Subproject 2: Spatio-temporal control of phagocytosis

Progress report

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Biological Physics Group:  
http://www3.imperial.ac.uk/biologicalphysics
Recent achievements

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A mechanical bottleneck explains the variation in cup growth during FcγR phagocytosis

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Biophysical Mechanism for Ras-Nanocluster Formation and Signaling in Plasma Membrane

Thomas Gurry1,2, Ozan Kahramanoğulları1,3, Robert G. Endres1,4,*
Biophysical aspects of phagocytosis

1. Size dependence (endocytosis vs phagocytosis)

Herant et al. (2006)

2. Shape dependence

Champion et al. (2006)

3. Elastic properties

4. Ligand density Zipper mechanism

Griffin et al. (1975), Swanson (2008)
Signalling in phagocytosis

Underhill & Ozinsky (2002)

Allen & Aderem (1996)
Objectives and Outline

1. Model of early signalling events in Fc-R phagocytosis
   (Jeroen van Zon, George Tzircotis, Emmanuelle Caron, Martin Howard)

2. Process algebra model of small GTPases and actin polymerization
   (Ozan Kahramanogullari, Luca Cardelli, Philippa Gardner)

3. Early signalling events using RNA interference (RNAi) and imaging
   (George Tzirkotis, Emmanuelle Caron)

4. Extension of model and new experiments (shape dependence)
   (Sylvain Tollis, George Tzircotis, Robert Endres)

5. Collaboration with other subprojects
   (George Tzircotis)

6. Future directions
Imaging of phagocytosis

Phagocytic assay

Time series data of FcR dynamics during uptake (imaging of 3µm particles)

0 min
4 min
10 min

Bead
Actin
FcR
Model of early events in Fc-R phagocytosis

PDE model of phagocytic cup formation couples membrane/cytoskeletal dynamics to receptor diffusion and signalling

Completely determined by bending modulus and cortical tension

Predicts mechanical bottle neck

Cup progression: actual data (grey bars) vs. model (red line)

Half cup is point of max resistance due to cortical tension. If force from actin can overcome this point, then cup completes.

→ Bimodal distribution
Further predictions

Tall/thin particles are phagocytosed, short/flat particles are not phagocytosed

Further experiments using 6µm beads show similar phagocytosis dynamics and excellent agreement with model.
Early signalling events using RNA interference

siRNA screen of Rho GTPases in macrophage cell line

RhoG is a possible universal regulator of phagocytosis – role in FcR and CR3
Phagocytosis confirmed in bone marrow derived macrophages
RhoG is required for actin polymerisation and localises to cups
Hierarchy of small GTPases

Probe for active RhoG localises to both FcR and CR3 phagocytic cups (except for signalling-dead mutant receptors)

GTPase hierarchy: preliminary data using siRNA suggests RhoG is upstream of Cdc42 and Rac2 in FcR, but downstream of RhoA in CR3

Future work:
Use of probes for detection active Rac/Cdc42 and RhoA localisation to further resolve hierarchy of GTPases
The Zipper mechanism

Zipper mechanism:
Unidirectional, sequential ligand-receptor interactions guide membrane around particle

FRAP points towards ratchet-like mechanism

Corbett-Nelson et al. (2006)
Model ingredients

Actin polymerizes at barbed end

Fluoresent speckle microscopy

Membrane energy: bending, surface tension, volume constraint

Ligand-receptor binding

Ji et al. (2008)

Helfrich (1973)
Minimal biophysical model for zipper mechanism

(1) Random, thermal membrane fluctuation.

(2a) If near particle, ligand-receptor binding leads to actin polymerization, stabilizing fluctuation $\rightarrow$ irreversible $\rightarrow$ ratchet.

(2b) If away from particle, no stabilization and membrane fluctuation may be reversed at a later time.
Active versus passive phagocytosis

Active

Passive

→ Active engulfment completes much faster and cups are more regular
Cup shape and variability

Shape

A  High \(K_p\)  Low \(\sigma\)
B  Low \(K_p\)  High \(\sigma\)
C  \(W_{\text{max}} = R_{\text{part}}\)
D  \(W_{\text{max}} = 3R_{\text{part}}\)
E  \(W_{\text{max}} = 4R_{\text{part}}\)

Physical parameters

Simulation parameters

Large variability

A
B
C
Experimental verification of predictions

Measure cup shape and variability from confocal images of wild-type (active), signalling mutant (active/passive), and cytochalasin-D treated cells (passive).

Preliminary data analysis of wild-type receptor:
Future directions

1. Shape dependence
2. Mechano-sensitivity during phagocytosis (squeezing of particle, soft vs stiff)
3. Later stages of phagocytosis (actin belt, motor proteins)
4. Zipper-like engulfment in other areas of biology

Collaboration with other subprojects

Sub-project 1 – Phagocytosis of *Campylobacter* – effect of glycosylation mutants (Emily Kay)

Sub-project 3 – Notch signalling and phagocytosis. Effect of Jgd stimulation on uptake (Anna Rose)
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