BCH4125

Cell Regulation and Control

Welcome!

Drs. Kristin Baetz & Adam Rudner
Regulation of the Cell Cycle from

START to Finish

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8 Lectures
course coordinator

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8 Lectures

We will actually start with mitosis and then move to the beginning of the cell cycle. This might seem a bit backward, but historically studies on mitosis revealed the critical regulators of the cell cycle. This information then guided the studies of G1 and S-phase.

Some topics covered in this class

using biochemical and genetic techniques to study cell biology

cell cycle regulation

chromosome segregation

cell cycle checkpoints

control of DNA replication

DNA replication

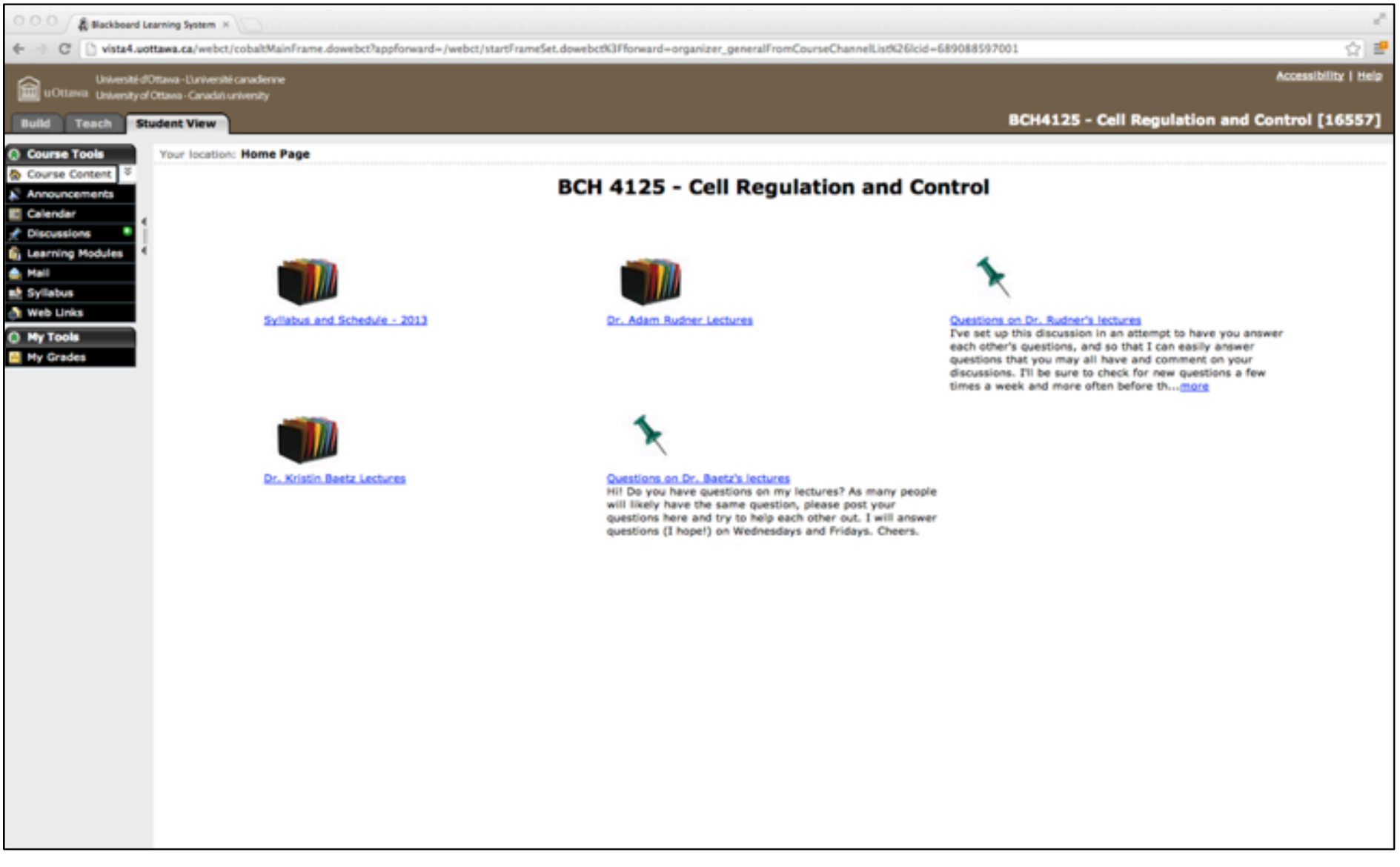
sister chromatid cohesion

primarily will focus on model systems (yeasts, egg extracts, and a bit on vertebrate cells).

Research papers are primary sources.

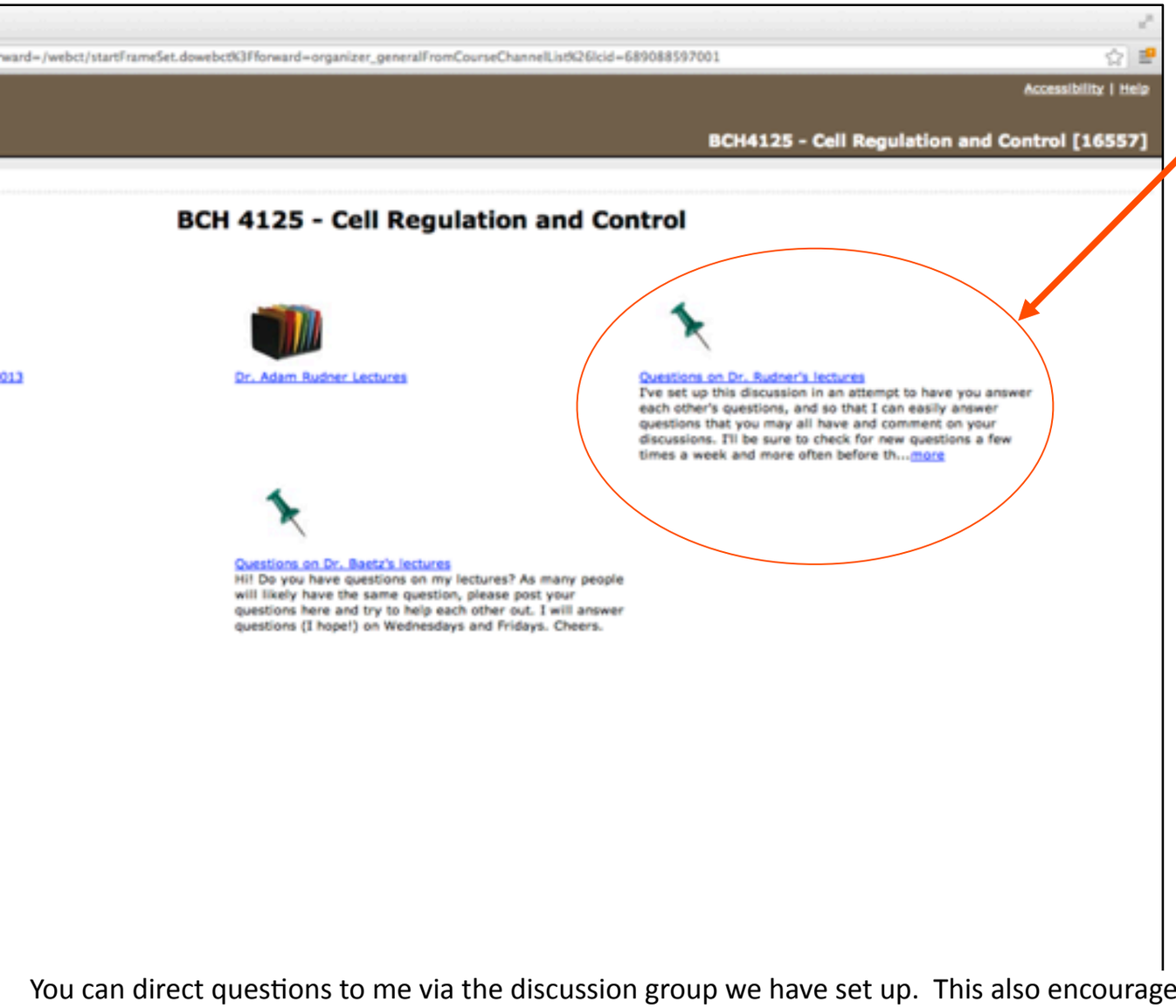
Goal is to apply the knowledge you gain in class to solve biological problems.

Very little memorization/regurgitation on exams.



You'll find everything on the blackboard site.

Questions?



Post on Discussion Groups for each Prof.

or

Contact Professor directly by e-mail

You can direct questions to me via the discussion group we have set up. This also encourages you to discuss questions amongst yourselves.

Questions?

Four ways:

post questions on Blackboard site

e-mail me

Thursday from 1:30 to 3 pm in Biochemistry office.

Set up a meeting with me and come out to RGN.

Although I strongly urge you to discuss the material amongst yourselves first, you can contact me directly, or we can meet in person.

Evaluation

20% 1st mini Exam

January 24th

Based on my first 5 lectures

30% 2nd Exam

February 14th

Based on all my lectures

20% 3rd mini Exam

Date TBA (likely early March).

Based on Dr. Baetz's first lectures

30% 4th Final Exam

During the final exam slots

Based on all of Dr. Baetz's lectures

The first mid-term is soon! So pay attention early on. The point of the early mid-term is so you can know if you are on track and you'll get a taste of the type of exams you'll see. We hope to have the marks posted for the first exam before the next class (on January 28th).

Past students have struggled on BCH4125 exams.

I will include one or two practice questions at the end of each lecture that we will discuss at the start of the next class.

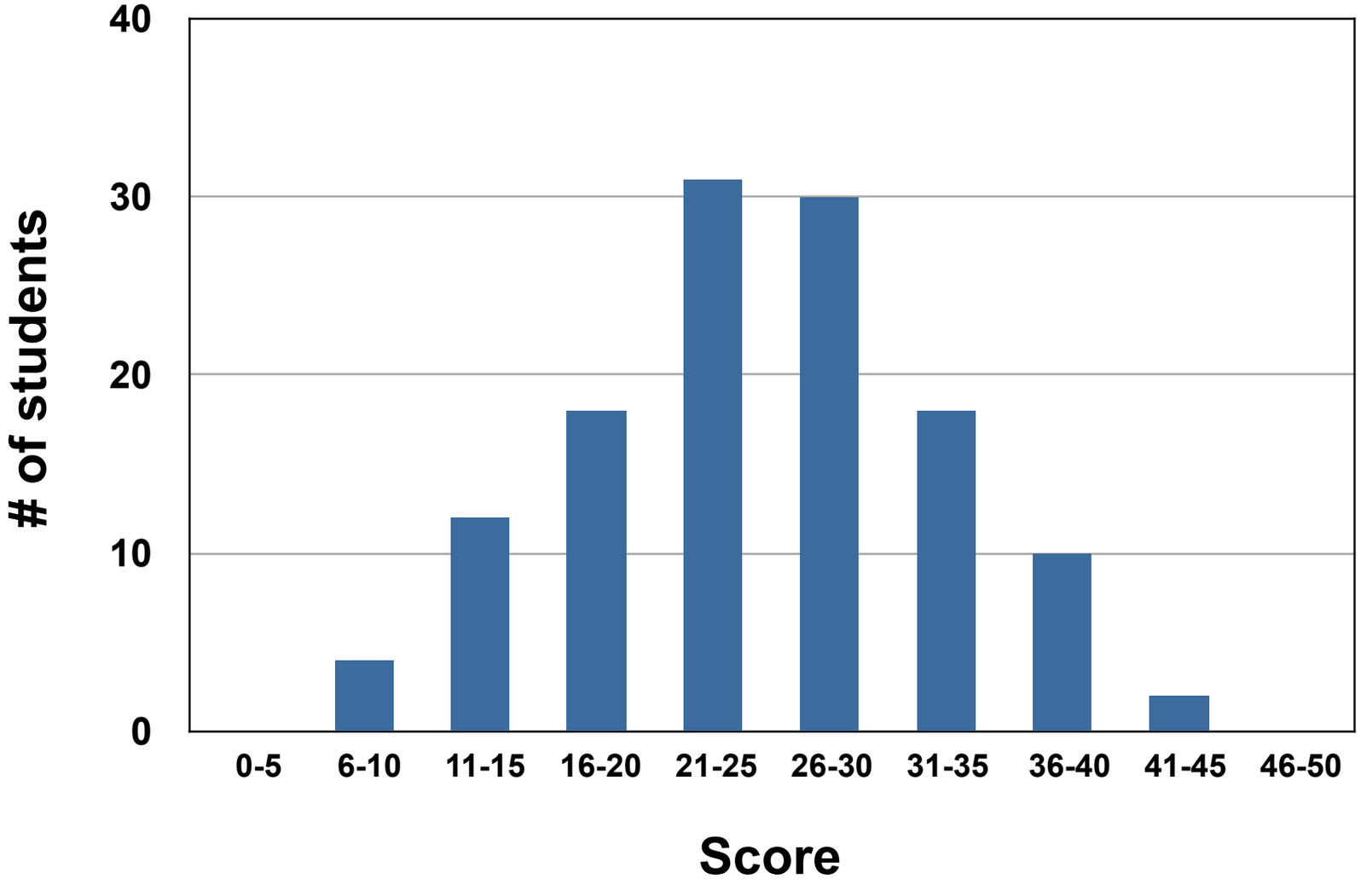
Spending time going over the lecture slides and notes before and after each class will help you engage with the material.

Ask questions!

Good luck!

Raw Scores

average: 25
high score: 42



The results from last year's midterm. Although we did curve the scores somewhat, it doesn't feel great getting a 50% or lower! Hopefully the class average will be higher. We've decided to include two shorter mid-terms to help you improve your marks.

Adam Rudner

My lab studies how cells regulate chromosome segregation and assemble chromosome structures

Contact me via the blackboard site or arudner@uottawa.ca

Slides

My lecture slides will be posted the evening before the lecture. Updates may appear after the lecture.

I will post the slides as pdf and powerpoint files, but the powerpoint may have formatting issues (I create the presentations in keynote and convert to powerpoint).

Two Midterms

mini-midterm on January 24th worth 20%, based on first five lectures

**longer midterm on February 14th worth 30%,
based on all my lectures**

Everything we talk about in the class could be on the exam

I expect you to read the papers I assign and understand them (not memorize each figure)

In general, I'm interested in testing if you can apply what you've learned to new problems.

There will be two midterms. The first will be short, so you'll have ample time to complete it, the second will be a bit longer.

Suggested papers

I will suggest one or two papers for each class that contain some of the experiments/concepts that I will discuss during the lecture. Obviously reading these before class will help you understand the material.

Required papers

There are three required papers for my lectures. These papers will help you focus on important techniques and concepts. You are responsible for being very familiar with the papers for the exam, though you are not expected to memorize the figures.

Paper 1

Russell, P., & Nurse, P. (1986). cdc25+ functions as an inducer in the mitotic control of fission yeast. *Cell*, *45*(1), 145–153.

This paper will be discussed in Lecture 3. A set of questions are posted with the paper to help guide you in reading and understanding the paper.

I suggest you read it once before Lecture 3, try to answer the questions, and then read it again after class.

I suggest you work together to answer the questions I assign for each paper.

Textbooks

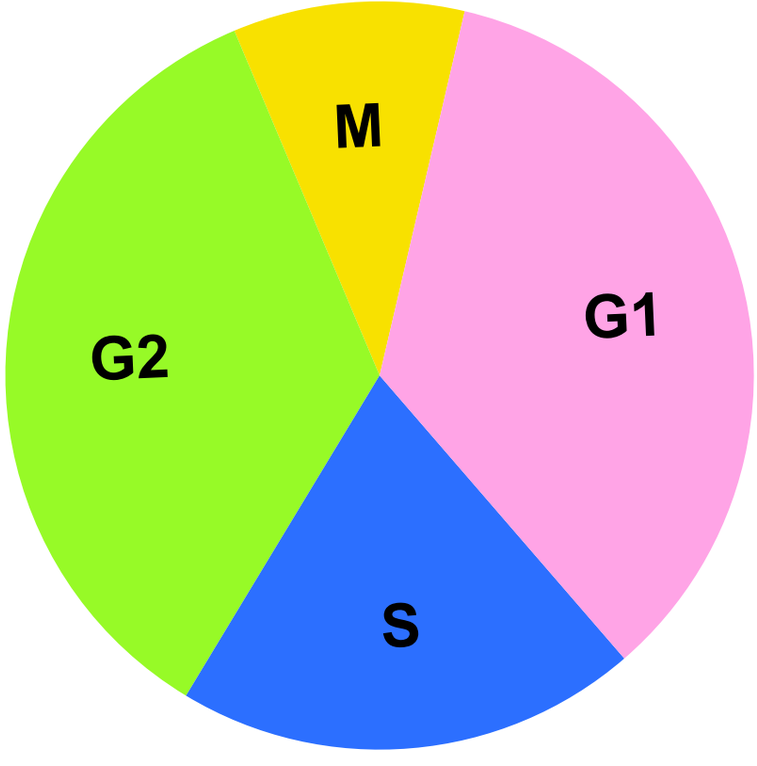
Molecular Biology of the Cell, Alberts et al. 5th Edition, 2008 (4th or 3rd may be OK, but a bit out of date). Key chapters are 17, 16 and 20.

The Cell Cycle: an introduction, Andrew Murray and Tim Hunt, 1993 (out of date, but a great introduction to the logic of the cell cycle)

The Cell Cycle: Principles of Control, David O Morgan, 2007 (very current, but mainly a reference book to get a short blurb on specific topics).

http://books.google.com/books?id=_7ygQAOK1DUC&printsec=frontcover&dq=the+cell+cycle&sig=ACfU3U3FQ3LxG8uDxE-gklwdfM9JlgXLIQ#PPP1,M1

Lecture 1 - Dominoes and Clocks



Outline of Today's Lecture

1. The events and logic of the cell cycle.

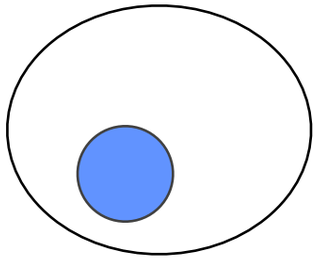
2. Cell fusion experiments suggested a key factor regulates mitosis.

3. The discovery of MPF in frogs.

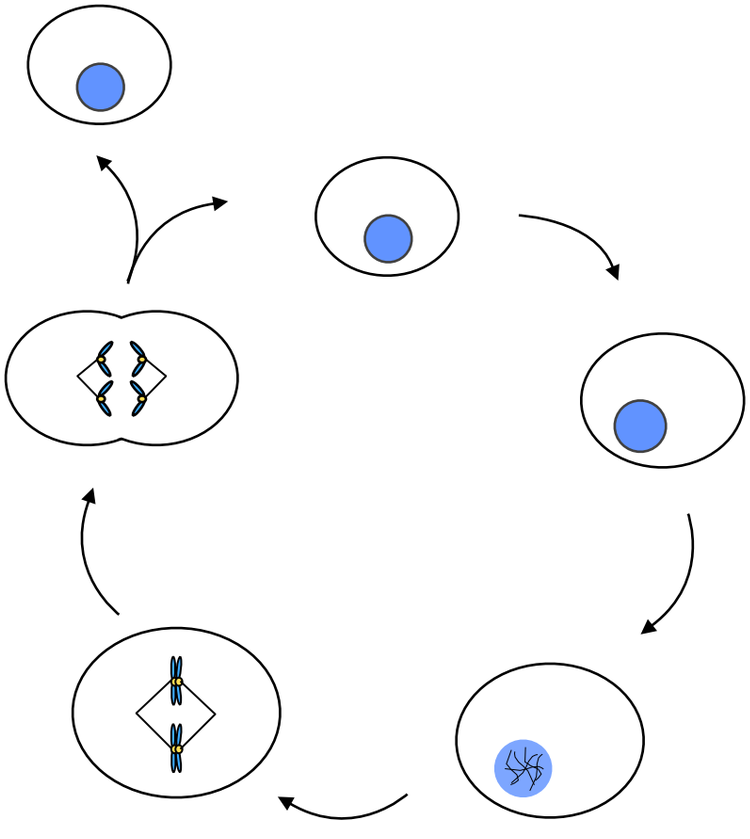
4. A brief introduction to using biochemistry to understand biological mechanism.

Outline of this lecture.

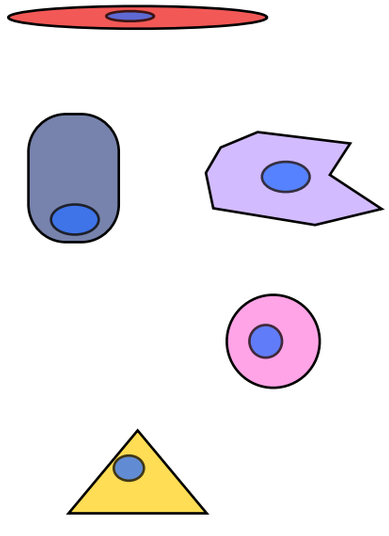
three choices for cells



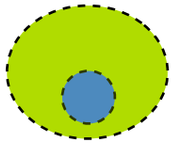
proliferation



differentiation



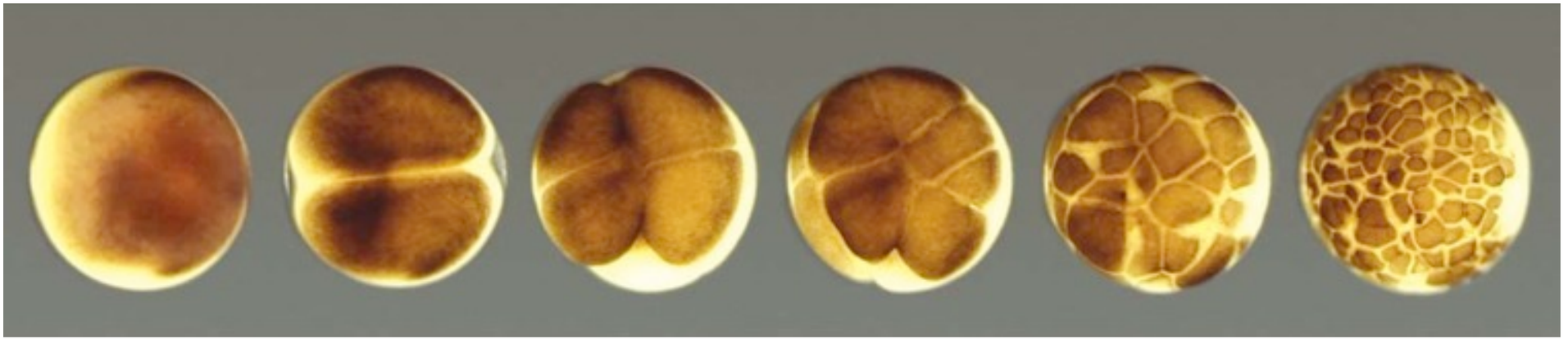
cell death



Cells have three choices: To proliferate, to differentiate and to undergo cell death. All three are critical during development. A fourth option is for cells to remain quiescent and not make any of these three choices.

Why study cell division?

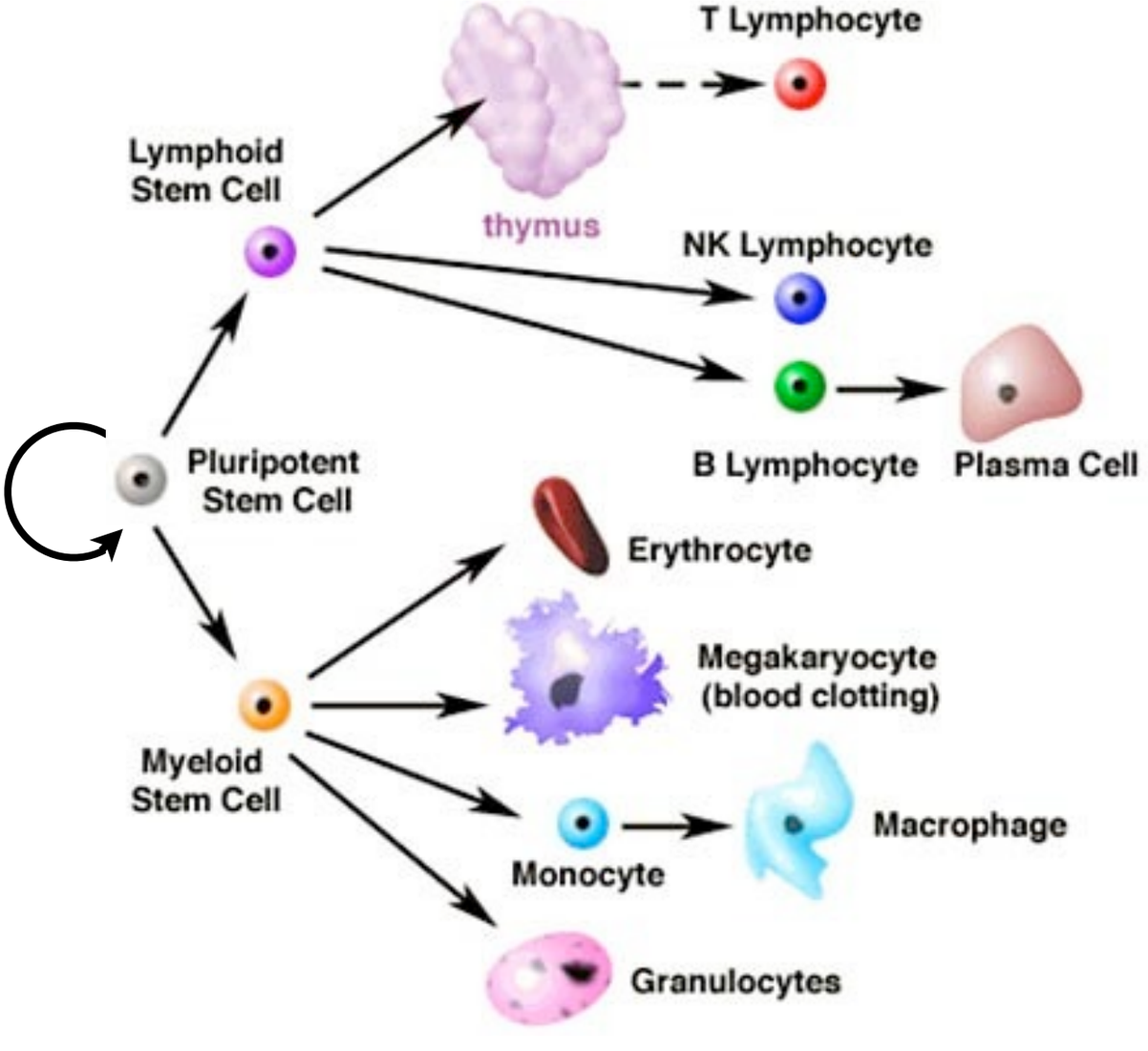
Development requires proliferation



Early development requires rapid cell proliferation. In the early development of amphibian embryos, a single large fertilized egg rapidly divides into thousands of smaller cells.
Photo from cover of The Cell Cycle, by David O Morgan.

Why study cell division?

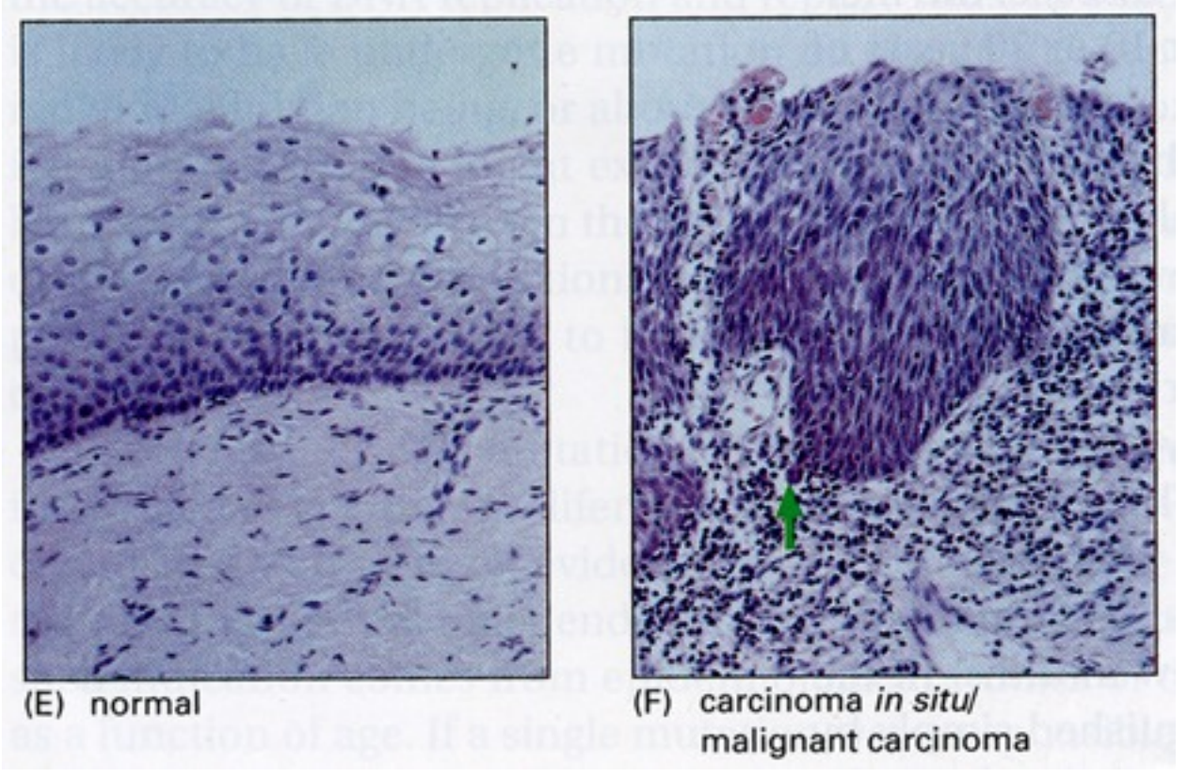
Stem cells retain the ability to proliferate



Stem cells retain the ability to proliferate. Understanding how this occurs in these specialized cells is still poorly understood, and is critical to the future success of stem cell therapy and regenerative medicine.
Photo from http://www.itmonline.org/arts/antler.htm

Why study cell division?

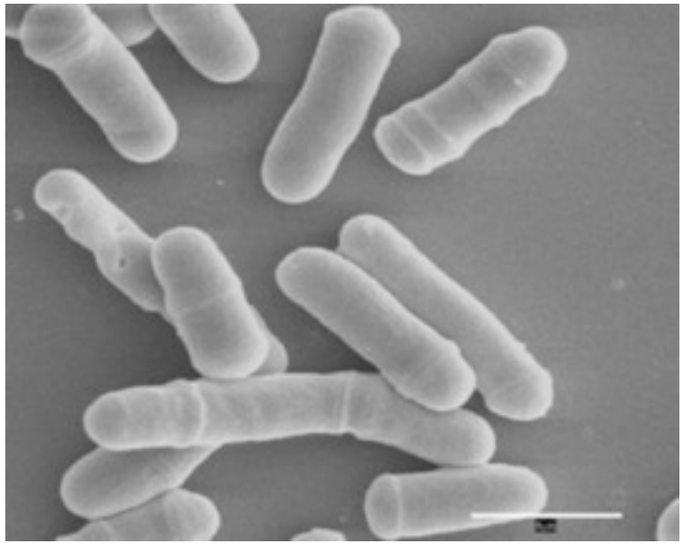
Cancer is a disease of excessive cell division

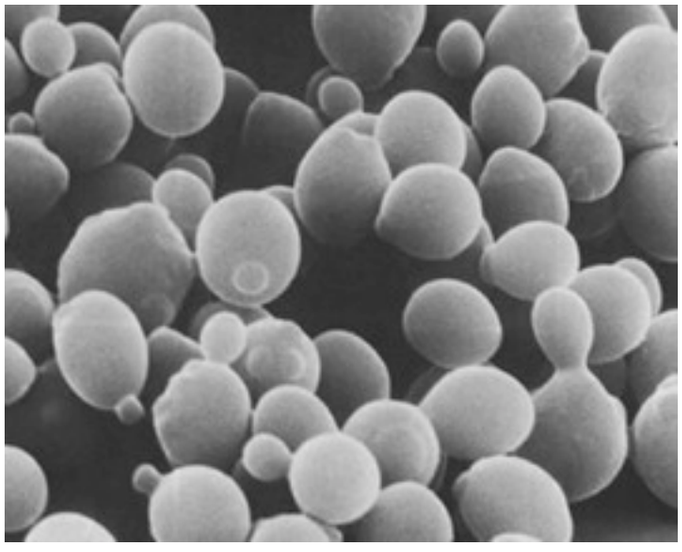


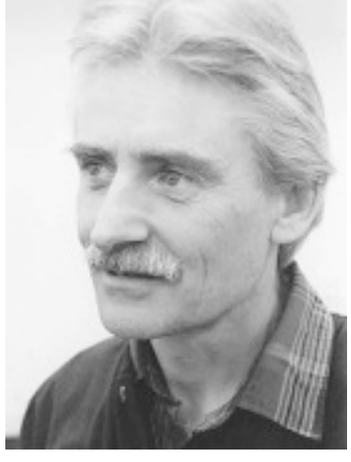
Cancer cells divide uncontrollably as is seen in this picture of cervical carcinoma. The dark spots are the nuclei of cells. Clearly the carcinoma has many many more cells than the normal tissue. The carcinoma is also invading into the tissue below the epithelium. Photo from MBOC, 3rd Edition.

Why study cell division?

A terrific scientific story



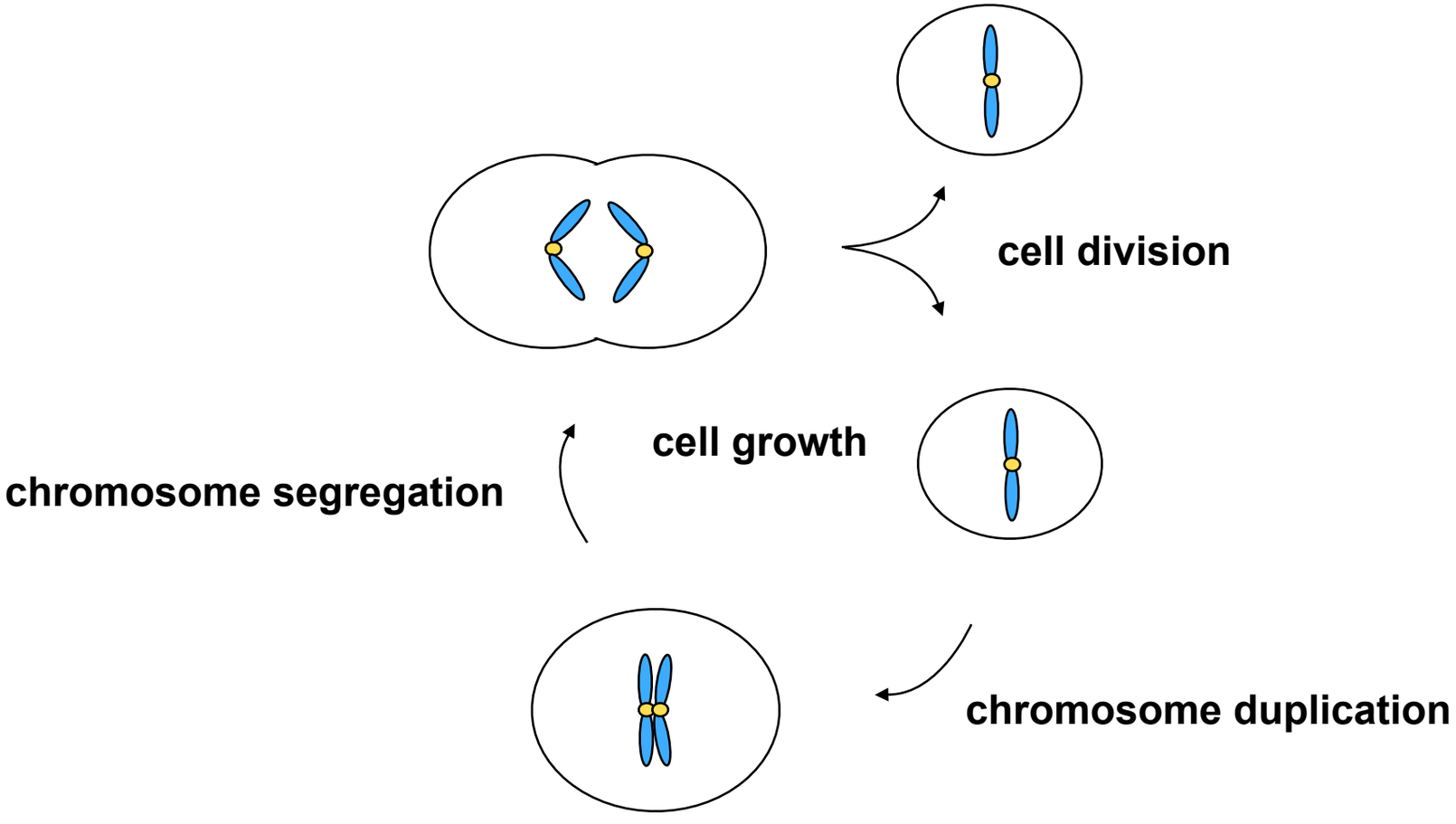




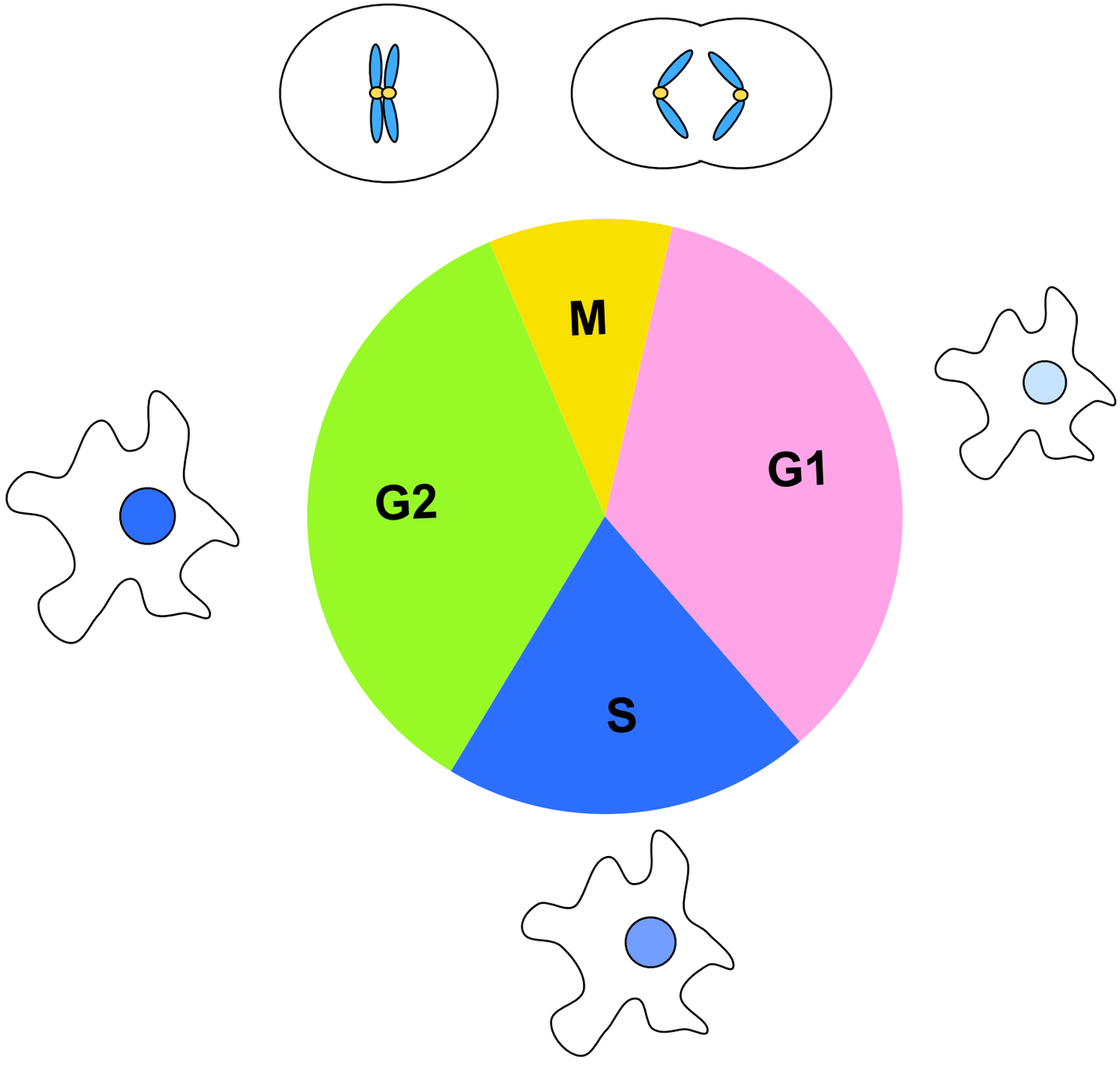
This lecture (and the next) will tell the story of how three different research programs led to the current view of how the cell cycle works. Work on amphibian and marine invertebrate oocytes, fission and budding yeast all led to the result that the inner workings of the cell cycle are conserved. In 2001, Tim Hunt, Paul Nurse and Lee Hartwell shared the Nobel prize in physiology and medicine for this work. My lectures will focus primarily on these three systems - though remember this process is highly conserved, and most of what we know about how cells grow and divide has come from work on model systems like these.

Four critical tasks

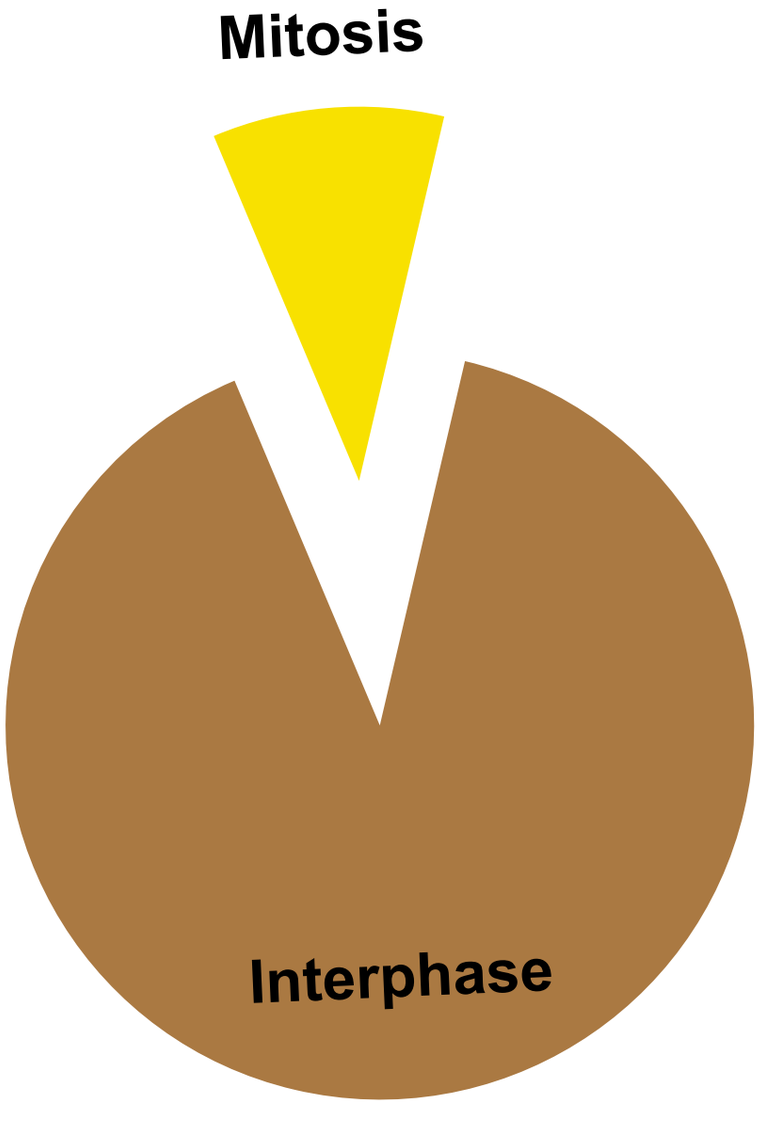
1. Grow
2. Duplicate chromosomes
3. Segregate chromosomes
4. Divide



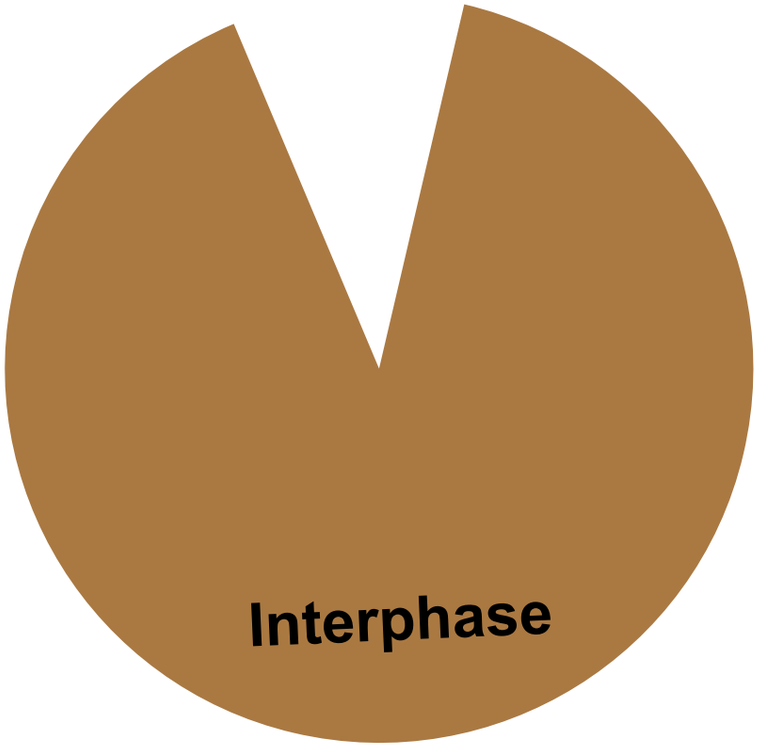
In the most simplified cell cycle there are four critical tasks that a cell must accomplish. Cells must double in size, duplicate their chromosomes, segregate their chromosomes and divide. Cell growth is a continuous process, while the three other events occur at distinct times during the cell cycle.

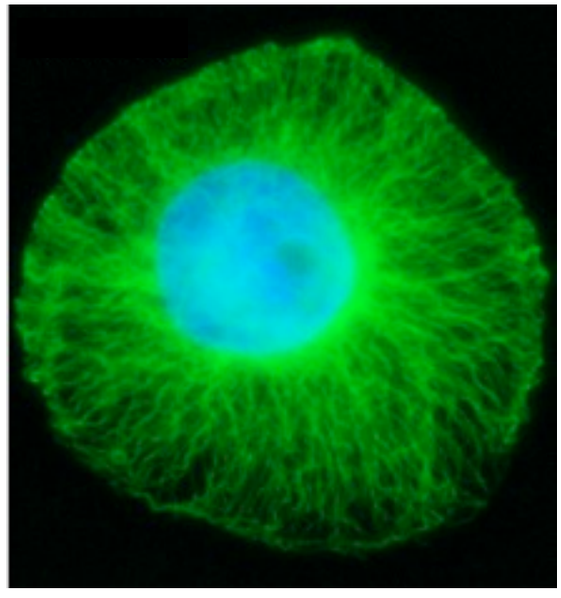


Yet another view of a simplified cell cycle. Cells transition from G1 (Gap or Growth) phase to S (DNA Synthesis) phase to G2 phase to M (Mitosis) phase. If we were actually looking at cells not much changes between G1, S and G2 (other than the cell increasing its mass), while dramatic events occur during mitosis. The cells round up, the nuclear envelope breaks down, chromosomes condense and are segregated on the mitotic spindle.

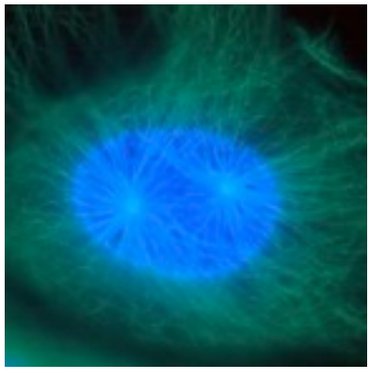


Often the cell cycle is broken up into Interphase and Mitosis. Interphase includes G1, S and G2. This distinction comes mainly from observations of cells - little changes during interphase.

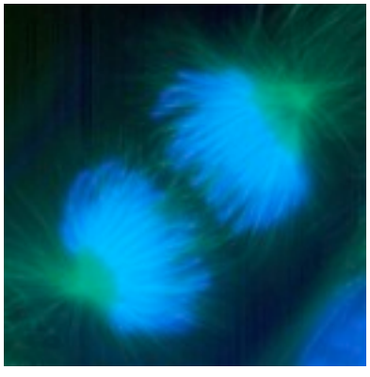




A picture of an interphase cell. The DNA (in the nucleus) is stained in blue, while the microtubules are stained in green. Interphase microtubules are largely cytoplasmic.
Photo comes from: http://mitchison.med.harvard.edu/research/researcharea.html?area=2

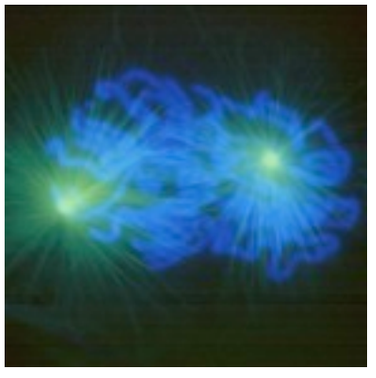


prophase

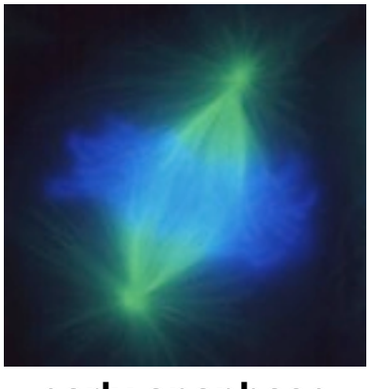


anaphase

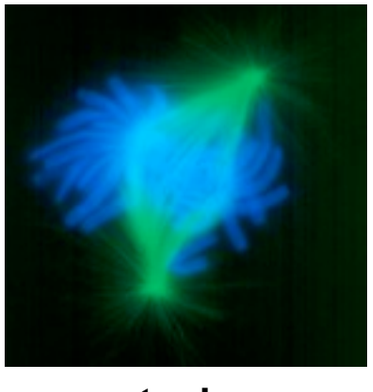
Mitosis



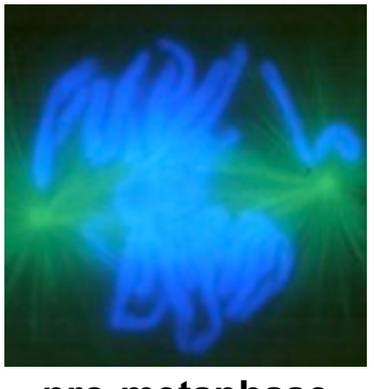
early pro-metaphase



early anaphase



metaphase

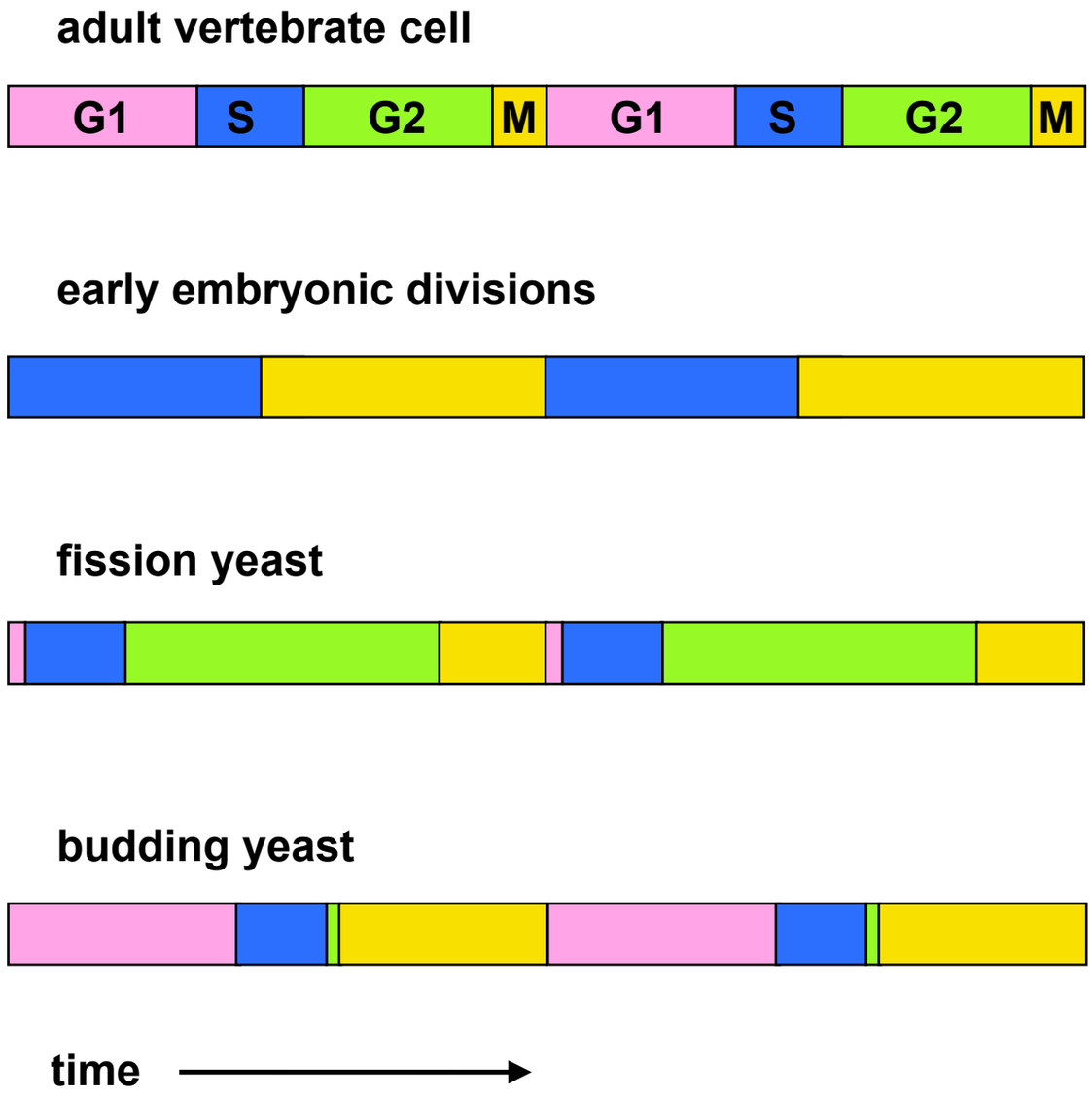


pro-metaphase

The stages of mitosis. Again, DNA is in blue, microtubules in green. Notice how the microtubule array has changed compared to interphase cells. In prophase microtubules are found in both the cytoplasm and the nucleus, the nuclear envelope begins breaking down and the spindle poles separate and nucleate the mitotic spindle. Condensation of chromosomes begins in early pro-metaphase, and de-condensation begins in early anaphase.

Photos are from Conly Rieder: http://www.wadsworth.org/bms/SCBlinks/web_mit2/res_mit.htm

Organisms organize their cell cycles differently



Four different cell cycles. The typical adult dividing vertebrate cell divides once every 24 hours. Mitosis is only about 1 hour long, while the three other phases are each 6-8 hours long. Early embryonic cycles are fast, as fast as 8 minutes in the fly, Drosophila melanogaster, and about 30 minutes in the frog, *Xenopus laevis*. These cycles have no G1 or G2 and switch between S phase and mitosis. Their main aim is to rapidly take a large fertilized egg (which is full of nutrients, mRNAs and proteins) and turn it into many smaller cells as rapidly as possible. Fission and budding yeast divide once every 75 to 120 minutes. Their cell cycle organization is quite different. Fission yeast have a long G2 phase, while an almost non-existent G1 phase. In fact S phase can initiate pretty much right after or even during cell division. Budding yeast have a long G1 phase, and virtually no G2 phase. In the budding yeast cycle the mitotic spindle will assemble during the end of DNA replication.

Four Coordination Problems in the Cell Cycle

1. Completion - how do cells know when one phase of the cycle is complete?

2. Alternation - how do cells keep the cycle in order?

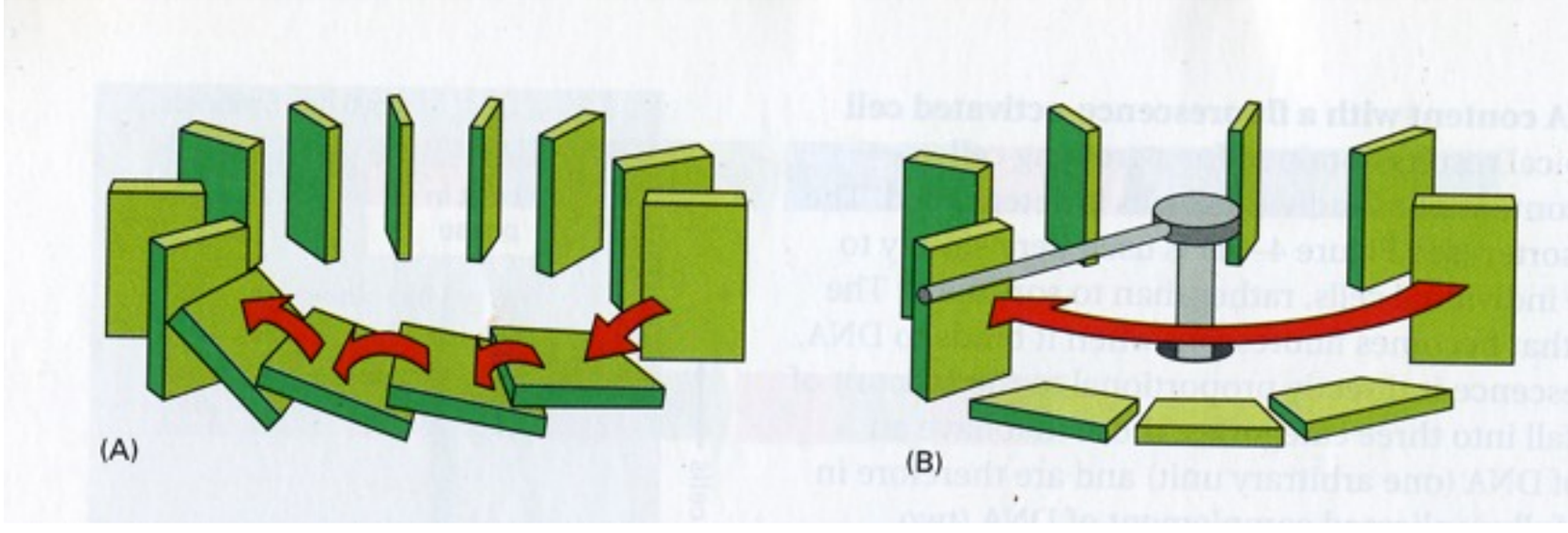
3. Coordinating size and division - how do cells maintain the size they want to be?

4. Coordinating development/apoptosis and division - how do cells grow into organs and organisms?

Four important questions that will be touched on during this course.

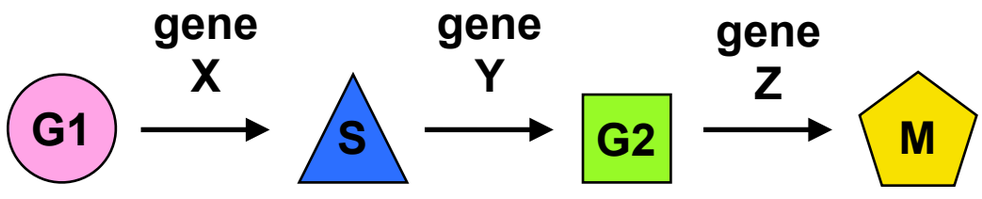
domino theory

clock theory

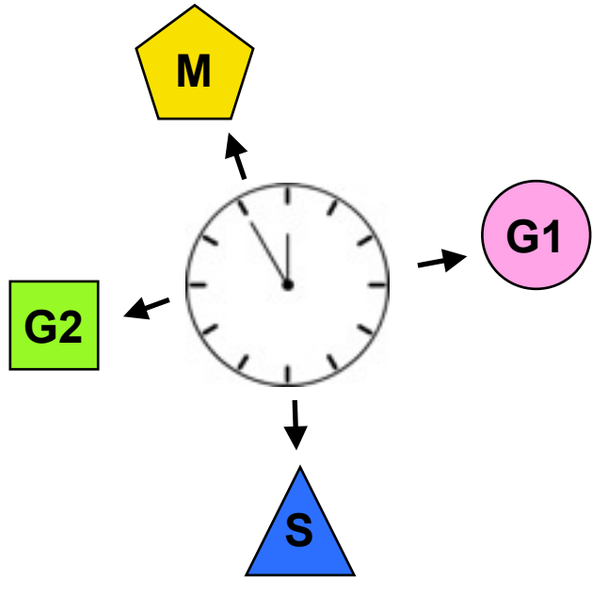


Two theories for how the cell cycle works. In the domino theory one event is dependent on completion of previous event. This model is based largely on models for bacteriophage assembly where the assembly of one structural protein depends on the assembly of a prior structural protein. Early researchers wondered if there would be a direct connection between the structural events of the cell cycle. In this model stages cannot be skipped - in the same way that if a domino is removed, the cascade of falling dominoes will end. In the clock theory a central regulator (or clock, or engine) controls the passage through different cell cycle phases. In this model there is no dependency between the different transitions and a given stage can be skipped. In the simplest example the engine would operate autonomously and there would be no feedback from completion of cell cycle events.

domino theory



clock theory



Another way to think about the domino and clock theory. In the domino theory one gene, here gene X, could control passage from G1 to S, and a different gene, gene Y, passage from S to G2. in this model S could never be skipped because the "substrate" of gene Y is S-phase. In the clock theory a central regulator, here the clock, controls passage through the cycle. The same basic mechanism controls passage through the entire cycle, so changes in the clock could change the order of the cycle. In this model the different events depend on the function of the clock, not on each other.

A third version

dependent pathway model

A → B → C → D → E → F

independent pathways model

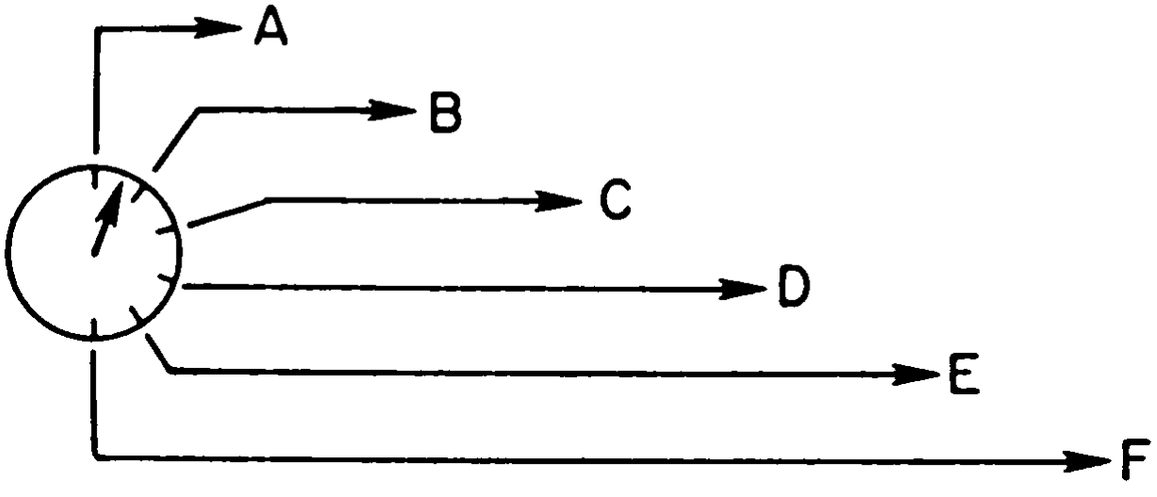


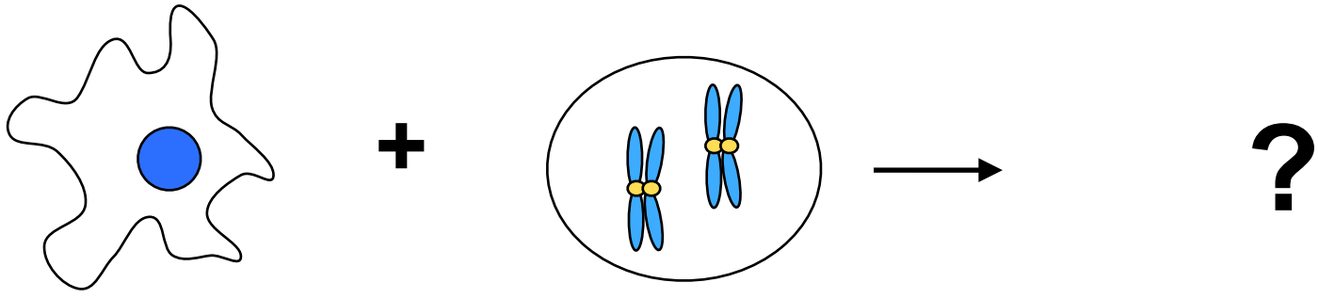
Fig. 2. Two models to account for the ordering of cell cycle events.

Hartwell, et al., 1974

Yet another depiction of the two models. This comes from the suggested reading for Thursday.

Cell fusion experiments gave first hints of the logic of the cell cycle

What happens when a G2 and a M cell are fused?



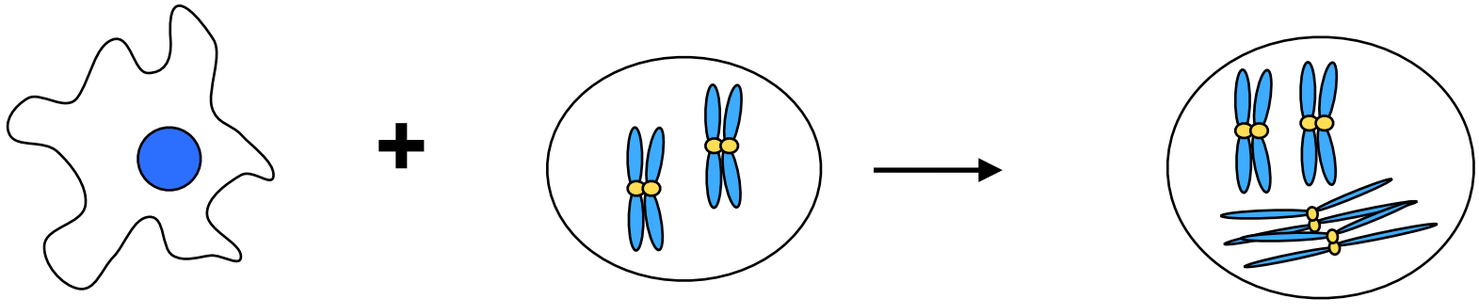
G2 **M**

Rao and Johnson, 1970
Johnson and Rao, 1970

Early experiments by Rao and Johnson fused cells at different stages of the cell cycle. These cell fusion don't often occur in nature, but by looking at what happens to the different nuclei in the fused cell (which is called a heterokaryon). These heterokaryons don't survive, and the point of the experiment is to look at them in the short term. What do you think happens when a G2 cell is fused with a mitotic cell?

Cell fusion experiments gave first hints of the logic of the cell cycle

The M nucleus prematurely induces mitosis in the G2 nucleus

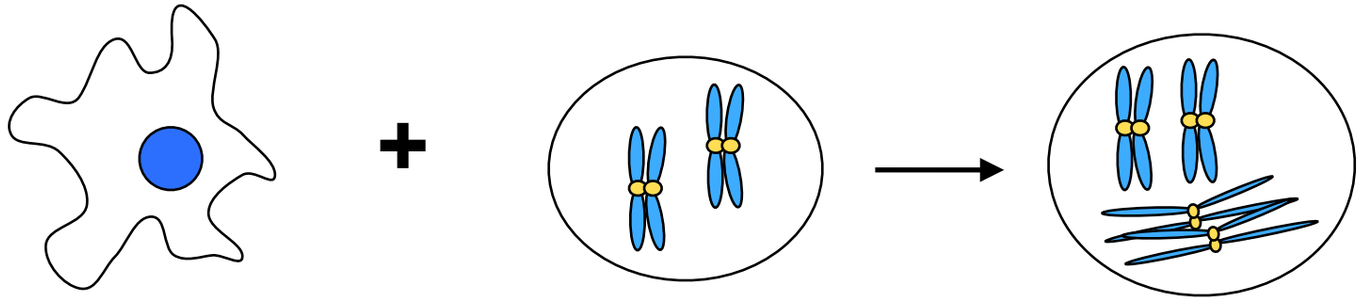


G2 **M**

Rao and Johnson, 1970
Johnson and Rao, 1970

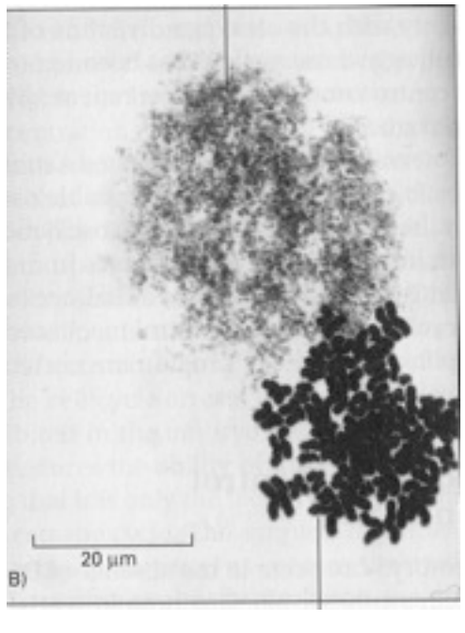
The G2 nucleus is driven immediately into mitosis and its chromosomes condense (though not perfectly), its nuclear envelope breaks down and it builds a mitotic spindle. This result led to the idea of mitosis promoting factor (MPF) - an activity in the mitotic cell that can drive the G2 cell into mitosis. MPF suggests that the clock theory may hold because the G2 cell immediately enters mitosis - potentially skipping important events in G2.

Mitosis Promoting Factor (MPF)



G1 + M

S + M



G2 + M

Rao and Johnson, 1970; Johnson and Rao, 1970

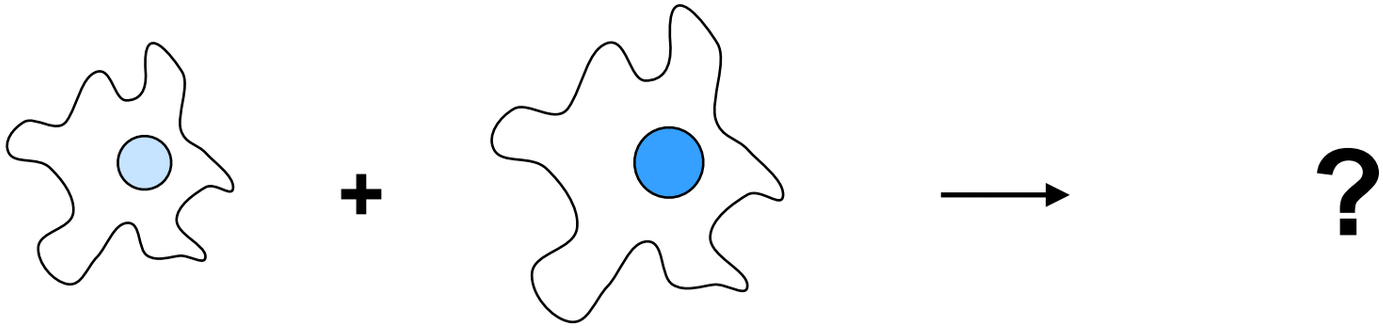
MPF will drive any cell, a G1, S or G2 cell into mitosis. The condensed G1 chromosomes only contain half the amount of DNA (they haven't been replicated) and condensing S phase chromosomes leads to their complete fragmentation probably due to unligated Okazaki fragments. The G1 and S phase fusions also support the clock theory: in the G1 + M experiment S-phase is completely skipped, and in the S + M experiment S-phase is not completed when the cells enter mitosis.
Photos come from original Rao and Johnson article.

Conclusions

1. Mitosis is “dominant” to other phases of the cell cycle. A factor or factors promote mitosis.

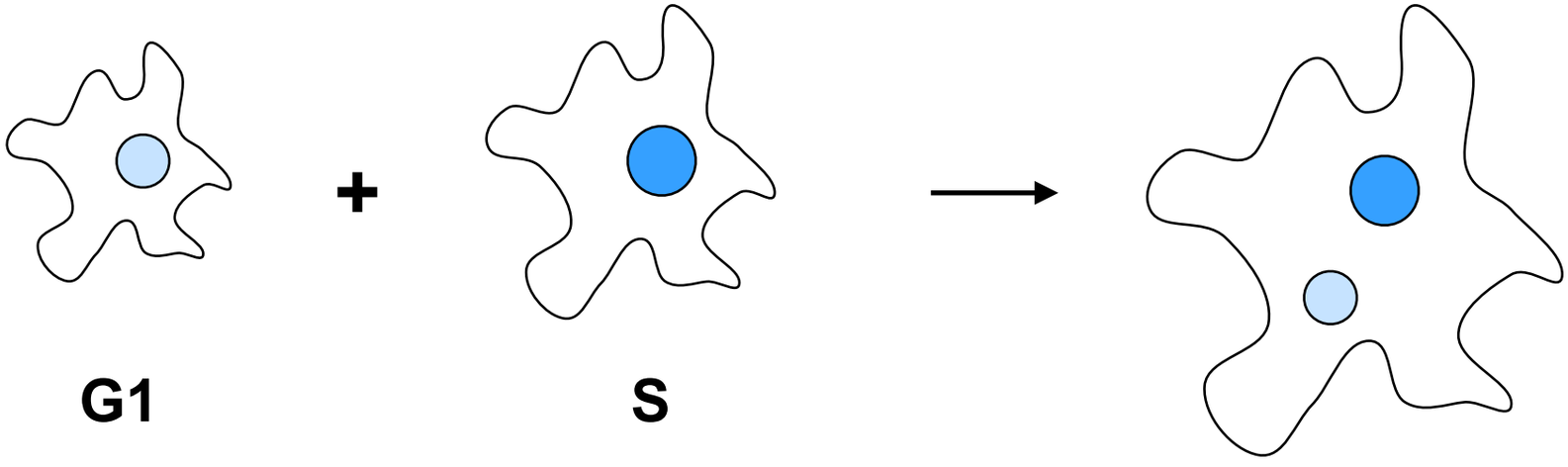
The main conclusions from these experiments.

What happens when a G1 and a S cell are fused?



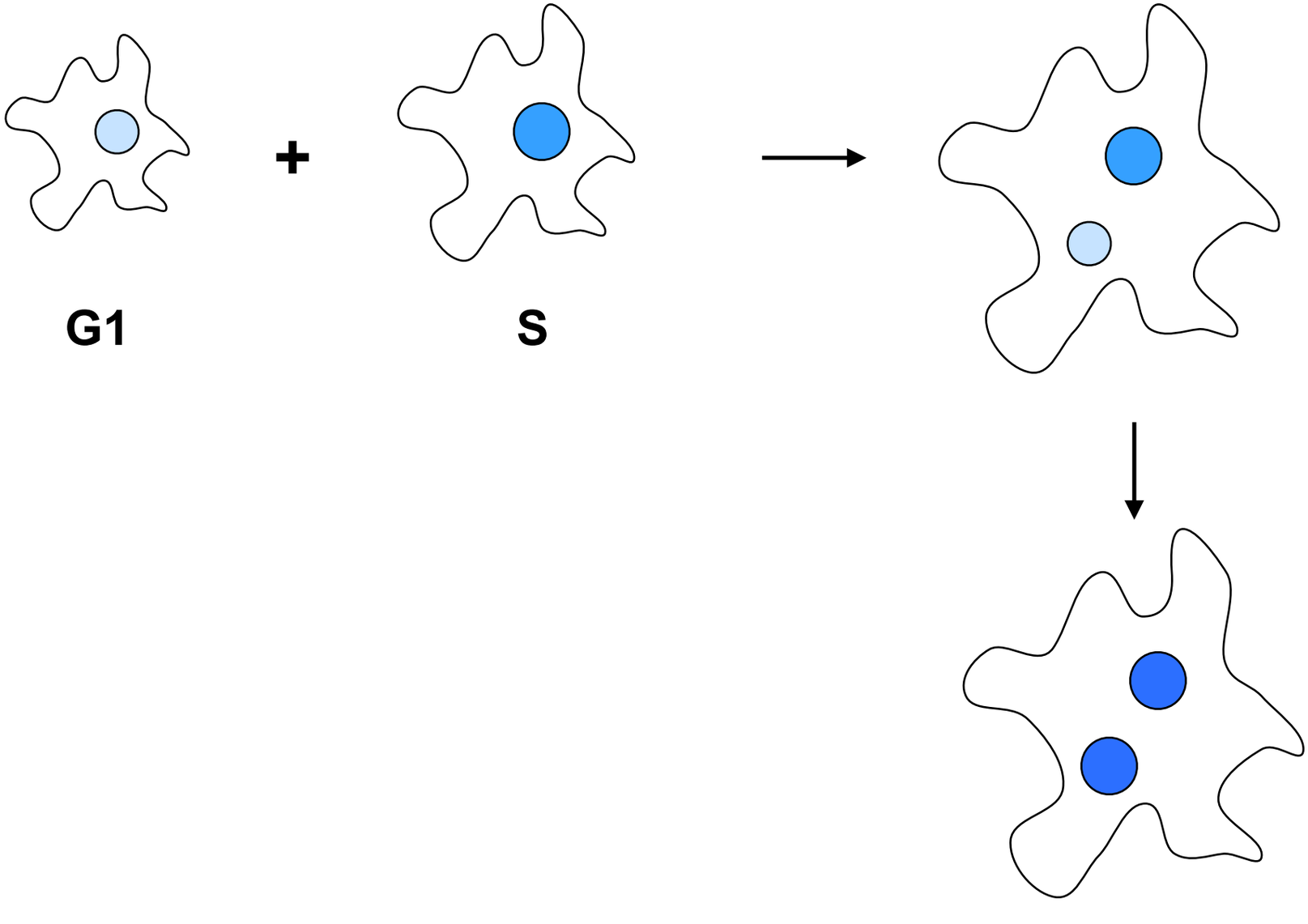
Rao and Johnson (the experimenters) also did the following experiment: they fused a G1 and G2 cell. They saw that....

S nucleus induces S-phase in G1 nucleus



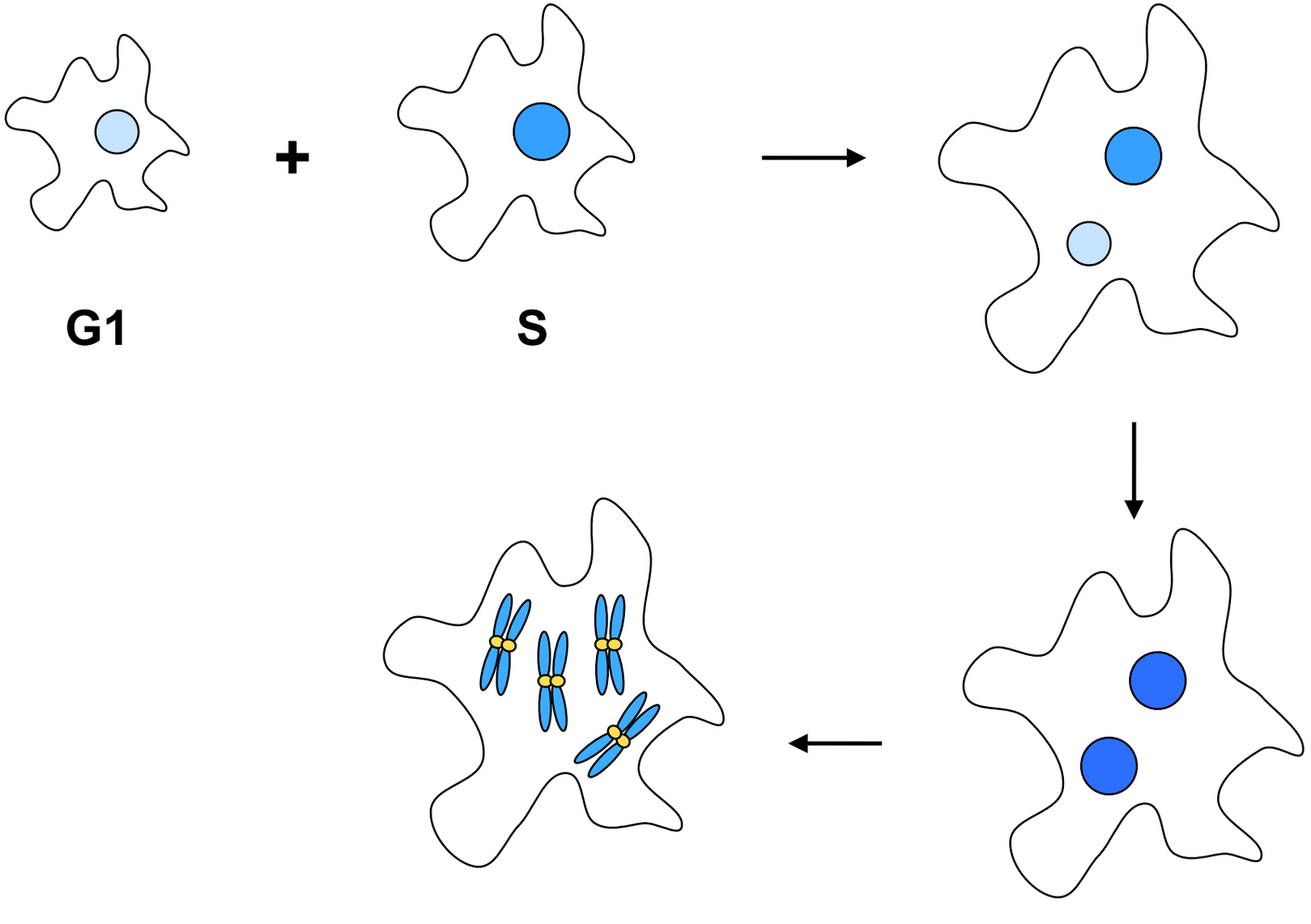
the G1 nucleus were driven into S-phase and the the S nucleus “waited” for the G1 nucleus to “catch up” before entering mitosis. When both nuclei were finished with replication, together they continued in the cell cycle.

S nucleus induces S-phase in G1 nucleus then waits for the G1 nucleus to “catch up”



the G1 nucleus were driven into S-phase and the the S nucleus “waited” for the G1 nucleus to “catch up” before entering mitosis. When both nuclei were finished with replication, together they continued in the cell cycle.

S nucleus induces S-phase in G1 nucleus then waits for the G1 nucleus to “catch up” before going into mitosis together



the G1 nucleus were driven into S-phase and the the S nucleus “waited” for the G1 nucleus to “catch up” before entering mitosis. When both nuclei were finished with replication, together they continued in the cell cycle.

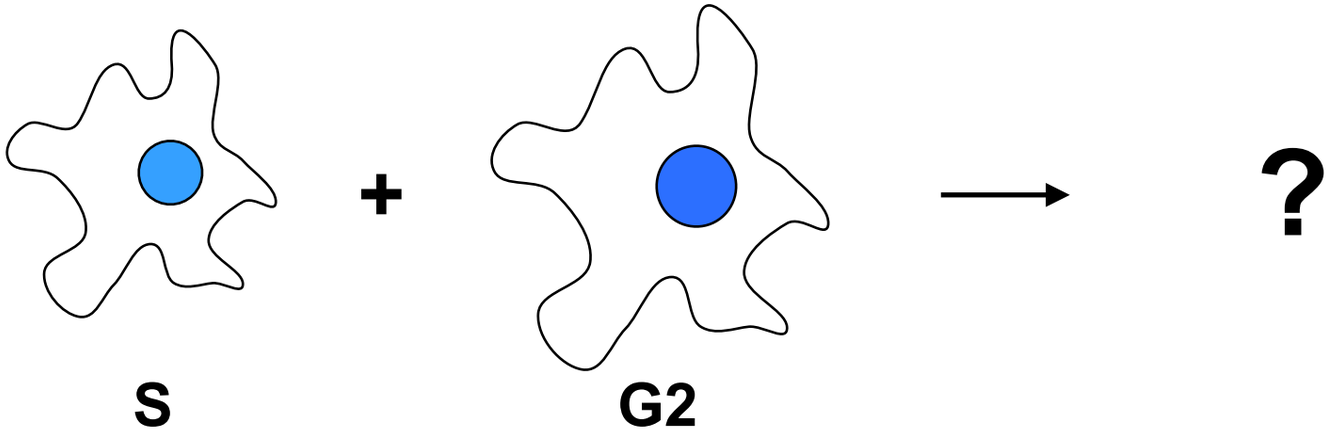
Conclusions

1. Mitosis is “dominant” to other phases of the cell cycle. A factor or factors promote mitosis.

2. S-phase is “dominant” to the G1 phase. A factor or factors can induce S-phase.

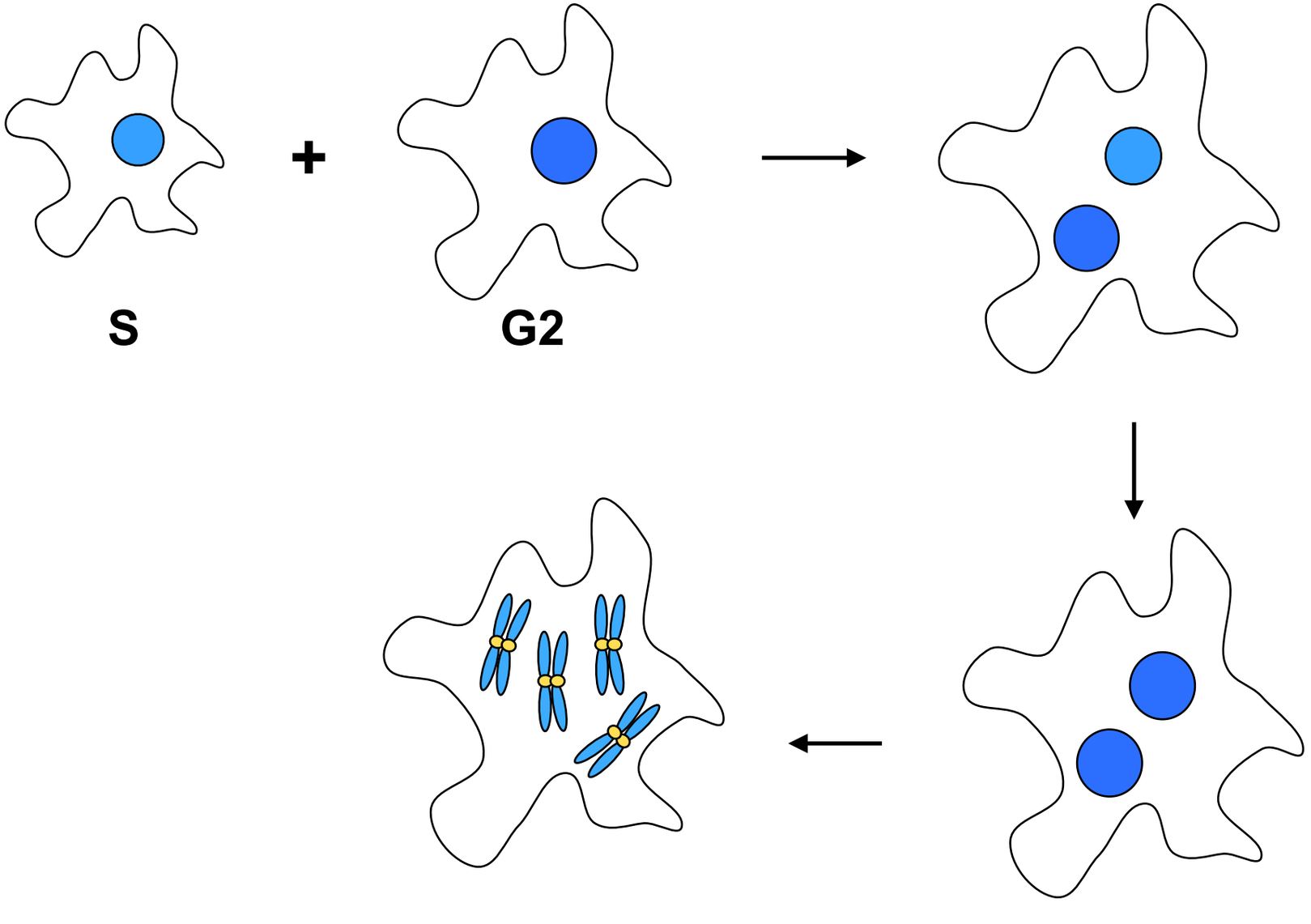
The main conclusions from these experiments.

What happens when a S and a G2 cell are fused?



Rao and Johnson (the experimenters) also did the following experiment: they fused a S and G2 cell. They saw that....

G2 nucleus does not undergo a second round of DNA replication, but “waits” for S nucleus. Suggests a block to re-replication.



The G2 nucleus was not driven into S phase, but it did wait for the S phase nucleus to finish replication before the 2 nuclei continued in the cell cycle.

Conclusions

1. Mitosis is “dominant” to other phases of the cell cycle. A factor or factors promote mitosis.

2. S-phase is “dominant” to the G1 phase. A factor or factors can induce S-phase.

3. Active DNA replication blocks entry into mitosis. The existence of feedback controls or “checkpoints.” Do checkpoints ensure completion of specific events in the cell cycle?

The main conclusions from these experiments.

Conclusions

1. Mitosis is "dominant" to other phases of the cell cycle. A factor or factors promote mitosis.

2. S-phase is "dominant" to the G1 phase. A factor or factors can induce S-phase.

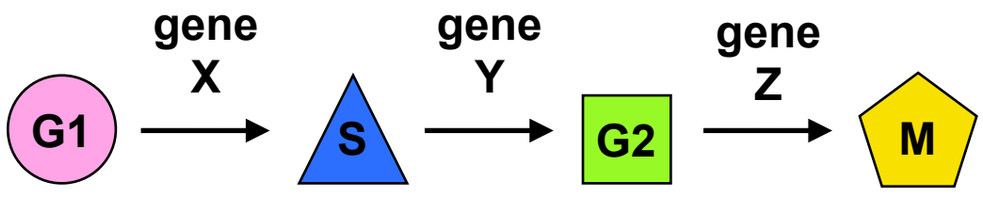
3. Active DNA replication blocks entry into mitosis. The existence of feedback controls or "checkpoints." Do checkpoints ensure completion of specific events in the cell cycle?

4. S-phase nucleus is <u>not</u> "dominant" to G2 nucleus. A block to re-replication? Does mitosis remove this block, allowing G1 nuclei to go into S-phase, and explain the alternation between S and M?

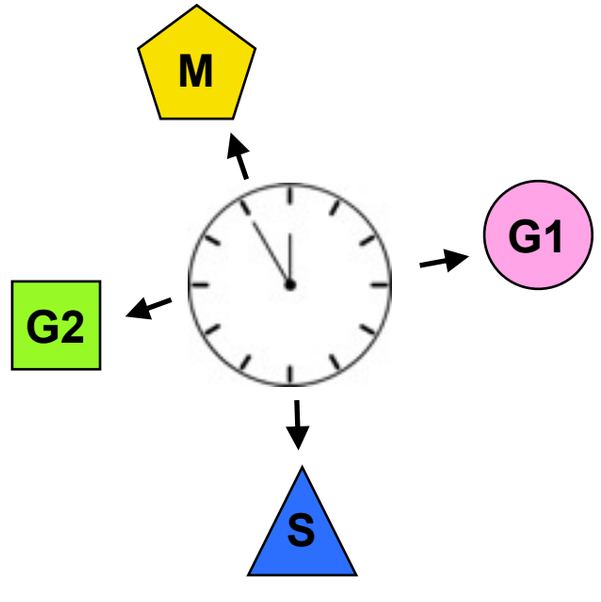
The main conclusions from these experiments.

Which model is best supported by these experiments?

domino theory

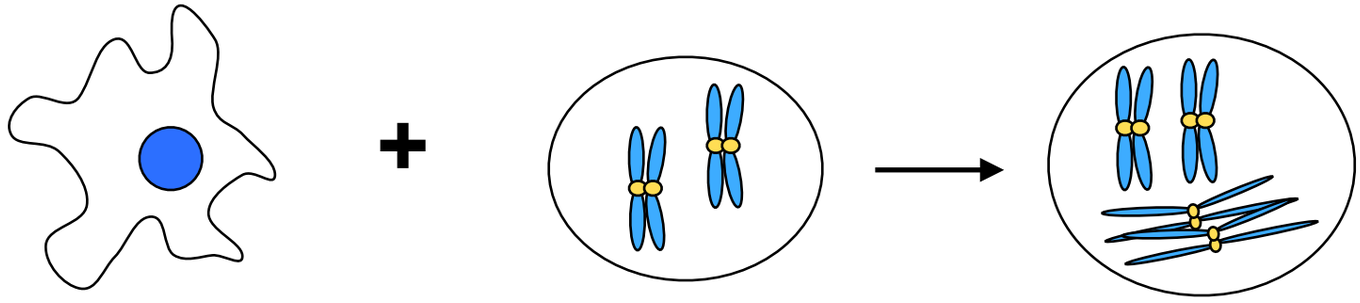


clock theory



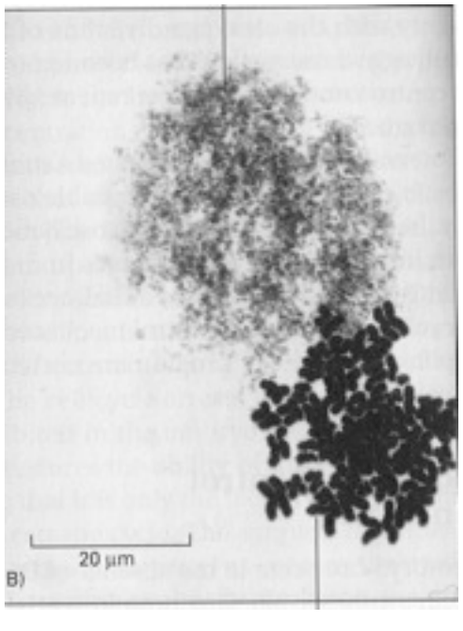
Which model is supported by each of these experiments?

Which model is supported by M fusions?



G1 + M

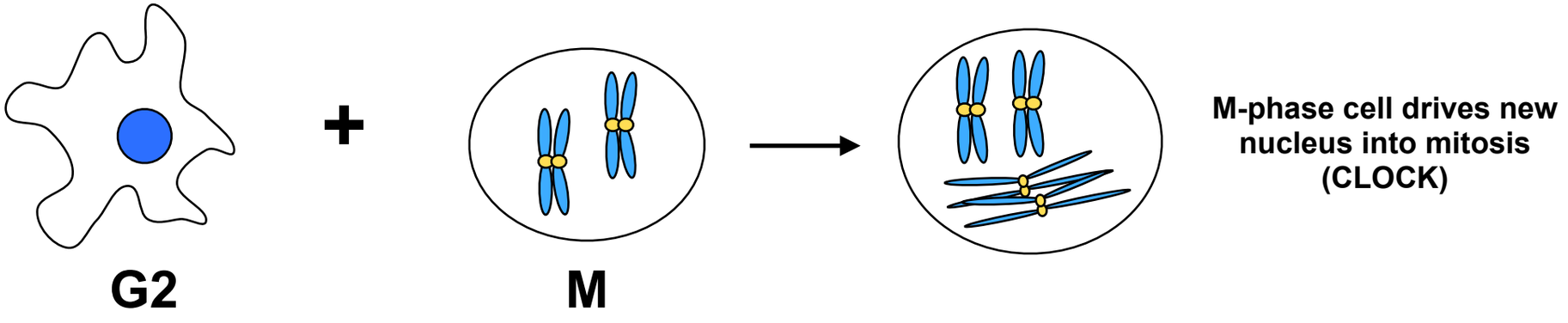
S + M



G2 + M

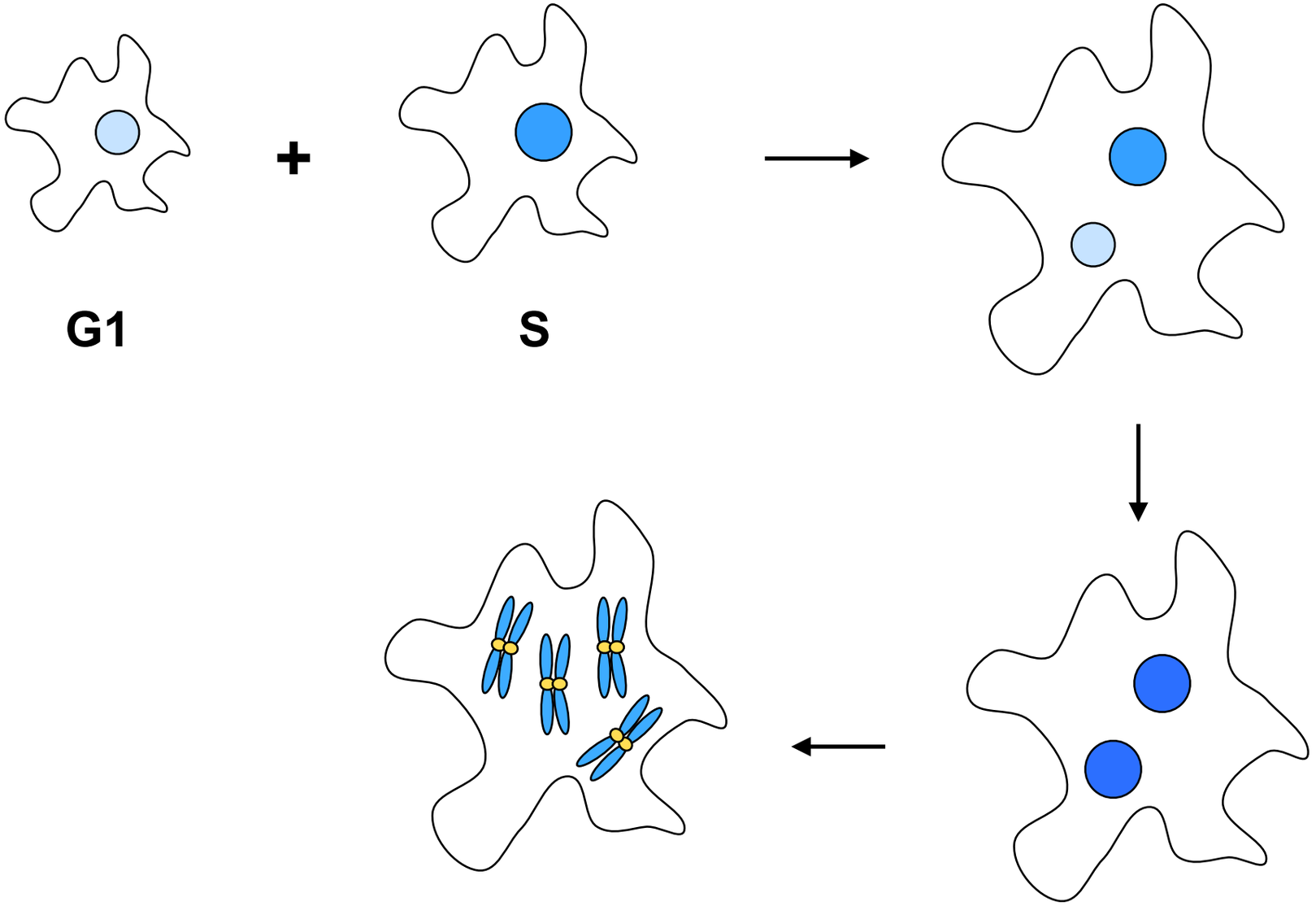
Which model is supported by M fusions?

Cell fusion experiments support both models



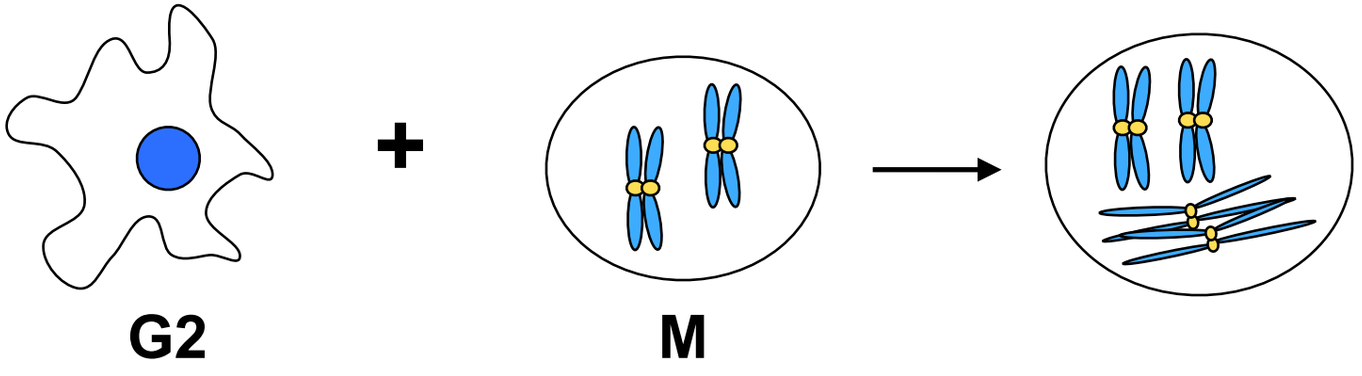
Cell fusion experiments support both models. The G2 + M fusions support the clock theory, the G1 + S fusions support both the clock and domino model, and the G2 + S fusions support the domino model.

Which model is supported by G1 + S fusions?

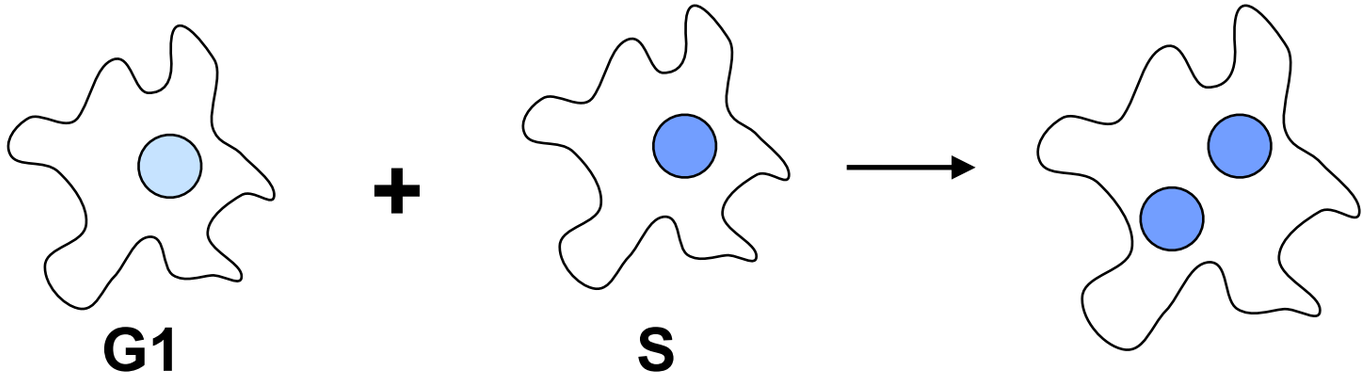


Which model is supported by G1+S fusions?

Cell fusion experiments support both models



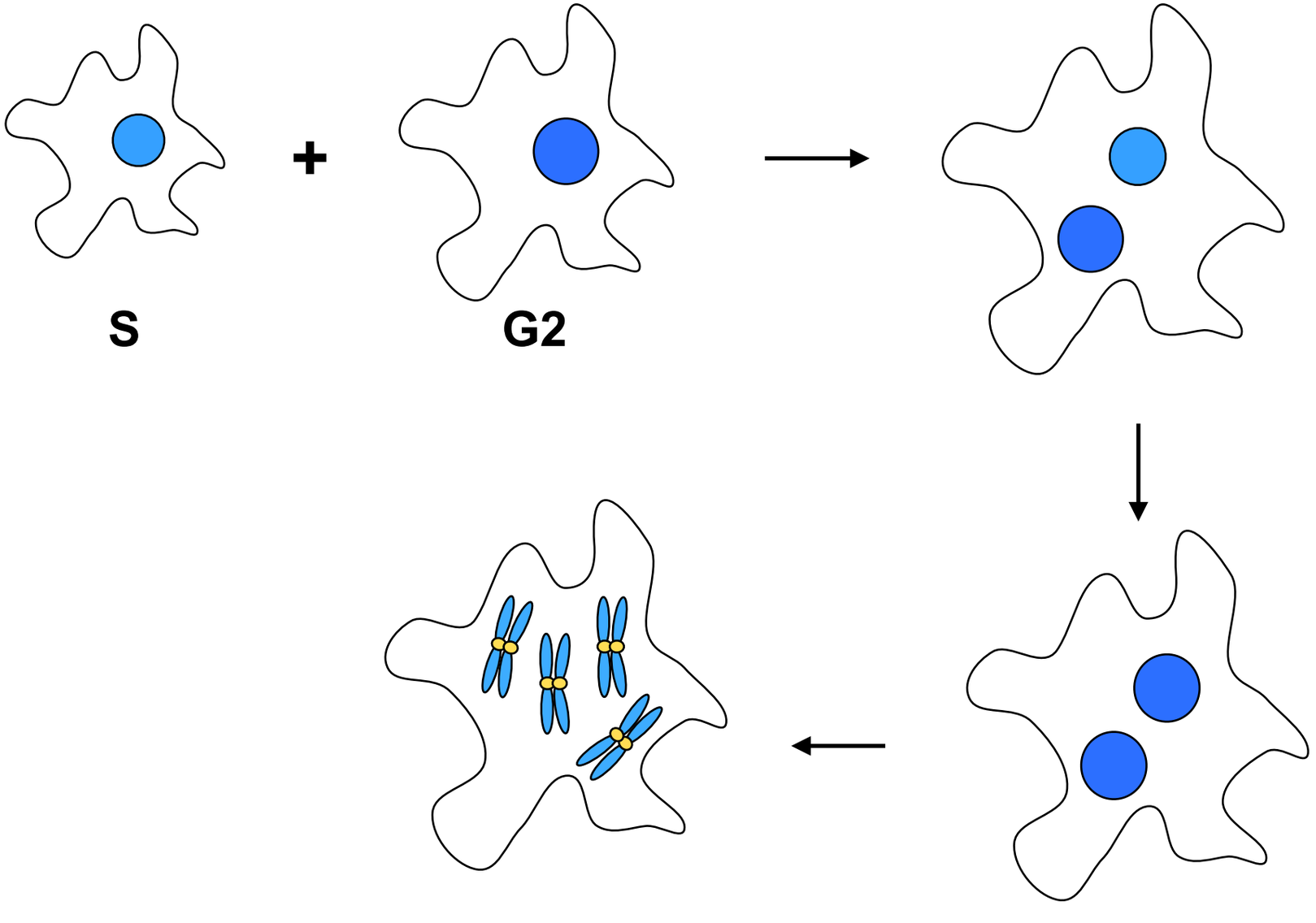
M-phase cell drives new nucleus into mitosis (CLOCK)



S-phase cell drives G1-phase nucleus into S-phase, but waits for it to complete DNA replication before proceeding into mitosis (BOTH)

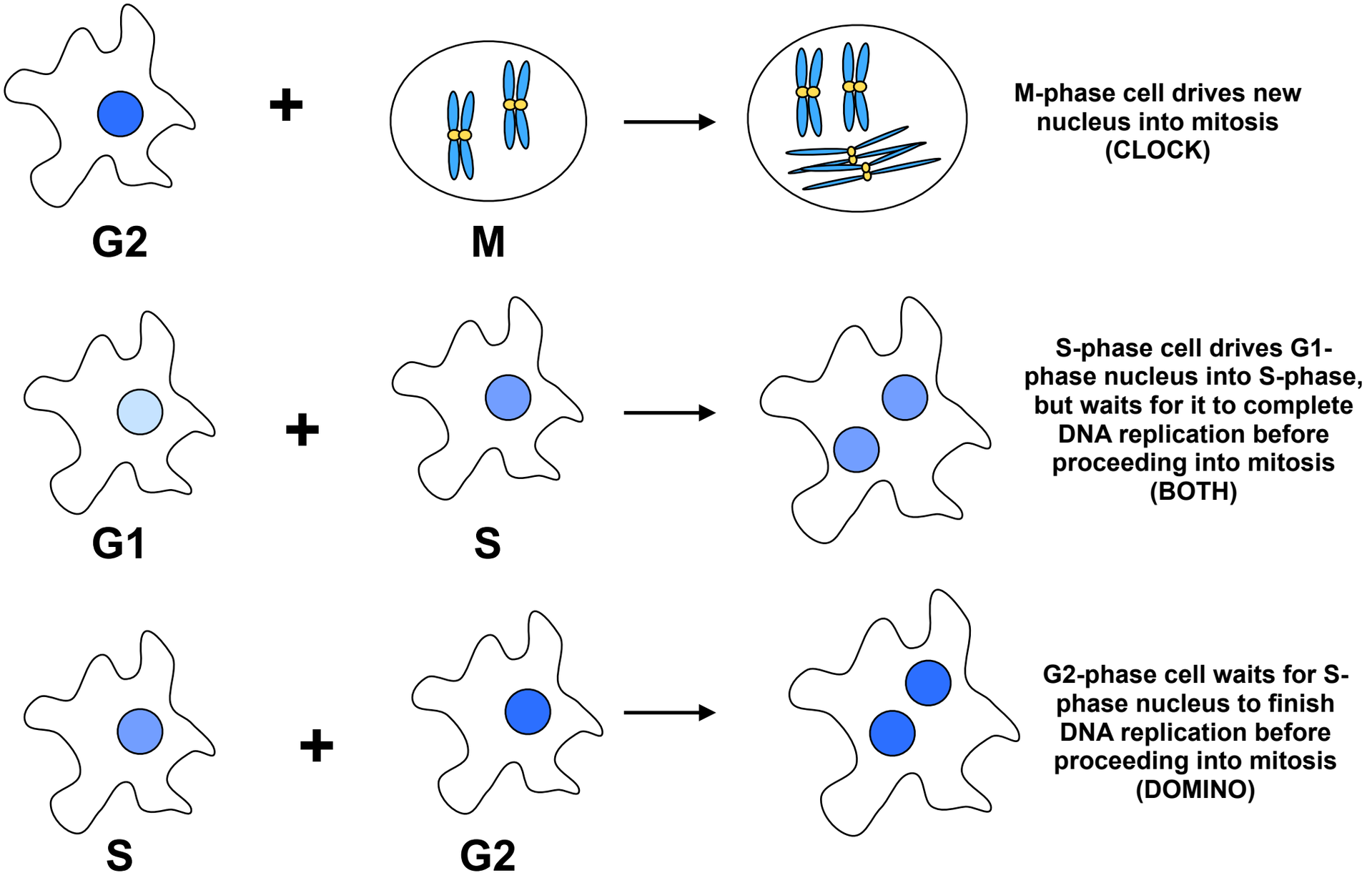
Cell fusion experiments support both models. The G2 + M fusions support the clock theory, the G1 + S fusions support both the clock and domino model, and the G2 + S fusions support the domino model.

Which model is supported by S + G2 fusions?



Which model is supported by S+G2 fusions?

Cell fusion experiments support both models

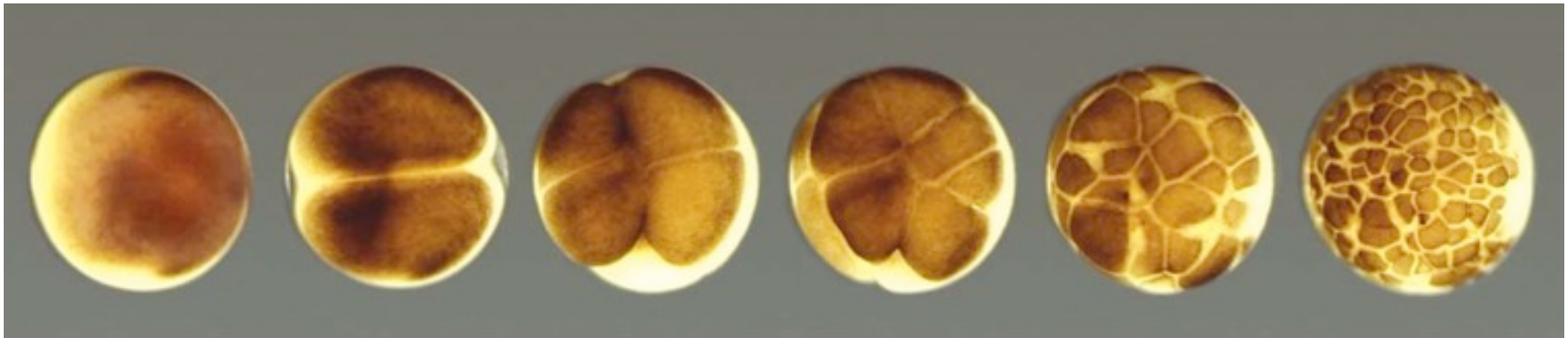


Cell fusion experiments support both models. The G2 + M fusions support the clock theory, the G1 + S fusions support both the clock and domino model, and the G2 + S fusions support the domino model.

Cell cycle biochemistry in frogs and invertebrates

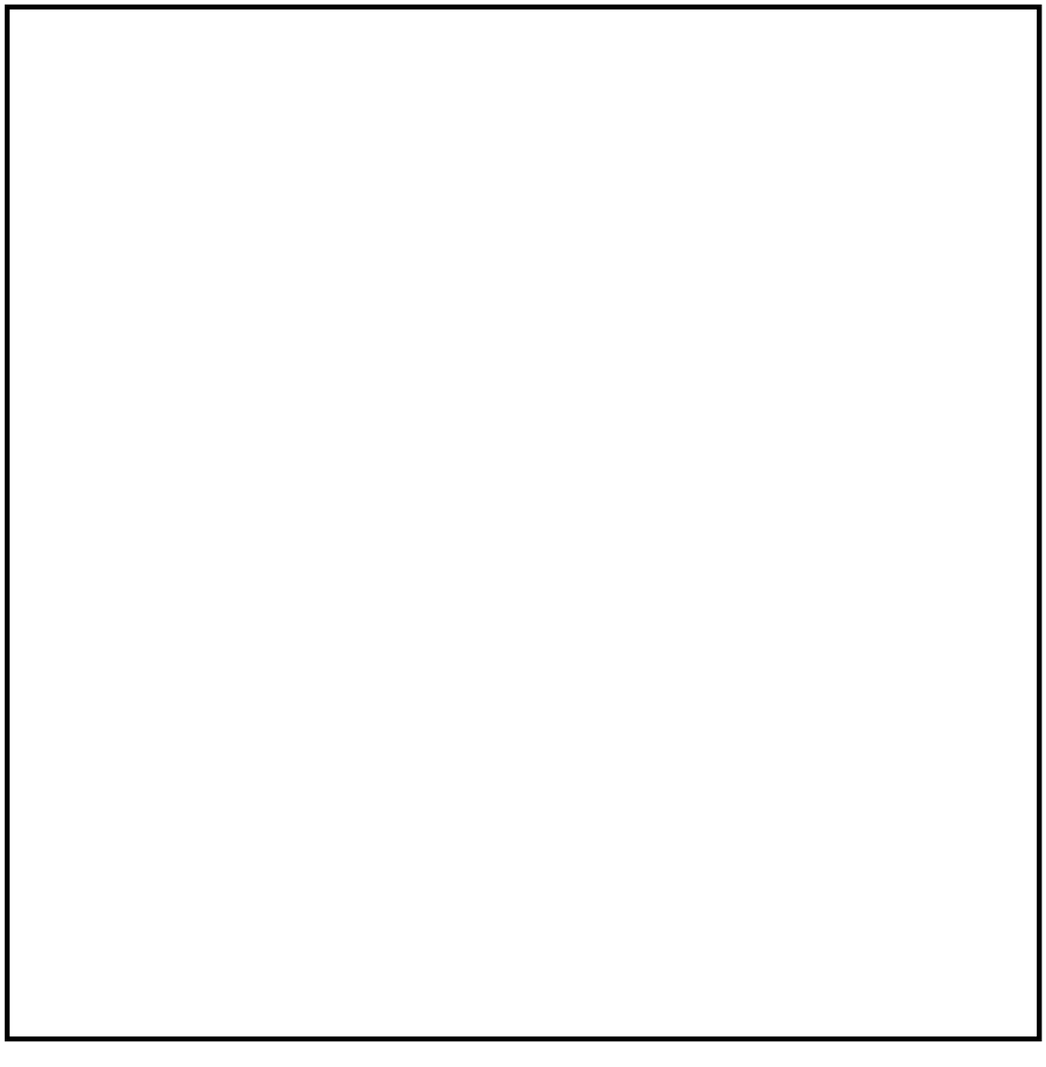






I'll next focus on experiments done in oocytes of frogs and marine invertebrates (sea urchins, clams and starfish).

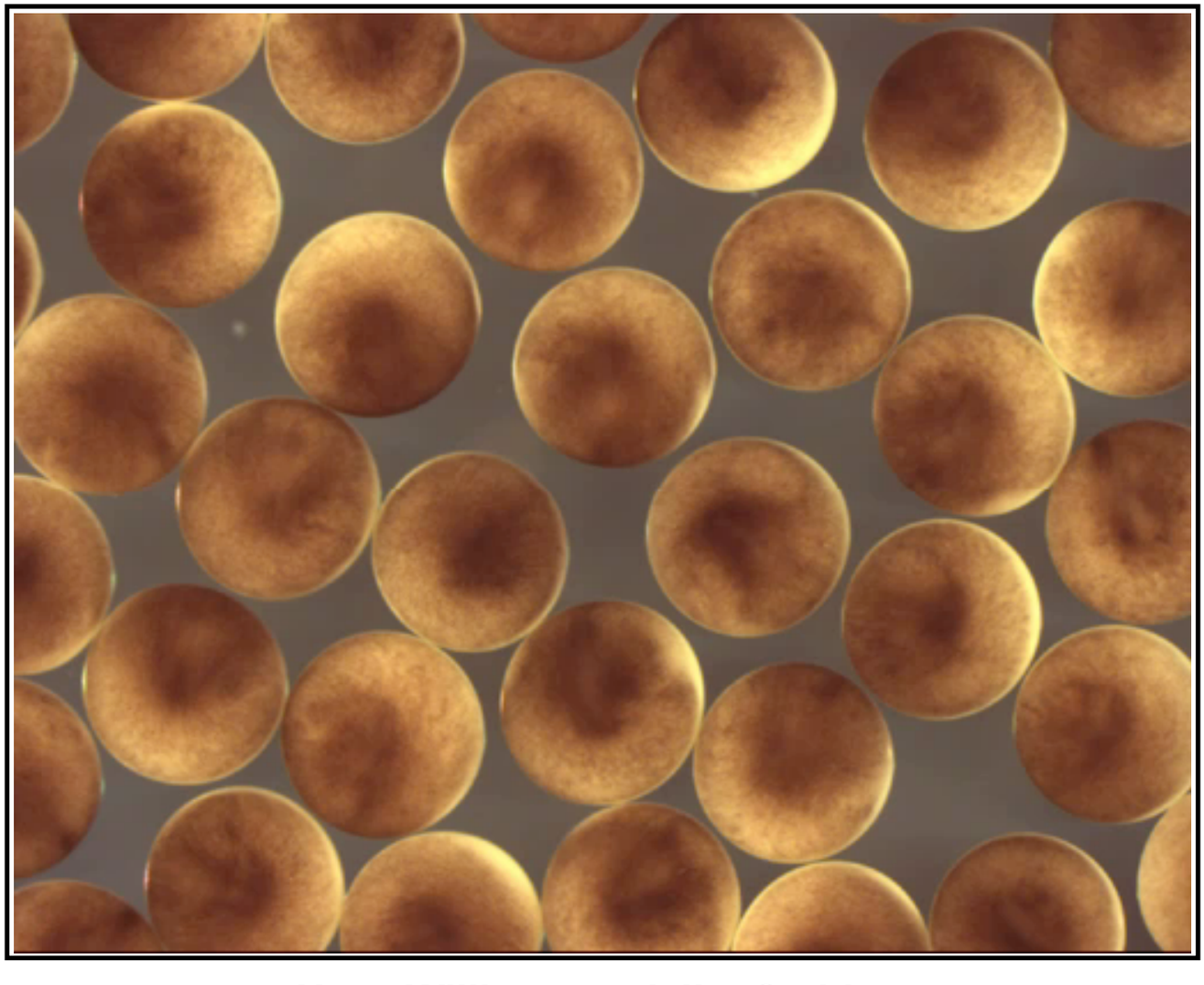
Early divisions are fast and synchronous



Huw Williams and Jim Smith

Early divisions are fast and synchronous.

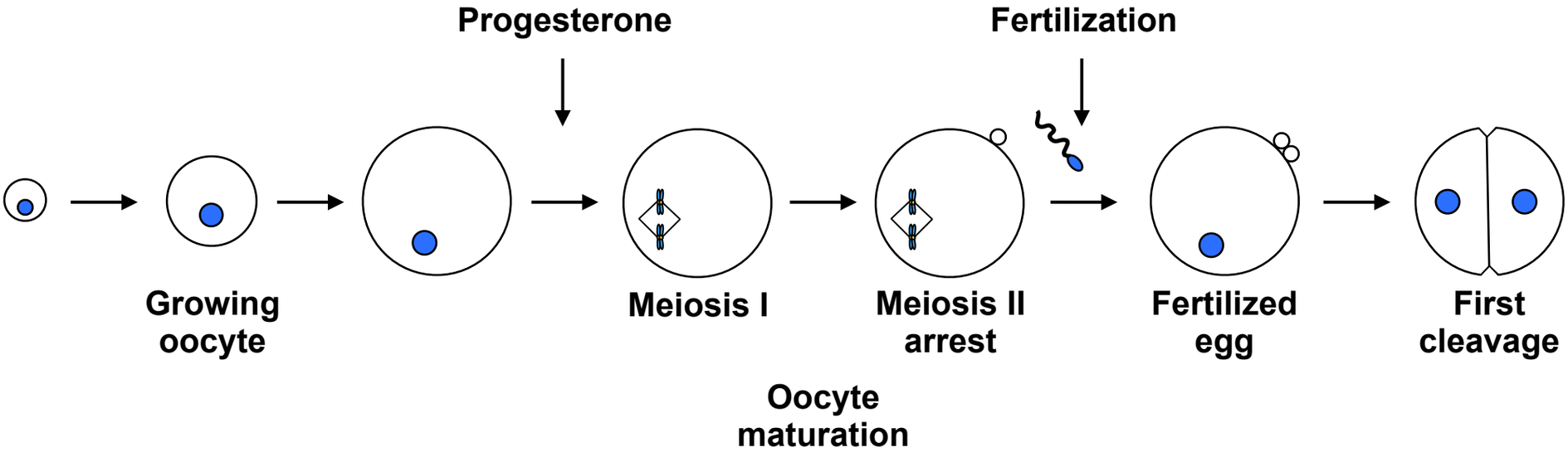
Early divisions are fast and synchronous
(notice the surface contraction waves)

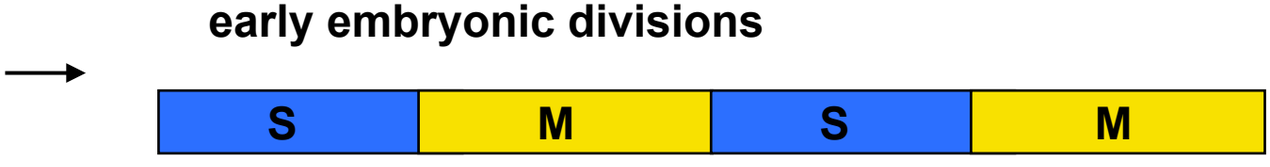


Huw Williams and Jim Smith

Early divisions are fast and synchronous.

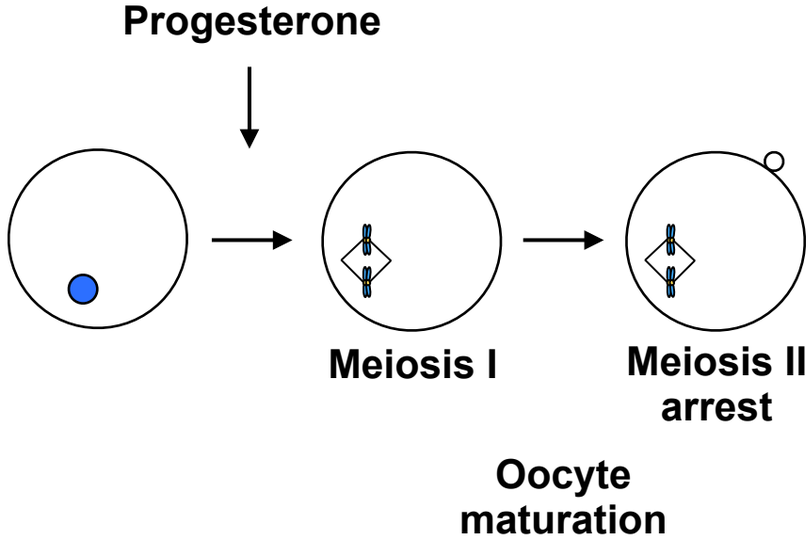
Early Development





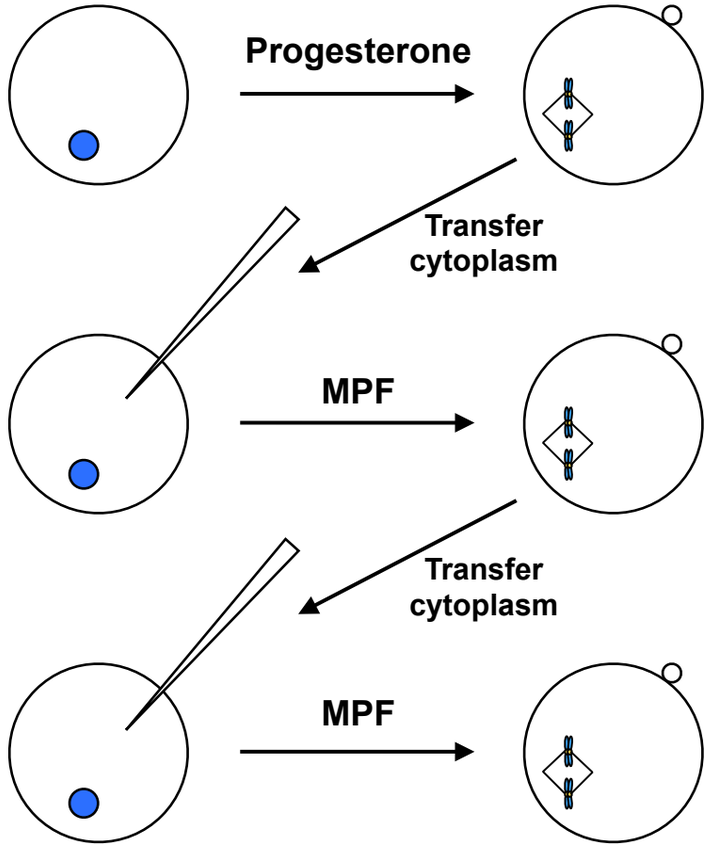
The meiotic divisions in frog oocytes. The immature oocyte grows until it reaches its mature size and they arrest in prophase of meiosis I. Oocyte maturation is triggered by the action of progesterone - this triggers the first meiotic division (Meiosis I), ejection of the first polar body, and arrest in metaphase of the second meiotic division (Meiosis II). Mature oocytes are then lain by the frog and await fertilization. At fertilization, the second meiotic division occurs, the second polar body is ejected and the female and male pro-nuclei fuse and the one-celled embryo begins its rapid embryonic divisions. This will come up a few times in my series of lectures, so its worth trying to remember this!

Researchers wondered if a cytoplasmic factor regulated oocyte maturation



Since oocyte maturation is triggered by a hormone, people wondered if there was a soluble cytoplasmic factor or factors that trigger this process.

Maturation Promoting Factor (MPF) is a cytoplasmic inducer of oocyte maturation

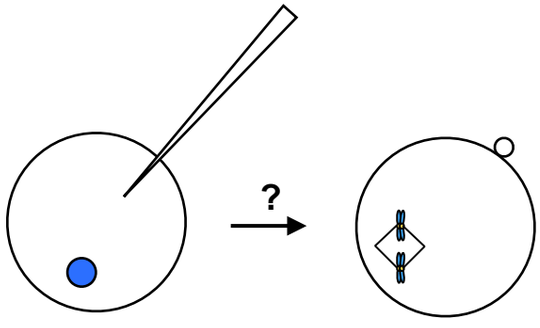


Yoshio Masui, Canada's contribution to early cell cycle research

Early researchers discovered that cytoplasm transferred from a mature oocyte into an immature oocyte would trigger oocyte maturation (independent of progesterone). The activity present in the cytoplasm was called Maturation Promoting Factor (MPF). Maturation triggered by MPF created more MPF, because the activity did not decrease with serial cytoplasmic transfers - suggesting that the MPF present in the first mature oocyte was not getting diluted during the transfers.

What is MPF?

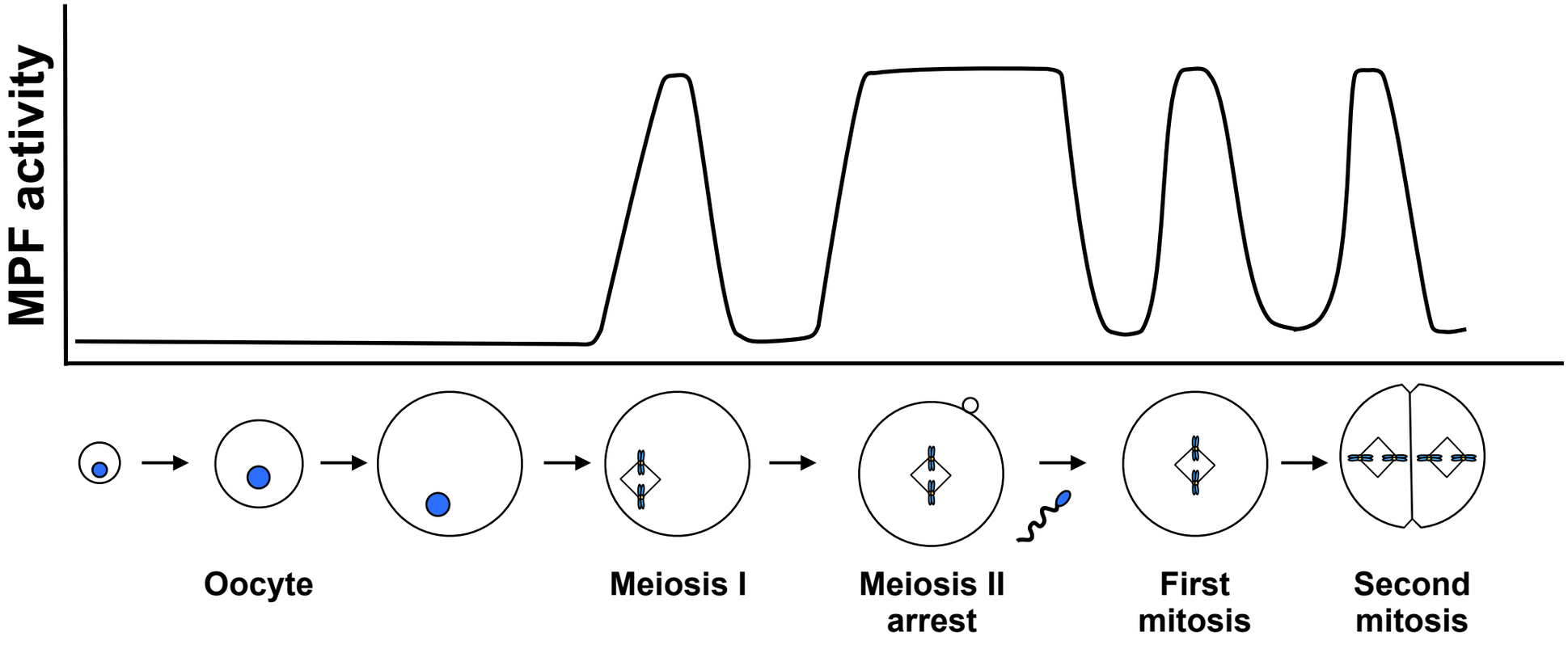
cytoplasmic transfer assay:



oocyte maturation

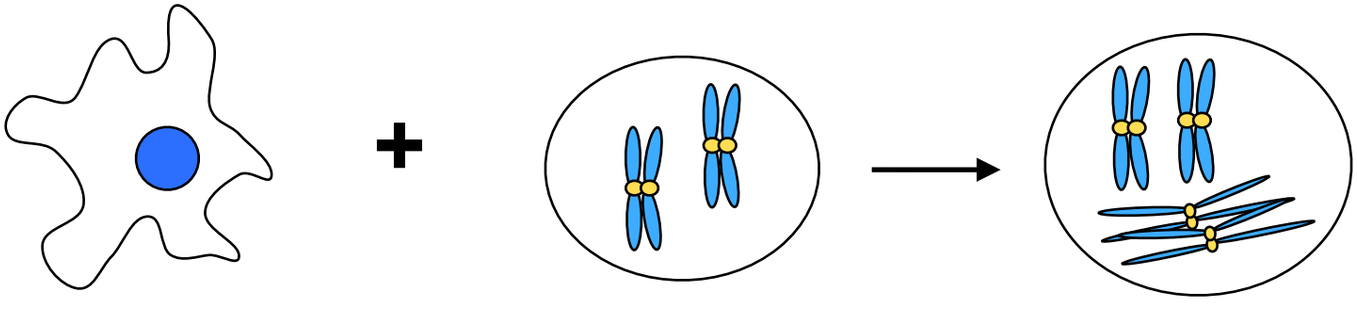
MPF was first defined as an activity, so biochemical methods seemed most appropriate. Extracts of eggs could be made, fractionated, and the fractions tested in the oocycte maturation assay. On paper a good idea, but it was exceedingly difficult because the maturation assay is so time consuming. Other methods (to be discussed) discovered the molecular components of MPF.

MPF activity is high in meiosis and mitosis.



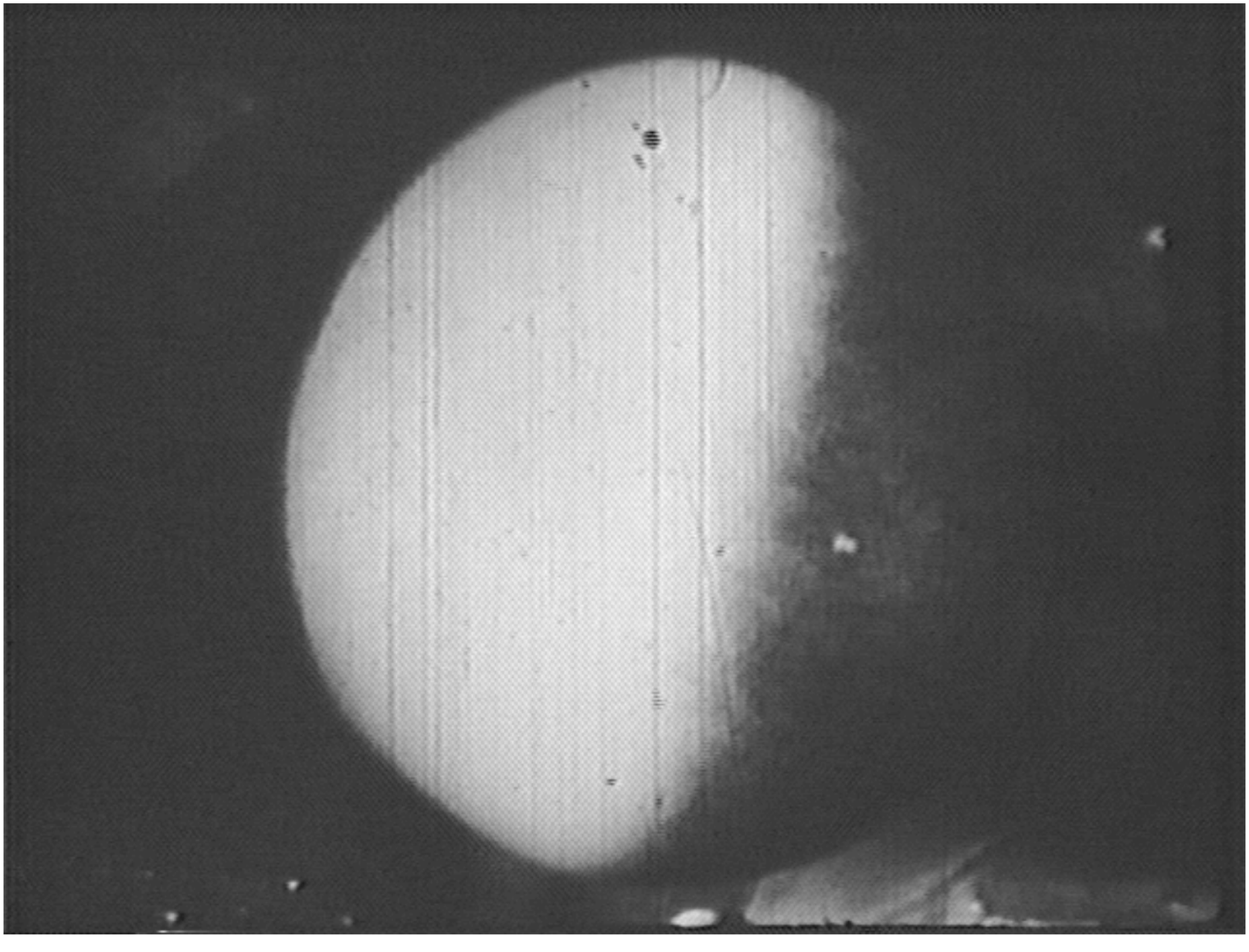
This assay did allow researchers to figure out when MPF was high in cells, and if it could be found in other cell types. They measured MPF activity at different stages of oogenesis and early embryonic divisions using the cytoplasmic transfer assay described on the previous slide. They found that MPF activity was high during metaphase of meiosis I, during the prolonged arrest in metaphase of meiosis II, and during metaphase of each embryonic division. In addition, cytoplasm from other cells in mitosis (other vertebrates and even yeast) contained MPF activity. The cytoplasmic transfer assay was also used to (partially) purify the protein components of MPF.

Are
Mitosis Promoting Factor (MPF)
and
Maturation Promoting Factor (MPF)
the same thing?



Mitosis Promoting Factor and Maturation Promoting Factor appear to have similar activities.

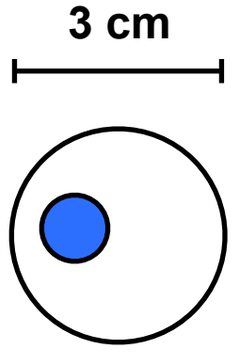
Other researchers became interested in early divisions of oocytes because they are synchronous, fast and happen in such large cells



Hara, Tydeman and Kirschner, 1980

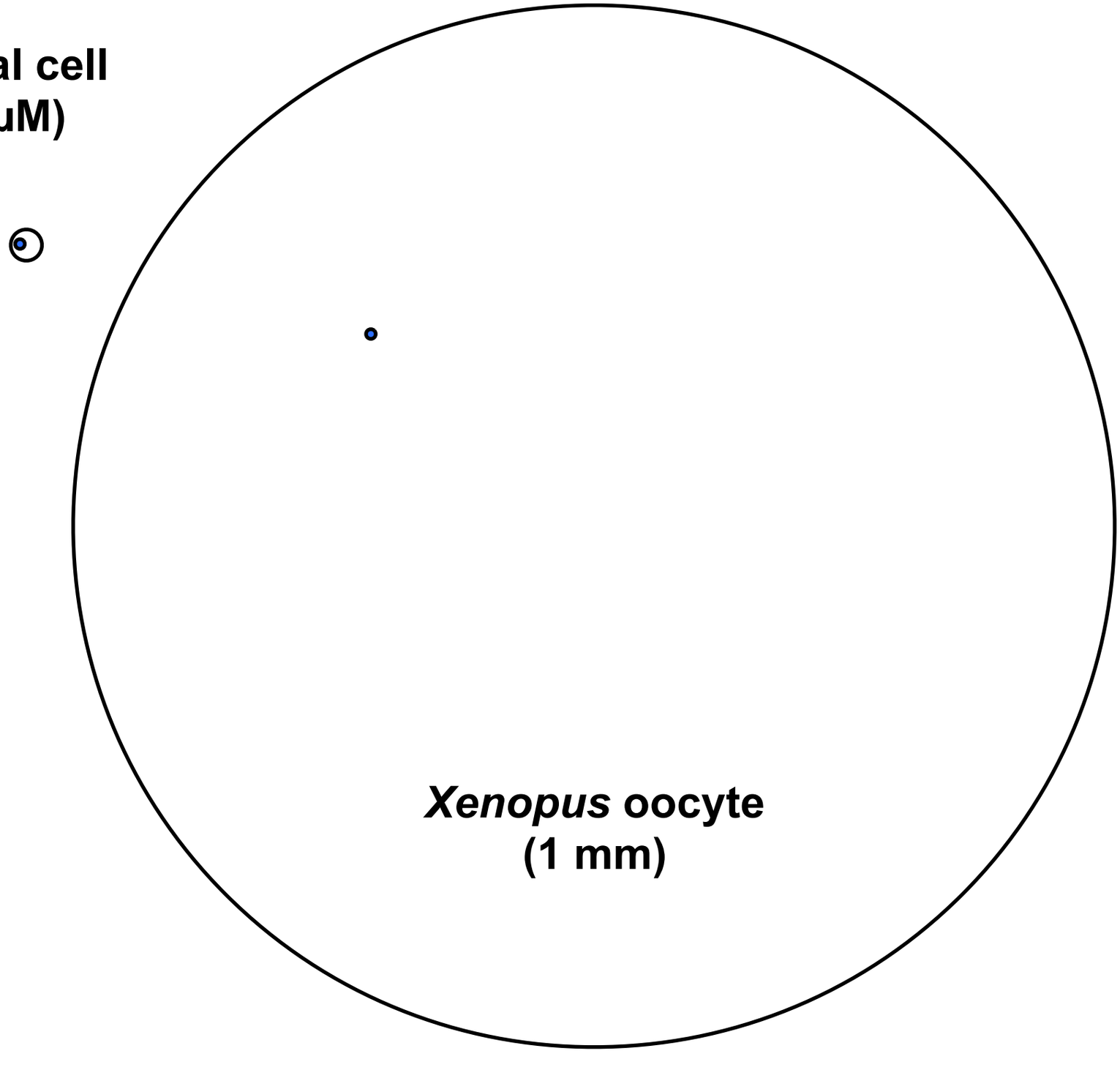
One of the first time-lapse movies of early cleavages in *Xenopus* embryos. Notice the sperm entry point and the surface contraction waves that initiate from this position.

Just how big are *Xenopus* oocytes?

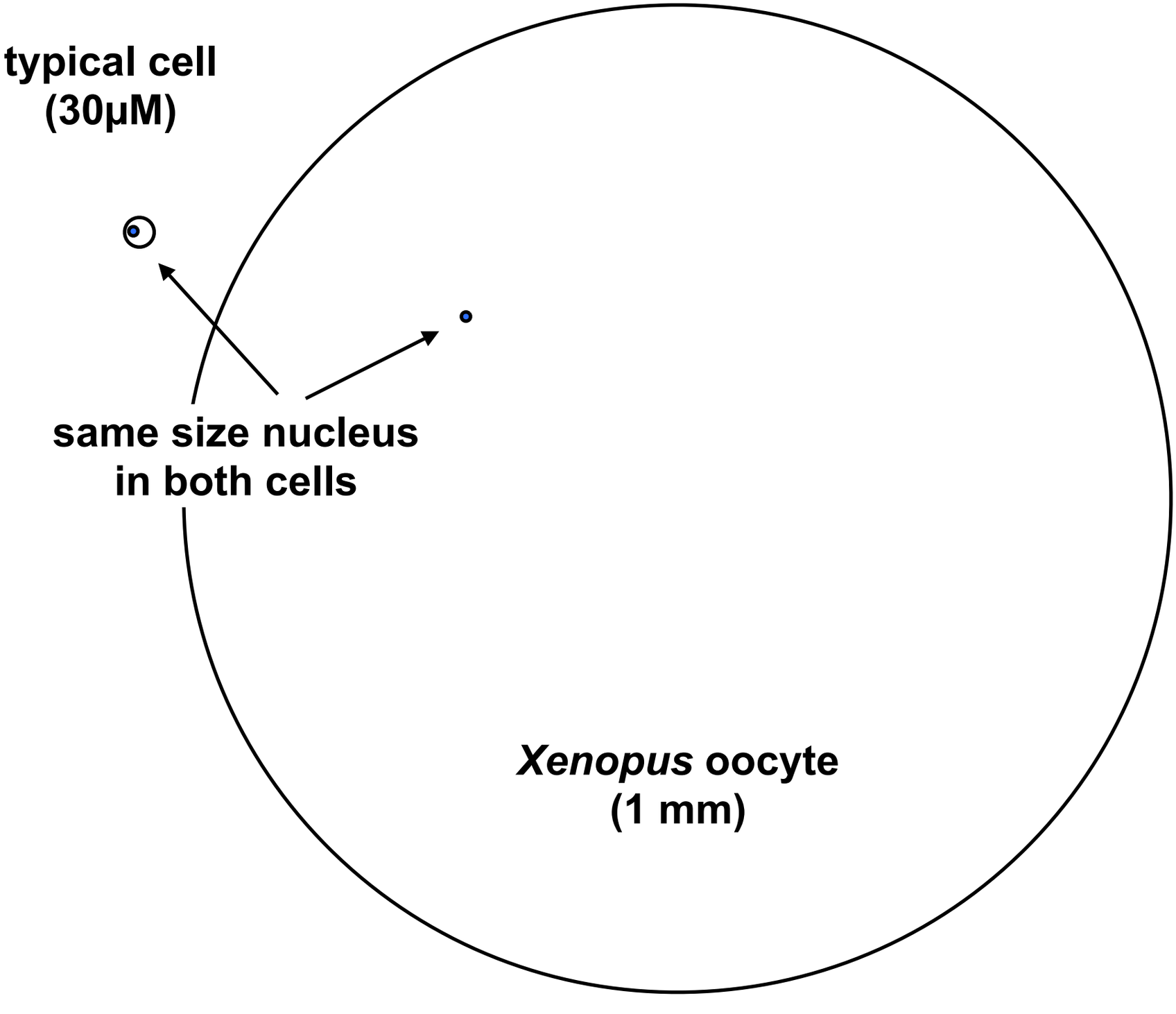


typical cell at 1000X magnification
(30µM diameter)

These cells are big. A typical skin cell is about 30µM.



A *Xenopus* oocyte is 1 mm in diameter.



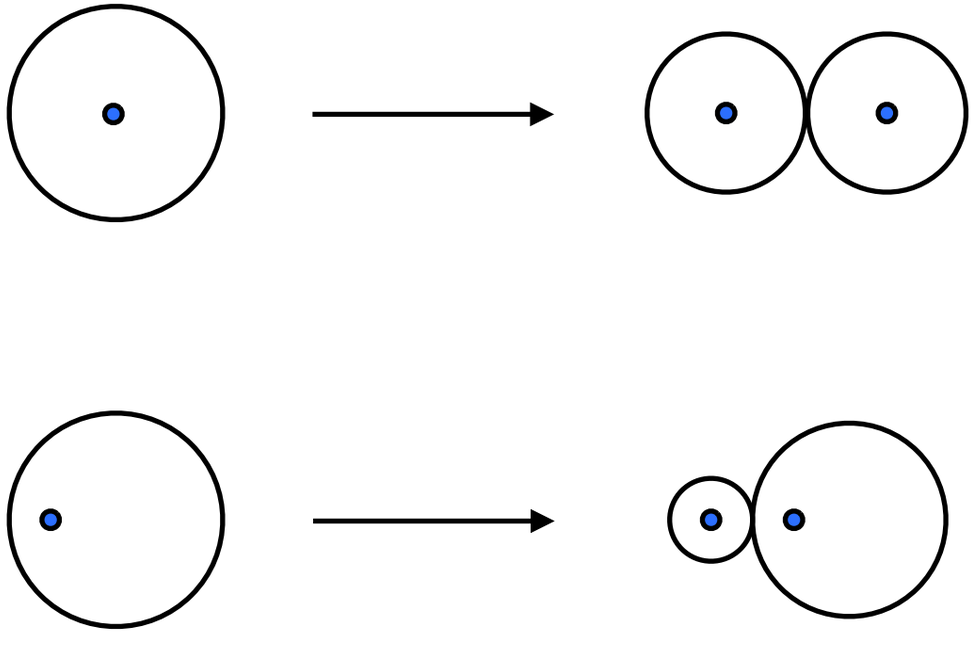
But the diameter in these two cells are about the same size.

	volume cytoplasm:nucleus (assuming a 10μM nucleus)
typical cell (30μM)	27
Xenopus oocyte (1 mm)	1,000,000

What influence does the nucleus have on early divisions in oocytes?

This size difference is even more exaggerated if you take the volume of the cells into account. One was to think about this is the ratio of cytoplasmic:nuclear volume. Its about 27 for skin cells, but 1,000,000 for the oocyte!

The nucleus is important for determining the plane of division

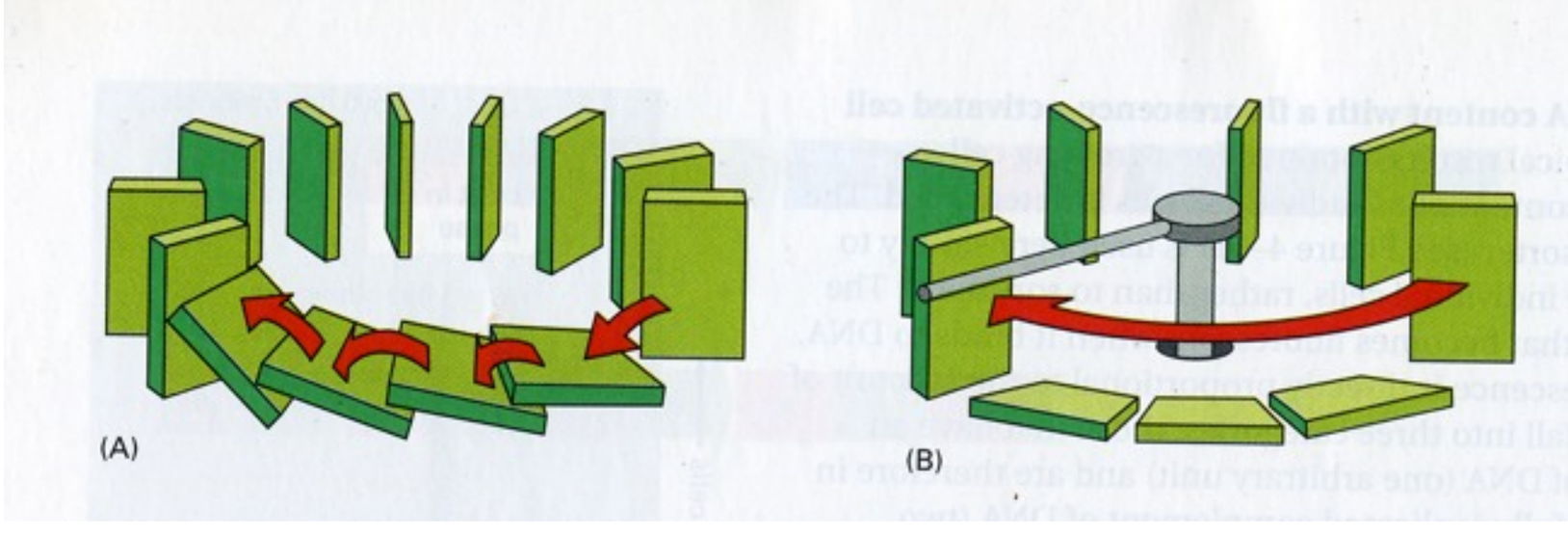


but do other nuclear events (replication, chromosome condensation, mitotic spindle assembly) affect progression through the cell cycle?

Researchers knew that the nucleus was important for determining the plane of division. This is apparent when the nucleus is not in the center of the cell. This is one of the ways asymmetric divisions are generated. Additional experiments were done that deliberately changed the position of the nucleus in oocytes and this could dramatically change the position of cleavage. But does the nucleus, and events in the nucleus, direct other key events in the cell cycle? This is the question that Hara et al. asked.

domino theory

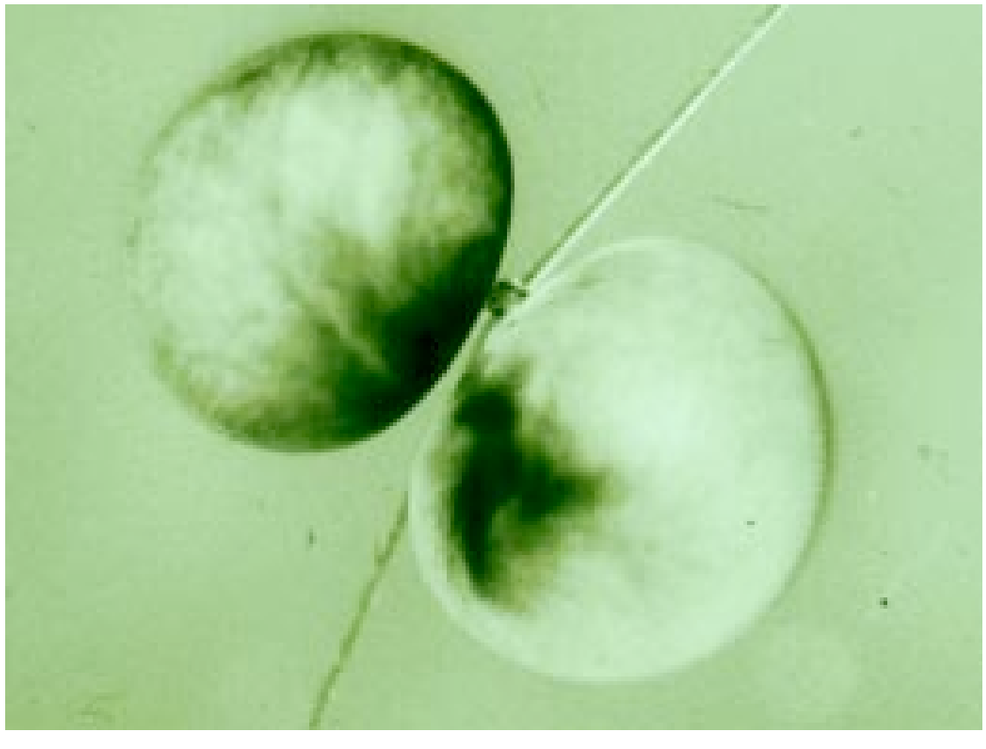
clock theory



The influence of the nucleus on the division plane supports which model?

Does synchronous division depend on the nucleus?

An enucleated zygote



Genesis of an enucleate fragment by constricting a *Xenopus* zygote with a baby hair.

Hara et al. wondered if the synchronous divisions depended on the nuclei of the embryo. The basic experimental set up was to create embryos that lacked a nucleus by splitting the embryo in half using a hair.

The enucleated embryo doesn't cleave

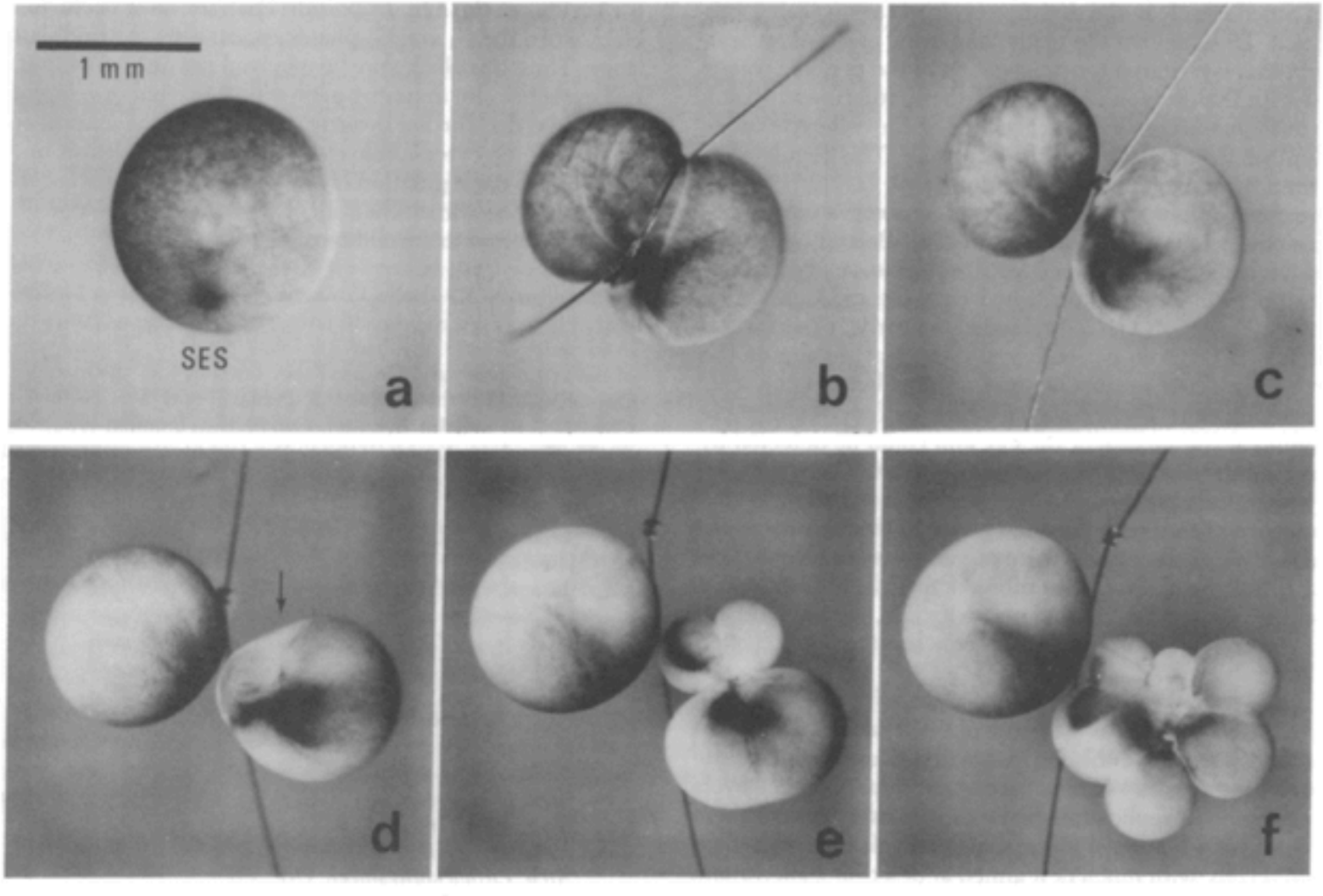
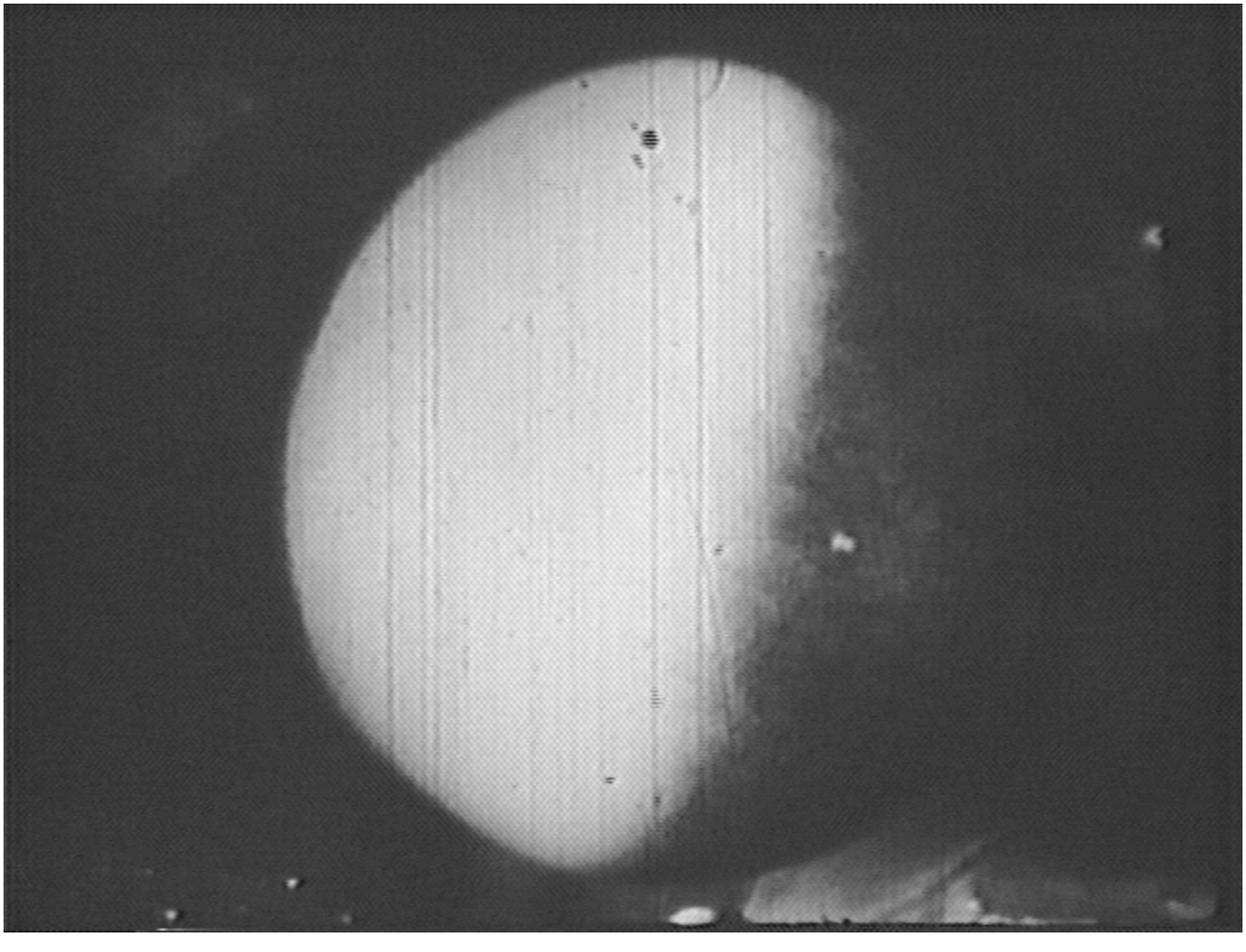


FIG. 4. Series of still pictures showing constriction of a fertilized egg with newborn human hair and cleavages in one of the two halves (25°C). (*a*) At 25 min after fertilization: vitelline membrane is still intact, and sperm entrance spot (SES) is clear. (*b*) At 38 min: halfway constriction after removal of vitelline membrane. (*c*) At 46 min: constriction is completed and two halves are separated. (*d*) At 64 min: beginning of first cleavage (arrow). (*e*) At 90 min: second cleavage is started. (*f*) At 116 min: third cleavage is started.

Hara, Tydeman and Kirschner, 1980

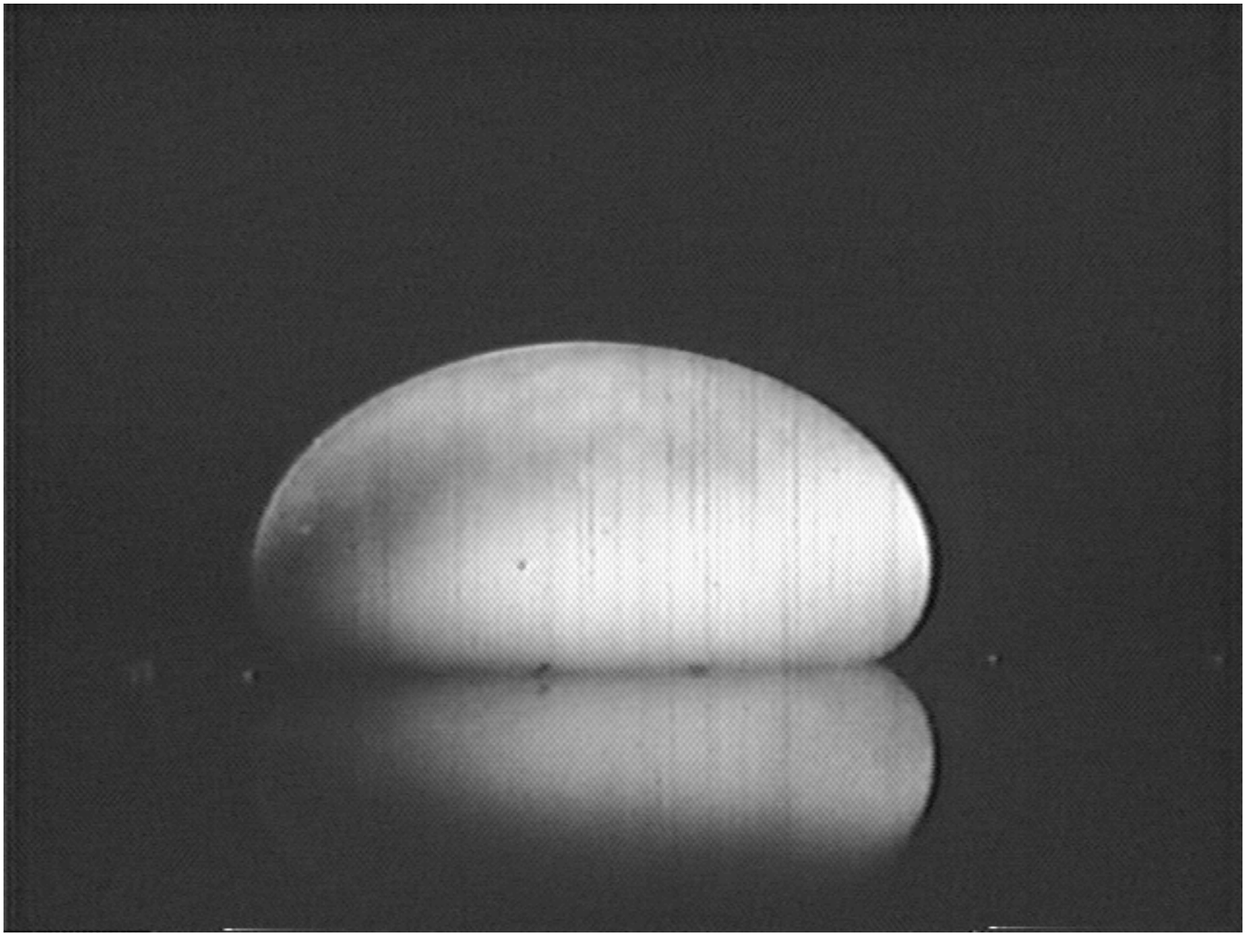
The data in the paper showing the enucleated embryo doesn't cleave, but the nucleated embryo does.

a normal embryo



Hara, Tydeman and Kirschner, 1980

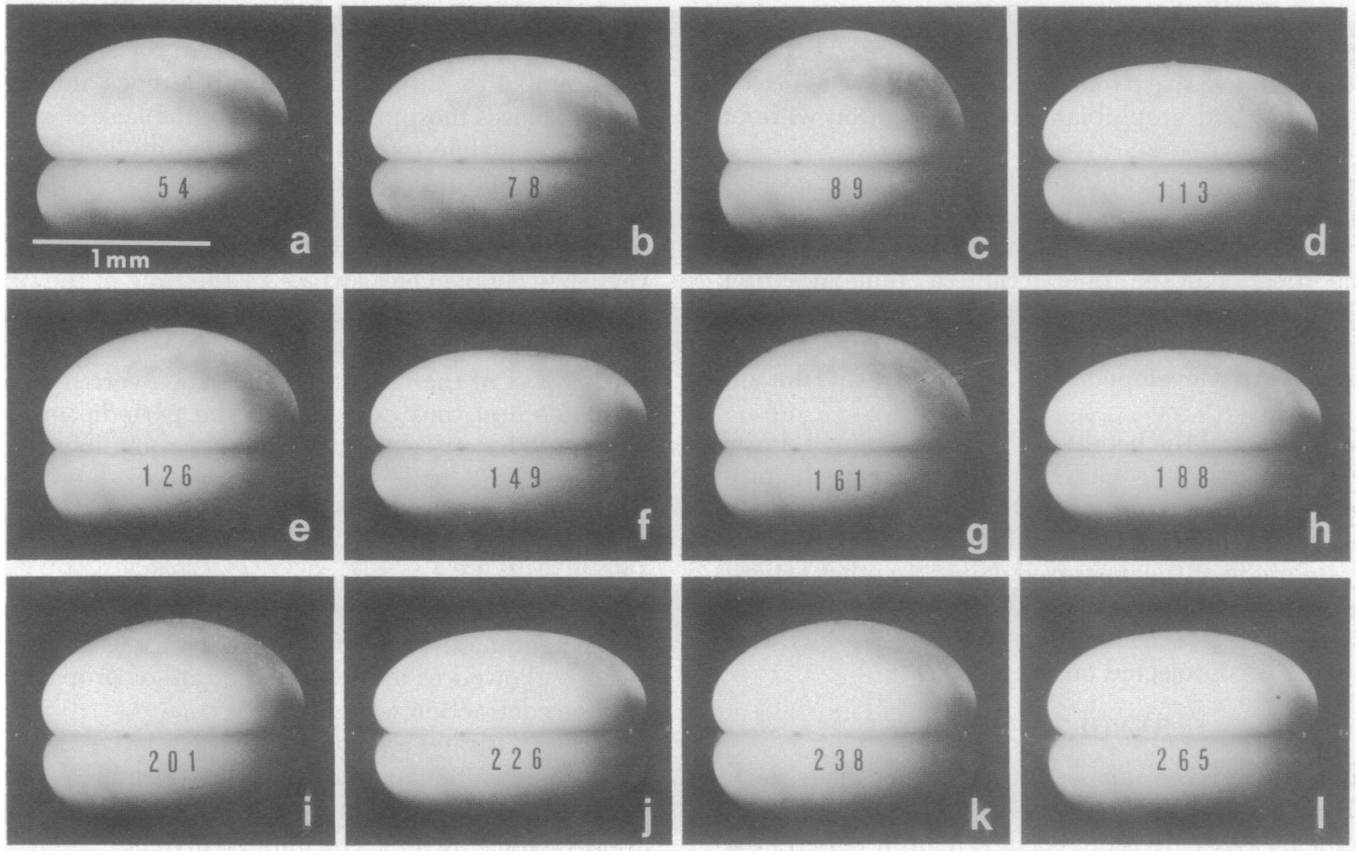
although the enucleated embryo doesn't cleave, it does bounce!



Hara, Tydeman and Kirschner, 1980

The bouncing enucleated embryo filmed by time lapse.

Although cytokinesis did not occur, surface contraction waves of enucleated zygotes follow the same timing as cleavage of normal zygotes. Is there a cytoplasmic factor that acts as a clock?



Hara, Tydeman and Kirschner, 1980

Enucleated zygotes still underwent surface contraction waves on the cell cortex even though they cannot divide. These waves happen at the same time as the sister zygote that contains the nucleus divides. This experiment suggested a cytoplasmic clock that regulates some aspects of the cell cycle.

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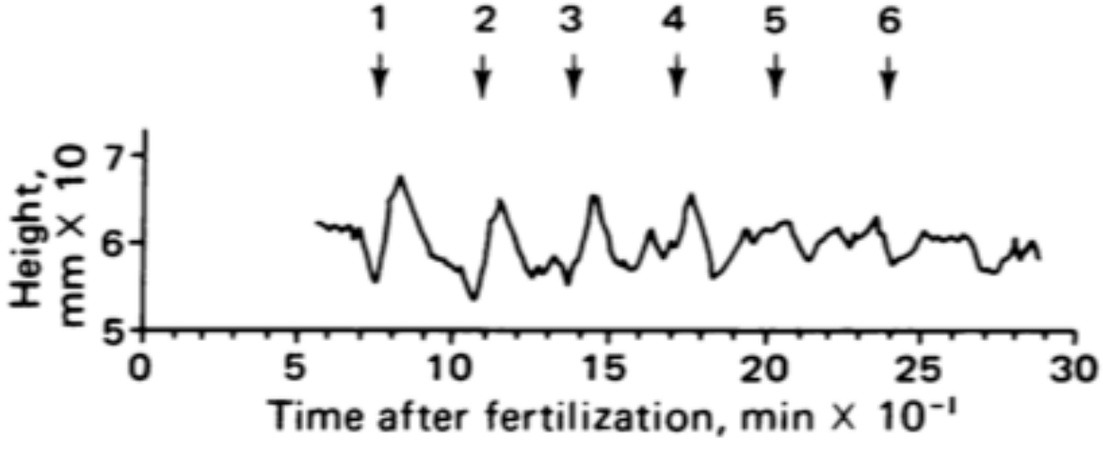
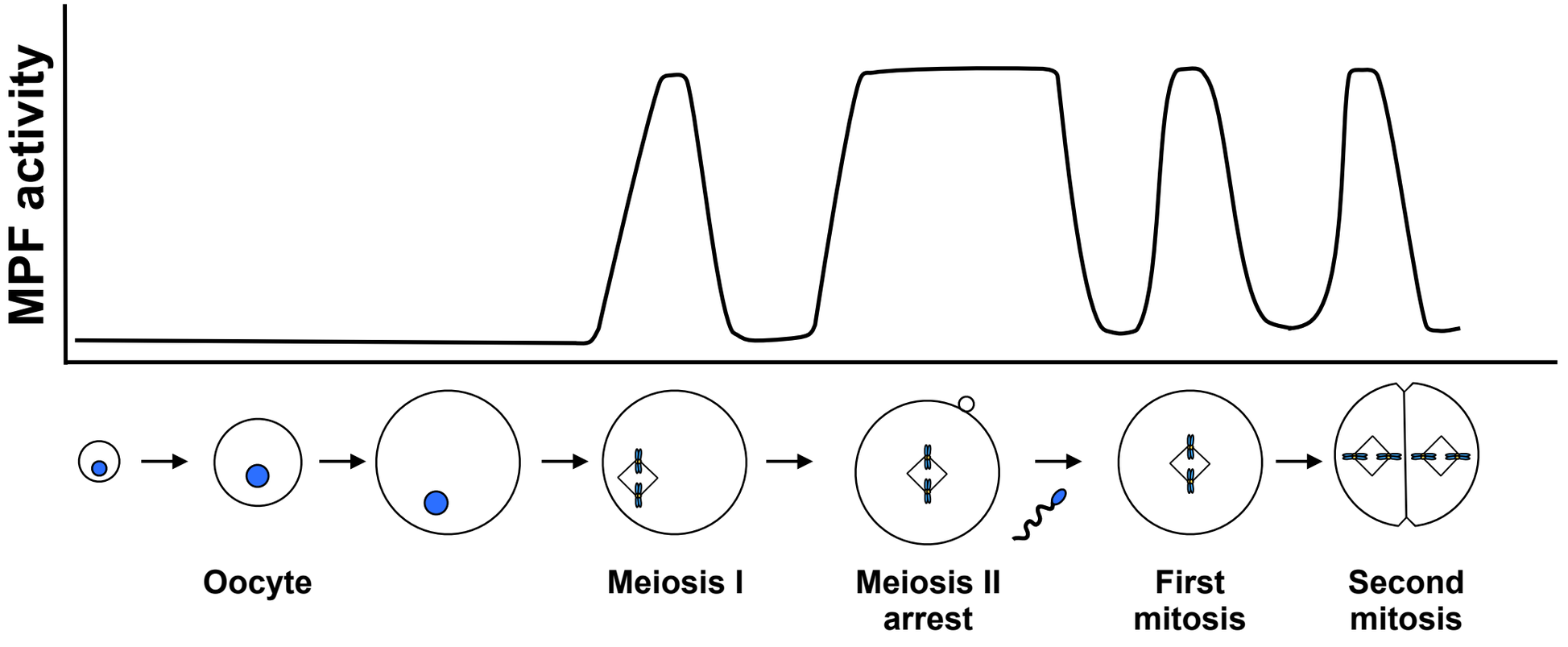


FIG. 5. Periodic surface activities in a noncleaving (non-nucleated) fragment of a fertilized egg prepared by constriction, expressed as changes in the height of the noncleaving fragment. Arrows 1–6 indicate times of onset of cleavage in the cleaving (nucleated) partner fragment (21°C).

Hara, Tydeman and Kirschner, 1980

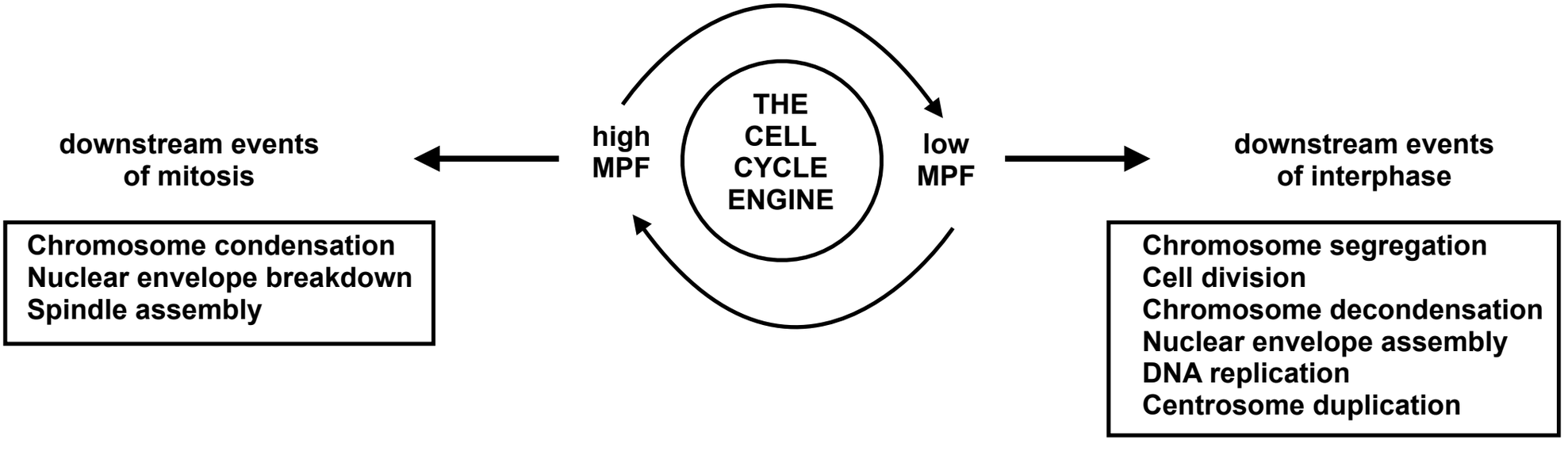
Enucleated zygotes still underwent surface contraction waves on the cell cortex even though they cannot divide. These waves happen at the same time as the sister zygote that contains the nucleus divides. This experiment suggested a cytoplasmic clock that regulates some aspects of the cell cycle.

MPF activity is high in meiosis and mitosis.
Is MPF the cytoplasmic factor that regulates synchronous divisions?



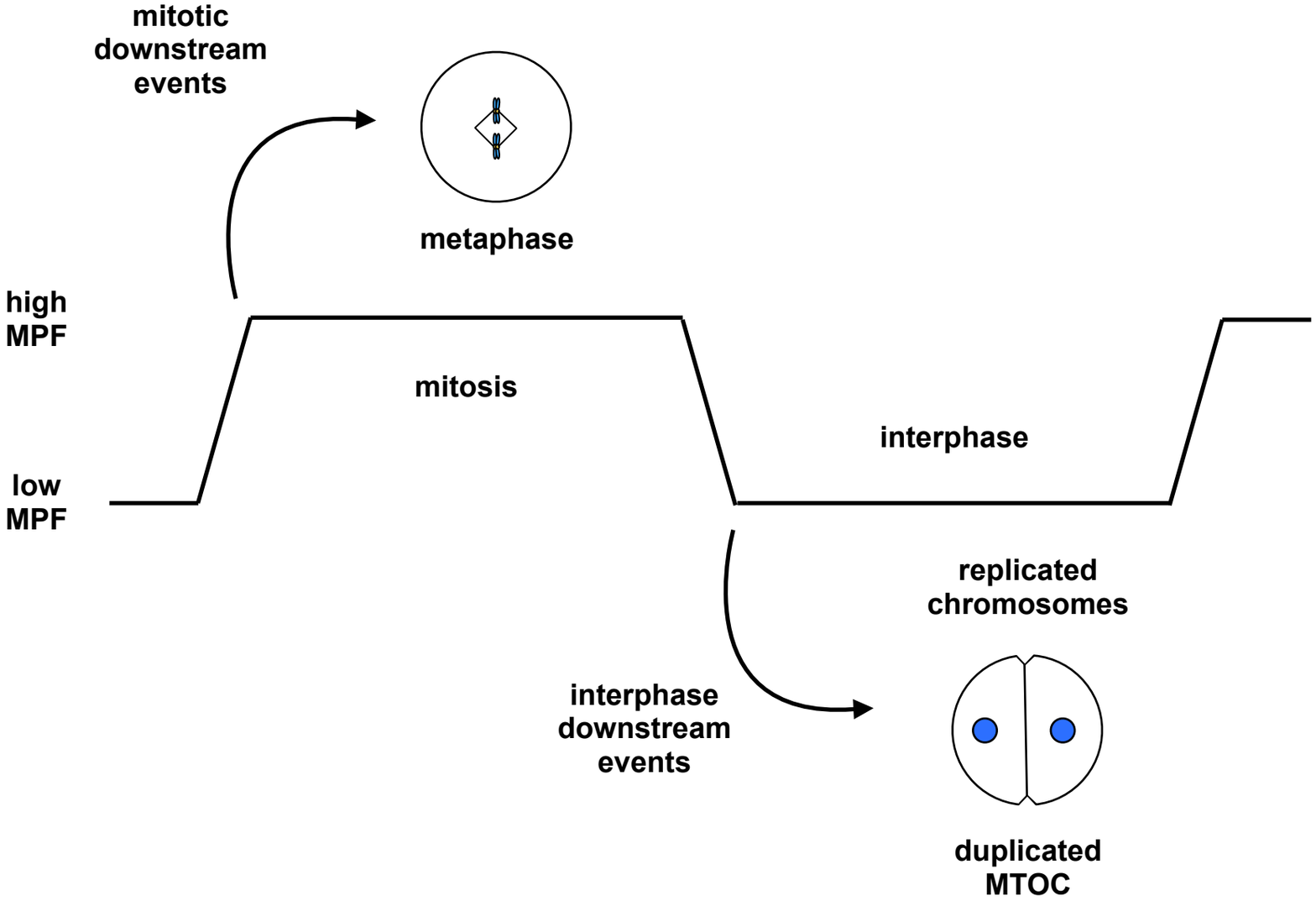
Is MPF the cytoplasmic factor that regulates synchronous divisions?

A simple model for how changes in MPF drive key cell cycle events



The idea of MPF led to the following model that hypothesized oscillations in MPF activity is the main driver of key cell cycle events.

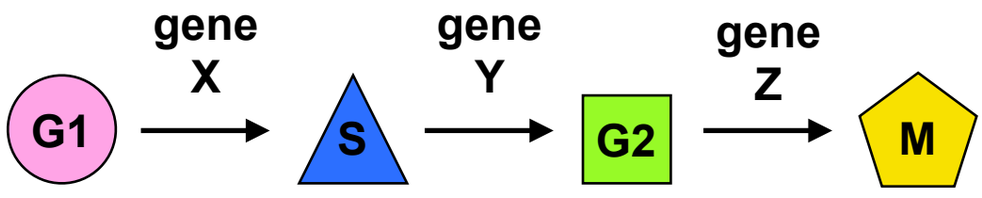
Another depiction of the model



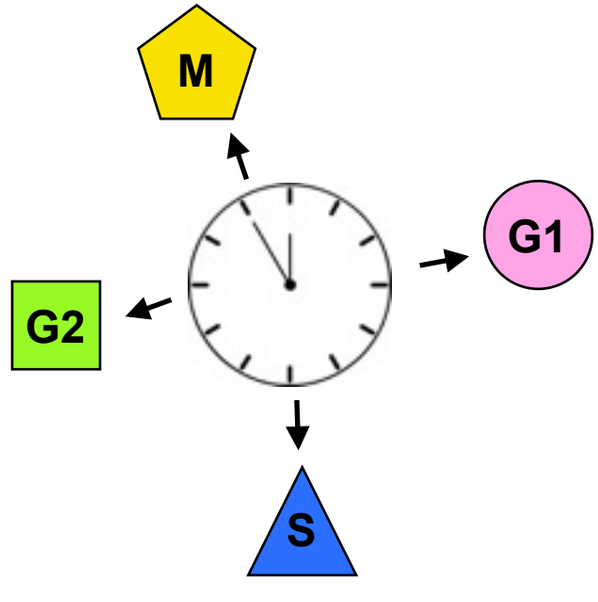
Another way of presenting the model.

Does MPF support the domino or clock theory?

domino theory

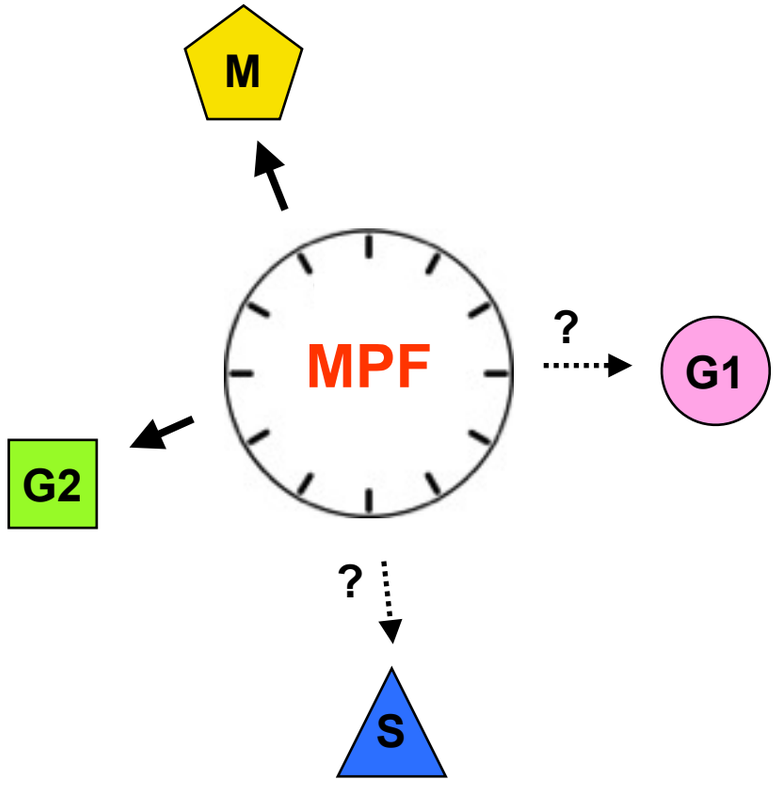


clock theory



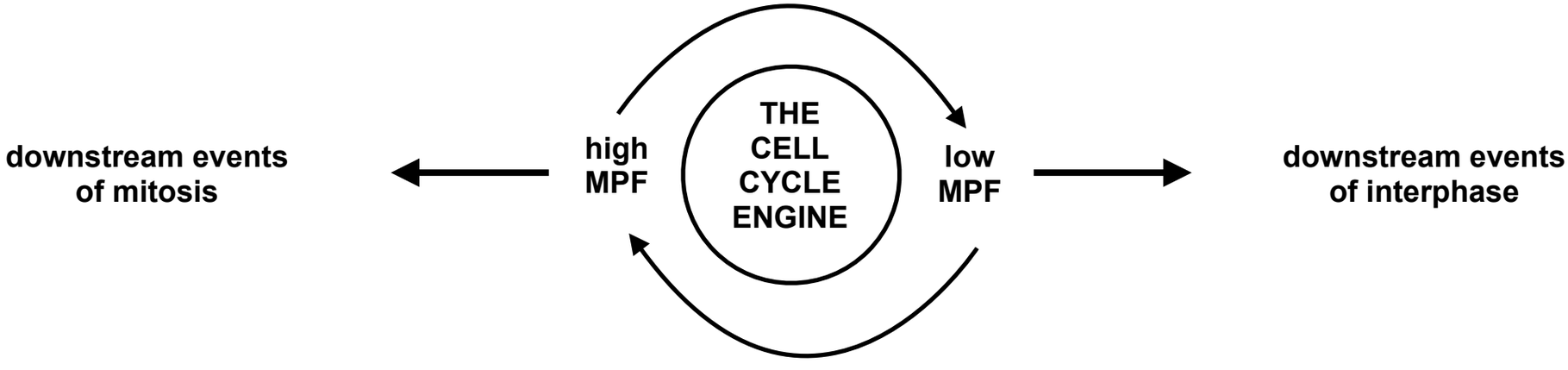
Which model is supported by MPF?

MPF is a key part of the clock



MPF clearly supports the clock theory. It was hypothesized to be the center of the “cell cycle engine”.

What is MPF?



Researchers next wondered what the molecular composition of MPF was. Is it a single protein? Many proteins? Finding MPF and manipulating it cells is the only way to really prove that it exists.

Next class:

the identity of MPF.....

suggested reading:

Hartwell, Culotti, Pringle and Reid. Genetic control of the cell division cycle in yeast. Science (1974) vol. 183 pp. 46-51.

and

The Demise of Bill and the Salvation of Doug

Next class we'll talk about how researchers started figuring out what MPF is. Two very different approaches were used: a biochemical approach that tried to purify MPF from extracts, and genetic approaches that sought to characterize how the cell cycle worked. Both ended up in the same place. The suggested reading for next time is a review by Lee Hartwell that explains how he used budding yeast to begin to decipher how the cell cycle functions, and a brief fiction piece that illustrates the strengths and weaknesses of biochemistry and genetics.

Additional Reading:

Rao et al. Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis. Nature (1970) vol. 225 (5228) pp. 159-64.

Johnson et al. Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei. Nature (1970) vol. 226 (5247) pp. 717-22.

Hara et al. A cytoplasmic clock with the same period as the division cycle in Xenopus eggs. Proc Natl Acad Sci USA (1980) vol. 77 (1) pp. 462-6. Paper showing surface contraction waves occur in activated enucleated embryos.

Kirschner. A visit to the Hubrecht laboratory. Int J Dev Biol (1999) vol. 43 (7) pp. 629-31. A short personal history of early *Xenopus* experiments.

Wasserman et al. A cytoplasmic factor promoting oocyte maturation: its extraction and preliminary characterization. Science (1976) vol. 191 (4233) pp. 1266-8. One of the many Masui papers on MPF.

Murray et al. Dominoes and clocks: the union of two views of the cell cycle. Science (1989) vol. 246 (4930) pp. 614-21

Some references. The short Kirschner paper is an easy read, and the Murray review is dense, but covers much of the same material I'm covering.

The suggested reading for this week in the Hara et al. paper which was the first indication that a cytoplasmic and soluble factor regulated cell cycle progression independent of nuclear events.

If you are interested or confused, I'd also strongly recommend the Chapter on the Cell Cycle in MBOC.

Rao and Johnson fused S and M phase cells together and saw that the M phase nucleus drove the S phase nucleus into mitosis. The photo below shows what the chromosomes from the fused cell looked like.

A. What happened to the S-phase nucleus' chromosomes (they are the upper ones)? Why?

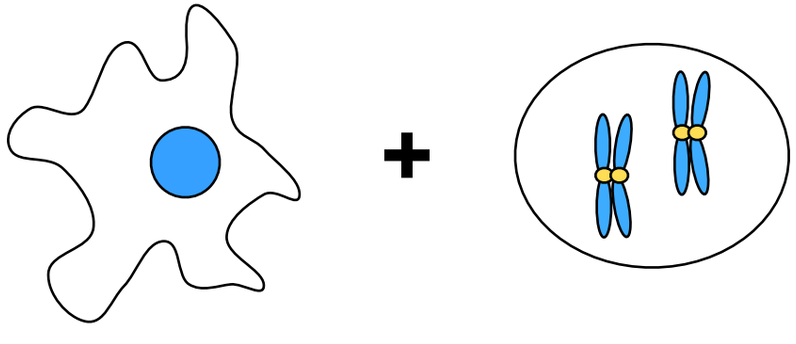
B. Propose a model for how an S-phase cell prevents mitosis from happening.

C. What would happen to a cell that has two rounds of S-phase with no intervening mitosis?

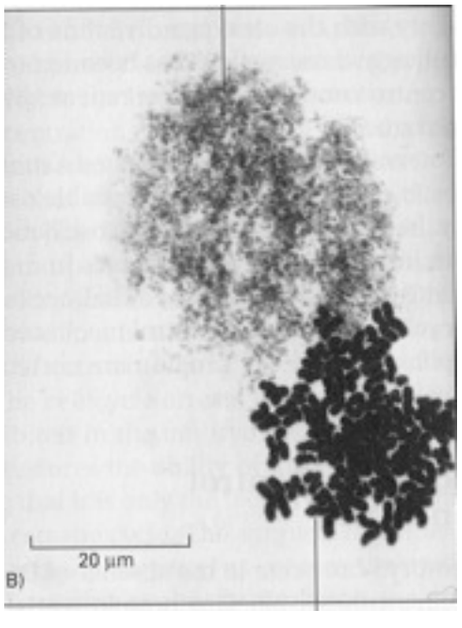
D. What would happen to a cell with two mitosis with no intervening S-phase?

E. What would happen to cells that don't undergo cytokinesis, but still alternate S and M phases?

F. Extra credit - can you think of examples of normal cells that behave as in B, C and D?



S **M**



S + M

Some sample questions that you can think about and be able to answer. Definitely discuss them with your friends. I will go over the answers in class on Thursday.

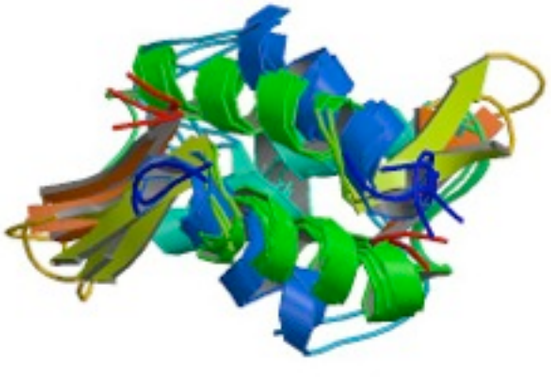
Early on researchers realized that MPF activity was associated with high levels of histone H1 kinase activity, and this was exploited as an easy assay when researchers were studying MPF. Histone H1 is the linker histone, which binds to the DNA between nucleosomes.

A. If histone H1 were a true substrate of MPF, what process do you think it might have regulated? Propose what MPF phosphorylation might do to histone H1 and chromatin structure.

B. An eager graduate student, Marion, maps 12 sites of phosphorylation (which are all serine and threonine residues) on histone H1 and mutates them to alanine. Why? What phenotype do you think she expects to see when she expresses this mutant version of histone H1?

C. Despite all her hard work, she sees no phenotype in her cells. Propose one reason why.

D. Do you think histone H1 could be the only target of MPF phosphorylation? Explain.

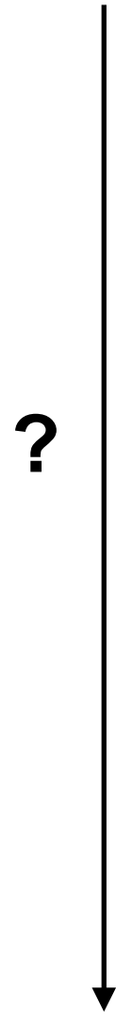


histone H1

Some sample questions that you can think about and be able to answer. Definitely discuss them with your friends. I will go over the answers in class on Thursday.

How to figure out the mechanism of a biological process?

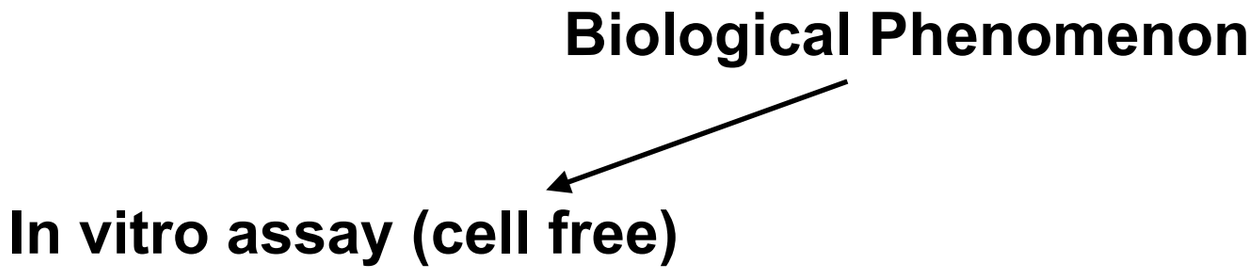
Biological Phenomenon



MECHANISM

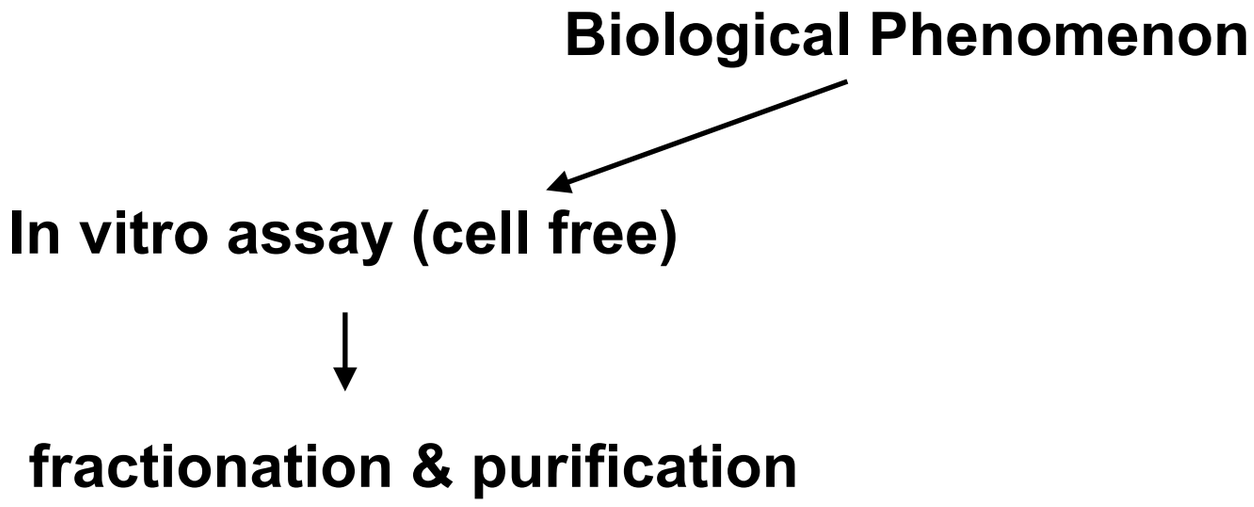
Before we get into the details of what MPF is, I wanted to talk briefly about how researchers go from biological phenomenon to mechanism, which is the goal of cell biological research.

Biochemical Approach:



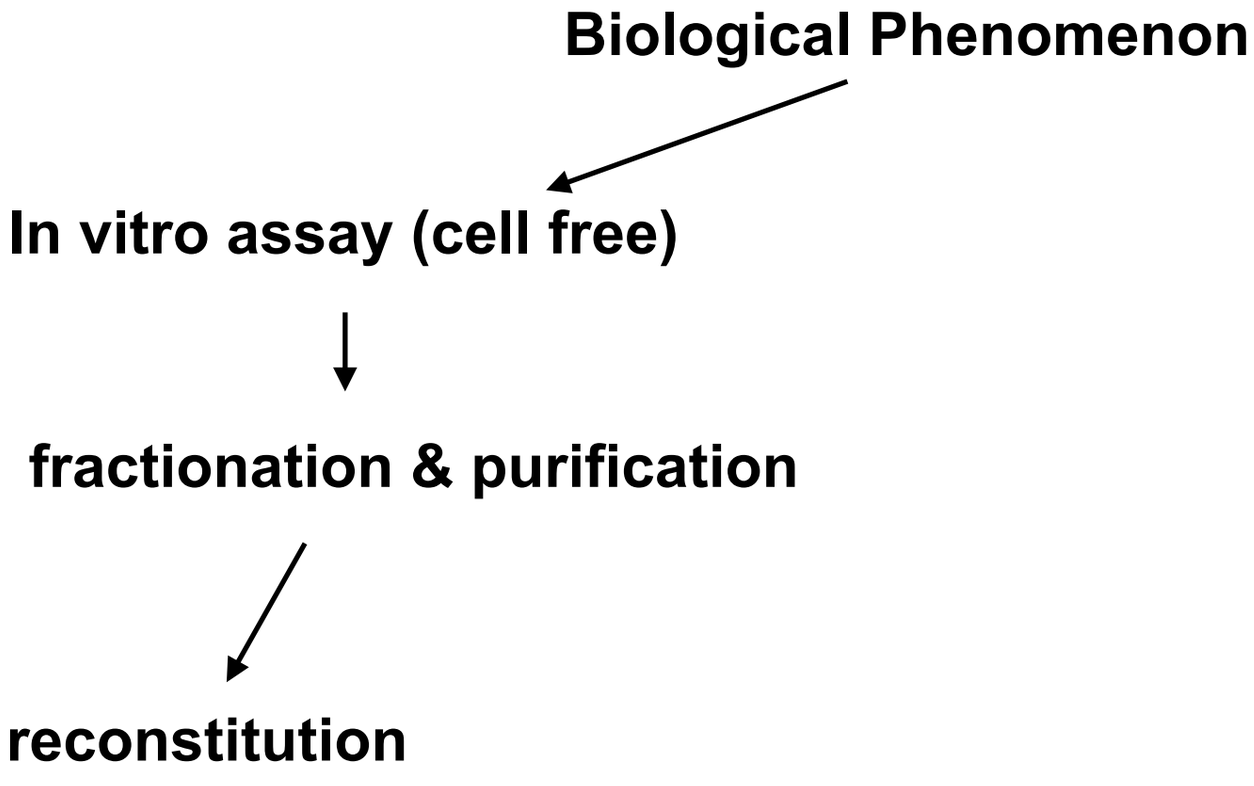
The biochemical approach begins by defining an in vitro assay, which is usually cell free and performed in a test tube, but in the case of MPF, is done on immature oocytes dissected from female frogs. In some respects the immature oocytes are acting like little tubes for this assay. The goal of any in vitro assay is to recapitulate some aspect of a biological process, and be set up in a way that allows for it to be dissected.

Biochemical Approach:



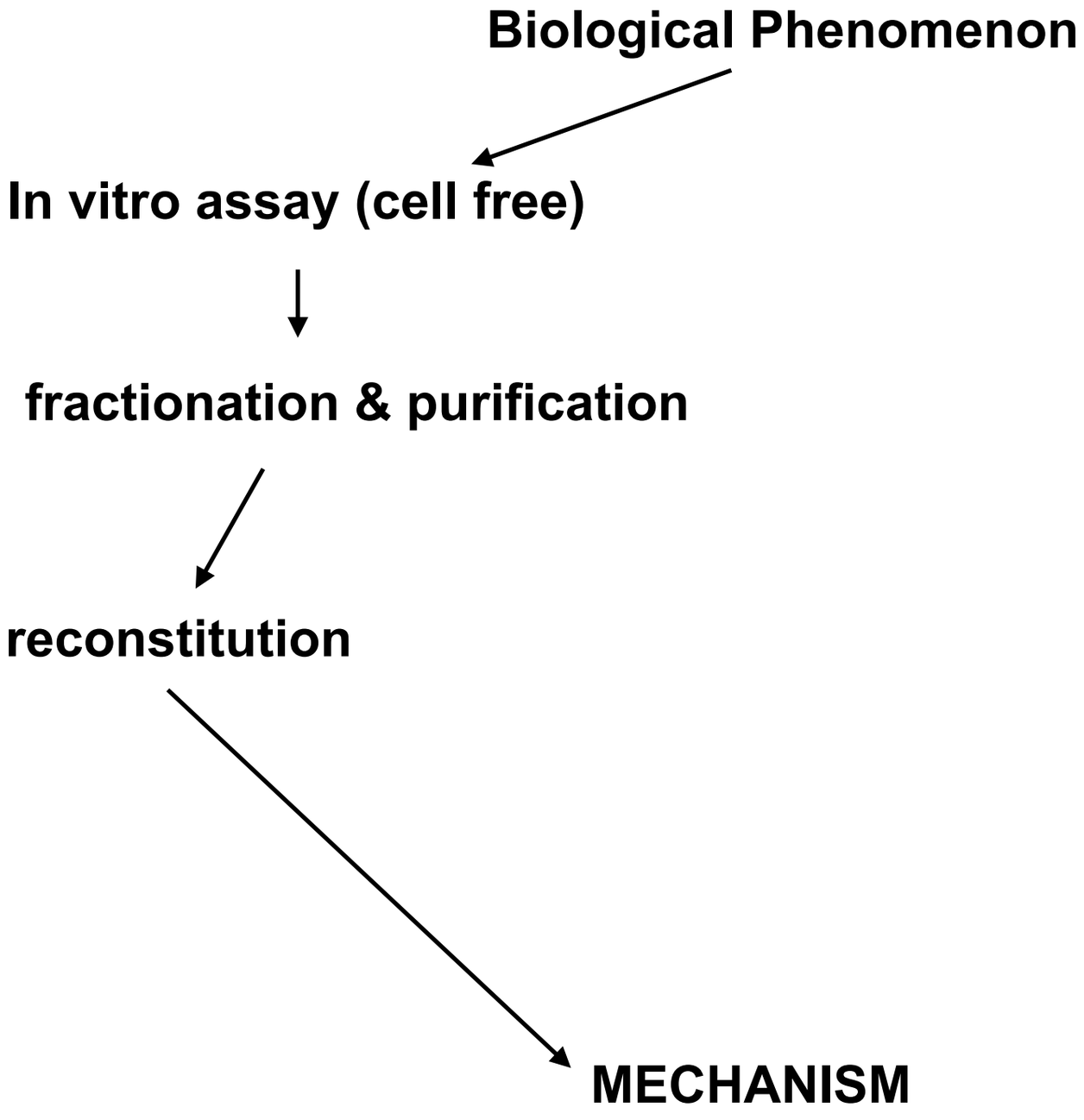
An assay is dissected by fractionation and then purification. Does the assay depend on one protein or factor, or many? Can that protein be purified to homogeneity and then identified? For any fractionation/purification you need an activity that you measure in an assay. For example for MPF, the activity was oocyte maturation and the assay was injecting cytoplasm or fractionated cytoplasm into immature oocytes. Early on people recognized that MPF was associated with histone H1 kinase activity, so people thought MPF was a protein kinase, so they often also measured H1 kinase activity (which is much easier than measuring oocyte maturation).

Biochemical Approach:



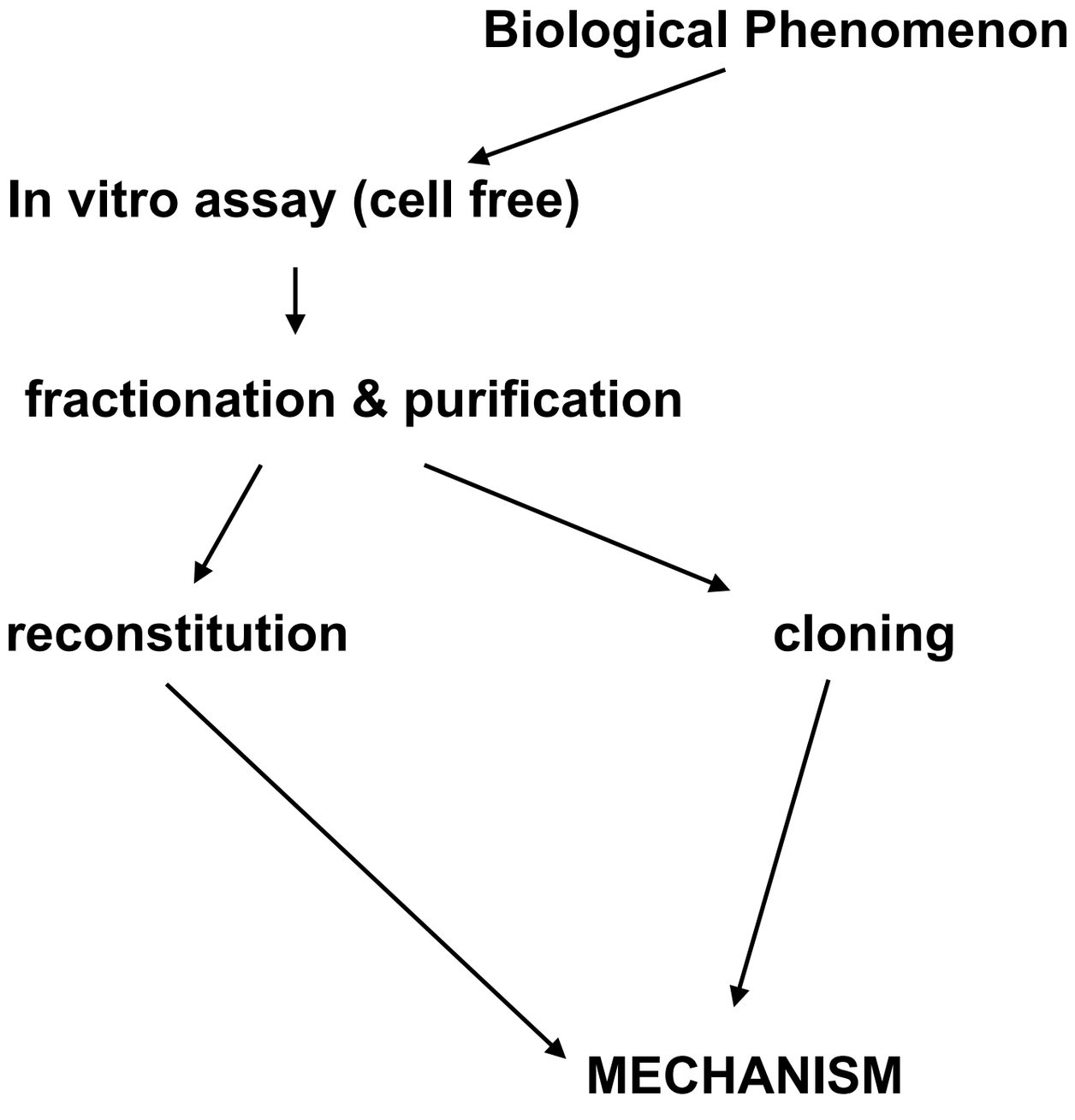
Once your factors are purified, the high road of biochemistry is to reconstitute your activity with pure components. This confirms that all you need to make a reaction go is the components you add. The classic example of this is Arthur Kornberg's reconstitution of DNA replication. Reconstitution tells you a lot about what factors are sufficient for a process, but doesn't necessarily tell you that particular proteins are necessary for the process in vivo.

Biochemical Approach:



The end goal of a biochemical approach is to tell you something about the molecular mechanism of a particular process. In the example of MPF, people wondered what sort of protein or proteins triggered oocyte maturation, and if the same proteins also triggered entry into mitosis. Knowing the activity of the proteins that composed MPF would open up the field of cell cycle research because it was assumed knowing what MPF was would lead to finding all the downstream targets that were needed for each cell cycle transition.

Biochemical Approach:



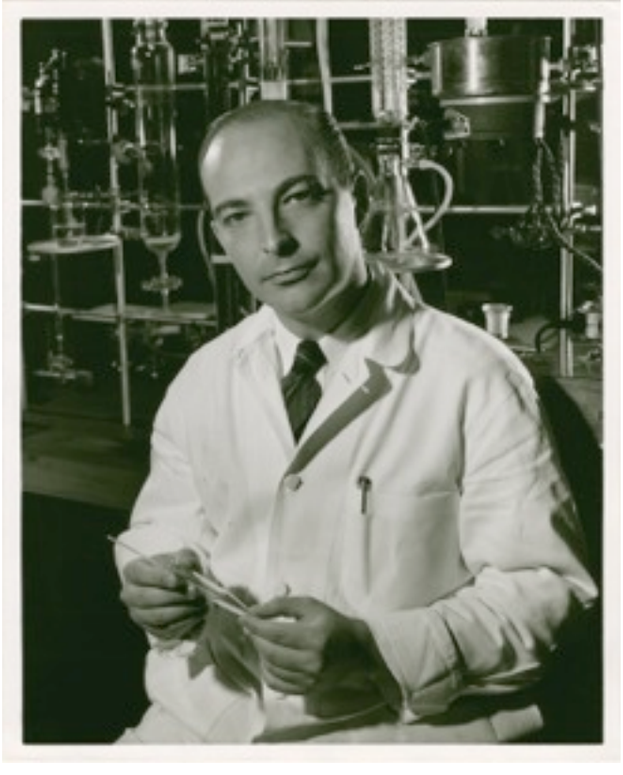
Fractionation and purification can also lead to the cloning of the gene or genes that encode proteins involved in a particular process. Its important to remember that sometimes the activities measured are not always proteins, so there isn't always a gene to clone! With current methods (in particular mass spectrometry) cloning of genes encoding candidate proteins happens quickly, and allows reconstitution and fractionation to proceed more rapidly.

Biological Phenomenon studied with Biochemistry

1. Metabolic enzymes.
How is glucose converted to glucose-6-phosphate?

2. DNA replication

You may be more used to Biochemistry helping to discover things like the metabolic enzymes. Things like hexokinase which converts glucose to glucose-6-phosphate. Researchers likely set up a simple assay for this process and purified the enzyme responsible. Purifying MPF uses biochemistry to understand a complicated cell biological process - oocycte maturation which probably requires 100 of proteins, but the maturation assay allowed researchers to look for key regulators of the process. The hope was knowing the identity of MPF would then lead to finding the proteins downstream that induce all the major changes during maturation.

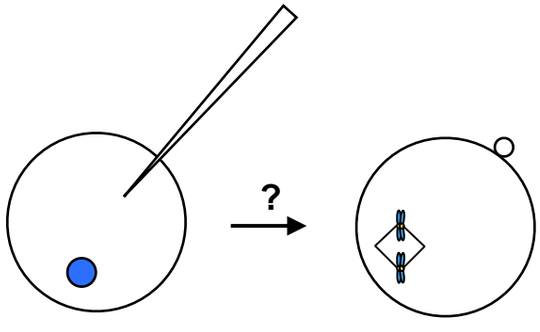


“Implicit in the devotion to purifying enzymes is the faith of a dedicated biochemist of being able to reconstitute in a test tube anything a cell can do.” Arthur Kornberg

Arthur Kornberg popularized this approach. He was the first person to try and reconstitute a very complicated cell biological process - DNA replication.

What is MPF?

In vitro assay ("cell free"):

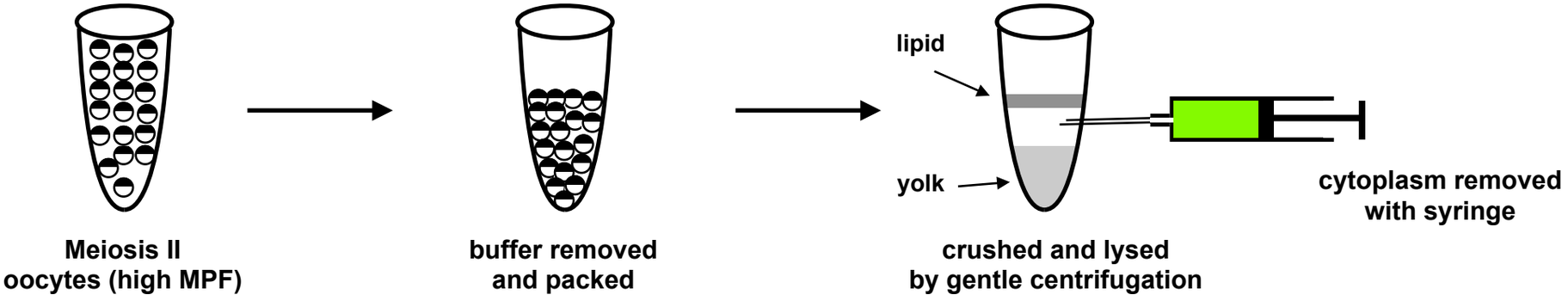


oocyte maturation

MPF was first defined as an activity, so biochemical methods seemed most appropriate. Extracts of eggs could be made, fractionated, and the fractions tested in the oocycte maturation assay. On paper a good idea, but it was exceedingly difficult because the maturation assay is so time consuming. Other methods (to be discussed) discovered the molecular components of MPF.

What is MPF?

material to fractionate:

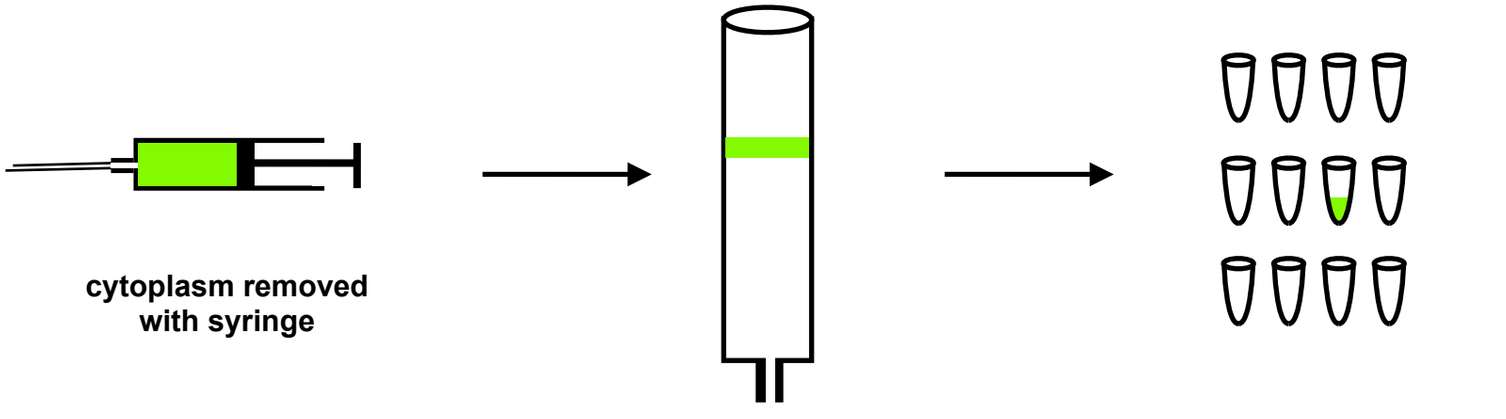


frog egg cytoplasm

The material used to fractionate was extracts of mature oocytes that are arrested in metaphase of meiosis II. People knew these had high MPF activity. The oocytes were collected, packed in a tube and then crushed by high speed centrifugation. The contents separate into three phases: lipid, cytoplasm and yolk. MPF was present in the cytoplasm which is a good thing because it is tricky to fractionate lipids and yolk!

What is MPF?

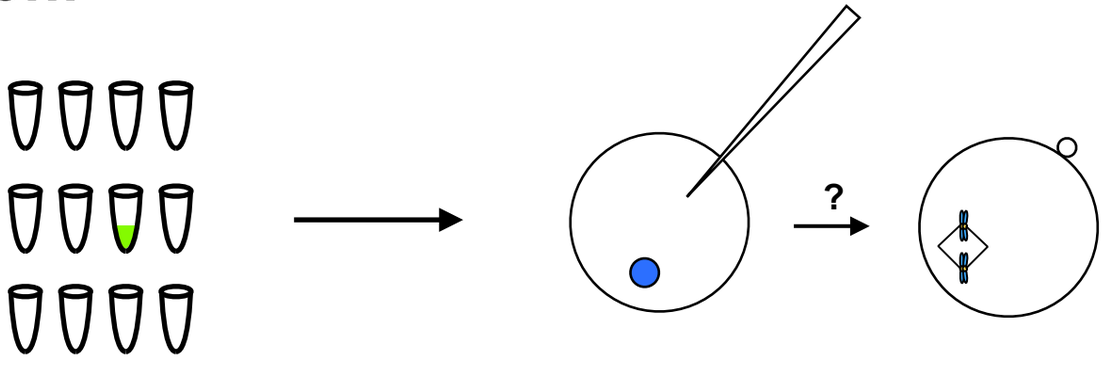
fractionation:



The cytoplasm was then fractionated primarily by column chromatography. I'm showing one column in this cartoon, but the actual purification involved many columns. Chromatography separates the components of the cytoplasm into different fractions based on intrinsic properties of proteins - charge, size, hydrophobicity.

What is MPF?

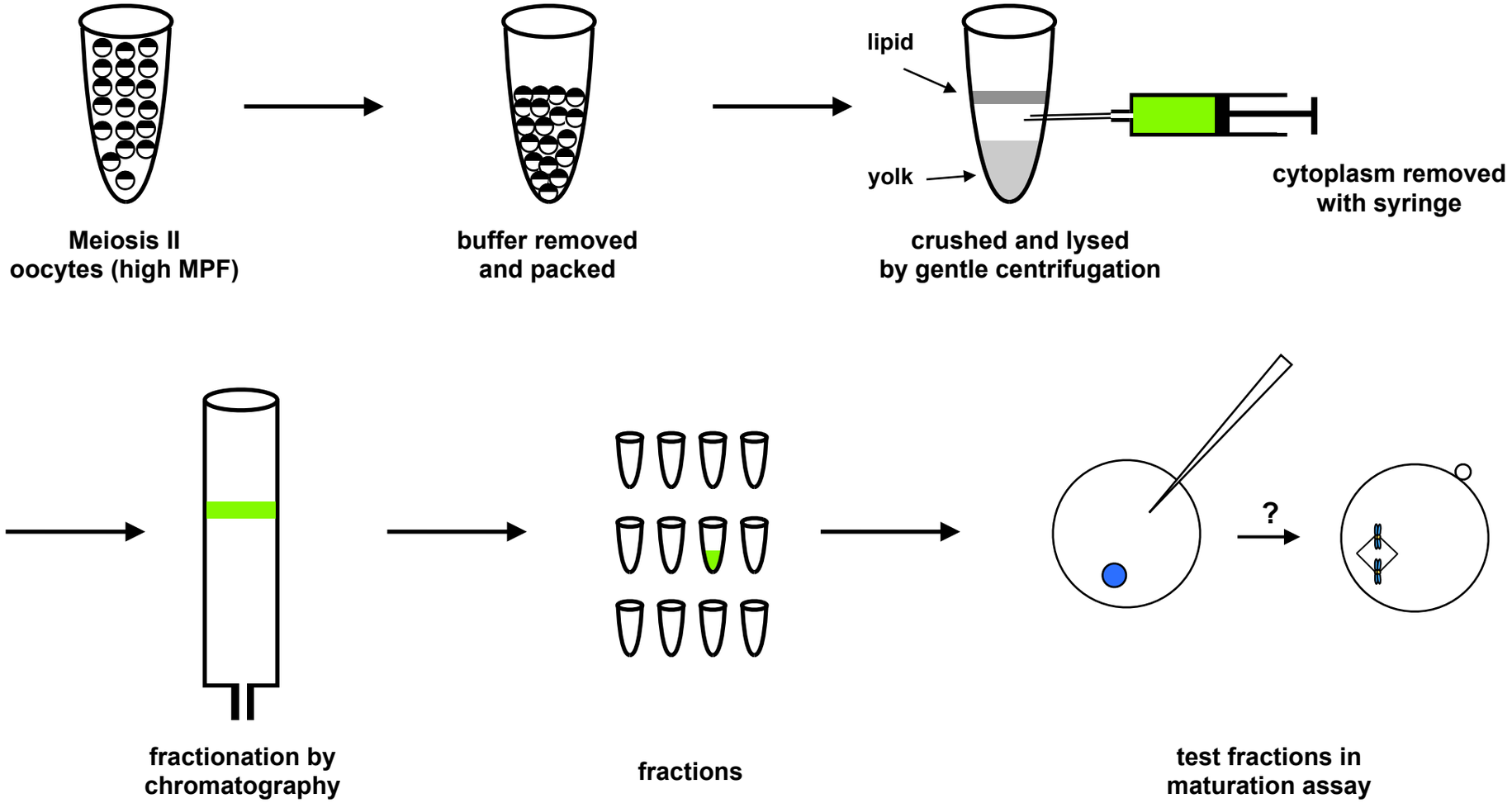
reconstitution:



fractionated extract **oocyte maturation**

Each fraction was then tested in the maturation assay. The hope was to eventually have a fraction that contained only MPF that could support oocyte maturation.

What is MPF?



Meiosis II
oocytes (high MPF)

buffer removed
and packed

crushed and lysed
by gentle centrifugation

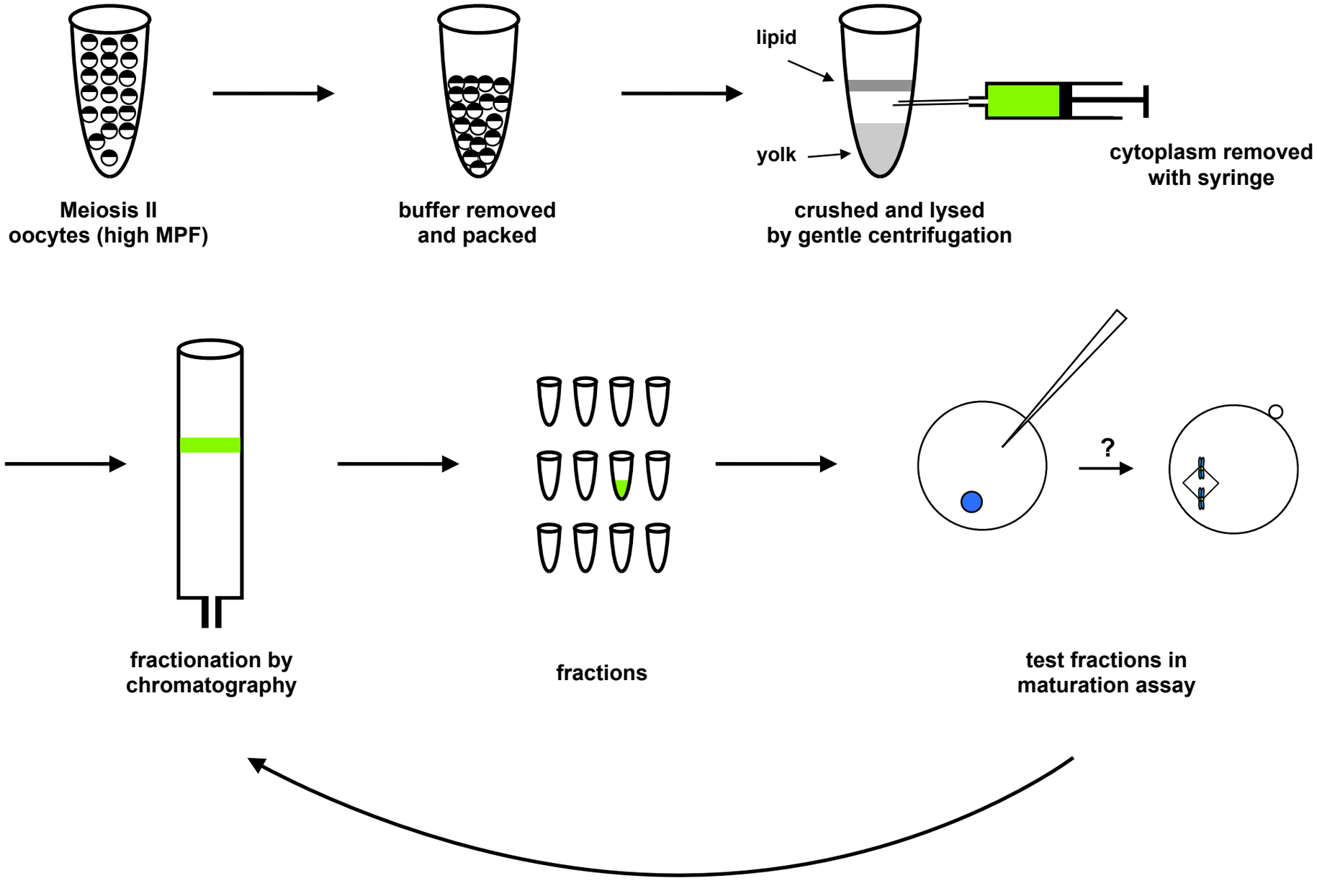
fractionation by
chromatography

fractions

test fractions in
maturation assay

Here is the whole work flow.

What is MPF?



The fraction that contained MPF was then fractionated further on additional columns.

An example of the best MPF purification scheme:
(though it was published a year after the identity of MPF was already determined!)

Table 1. Copurification of MPF and of the M Phase–Specific H1 Histones Kinase from Starfish Oocytes

Step	Total Protein (mg)	Total Activity		Specific Activity		Recovery (%)	
		H1 Kinase (Units × 10^3)	MPF (Units × 10^{-5})	H1 Kinase (Units × 10^3/mg)	MPF (Units × 10^{-3}/mg)	H1 Kinase	MPF
Supernatant (100,000 × g)	4800	2448	250	0.51	5	100	100
DEAE cellulose	1100	2398	210	2.2	19	98	85
Hydroxylapatite	254	1152	140	4.5	55	47	55
Phosphocellulose	100	1050	150	10.5	150	43	60
TSKG 3000 SWG	9	463	50	51	550	19	20
Mono Q	0.85	128	10	150	1750	5.2	4
Mono S	0.042	22	2	520	4800	0.9	0.8

One unit of H1 kinase activity corresponds to 1 μmol of ^{32}P transferred per min at 25°C. One unit of MPF corresponds to the amount of MPF required in 50 nl of a microinjected sample for 50% of the recipient Xenopus oocytes to undergo GVBD (Wu and Gerhart, 1980).

Labbe et. al., Cell 1989

This is a table showing the best purification of MPF that was performed. After a high speed spin and six columns MPF was purer, but not pure enough to identify the components of MPF. Note that H1 kinase activity was measured in parallel with oocyte maturation activity. At the time researchers were pretty sure they were the same thing, but not yet positive.