Cell Division & Cell Linage II

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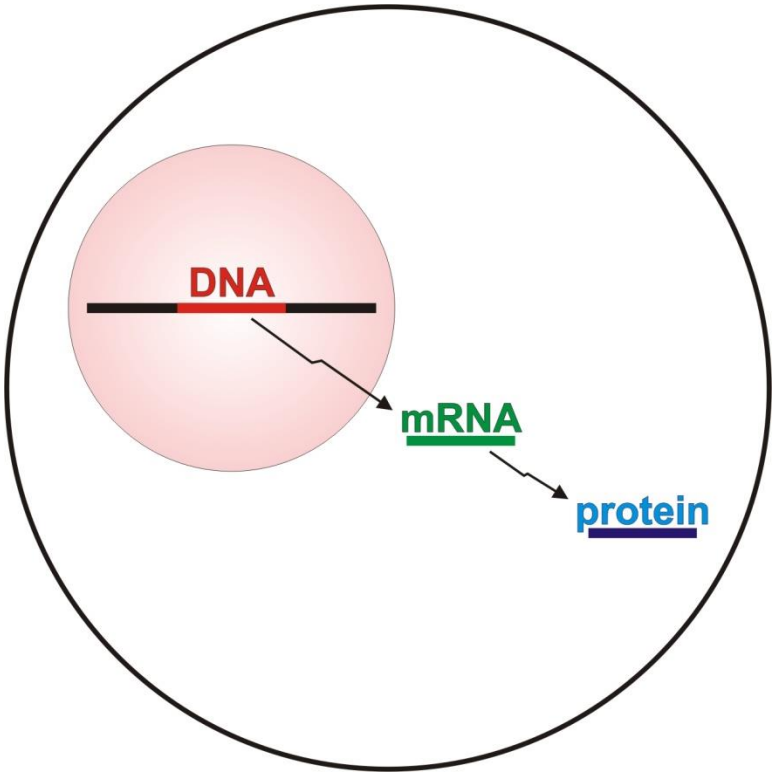
Course News

There is no class on Friday!

Methods for Detecting Gene Expression in the Developing Nervous System



Methods for Detecting Gene Expression in the Developing Nervous System

technique	*detects*	*key reagent*	*resolution*
immunohistochemistry	protein (and other molecules)	antibody	cellular
in situ hybridization	mRNA	cDNA probe	cellular
RT-PCR	mRNA	primers	tissue (or single cell)
northern blot	mRNA	cDNA probe	tissue
western (immuno) blot	protein	antibody	tissue
transgenic	promoter activity	reporter gene	cellular

Miscellaneous Facts Regarding Cell Division

- Differentiated neurons cannot divide.
- In warm-blooded vertebrates, most neurons are produced during development. In humans, most neuron production is complete by two years of age.
- There are isolated examples of continued neuron production throughout life in warm-blooded animals; e.g.
  - Subventricular zone of the mammalian forebrain
  - Subgranule zone of the dentate gyrus of the hippocampus in mammals
  - Olfactory receptor neurons in the nasal epithelium
  - Neurons in the song control nuclei in some species of song birds during Spring

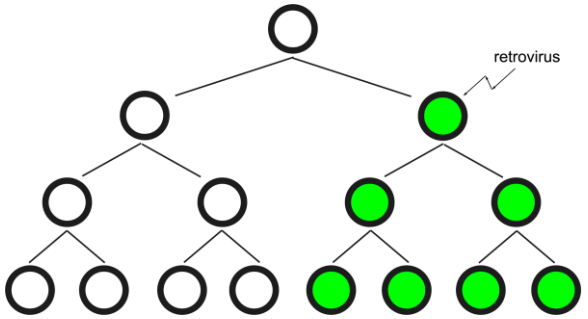
Miscellaneous Facts

- Many cold-blooded vertebrates exhibit continued growth of the nervous system including addition of new neurons throughout the life of the organism.
- Large-scale regeneration of lost neurons is not observed in warm-blooded vertebrates but is often possible in cold-blooded vertebrates.

Lineage Tracing

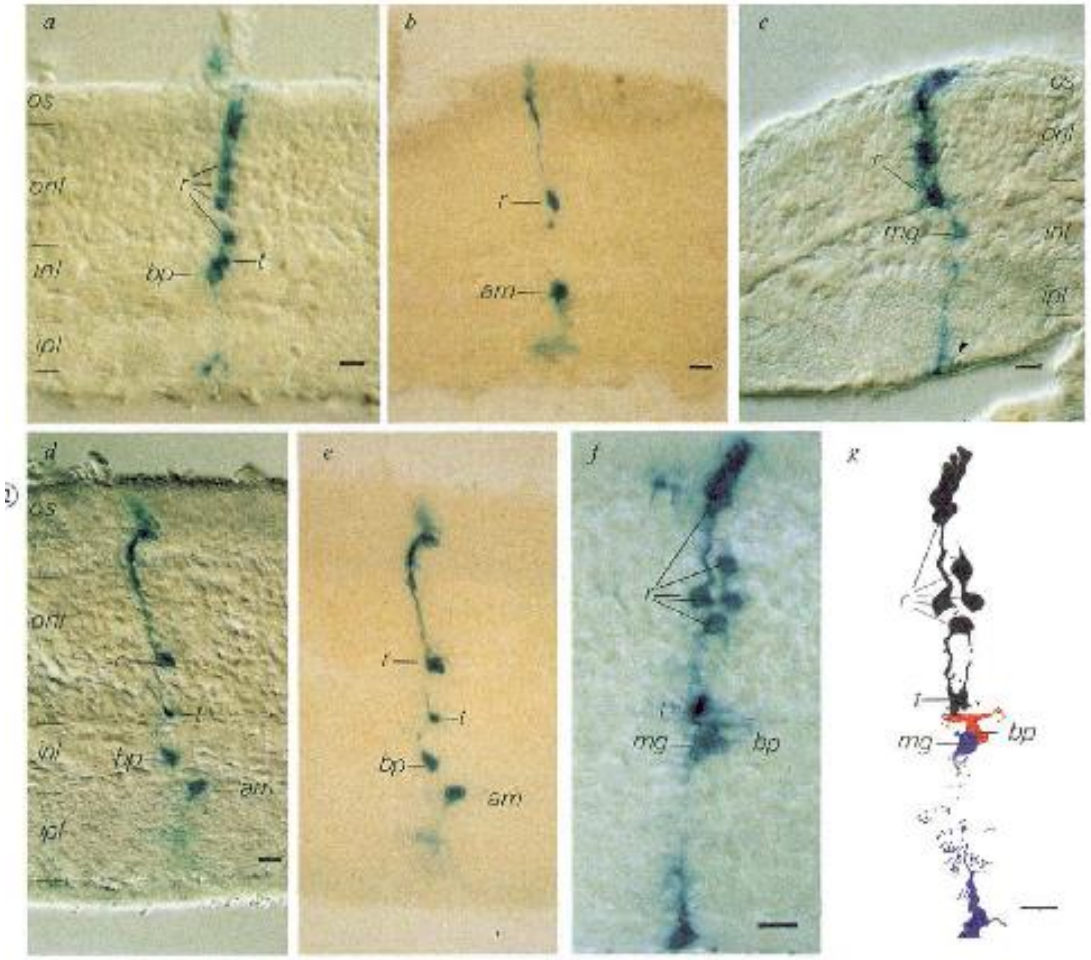
- Dividing cells can be stablely transfected with a reporter gene; the transgene will be inherited by all the progeny of that cell.

- The reporter gene is introduced with a retrovirus that infects dividing cells selectively.



Lineage Tracing

- Individual progenitor cells typically give rise to multiple cell types.

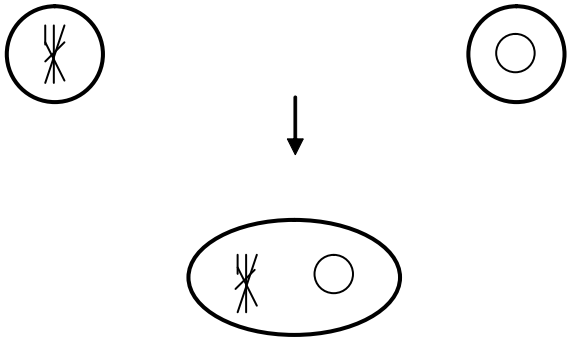


[Turner & Cepko (1987) Nature 328]

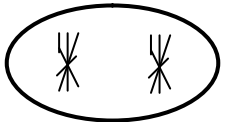
Regulation of the Cell Cycle

Mitosis Promoting Factor (MPF)

- Fuse an M-phase cell with a G_1, S or G_2-phase cell

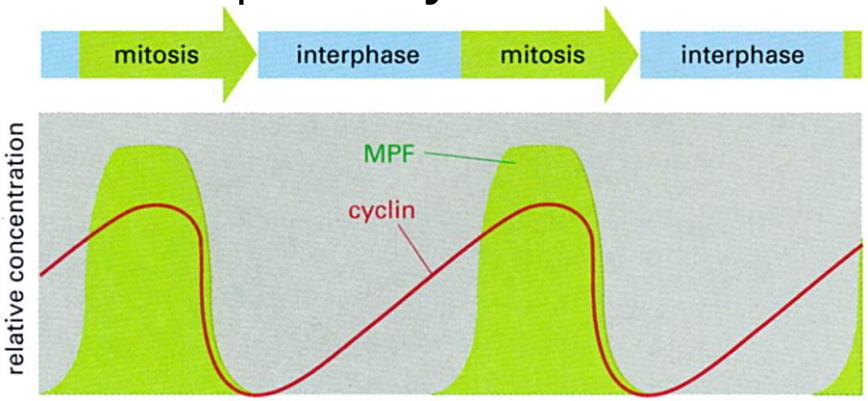


and the G_1, S or G_2-phase nucleus immediately enters M-phase.



Cyclin

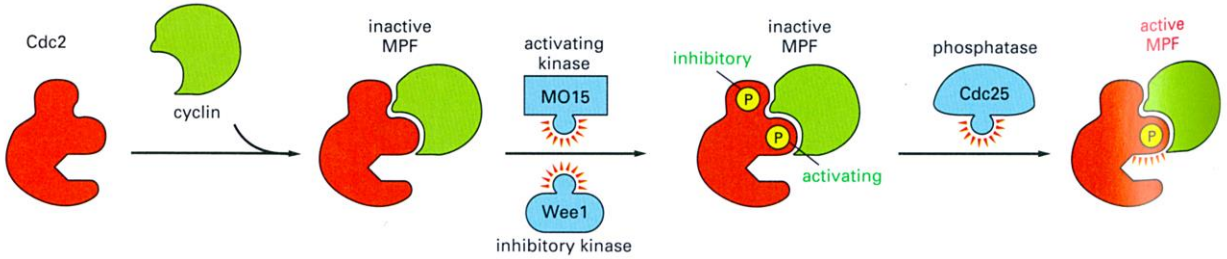
- MPF activity was found to correlate with the level of expression of the protein **cyclin**.



- Cyclin is required for M phase to begin, but cyclin alone does not initiate M phase.

Cyclin-Dependent Protein Kinase (cdk)

- MPF also contains a **cyclin-dependent kinase** (**cdk**).
- Cdk requires binding to cyclin to function.
- Its activity is also regulated by phosphorylation and dephosphorylation.



MPF Function

- Active MPF phosphorylates many cell components involved in M phase.

  e.g. Active MPF phosphorylates nuclear lamin, which results in its depolymerization, thus leading to breakdown of the nuclear membrane.

Cyclin Degredation

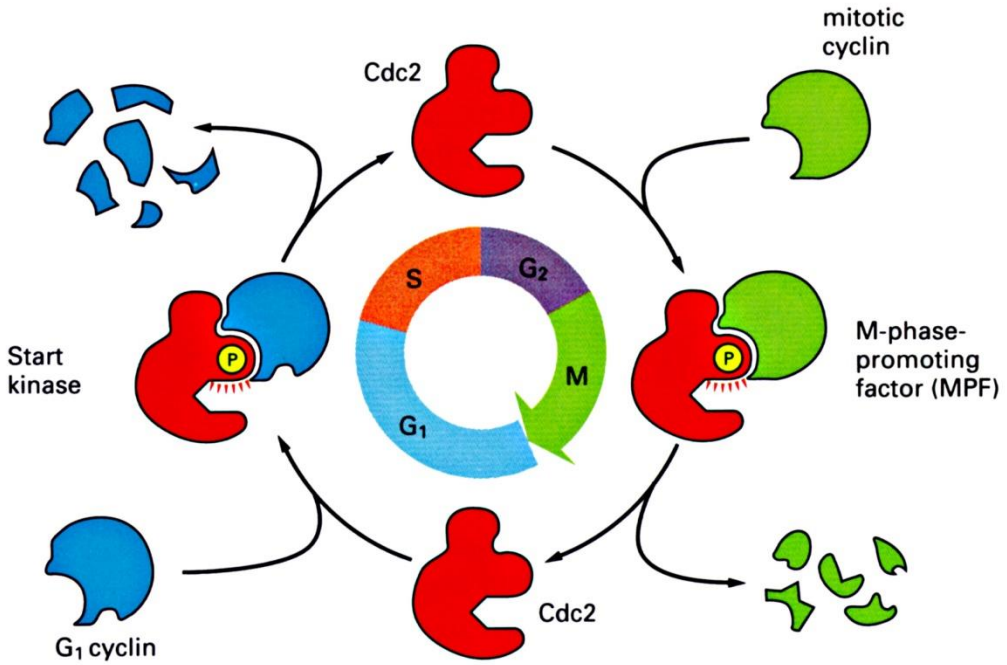
- Cyclin is destroyed by proteases at the metaphase-anaphase transition.

- A signal sequence in the cyclin protein is an attachment site for ubiquitin, which targets the protein for degredation.

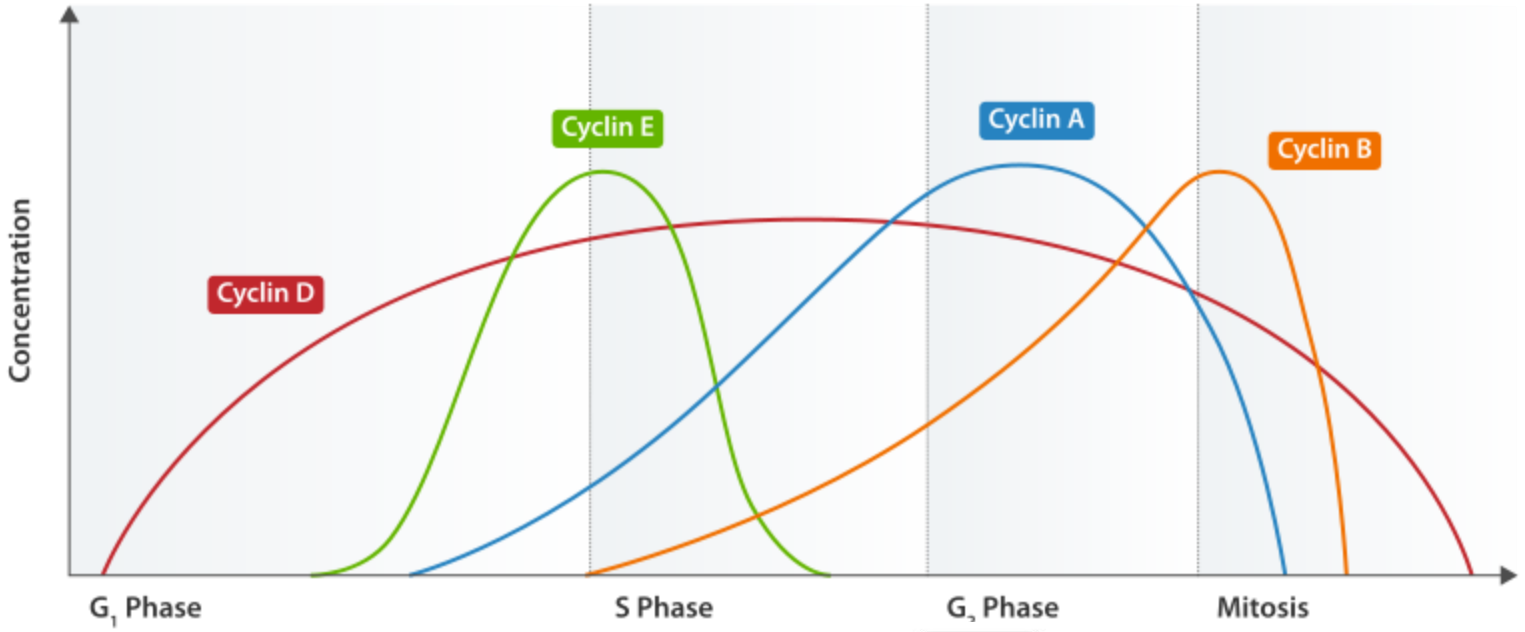
Yeasts have one kinase.

- Yeast have one kinase that regulates entry into both S- and M-phases but with different cyclins.

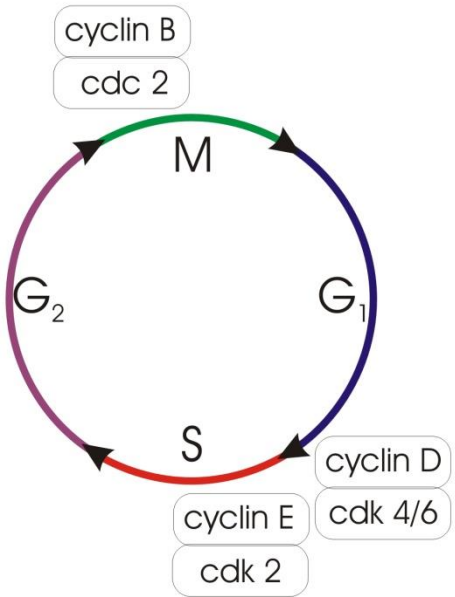
- The specific cyclin determines the substrates of the kinase.



Vertebrates have multiple kinases and cyclins that regulate each step of the cell cycle.



Different cyclins use different cdk's and have different substrates.



Tumor Suppressor Genes

- Tumor suppressor genes, such as **retinoblastoma protein (Rb)**, maintain cells in G_1 (G_0):

  - The Rb gene was found mutated in children with retinal tumors.

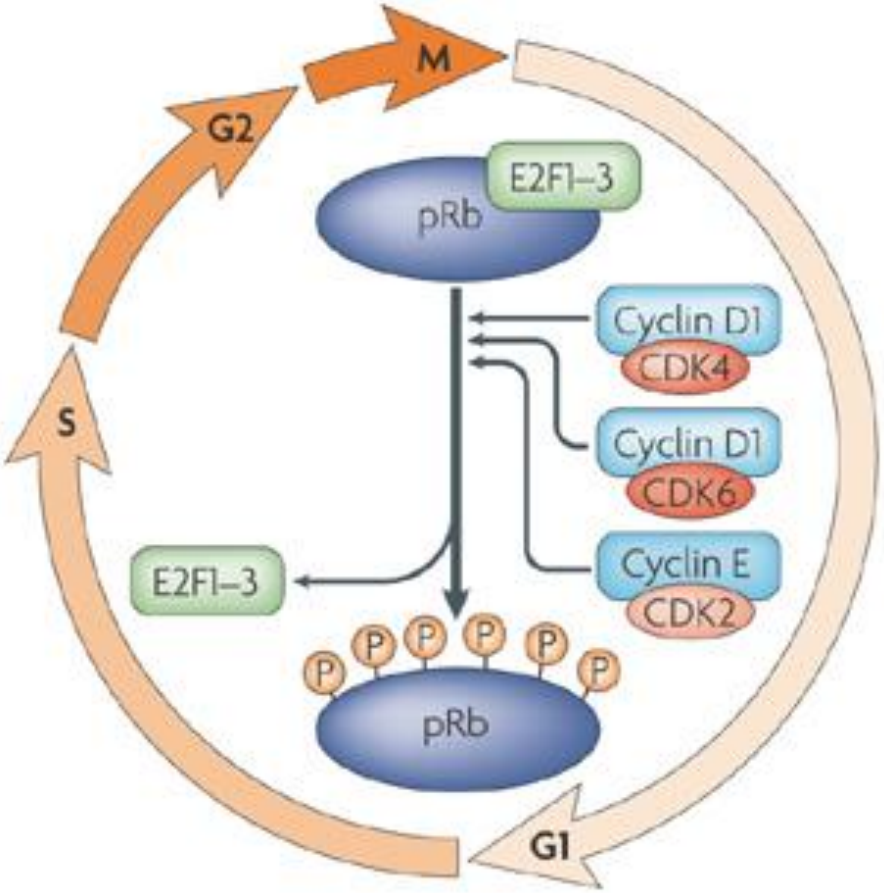
  - In its active (dephosphorylated) state, Rb inhibits E2F transcription factors, which are required for entry into S-phase.

  - Active cyclinD+cdk4/6 and cyclinE+cdk2 inactivates (phosphorylates) Rb.

  - E2F transcription factors promote expression of proteins needed for S-phase such as DNA polymerase. Activation of E2F is probably the 'restriction point'.

  - Rb is dephosphorylated during M-Phase.

Tumor Suppressor Genes



Growth Factors

- Most cell types require certain growth factors to divide; over 50 factors have been identified

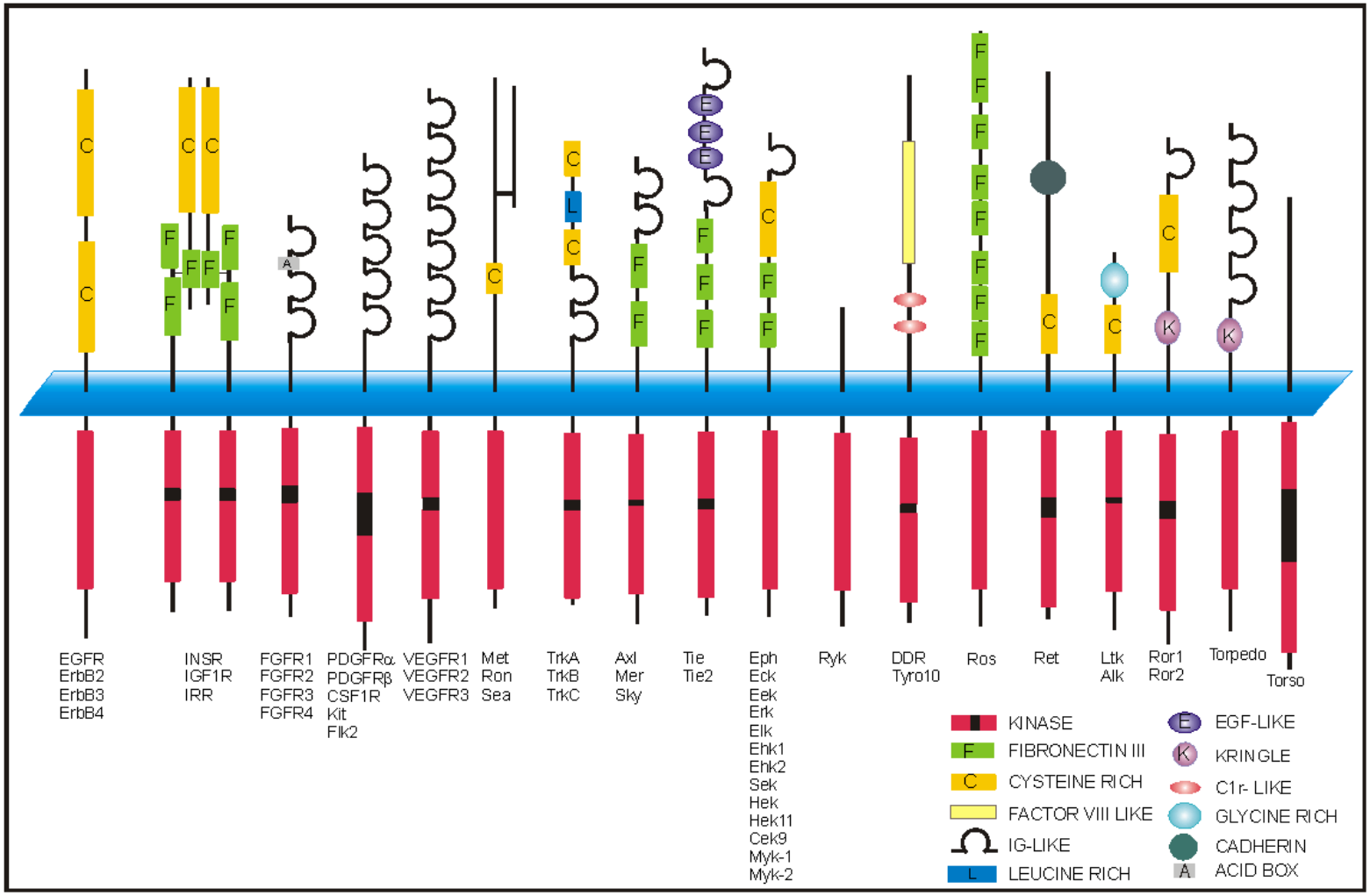
  e.g. The rate of division of 3T3 cells in culture is directly proportional to the amount of serum in the medium, which contains PDGF.

  3T3 cells divide in defined medium as long as insulin and epidermal growth factor (EGF) are present.

- Most neural progenitor cells divide in response to EGF or fibroblast growth factor (FGF).

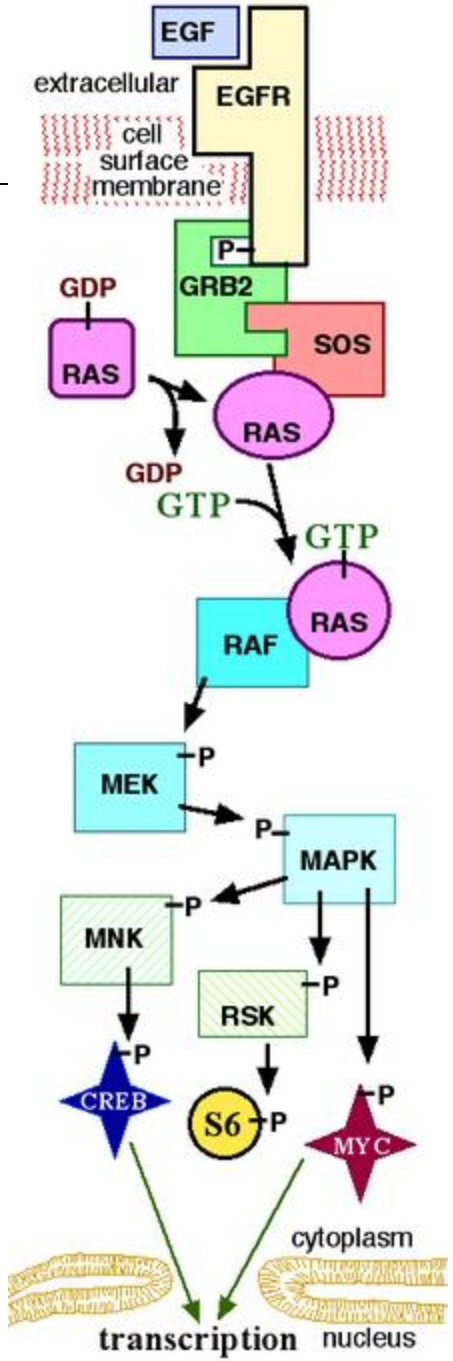
Growth Factors

- Growth factors activate specific receptors; many growth factor receptors are receptor tyrosine kinases (RTKs).

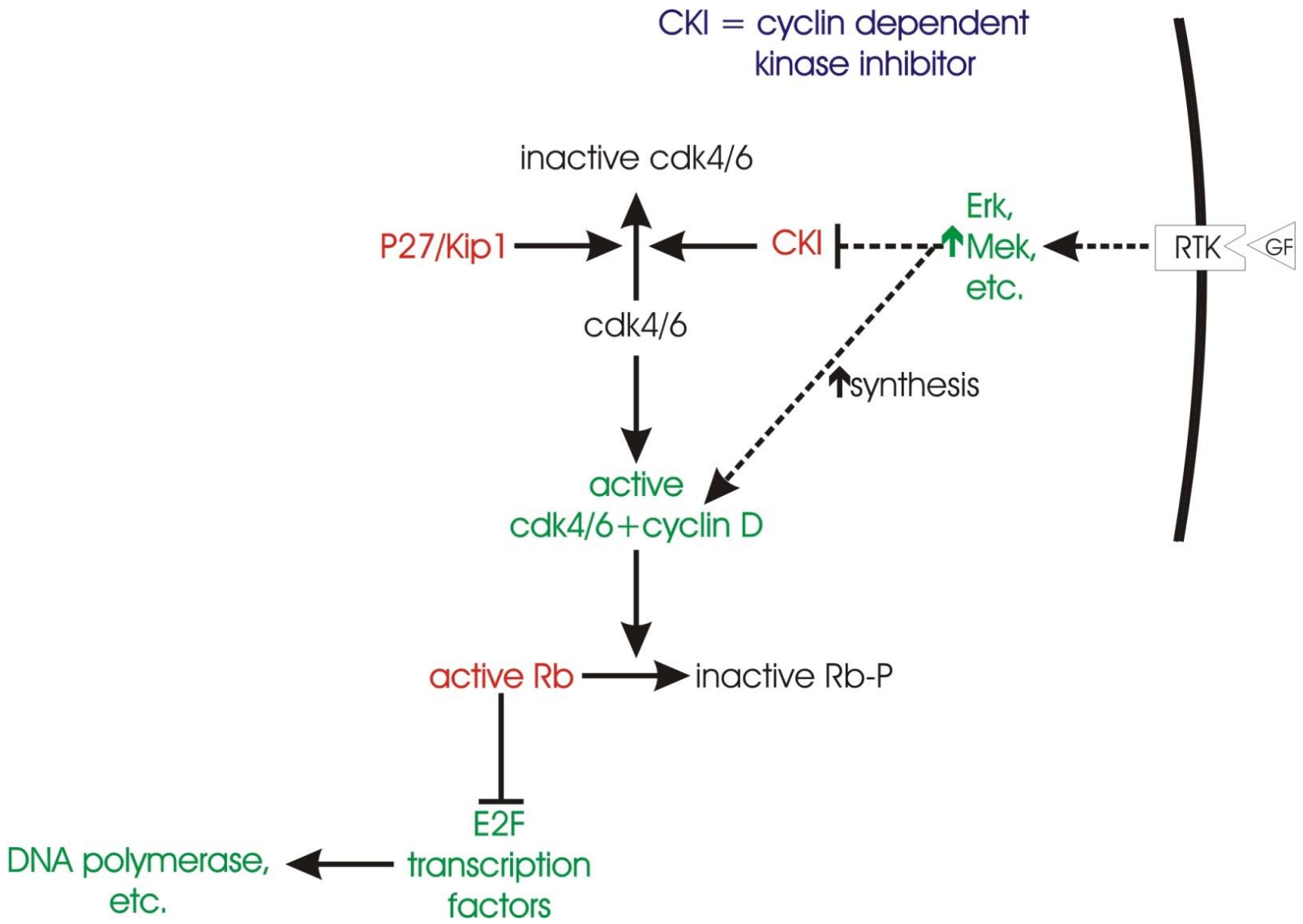


Growth Factors

- Ligand binding to growth factor receptors activates the MAPK (Erk) pathway, which leads to synthesis and activation of cyclin D, which in turn phosphorylates (inactivates) Rb.



Growth Factors



Growth Factors

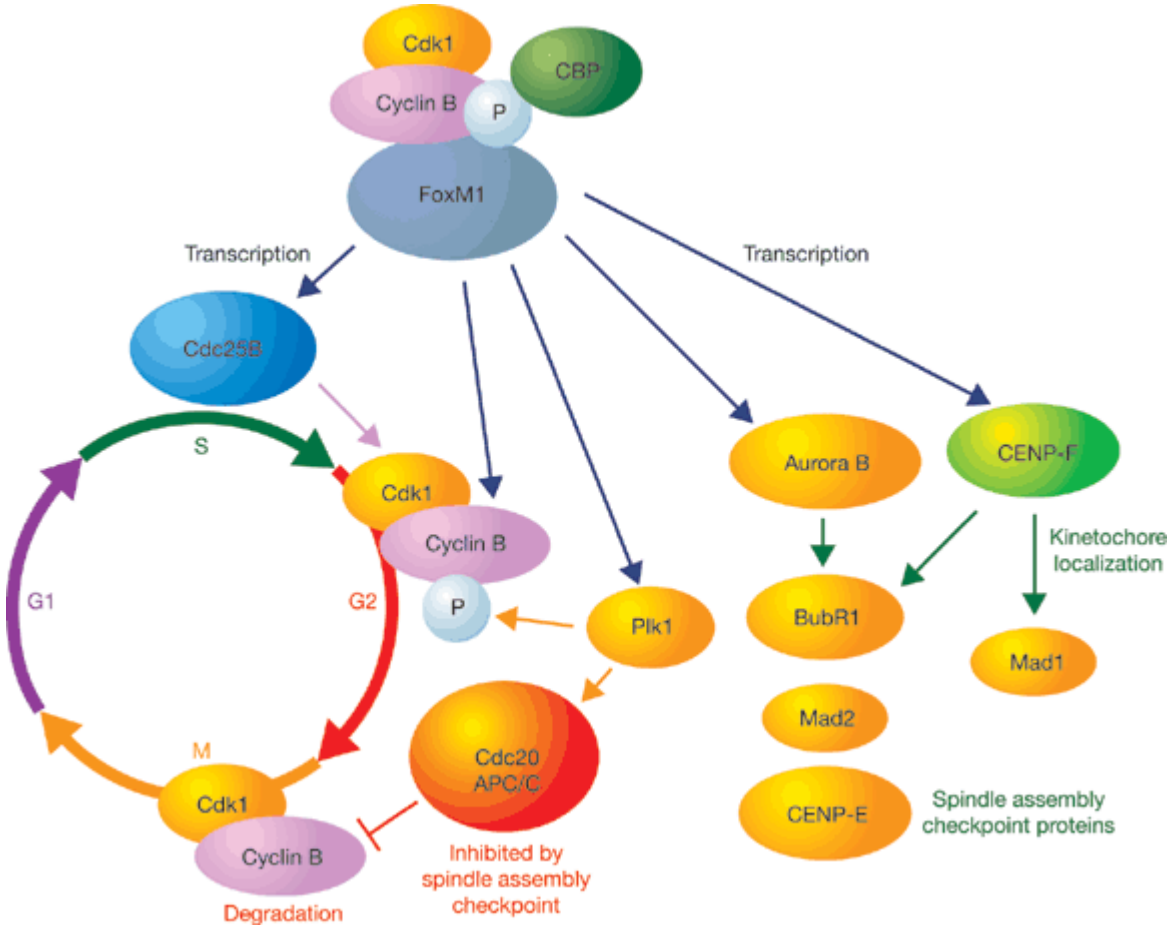
- In addition to traditional growth factors, other factors are mitogenic to specific populations of neural progenitor cells.

  e.g. Shh promotes division of external granule cells in the developing cerebellum.

Regulation of cell division is much more complicated …

Several dozen proteins regulate progress through each stage of the cell cycle.

Regulation of cell division is much more complicated …



Inhibition of the Cell Cycle

- Some factors, such as p27/Kip1, arrest cells in G_1 (G_0) by inhibiting cdk's.

Notch Signaling

- Activation of the cell surface receptor, Notch, can block progenitor cells from differentiating and promote cell division:

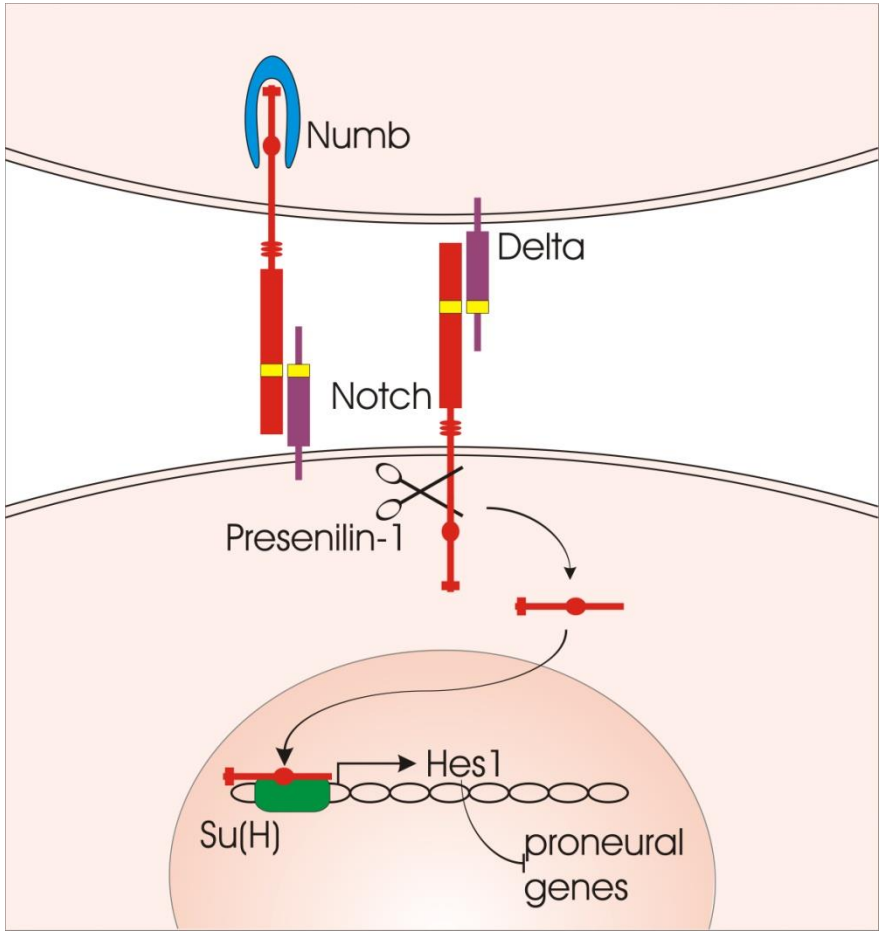
  - Transfection of early neuroepithelium with constitutively active Notch (i.e. the intracellular domain) or overexpression of Delta blocked differentiation and resulted in prolonged symmetric division of the progenitor cells.

  - Blocking Notch resulted in premature neuronal differentiation.

- Notch ligands include Delta and Jagged.

- Notch is expressed by neural progenitor cells.

Notch Signaling



Position Influences Cell Division

- Even prior to significant cell differentiation, different regions of the nervous system exhibit different amounts of cell division.

- For example, alar and basal plates of the spinal cord have more division than roof or floor plates; there are also differences along the anterior-posterior length of the neural tube.

- These differences are controlled by expression of position-specific transcription factors.