TECHNOLOGY

Novel strategies for engineering foreign protein-encoding cassettes into bacterial chromosomal DNA

OVERVIEW

Loss of protein expression is a major problem in protein therapy and live vector vaccination, wherein loss of therapeutic dosage often results in treatment failure. Plasmid DNA, when used as a pharmaceutical agent, allows for real-time protein expression, but is easily lost during bacterial multiplication. In order to achieve sufficiently immunogenic levels of antigen over a period of time, the inventors have developed a strategy to insert multiple copies of foreign antigen expressing gene(s) cassettes at multiple loci within the bacterial chromosome. The modified chromosomal DNA, when packaged in an attenuated strain of bacteria, will work as a live vector vaccine or could be applied to produce recombinant proteins and peptides with anti-microbial, cancer or parasitic and a host of other therapeutic actions. More importantly, this technology allows for simultaneous delivery of multiple antigens within one live vector, thus truly bringing the concept of "multivalent" live vector vaccines to the forefront of disease control. A synthetic codon-optimized GFP-encoding gene cassette was inserted into multiple chromosomal loci of choice in an attenuated Salmonella enterica serovar Typhi (S. Typhi) vaccine candidate. Depending on the site of integration, gene expression can be regulated by osmolarity, S typhi growth-rate (guaBA locus), environmental stress (htrA locus) or for transport out of the cell (clyA locus). Using this elegant strategy, the inventors have made a few important findings: a) antigen cassettes integrated into multiple chromosomal loci do not cause further attenuation of the vaccine strain b) multiple integration strategy results in higher antigen expression level compared to a single site integration c) the choice of site of chromosomal integration allows superior control of antigen expression levels, timing and secretion across the bacterial outer membrane.

ADVANTAGES

Live vector vaccines are capable of expressing multiple antigens from both chromosomal and plasmid-based expression systems from a single strain. The biggest advantage offered by this strategy is that the antigen expression levels and live vector propagation are "in sync," hence, the problem of "over attenuation" due to metabolic stress induced by antigen production does not arise. In addition, the protein expression is maintained without the need for antibiotic selection. Vaccine failure also often results from loss of low-copy number plasmids or due to loss of high-copy number plasmid often seen with lack of selective pressure. This problem is dodged with the expression of the foreign antigen on chromosomal DNA. Hence, this strategy is likely to overcome the loss of gene dosage problem currently constraining the field of live bacteria vaccines.

STAGE OF DEVELOPMENT

Mouse immunogenicity and challenges experiments are being conducted. The inventors are also actively engaged in development of live vector vaccine targeted towards specific diseases and infections.

R&D REQUIRED

Further studies required to gain proof of concept for functionality in animals.

LICENSING POTENTIAL

UMB seeks partners to develop and use this technology for vaccines and possibly, "finely tuned" chromosomal protein expression systems.

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PATENT STATUS

Canada 2,889,069 issued 01/26/2021, US CON 10,010,596 issued 07/03/2018, US CIP 9,446,113 issued 09/20/2016

LICENSE STATUS

Available for License

CATEGORIES

- Vaccines

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EXTERNAL RESOURCES

- Novel methods for expression of foreign antigens in live vector vaccines.
- Salmonella enterica serovar Typhi live vector vaccines finally come of age.

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