Using Advanced Live Imaging to Visualize How the Embryo Forms in Real Time

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LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:

- Describe how imaging techniques can be used to reveal mechanism controlling embryonic development.
- Identify key dynamic processes and morphogenetic events patterning the early mammalian embryo.

DISCLOSURE

- I have no commercial and financial relationships with commercial or financial interests and/or other relationships with manufacturers of pharmaceuticals, laboratory supplies, and/or medical devices.
Imaging the Dynamics that Form the Preimplantation Embryo

Nicolas Plachta

The Challenge

Early Mouse Embryo

Human embryo
Transcription Factors & Cell Fate

Oct4  Sox2  Nanog
Cdx2  Eomes  Tead4  Gata4/6

Levels & Lineages Uncorrelated
TF Function
Levels
Dynamics

Plachta et al.
Nat Cell Biol (2011)

TF–DNA Binding Model
Oct4-paGFP

Photo-Activatable FCS

Correlation time
Correlation

Diffusion
Fractions
Brownian

Fluctuations
Time

Kaur et al. Nature Communications (2013)

TF–DNA Dynamics In Vivo

Normalized G(t)

0.4

3D diffusion

Fit

Non-specific

Specific

White et al. Cell (2016)

First Differences at 4-Cell Stage

White et al. Cell (2016)
Sox2–DNA Binding Predicts Fate

More Sox2–DNA
Less Sox2–DNA

White et al., Cell (2016)

summary

New ways to reveal TF–DNA binding in vivo

Histone methylation controls TF–DNA binding

TF–DNA binding predicts fate

Cell Shape

Embryo Compaction
Filopodia in Living Embryo

E-cad–GFP

10 μm

Fierro-Gonzalez et al.

SEM

Filopodia in Living Embryo

E-cad–GFP

45 min

Fierro-Gonzalez et al.

F-actin

Membrane

SEM

Fierro-Gonzalez et al.
Filopodia Grow when Cells Elongate

Filopodia Retract when Cells Divide
Pre 5 μm Filopodia Ablation Releases Tension

Post 2D live embryo

E-cad Controls Filopodia, Shape & Compaction

Control E-cad siRNA

Injected cells Filopodia control compaction

In vivo system to study filopodia
Spatially Orientated Divisions

Membrane Segmentation

Apical Constriction Forms Inner Mass

Mechanical Forces?

How to Measure Tension?
Cell Polarity Model

"...polarity is asymmetric inherited..."
Actin Rings Zipper Along Junctions

Mouse

Human

cavity
cavity

F-actin
Zippering Forms Tight Junctions

GFP-ZO1 / RFP-Utrophin

Cell (2018)

Cell (2018)
How Microtubules Organize In Vivo?

Many cells

Mouse embryo

Centrosome

MTOC

DNA

α-tubulin

Microtubule bridge
**summary**

Sister cells connected by bridge

Bridge is a non-centrosomal MTOC

MTOC directs transport