

innovations

from The University of Vermont

TITLE: DNA DELIVERY SYSTEM: RIP60 NUCLEIC ACID COMPOSITION AND USES

INVENTOR: Nicholas Heintz

DESCRIPTION: Using a one hybrid screen in yeast, we cloned a protein called RIP60 that binds to the dhfr origin of replication. The protein is 62 kd in length and contains three clusters of zinc fingers (ZFs) that we call hands Z1, Z2, and Z3. The central Z2 cluster contains ZFs 6-8; it is located adjacent to a proline-rich region that we believe is involved in protein multimerization. Using ligation enhancement assays and atomic force microscopy (AFM), we showed that Z2 will support DNA looping between two RIP60 binding site, an activity that we had demonstrated previously for the full length protein.

We then went on and used AFM to examine binding of Z2 to linear, supercoiled, and relaxed circular DNA. Z2 has relaxed binding specificity as compared to the full length protein, and it bound all these substrates avidly. We then looked at the ability of Z2 to bind to large bacterial artificial chromosomes (BACs). Z2 binds at multiple dispersed sites, and then condenses the BAC DNA into a structure with a protein core and multiple DNA loops (the structure looks like a flower with 6-8 petals). Using cationic lipids, we can then condense the loops onto the protein core, resulting in a spherical particle we think of as an inside-out virus. Remarkably, cells ingest these molecules with very high efficiency. It is trivial for us to introduce a 150 kb BAC into cells in culture at 25% efficiency. There is no need for specific sequences in the DNA, and it can be in a linear, relaxed, or supercoiled state.

The presence of the 26 kd OST purification tag on Z2 does not inhibit DNA condensation or uptake, suggesting that we can engineer fusion proteins with amino terminal cell recognition domains (e.g. toxins, growth factors, etc) that would allow targeting to specific cell types. Moreover, the true value of this technique is that you can put an entire gene into cells, including boundary elements, promoter and enhancers, exons and introns, and 3' flanking sequences. Introduction of the entire locus obviates all regulatory considerations - all of the regulatory signals within and without the gene are present in their native form. Coupled with the work of Hunt Willard, which shows large episomes are stable in the absence of selection when they contain centromeric repetitive DNA, we expect that we could use this method for gene therapy. No viruses, no integration, no heterologous promoters or other regulatory elements - just the native gene in an episomal state.

ADVANTAGE OF THE TECHNOLOGY: We therefore think the Z2 core provides a highly flexible platform for the delivery of large DNA molecules into cells. In addition, work by many labs shows that the DNA itself can be decorated with peptides or other conjugates, again for cell targeting, increasing stability, etc. The method is simple beyond belief - mix soluble protein with DNA, incubate 15 min at room temperature, and then feed to cells. The protein is not toxic and the cells are 100% viable.

PROPRIETARY POSITION: Two patents have been applied for (use and composition)