

**Molecular Cell Biology**

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**Module 6**

**Cell Division, Cell Cycle, Cell Growth and Differentiation, Programmed  
Cell Death**

**Lecture 3**

**Cell Growth and Differentiation**

## **Cell Growth**

There are different phases of cell growth in multicellular organisms

- Cell division
- Cell enlargement
- Cell differentiation

### **What is growth?**

- Cell growth is the phenomenon by which cells increase their weight/mass and size.
- Animal cells are  $\approx$ 10 to 20  $\mu\text{m}$  in diameter with a lot of variation.
- red blood cells ( $\approx$ 5  $\mu\text{m}$  in diameter).
- motor neurons can grow 100's of micrometers in length.
- $\approx$ 70% of the weight of a cell is water
- Nucleic acids, proteins, polysaccharides, and lipids ( $\approx$ 25%)
- trace amounts of ions and small molecules
- Animal cellular dry mass is from proteins, which makes up about 18% of the total cell weight
- physical, chemical, and biologic factors can affect cell size.
- In some cells, persistent DNA replication in the absence of cell division (called endoreplication) can increase their cell size.
- Example: Megakaryoblasts, which mature into granular megakaryocytes
- This is found not only in animals but also in plants, and single-celled organisms.
- Adipocytes can grow to  $\approx$ 85 to 120  $\mu\text{m}$  by accumulating intracellular lipids.
- However, neurons and cardiac muscle cells, cease dividing and grow without increasing their DNA content.
- These cells proportionately increase their macromolecule content.

## **Cell Size**

- Cell size is a fundamental feature that contributes to function in multicellular organisms and to fitness in the context of unicellular organisms.
- Size imposes constraints on cellular design
- When cells grow larger, passive diffusion may limit intracellular transport and the decreased surface area to volume ratio may make nutrient uptake limiting for cell growth
- Cell size homeostasis in proliferating cells requires a coordination of growth with division, such that on average each cell division is accompanied by a doubling in cell mass
- In post-mitotic cells, such as neurons, the maintenance of cell size requires that no net cell growth occurs

## **Cell volume**

- Cell volume increases with ploidy as observed in a wide variety of eukaryotic cells from yeast to mice.
- Increased ploidy may be increasing nuclear volume, chromatin content, or the expression of unknown genes
- Ploidy increases are needed to prevent genomic DNA from becoming a limiting factor for cell growth.
- Increased ploidy helps to create specialized cell types and to pattern tissues, such as the larval salivary gland in *Drosophila*, and muscle fibers, megakaryocytes, and giant trophoblast cells in mammals

## **Nucleo-cytoplasmic ratio**

- The nucleo-cytoplasmic ratio plays a critical role in metazoan embryonic development
- In many animals, fertilization is followed by a series of rapid and synchronous cleavage divisions that section the huge zygote into thousands of smaller cells.
- After a certain number of divisions cell cycle times lengthen and become asynchronous

- This mid-blastula transition occurs when cells reach a particular nucleo-cytoplasmic ratio.
- Thus, haploid embryos compensate for their decreased nucleo-cytoplasmic ratio by going through exactly one extra cleavage division
- Similarly, artificially increasing the nucleo-cytoplasmic ratio results in fewer cleavage divisions.

### **What is cell division?**

- Cell growth and cell division can be distinguished in dividing cells.
- In mammalian cells, growth occurs in the G<sub>1</sub> phase of the cell cycle and is tightly coordinated with S-phase (DNA synthesis) and M phase (mitosis).
- Growth factors, hormones, and nutrients are the external cues for cells to grow.
- It is hypothesized that once a threshold cell size is attained, cells irreversibly commit to at least one round of division, thus achieving adequate size is a prerequisite for DNA synthesis and mitosis.
- Deprivation of nutrients and other growth signals, as might be the case in the nutrient-, and oxygen-, starved regions of an advancing tumor, may encourage normal cells to exit the cell cycle into a resting or G<sub>0</sub> state.
- Mutations resulting in deregulation of a cell's ability to sense nutrients or growth factors may thus provide tumor cells with a selective growth advantage.

### **Cell growth and the cell cycle**

- The dependency of cell cycle progression on growth
- The dependency of growth on cell cycle progression,
- The coordinate control of growth and cell cycle progression,
- or the complete intertwining of growth and cell cycle progression are important for this regulation
- Blocking cell growth in eukaryotes by nutrient or growth factor deprivation results in a cell cycle arrest, usually in G<sub>1</sub> phase
- Nutrient deprivation or treatment with translation inhibitors leads to a lengthening of the cell cycle in G<sub>1</sub> phase

- Similarly, abundant nutrients or overactivation of growth regulatory pathways can typically shorten the length of the G<sub>1</sub> phase

## Cell Differentiation

### Some terms first

- The fate of a cell is a description about what happens to it during normal development.
- Fate map is a diagram of an organism at an early stage of development that indicates the fate of each cell or region at a later stage of development.
- The developmental potential, or potency, of a cell describes the range of different cell types it can become.
- The zygote and its very early descendants are **totipotent** - these cells have the potential to develop into a complete organism.
- Totipotency is common in plants, but is uncommon in animals after the 2-4 cell stage.
- As development proceeds, the developmental potential of individual cells decreases until their fate is determined.

### Determination and Differentiation

- The **determination** of different cell types (cell fates) involves progressive restrictions in their developmental potentials.
- A cell obviously has many choices while undertaking changes say during development or other cellular processes/programs.
- When a cell “chooses” a particular fate, it is said to be determined, although it may not be very different morphologically from its neighbors.
- Determination implies a stable change - the fate of determined cells does not change.
- Differentiation follows determination, as the cell elaborates a cell-specific developmental program.
- Differentiation results in the presence of cell types that have clear-cut identities and functions, such as muscle cells, nerve cells, and skin cells.

## **Differentiation**

- A cell that is able to differentiate into all cell types of the adult organism is known as pluripotent.
- Such cells are called embryonic stem cells in animals and meristematic cells in higher plants.
- A cell that is able to differentiate into a total organism with all cell types, including the placental tissue, is known as totipotent.
- In mammals, only the zygote and subsequent blastomeres are totipotent, while in plants many differentiated cells can become totipotent
- For example cancer cells can be graded differently depending on their level of differentiation as poorly differentiated, moderately differentiated and well differentiated.

## **Cellular Differentiation**

- It is the process by which a cell acquires or develops certain properties and functions or capabilities and becomes a more specialized cell type
- Differentiation occurs when a simple zygote turns in to a complex system of tissues and cell types
- Adult stem cells divide and create fully differentiated daughter cells during tissue repair and during normal cell turnover
- Size, shape, membrane potential, metabolic activity, and responsiveness to signals of a cell change drastically during differentiation
- These changes are mostly because of highly controlled gene expression
- With a few exceptions, cellular differentiation almost never involves a change in the DNA sequence itself
- Thus, different cells can have very different physical characteristics despite having the same genome.

### **No difference in DNA content between cells**

- In a living organism, each one of its cells contains the same DNA
- However, we all know that we have many different types of cells and tissues

### **How to account for this?**

- The answer lies in the way the DNA is expressed in a given type of cell/tissue
- Specific combination of genes turned on (expressed) or turned off (repressed) determines cellular morphology (shape) and function.
- That is why a liver cell is distinct in its shape and function from those of a muscle or brain cell.
- This process of gene expression is regulated by cues/signals from both within and outside cells, and the interplay between these cues and the genome affects essentially all processes that occur during embryonic development and adult life
- How can we say for sure that all of the cells within a multicellular organism contain the same genome?
- As you know a single cell (zygote) with a half-genome from each parent is the starting point for our genesis
- This single cell quickly divides and new cells begin to differentiate, or become different from each other
- This process of differentiation occurs in a wide variety of cell types (e.g., skin, liver, muscle, etc.)

### **No difference in DNA content between cells- Evidences**

- This is not accompanied by any permanent loss of genetic material.
- If they get the right environment, fully differentiated cell types are also capable of producing the whole organism/animal.
- This was first shown by transplanting the nucleus of an adult frog skin cell into an enucleated donor embryo, leading to the development of a cloned adult frog
- Later, the intact complete genome of a differentiated cell was used in the cloning of the famous sheep Dolly, showing that in mammals genes are not lost during development, so they must therefore be regulated.

- Hence we now know that the specialized, differentiated cell types of the adult body contain a genome as complete as that of an embryo.
- Thus changes in gene expression, rather than losses of genetic material, play a key role in guiding and maintaining cell differentiation.
- Differential gene expression is not a result of differential loss of the genetic material.
- That is, genetic information is not lost as cells become determined and begin to differentiate.
- In fact, even the nuclei of adult cells contain all of the information needed for the construction of an entire organism, if provided with the proper cytoplasmic components.
- The cloning of Dolly from an adult cell is one of the best convincing evidences available in support of differential gene expression.

### **Dedifferentiation and Redifferentiation**

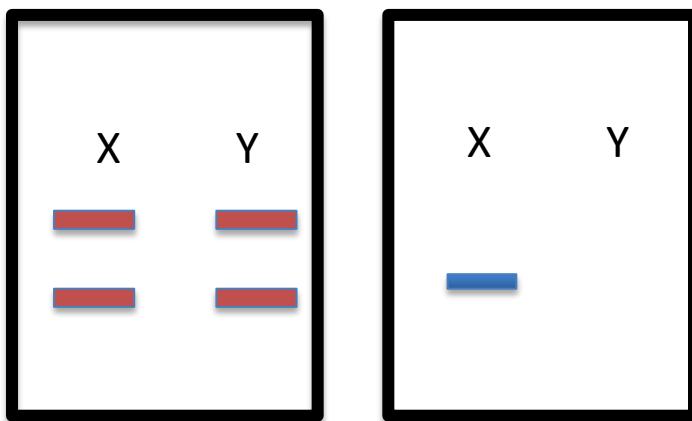
- Dedifferentiation is a cellular process that often occurs in worms and amphibians
- In this process a cell reverts back to its original or some earlier developmental stage from a partially differentiated or terminally differentiated condition.
- Dedifferentiation also occurs in plants.
- The fully differentiated cells sometimes lose some of their properties such as a change in shape or protein expression etc
- Dedifferentiation may be considered as an aberration of the normal development cycle that results in cancer
- It can also be a part of immune response lost during evolution
- Chemicals such as Reversine can induce dedifferentiation in myotubes
- Such dedifferentiated cells are sometimes able to **redifferentiate** into osteoblasts and adipocytes

## Gene expression regulates differentiation

- Gene expression is regulated by factors both extrinsic and intrinsic to the cell.
- Cell-extrinsic factors include environmental cues, such as small molecules, secreted proteins, temperature and oxygen.
- Intrinsic factors include growth factors, morphogens, cytokines or signaling molecules
- Signaling causes changes in transcription or expression of genes that may include turning off or on of some genes. This can influence the cell fate and cell functions.
- In addition, gene expression changes can lead to changes in an entire organism, such as molting in insects.

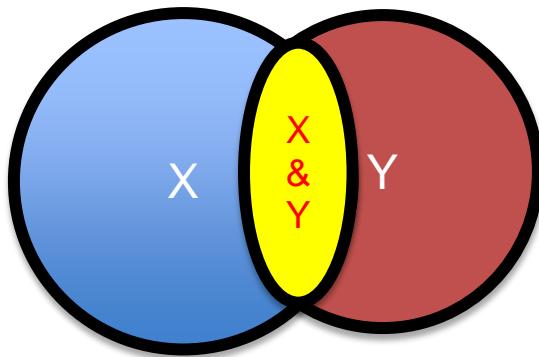
## Gene expression differs between two cell types

Southern blot      Northern blot



X and Y are two cell types that contain the same DNA but RNA expression differs

**Two cell types have unique as well as shared gene expression**



Cell types X and Y share house keeping genes and also have unique set of genes

#### **Gene expression regulates differentiation**

- DNA can be modified in a way that affects gene expression without any changes in its order of nucleotide sequence (epigenetic change).
- Chromatin modification can affect gene expression by changing the accessibility of genes to transcription factors, in either a positive or a negative manner.
- Two major classes of such chemical modifications include DNA methylation and histone modification (methylation and/or acetylation).

#### **Chromatin modifications modulate differentiation**

- Changes in chromatin modification play an important role in regulating gene expression during developmental cell-type specification as well.
- For example, chromatin-modifying proteins play an essential role in muscle cell differentiation via interactions with key muscle-promoting transcription factors MyoD and MEF.
- This example provides evidence for a link among chromatin modifications, transcription factors, and, ultimately, cell-fate-specific changes in gene expression.
- Chromatin can become mismodified in certain cancers.

- Moreover, chromatin modifications are usually erased and reset during the production of gametes, such that the adult program of intrinsic cues is replaced with a program more suited to embryonic development.

### **Chromatin modifications in embryonic cells**

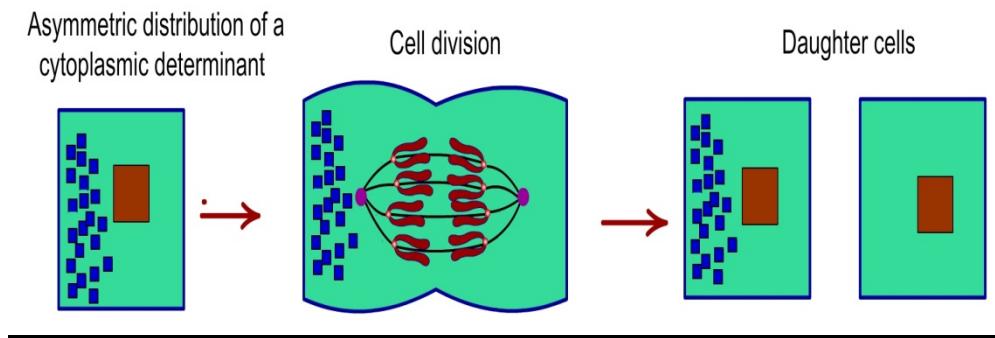
- Embryonic cell types contain a specific set of chromatin modifications and this is different in adult cell types.
- A repressive transcription factor can be inactivated by chromatin modification.
- Alternatively, chromatin modifications (especially histone acetylation) can cause the DNA to become open and, therefore, more accessible to the transcription.
- Embryonic cell types have been found to contain chromatin modifications with both silencing and promoting characteristics.
- These bivalent (two-directional) markers assist the embryonic cells to choose to remain embryonic (as in an embryonic stem cell) or to differentiate (as in normal development).
- Thus cell-cell signaling and epigenetic changes together can dictate the course of cell differentiation.

### **Role of other chromatin proteins**

- Apart from the histones themselves, other chromatin proteins have been implicated in the maintenance of expression states during development.
- One such class of proteins, is called the Polycomb group
- Their role in maintaining gene expression patterns in body segments of the fruit fly *Drosophila* is well known
- Polycomb group complexes in flies have histone methyltransferase activity
- Roles for specific plant Polycomb group proteins in leaf, flower and seed development, as well as in floral induction are being identified

## Asymmetric segregation of cellular determinants

- Asymmetric segregation of cellular determinants is based on the asymmetric localization of cytoplasmic molecules (usually proteins or mRNAs) within a cell before it divides
- During cell division, one daughter cell receives most or all of the localized molecules, while the other daughter cell receives less (or none) of these molecules
- This results in two different daughter cells, which then take on different cell fates based on differences in gene expression such as the asymmetrically expressed maternal cytoplasmic determinants
- The localized cytoplasmic determinants are often mRNAs encoding transcription factors, or the transcription factors themselves
- The distinct cytoplasm inherited will have a distinct pattern of differentiation.



## Signals and gene expression

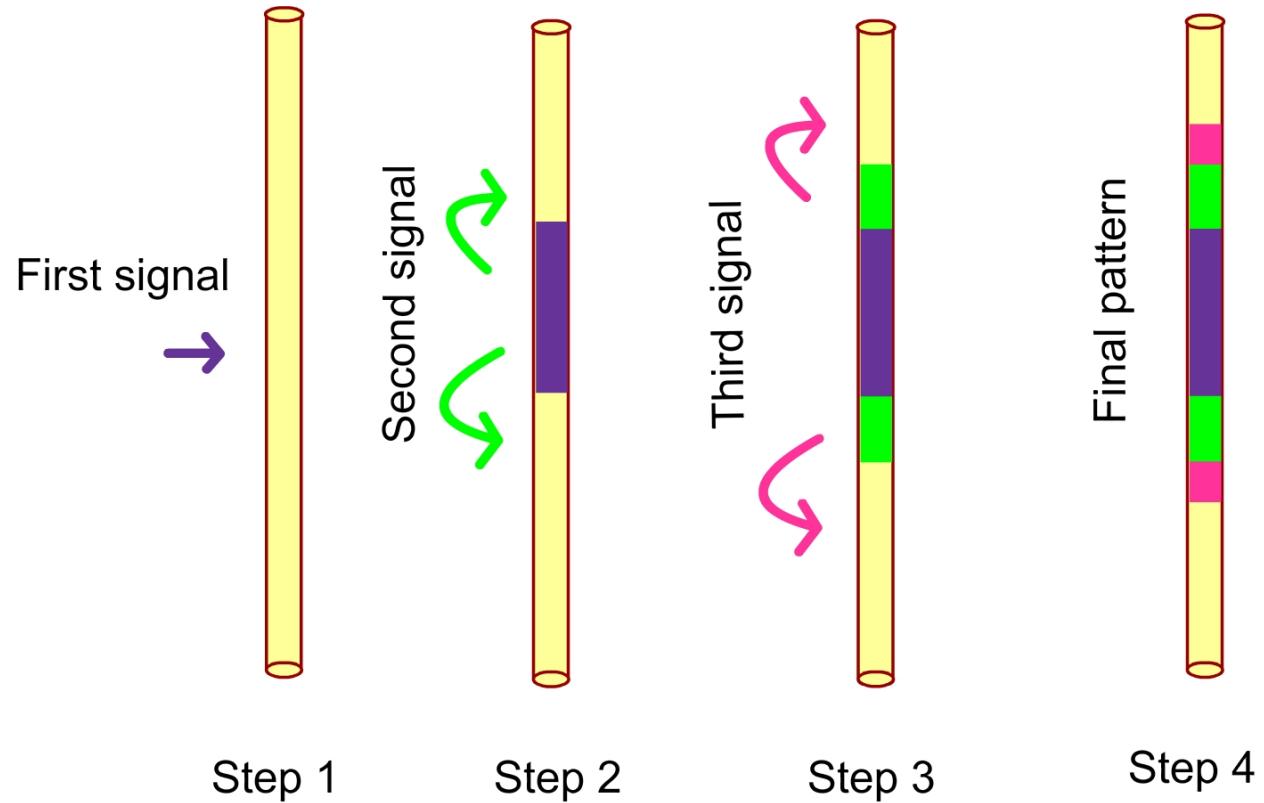
- Cells become different from one another as a result of inductive signals coming either from other cells or from their external environment.
- There are three mechanisms by which signals can be passed between cells.
- In the first mechanism, a diffusible signal is sent through the extracellular space, and is received by a cell-surface receptor, which further transmits the signals by way of second messengers.

- In the second mechanism, cells directly contact each other through transmembrane proteins located on their surfaces.
- In the third mechanism, the cytoplasm of two cells is connected through gap junctions, allowing the signal to pass directly from one cell to another cell. In plants, direct connections between cells are called plasmodesmata

### **Pattern formation**

- Pattern formation concerns the processes by which cells acquire positional information
- There are two general models for how patterns form: use of a **morphogen gradient**, and **sequential induction**.
- The **morphogen gradient** model involves the production and release of a diffusible chemical signal called a **morphogen**.
- Morphogen release creates a concentration gradient, with high concentrations of morphogen close to the source, and low concentrations farther away from the source.
- Exposure to different threshold levels of morphogen leads to different cell fates.
- In this way, different amounts of **one** chemical signal can create a complex pattern.

## Pattern formation



## **Study Questions**

1. Bring out the differences between differentiation and dedifferentiation.
2. What is pattern formation?
3. Gene expression is modified by
  - a) ATP
  - b) DNA methylation
  - c) DNA phosphorylation
  - d) None of these
4. Match the following

Northern blot	Totipotent
Southern blot	Diploid
Zygote	RNA
Plant cells	DNA

5. DNA replication in the absence of cell division is called -----.