Cell Cycle Regulation

The cell cycle involves a series of carefully controlled events resulting in DNA duplication and cell division. Progression through each of the four distinct phases (G₁, S, G₂ and M) is carefully controlled by the sequential formation, activation and subsequent degradation or modification of a series of cyclins and their partners, the cyclin-dependent kinases (CDKs). In addition, a further group of proteins, the cyclin-dependent kinase inhibitors (CDKIs) are important for co-ordination of each stage. The transition from one stage to the next is regulated at a number of checkpoints which prevent premature entry into the next phase of the cycle. The degradation of various cyclins occurs at each checkpoint and it is this mechanism together with interaction of the CDKIs which allows the cell to enter the next phase.

Cyclins

At least 13 mammalian cyclins have now been identified, each one of which is required at a different stage of the cell cycle. They all contain a homologous region of about 100 amino acids called the cyclin box and it is this region of the protein which binds to the appropriate CDK. Each cyclin undergoes a characteristic pattern of synthesis and degradation dependent on the stage of the cycle at which it acts. The cyclins are broadly classified into G₁ and mitotic cyclins, according to the stage of the cycle during which they are produced. The G₁ cyclins are relatively short lived proteins and are degraded via their PEST sequences which lie C terminal to the cyclin box. The mitotic cyclins are longer lived and are degraded, prior to entry into mitosis, by proteinases via an ubiquitin-dependent pathway via the 'destruction' box located N terminal to the cyclin box. Cyclin degradation results in CDK inactivation.

CDKs

At least 6 mammalian CDKs have so far been identified. The CDKs are activated by forming complexes with cyclin partners and by a pattern of phosphorylation and dephosphorylation at specific residues. CDKs are activated by phosphorylation of a conserved threonine residue at position 160 and by cyclin binding. E.g., Phosphorylation of the CDKs CDC2, CDK2 and CDK4 is carried out by the p40^mol5 protein which in turn is activated by cyclin H. In addition, phosphorylation also may causes inhibition of cyclin/CDK complexes, E.g., the two CDKs, CDC2 and CDK2, are inactivated throughout interphase by phosphorylation on threonine 14
or tyrosine 15 by the wee1/mik1 protein kinase. At the end of G\textsubscript{1}, the product of the CDC25 gene, activates the kinase by dephosphorylation of these residues.

**CDKIs**

There are at least 7 different CDKIs in mammalian cells which belong to two different classes. The first class comprises p21, p27 and p57 which preferentially bind to the G\textsubscript{1}/S class of CDKs. The second class of CDKs, referred to as the INK4 (Inhibitor of CDK4) family, is comprised of ankyrin repeat proteins and includes p15, p16, p18 and p19. These inhibitors act on cyclin D complexed either to CDK4 or CDK6.

**Control of the cell cycle**

Each of the cyclin-CDK complexes, together with the CDKIs, are responsible for controlling different stages of the cell cycle by preventing progression through checkpoints in the presence of DNA damage.

**Late G\textsubscript{1} Checkpoint**

The D type cyclins are linked to the regulation of the first checkpoint at G\textsubscript{1}/S. They are synthesised in response to growth factors and are very short lived. The D type cyclins are found in partnership with four kinases, CDK2, -4 -5 and -6 although CDK4 appears to be the main partner in most cell types. Activation of CDK4 by complexing with cyclin D and phosphorylation on threonine 160 drives cells through this checkpoint by phosphorylation of the Rb protein which releases the E2F transcription factor allowing it to activate genes necessary for DNA synthesis.

**G\textsubscript{1}/S phase**

The E type cyclins are believed to act after the D type and to be important for the initiation of DNA replication. Cyclin E is expressed towards the end of G\textsubscript{1}, and complexes with CDK2 to activate it. As with cyclin D-CDK4, phosphorylation of the threonine residue (160) is necessary for activation. After cells have entered S phase, cyclin E is rapidly degraded and CDK2 is released to be complexed by cyclin A at the next stage.

Normally, cells which have suffered DNA damage are prevented from entering S phase and are blocked at G\textsubscript{1}, a p53-dependent process through its transcriptional regulation of the cyclin-dependent kinase inhibitor, p21. Activation of p53 by DNA damage results in
increased p21 levels. It can then bind to a number of cyclin-CDK complexes including cyclin D-CDK4, cyclin E-CDK2 and cyclin A-CDK2 thereby preventing phosphorylation of pRb causing the cell cycle to arrest in G₁.

**S phase**

Once cells enter S phase, a further set of cyclins and CDKs are required for continued DNA replication. In mammalian cells, cyclin A-CDK2 performs this function. Cyclin A is expressed from S phase through G₂ and M. Cyclin A binds to two different CDKs. Initially during S phase it is found complexed to CDK2 and during G₂ and M it is complexed to CDC2.

**Mitosis**

Entry into the final phase of the cell cycle, mitosis, is signalled by the activation of the cyclin B-CDC2 complex. This complex accumulates during S and G₂ but is kept in the inactive state by phosphorylation of tyrosine 15 and threonine 14 residues, a process regulated by the weeI/mikI kinases. At the end of G₂, the CDC25c phosphatase is stimulated to dephosphorylate these residues thereby activating CDC2. Cyclin B is located in the cytoplasm during interphase but is translocated to the nucleus at the beginning of mitosis. Cyclin B-CDC2 plays a major role in controlling the rearrangement of the microtubules in mitosis. In addition, the complex plays a role in disassembling the nucleus and allowing the cell to round up and divide.

There is one final checkpoint which occurs at the end of metaphase and at this point the correct assembly of the mitotic apparatus and the alignment of chromosomes on the metaphase plate are monitored. Normal cells arrest at this point if there are any defects, whereas in tumour cells abnormalities of spindle formation are found, suggesting that the checkpoint control is lost. Cyclin B is degraded as cells enter into anaphase. Re-establishment of interphase can then be initiated.

**Cell Cycle Genes and cancer**

**Cyclin D1**

Cyclin D1 is encoded by the CCND1 gene on chromosome11q13 and is identical to the PRAD1 proto-oncogene. Its overexpression is associated with a number of tumours such as esophageal, breast and gastric cancers. Chromosome rearrangements involving 11q13 are
found in several tumors such as parathyroid adenomas and B-cell lymphomas. In these cases, the rearrangement results in overexpression of the gene by bringing it respectively under the influence of the parathyroid promoter or the immunoglobulin heavy chain enhancer.

**Cyclin D2**

Cyclin D2 is encoded by the CCND2 gene on chromosome 12p13 and has been identified as the VIN1 proto-oncogene and has been shown to be amplified in human colorectal cancer.

**Cyclin A**

Cyclin A was one of the first cyclins to be implicated in tumour development. The cyclin A gene has been found to be the site of integration of HBV in a case of hepatocellular carcinoma resulting in the production of a chimeric protein absent of the 'destruction' box necessary for the degradation of the cyclin in mitosis. However, gene rearrangements involving cyclin A have rarely been observed in other liver tumours and there have been few reports of other abnormalities of cyclin A in tumors from other sites.

**CDK4**

CDK4 has also been shown to have a potential role in tumorigenesis as the gene encoding this protein, located on 12q13, has been shown to be amplified in sarcomas and gliomas.

**CDKIs**

The CDKIs have also been shown to be involved in tumour development. The CDK4/6 inhibitor, p16, acts by competing directly with cyclin D, thereby preventing these kinases from phosphorylating the Rb protein. p16 gene aberrations are frequently found in a wide range of cancers. p16 mutations occur at high frequencies in biliary tract and esophageal cancers. Homozygous deletions at the p16 locus occur commonly in gliomas, nasopharyngeal carcinomas, acute lymphocytic leukaemias, sarcomas and bladder and ovarian tumours. Pancreatic, head and neck and non-small-cell lung carcinomas sustain both p16 deletions and mutations.