

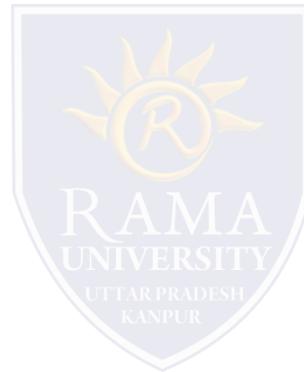


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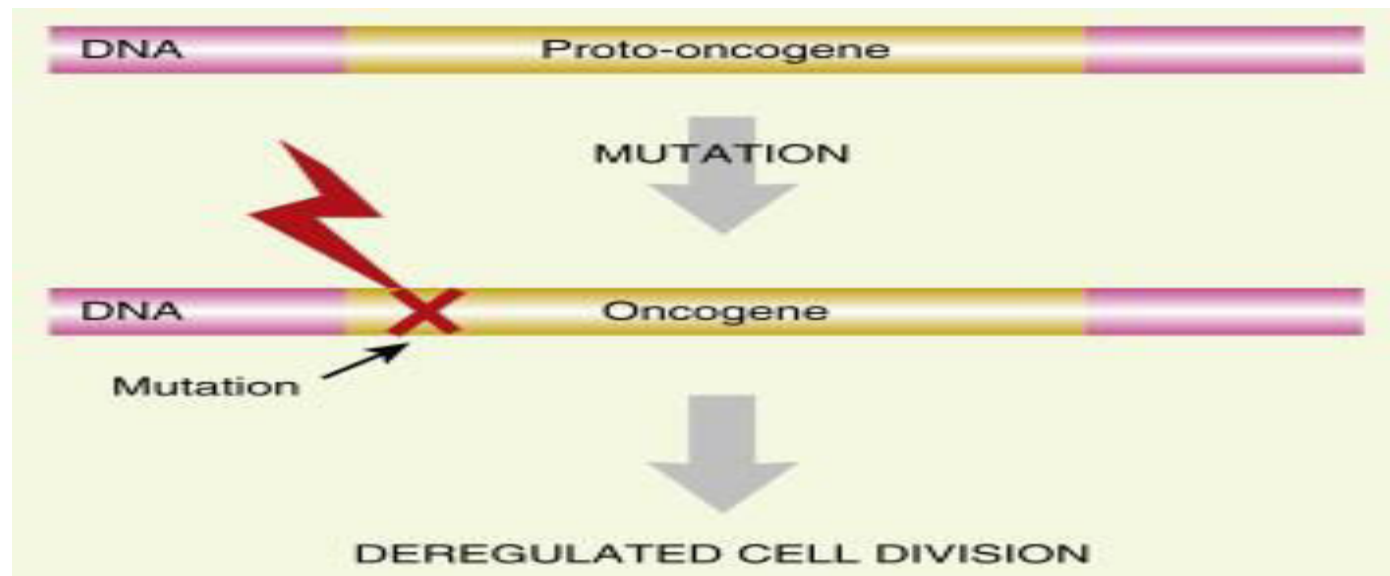
Content outline



Oncogene, Tumor suppressor gene

Oncogenes:

An oncogene is a sequence of deoxyribonucleic acid (DNA) that has been altered or mutated from its original form, the proto-oncogene (promotes cell growth). These genes encode a protein that is capable of transforming cells in culture or inducing cancer in animals.



Proto-Oncogene

- A normal gene which, when altered by mutation, becomes an oncogene that can contribute to cancer
- Some proto-oncogenes provide signals that lead to cell division. Other proto-oncogenes regulate programmed cell death (apoptosis).

Oncogene in human tumors : Ras gene family

- The first human oncogene identified in gene transfer assays was subsequently identified as the human homolog of the *rasH* oncogene of Harvey sarcoma virus. Three closely related members of the ras gene family (*rasH*, *rasK*, and *rasN*) are the oncogenes most frequently encountered in human tumors. These genes are involved in approximately 20% of all human malignancies, including about 50% of colon and 25% of lung carcinomas.
- The *ras* oncogenes are not present in normal cells; rather, they are generated in tumor cells as a consequence of mutations that occur during tumor development. The *ras* oncogenes differ from their proto-oncogenes by point mutations resulting in single amino acid substitutions at critical positions.
- The first such mutation discovered was the substitution of valine for glycine at position 12. Other amino acid substitutions at position 12, as well as at positions 13 and 61, are also frequently encountered in *ras* oncogenes in human tumors.
- The *ras* genes encode guanine-nucleotide binding proteins that function in transduction of mitogenic signals from a variety of growth factor receptors

- The activity of the Ras proteins is controlled by GTP or GDP binding, such that they alternate between active (GTP-bound) and inactive (GDP-bound) states. The mutations characteristic of *ras* oncogenes have the effect of maintaining the Ras proteins constitutively in the active GTP-bound conformation.
- In large part, this effect is a result of nullifying the response of oncogenic Ras proteins to GAP (GTPase-activating protein), which stimulates hydrolysis of bound GTP by normal Ras. Because of the resulting decrease in their intracellular GTPase activity, the oncogenic Ras proteins remain in the active GTP-bound state and drive unregulated cell proliferation.
- Oncogenes are activated in human tumors via three distinctive mechanisms: (1) Translocation; (2) Amplification; (3) Point mutation. The table lists the various oncogenes of human tumors.

Tumor suppressor gene

- Oncogenes drive abnormal cell proliferation as a consequence of genetic alterations that either increase gene expression or lead to uncontrolled activity of the oncogene-encoded proteins.
- Tumor suppressor genes represent the opposite side of cell growth control, normally acting to inhibit cell proliferation and tumor development.
- Tumor-suppressor genes normally restrain growth, so mutations that inactivate them allow inappropriate cell division. This gene encodes cellular proteins that normally function to prevent tumor development, and retinoblastoma typifies the behavior of a classical tumor suppressor gene.
- Normally, Fusion of tumor cells with normal cells yields hybrids that contain chromosomes from both parents. Such hybrids are usually non-tumorigenic. The first tumor suppressor gene was identified by studies of retinoblastoma, a rare childhood eye tumor.

Tumor suppressor gene from humans

A number of tumor suppressor genes have been identified in human. Table 18.5 list the various tumor suppressor genes identified in human.

TABLE 18.5 Representative Tumor Suppressor Genes

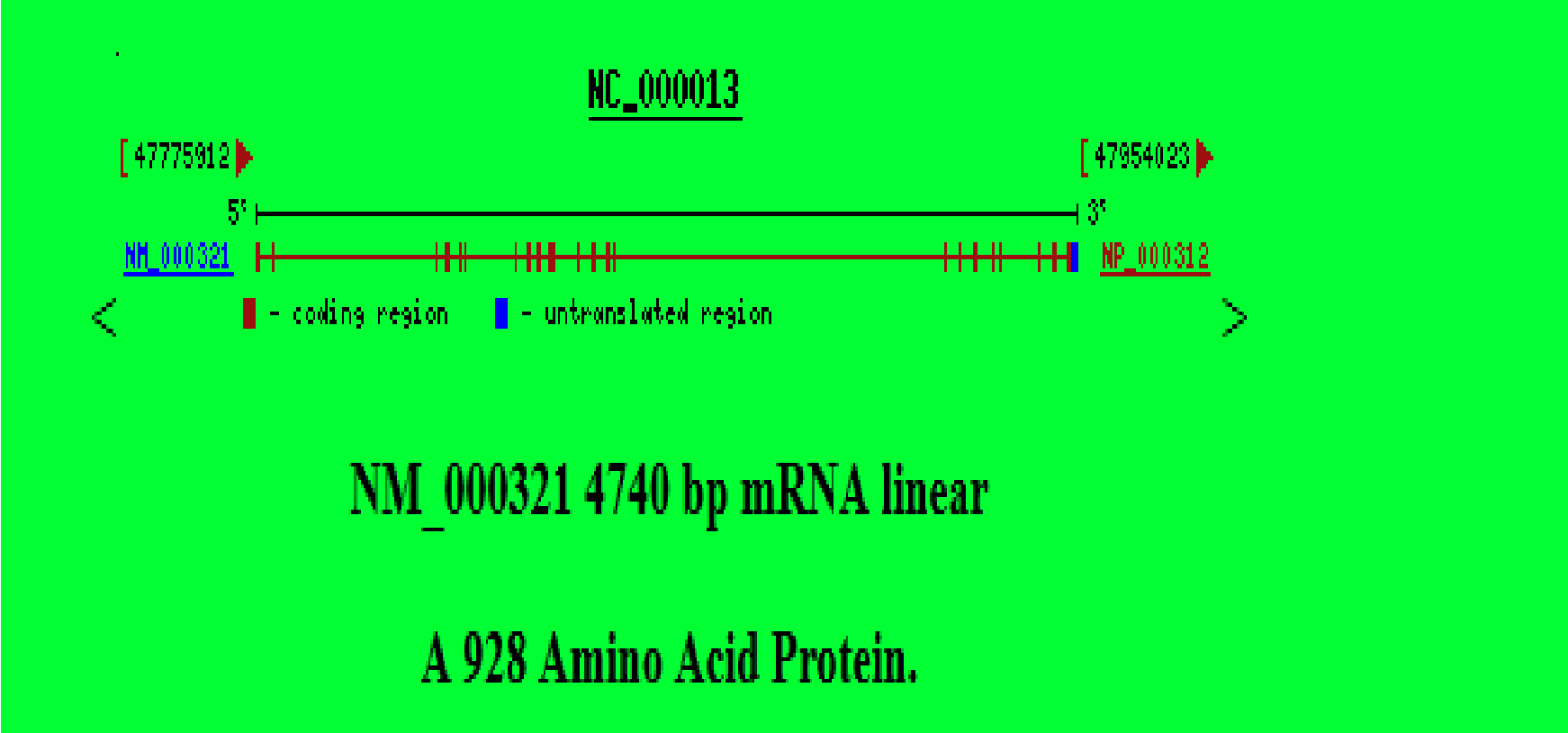
Gene	Type of cancer
<i>APC</i>	Colon/rectum carcinoma
<i>BRCA1</i>	Breast and ovarian carcinomas
<i>BRCA2</i>	Breast carcinoma
<i>INK4</i>	Melanoma, lung carcinoma, brain tumors, leukemias, lymphomas
<i>NF1</i>	Neurofibrosarcoma
<i>NF2</i>	Meningioma
<i>p53</i>	Brain tumors; breast, colon/rectum, esophageal, liver, and lung carcinomas; sarcomas; leukemias and lymphomas
<i>PTCH</i>	Basal cell carcinoma
<i>PTEN</i>	Brain tumors; melanoma; prostate, endometrial, kidney, and lung carcinomas
<i>Rb</i>	Retinoblastoma; sarcomas; bladder, breast, and lung carcinomas
<i>Smad2</i>	Colon/rectum carcinoma
<i>Smad4</i>	Colon/rectum carcinoma, pancreatic carcinoma
<i>TβRII</i>	Colon/rectum carcinoma, gastric carcinoma
<i>VHL</i>	Renal cell carcinoma
<i>WT1</i>	Wilms' tumor

Image source : The Molecular biology of Cell, Cooper

Retinoblastoma (Rb) : Structure

The RB transcript is encoded in 27 exons dispersed over about 200 kilobases (kb) of genomic DNA. The length of individual exons ranges from 31 to 1889 base pairs (bp). The largest intron spans greater than 60 kb and the smallest one has only 80 bp. Deletion of exons 13-17 is frequently observed in various types of tumors, including retinoblastoma, breast cancer, and osteosarcoma, and the presence of a potential "hot spot" for recombination in the region is predicted. A putative "leucine-zipper" motif is exclusively encoded by exon 20. Transcription of RB is initiated at multiple positions and the sequences surrounding the initiation sites have a high G + C content. A typical upstream TATA box is not present. A region as small as 70 bp is sufficient for RB promoter activity. Several direct repeats and possible stem-and-loop structures are found in the promoter region. Several features of the RB promoter are reminiscent of the characteristics associated with many "housekeeping" genes, consistent with its ubiquitous expression pattern. sufficient for RB promoter activity. Several direct repeats and possible stem-and-loop structures are found in the promoter region. Several features of the RB promoter are reminiscent of the characteristics associated with many "housekeeping" genes, consistent with its ubiquitous expression pattern.

Size and structure of Rb genes



NM_000321 4740 bp mRNA linear

A 928 Amino Acid Protein.

Function and mechanism of Rb gene

- The Rb protein is a tumor suppressor, which plays a pivotal role in the negative control of the cell cycle and in tumor progression. It has been shown that Rb protein (pRb) is responsible for a major G1 checkpoint, blocking S-phase entry and cell growth.
- The retinoblastoma family includes three members, Rb/p105, p107 and Rb2/p130, collectively referred to as 'pocket proteins'. The pRb protein represses gene transcription, required for transition from G1 to S phase, by directly binding to the transactivation domain of E2F and by binding to the promoter of these genes as a complex with E2F.
- pRb represses transcription also by remodeling chromatin structure through interaction with proteins such as hBRM, BRG1, HDAC1 and SUV39H1, which are involved in nucleosome remodeling, histone acetylation/deacetylation and methylation, respectively. Loss of pRb functions may induce cell cycle deregulation and so lead to a malignant phenotype.
- Visible deletions of chromosome 13q14 were found in some retinoblastomas, suggesting that loss (rather than activation) of the *Rb* gene led to tumor development.

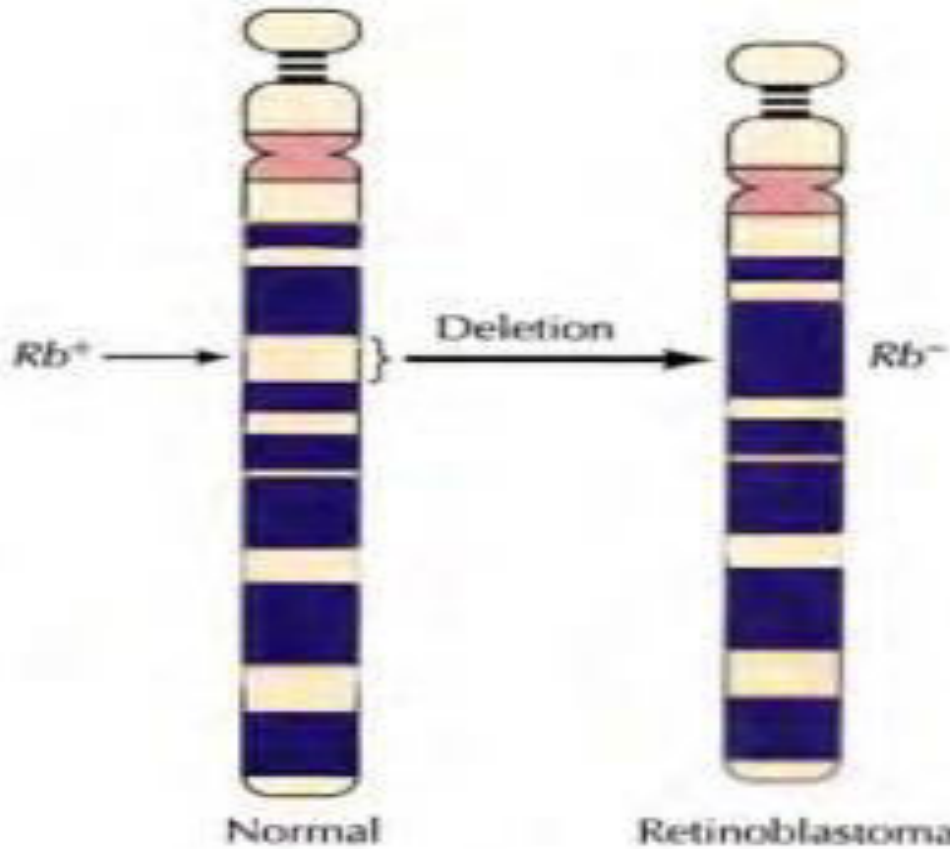
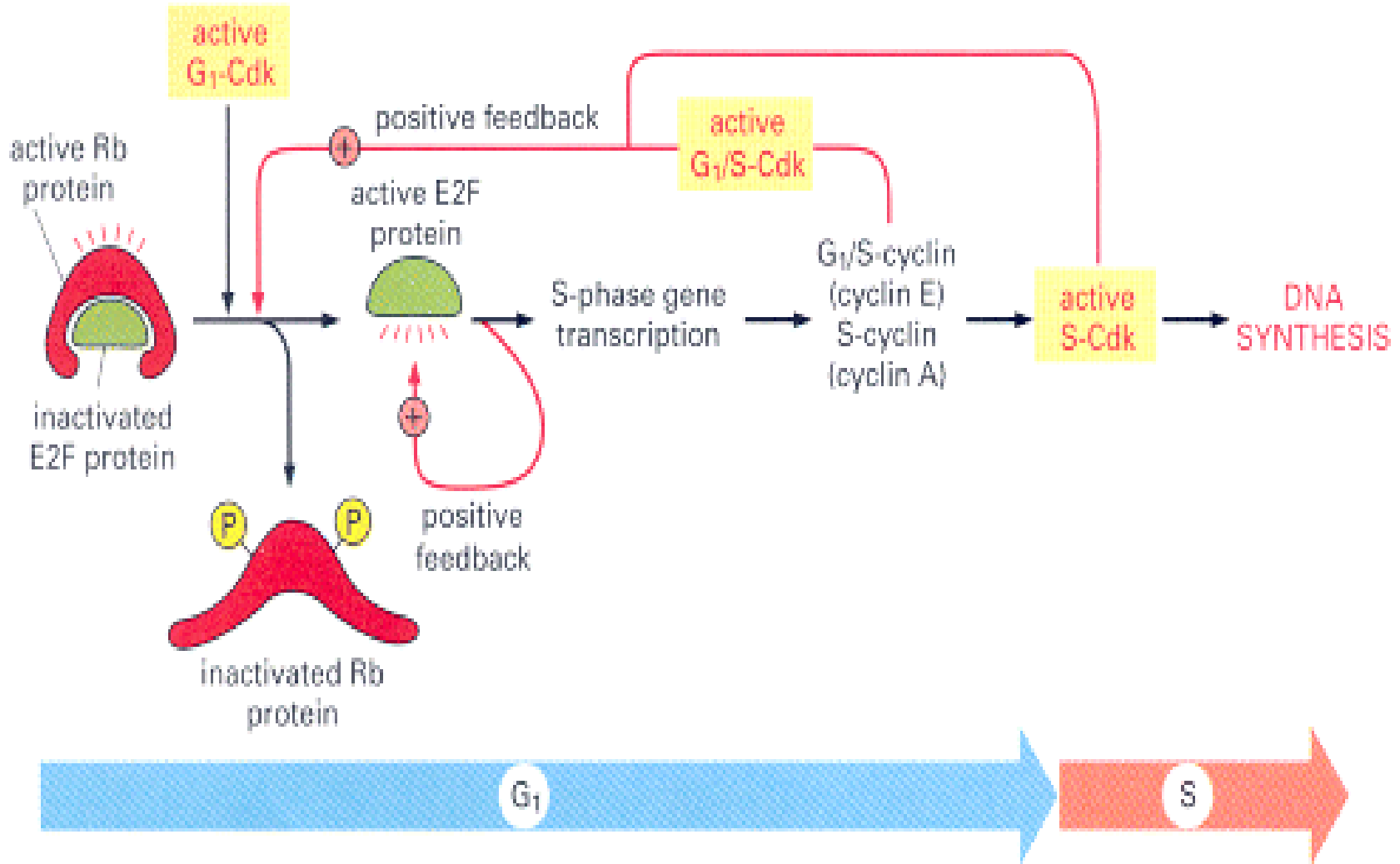
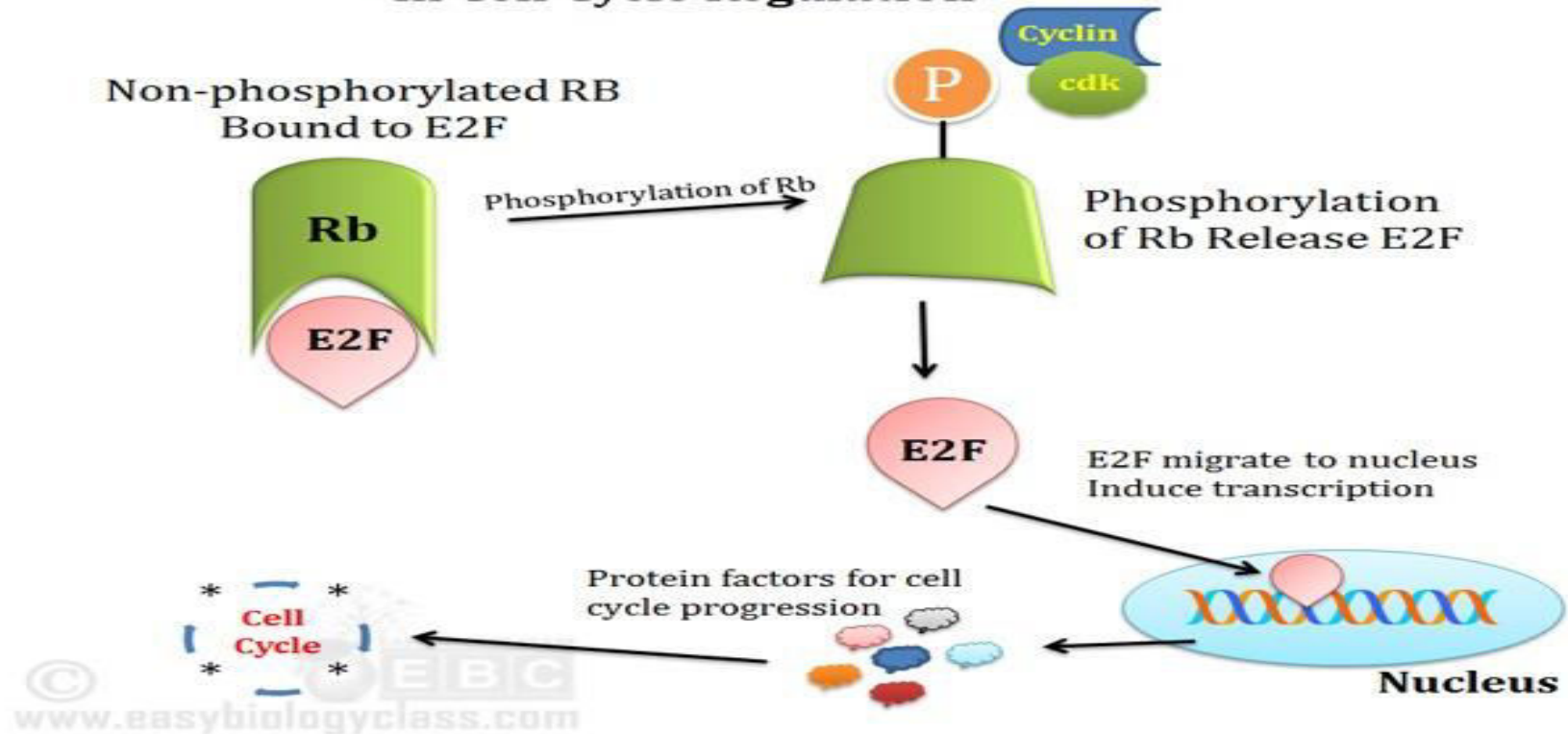


Figure: *Rb* deletions in retinoblastoma: Many retinoblastomas have deletions of the chromosomal locus (13q14) that contains the [*Rb* gene](#).



The complete loss of Rb does not immediately cause increased proliferation of other cell types, in part because Hct1 and p27 (two other proteins) provide assistance in G1 control, and in part because other cell types contain Rb-related proteins that provide backup support in the absence of Rb (14). It is also likely that other proteins, unrelated to Rb, help to regulate the activity of E2F.

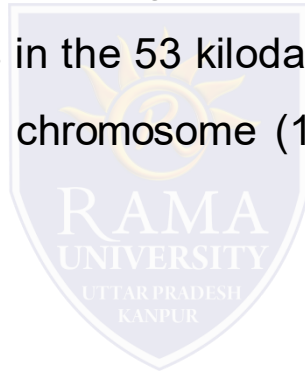
Mechanism of Action of **Rb (Retinoblastoma)** Protein in Cell Cycle Regulation



Mechanism of action of pRb gene (<https://www.easybiologyclass.com/tumor-suppressor-gene-rb-and-its-role-in-cell-cycle-and-cancer/>). The phosphorylation and dephosphorylation is mediated by cyclin D/ CDK4/CDK6 family.

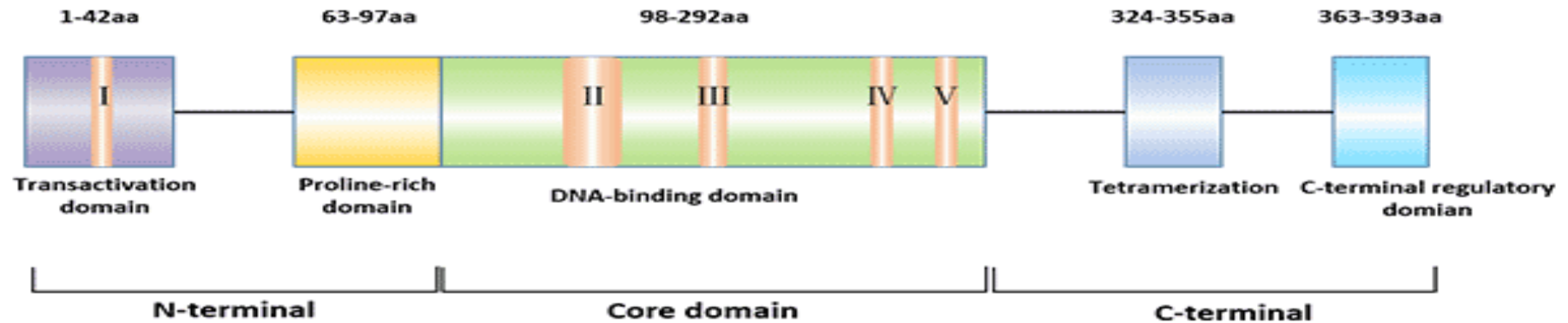
P53 Tumor suppressor gene

- **p53**, also known as **TP53** or **tumor protein** (EC :2.7.1.37) is a gene that codes for a protein that regulates the cell cycle and hence functions as a tumor suppression. It is very important for cells in multicellular organisms to suppress cancer. P53 has been described as "the guardian of the genome", referring to its role in conserving stability by preventing genome mutation. The name is due to its molecular mass: it is in the 53 kilodalton fraction of cell proteins. The human p53 gene is located on the seventeenth chromosome (17p13.1).



Structure of p53

Structure of p53 gene

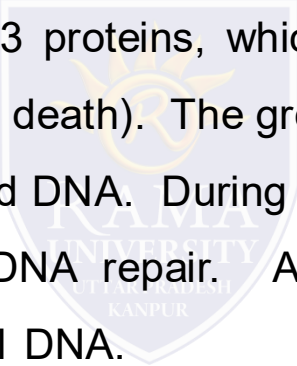


- The p53 protein is a phosphoprotein made of 393 amino acids. It consists of four units (or domains):
- The N-terminal region of p53 plays a role in recruiting transcription proteins. Adenovirus E1B protein, human [MDM2](#), and hepatitis b virus X protein can bind to the N-terminus of p53 and inhibit its transcriptional activation function. The N-terminus also has a proline-rich region and may be involved in apoptosis regulated by p53.

- The intermediate region of p53 protein (102 ~ 292aa) contains four conserved regions, where 80% ~ 90% of the mutations caused by tumor cells occur.
- The C-terminus of p53 (300-393aa) contains a variable junction region that connects the central core region to the C-terminal region; there is also a tetramerization region. There are also three nuclear localization signals (NLS) at the C-terminus of p53: NLS1 (316-325aa), NLS2 (369-375aa), and NLS3 (379-384aa). The C-terminus is a flexible unfolded region that binds to DNA non-specifically and acts as a repressor in the regulation of other genes.
- The spatial structure of p53 protein determines its biological function
- Wild-type p53 is a labile protein, comprising folded and unstructured regions which function in a synergistic manner. p53 protein has been voted molecule of the year

Function and mechanism of p53

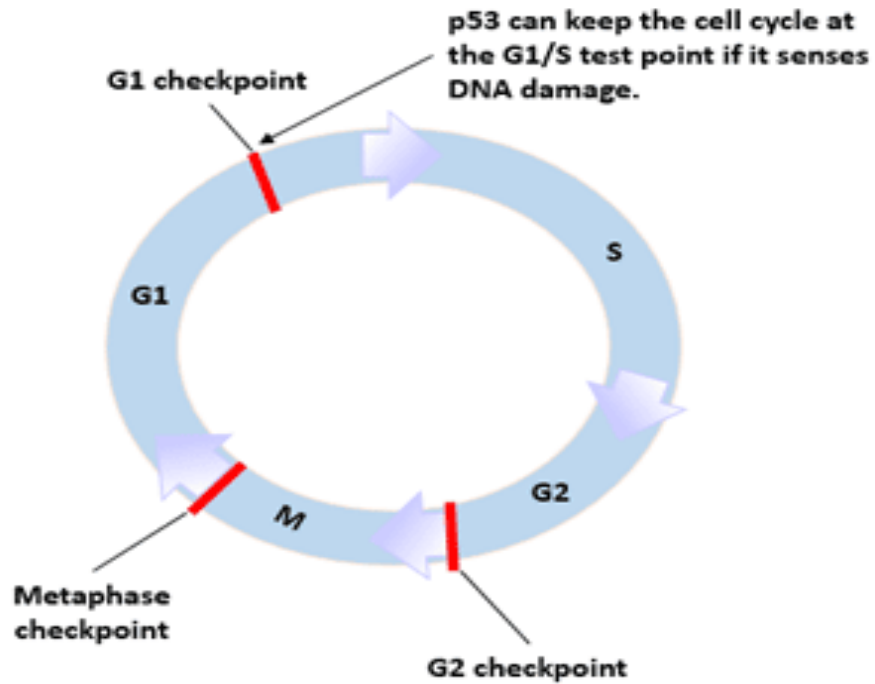
It plays an important role in cell cycle control and apoptosis. Defective p53 could allow abnormal cells to proliferate, resulting in cancer. As many as 50% of all human tumors contain p53 mutants. In normal cells, the p53 protein level is low. DNA damage and other stress signals may trigger the increase of p53 proteins, which have three major functions: **growth arrest, DNA repair** and **apoptosis** (cell death). The growth arrest stops the progression of cell cycle, preventing replication of damaged DNA. During the growth arrest, p53 may activate the transcription of proteins involved in DNA repair. Apoptosis is the "last resort" to avoid proliferation of cells containing abnormal DNA.



Role of p53

p53 and Cell Cycle Regulation

- In addition to being in the form of p53 protein, p53 is present in cells as a potential untranscribed form. The untranscribed form of p53 is only activated to produce biological functions. p53 protein can be activated by DNA damage. The upstream factor of p53 recognizes DNA-damaged proteins.
- In addition, p53 can also bind directly to the ends and damage sites of DNA, thereby being phosphorylated or receiving other signals to generate activity. In addition to DNA damage, tissue hypoxia also increases and activates p53 concentration.
- p53-mediated downstream processes mainly include:
 - Make the cells stop at the cell cycle test point
 - Make the cells go to apoptosis
- p53 gene has the function of blocking cell cycle. The expression or ectopic expression of p53 after DNA damage keeps the cell cycle at the G1/S test point.



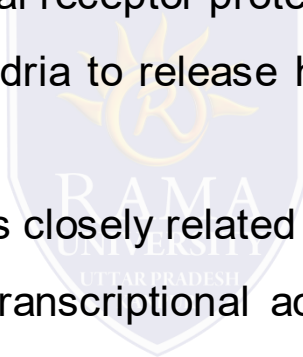
p53 and DNA Damage Repair

The p53-regulated DNA damage repair process mainly includes Nucleotide Excision repair (NER), Base excision repair (BER), Nonhomologous end-joining (NHEJ) and transcriptional activation pathways and Homologous recombination by both transactivation-dependent and-independent pathways

p53 and Apoptosis

•p53 gene has the effect of promoting cell apoptosis. p53 protein can induce the apoptosis of malignant tumor cells, thus damaging the function of tumor cells and eventually causing tumor atrophy or disappearance. It can regulate apoptosis through proteins such as Bax/[Bcl-2](#), [Fas/Apo1](#), and induce apoptosis through death signal receptor proteins such as TNF receptor and Fas protein. p53 can also directly stimulate mitochondria to release highly toxic oxygen free radicals to induce apoptosis .

•The role of p53 in promoting apoptosis is closely related to its tumor suppressor function. Apoptotic mechanisms regulated by p53 include transcriptional activation and non-transcriptional activation pathways.



References and Further reading

1. http://dpuadweb.depauw.edu/cfornari_web/DISGEN/retinoblastoma_website/public_html/protein.htm
2. <https://www.ncbi.nlm.nih.gov/books/NBK9894/>
3. <https://www.slideshare.net/gangadharchatterjee/molecular-basis-of-cancer-part-1>
4. The cell. Geoffrey cooper

