Hypothesis

Encapsulated Bacteria with a Light-Repressed Deadman Switch for Liver Gene Delivery

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Encapsulated Bacteria with a Light-Repressed Deadman Switch for Liver Gene Delivery

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Abstract: Adeno-associated viruses (AAVs) have been used for liver gene therapy. Hemgenix and Roctavian are AAV-based treatments for hemophilia B and A, respectively. They cost $3.5 million and $2.9 million per dose, respectively. While AAV vectors may eventually be cheaper to mass produce, a bacterium-based DNA delivery system might be much cheaper for patients. Also, this approach would allow for the delivery of much larger DNA packages. Such a bacterial system may now be feasible, and a prototype for the liver could possibly be developed immediately.

Keywords: Bacterial capsule; caged luciferin/luciferase; CY3PA; light-activated allosteric protein switch; Deadman switch; and bactofection

Introduction

Adeno-associated viruses (AAVs) can only encode ~5 kb of DNA maximum, which is not sufficient to cure certain individuals with genetic disorders wherein long stretches of nucleotides are affected, e.g., at least some cases of Duchenne muscular dystrophy [1–6].

According to Dr. Anzalone *et al*., "…in principle prime editing can correct up to ~89% of the 75,122 pathogenic human genetic variants in ClinVar…"[7]. New prime editing-based techniques can almost surely cover even more variants[8]. However, multiple proteins/RNAs/DNA molecules (or at least a single, large DNA molecule) may be required in certain cases when more complex molecular machinery must be employed, e.g., if heterochromatin is an issue.

Also, the cost of producing sufficiently high titers of AAV vectors and other viral vectors for therapeutic purposes is very steep[9–11].

Lipid nanoparticle (LNP)-encapsulated CRISPR ribonucleoproteins (RNPs) have been successfully utilized for gene delivery and editing in the liver and somewhat in the lungs[12,13]. However, current LNP formulations at least cannot effectively reach or target cells in the central nervous system (CNS), heart, or kidneys after intravenous injection[13,14]. Additionally, the larger the LNP - the less effective it is at extravasation. Thus, it cannot encapsulate large cargos unless targeted to the liver or spleen. Finally, LNP production costs are still substantial, and scaling up the production of new ionizable lipids is not that facile[15–18].

Intrathecal or intracerebroventricular administration of RNPs, as well as direct intraparenchymal injection of RNPs into the striatum, does not result in widespread CNS editing[19].

A synthetic bacterial vector with low immunogenicity that only delivers to particular organs or organ systems would be of use for multiple reasons:

1. It would be cheap.
2. It could deliver large DNA constructs to use as homologous repair templates or large serine recombinase/CRISPR transposase cargo for individuals with genetic disorders wherein long stretches of nucleotides are affected.
3. May be able to extravasate at least moderately efficiently in most anatomical locales - and then specifically proliferate in the target organ or organ system[20,21]. (If necessary - intraparenchymal injections could be employed.)

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4. Flagellar motility of the vector allows for autonomous, widespread delivery throughout the organ or organ system[22].

Prototype for the Liver

Recently, a new system was developed for the programmable encapsulation of microbes to attenuate the immune response and enhance multifocal tumor therapy. An *Escherichia Coli* Nissle 1917 or a *Salmonella* Typhimurium vector could be encapsulated to prevent cytotoxic protein secretion from affecting the tissue environment and substantially attenuate the immune response[23]. It can also be myristoylation negative[24]. One could employ an anti-sepsis small molecule, at least in a mouse model[25]. Dexamethasone would be used to further suppress the immune response and induce the expression of CY3PA in the liver. The bacterial vector could then be administered. Finally, one would periodically administer a caged luciferin molecule that is uncaged by CY3PA[26]. The bacterial vector would express firefly luciferase and have a Deadman switch that is sensitive to light[27–31]. Bacteria that stray outside of the liver, where the luciferin is uncaged, would quickly stop replicating and lyse.

When the bacteria in the liver reach a high enough population level (either visualized with an MRI-based reporter gene[32] or just after an experimentally-determined period of time), they can be induced via small molecule to produce a large quantity of CRISPR RNPs[19,33] and lyse.

Theoretically, *E. coli* Nissle 1917 is endotoxin-free. However, to prevent possible issues from mass lysis, perhaps the small molecule should stop motility immediately - while the gene circuit for lysis can be noisy[34].

Another option would be to use a second Deadman switch; a molecule that the bacteria are exposed to in solution prior to intravenous injection would be gone after injection, and eventually they will just stop replicating and lyse naturally.

Bactofection is a method that has been used for DNA transfer to target cells. It involves invasion of a facultative intracellular bacterium into the a target cell, endosomal rupture, and finally lysis[35]. One of the primary roadblocks to bactofection in vivo has been the immune response to the bacteria.

Notably, only two genes may be required to enable this strategy using *E. coli* Nissle 1917: the *Yersinia pseudotuberculosis* invasin and listeriolysin O[36,37].

Having a thick capsule may hinder hepatocyte uptake, however[38]. If so, the bacteria could perhaps divide several times after halting capsule production and overexpressing an eliminase to shed its capsule[39], enter hepatocytes[40], escape the endosome or vacuole[36,37,41], and then lyse.

To increase the efficiency of bactofection, the *Listeria monocytogenes actA* promoter can be used to drive phage lysin production when the bacteria enter the cytosol of target cells[42]. Using a linear plasmid in combination with NLS-containing proteins that bind said plasmid could substantially increase the efficiency, as linear DNA may be taken up more easily through nuclear pore complexes[43,44]. Moreover, it may be possible to employ a mechanism involving asymmetric division of the vector inside target cells, wherein one of the progeny cells remains in a vacuole and the other lyses to release its cargo[45,46]. The other progeny cell could continue to asymmetrically divide until at least one copy of the DNA construct reaches the host cell nucleus, at which point expression of an artificial gene product would cause it to lyse.

Finally, to strengthen this system, prior to asymmetric division, the bacterium could potentially be allowed to undergo replication up until a tolerable copy number, then being restrained by quorum sensing.

Bacterial entry and release of a replicating RNA vector may also help in the case of RNP delivery, to enhance gene editing efficiency[47–49].

For diseases like Hemophilia A and B, not every hepatocyte nucleus would need a copy of the gene construct in order to achieve a curative status, which is helpful with regard to bactofection efficiency and immunogenicity.

Other Organs and Organ Systems

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We could identify other enzymes specific to an organ or organ system and develop caged luciferins for those as well. Alternatively, the enzyme could be an organ or organ system-specific extracellular protease, for example. The protease could directly or indirectly activate regulated intramembrane proteolysis[50]. Or, it could cleave a pro-peptide that activates a two-component regulatory system[51]. The benefit of either of those approaches is that an exogenous small molecule would not be required for the continued survival and replication of the vector.

Bacteria can also cross the blood-brain barrier after intravenous injection [21,52].

There is a caged luciferin that can be uncaged in the brain - but it also is uncaged in the kidneys [53]. A better strategy, for now at least, may be to administer a small molecule that is blood-brain barrier-impermeable and that kills any of the synthetic bacteria in the periphery.

In a paper by Dr. Antas *et al*., it was said that "Although nonviral NPs may tackle the main limitations of their viral counterparts, they have thus far failed to compete with the transduction efficiency achieved by viral vectors in the retina"[54]. Bactofection might be better than AAVs for ocular genetic diseases [55,56]. A Deadman switch could be used to allow limited bacterial replication in the eye via a small molecule present in an eye-drop solution or micro-drug reservoir [57].

Identifying tissue-specific enzymes should not be very difficult nowadays [58].

Conclusions

Encapsulated bacteria with a light-repressed Deadman switch could be used for liver gene delivery. They may be much cheaper than AAV-based liver gene therapies, and even LNPs. It would also allow for the delivery of large DNA packages. In the future, other organs that are harder to reach with gene therapy or editing components, like the heart or brain, could be treated with this therapy.

References

1. Spinner NB, Loomes KM, Krantz ID, Gilbert MA. Alagille Syndrome. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Bean LJ, et al., editors. GeneReviews®, Seattle (WA): University of Washington, Seattle; 1993.
2. Stoller JK, Hupertz V, Aboussouan LS. Alpha-1 Antitrypsin Deficiency. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Bean LJ, et al., editors. GeneReviews®, Seattle (WA): University of Washington, Seattle; 1993.
3. Myerowitz R. Tay-Sachs disease-causing mutations and neutral polymorphisms in the Hex A gene. Hum Mutat 1997;9:195–208. https://doi.org/10.1002/(SICI)1098-1004(1997)9:3<195::AID-HUMU1>3.0.CO;2-7.
4. Butchbach MER. Genomic Variability in the Survival Motor Neuron Genes (SMN1 and SMN2): Implications for Spinal Muscular Atrophy Phenotype and Therapeutics Development. International Journal of Molecular Sciences 2021;22:7896. https://doi.org/10.3390/ijms22157896.
5. Daiger S, Sullivan L, Bowne S. Genes and mutations causing retinitis pigmentosa. Clin Genet 2013;84:10.1111/cge.12203. https://doi.org/10.1111/cge.12203.
6. Duan D, Goemans N, Takeda S, Mercuri E, Aartsma-Rus A. Duchenne muscular dystrophy. Nat Rev Dis Primers 2021;7:1–19. https://doi.org/10.1038/s41572-021-00248-3.
7. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 2019;576:149–57. https://doi.org/10.1038/s41586-019-1711-4.
8. Zheng C, Liu B, Dong X, Gaston N, Sontheimer EJ, Xue W. Template-jumping prime editing enables large insertion and exon rewriting in vivo. Nat Commun 2023;14:3369. https://doi.org/10.1038/s41467-023-39137-6.
9. Collins LT, Ponnazhagan S, Curiel DT. Synthetic Biology Design as a Paradigm Shift toward Manufacturing Affordable Adeno-Associated Virus Gene Therapies. ACS Synth Biol 2023;12:17–26. https://doi.org/10.1021/acssynbio.2c00589.
10. Becker Z. Sporting a $3.5M price tag, CSL and uniQure's hemophilia B gene therapy crosses FDA finish line. Fierce Pharma 2022. https://www.fiercepharma.com/pharma/csl-and-uniqures-hemophilia-b-gene-therapy-scores-approval-35-million-price-tag (accessed December 9, 2023).
11. Bluebird Bio Secures Deal with Large Commercial Payer for Lyfgenia Amid Price Concerns. BioSpace n.d. https://www.biospace.com/article/bluebird-bio-secures-deal-with-large-commercial-payer-for-lyfgenia-amid-price-concerns/ (accessed January 4, 2024).
12. Wei T, Sun Y, Cheng Q, Chatterjee S, Traylor Z, Johnson LT, et al. Lung SORT LNPs enable precise homology-directed repair mediated CRISPR/Cas genome correction in cystic fibrosis models. Nat Commun 2023;14:7322. https://doi.org/10.1038/s41467-023-42948-2.

13. Chen K, Han H, Zhao S, Xu B, Yin B, Trinidad M, et al. Lung and liver editing by lipid nanoparticle delivery of a stable CRISPR-Cas9 RNP 2023:2023.11.15.566339. https://doi.org/10.1101/2023.11.15.566339.
14. Behr M, Zhou J, Xu B, Zhang H. In vivo delivery of CRISPR-Cas9 therapeutics: Progress and challenges. Acta Pharmaceutica Sinica B 2021;11:2150–71. https://doi.org/10.1016/j.apsb.2021.05.020.
15. Wang X, Liu S, Sun Y, Yu X, Lee SM, Cheng Q, et al. Preparation of selective organ-targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. Nat Protoc 2023;18:265–91. https://doi.org/10.1038/s41596-022-00755-x.
16. Verma M, Ozer I, Xie W, Gallagher R, Teixeira A, Choy M. The landscape for lipid-nanoparticle-based genomic medicines. Nat Rev Drug Discov 2023;22:349–50. https://doi.org/10.1038/d41573-023-00002-2.
17. Shepherd SJ, Han X, Mukalel AJ, El-Mayta R, Thatte AS, Wu J, et al. Throughput-scalable manufacturing of SARS-CoV-2 mRNA lipid nanoparticle vaccines. Proc Natl Acad Sci U S A n.d.;120:e2303567120. https://doi.org/10.1073/pnas.2303567120.
18. Mehta M, Bui TA, Yang X, Aksoy Y, Goldys EM, Deng W. Lipid-Based Nanoparticles for Drug/Gene Delivery: An Overview of the Production Techniques and Difficulties Encountered in Their Industrial Development. ACS Mater Au 2023;3:600–19. https://doi.org/10.1021/acsmaterialsau.3c00032.
19. Stahl EC, Sabo JK, Kang MH, Allen R, Applegate E, Kim SE, et al. Genome editing in the mouse brain with minimally immunogenic Cas9 RNPs. Molecular Therapy 2023;31:2422–38. https://doi.org/10.1016/j.ymthe.2023.06.019.
20. Tan X, Petri B, DeVinney R, Jenne CN, Chaconas G. The Lyme disease spirochete can hijack the host immune system for extravasation from the microvasculature. Mol Microbiol 2021;116:498–515. https://doi.org/10.1111/mmi.14728.
21. Sun R, Liu M, Lu J, Chu B, Yang Y, Song B, et al. Bacteria loaded with glucose polymer and photosensitive ICG silicon-nanoparticles for glioblastoma photothermal immunotherapy. Nat Commun 2022;13:5127. https://doi.org/10.1038/s41467-022-32837-5.
22. Toley BJ, Forbes NS. Motility is Critical for Effective Distribution and Accumulation of Bacteria in Tumor Tissue. Integr Biol (Camb) 2012;4:165–76. https://doi.org/10.1039/c2ib00091a.
23. Harimoto T, Hahn J, Chen Y-Y, Im J, Zhang J, Hou N, et al. A programmable encapsulation system improves delivery of therapeutic bacteria in mice. Nat Biotechnol 2022;40:1259–69. https://doi.org/10.1038/s41587-022-01244-y.
24. Stritzker J, Hill PJ, Gentschev I, Szalay AA. Myristoylation negative msbB-mutants of probiotic E. coli Nissle 1917 retain tumor specific colonization properties but show less side effects in immunocompetent mice. Bioeng Bugs 2010;1:139–45. https://doi.org/10.4161/bbug.1.2.10286.
25. Gawish R, Maier B, Obermayer G, Watzenboeck ML, Gorki A-D, Quattrone F, et al. A neutrophil–B-cell axis impacts tissue damage control in a mouse model of intraabdominal bacterial infection via Cxcr4. eLife n.d.;11:e78291. https://doi.org/10.7554/eLife.78291.
26. Yevtodiyenko A, Bazhin A, Khodakivskyi P, Godinat A, Budin G, Maric T, et al. Portable bioluminescent platform for in vivo monitoring of biological processes in non-transgenic animals. Nat Commun 2021;12:2680. https://doi.org/10.1038/s41467-021-22892-9.
27. Mi Z, Yao Q, Qi Y, et al. *Salmonella*-mediated blood–brain barrier penetration, tumor homing and tumor microenvironment regulation for enhanced chemo/bacterial glioma therapy. Acta Pharmaceutica Sinica B 2023;13(2):819–833; doi: 10.1016/j.apsb.2022.09.016.
28. Dash R, Holsinger KA, Chordia MD, et al. Bioluminescence-Based Determination of Cytosolic Accumulation of Antibiotics in Escherichia coli. ACS Infect Dis 2024; doi: 10.1021/acsinfecdis.3c00684.
29. Chan CTY, Lee JW, Cameron DE, Bashor CJ, Collins JJ. "Deadman" and "Passcode" microbial kill switches for bacterial containment. Nat Chem Biol 2016;12:82–6. https://doi.org/10.1038/nchembio.1979.
30. Branchini BR, Ablamsky DM, Rosenman JM, Uzasci L, Southworth TL, Zimmer M. Synergistic Mutations Produce Blue-Shifted Bioluminescence in Firefly Luciferase. Biochemistry 2007;46:13847–55. https://doi.org/10.1021/bi7015052.
31. Nash AI, McNulty R, Shillito ME, Swartz TE, Bogomolni RA, Luecke H, et al. Structural basis of photosensitivity in a bacterial light-oxygen-voltage/helix-turn-helix (LOV-HTH) DNA-binding protein. Proc Natl Acad Sci U S A 2011;108:9449–54. https://doi.org/10.1073/pnas.1100262108.
32. Allouche-Arnon H, Khersonsky O, Tirukoti ND, Peleg Y, Dym O, Albeck S, et al. Computationally designed dual-color MRI reporters for noninvasive imaging of transgene expression. Nat Biotechnol 2022;40:1143–9. https://doi.org/10.1038/s41587-021-01162-5.
33. Yu S, Yang H, Li T, Pan H, Ren S, Luo G, et al. Efficient intracellular delivery of proteins by a multifunctional chimaeric peptide in vitro and in vivo. Nat Commun 2021;12:5131. https://doi.org/10.1038/s41467-021-25448-z.
34. Kannoly S, Gao T, Dey S, Wang I-N, Singh A, Dennehy JJ. Optimum Threshold Minimizes Noise in Timing of Intracellular Events. iScience 2020;23:101186. https://doi.org/10.1016/j.isci.2020.101186.

 doi:10.20944/preprints202404.1631.v4

35. Sultana A, Kumar R. Modified bactofection for efficient and functional DNA delivery using invasive *E. coli* DH10B vector into human epithelial cell line. Journal of Drug Delivery Science and Technology 2022;70:103159; doi: 10.1016/j.jddst.2022.103159.
36. Grillot-Courvalin C, Goussard S, Huetz F, et al. Functional gene transfer from intracellular bacteria to mammalian cells. Nat Biotechnol 1998;16(9):862–866; doi: 10.1038/nbt0998-862.
37. Zare M, Farhadi A, Zare F, et al. Genetically engineered E. coli invade epithelial cells and transfer their genetic cargo into the cells: an approach to a gene delivery system. Biotechnol Lett 2023;45(7):861–871; doi: 10.1007/s10529-023-03387-7.
38. Schembri MA, Dalsgaard D, Klemm P. Capsule Shields the Function of Short Bacterial Adhesins. J Bacteriol 2004;186(5):1249–1257; doi: 10.1128/JB.186.5.1249-1257.2004.
39. Hickey AM, Bhaskar U, Linhardt RJ, et al. Effect of eliminase gene (elmA) deletion on heparosan production and shedding in Escherichia coli K5. J Biotechnol 2013;165(3–4):175–177; doi: 10.1016/j.jbiotec.2013.03.018.
40. Raman V, Van Dessel N, Hall CL, Wetherby VE, Whitney SA, Kolewe EL, et al. Intracellular delivery of protein drugs with an autonomously lysing bacterial system reduces tumor growth and metastases. Nat Commun 2021;12:6116. https://doi.org/10.1038/s41467-021-26367-9.
41. Brumell JH, Tang P, Zaharik ML, Finlay BB. Disruption of the Salmonella-Containing Vacuole Leads to Increased Replication of Salmonella enterica Serovar Typhimurium in the Cytosol of Epithelial Cells. Infect Immun 2002;70:3264–70. https://doi.org/10.1128/IAI.70.6.3264-3270.2002.
42. Pilgrim S, Stritzker J, Schoen C, et al. Bactofection of mammalian cells by Listeria monocytogenes: improvement and mechanism of DNA delivery. Gene Ther 2003;10(24):2036–2045; doi: 10.1038/sj.gt.3302105.
43. Johansson P, Lindgren T, Lundström M, et al. PCR-generated linear DNA fragments utilized as a hantavirus DNA vaccine. Vaccine 2002;20(27):3379–3388; doi: 10.1016/S0264-410X(02)00265-7.
44. Zhu J, Batra H, Ananthaswamy N, et al. Design of bacteriophage T4-based artificial viral vectors for human genome remodeling. Nat Commun 2023;14(1):2928; doi: 10.1038/s41467-023-38364-1.
45. Molinari S, Shis DL, Bhakta SP, et al. A synthetic system for asymmetric cell division in Escherichia coli. Nat Chem Biol 2019;15(9):917–924; doi: 10.1038/s41589-019-0339-x.
46. Eswarappa SM, Negi VD, Chakraborty S, et al. Division of the Salmonella-Containing Vacuole and Depletion of Acidic Lysosomes in Salmonella-Infected Host Cells Are Novel Strategies of Salmonella enterica To Avoid Lysosomes. Infect Immun 2010;78(1):68–79; doi: 10.1128/IAI.00668-09.
47. Singer ZS, Pabón J, Huang H, et al. Engineered bacteria launch and control an oncolytic virus. bioRxiv 2023;2023.09.28.559873; doi: 10.1101/2023.09.28.559873.
48. Mc Cafferty S, De Temmerman J, Kitada T, et al. In Vivo Validation of a Reversible Small Molecule-Based Switch for Synthetic Self-Amplifying mRNA Regulation. Molecular Therapy 2021;29(3):1164–1173; doi: 10.1016/j.ymthe.2020.11.010.1.
49. Perkovic M, Gawletta S, Hempel T, et al. A trans-amplifying RNA simplified to essential elements is highly replicative and robustly immunogenic in mice. Mol Ther 2023;31(6):1636–1646; doi: 10.1016/j.ymthe.2023.01.019.
50. Heinrich J, Wiegert T. Regulated intramembrane proteolysis in the control of extracytoplasmic function sigma factors. Research in Microbiology 2009;160:696–703. https://doi.org/10.1016/j.resmic.2009.08.019.
51. Brink KR, Hunt MG, Mu AM, et al. An E. coli display method for characterization of peptide-sensor kinase interactions. Nat Chem Biol 2023;19(4):451–459; doi: 10.1038/s41589-022-01207-z.
52. Arbab M, Matuszek Z, Kray KM, Du A, Newby GA, Blatnik AJ, et al. Base editing rescue of spinal muscular atrophy in cells and in mice. Science 2023;380:eadg6518. https://doi.org/10.1126/science.adg6518.
53. Mofford DM, Adams STJr, Reddy GSKK, Reddy GR, Miller SC. Luciferin Amides Enable in Vivo Bioluminescence Detection of Endogenous Fatty Acid Amide Hydrolase Activity. J Am Chem Soc 2015;137:8684–7. https://doi.org/10.1021/jacs.5b04357.
54. Antas P, Carvalho C, Cabral-Teixeira J, Lemos L de, Seabra MC. Toward low-cost gene therapy: mRNA-based therapeutics for treatment of inherited retinal diseases. Trends in Molecular Medicine 2023;0. https://doi.org/10.1016/j.molmed.2023.11.009.
55. Yiu G, Chung SH, Mollhoff IN, et al. Suprachoroidal and Subretinal Injections of AAV Using Transscleral Microneedles for Retinal Gene Delivery in Nonhuman Primates. Mol Ther Methods Clin Dev 2020;16:179–191; doi: 10.1016/j.omtm.2020.01.002.
56. Chuah J-A, Matsugami A, Hayashi F, et al. Self-Assembled Peptide-Based System for Mitochondrial-Targeted Gene Delivery: Functional and Structural Insights. Biomacromolecules 2016;17(11):3547–3557; doi: 10.1021/acs.biomac.6b01056.
57. Than A, Liu C, Chang H, et al. Self-implantable double-layered micro-drug-reservoirs for efficient and controlled ocular drug delivery. Nat Commun 2018;9(1):4433; doi: 10.1038/s41467-018-06981-w.
58. Yang RY, Quan J, Sodaei R, Aguet F, Segrè AV, Allen JA, et al. A systematic survey of human tissue-specific gene expression and splicing reveals new opportunities for therapeutic target identification and evaluation 2018:311563. https://doi.org/10.1101/311563.

 doi:10.20944/preprints202404.1631.v4