Title: Developing Magnetic Resonance Reporter Gene Imaging: Co-Expression of Magnetotactic Bacteria Genes mamI and mamL

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Structured Abstract:

Introduction: With its superb spatial and temporal resolution, magnetic resonance imaging (MRI) has great potential to track cellular activities that define early stages of disease. To improve molecular imaging techniques, we are developing MRI reporter gene expression based on the magnetosome. In magnetotactic bacteria (MTB), magnetosome formation allows cells to concentrate and compartmentalize iron biominerals in membrane-enclosed vesicles. While the entire process is regulated by numerous genes, we have selected a subset of genes, mamI, mamL, mamB, and mamE, deemed essential for initial stages of magnetosome formation. While mamI, mamL, and mamB have roles in designating the magnetosome vesicle, they may also provide docking site(s) for additional proteins, like mamE, that facilitate biomineralization. Refining the development of magnetosome-like nanoparticles will provide an endogenous magnetic resonance (MR) label in which iron contrast may be fine-tuned.

Hypothesis: In mammalian cells, MamI and MamL co-localize on an intracellular membrane and interact to initiate formation of a rudimentary magnetosome-like nanoparticle.

Methods: MTB genes mamI and mamL were cloned from M. magneticum sp. AMB-1 genomic DNA by PCR and inserted into vectors with fluorescent protein tags (pEGFP and ptdTomato, respectively) to create Mam fusion proteins. GFP-MamI and Tomato-MamL were stably expressed in human MDA-MB-435 melanoma cells—alone and in combination—using antibiotic selection and enriched with fluorescence-activated cell sorting (FACS). Expression of Mam fusion proteins was verified by Western blot. Subcellular location and co-localization of fusion proteins were examined with confocal microscopy (Nikon A1R Confocal Laser Microscope) and fluorescence correlation spectroscopy (FCS, Evotec Insight Confocal Instrument).

Results: When expressed alone, GFP-MamI formed a net-like fluorescence pattern while Tomato-MamL showed punctate fluorescence that exhibited motion in live cells. When co-expressed, the fluorescence signal co-localized in a punctate pattern and, unexpectedly, exhibited motion that was similar to that displayed by Tomato-MamL alone. FCS indicated that when the fusion proteins were co-expressed, GFP-MamI/Tomato-MamL fluorescent particles were associated with larger and slower-diffusing intracellular structures compared to their individually-expressed fluorescent particles.

Discussion: We have shown that over-expression of MTB proteins MamI and MamL are compatible with a mammalian cell system. Confocal fluorescence microscopy and FCS data demonstrated that these integral membrane proteins co-localize in an intracellular compartment, as expected of a rudimentary magnetosome-like particle. Future work involves characterizing the expression and interaction of additional MTB genes such mamE and mamB in MDA-MB-435 cells, and evaluating their influence on cellular iron content and MR signal.