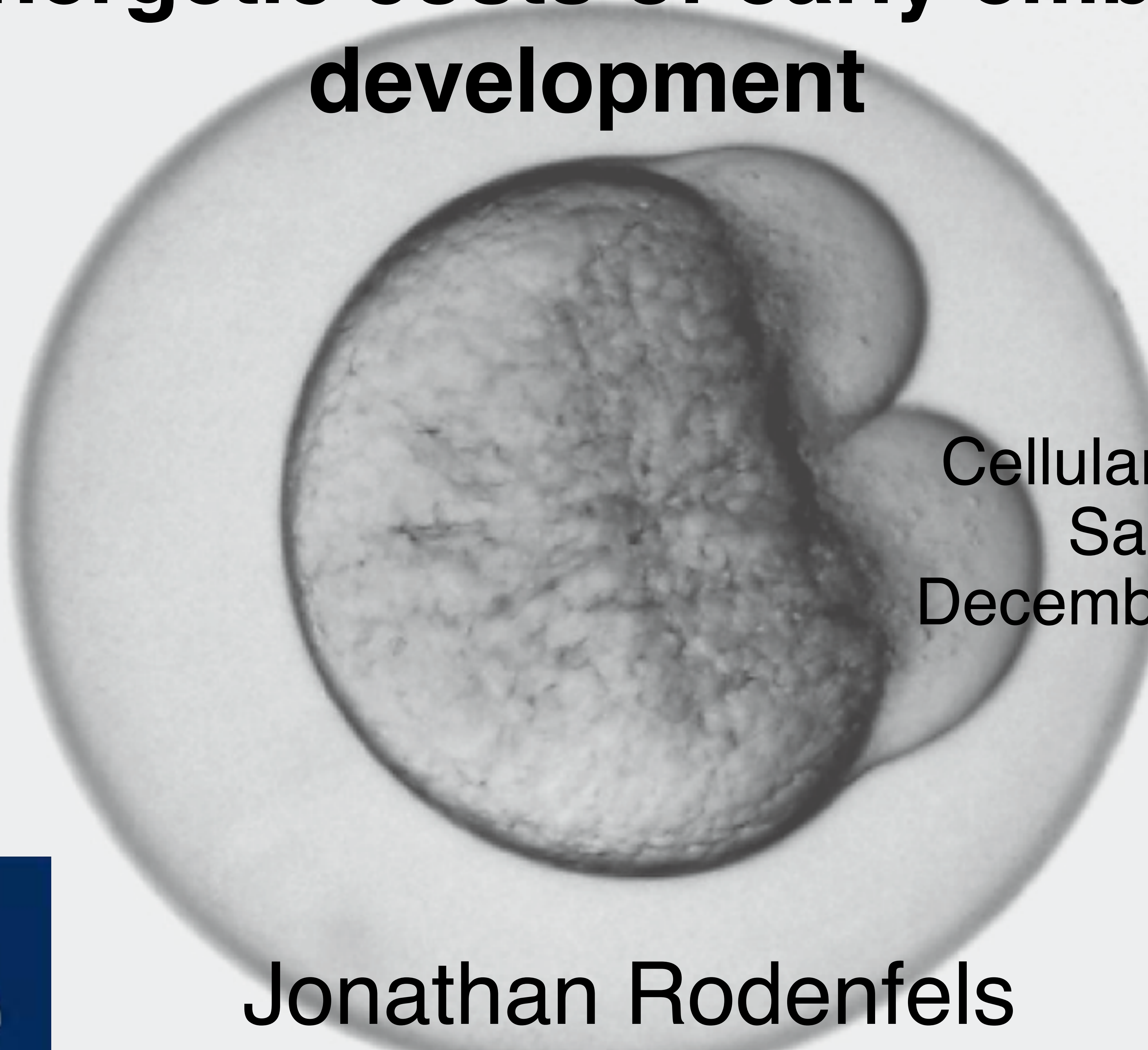
The energetic costs of early embryonic development



KITP
Cellular Energetics
Santa Barbara
December 10, 2019

Jonathan Rodenfels
Molecular Biophysics & Biochemistry
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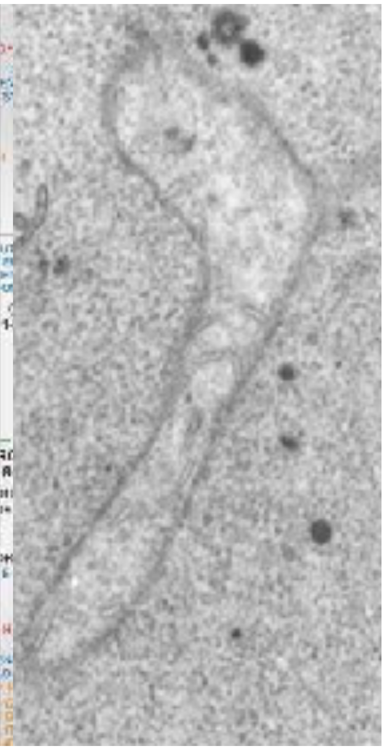
Yale UNIVERSITY

EMBO

NIH

Karla Neugebauer

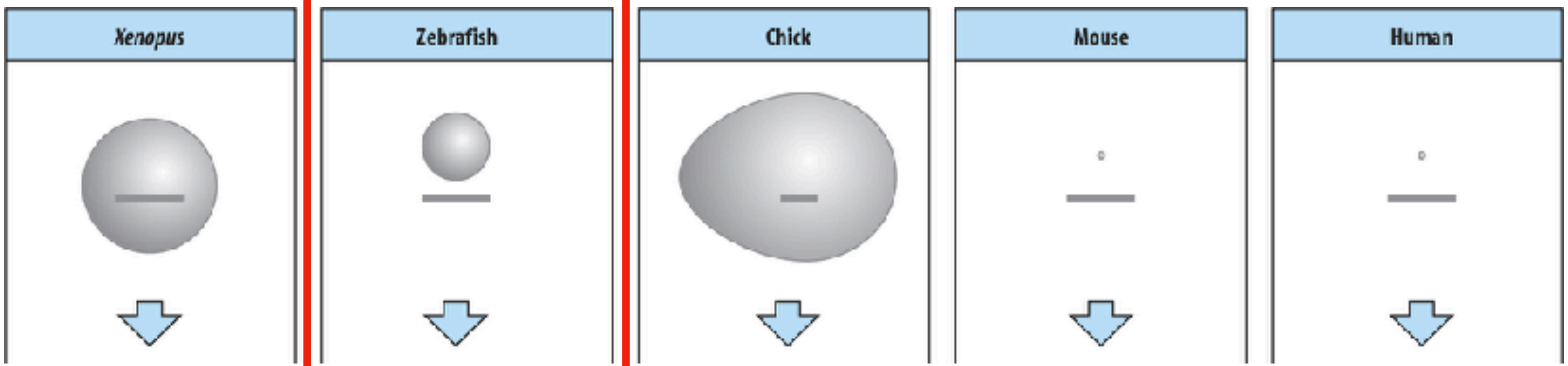
Jonathon Howard




Outline

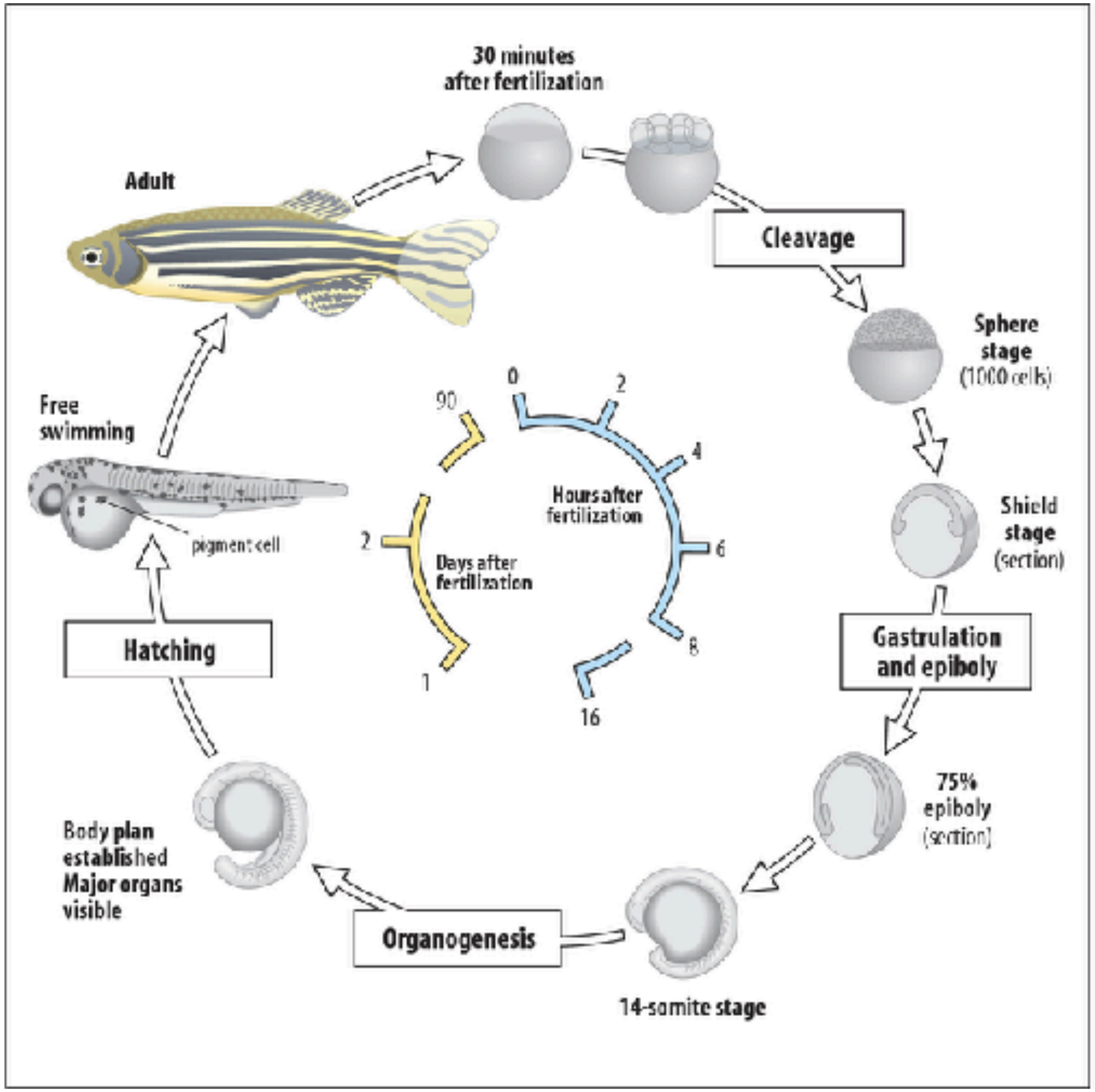
1. Developmental biology- An introduction
2. Metabolism and development
3. Calorimetry and the energetic costs of early embryonic development

Developmental biology - An introduction



Differential cellular behaviors (division, differentiation, growth, patterning, movement) → **The emergence of organized structures (tissues, organs,…)**

Development biology - An introduction



Event (E): Cleavage
Principle (P): Cell division
Outcome (O): Multicellularity

E/P: Growth
O: Maturity

E: Gastrulation
P: Morphogenesis/
Developmental mechanics
O: Formation of
3 germ layers

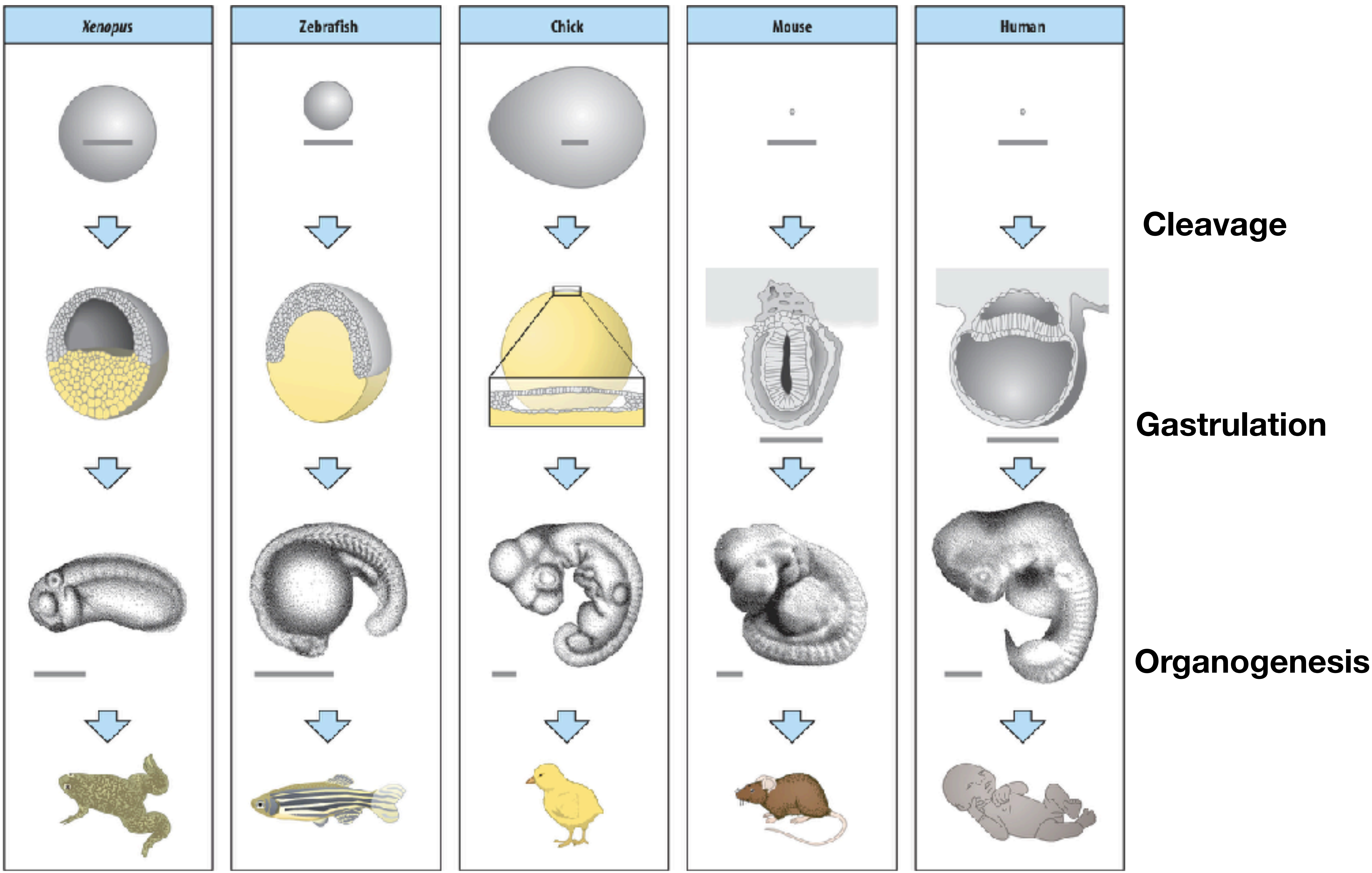
E: Organogenesis
P: Cell differentiation
O: (Muscle, Blood, nerves, etc..)

8 s counter:

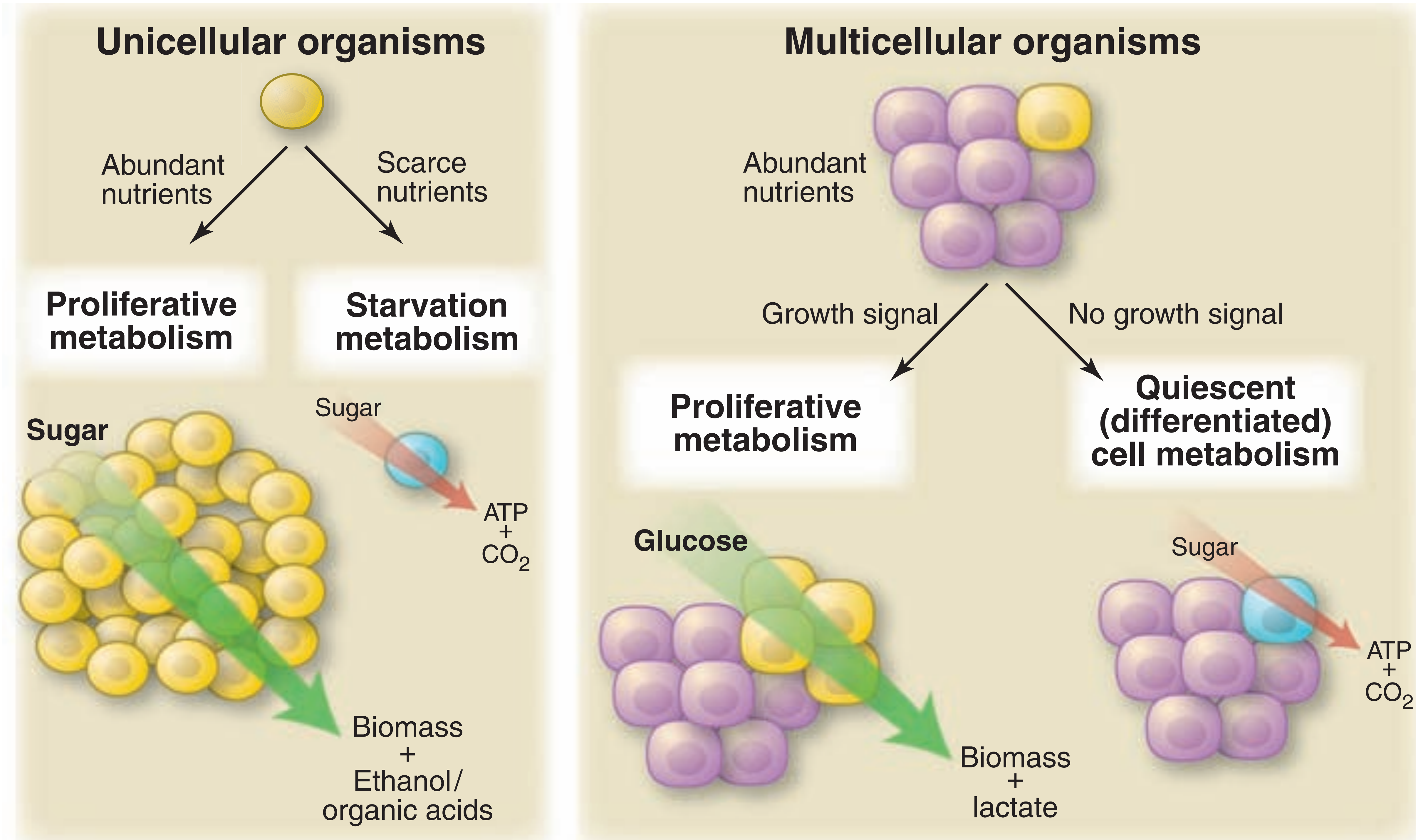
Time is from 0:45 to 17:00 hrs post fertilization (nonlinear replay speed)

Movie: RO Karlstrom & DA Kane, Development 1996

Development biology - An introduction

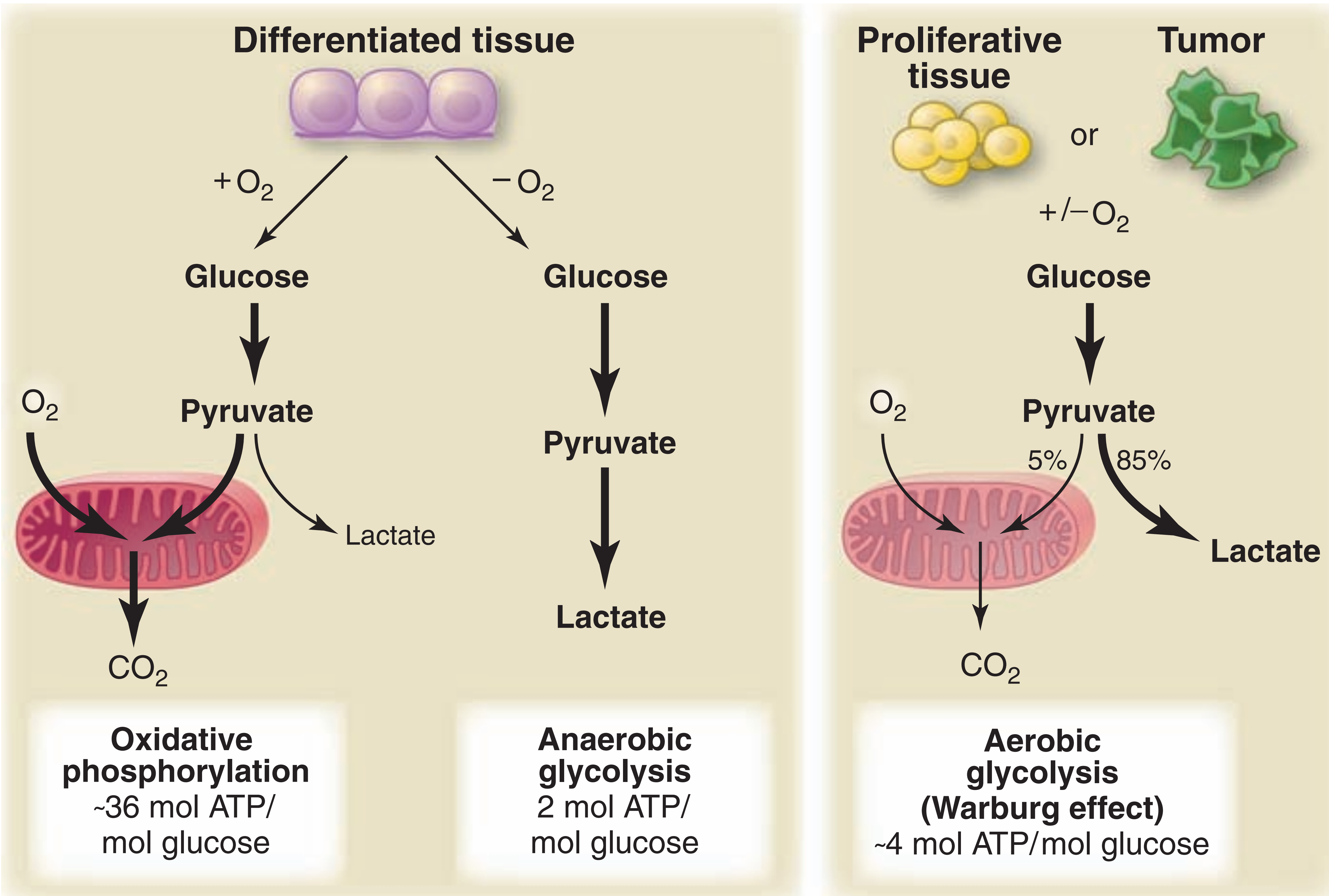


Regulation of metabolism in time and space



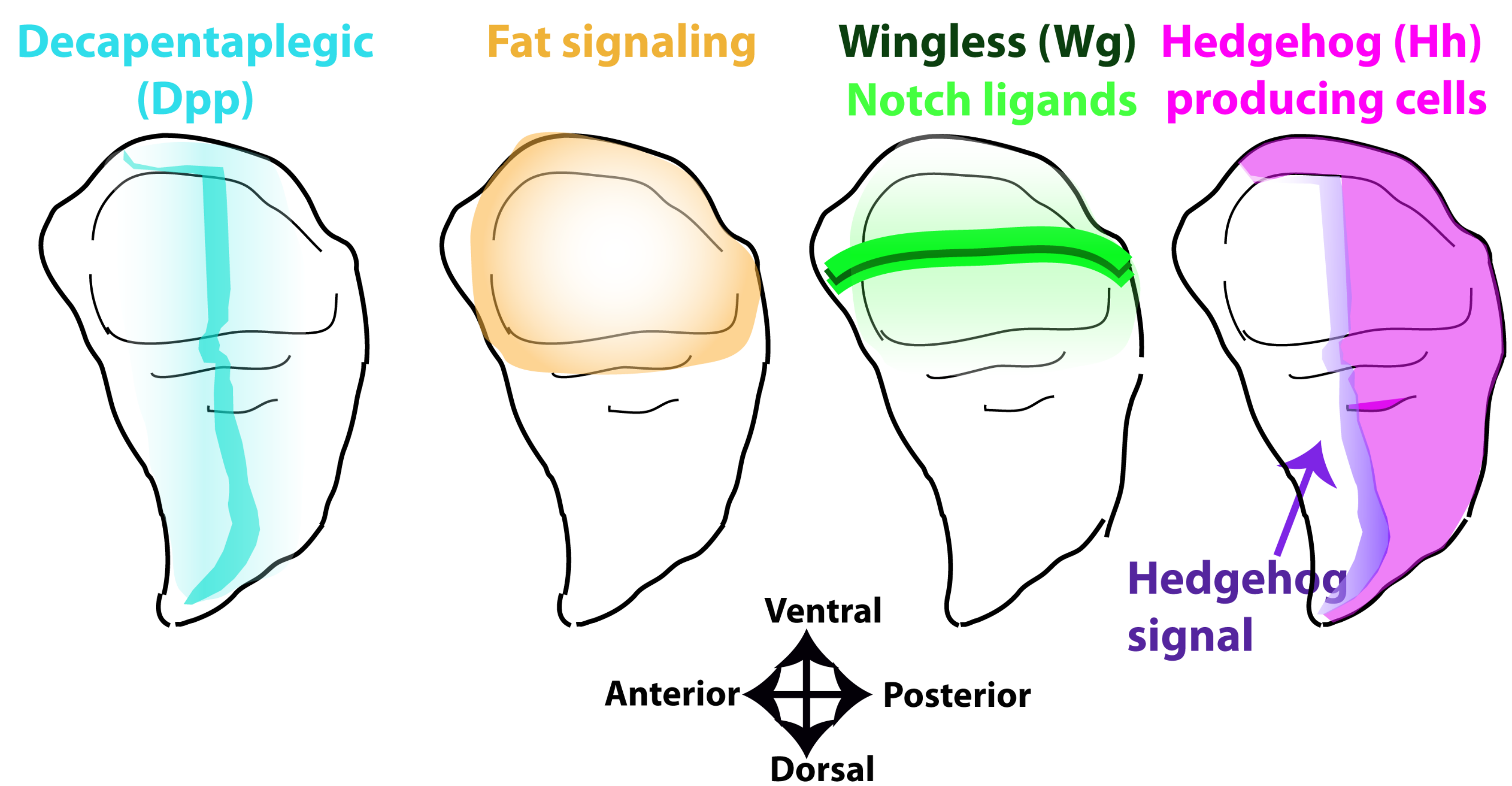
Vander Heiden et al. 2009

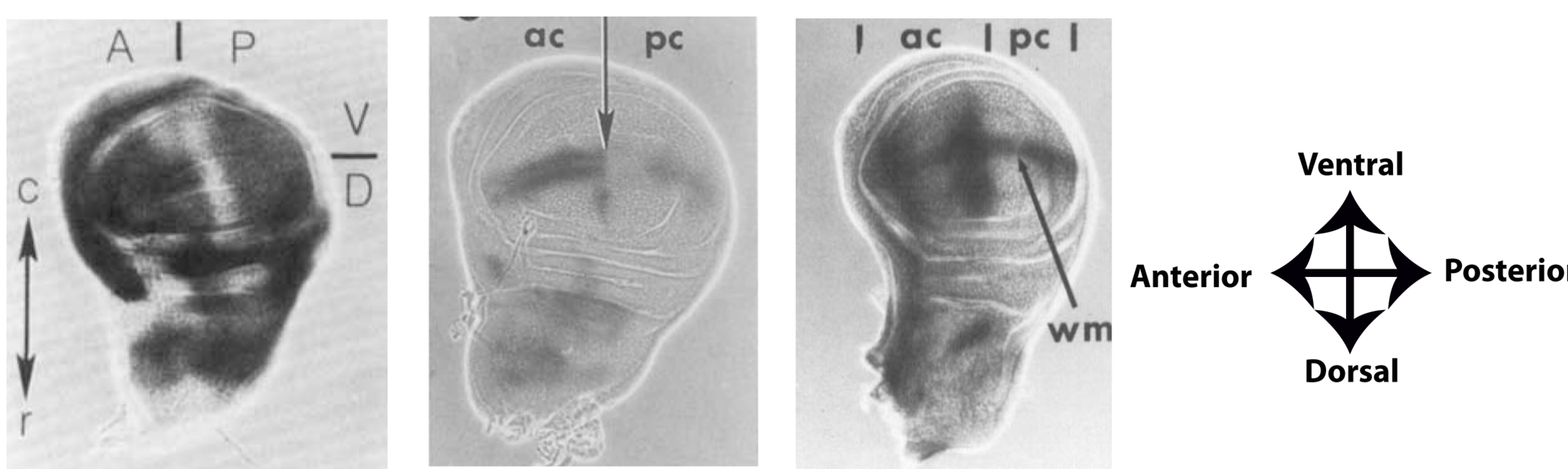
Regulation of metabolism in time and space



Vander Heiden et al. 2009

Regulation of metabolism in time and space

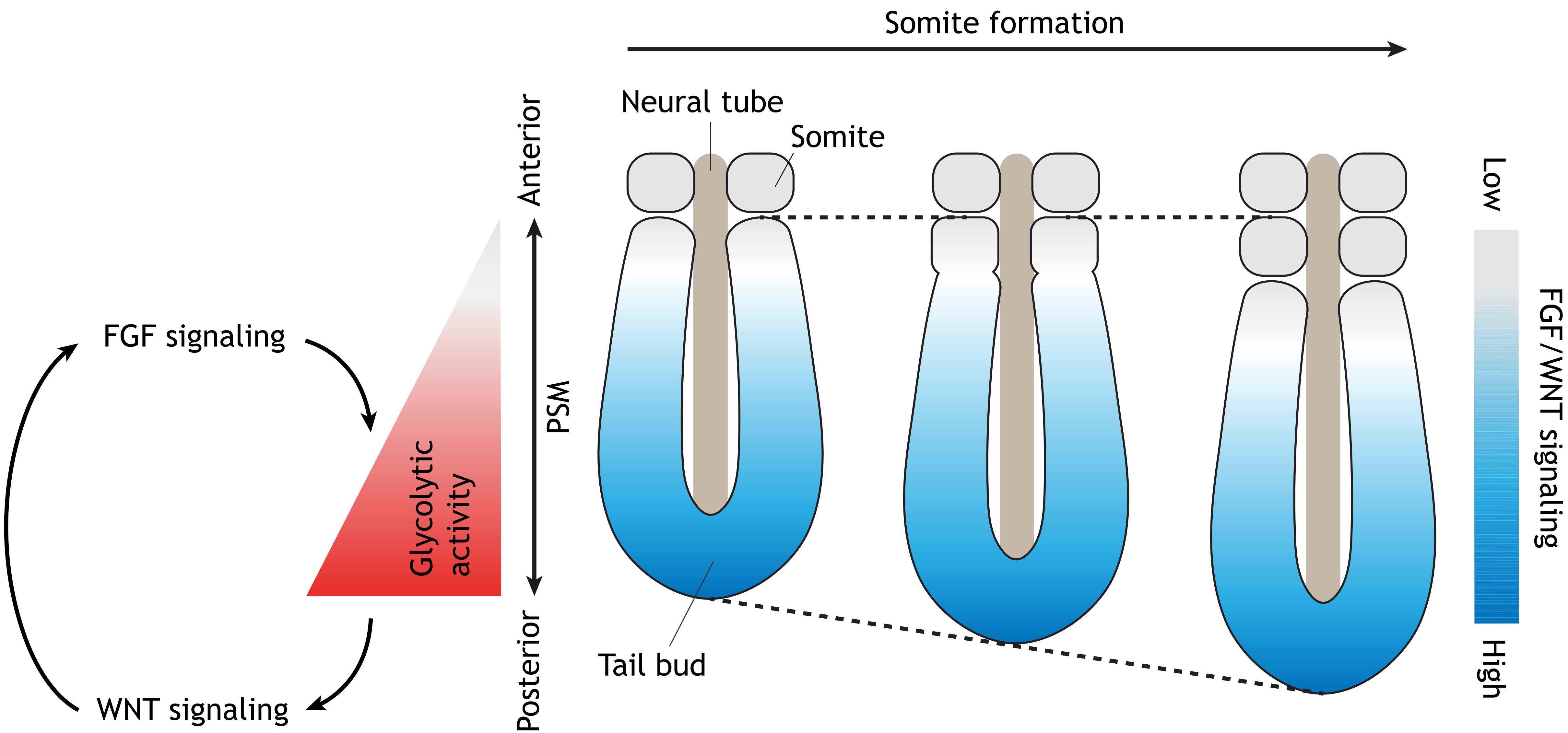




IDH1 G6PD and 6PGD Aldehyde Oxidase

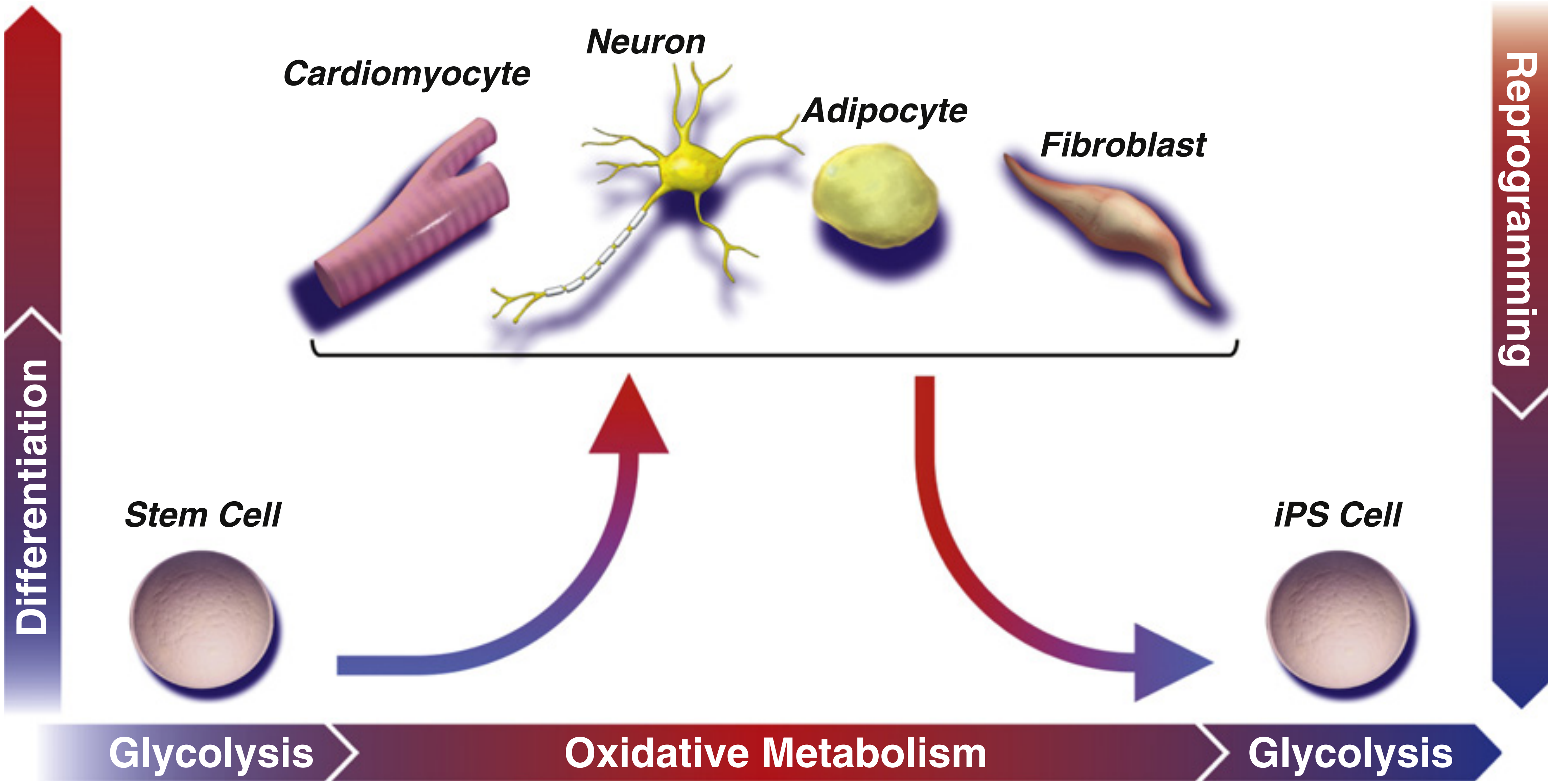
Eaton S, personal communication, Cunningham et al. 1983, Cunningham & Kuhn, 1977, 1986

Regulation of metabolism in time and space



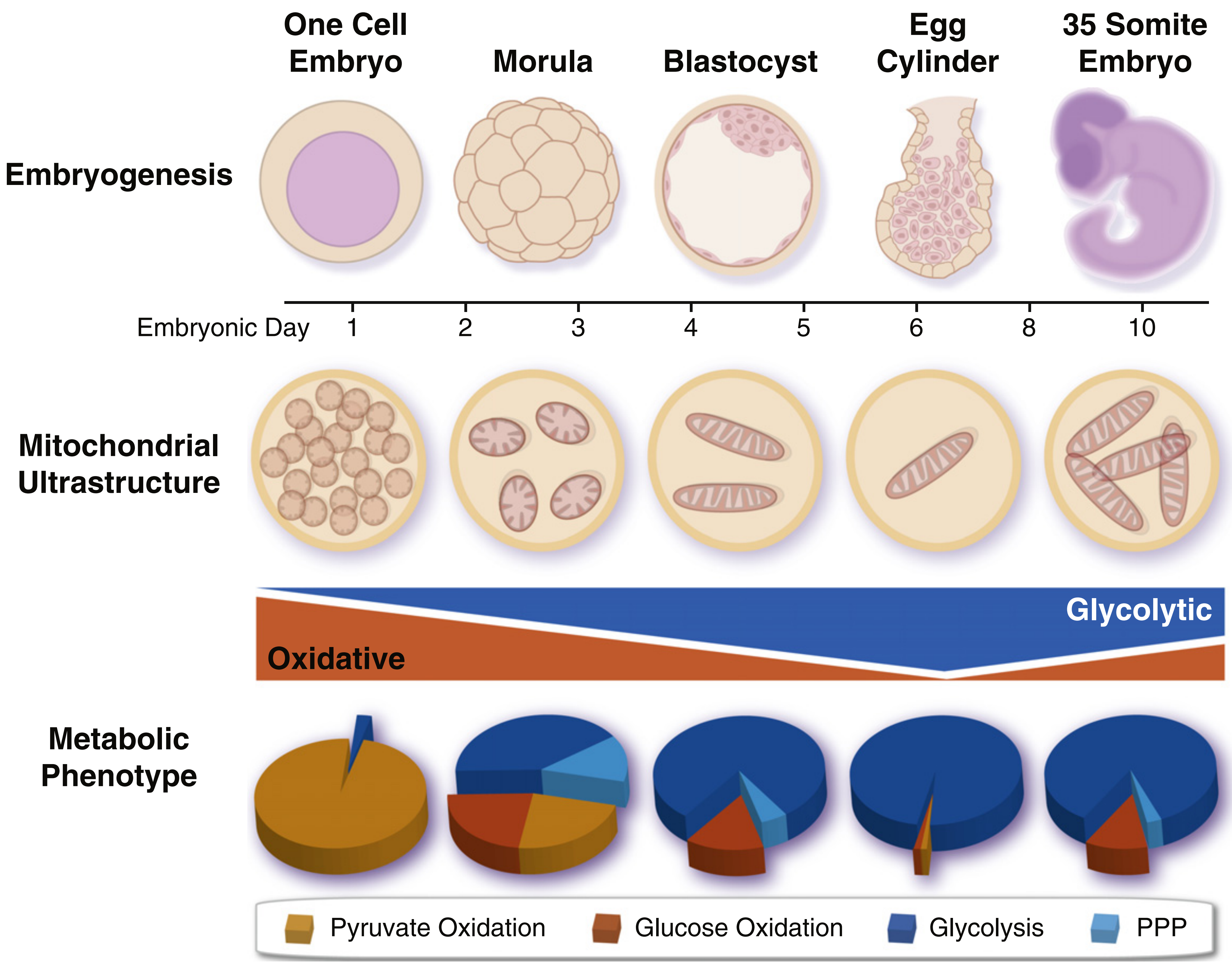
Miyazawa et al. 2018

Regulation of metabolism in time and space



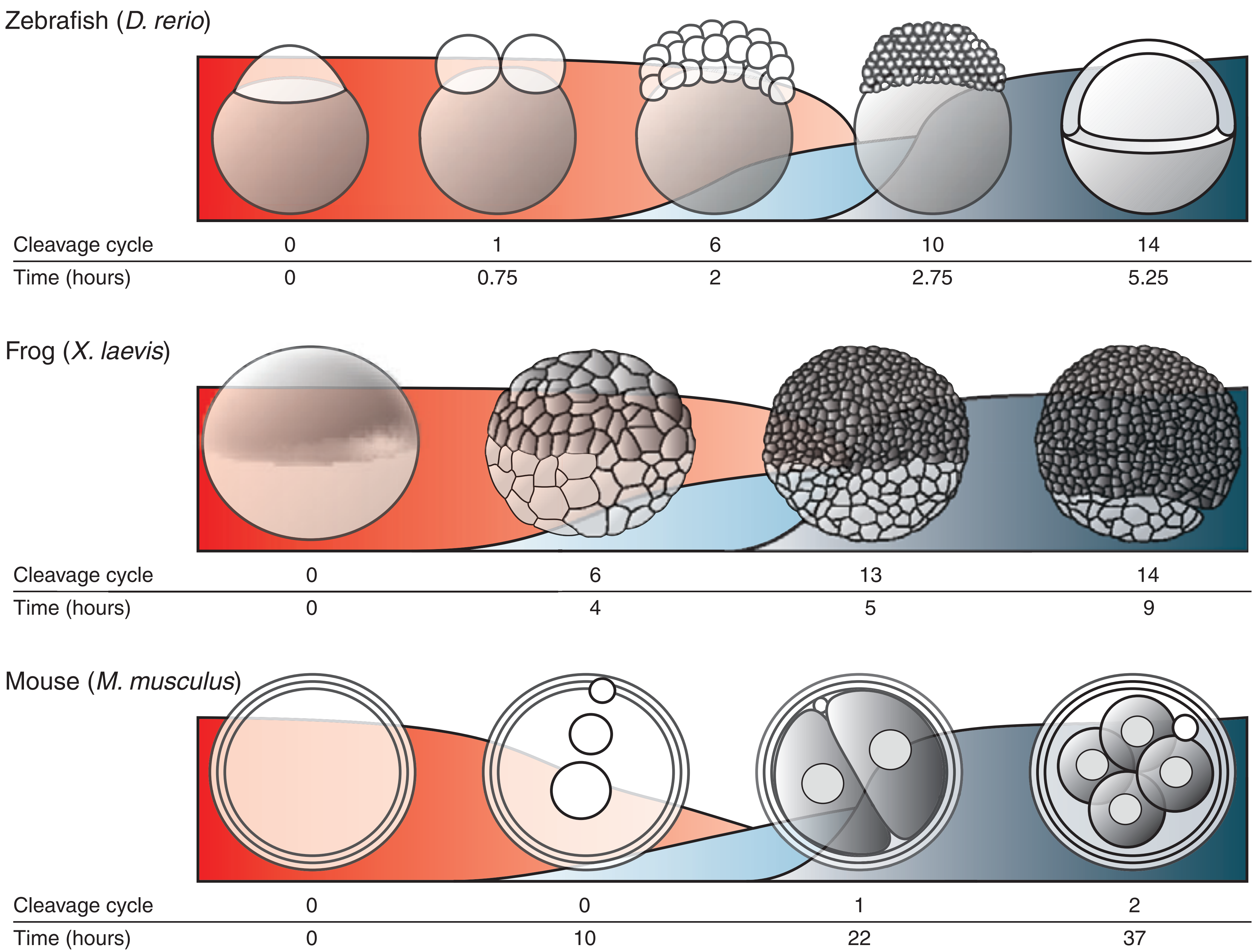
Folmes et al. 2012

Metabolism during early embryonic development



Folmes et al. 2012

Zygotic genome activation



Tadros & Lipshitz et al. 2009

RESOURCE | VOLUME 149, ISSUE 6, P1393-1406, JUNE 08, 2012

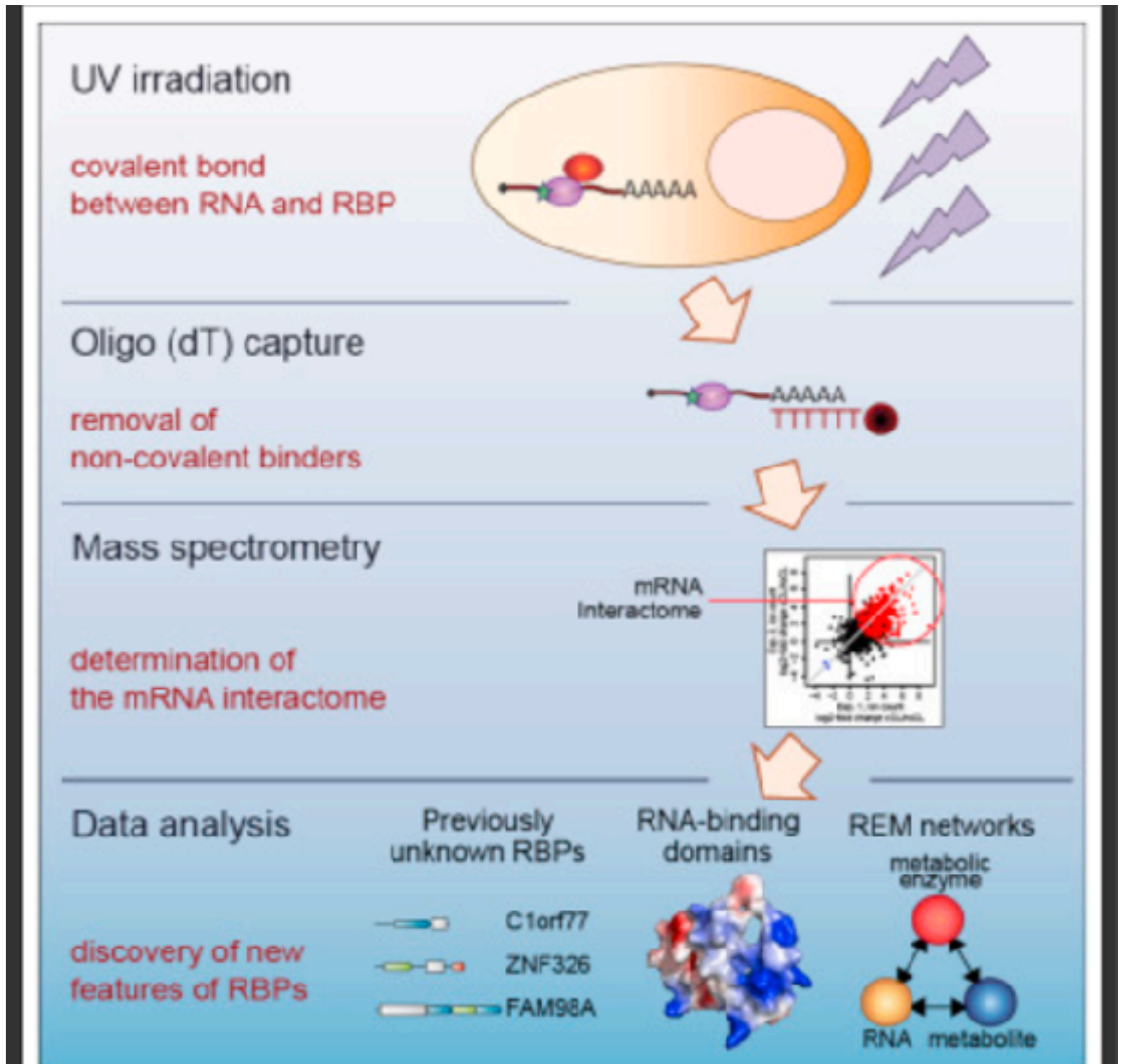
PDF Figures Save Share Reprints Request

Insights into RNA Biology from an Atlas of Mammalian mRNA-Binding Proteins

Alfredo Castello [1] • Bernd Fischer [1] • Katrin Eichelbaum • ... Lars M. Steinmetz • Jeroen Krijgsveld • Matthias W. Hentze • Show all authors • Show footnotes

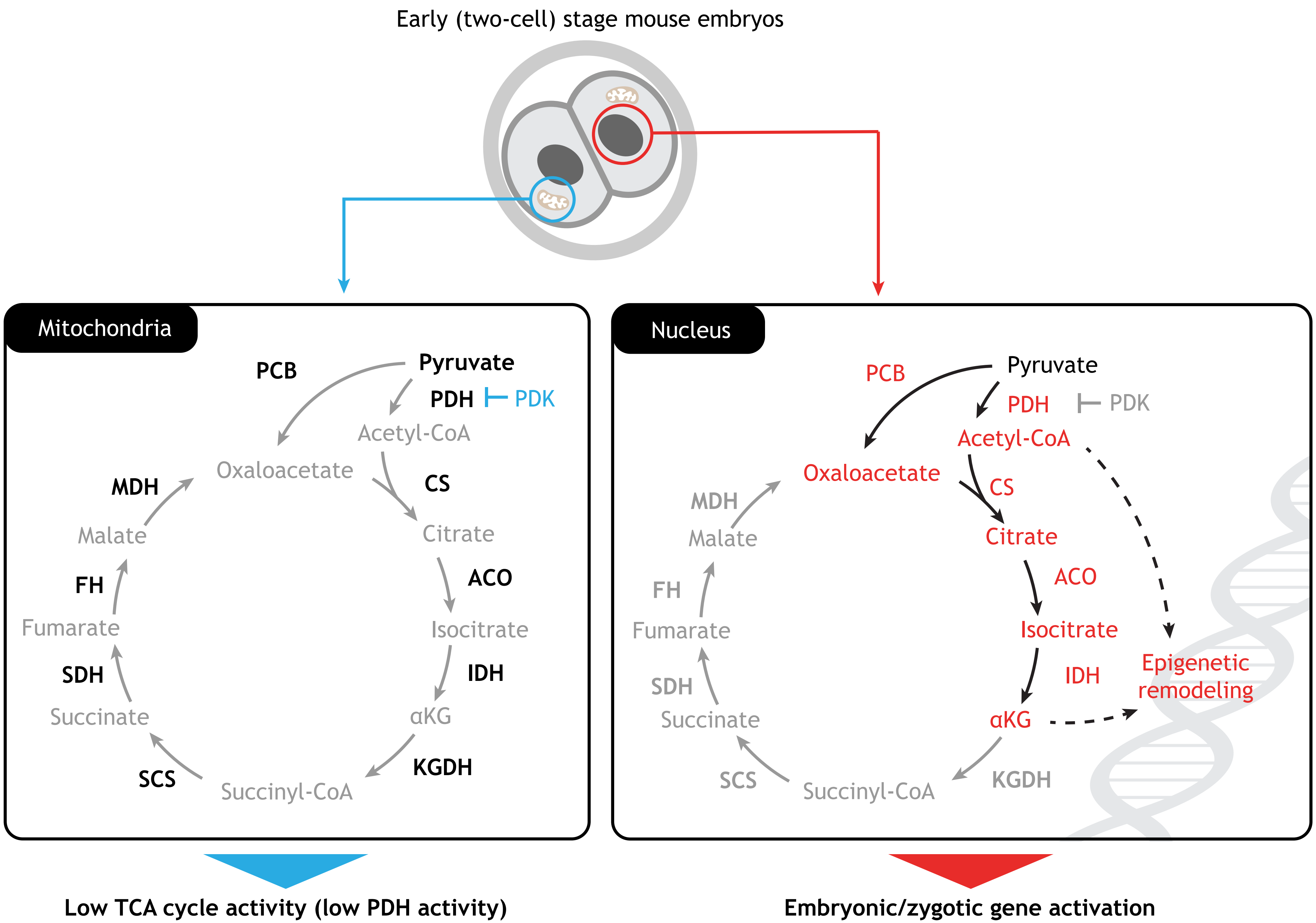
Open Archive • Published: May 31, 2012 • DOI: https://doi.org/10.1016/j.cell.2012.04.031

PlumX Metrics



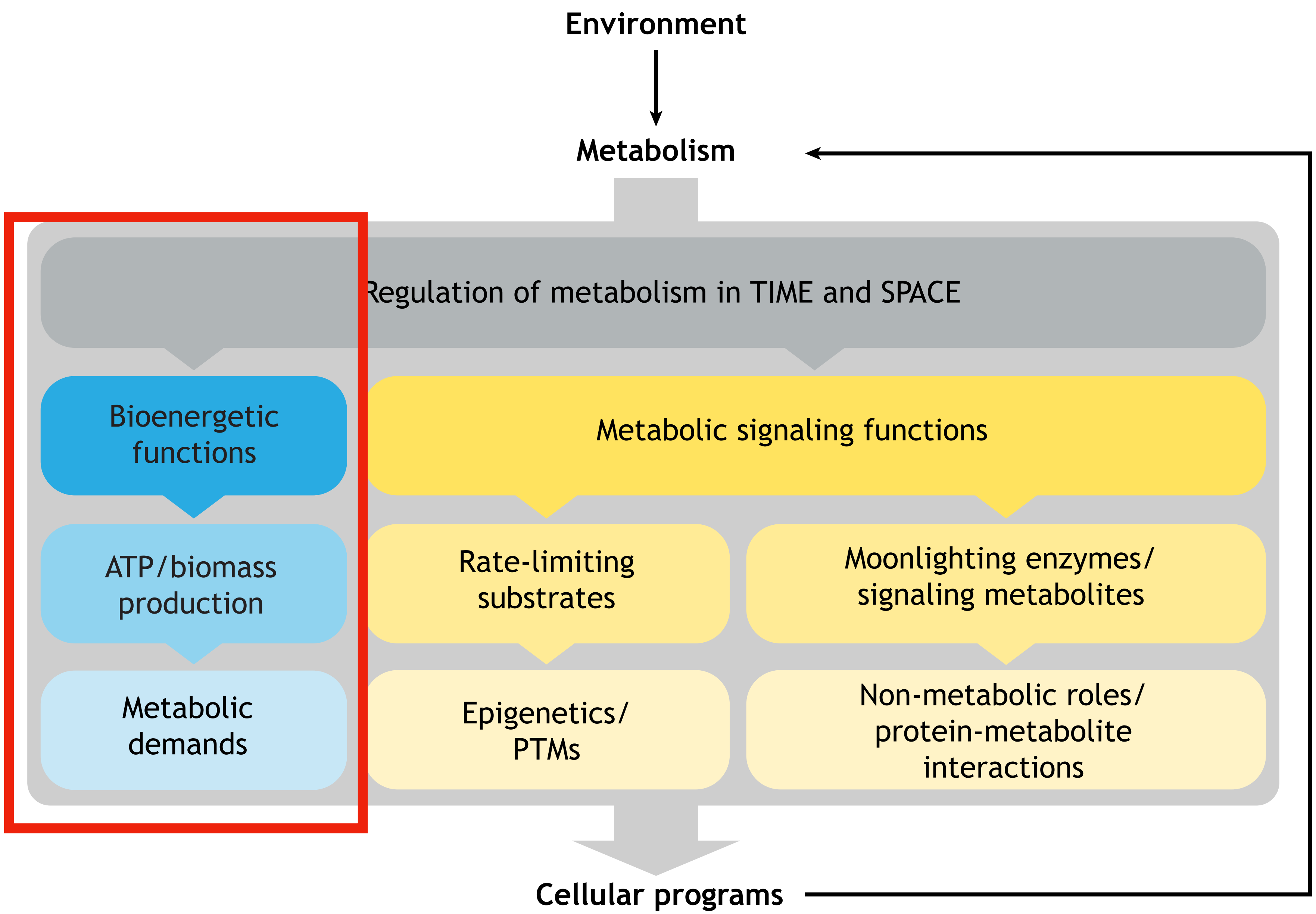
Castello et al. 2012

Metabolic signaling function during zygotic genome activation



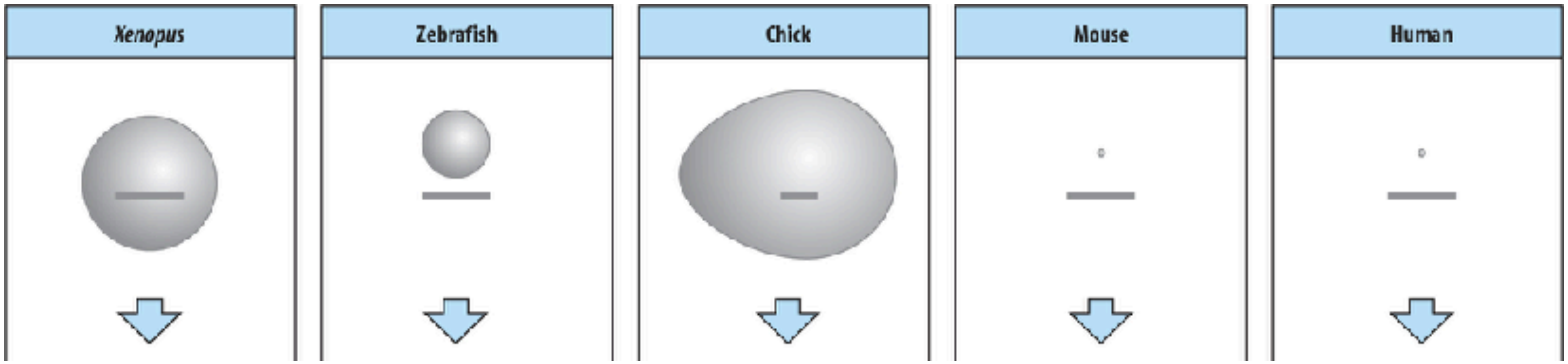
Miyazawa et al. 2018

Regulation of metabolism in time and space



An emerging view emphasizes that bioenergetic activities are highly regulated, in both time and space, in order to match context- dependent cellular demands (Vander Heiden and DeBerardinis, 2017). …(2 sentences about Warburg metabolism)….Nonetheless, this intricate regulation of a particular metabolic program exemplifies the tight adjustment of energy metabolism to specific metabolic demands.

Miyazawa et al. 2018

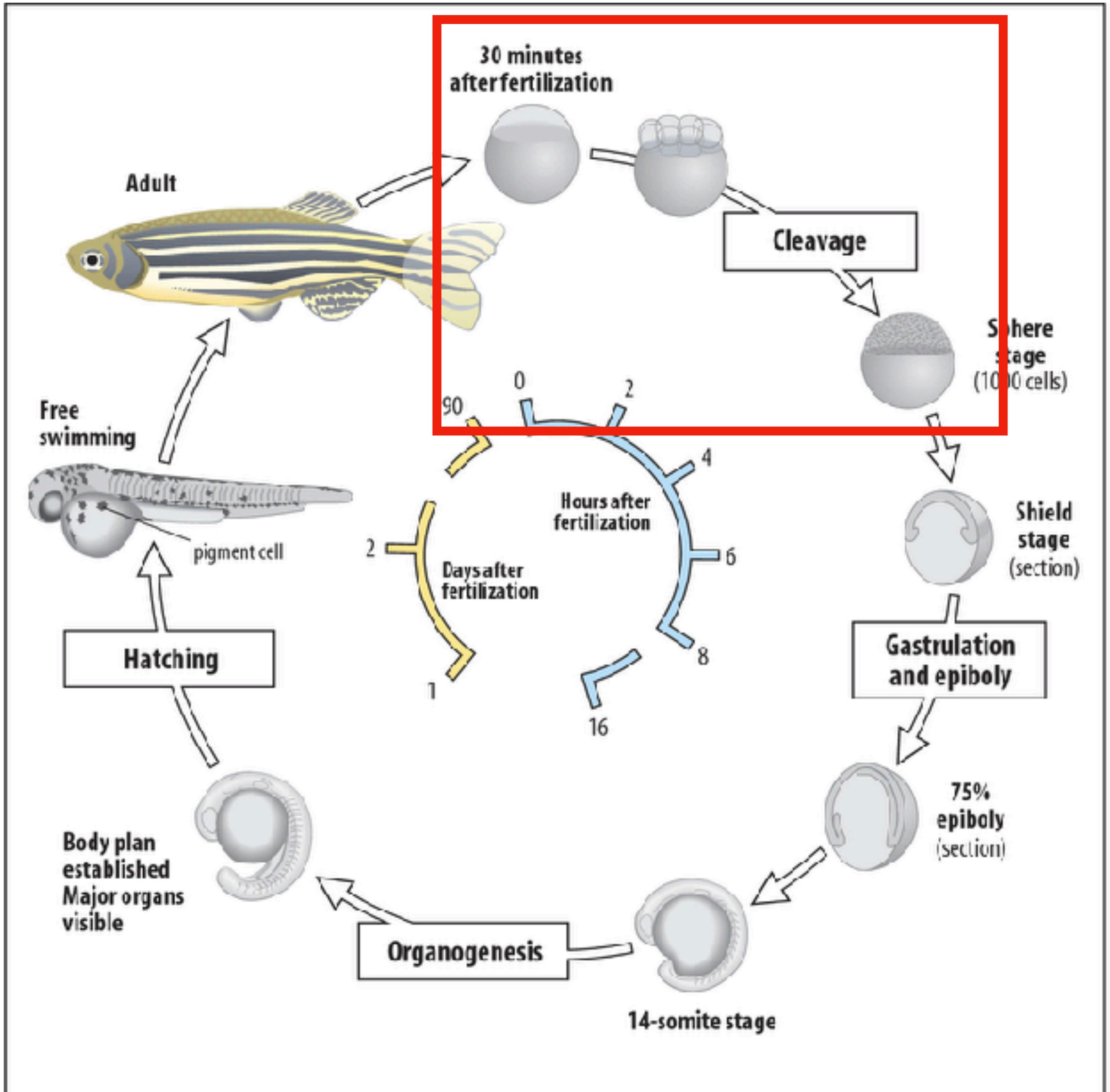


What is the free energy cost of a single cell developing into a complex multicellular organism?

&

How does cellular metabolism fulfill this cost and “drive” development?

Cleavage stage - A model system to study the energetics of embryonic development

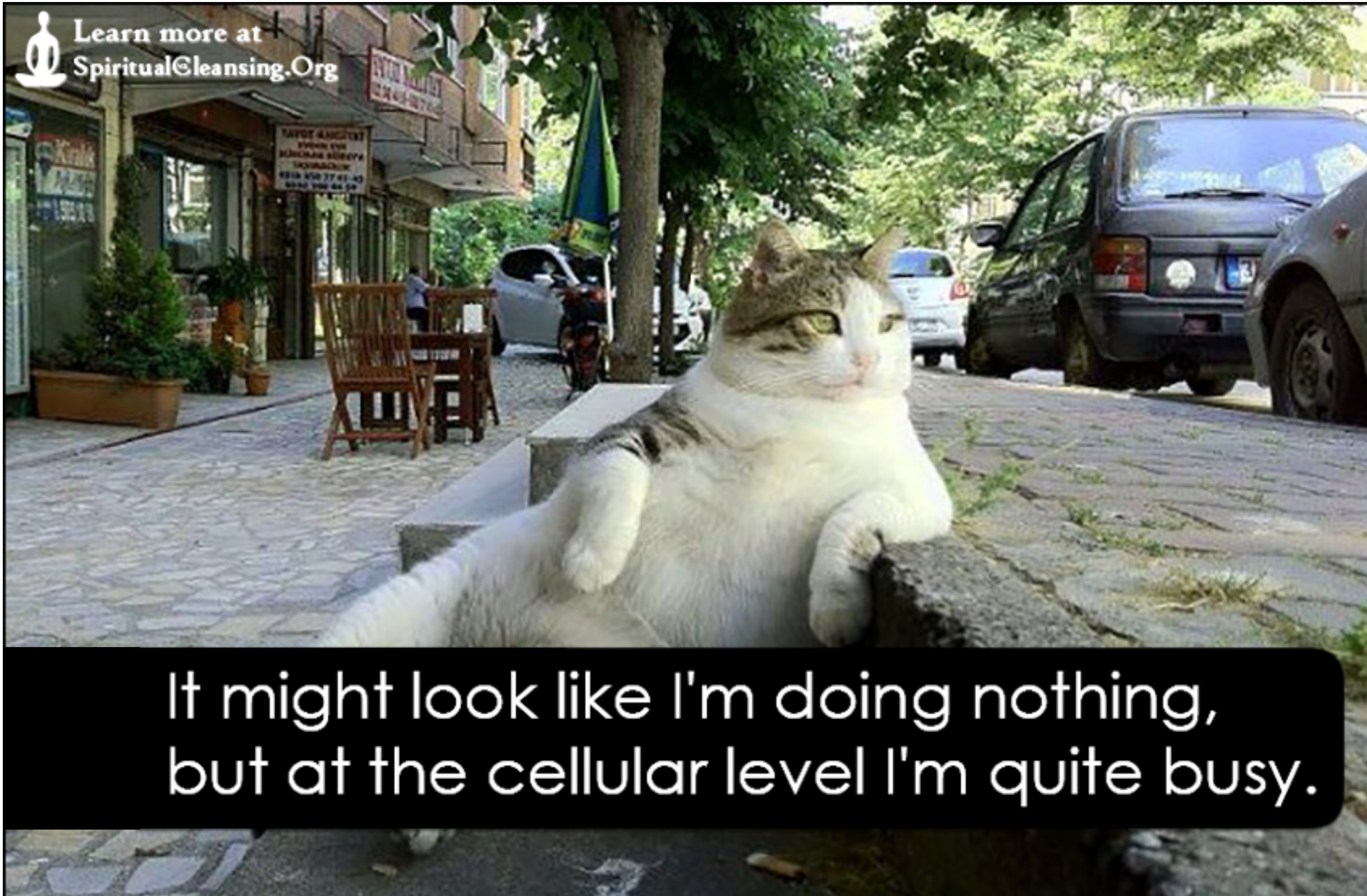


Event (E): Cleavage
Principle (P): Cell division
Outcome (O): Multicellularity

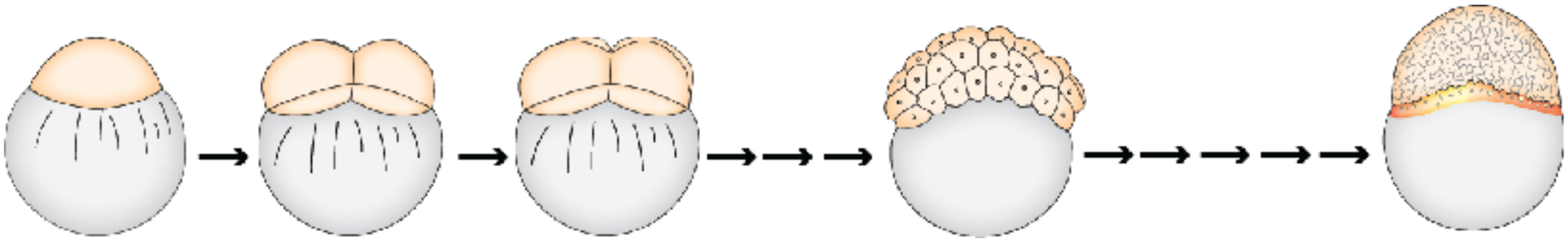
Reductive cleavage stage
(Early divisions without volumetric growth)

Synchronous up to the tenth division

Movie: RO Karlstrom & DA Kane, Development 1996


Learn more at
SpiritualCleansing.Org
It might look like I'm doing nothing,
but at the cellular level I'm quite busy.

What keeps the embryo busy during cleavage stage?

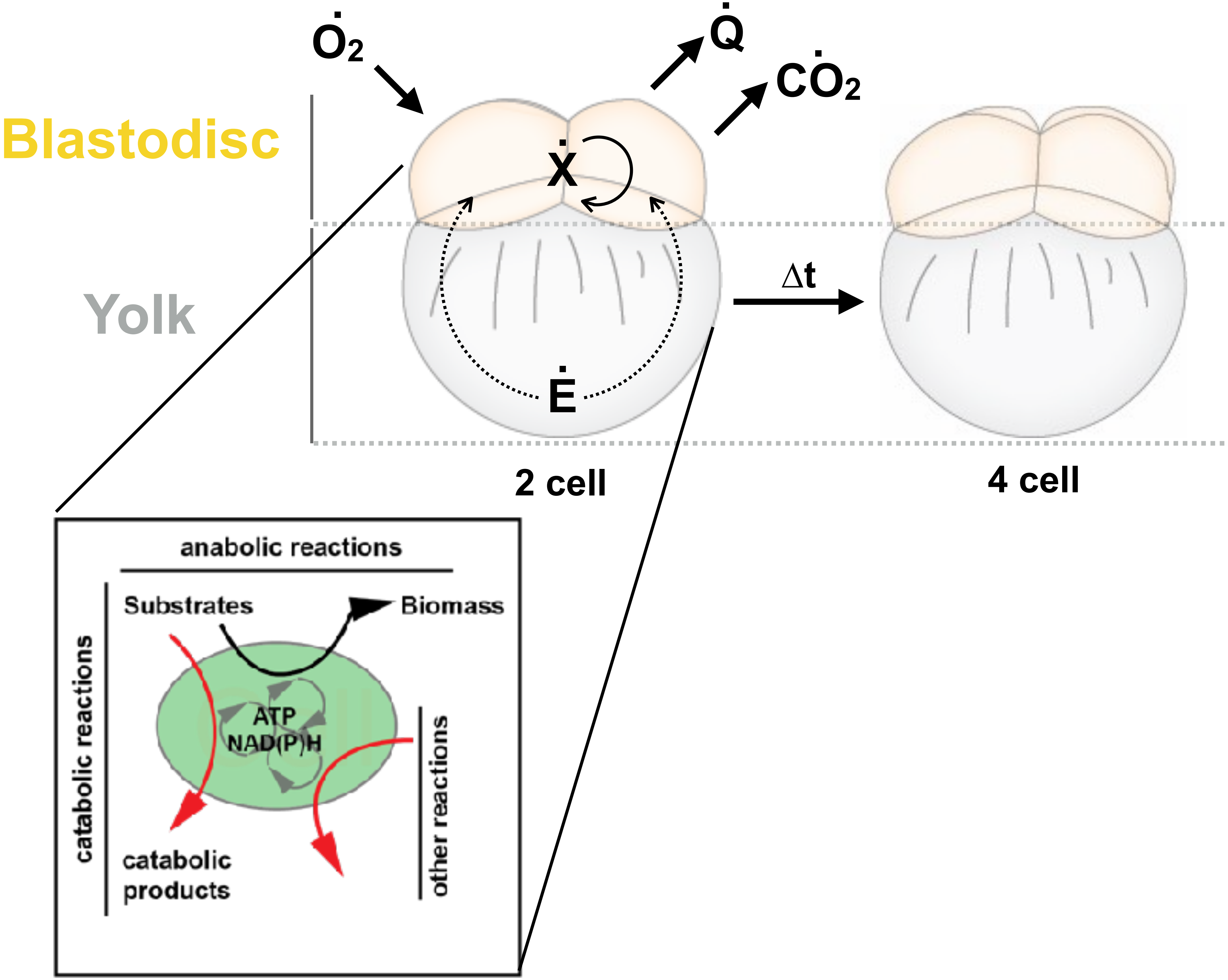


Making more cells!!!

- Embryonic volume is ~ constant
- synchronous cleavage divisions ~ every 15 min until cycle 10 (~1000 cells stage)
- Minimal cell cycle comprised of DNA-replication (S-phase) and chromosome segregation (M-phase)
  - DNA replication (exponential increase in genome number)
  - assemble and disassemble cellular machineries (chromatin, spindles)
  - generate forces
- Increase in plasma membrane
- Protein assembly/synthesis
- zygotic genome activation (start of embryonic transcription)
- ….

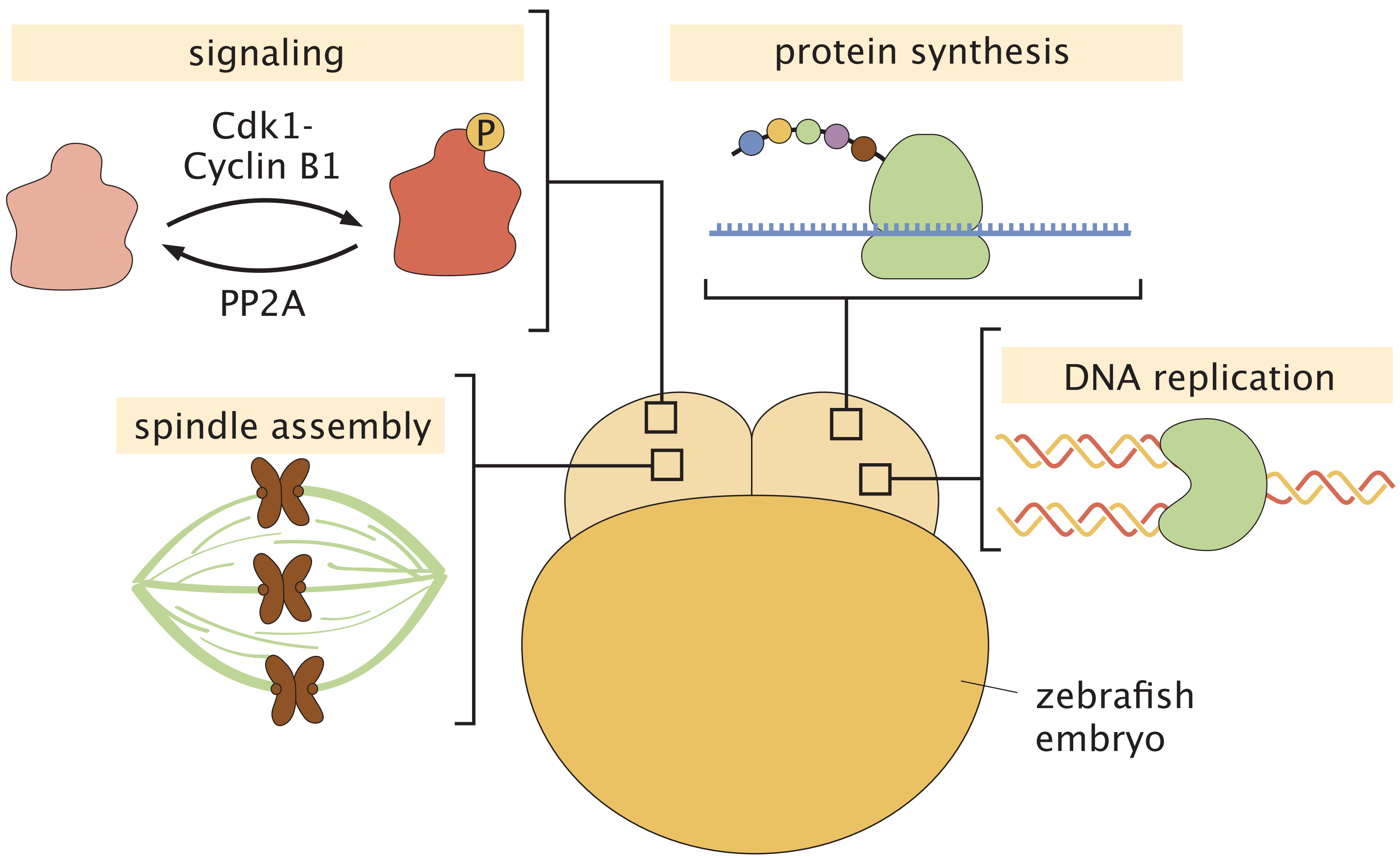
Need: energy & precursors (nucleotides, fatty acids, and amino acids)

Cleavage stage - A model system to study the energetics of embryonic development



Cleavage stage - A model system to study the energetics of embryonic development

$$\dot{Q}_{tot}(t) = \dot{Q}_{spindle}(t) + \dot{Q}_{signal}(t) + \dot{Q}_{protein}(t) + \dot{Q}_{replication}(t) + \dot{Q}_{other}(t)$$



Foster P, Razo M & Phillips, R, *Dev Cell* 2019

Conservation of energy

$$dU = \delta Q - \delta W$$

δQ = heat added to system
δW = work done by the system

Enthalpy

$$H = U + PV$$

Heat and enthalpy

$$dH = \delta Q$$

(constant *P* and no additional work)

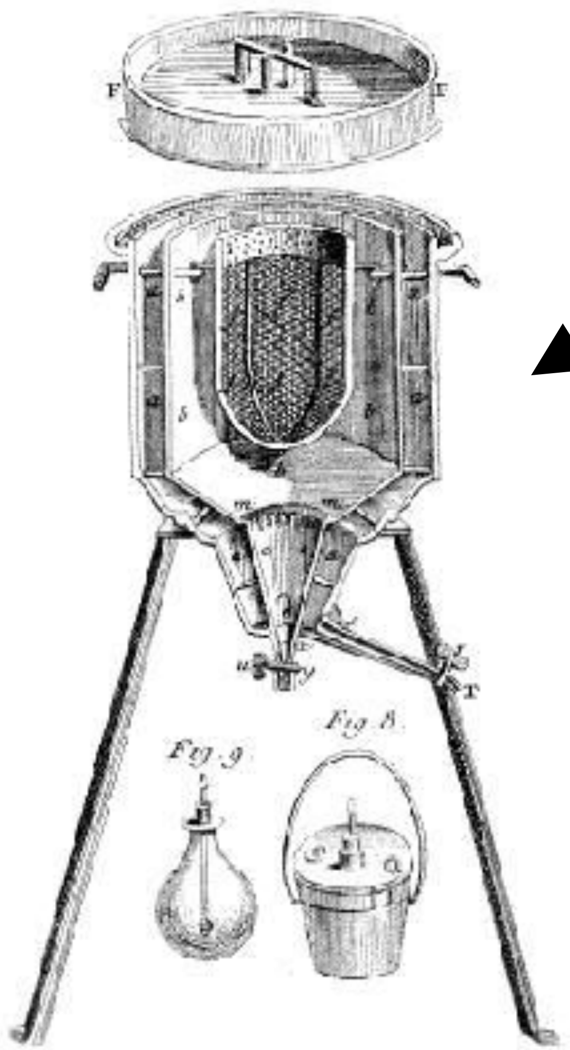
$$\frac{dH}{dt} = \frac{dQ}{dt}$$

Gibbs free energy

$$G = H - TS$$

Heat dissipation can be measured by calorimetry

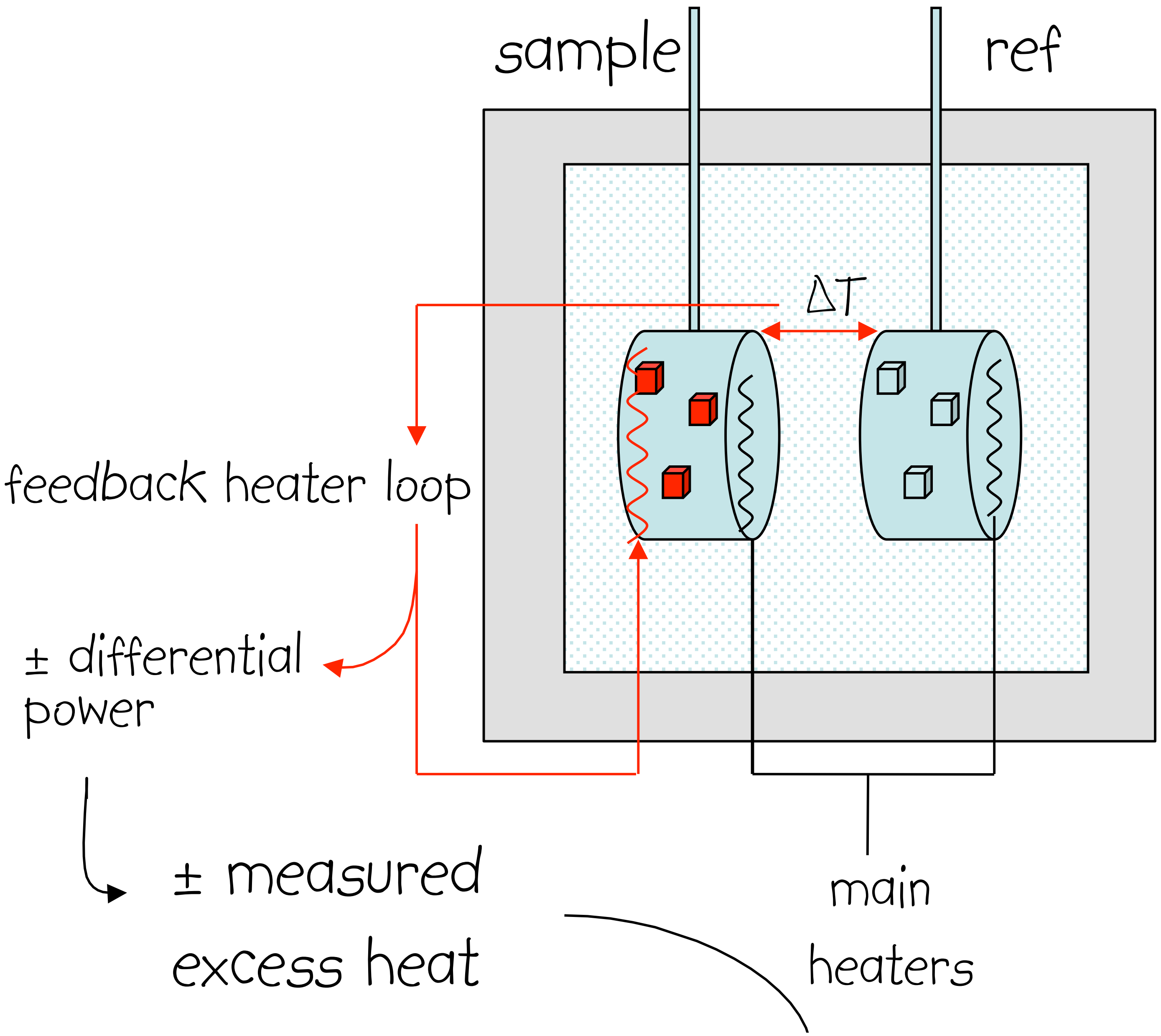
Calorimetry was one of the earliest techniques reported in the ‘literature’ and is now used in many areas



“la respiration est donc une combustion”
-
Respiration is in fact a combustion

Lavoisier & Laplace’s ice calorimeter
1780’s

Power Compensation Biocalorimeters

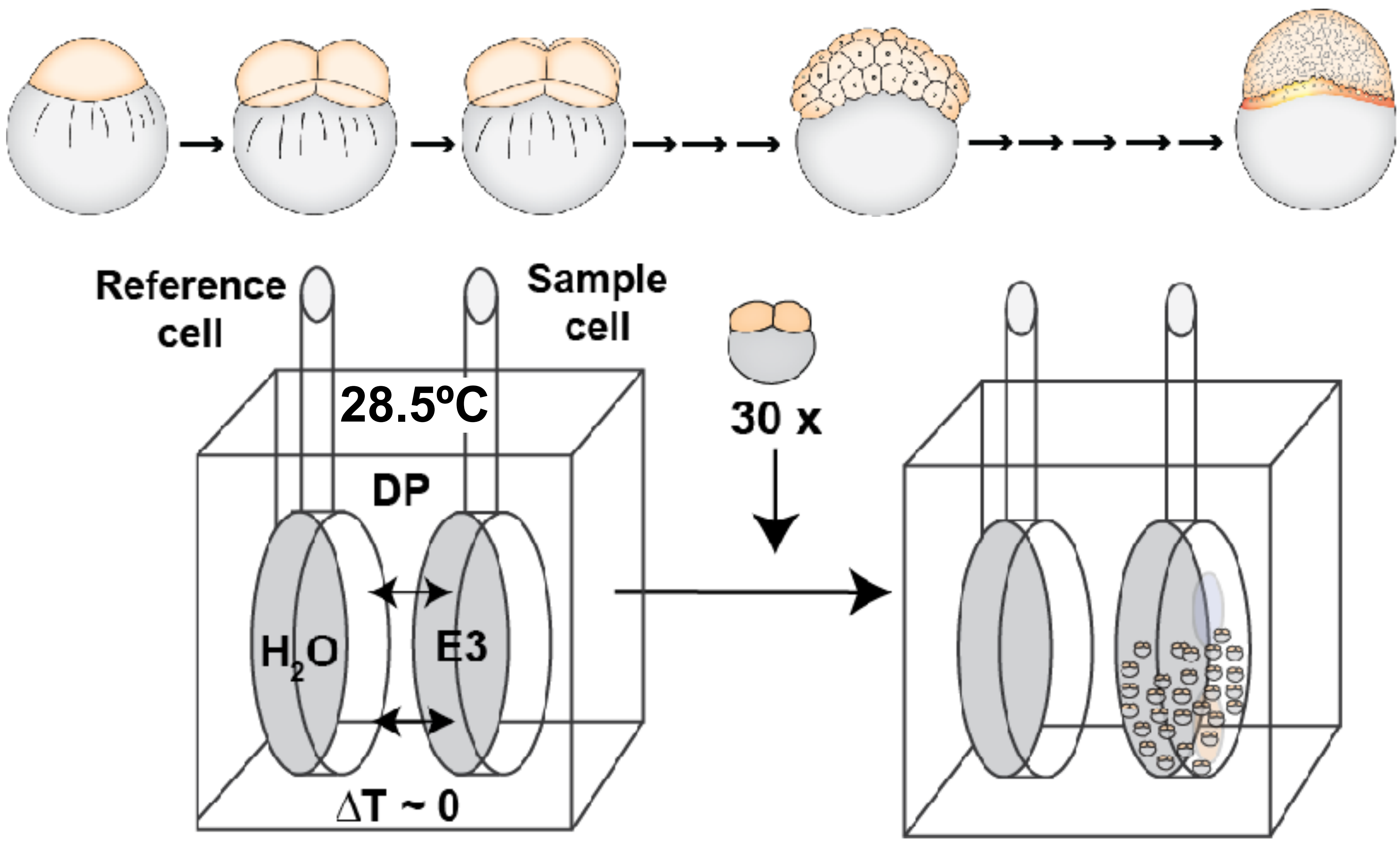


Difference in temperature relative to an ‘identical’ reference cell, measured by very precise thermopile, is kept constant by the calorimeter in a feedback loop controlling electrical heating to the sample cell.

Increases or decreases in differential power in this circuit are directly proportional to the excess heat taken up or given off during ‘reactions’

Image from Chris Johnson, MRC, Biological calorimetry

Measurement of embryonic heat dissipation by isothermal calorimetry



Malvern MicroCal VP-ITC

(usually used for protein-protein interactions)

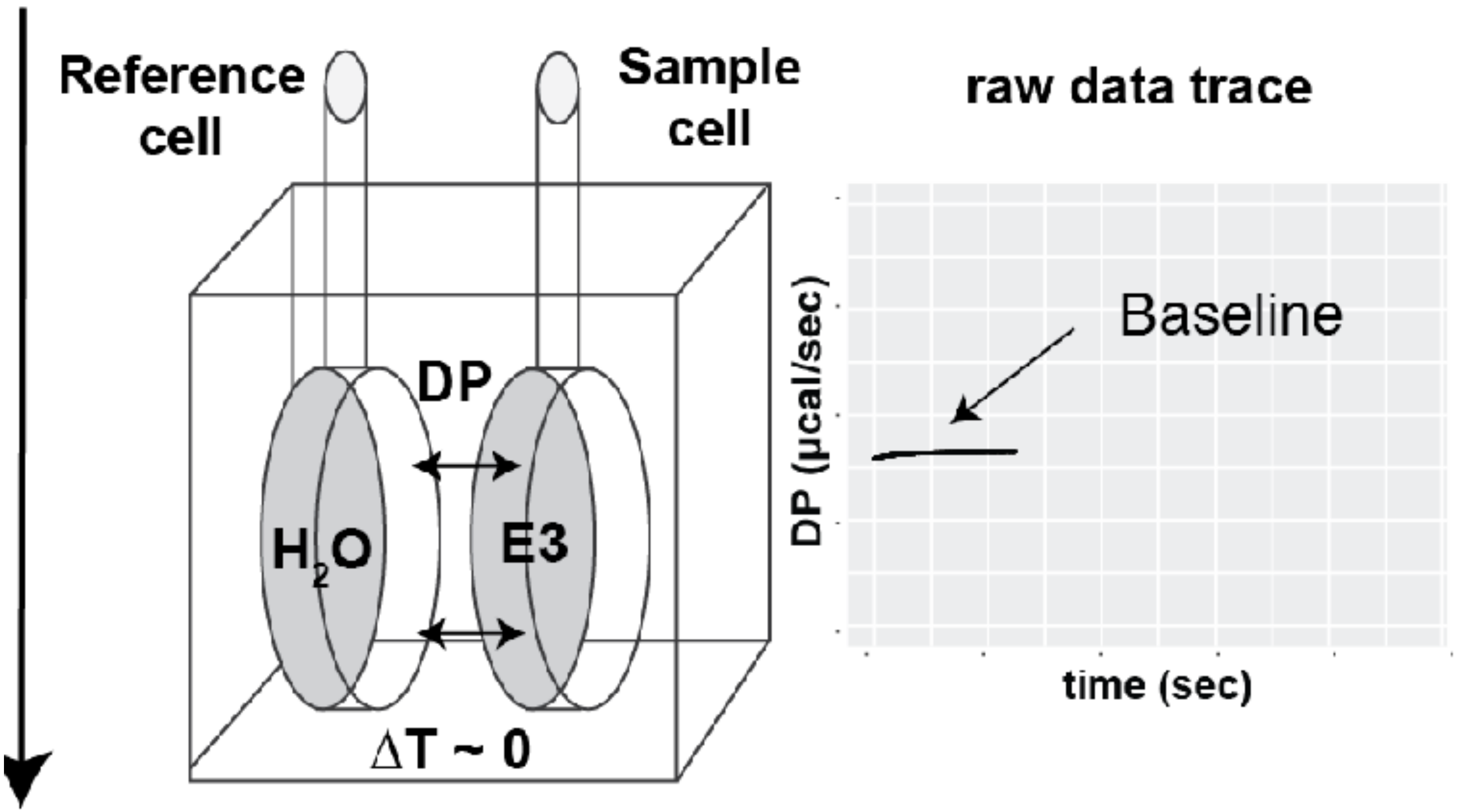
Eggs staged at 2-cell cleavage

(within 3 minutes)

Rodenfels *et al.*, *Dev. Cell* 2019

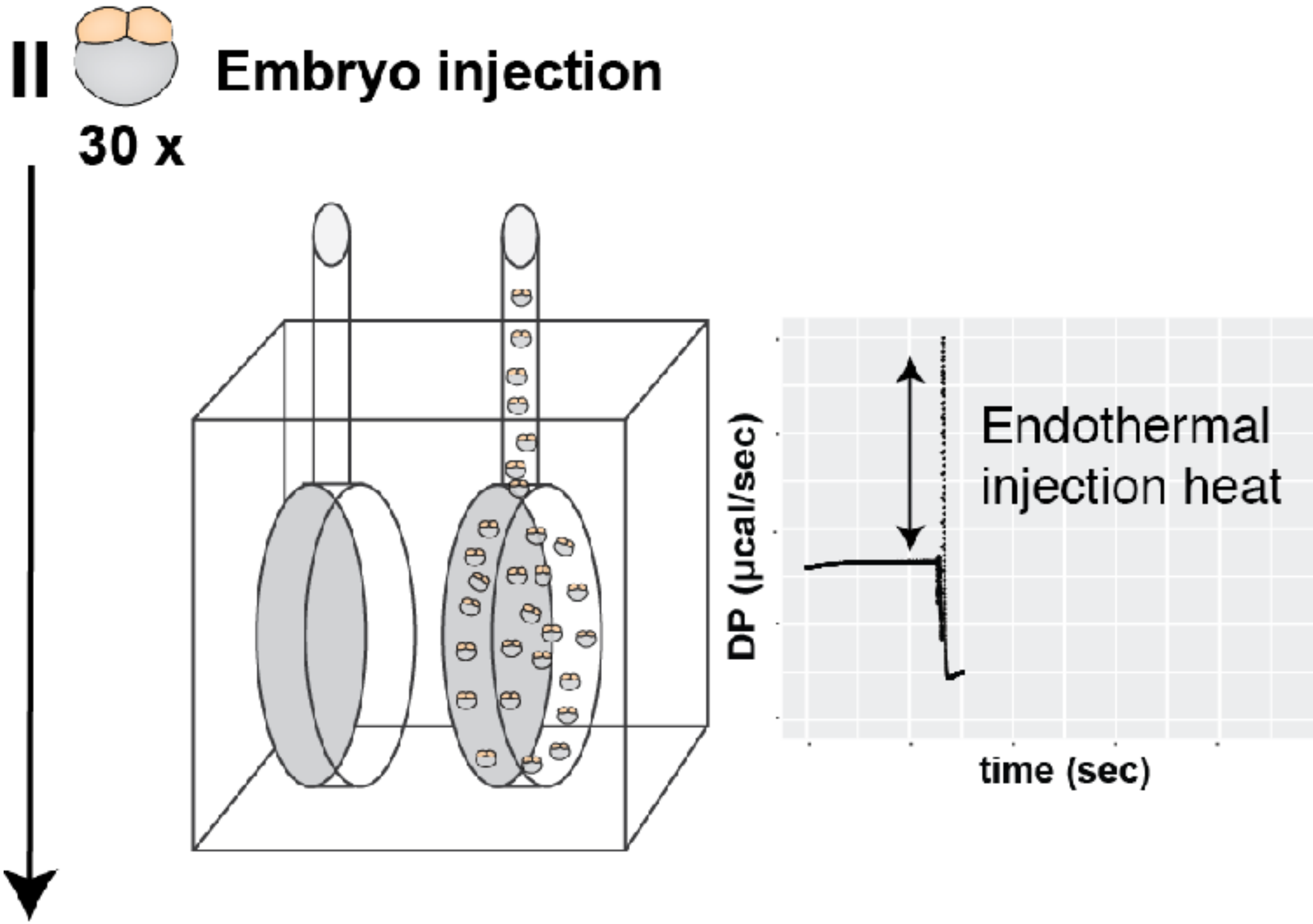
Measurement of embryonic heat dissipation by isothermal calorimetry

Equilibration & baseline establishment



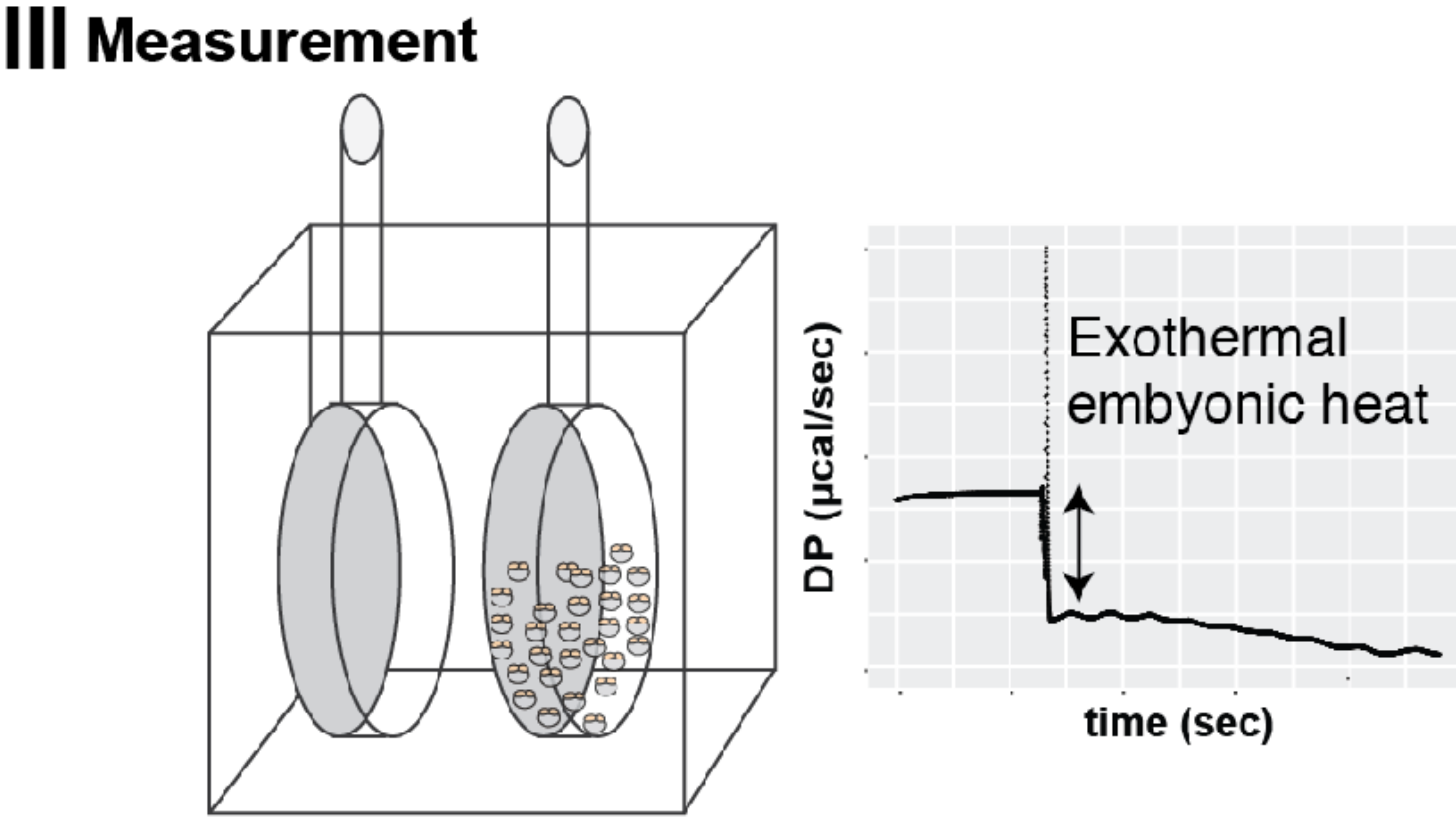
Rodenfels *et al.*, *Dev. Cell* 2019

Measurement of embryonic heat dissipation by isothermal calorimetry



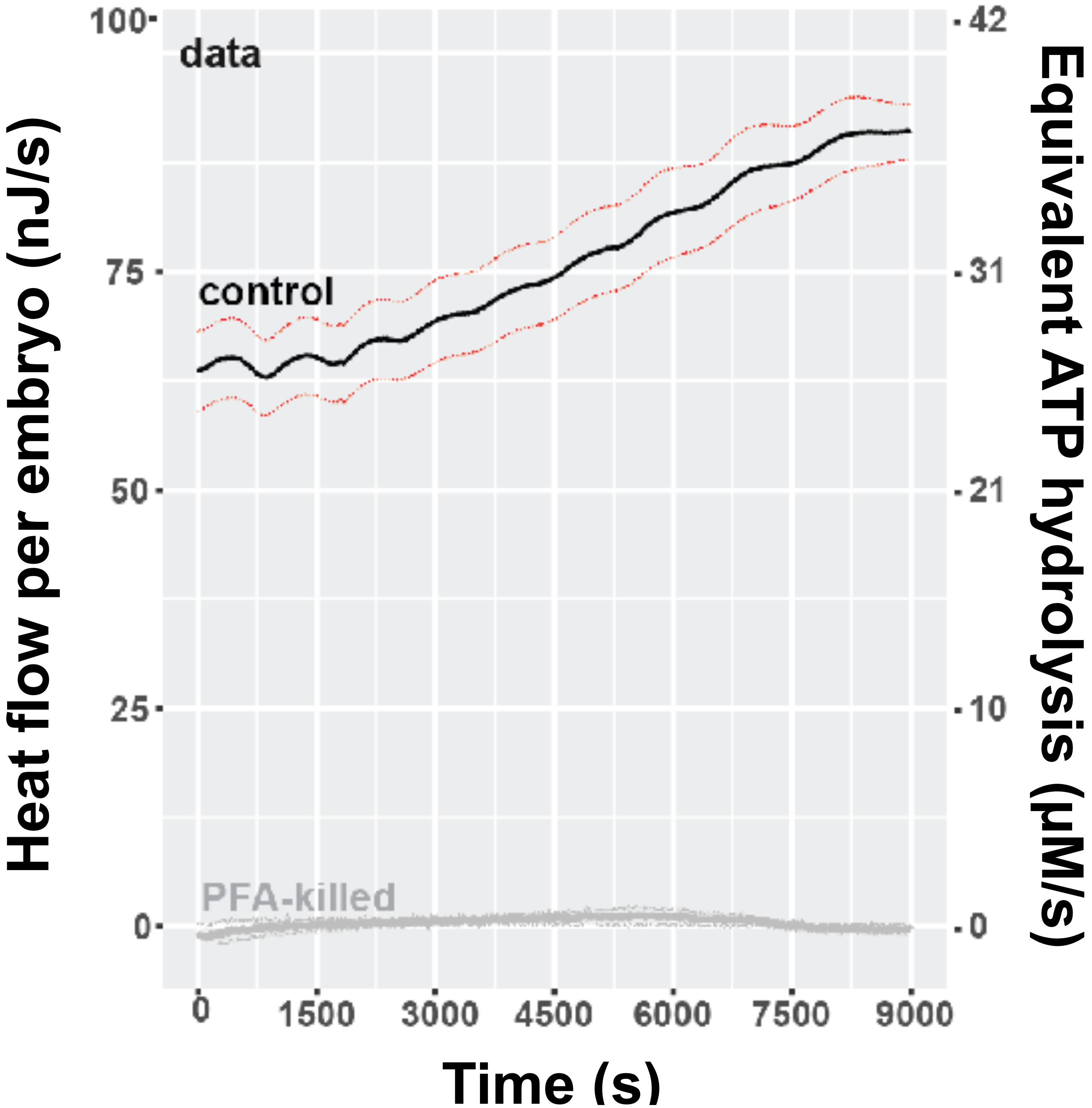
Rodenfels *et al.*, *Dev. Cell* 2019

Measurement of embryonic heat dissipation by isothermal calorimetry



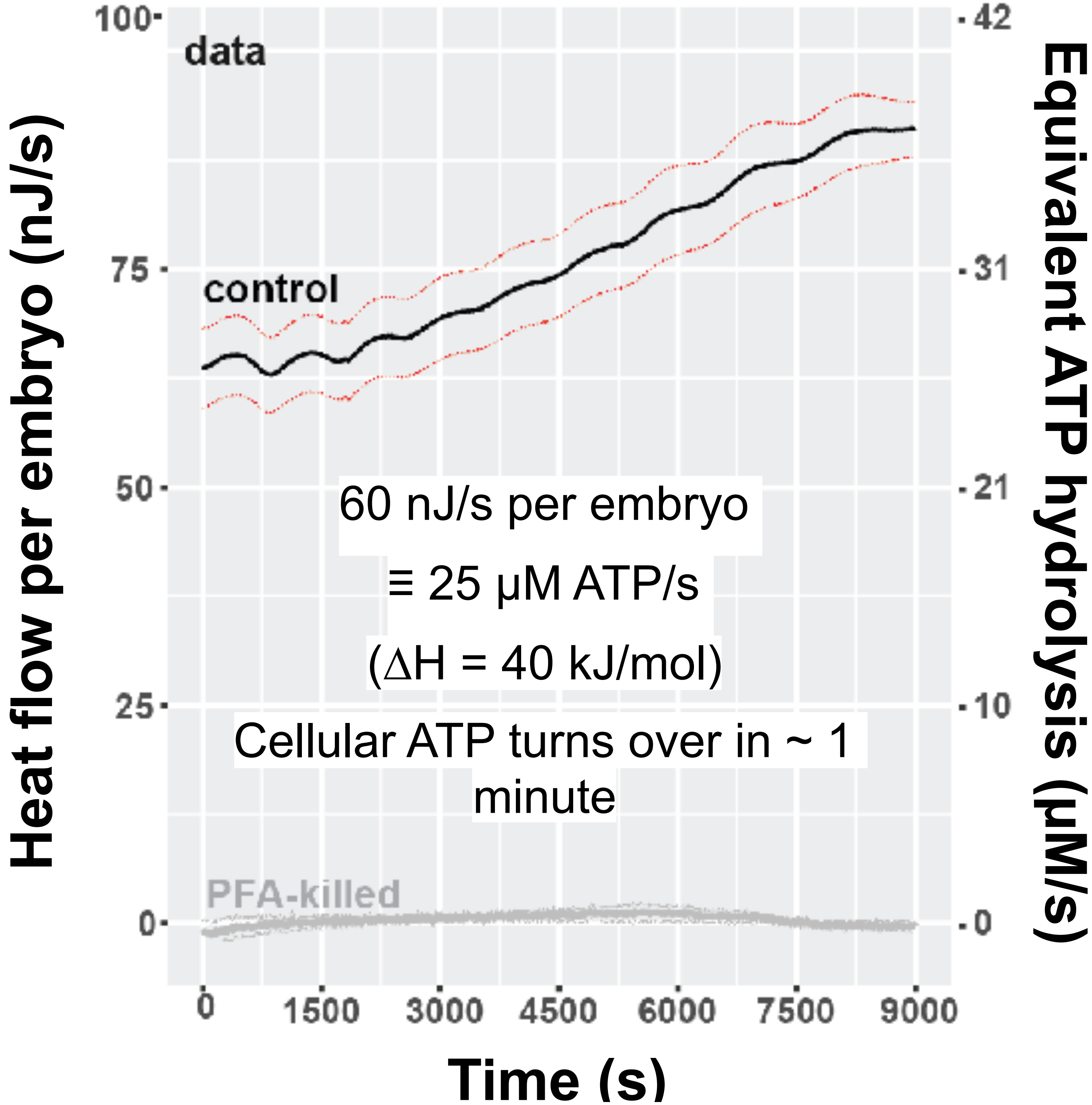
Rodenfels *et al.*, *Dev. Cell* 2019

Heat flows out of the embryo



Rodenfels *et al.*, *Dev. Cell* 2019

Heat flow equals change in enthalpy



Conservation of energy

$$dU = \delta Q - \delta W$$

δQ = heat added to system
δW = work done by the system

Enthalpy

$$H = U + PV$$

Heat and enthalpy

$$dH = \delta Q$$

(constant *P* and *V* and no additional work)

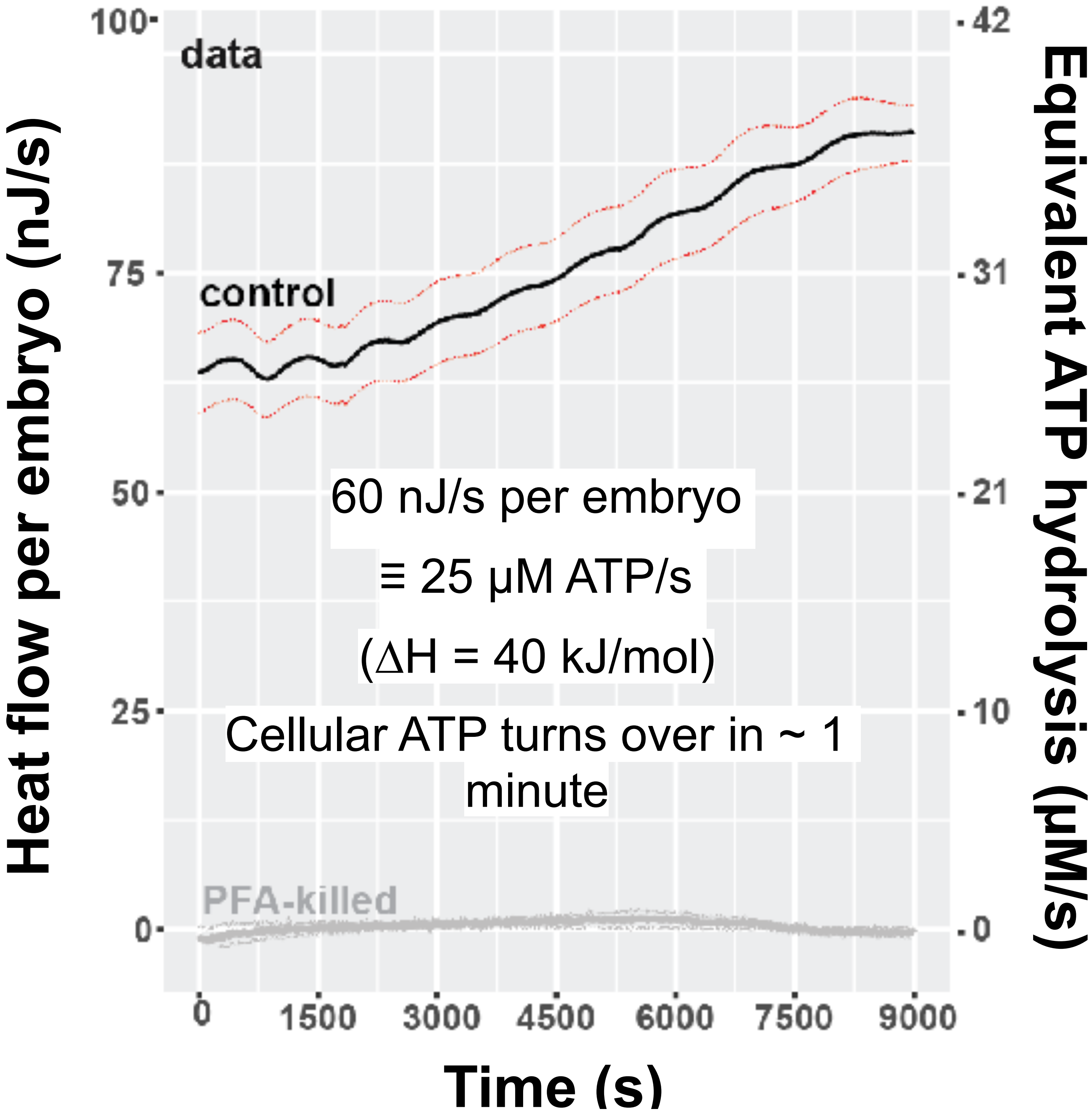
$$\frac{dH}{dt} = \frac{dQ}{dt}$$

Gibbs free energy

$$G = H - TS$$

Rodenfels *et al.*, *Dev. Cell* 2019

What are the embryos doing with their ATP?

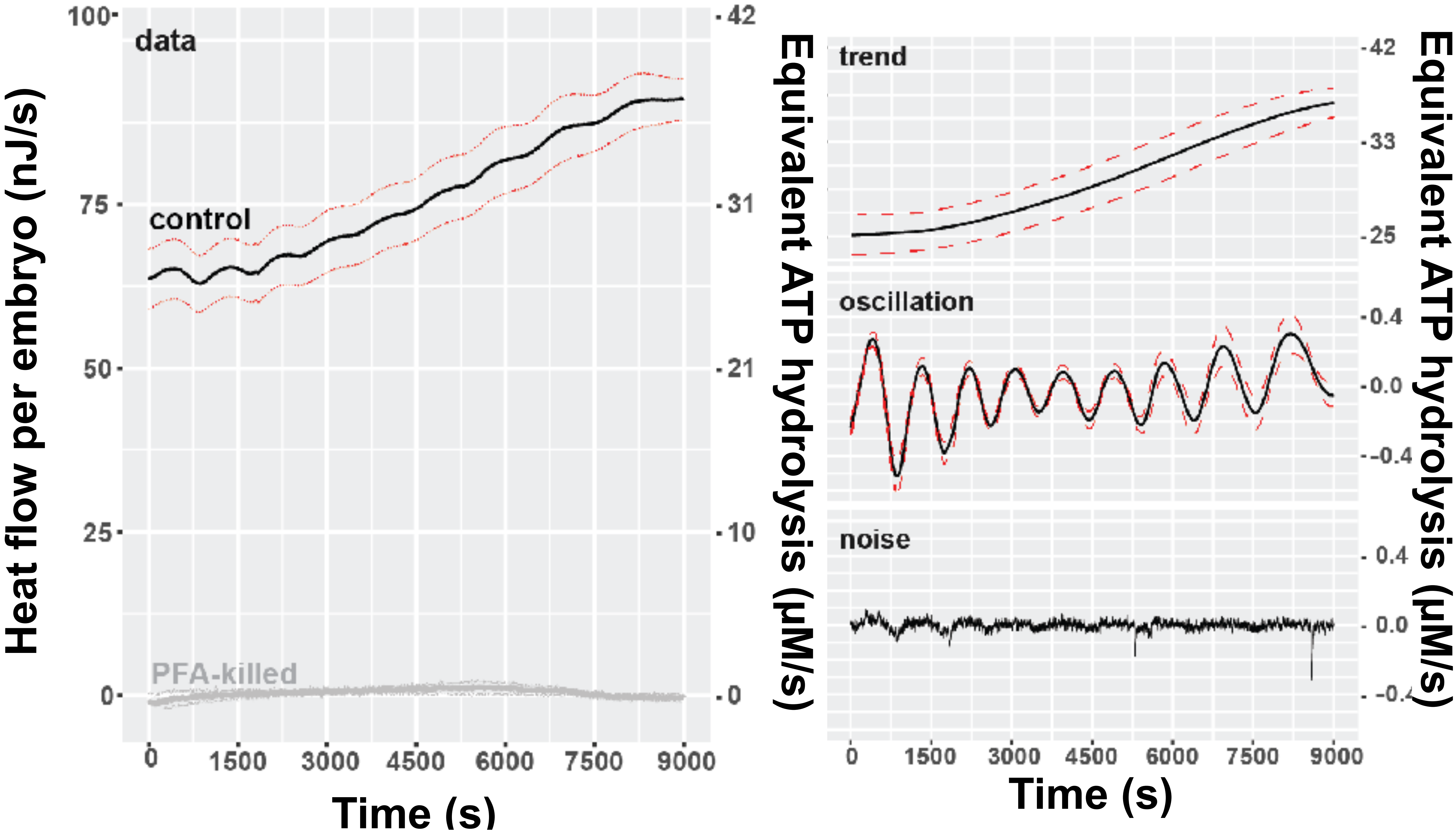


Making more cells!!!

- DNA replication (exponential increase in genome number)
- increase in plasma membranes
- protein synthesis
- assemble and disassemble cellular machineries (chromatin, spindles)
- generate forces
- zygotic genome activation

Rodenfels *et al.*, *Dev. Cell* 2019

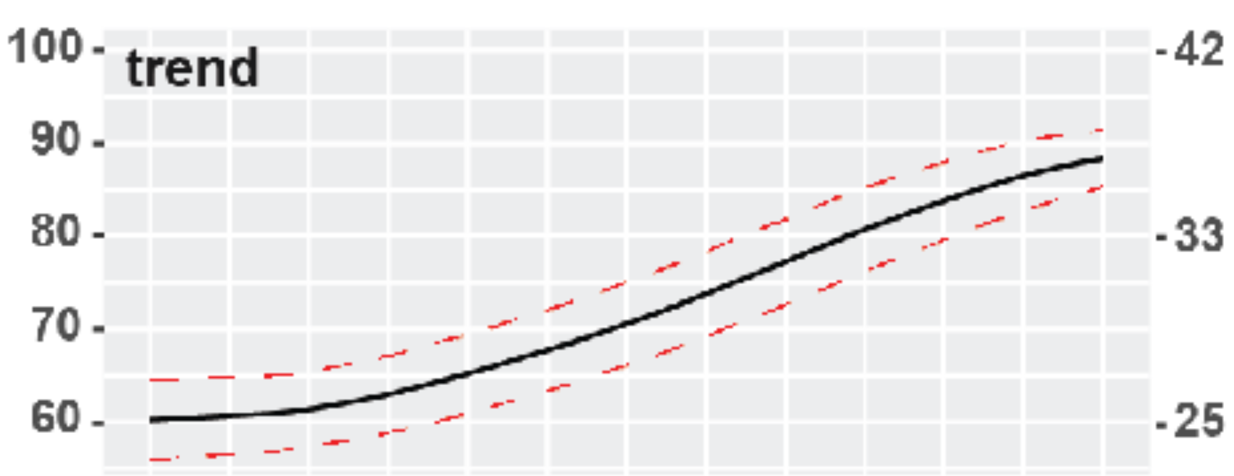
Embryonic heat flow is composed of an increasing trend and an oscillatory component



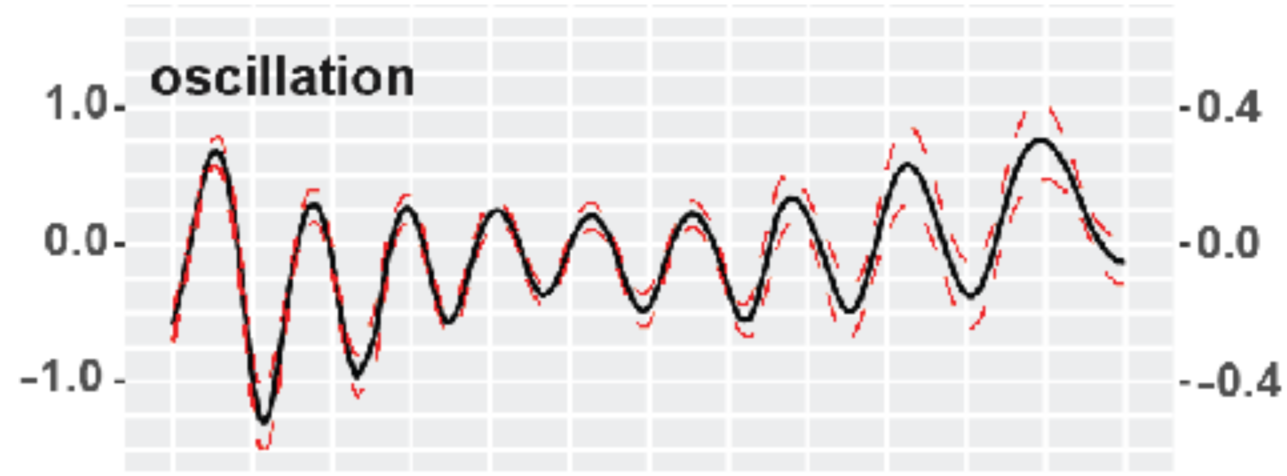
Rodenfels *et al.*, *Dev. Cell* 2019

Embryonic heat flow is composed of an increasing trend and an oscillatory component

Increasing component

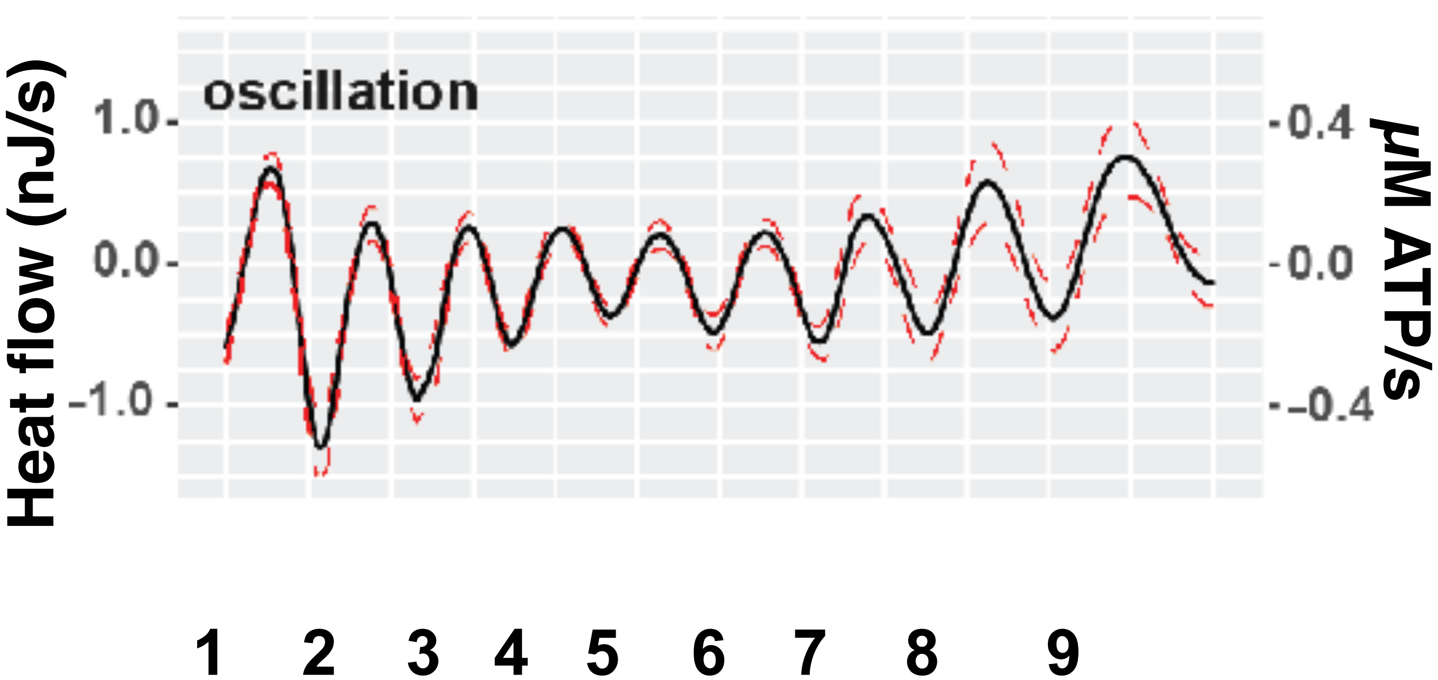


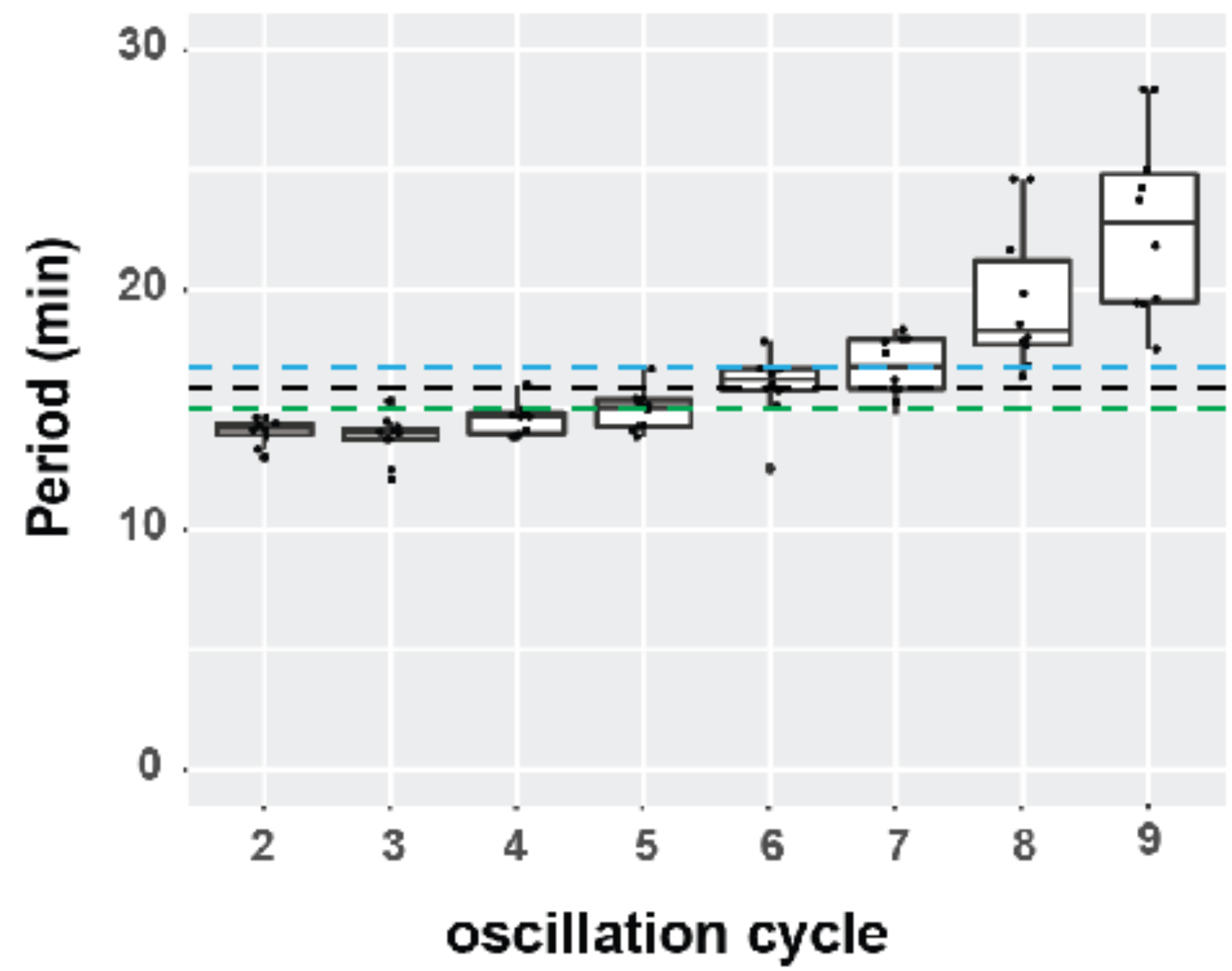
Oscillatory component



Rodenfels *et al.*, *Dev. Cell* 2019

Heat flow oscillates in conjunction with the cell cycle

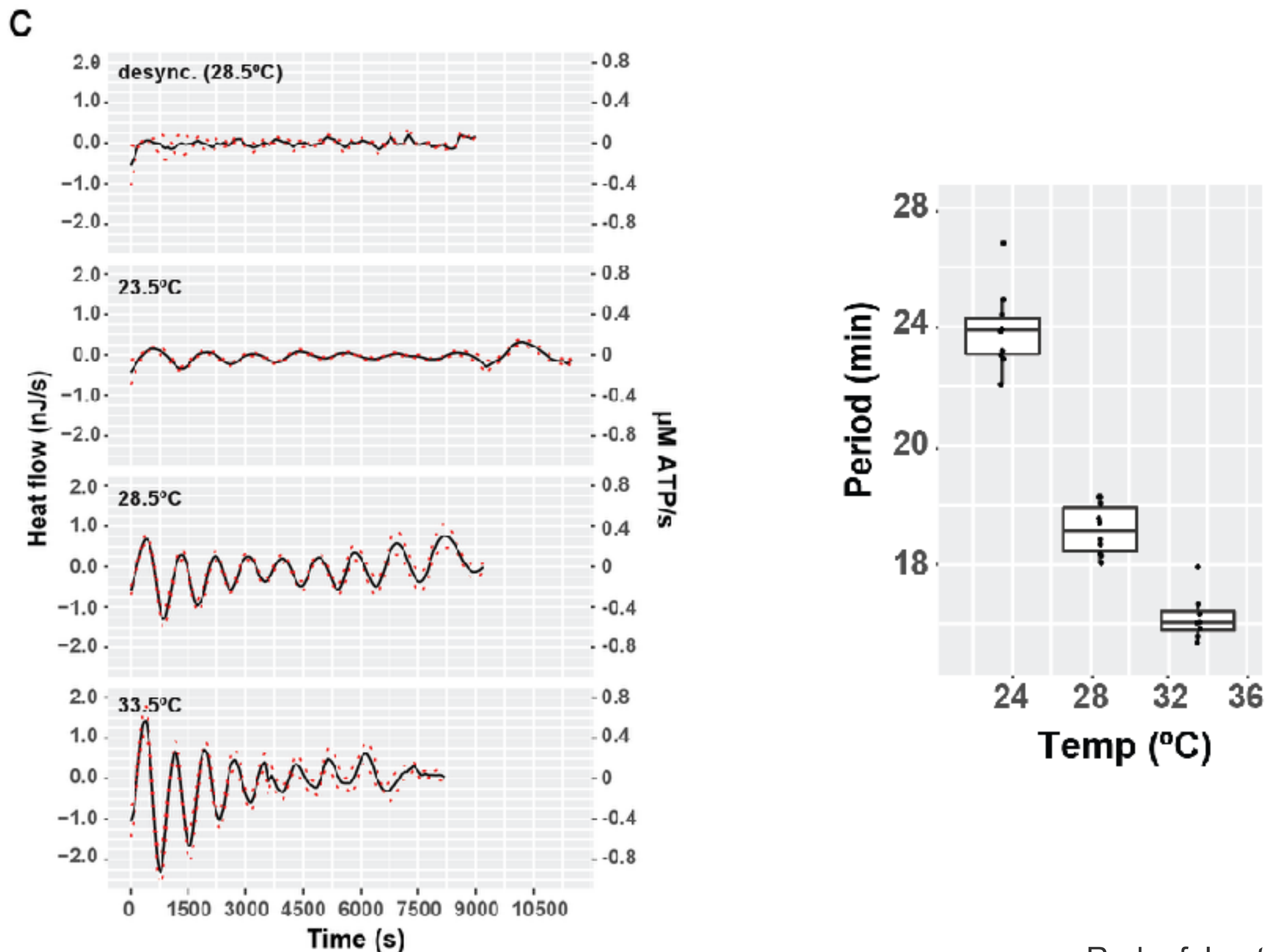




The cell cycle period is roughly 15 minutes, increasing with cycle number

Rodenfels *et al.*, *Dev. Cell* 2019

The oscillatory component has the same temperature sensitivity as the cell cycle

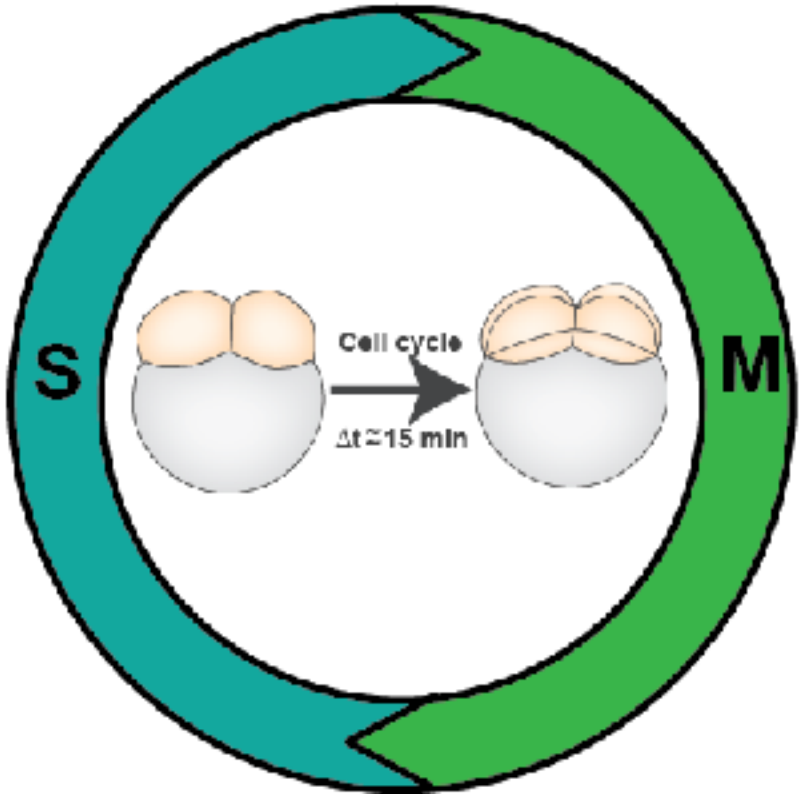


Rodenfels *et al.*, *Dev. Cell* 2019

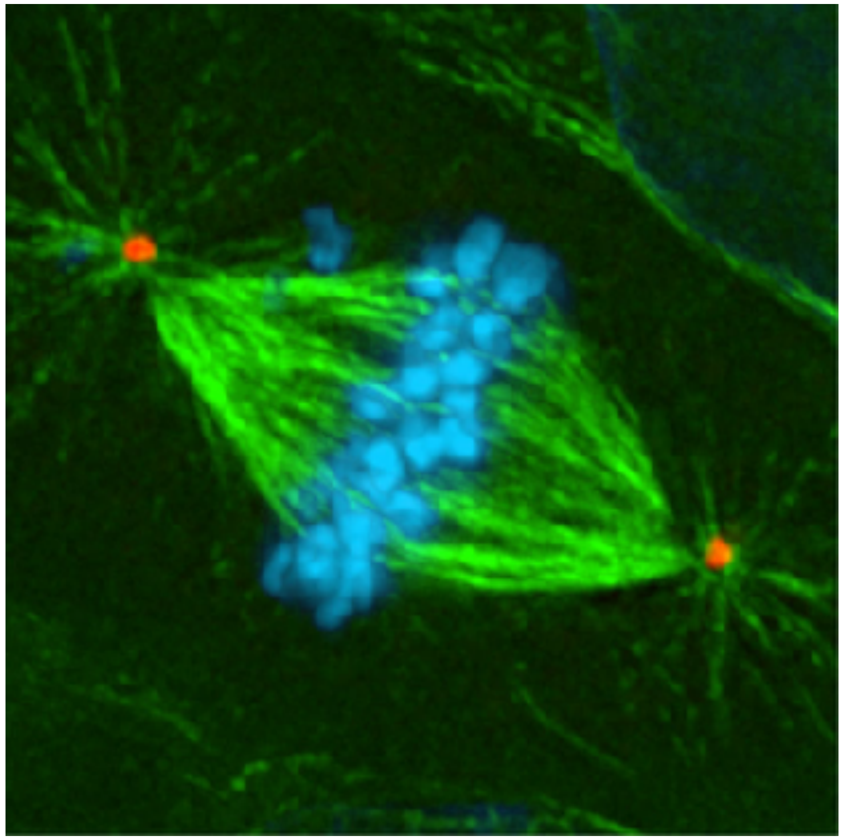
What cellular processes cause the oscillations?

DNA-replication during S-phase

Drew Berry



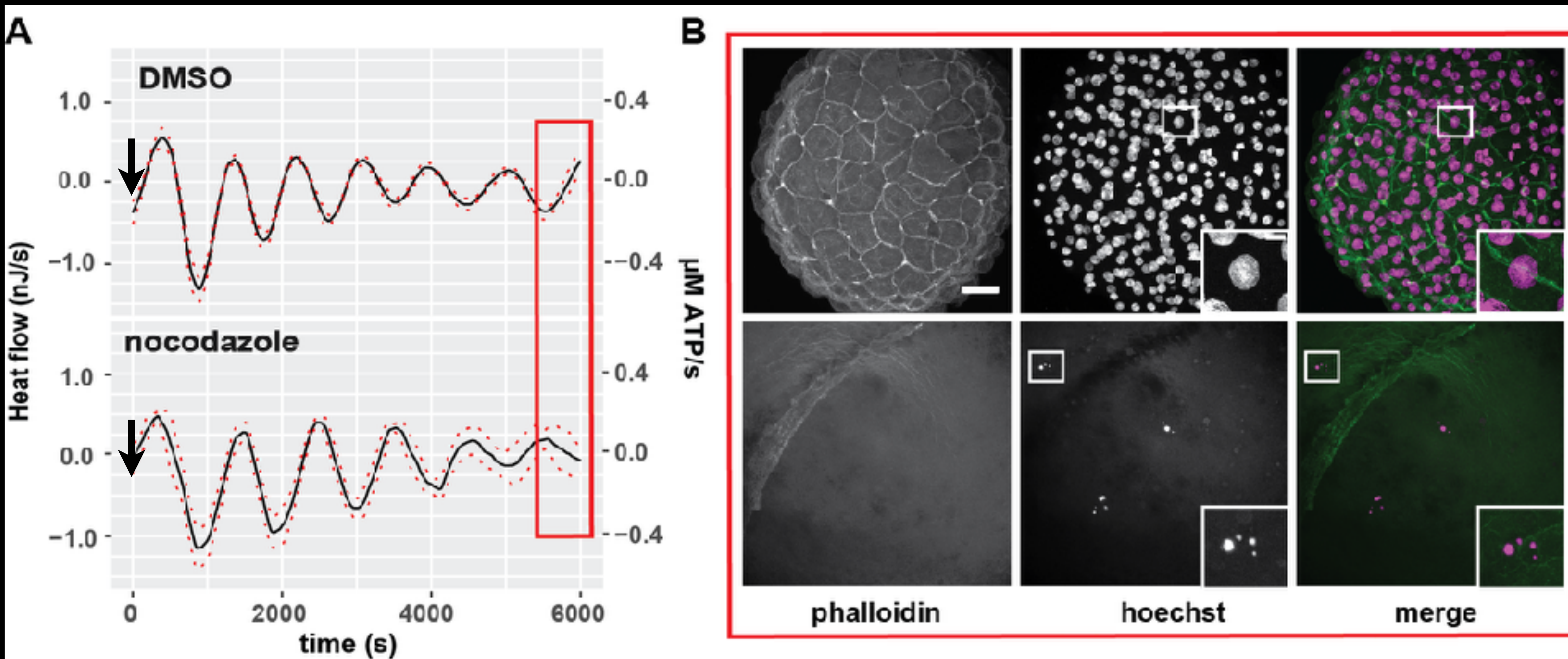
Chromosome segregation during M-phase



Duane Compton

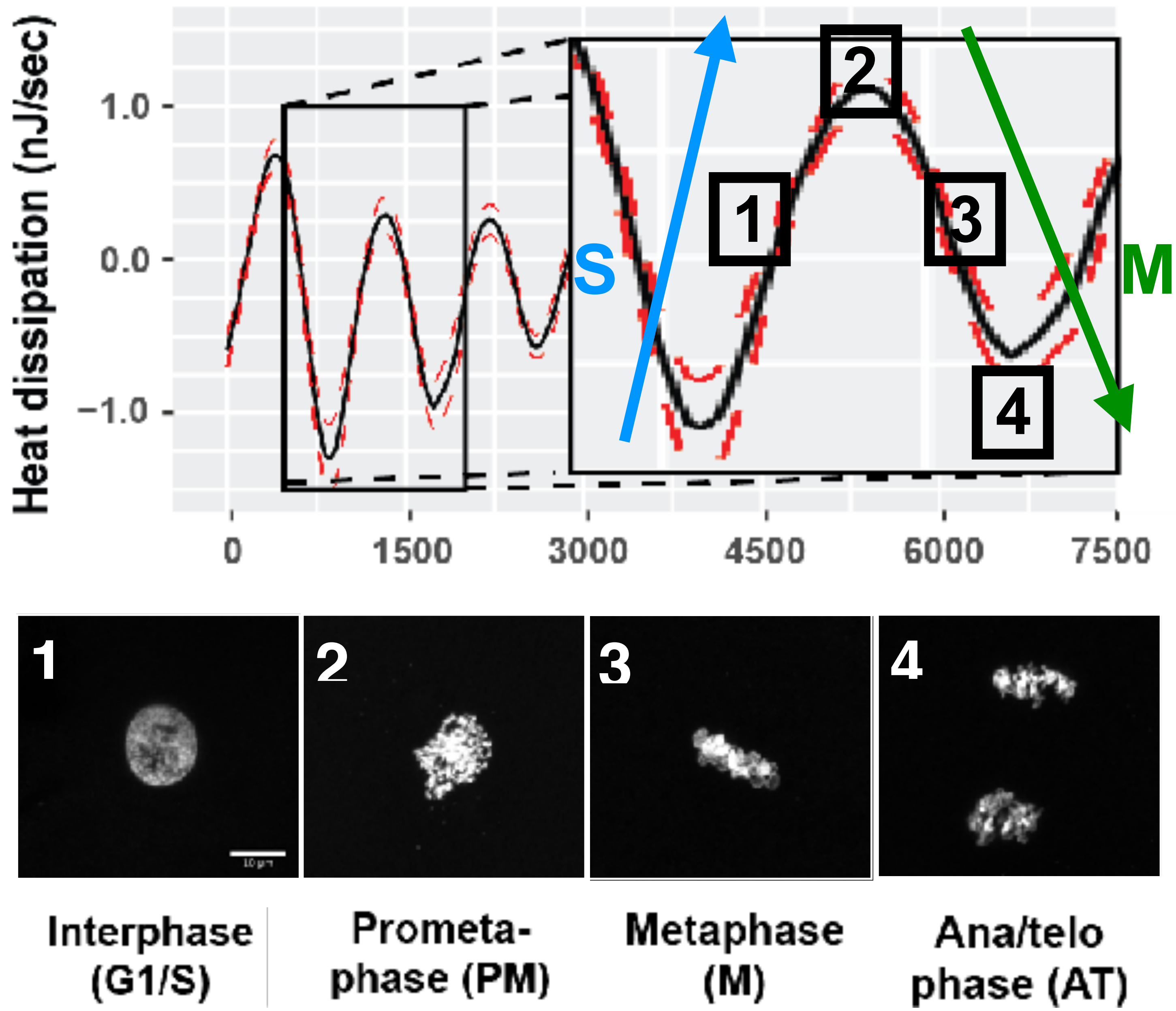
Other biochemical processes dependent on the cell cycle: cell division, cyclin degradation, others?

Oscillatory heat flow is not blocked by nocodazole



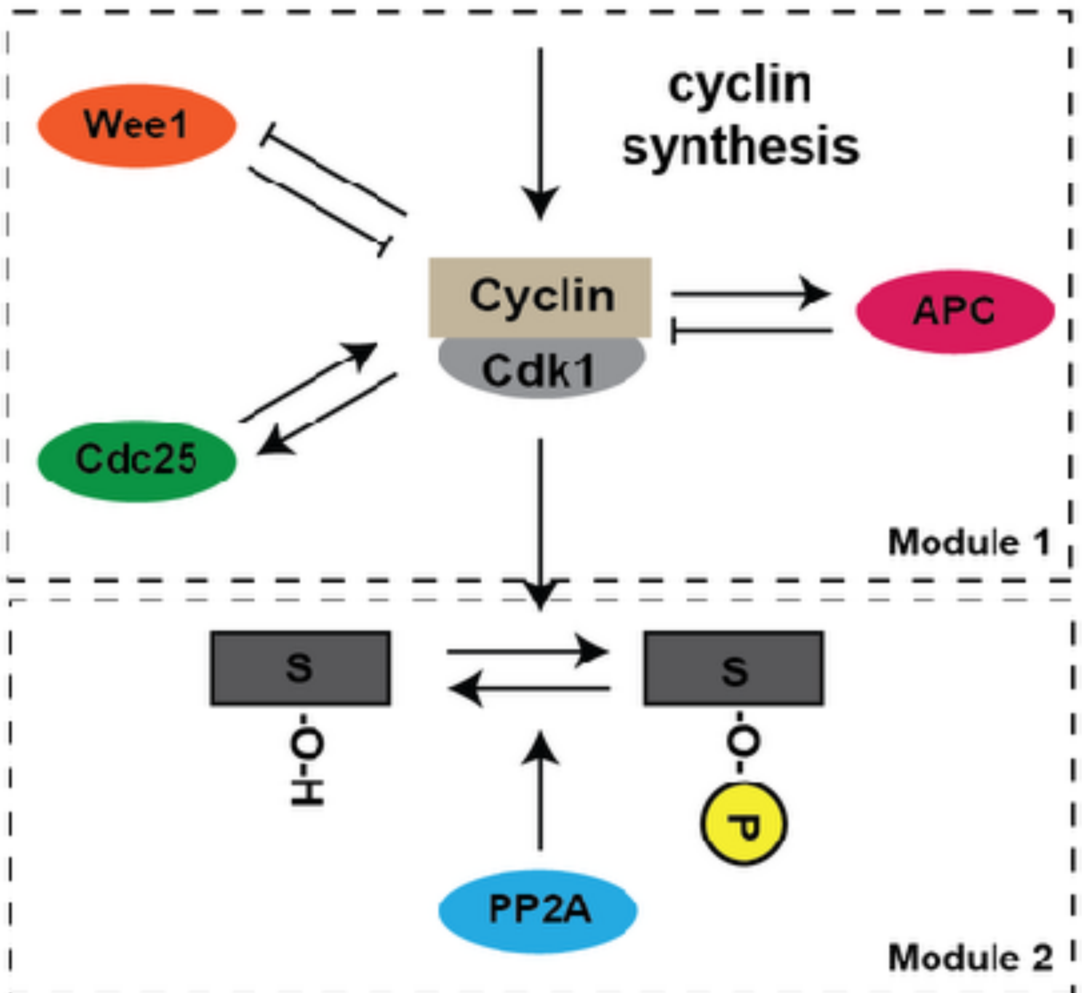
Nocodazole blocks DNA replication and mitosis

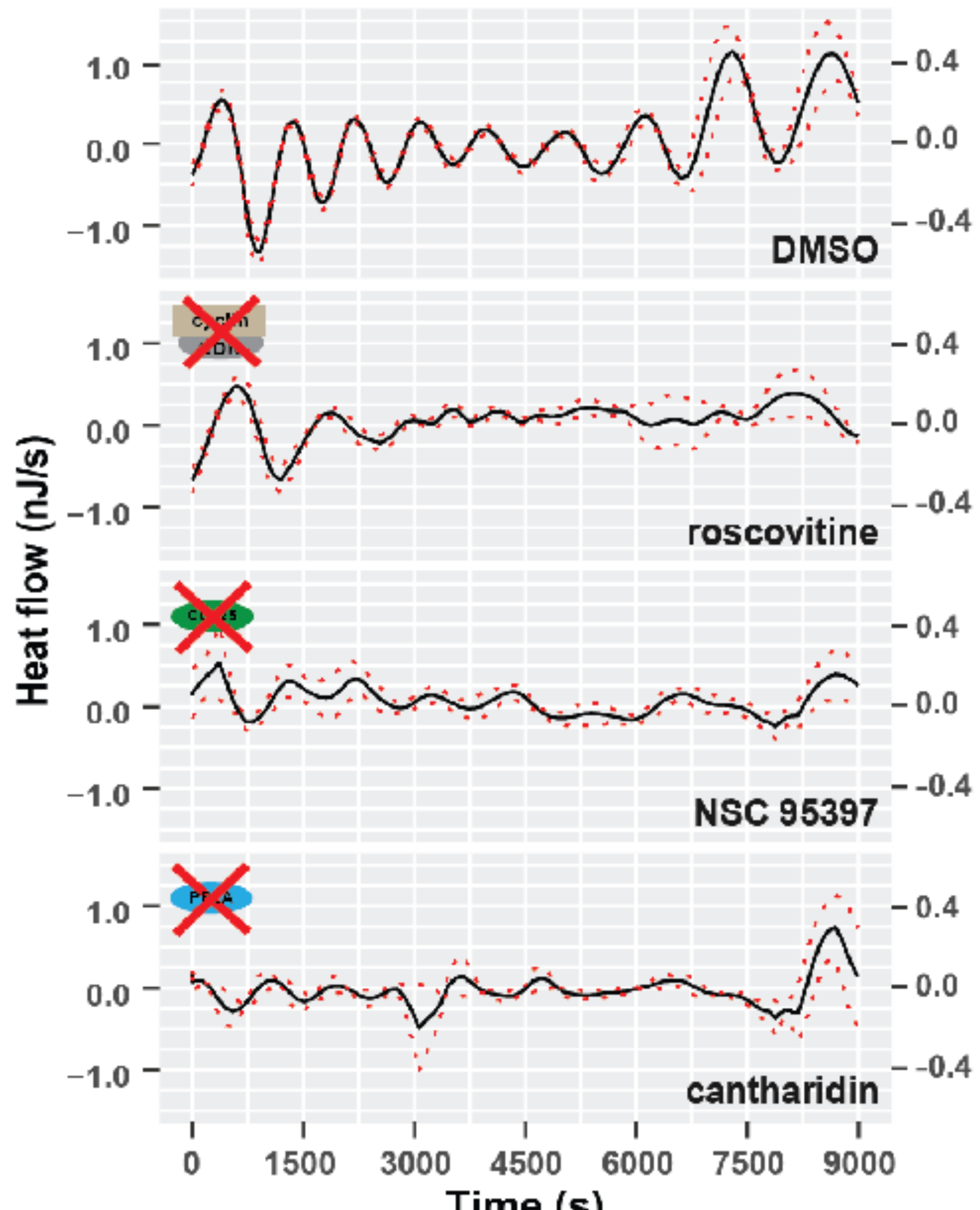
Embryonic heat flow peaks during mitotic entry and troughs during mitotic exit



Rodenfels *et al.*, *Dev. Cell* 2019

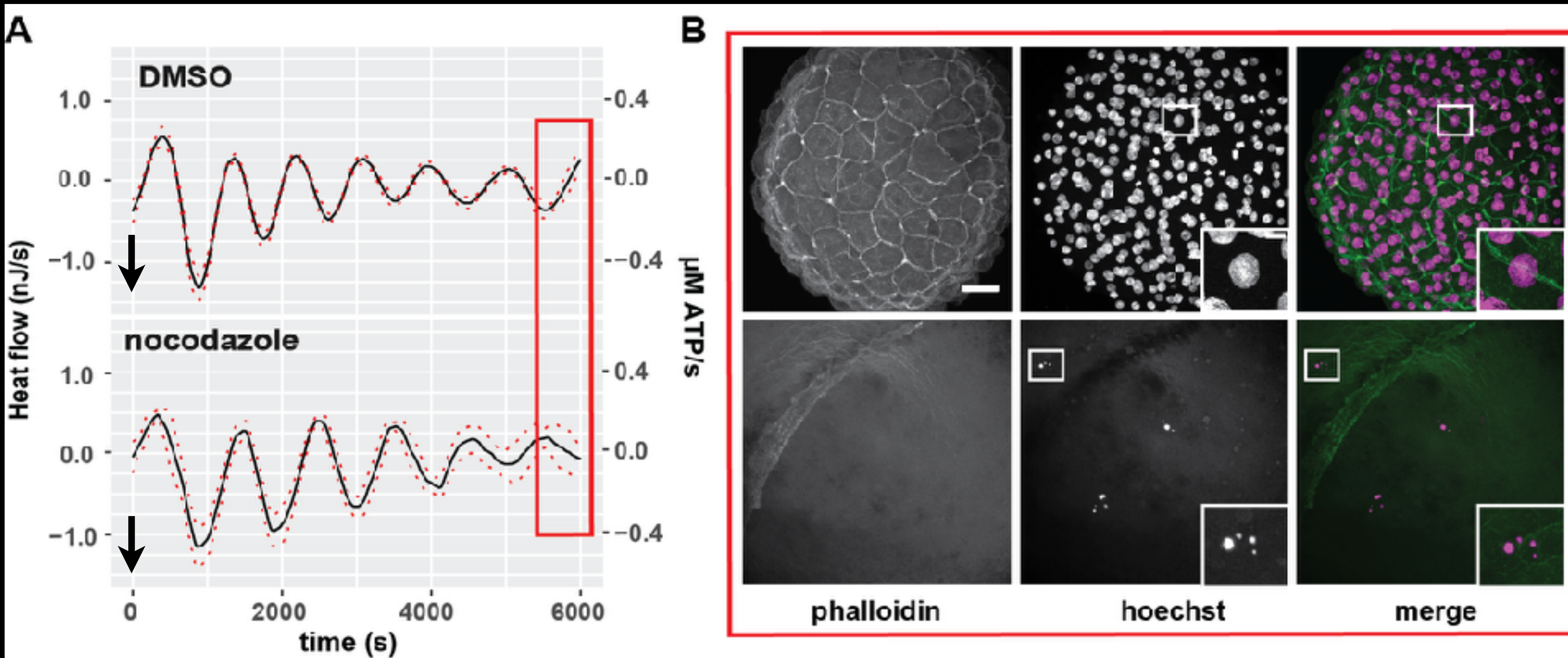
Oscillatory heat flow depends on cell cycle signaling





Rodenfels *et al.*, *Dev. Cell* 2019

Oscillatory heat flow is not blocked by nocodazole



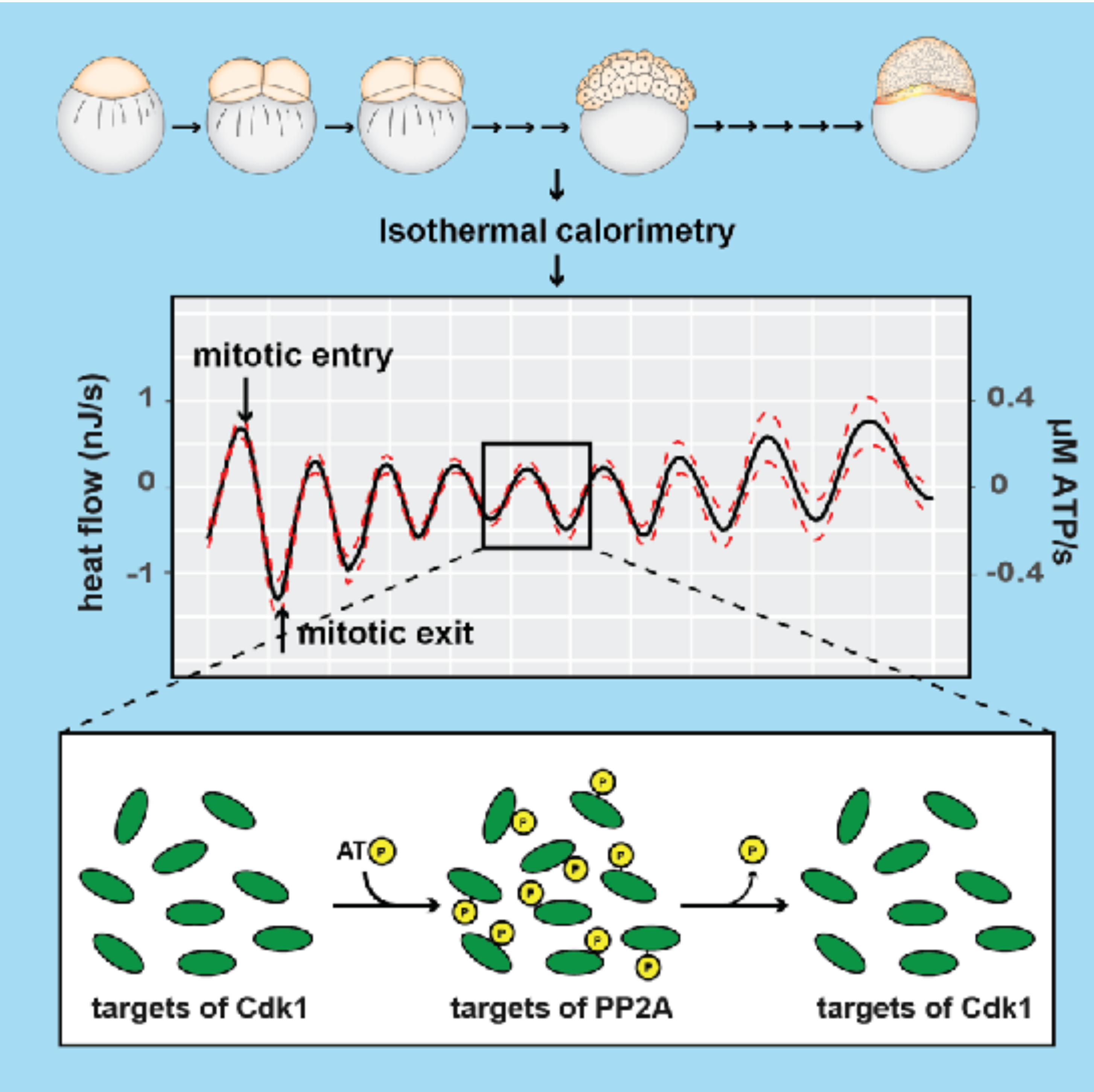
Nocodazole blocks DNA replication and mitosis

Energetic cost estimates for cell cycle-dependent processes during cleavage stage development

- Measured average oscillatory amplitude of **~ 1 nJ/s = ~ 400 nM ATP/s**

Process	ATP equivalents
DNA replication (~ 1.5 x 10^9 bp, w/o histone translation)	~ 0.6 nM ATP/s
DNA replication (~ 1.5 x 10^9 bp, w/ histone translation)	~ 2 nM ATP/s
cyclin B1 translation	does not oscillate ~ constant
Degradation of 60 nM cyclin B1 bound to cdk1	~ 50 nM ATP/s
tubulin turnover (upper limit, 1024 cell stage)	~ 1000 nM GTP/s
Cdk1-dependent phosphorylation & dephosphorylation reactions	~ 40 - 180 nM ATP/s
Turnover of protein phosphorylation during *X.laevis* egg activation	~ 80 nM ATP/s

The cell cycle biochemical oscillator is likely to contribute substantially to the heat oscillations



1. The phase is right (CDK1 activity peaks with the heat)
2. Cell cycle blockers block the heat oscillations
3. CDK1 oscillations are not blocked by nocodazole and heat oscillations continue
4. Modeling of the cell cycle and energetic cost estimates reveal that cyclin degradation and phosphorylation/dephosphorylation cycles are expected to contribute 100 - 250 nM ATP/s of the 400 nM ATP/s enthalpy oscillations.

More to read:

Rodenfels, J.*, K.M. Neugebauer* and J. Howard. March 11, 2019. Heat oscillations driven by the embryonic cell cycle reveal the energetic costs of signaling. Developmental Cell 48: 646-658. *co-corresponding authors

Why does the zebrafish embryo pay so much energy for its cell cycle oscillator?

Inherent cost of signaling is very high?

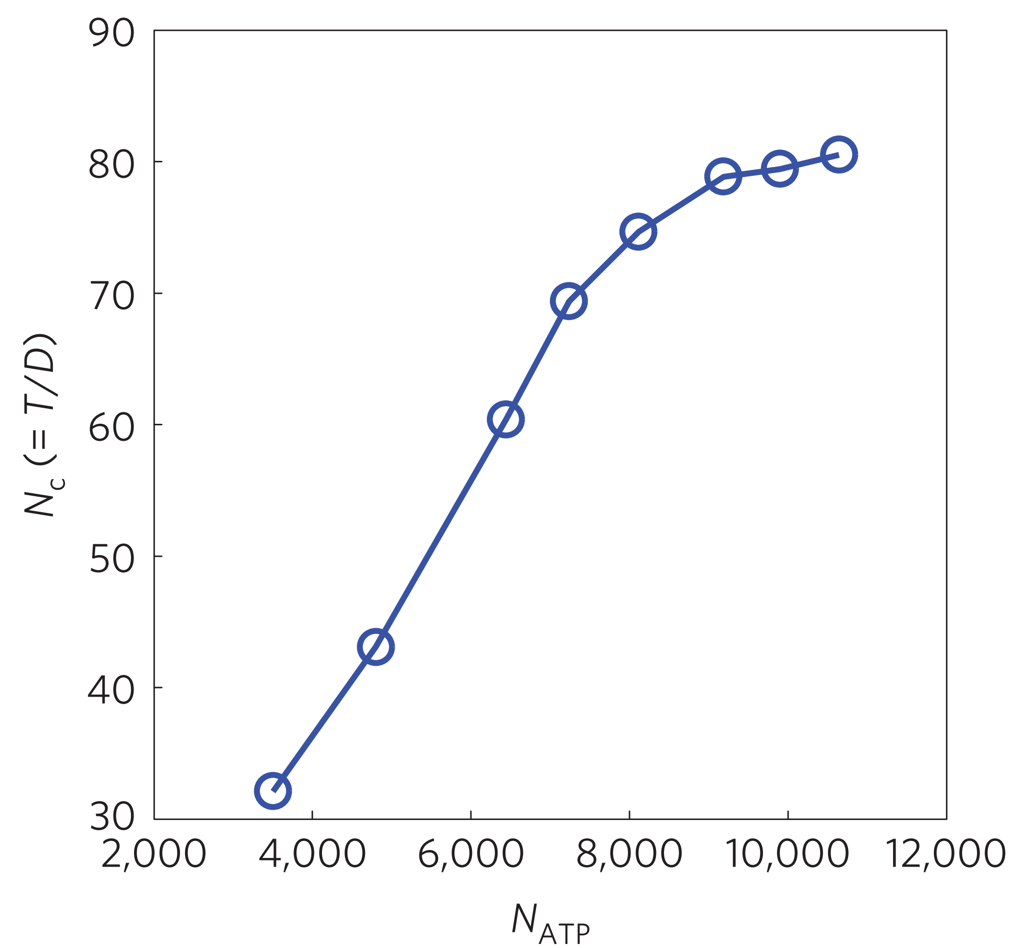
Need a theory (e.g. Cao, Wang, Quyang & Tu 2015), but difficult to apply to the heat oscillations in embryos

Need single embryo measurements & a biochemical system

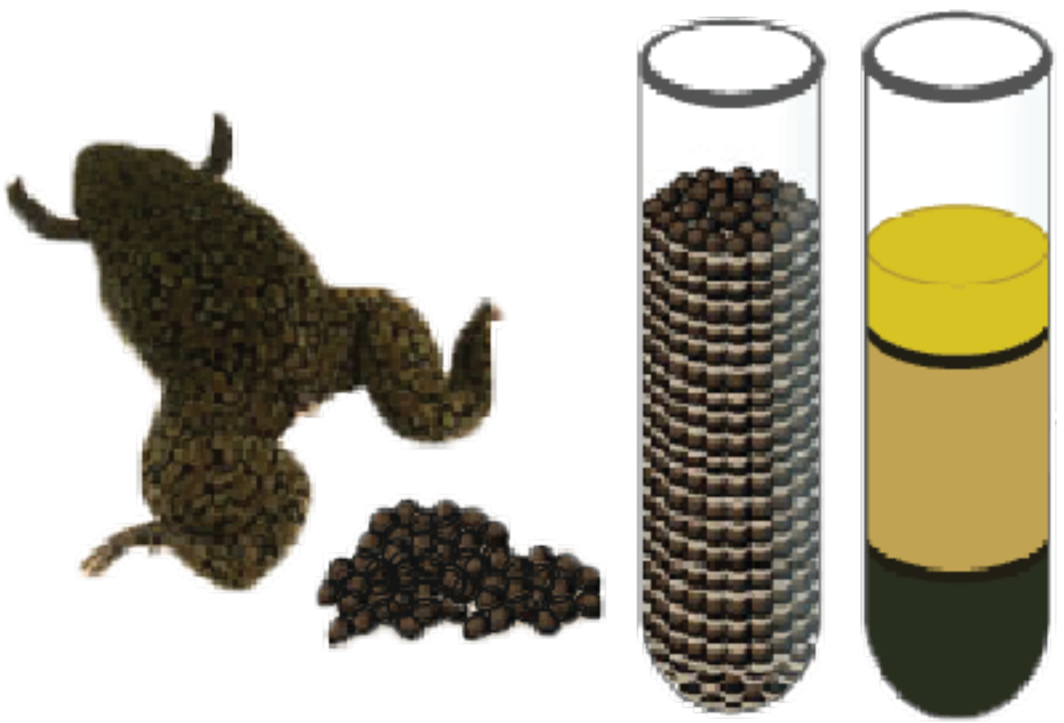
ARTICLES
PUBLISHED ONLINE: 27 JULY 2015 | DOI: 10.1038/NPHYS3412
nature physics

The free-energy cost of accurate biochemical oscillations

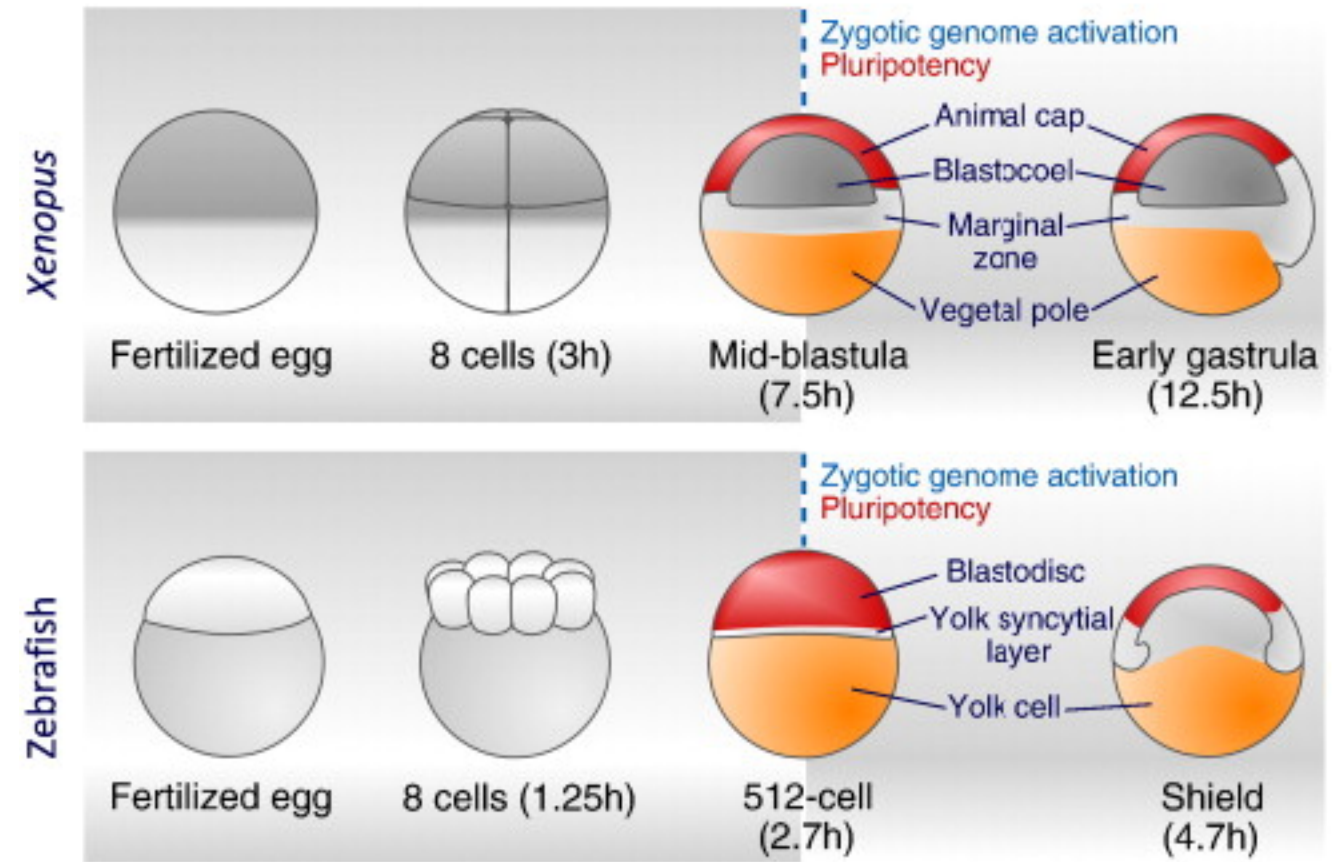
Yuansheng Cao[1], Hongli Wang[1], Qi Ouyang[1,2]* and Yuhai Tu[3]*



Can we measure heat flow in single embryos?

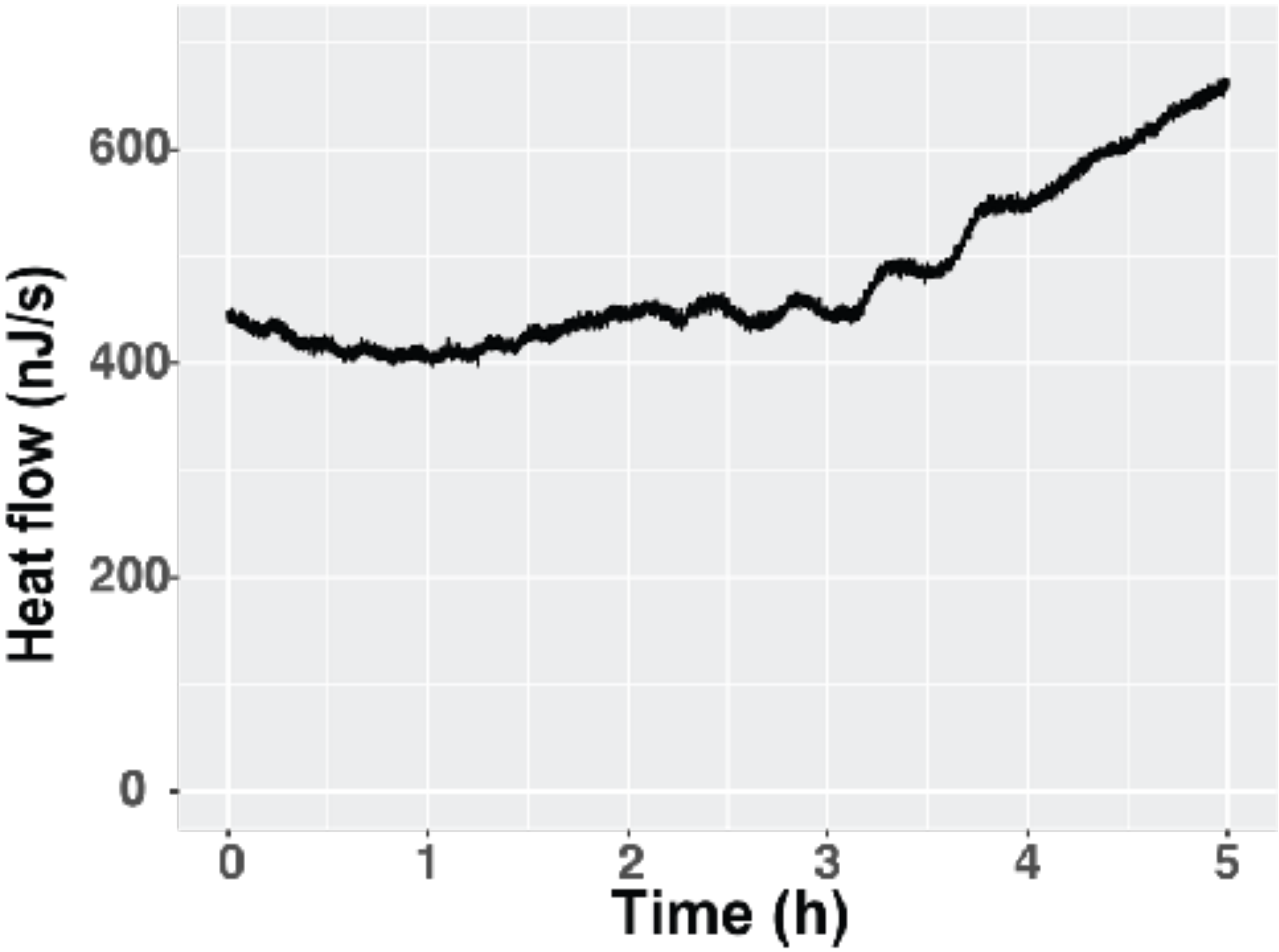


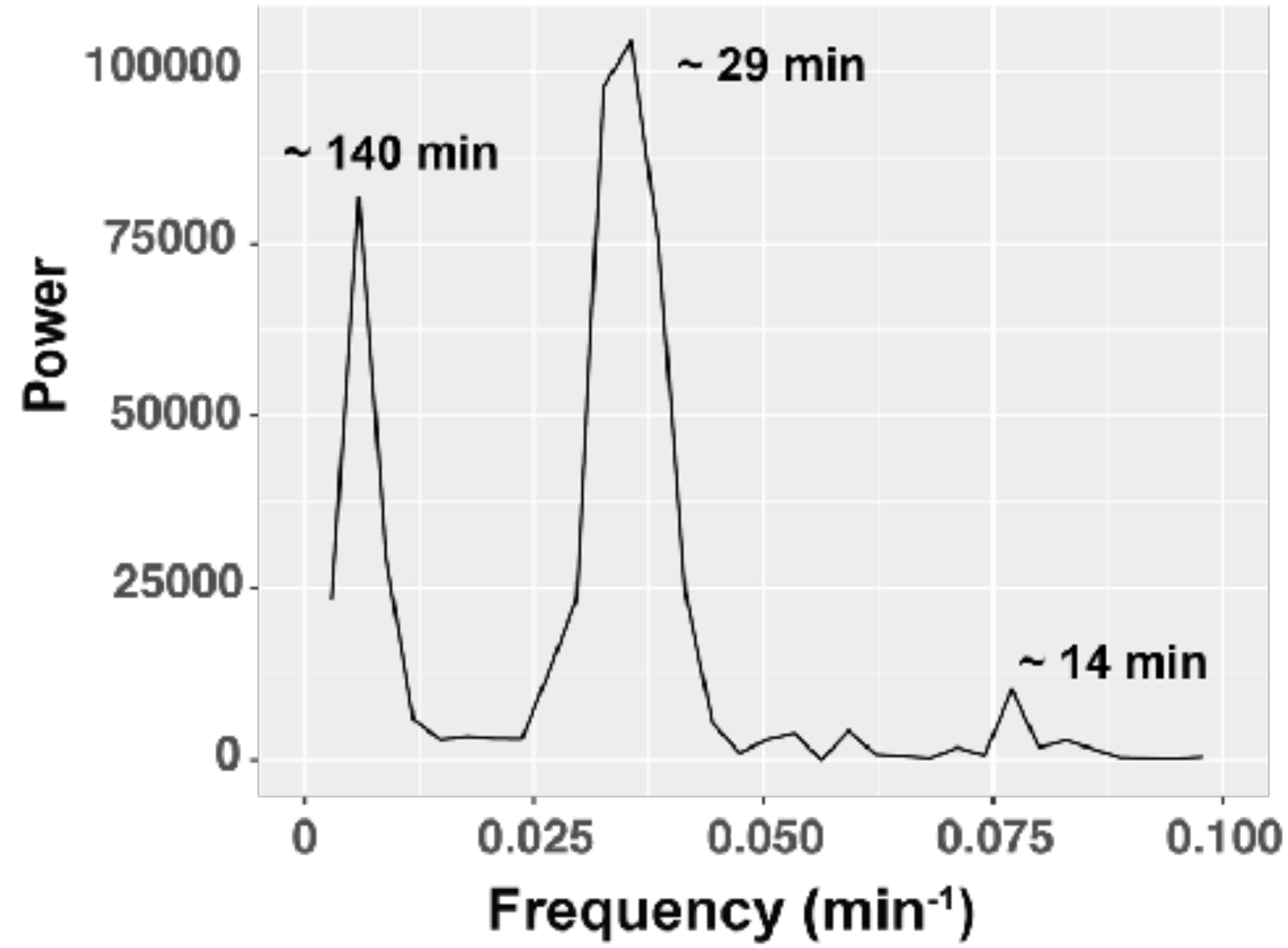
Bradley T. French & Aaron F. Straight *et al.*, 2017



Paranjpe & Veenstra 2015

Heat oscillations are conserved in single frog embryos

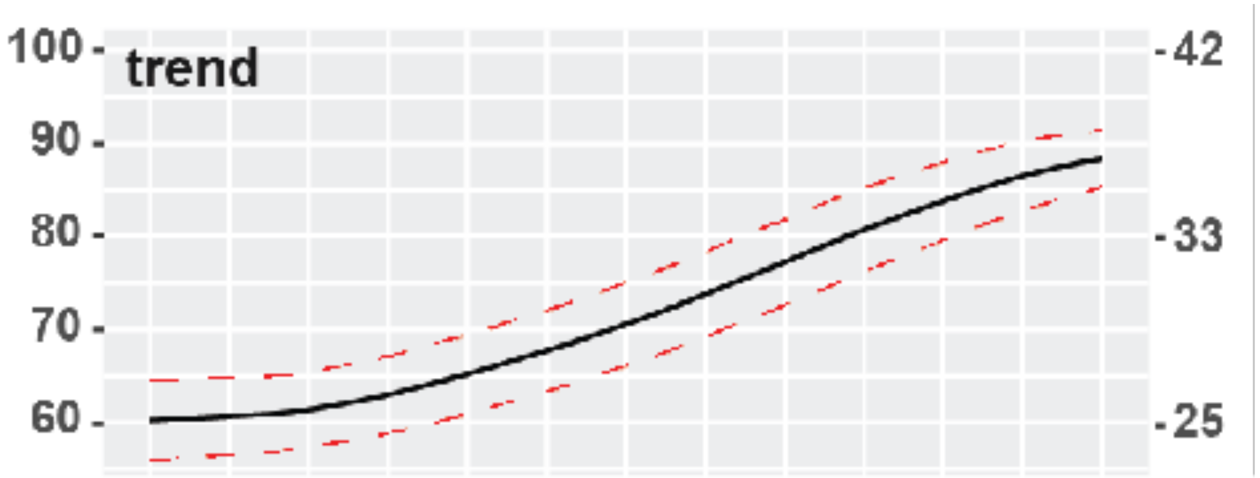




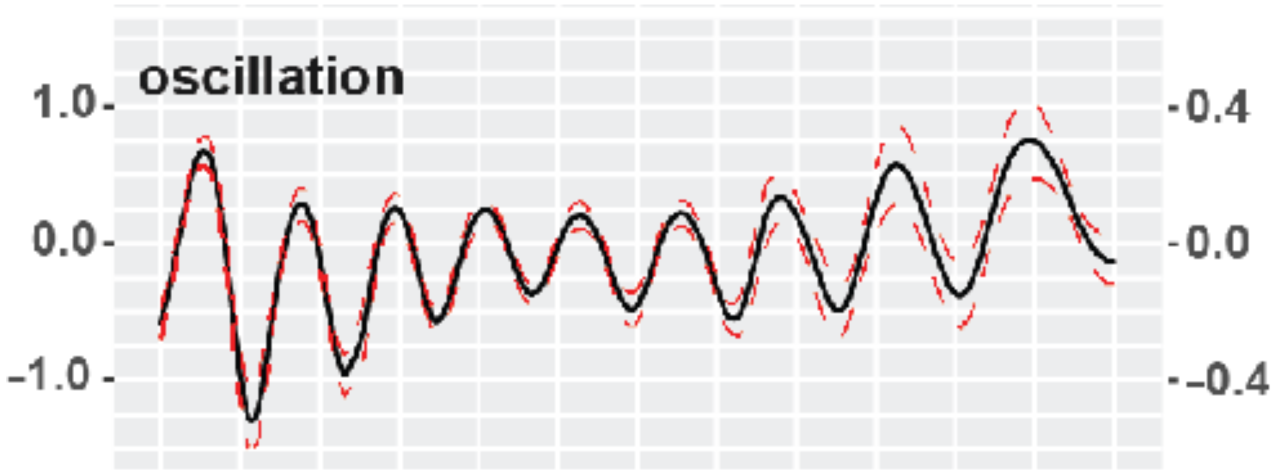
Xenopus laevis, 22 degrees C

Embryonic heat flow is composed of an increasing trend and an oscillatory component

Increasing component



Oscillatory component



Rodenfels *et al.*, *Dev. Cell* 2019

Cell proliferation drives increasing heat dissipation

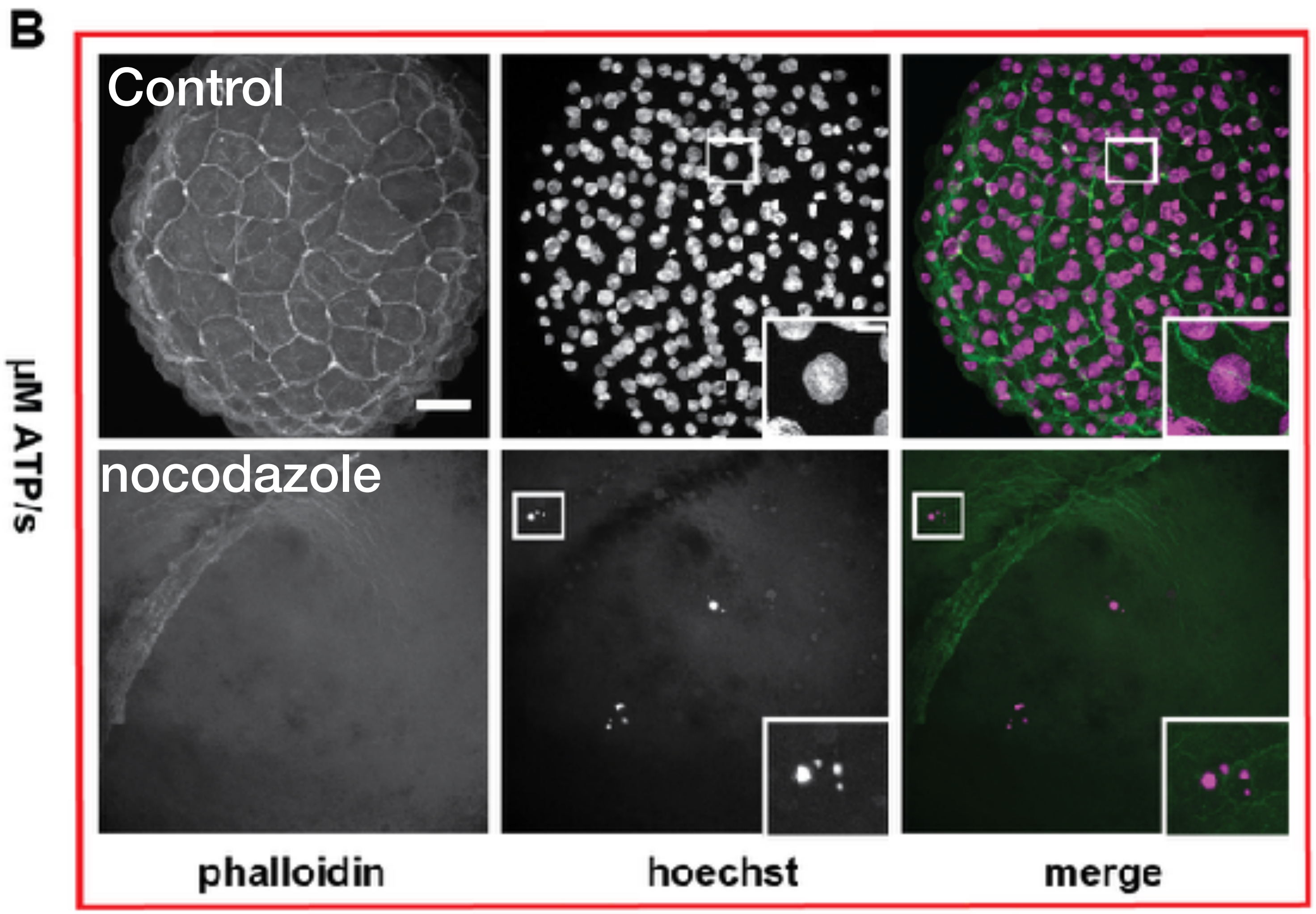
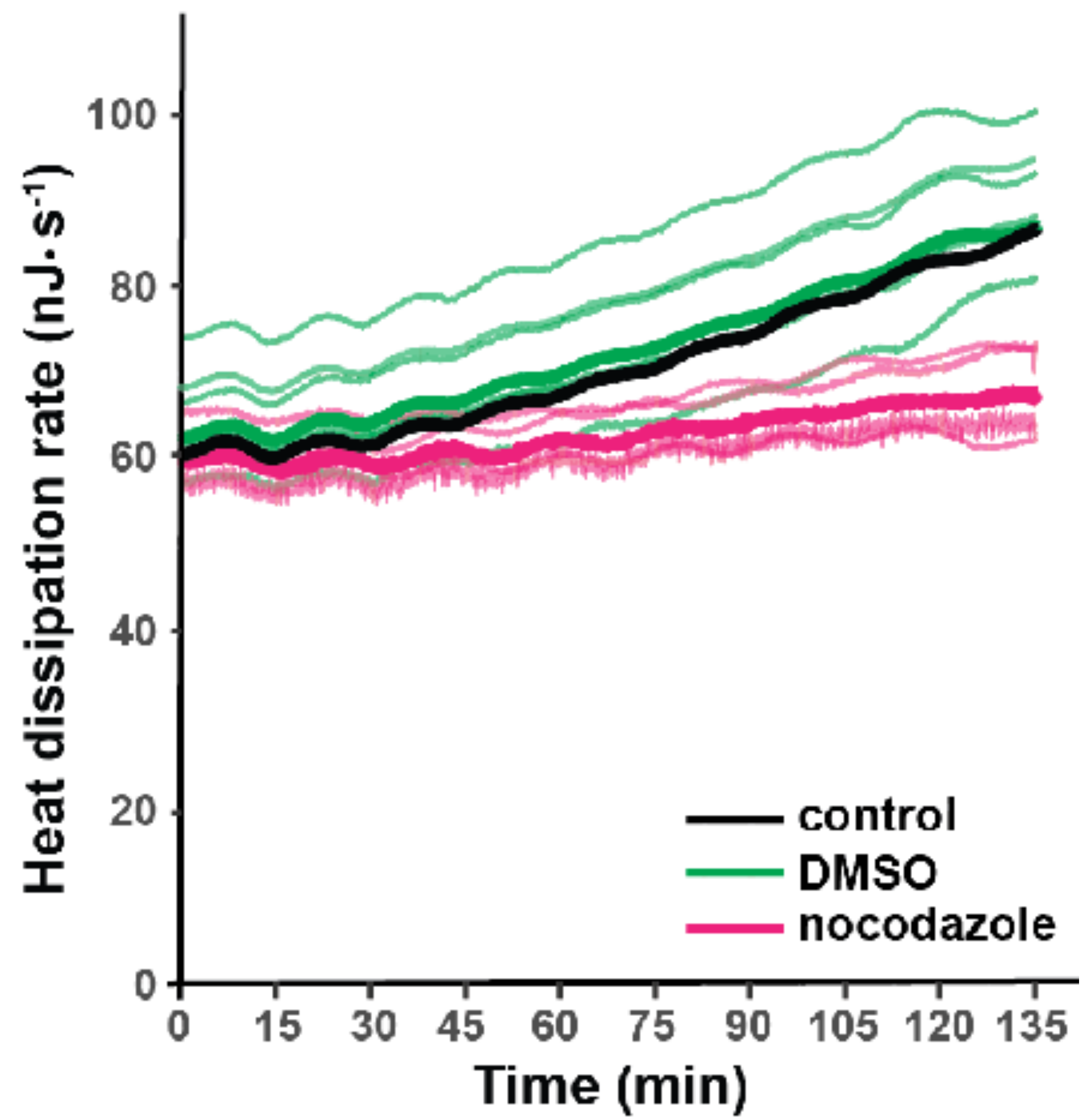


Fig 2. Rodenfels *et al.*



Rodenfels et al. bioRxiv, 2019

Does the increase in heat dissipation scale with cell number?

$$\dot{Q}(t) = A + B \cdot f\big(N(t)\big) = A + B \cdot f\big(2^{n(t)}\big) = A + B \cdot f\big(2^{t/T+1}\big) \qquad \text{Eq. 1}$$

If proportional
to the number of cells

$$f\big(2^{t/T+1}\big) \propto 2^{t/T}$$

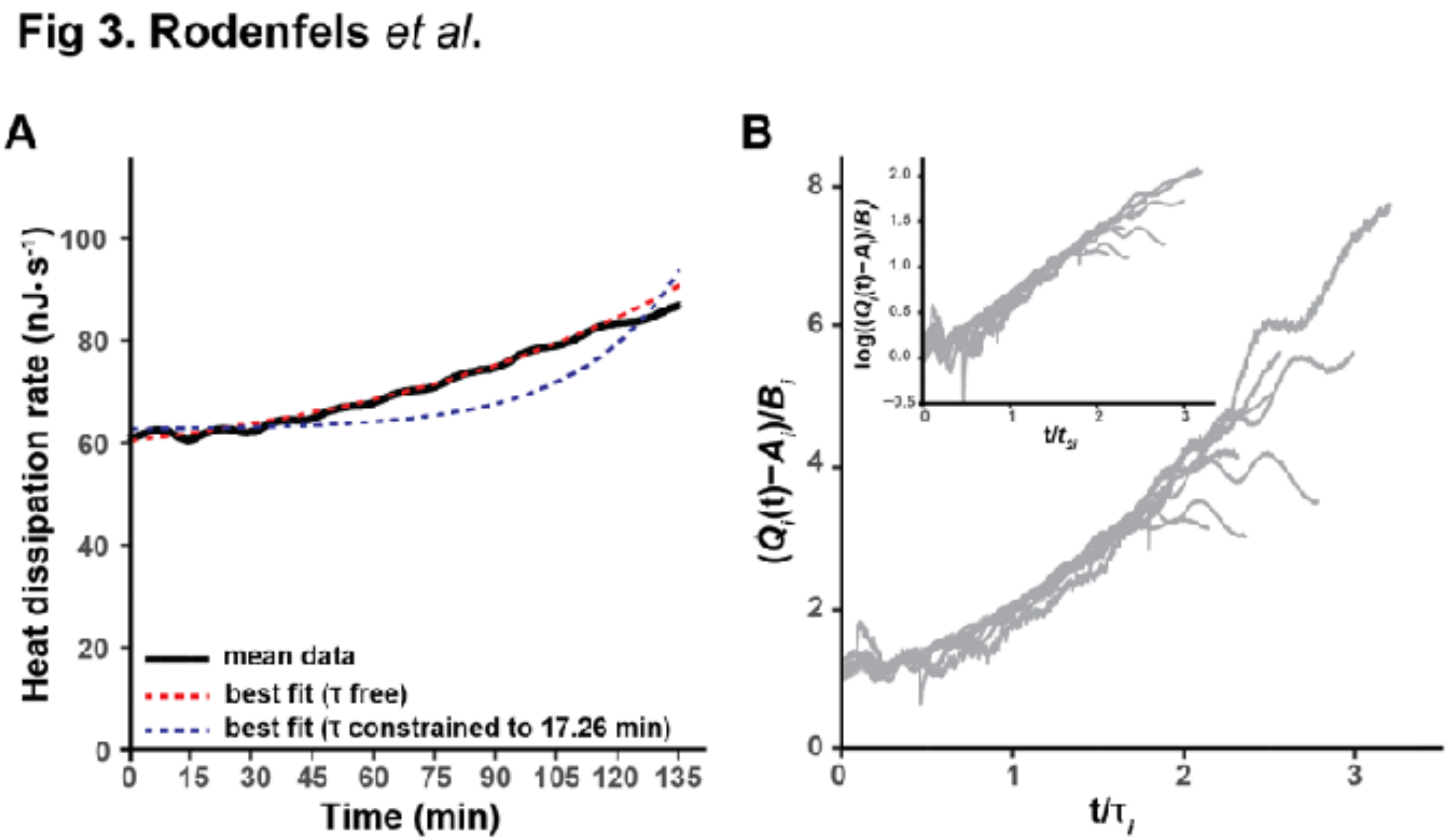
If proportional
to the rate of increase in cell number

$$f(t) \propto (1/T)2^{t/T}$$

Rodenfels et al. bioRxiv, 2019

Heat dissipation scales with cell number but increases 3x more slowly

$$\dot{Q}(t) = A + B \cdot 2^{t/\tau} \qquad \text{Eq. 2}$$



Parameter	
Temperature	**28.5ºC**
Volume term, ***A***	$52 \pm 12\ \text{nJ} \cdot \text{s}^{-1}$
Area prefactor, ***B***	$8.2 \pm 3.2\ \text{nJ} \cdot \text{s}^{-1}$
Heat doubling time, ***τ***	$60.4 \pm 10.4\ \text{min}$
Cell doubling time, ***T***	$17.2 \pm 0.8\ \text{min}$

Rodenfels et al. bioRxiv, 2019

Slower heat doubling time is consistent with being proportional to total cell surface area

<u>Surface-area model</u>

The surface-area model assumes that
(i) The cells are closed spheres.
(ii) The total volume ($60 \cdot 10^6$ µm³) remains constant over the cleavage stage
(iii) Cell division time is constant

$$V_0 = \frac{4\pi}{3} R_0^3 \qquad \text{Eq. 3}$$

$$V_n^{tot} = N_n V_n = 2^n \frac{4\pi}{3} R_n^3 \qquad \text{Eq. 5}$$

$$V_n = V_0$$

$$S_0 = 4\pi R_0^2 \qquad \text{Eq. 4}$$

$$R_n = \frac{R_0}{2^{n/3}} \qquad \text{Eq. 6}$$

$$S_n^{tot} = N_n S_n = S_0 \cdot 2^{n/3} \qquad \text{Eq. 7}$$

$$S_t = S_{t/T+1} = S_0 2^{1/3} 2^{t/3T} = S_1 2^{t/3T}$$

Slower heat doubling time is consistent with being proportional to total cell surface area

$$f = \beta S_n^{tot} + \gamma d S_n^{tot} / dt$$

$$\dot{Q}(t) = A + B \cdot f\big(N(t)\big) = A + B \cdot f\big(2^{n(t)}\big) = A + B \cdot f\big(2^{t/T+1}\big) \qquad \text{Eq. 1}$$

$$Q(t) = A\, f = \beta S_n^{tot} + \gamma d S_n^{tot} / dt = A + \beta S_1 \cdot 2^{\frac{t}{3T}} + \gamma \frac{S_1}{3T} \ln 2 \cdot 2^{\frac{t}{3T}}$$

$$\dot{Q}(t) = A + \left(\beta + \gamma \frac{\ln 2}{3T}\right) S_1 \cdot 2^{t/3T}$$

embryo volume

embryo surface area

increase in embryo surface area

Rodenfels et al. bioRxiv, 2019

Slower heat doubling time is consistent with being proportional to total cell surface area

$$B = \left(\beta + \gamma \frac{\ln 2}{3T}\right) S_1 \qquad \text{Eq. 3}$$

Table 3: Estimated energetic parameters at 28.5 °C

Parameter	**Estimates**		**Values from fits**
Volume term[1], $\boldsymbol{A}$	$90\ \mathrm{nJ \cdot s^{-1}}$		$52 \pm 12\ \mathrm{nJ \cdot s^{-1}}$
Area pre-factor[2], $\boldsymbol{B}$	β (maintenance)	γ (production)	
	β_{ATPase}[3] $= 0.74$ to $7.4 \cdot 10^{-3}$ $\mathrm{pJ \cdot s^{-1} \cdot \mu m^{-2}}$	γ_{lipid}[4] $= 0.12$ to 24 * $\mathrm{pJ \cdot \mu m^{-2}}$	
	$\beta_{\mathrm{turnover}}$[5] $= 0.02 \cdot 10^{-3}$ $\mathrm{pJ \cdot s^{-1} \cdot \mu m^{-2}}$	$\gamma_{\mathrm{protein}}$[6] $= 4.3$ to 128* $\mathrm{pJ \cdot \mu m^{-2}}$	
	$B = \left(\sum \beta_{\mathrm{i}} + \frac{\ln(2)}{3T} \sum \gamma_{\mathrm{i}}\right) \cdot S_1 = 1.6$ to $40\ \mathrm{nJ \cdot s^{-1}}$* (see[7],[8])		$8.2 \pm 3.2\ \mathrm{nJ \cdot s^{-1}}$

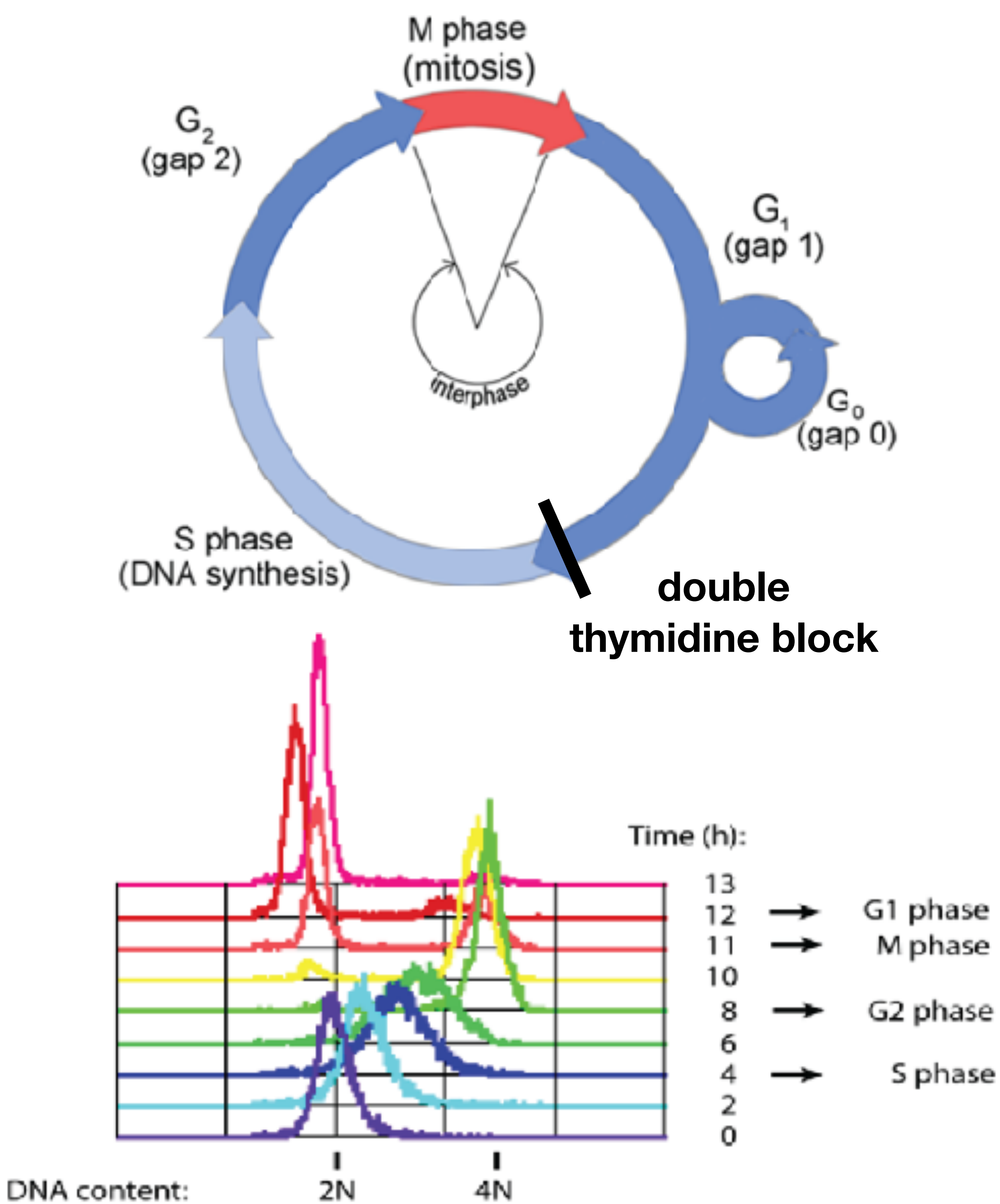
Rodenfels et al. bioRxiv, 2019

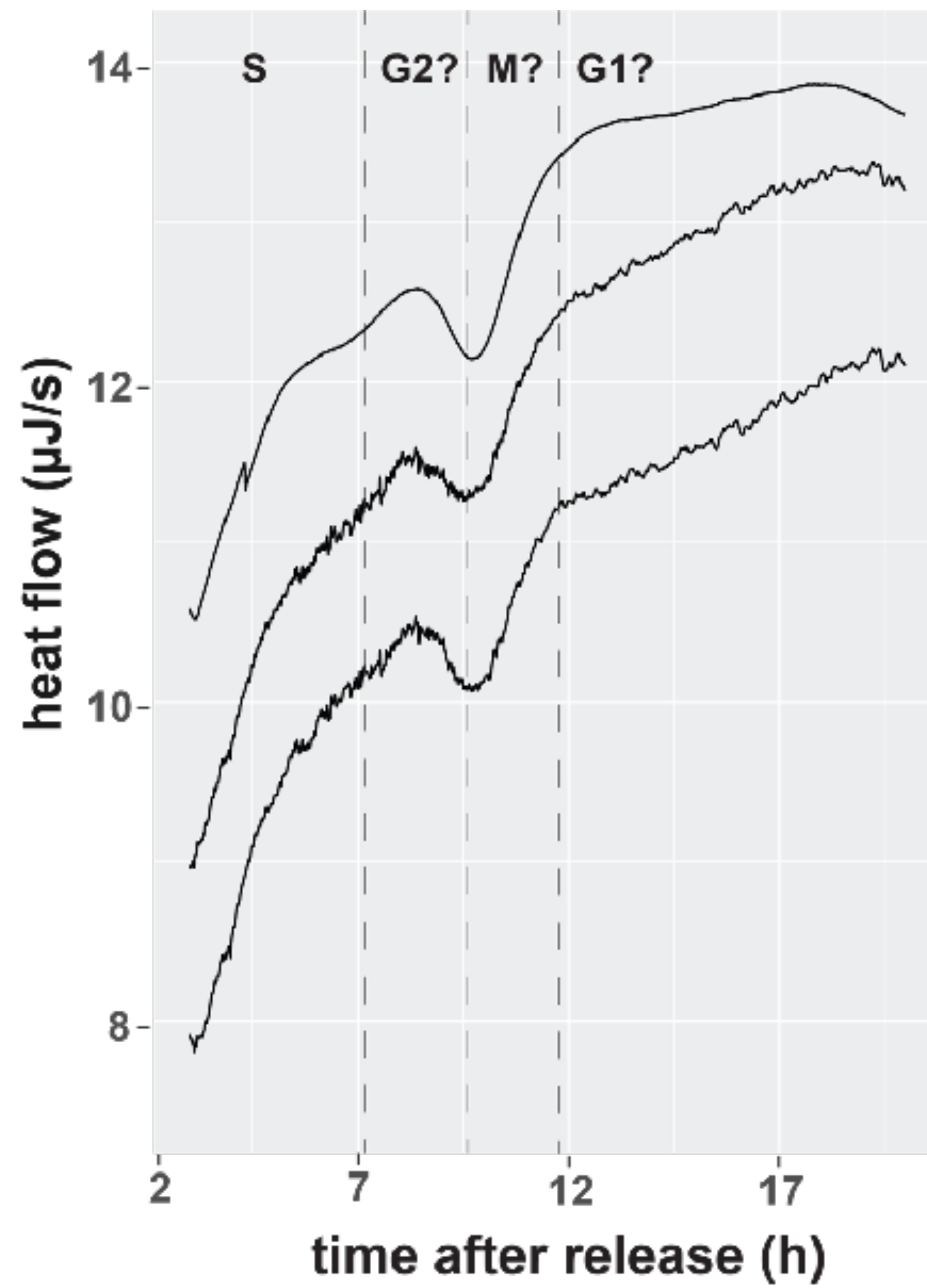
Summary

- **Increase in heat dissipation is not due to volumetric growth**
- **Increase in heat dissipation is due to some aspect of cell proliferation**
- **The increase scales with total cell surface area rather than total cell number**
- **Calculated energetic cost of maintaining and assembling plasma membranes and associated proteins likely accounts for the increase in heat dissipation**

Rodenfels et al. bioRxiv, 2019

Synchronized HeLa cells display dynamic heat dissipation during the cell cycle





http://cyberbridge.mcb.harvard.edu/mitosis_3.html

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EMBO

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