao, H. R. Lo, C. P. Wu, and Y. C. Chao*. 2009. RING and coiled-coil domains of baculovirus IE2 are critical in strong activation of the cytomegalovirus major immediate early promoter in mammalian cells. Journal of Virology 83:3604-16.

3. Wu, Y. L., C. P. Wu, H. Tang, C. H. Chang, H. H. Chen, S. T. Lee and Y. C. Chao*. 2010. The early gene *hhi1* reactivates latent HzNV-1 virus in the latently infected cells. Journal of Virology 84: 1057-1065.

4. Wu, C. P., Y. R. Huang, J. Y. Wang, Y. L. Wu, H. R. Lo, and Y. C. Chao*. 2010. AcMNPV LEF-2 is a capsid protein required for amplification but not initiation of viral DNA replication. Journal of Virology 84: 5015-5024.

5. Wu, Y. L., and Y. C. Chao*. 2011. HzNV-1 Viral gene *hhi1* induces apoptosis which is blocked by Hz-*iap2* and a non-coding gene *pag1*. Journal of Virology 85:6856-66.

6. Wu, Y. L., C. P. Wu, C. Y. Y. Liu, W. C. Hsu, E. C. Wu, and Y. C. Chao*. 2011. A non-coding RNA of HzNV-1 virus establishes latent viral infection through microRNA. Scientific Reports 1: article 60.