How eukaryotic cells can be made para- or even ferromagnetic

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Content

- **A single-gene approach**
  - transporters
  - catalysts
  - sequesters
  - viral capsides
  - limited success
- **A multi-gene approach**
  - magnetosomes
  - metabolic control
- **Magnetite formation conditions**
  - biomimetics
  - design from scratch

Marchella Piery "Love Attraction"
Brush, 40"x30", Oil
MagA & DMT1 transporters

- Mouse 2B5 cells with MagA produced iron-oxide nanoparticles in vesicles, resembling endosomes
- Prussian blue staining detected Fe$^{3+}$ ions
- The effect of DMT1 expression in HEK293 cells on the spin-lattice relaxation time (T1) is shown by measuring the Mn-dose dependent relaxation rate, $R_1 = 1/T_1$

(Bartelle et al, 2013)

(Zurkiya et al, 2009)
C-terminus of Mms6 protein forms magnetic nanoparticles \textit{in vitro}

(a) Mms6 protein (b-e) Mms6 peptides (f) absence of Mms6 (Amemiya et al, 2007; Arakaki et al, 2010)

(A) Buffer (B) Mms6 (C) Mms6(A131C) (D) Mms6(A133C) (Feng et al, 2013)
Nanomagnet toolkit

Single magnetic domain particles for various materials (Chung et al. 2004)

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<th>Material</th>
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<th>size (nm)</th>
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<tr>
<td>Magnetosomes</td>
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Dps & ferritin

Iron-related redox functions of Dps:

- $O_2 + Fe^{3+} \rightarrow Fe^{2+} + O_2$ (Iron reduction)
- $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH$ (Fenton reaction)
- $O_2 + H_2O_2 \xrightarrow{Fe \text{ catalysis}} OH + OH^- + O_2$ (Haber-Weiss reaction)
- $2Fe^{2+} + H_2O_2 + 2H^+ \rightarrow 2Fe^{3+} + 2H_2O$ (Peroxidase activity)

- 24 ferritin subunits
- 8 three-fold channels providing a pathway of $Fe^{2+}$ to the interior
- H-chain is necessary for the oxidation (ferroxidase centre)
- L-chain for the mineralization (nucleation centre)
Fine structure of ferritin

- 12 nm spherical complex with 8 nm cavity
- clusters of **ferrihydrite, magnetite** and **hematite**
- up to 4500 iron atoms with total magnetic moment ~250-400 $\mu_B$
- helix bundle and interior surface control the catalytic properties of ferritin
- N-terminus and BC-loop control the aggregation of ferritin complexes

(Ha et al, 1999; Goodsell, 2002; Brem et al, 2006; Crichton, Declercq, 2010)
A chimeric ferritin with enhanced iron loading

- Human H- and L-ferritin chains were fused into one subunit to complement L and H functionality
- L*H ferritin chimera demonstrated the improved iron loading ability and T2 relaxation compared to *wt* ferritin

(Iordanova et al, 2010)
Synthetic pathways using apoferritin

- The oxidation of iron to form **ferrihydrite** is dependent on Fe/protein ratio:
  - when 1 Fe$^{2+}$ ion per subunit, then the reaction (1)
  - when more than 10 Fe$^{2+}$ ions per subunit, then the reaction (2)

(Klem et al, 2005)
Capsides

Icosahedral plant viruses

BMV  CCMV  CPMV

Icosahedral bacteriophages

MS2  Qβ

- The core proteins of plant viruses and bacteriophages can be genetically modified to be expressed in form of ghost virus particles available for internal mineralization

- pro and contra arguments for using viruses:
  - the large size of capsids in comparison to ferritin and the small number of coding genes versus mamAB operon
  - an uncertain redox potential, unpredictable expression and misfolding, as well as possible wrong assembling in a heterologous environment
Engineering viral cage for the synthesis of constrained nanomaterials

(a) 180 (60x3) subunits of CCMV cage
(b) ~20 nm cavity of the viral cage

- Basic residues on the N-terminus of the CCMV coat protein were replaced by glutamic acids leading to mineralization:
  - (left) TEM of the lepidocrocite (γ-FeO(OH)) core of CCMV
  - (right) spectroscopy of mineralized CCMV showing Fe (yellow) surrounded by N (blue) of the protein shell

(Douglas et al, 2002; Liepold et al, 2005)
Structural transitions in CCMV

- Cryo TEM and image reconstruction of CCMV:
  - (left) close conformation ($\text{pH} \leq 6.5$, with metal ions)
  - (right) open conformation ($\text{pH} \geq 6.5$, without metal ions)

(Liepold et al, 2005)
Viral capsids as MRI contrast agents

- Cutaway view of the interior of CCMV
  - blue is the six-fold regions
  - red is five-fold regions

- A nine-residue peptide, from the Ca$_2$-binding protein **calmodulin** (CAL) was genetically fused to the N-terminus of CCMV core protein:
  - the first 20 amino acids are shown for both the unmodified and genetically modified viral subunit
  - the underlined 12 residues are responsible for Gd$^{3+}$ binding

(Liepold et al, 2007)
Nanoparticles synthesized by engineered *Escherichia coli*

(a) CdSeZn  
(b) PrGd  
(c) CdCs  
(d) FeCo  
(e) Au  
(f) Ag  
(g) freeze dried *E.coli* cells containing NPs

- The NPs were formed by using the metal binding proteins *phytochelatin synthase* (PCS) and *metallothionein* (MT) expressed in *E.coli*

(Park et al, 2010)
“Magnetic“ genes in *Magnetospirillum gryphiswaldense*

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(Lohsse et al, 2011)
mamAB gene cluster in
*Magnetospirillum magneticum* AMB-1

- mamAB gene cluster is essential for
  - magnetosome membrane biogenesis
  - iron delivery and magnetite production
  - magnetosome chain assembly

(A) 14 regions of the magnetosome island labeled R1–R14

(B) organization of mamAB gene cluster, R5

(Murat et al, 2010)
Magnetosome formation

- Magnetosomes are formed step by step:
  - invagination of the cytoplasmic membrane and magnetosome-specific proteins localization
  - assembly of the vesicles into a chain
  - iron transport and biomineralisation of the magnetite in magnetosomes

- All the stages are under strict genetic control

(Komeili, 2012)
Minimal *mamAB* operon

- A basic set of genes from *Magnetospirillum magneticum* AMB-1 depends strongly on environment conditions:
  - *mam*\{I, E, M, O, Q, B\} *in vitro*
  - *mam*\{H, I, E, L, M, N, O, P, A, Q, B\} *in vivo*:

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**mamAB** homologies with various genomes (tblastn alignment score)

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</table>
Bio-magnetization in *Saccharomyces cerevisiae*

- The **vacuolar iron transporter Ccc1p** plays a major role in iron sequestration.
- The *ccc1Δ* showed increased magnetization compared to *wt*, suggesting that non-vacuolar iron may have more magnetic contribution than iron in vacuoles. The synergistic effect of ferritin and *ccc1Δ* can be explained by higher availability of iron to ferritin in the cytosol.
- *Wt* cells with round particles associated with vacuoles, while *ccc1Δ* cells with aggregates within mitochondria.

(Nishida, Silver, 2012)
Ferritin-based magnetization of mammalian cells

- To maximize cellular iron uptake enabled efficient iron mineralization, the **ferritin** and **DMT1** proteins expression with 3 mM ammonium iron(II) sulfate in culture medium were used:
  - 2.9 pg iron content
  - 6.9x10^6 ferritin complexes
  - total magnetic moment ~1.7 to 2.7x10^9 µ_B per a cell

- The cells moved in a magnetic field and were separated using a permanent magnet

(Deans et al, 2006; Kim et al, 2012)
Single magnetic domain of magnetite

- **Magnetite**, Fe$_3$O$_4$=[Fe$^{3+}$]$_A$[Fe$^{3+}$Fe$^{2+}$]$_B$O$_4$
  - A - tetrahedral B - octahedral; A+B=4 $\mu_B$
  - $\alpha$=8.4 Å; 8x4=32 $\mu_B$
  - $\alpha$=1 nm; 54 $\mu_B$
  - $\alpha$=10 nm; 54x10$^3$ $\mu_B$
  - $\alpha$=50 nm; 6.7x10$^6$ $\mu_B$

- A cubic particle of 50 nm will be “superparamagnetic” (=6.7x10$^6$ $\mu_B$)

- The magnetosome size between 35 and 120 nm corresponds to a stable magnetic domain with the magnetic moment ~2.3-93x10$^6$ $\mu_B$

- Such crystals of magnetite are the smallest particles of this mineral that are permanently magnetic at ambient temperature

- Smaller nanoparticles will not allow permanent magnetization and will be useless to bacteria for magnetotaxis

- ~20-30 “cubic” magnetosomes/eukaryotic cell for a magnetic separation
Magnetization of *Magnetovibrio blakemorei* MV-1

- **Magnetization loops** $M(H)$ were measured at 5 K in frozen cell suspension of strain MV-1, whose magnetosomes contain magnetite nanoparticles.

- The diamagnetic background from cells of a nonmagnetic mutant strain of MV-1 that does not contain magnetite was subtracted from the *wt* magnetic strain.

- Saturation at 0.003 (emu) $= 3.2348 \times 10^{17}$ (µB)

*Prozorov et al, 2007*
Iron reaction pathway in magnetosomes, where Fe$^{2+}$ and Fe$^{3+}$ are bound by organic substrates (A unknown, B ferritin)

- uptake of Fe$^{2+}$ or Fe$^{3+}$ ions using organic substrates
- localization in the membrane-associated ferritin to be released in the magnetosome
- coprecipitation of Fe$^{2+}$ and Fe$^{3+}$ ions within the magnetosome

The Pourbaix diagram of iron in magnetosome
- magnetite formation is possible in the narrow window: pH=8-14 and Eh=-0.3 - -0.7 V

(Faivre, Schüler, 2008)
Mitochondria as a “factory” for magnetite

- A designer has to follow pH=8.0-9.0 and Eh=-300 - -400 mV
- The intermembrane space of mitochondrion with pH=6.9
- The mitochondrial matrix with pH=7.8 and $\Delta \Psi$=-160 - -170 mV
- When one put a magnetite formation system into the mitochondrial matrix, it will work against the $H^+$ gradient, i.e. against the electron transport chain and against the ATPase
- When one makes it in the intermembrane space, the system will not work because of low pH and positive Eh

(Porcelli et al, 2005)
Cautions & perspectives

- Design that allows the magnetite \((\text{Fe}_3\text{O}_4)\) formation in vesicles with pH=8.0-9.0 and Eh=-0.3 - -0.4 V
  - protons pumping, e.g. by MamN
  - the ratio of Fe\(^{2+}/\text{Fe}^{3+}\) regulation, e.g. by MamP
  - proteins affecting the redox value, such as MamH, MamX, MamZ, Nap, NirS... are used in

- Iron transporters (DMT1, MagA, FeoB), ferritin like proteins, viral capsides, and magnetosome genes are available building blocks to tackle the problem

- Additional parts and tools, such as the iron binding proteins, bacterial microcompartments, ferroporin, transferrin, siderophores, sHSP, lumazine synthase and phage display

- Alternative design using other magnetic materials (Co, FePt, CoFe\(_2\text{O}_4\))

- Fixation of nanoparticles with aligned magnetic moments in a matrix mimicking thalassemic tissues, genetic hemochromatosis and Alzheimer's diseases

- Engineering from the bottom up at the reasonable level of complexity


Many thanks to Prof. Gil Westmeyer suggesting this topic