Animal Tissue Culture

SQG 3242

Biology of Cultured Cells

Dr. Siti Pauliena Mohd Bohari

The Culture Environment

- Changes of Cell's microenvironment needed that favor the spreading, migration, and proliferation of unspecialized progenitor cells rather than the expression of differentiated functions.
- Cell – cell interaction and cell –matrix interaction are reduced because the cells lack the heterogeneity and three dimensional architecture found *in vivo*
- Many hormonal and nutritional stimuli are absent
- Influences of environment on the culture is expressed via 5 routes:
  - The nature of substrate
  - Degree of contact with other cells
  - Physicochemical and physiological constitution of medium
  - The constitution of gas phase
  - The incubation temperature

Cell adhesion

- Most cell from solid tissue grow as adherence monolayer.
- Thy need to attach and spread out on the substrate before start to proliferate.
  - Glass-slight negative charge
  - Plastic- treated with an electric ion discharge
- Cell adhesion mediated by specific sell surface receptor for molecules in the extracellular matrix

Cell Adhesion molecule

Three major classes of transmembrane proteins:

- CAMs(Ca2+ independent) and cadherin (Ca2+ dependent) involved in interaction between homologous cells.
- Intergrin – Receptor for matrix molecules such as fibronectin, entactin, laminin, and collagen, which bind to them via a specific motif usually containing arginine-glycine-aspartic acid (RGD).
- Transmembrane proteoglycans-interact with matrix constituent such as other proteoclycans or collagen, but not via RGD motif.

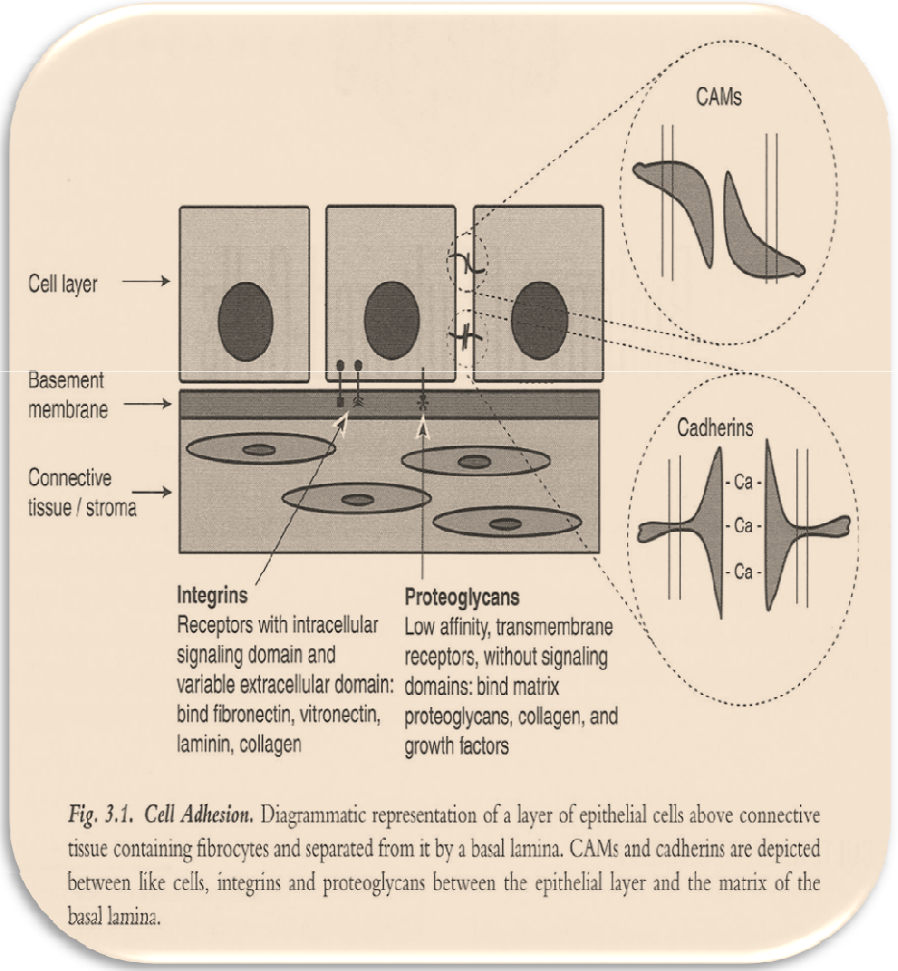


Fig. 3.1. Cell Adhesion. Diagrammatic representation of a layer of epithelial cells above connective tissue containing fibrocytes and separated from it by a basal lamina. CAMs and cadherins are depicted between like cells, integrins and proteoglycans between the epithelial layer and the matrix of the basal lamina.

Intercellular junctions

- The role of the junction varies between mechanical such as
  - Desmosomes and adherens between junctions, which hold epithelial cells together
  - Tight junction, which seal he space between cells.
  - Gaps junction which allow ions, nutrients, and small signaling molecules.
- As epithelial cell differentiate in confluent culture they form an increasing number of desmosomes and if some morphological organization occurs, can form junctional complexes - difficult to disaggregate.
- As many of the adhesion molecule within this these junctions depend on Ca2+ ions, chelating agent as EDTA is often in trypsin added before disaggregation.

Extracellular matrix

- Intercellular spaces in tissues are filled with extracellular matrix(ECM)
- The constituent of ECM depends on the cell types.
- The complexity of ECM is a significant component in the phenotypic expression of the cells attached to it
- ECM control its composition and, in turn the composition of the ECM regulates the cell phenotype.
- Cultured cell line are allowed to generate their own ECM, but primary culture and propagation of some specialized cells, and the induction of their differentiation, may require exogenous provision of ECM.

- Two components of interaction of ECM:
  - Adhesion-to allow attachment and spreading that are necessary for cell proliferation.
  - Specific interaction-reminiscent of the interaction of an apithelial cell with basement membrane, with other ECM constituents or with adjecent tissue

- The use of ECM constituent can be highly beneficial in enhancing cell survival, proliferation, or differentiation but unless recombinant molecules are used.

Components of ECM ???

Cytoskeleton

- Cell adhesion molecules are attached to element of cytoskeleton.
- The attachment of integrins to actin microfilaments via linker proteins is associated with reciprocal signaling between cell surface and the nucleus.
- Cadherin can also link to the actin cytoskeleton in adherens junctions, mediating changes in cell shape.
- Demosomes, which also employ cadherins, link to the intermediate filament via an intracellular plaque.
- Intermediate filament are specific to cell lineages and can be used to characterized them.
- The microtubules are the 3rd component of cytoskeleton
  - Function: Cell motility and intracellular trafficking of micro-organelles

Cell motility

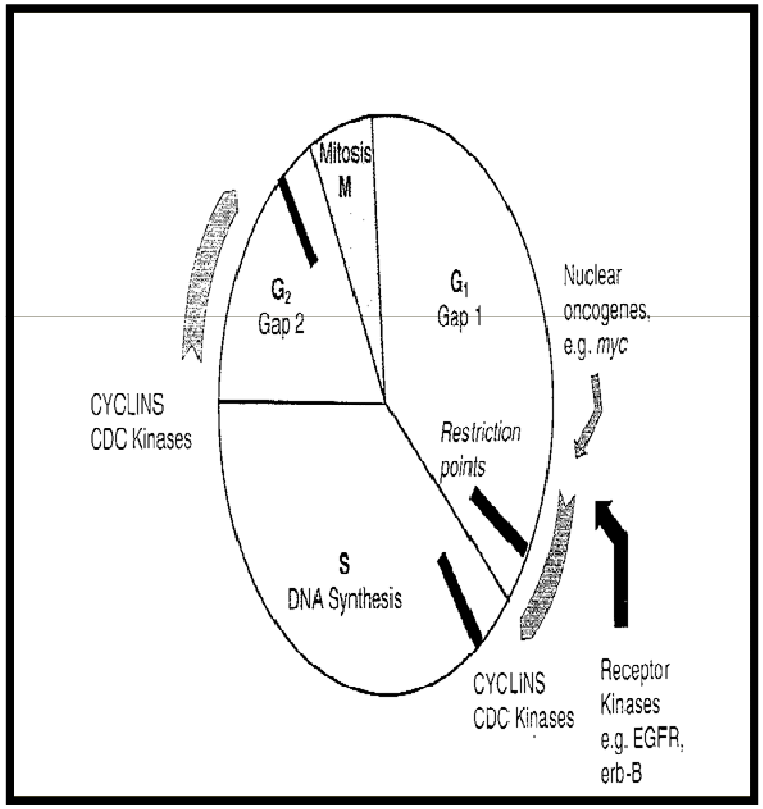
- Culture cells are capable of movement on a substrate.
- The most motile are fibroblast at a low cell density when cell are not in contact
- The least motile are dense epithelial monolayers.

Cell proliferation

- Cell cycle

•A process of division allowing the duplication of cells a cycle: end products (daughter cells) are the same as starting products (mother cells)

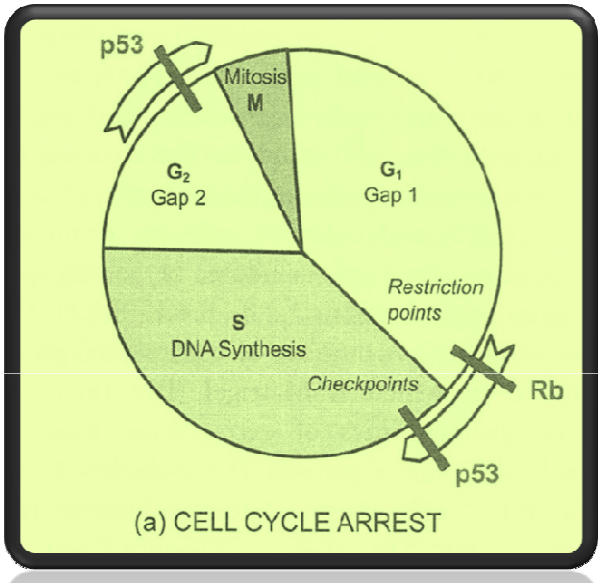
•Cell cycle is made up of four phases
1.Interphase (longest part of the cell cycle)
2.G1 (Growth 1)
3.S (Synthesis) - DNA copies
4.G2 (Growth 2) - cell prepares for division



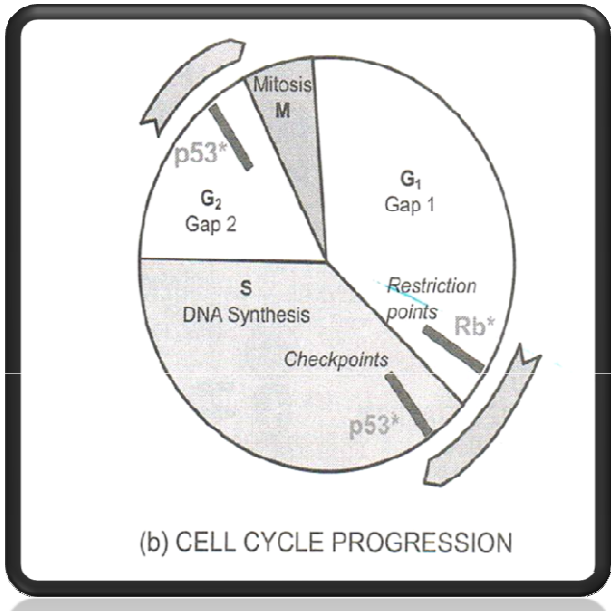
Control of cell proliferation

- Entry into the cell cycle is regulated by signal from the environment.
- Low cell density- leaves cells with free edge and renders them capable of speading-permit their entry into cell cycle in the presence of mitogenic growth factors
- High cell density – inhibit the proliferation of normal cells. Initiate by cell contact and is accentuated by crowding and resultant change in shape of the cell and reduced spreading
- Intracellular control is mediated by positive acting factors such as the cyclins, which are up regulated by signal transduction cascades activated by phosphorylation intracellular domain of the receptor when it is bound to growth factor

- Negative acting factor such as p53, p16, or the Rb gene product block the cycle progression at restriction point or check point.
- Link between extracellular control element and intracellular effectors is made by cell membrane receptor and signal transduction pathway



The cell cycle is arrested at restriction points or checkpoint by the action of Rb,p53, and other cell cycle inhibitor



When these inhibitor gene are inactivated usually by phosphorylation, cell proceed round the cycle

Cell cycle inhibition and progression

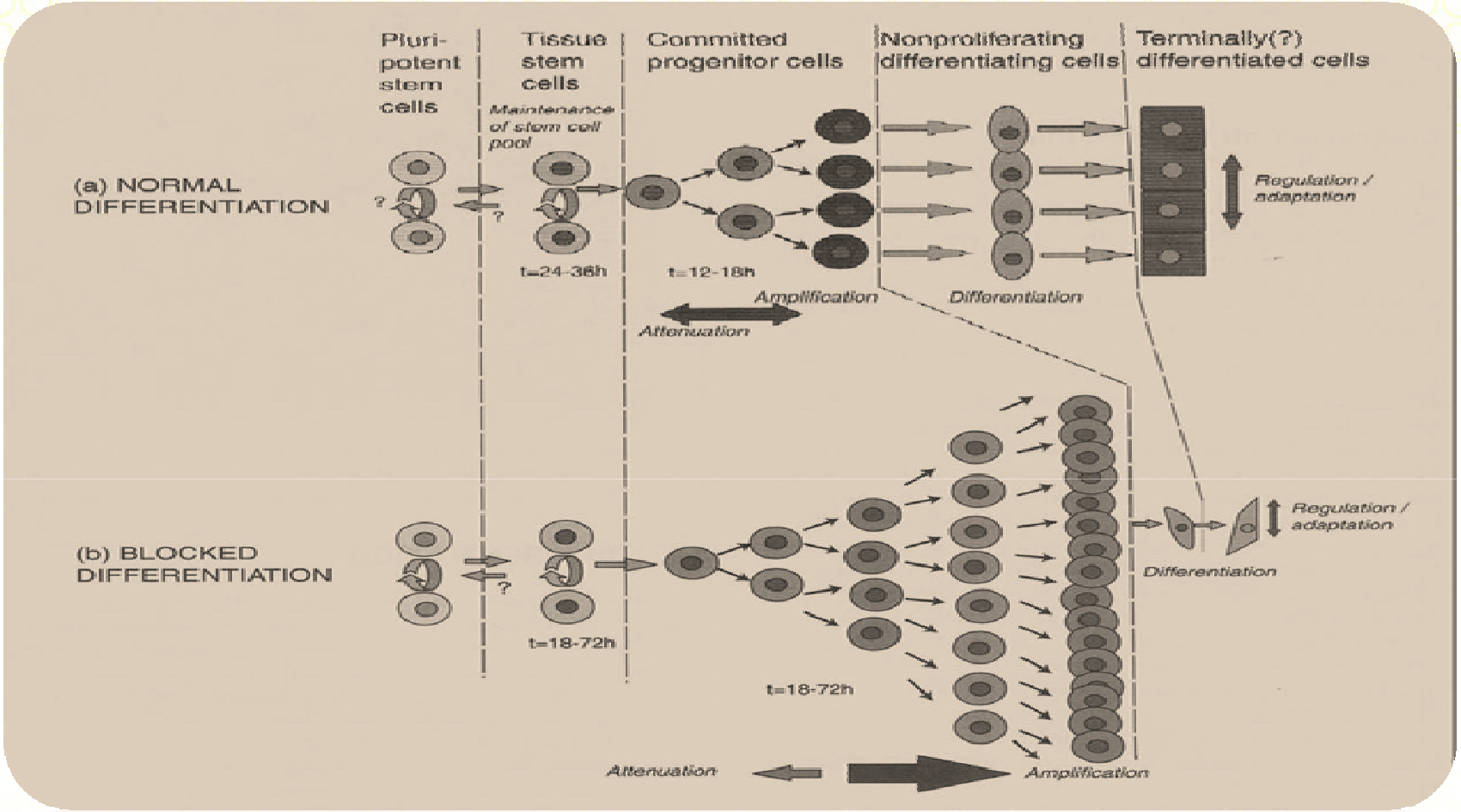
Differentiation

- Condition required or the induction of cell density:
  - A high cell density
  - Enhanced cell-cell and cell-matrix interaction
  - Presence of various differentiation factor

- If cell differentiation is required, two condition need to be applied
  - Optimize cell proliferation
  - Optimize cell differentiation

Maintained cell differentiation

- Normally specific cell functions are retained longer when the 3 dimensional structure o the tissue is retained, as in organ culture.
- Organ culture can not be propagate, must be prepared *de novo.*
- *Matrigel* –reproduce characteristic of extracellular matrix but are undefined
- Expression of the differentiated phenotype may also required maintenance in the appropriate selective medium with appropriate soluble inducers and in the absent of serum.
- The development of normal tissue unction in culture would facilitate the investigation of pathological behavior.



Stem cells

Dedifferentiation

- The differentiated cells lose their specialized properties in vitro but it is often unclear whether
  - Wrong lineage of cells is selected in vitro
  - Undifferentiated cells of the same lineage over grow terminally differentiated cells of reduced proliferative capacity
  - The absence of appropriate inducers (hormone cell or matrix interaction) causes an adaptive and potential reversible, loss of differentiated properties.
- Continuous proliferation will favor undifferentiated precursors.
- Dedifferentiation implies that the specialized properties of the cell are lost by conversion to a more primitive phenotype.
- Deadaptation implies that the synthesis of specific product or other aspect of specialized function are under regulatory control by hormone, cell-cell interaction, and cell-matrix interaction

Cell signaling

- Signal that reach the cell from another tissue via the systemic vasculature are called endocrine and those that diffuse from adjacent cells without entering the bloodstream are called paracrine.
- Signal that arise in a cell type different from the responding cells are heterotypic paracrine
- A cell can also generate its own signaling factor that bind to its own receptor called autocrine signaling.
- Under normal condition with basal media in vitro, only autocrine and homocrine signaling will occur
- The failure of many culture to plate with a high efficiency at low cell densities may be due in part, to the dilution of one or more autocrine and homocrine factor.

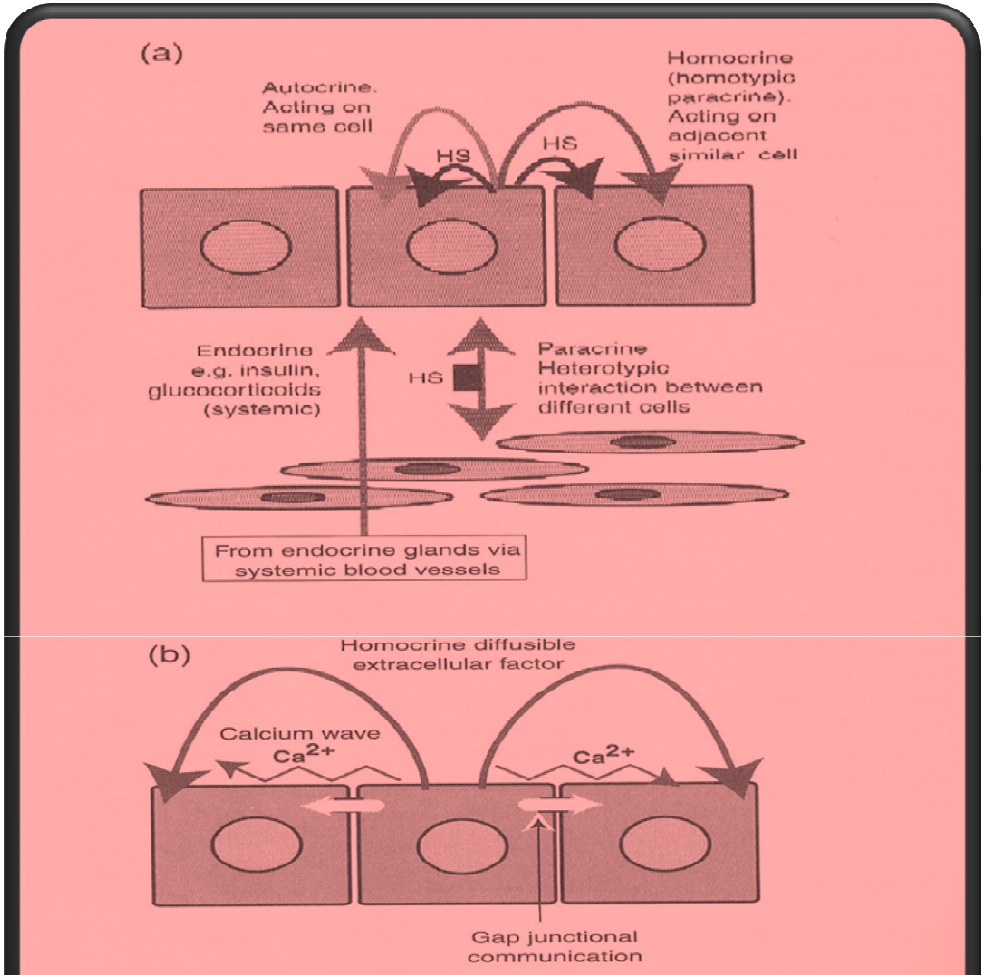


Fig. 3.7. Cell Interaction and Signaling. Routes of interaction among cells. (a) Factors influencing the behavior of a cell include endocrine hormones from the vasculature, paracrine factors from the stroma, homocrine factors from adjacent similar cells, and autocrine factors from the cell itself. Matrix, soluble, and cell-associated heparan sulfate (HS) may help the activation, stabilization, and/or translocation of paracrine factors. (b) Uniformity of response in target tissue is improved by gap junctional communication, by calcium signaling, and, possibly, by homocrine factors from the stimulated cell.

Energy Metabolism

- Carbon source-glucose for glycolysis, generating lactic acid as end product
- Normal culture-low O_2
- Absence of appropriate carrier such as hemoglobin, raising O_2 tension-generate radical species that are toxic to the cells
- This result in anaerobic conditions and use of glycolysis for energy metabolism.
- However, citric cycle remains active, and it has became apperent that amino acid can be utilized as carbon source by oxydation to glutamate.
- Deamination of glutamine tend to produce ammonia which is toxic and can limit cell growth,
- Use of dipeptides can minimize the production of ammonia and more stable in medium

References

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