BHARATHIDASAN UNIVERSITY
Tiruchirappalli- 620024,
Tamil Nadu, India

Programme: M.Sc., Biochemistry
Course Title : Cell biology
Course Code :BC105DCE

Unit-4
CELL CYCLE

Dr. M. Anusuyadevi Jayachandran
Professor
Department of Biochemistry

Cell cycle control system governs progression at three major regulatory transitions:-

First - at the start in Late G1 (where cell commits to cell-cycle entry and chromosome duplication)

Second - at G2/M transition (Where control system triggers early mitotic events that lead to chromosome alignment on mitotic spindle in metaphase)

Third - at Metaphase - Anaphase transition (where the control system stimulates sister-chromatid separation leading to completion of mitosis and cytokinesis)

CYCLIN DEPENDENT KINASES

Cdks are family of protein kinases that rise and fall as the cell progresses through the cell cycle - leading to cyclical changes in the phosphorylation of intracellular proteins that initiate or regulate the major events of the cell cycle.

Ex: An increase in Cdk activity at G2/M transition, for ex: increases the phosphorylation of proteins that control chromosome condensation, nuclear envelope breakdown, spindle assembly and other events that occur in early mitosis.

Regulators of Cdks are the proteins known as cyclins.

The levels of Cdk proteins, by contrast, are constant. Cyclical changes in cyclin protein levels result in the cyclic assembly and activation of cyclin-Cdk complexes at specific stages of the cell cycle.

4 CLASSES OF CYCLINS

G1/S cyclins activate Cdks in late G1 and thereby help trigger progression to cell cycle entry. Their levels fall in S phase

S-Cyclins - bind Cdks soon after progression through start - help chromosome duplication. Their levels remain elevated until mitosis and also contribute to control some of early mitotic events.

M-Cyclins - activate Cdks that stimulate entry into mitosis at the G2/M transition. M-Cyclin levels fall in the mid-mitosis.

In most cells, a fourth class of cyclins, the G1-cyclins, help govern the activities of the G1/S cyclins, that control progression through start in the late G1.

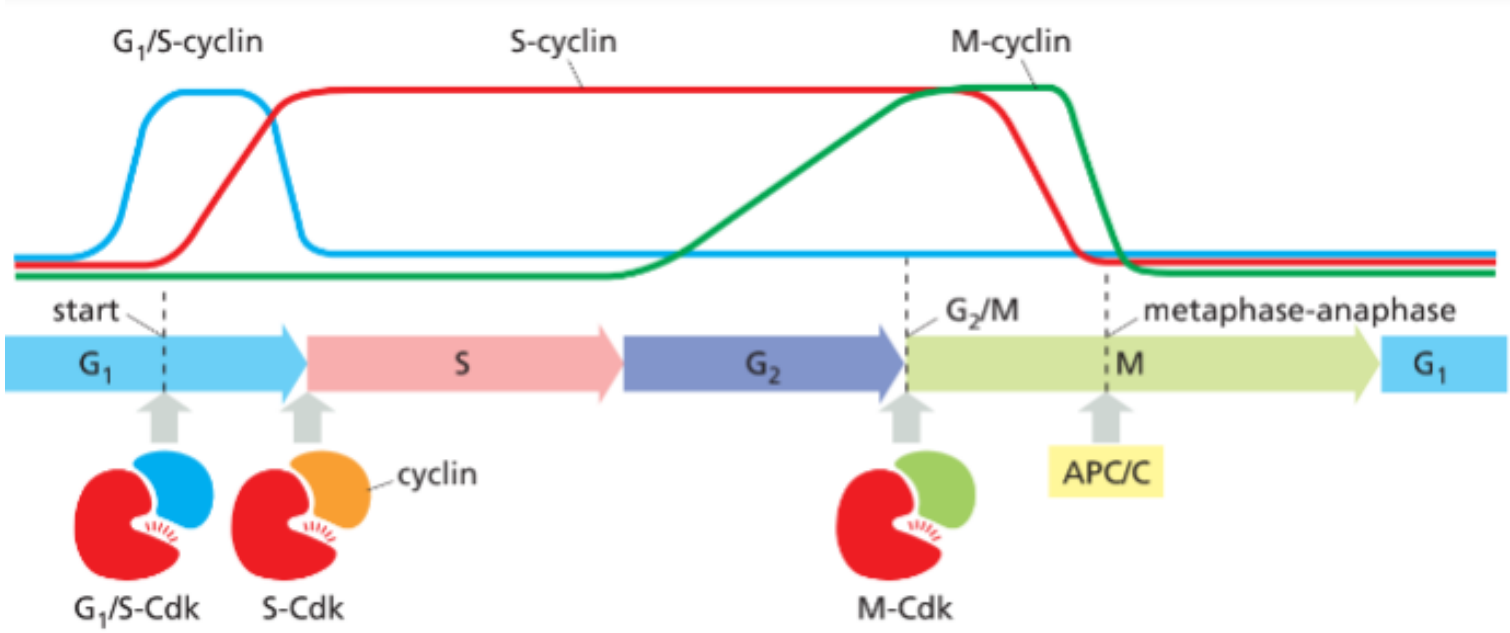
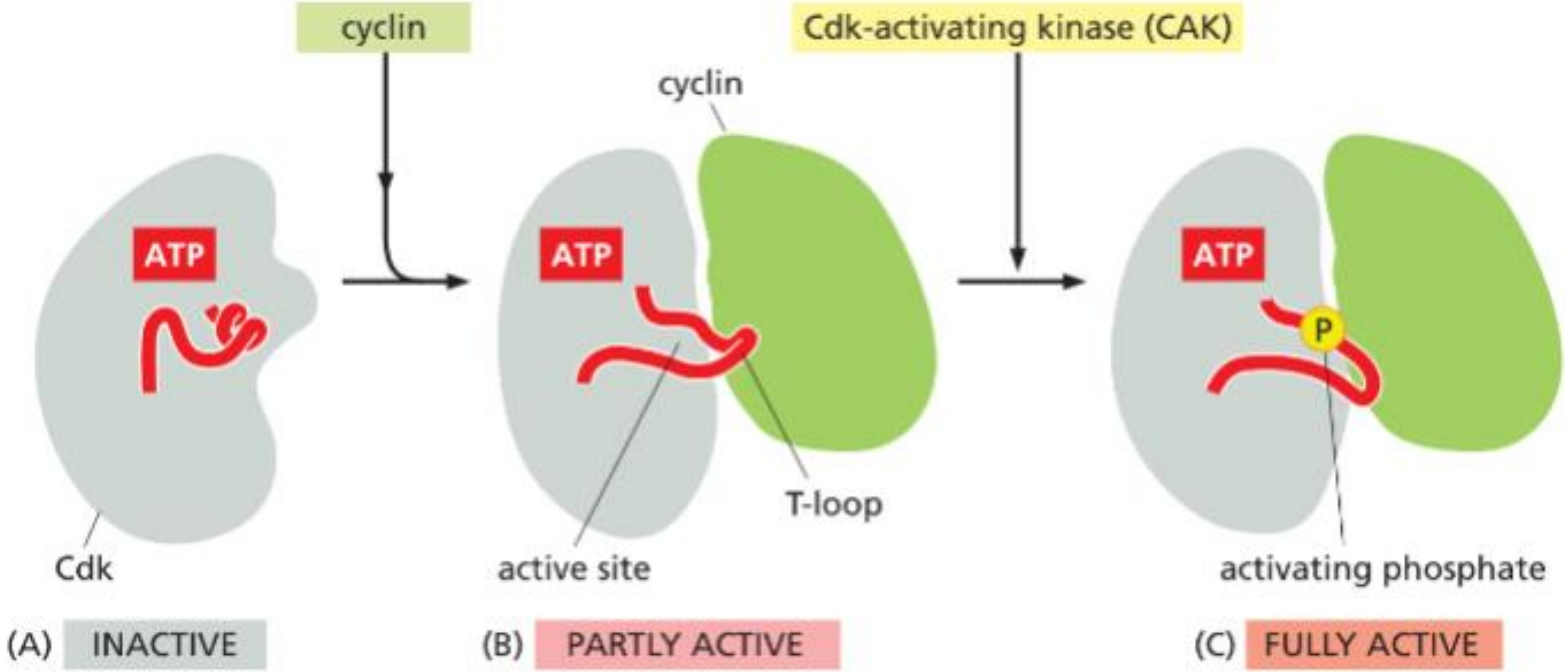

G1/S-cyclin
S-cyclin
M-cyclin
start
G2/M
metaphase-anaphase
G1
S
G2
M
G1
cyclin
APC/C
G1/S-Cdk
S-Cdk
M-Cdk

TABLE 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

	Vertebrates		Budding yeast	
Cyclin–Cdk complex	Cyclin	Cdk partner	Cyclin	Cdk partner
G_1-Cdk	Cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	Cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	Cyclin A	Cdk2, Cdk1**	Clb5, 6	Cdk1
M-Cdk	Cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

* There are three D cyclins in mammals (cyclins D1, D2, and D3).
** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.

Cdk activating kinase (CAK) - phosphorylates amino acid near the entrance of Cdk active site

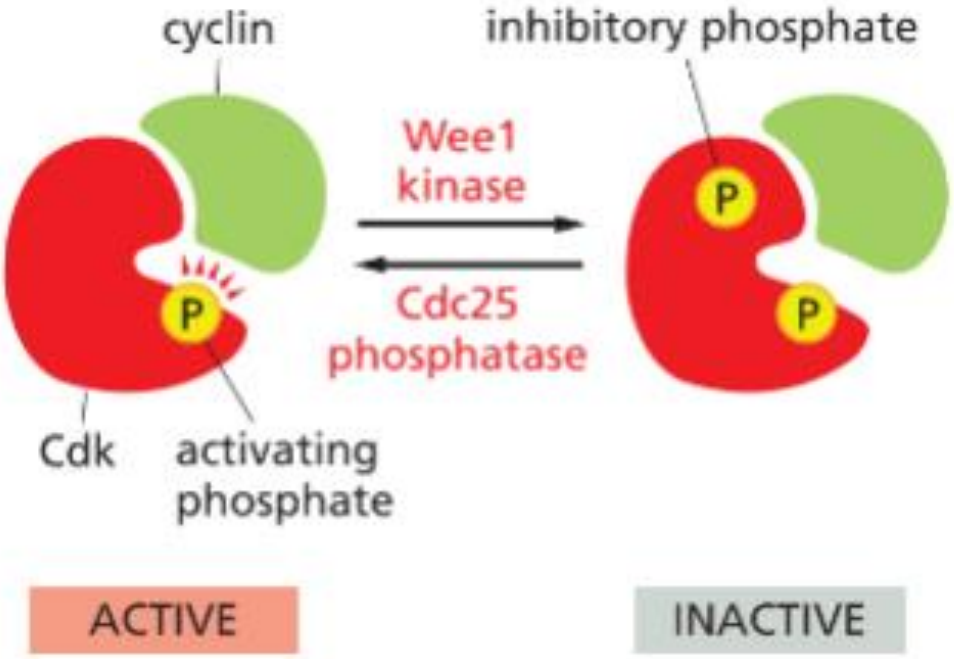


Cdk activity can be suppressed by inhibitory phosphorylation and Cdk inhibitor proteins (CKI)

The rise and fall of cyclin levels is the primary determinant of Cdk activity during the cell cycle.

Phosphorylation at amino acid of kinase active site inhibits the activity of Cyclin-Cdk complex. Ex: PO4 by Wee1 kinase inhibits Cdk while dephosphorylation by Cdc25 phosphatase increases its activity

Cdk INHIBITOR PROTEINS (CKI) - Wee1 phosphorylates two closely spaced sites above the active site



Other mechanism include Cdk inhibitor proteins (CKIs) inactivates cyclin-cdk complexes by stimulating large rearrangement in the structure of the Cdk active site, rendering it inactive

Cells use CKIs primarily to help govern the activities of G1/S - and S-Cdks early in the cell cycle.

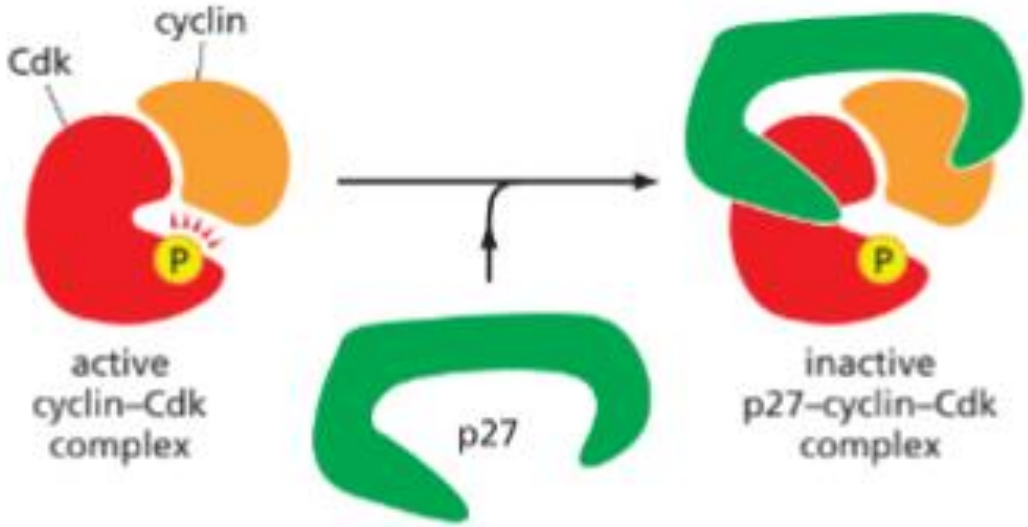


Figure 17–14 The inhibition of a cyclin–Cdk complex by a CKI. This drawing is based on the three-dimensional structure of the human cyclin A–Cdk2 complex bound to the CKI p27, as determined by x-ray crystallography. The p27 binds to both the cyclin and Cdk in the complex, distorting the active site of the Cdk. It also inserts into the ATP-binding site, further inhibiting the enzyme activity.

Regulated proteolysis triggers Metaphase - Anaphase transition - M-A phase is triggered not by phosphorylation, rather by protein destruction leading to final stages of cell division.

APC/C - Anaphase promoting complex / cyclosome - a member of ubiquitin ligase family of enzymes - that stimulates the proteolytic destruction of specific regulatory proteins.

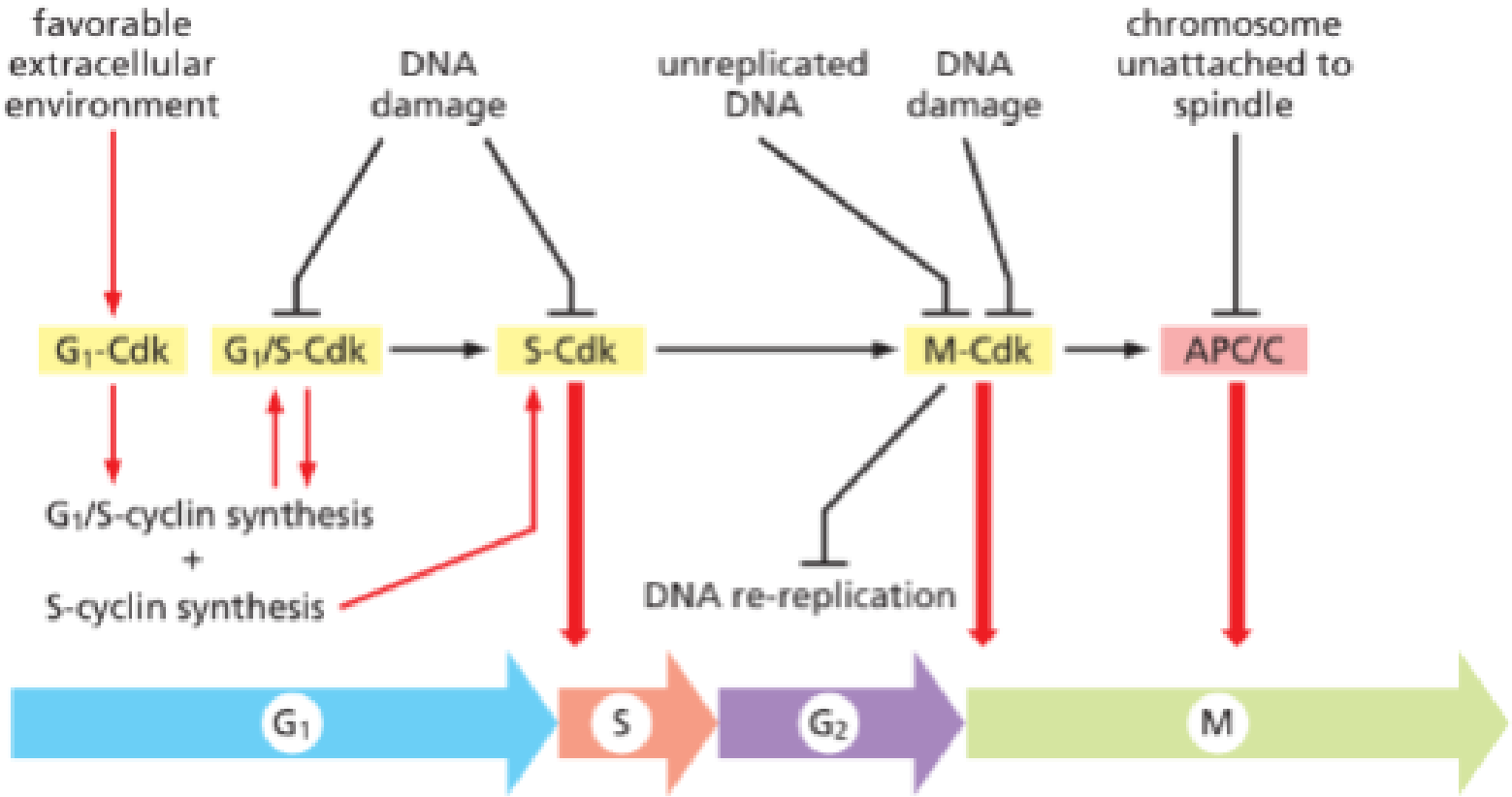
APC poly ubiquitinylate specific target proteins, and destructs in proteosomes.

APC/C catalyses the ubiquitylation of
i) securin - that protect protein linkages that hold sister-chromatid pairs together in early mitosis

Destruction of securin in metaphase activates a protease that separates the sisters and unleashes anaphase.
ii) S- and M - cyclins are the second major targets of APC/C - help completion of M phase, including the final steps in mitosis and then cytokinesis.

TABLE 17–2 Summary of the Major Cell Cycle Regulatory Proteins	
General name	Functions and comments
Protein kinases and protein phosphatases that modify Cdks	
Cdk-activating kinase (CAK)	Phosphorylates an activating site in Cdks
Wee1 kinase	Phosphorylates inhibitory sites in Cdks; primarily involved in suppressing Cdk1 activity before mitosis
Cdc25 phosphatase	Removes inhibitory phosphates from Cdks; three family members (Cdc25A, B, C) in mammals; primarily involved in controlling Cdk1 activation at the onset of mitosis
Cdk inhibitor proteins (CKIs)	
Sic1 (budding yeast)	Suppresses Cdk1 activity in G_1; phosphorylation by Cdk1 at the end of G_1 triggers its destruction
p27 (mammals)	Suppresses G_1/S-Cdk and S-Cdk activities in G_1; helps cells withdraw from cell cycle when they terminally differentiate; phosphorylation by Cdk2 triggers its ubiquitylation by SCF
p21 (mammals)	Suppresses G_1/S-Cdk and S-Cdk activities following DNA damage
p16 (mammals)	Suppresses G_1-Cdk activity in G_1; frequently inactivated in cancer
Ubiquitin ligases and their activators	
APC/C	Catalyzes ubiquitylation of regulatory proteins involved primarily in exit from mitosis, including securin and S- and M-cyclins; regulated by association with activating subunits Cdc20 or Cdh1
Cdc20	APC/C-activating subunit in all cells; triggers initial activation of APC/C at metaphase-to-anaphase transition; stimulated by M-Cdk activity
Cdh1	APC/C-activating subunit that maintains APC/C activity after anaphase and throughout G_1; inhibited by Cdk activity
SCF	Catalyzes ubiquitylation of regulatory proteins involved in G_1 control, including some CKIs (Sic1 in budding yeast, p27 in mammals); phosphorylation of target protein usually required for this activity

An overview of the cell cycle control system



MODEL ORGANISM TO STUDY CELL CYCLE

The most commonly used model organisms are

1. Unicellular yeasts,
2. The early embryos of frogs and fruit flies and
3. Mammalian cells in cell culture

Yeast includes the models of great importance for genetics. Viz., Saccharomyces cerevisiae; Schizosacchromyces pombe; Neurospora crassa etc.,

The budding yeast Saccharomyces cerevisiae and the fission yeast Schizosaccharomyces pombe are unicellular and considered to be among the best genetically tractable organisms for a comprehensive understanding of biology.

Budding yeast was the eukaryote whose genome was sequenced first in 1996 followed by the genome sequence of fission yeast, which was completed in 2002.

Allthough started later, S.pombe has been particularly influential in studies of cell cycle regulation, DNA damage/ repair mechanisms and chromosome dynamics, including RNA interference.

Furthermore, the fission yeast S.pombe harbors less genome duplication compared with other eukaryotes and holds the smallest sequenced eukaryotic genome, which led to its popularity as a eukaryotic model in the last decade.

Both budding yeast and fission yeast are able to exist in either a diploid or a haploid state.

Although most strains of yeast used in laboratory are haploid strains, in the wilderness, budding yeast tend to live in as diploid state, whereas fission yeast tend to stay in haploid state.

The proportion of the life cycle of budding yeast that they spend in the diploid state or haploid state varies, depending on the environment. When nutrient are plentiful, budding yeast proliferates as diploid cells. If starved, they undergo meiosis to form haploid spores.

In contrast, fission yeast typically proliferate as haploid cells. They fuse in response to starvation to form diploid cells and these diploid cells promptly undergo meiosis and sporulation.

Advantages of using yeast as model organism:

These two yeast system are non-pathogenic and are thus safe to handle

Their reproduction cycle are fast and easy to monitor with doubling times from 90 minutes for wild type cells in rich medium to a few hours for mutant cells

Distinct features of the cell cycles in S.cerevisiae and S. pombe

Both undergo closed mitosis - nuclear envelope doesnot breakdown

In contrast, most multicellular/ eukaryotic organism undergo open mitosis

Although both are yeasts, they exhibit distinct features for cell division.

Fission yeast undergo typical eukaryotic cell cycle with consecutive G1, S, G2 and M Phases. The G2/M phase is fuzzy, as spindle assembly starts to occur in S phase. Therefore the control of G2/M phase is more visible in fission yeast.

While G1/S transition start is the major control of cell cycle in budding yeast S. cerevisiae.

Cdc mutants in different models of yeast help learn cell cycle processes.

Using this genetic approach, researchers identified a cast of vital players in cell cycle control, including the genes encoding Cdc2, Wee1 protein kinases, as well as Cdc25 tyrosine phosphatase. The discoveries also provide the basis for the concept of checkpoint control of cell division

Early embryo of frog

Eggs of frog Xenopus laevis are a special type of cell very useful to study cell cycle.

The eggs of amphibians, marine invertebrates, and insects are large cells and they divide very rapidly following fertilization in early embryo development.

DISTINGUISHING feature of early embryonic cell cycle in frog - no cell growth occurs and each daughter cell produced from the cell division is half the size of the parent cell.

Therefore compared to the standard cell division, the duration of frog egg cycle is extraordinarily short, only consisting of alternating S phase and M phase without intervening G1 and G2 phase.

Because of the specialized rapid cell division of the frog embryo, they are very useful for studying the mechanisms of interphase–M phase transition.

The early development of the frog X. laevis embryo provides a particularly powerful system to analyze the factors that drive cells into mitosis.

Since the 1970s a lot of what we know about the control mechanisms of the mitotic cell cycle has been learned from the studies on the interphase–M phase transition in the frog egg system

A fully grown oocyte arrests in G2, when triggered by hormone, the oocyte matures into an egg and arrests in metaphase of the meiosis II. Fertilization releases the metaphase arrest, so that the egg completes its second meiotic division and enters the interphase of the first embryonic cell cycle. Since an immature oocyte arrests in meiotic G2, whereas a mature egg arrests in meiotic M phase, the abundant source of the cytoplasm can be extracted from these embryonic cells at defined stages of the cell cycle.

Moreover, because of their big size, they are amenable for injecting materials such as a molecule of interest or protein lysates into them, or for extracting out of the cytoplasm.

When M-phase cytoplasm from a mature egg is injected into a G 2-phase immature oocyte, the oocyte is driven into M phase and completes its maturation

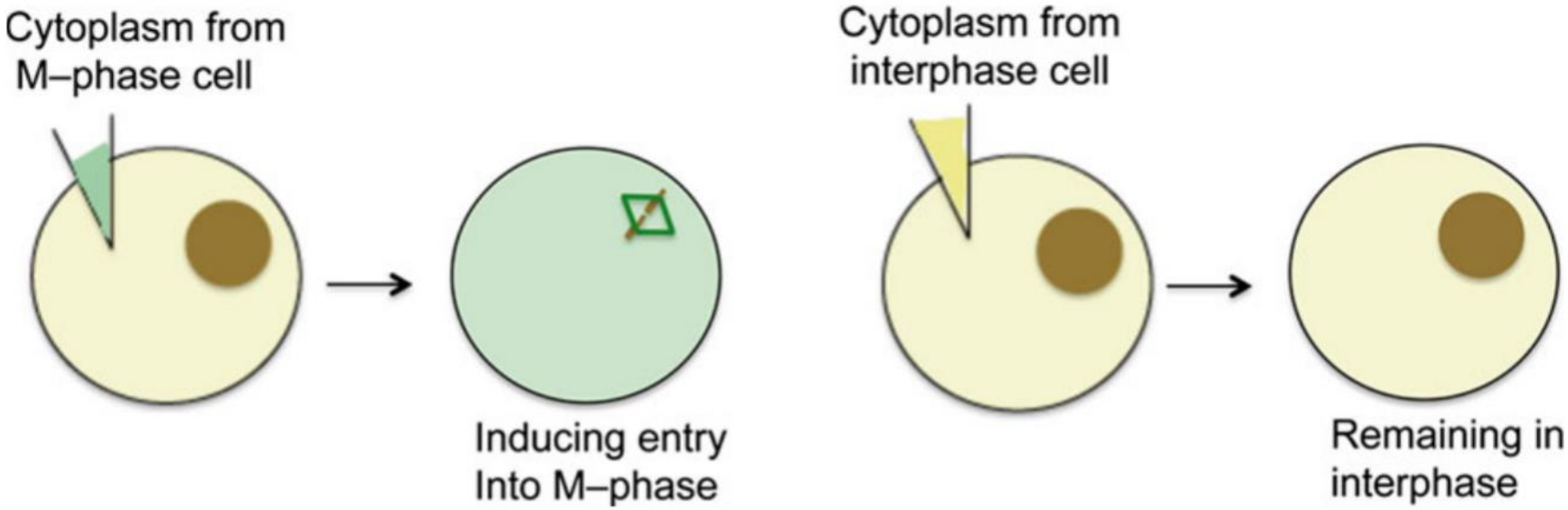


Fig. 13 Oocyte injection experiment. Injection of the cytoplasm from a mature egg in M phase into an immature oocyte in interphase induces the oocyte to become mature and enter M phase

CELL FUSION EXPERIMENT

Mammalian cells are generally not as large as frog oocytes; therefore, it is not as easy to use them for cytoplasmic injections. However, we can perform a logically equivalent test by fusing a mitotic cell with an interphase cell, so that the nucleus of the interphase cell is exposed to any active components present in the cytoplasm of the mitotic cell. In such experiments the interphase cell is directly driven into mitosis, no matter whether it is in G 1, S, or G 2, and whether it has replicated its DNA or not. This cytoplasmic activity is also named MPF—M-phase-promoting factor. It became clear several years later that MPF plays a general role in mitotic induction in somatic cells of all eukaryotic cells from yeasts to humans.

DISCOVERING CYCLINS

Protein synthesis was examined in sea urchin eggs by Tim Hunt. The fertilized eggs were incubated with water containing the radioactive amino acid, 35S-methionine. Samples were removed at allocated time points and analyzed by SDS-PAGE (polyacrylamide gel electrophoresis). The experiment revealed a novel class of proteins, appearing in a periodic fashion, although most proteins in sea urchin eggs accumulate continuously after fertilization. The family of oscillating proteins increases steadily during interphase until the metaphase–anaphase transition, at which they are suddenly abolished. The proteins are thus given the name of cyclin because of their characteristic cycling pattern during the cell cycle

It is this model organism that enables researchers to biochemically purify MPF and functionally identify the key regulators of the cell cycle including, Cdc2, later named as cyclin-dependent kinase1 (Cdk1), Cdc25 tyrosine phosphatase, and Wee1 tyrosine kinase.

FRUIT FLY - Drosophila melanogaster

Fruit Fly Drosophila melanogaster is a valuable model organism for cell cycle studies. The components of the cell cycle control system in Drosophila are structurally and functionally similar to humans.

The generation time of Drosophila is 2 weeks and it is relatively easy to grow and maintain them in a laboratory.

Drosophila has a genome size of about 14,000 genes, which is 2–3 times of yeasts and about half the number in humans.

An early Drosophila embryo undergoes rapid and synchronous nuclear divisions after the fusion of egg and sperm nuclei to give rise to a zygote nucleus. These divisions each last less than 10 min and proceed without gap phases, resulting in a syncytium, in which many nuclei share the same cytoplasm. The nuclei subsequently move to the surface of the embryo after nine divisions and start cytokinesis to form about 6000 cells at the end of the 13th division.

The synchronous progression of the nuclear division in early Drosophila embryos provides a good resource to isolate important regulators of the cell cycle

MAMMALIAN CELLS

Although the fundamental principles of cell cycle control are studied effi ciently in simpler systems including yeasts and Drosophila, it cannot supersede the research in the mammalian cell cycle. Only in mammalian cells can we ultimately decipher the complex circuits regulating the cell cycle.

However, in complex multicellular organisms such as humans, various cells divide at very different rates. The cells that line our intestine live only 3 days and must be constantly replaced by the division of precursor cells. On the other hand, the life span of liver cells is more than a year, thus cell division in this organ is rare.

The cell cycle in mammalian cells varies greatly. The variability in the length of the cell cycle of different cells occurs mainly in G 1 and G 2. It refl ects the ability of cells to exit from the cell cycle during either G 1 or G 2 phase

Many cells can withdraw from the cell cycle, entering G 0 or a stable G 2 arrest. Cells in G 0 have left the cycle after division but before the restriction point at the G 1–S transition. These cells account for most of the non-growing, non-proliferating cells in the human body. Some cells such as epidermal cells leave the cycle during G 2 and arrest without growth or proliferation

It is difficult to study cell proliferation in intact multicellular animals; therefore, most studies of cell cycle control are performed on cells proliferating in culture.

The tissue culture studies on the cell cycle contribute to our understanding of cancer development at a cellular level. Insights into the regulation of cell growth and proliferation in mammals have been provided by studies of cell lines and transformed cancer cells in cell culture.