Antennapedia-Green Fluorescence Fusion Protein Uptake by Human Umbilical Vein Endothelial Cells

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Background and Objectives:
Antennapedia (AN) is a small peptide that can chaperon macromolecules across cell membranes through a mechanism that is poorly understood. We investigated its ability to chaperon the Green Fluorescence Protein (GFP) into Human Umbilical Vein Endothelial Cells (HUVECs).

Methods:
AN DNA was fused to the 3' end of Glutathione-S-Transferase (GST) in the pGEX 6p1 vector. The gene encoding GFP was ligated to AN to produce a GST-AN-GFP fusion gene. The fusion protein was expressed in the BL-21 strain of *E. coli* and affinity purified on a glutathione sepharose column. AN-GFP was cleaved while the fusion protein was bound, and was eluted from the column.

Results:
The predicted size of the protein, approximately 32.8 KDa, was detected on immumnblots of the purified lysate. Fluorescence microscopy of HUVECs incubated for one hour with 1mM fusion protein revealed that AN-GFP was taken up by the cells.

Discussion and Conclusions:
The fusion protein is nearly twice the molecular weight of proteins that have previously been chaperoned into HUVECs by AN. Our work extends the repertoire of cells that have been able to internalize AN-GFP and demonstrates that AN can chaperon proteins of at least 30 KDa into HUVECs.

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