Artificial cells with Xenopus leavis eggs cell-free extracts

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SCOPE OF THE METHOD

The Method relates to	Other: Cell cycle fundamental research
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	Xenopus laevis frog
Type of cells/tissues/organs	eggs

DESCRIPTION

Method keywords

Microfluidic devices
droplets
eggs
cell free extracts
microemulsion
ultracentrifuge
fluorescence microscope
timelapse

Scientific area keywords

cell biology
cell cycle
Droplet-based microfluidics
cytoskeleton

Method description

The large (1mm diameter), easily accessible eggs of the frog Xenopus laevis (100-1000 eggs at once per frog) offer the opportunity to reconstitute cell cycle events *in-vitro* by generating cell-free extracts which retain all the biochemical components that regulate cell cycle progression, as well as all the organelles and cytoskeletal networks. In summary, ovulation is induced in frogs by subcutaneous injection of chorionic gonadotropin. After about 16 hours, eggs are collected, inspected for quality, washed, and processed using several centrifugation steps to obtain the cytoplasmic cell-free extract. Extracts can either be arrested in a cell-cycle phase, or being cycling (i.e., oscillating between interphase and mitosis). Cycling extracts are obtained by activating the eggs with calcium ionophore, which mimics fertilization and activates the biochemical processes of the cell cycle. To mimic cellular behavior, experiments require cell-sized compartments with realistic shapes and boundaries. This is achieved in two distinct ways: (i) by encapsulating extracts in surfactant-stabilized droplets, termed artificial cells, formed by vortexing frog egg extracts with surfactants and oil; (ii) by generating droplets using droplets-microfluidics. Cell cycle event are observed and recorded using timelapse fluorescence microscopy.

Lab equipment

Cell-free extract preparation:
- ultracentrifuge

Droplets generation:
- vortex or
- microfluidic chips for droplets production
- microfluidic pumps
- microscope with high-speed camera for tracking droplets production

Imaging:
- fluorescence microscope for timelapse
- multichannel
- multipoint microscopy

Method status

History of use
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- Frogs are injected with hormones once every 3 months with a minimally invasive procedure,
- Frogs can be kept in the lab for 5 years (or longer if quality of the eggs is not compromised),
- 100-1000 eggs per frog allows to obtain up to 2 mL of cell-free extract,

- Cell cycle events in cycling cell-free extracts are fast. A cell cycle lasts about 30min/1h (this is because in the early embryo cellular cleavages have a period of 30 min). When imaging for 18h, several cell cycle events can be observed,
- This method is well established since the '80s, and there is a lot of literature.

Challenges

- Apoptotic eggs must be removed to avoid compromising the experiment and obtaining a non-functional extract,
- During heatwaves it is recommended to work in temperature controlled rooms, because the cell-free extract quickly becomes apoptotic above 25 degrees,
- During heatwaves the quality of eggs may drop, as well as their quantity, even though the temperature at which frogs are housed is kept under control all year long.

Future & Other applications

Cell free extracts are not only used for studying cell cycle regulation. They have been used also for studying cytoskeletal structures (e.g., mitotic spindles).

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

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