Mechanically Induced Catalytic Amplification Reaction for Readout of Receptor-Mediated Cellular Forces

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Motivation

The advent of PCR and ELISA have revolutionized molecular biology over the past few decades. However, similar tools to study the physical aspect of cells do not exist. A new technique to catalytically translate forces generated by a cell receptor into an easily quantifiable, amplified chemical signal (output) may bridge this gap, and development of such a technique would facilitate screening of drug candidates that impair cell mechanics, rather than cell viability.

Mechanical force

Conformational change of the DNA “spring”

Catalytic reaction


Approach – Mechanically Induced Catalytic Amplification Reaction (MCR)

i) Primer Amplification is Highly Specific on substrates

- Non-blocked primer or scramble ssDNA was fabricated on substrate and subjected to MCR or fluorescent in situ hybridization.
- An enhancement factor of 102±4 was observed when comparing the sample amplified by MCR with the non-amplified sample (FISH)

- Integrin mediated forces generated a high density of exposed primers available for readout through MCR. Controls showed significantly lower signal compared to 12 pN and 56 pN duplexes

- Two chemically identical duplexes with different mechanical tolerances were immobilized on substrates and labelled with cRGDfK to support cell adhesion

- Loss in Cy3B signal closely matched the cell adhesion footprint as indicated by RICM

- Cells ruptured more of the 12 pN duplexes on the substrates

ii) Integrin Tension is Sufficient to Mechanically Expose the Surface Immobilized Primers

- Integrin mediated forces generated a high density of exposed primers available for readout through MCR. Controls showed significantly lower signal compared to 12 pN and 56 pN duplexes

- Blebbistatin, a non-muscle myosin II ATPase inhibitor, inhibits the MCR signal in a dose-dependent manner

- Blocking different integrins using antibodies showed differential MCR response

iii) Integrin Tension is Sufficient to Mechanically Expose the Surface Immobilized Primers

Results

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iv) MCR signal reports Inhibitor and Antibodies that Impair Integrin Tension

Acknowledgement

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...check out my other work in using DNA-based tension probes to study T cells