Genetics & molecular biology

◉ Sheet ○ Slide

Number:	34
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This sheet is written depending on the recorded lecture from section 2 with the help of the uploaded video on YouTube.

In this section from our course we'll talk about cell differentiation, cell cycle proliferation and cell death. Let's start with differentiation. Any specialized cell; to be specialized it will undergo a process known as differentiation; firstly, a general cell formed then it becomes a special cell to do specific function. All the cells come from stem cells –there is no specific function for it except it will give us all the specialized cells after differentiation.

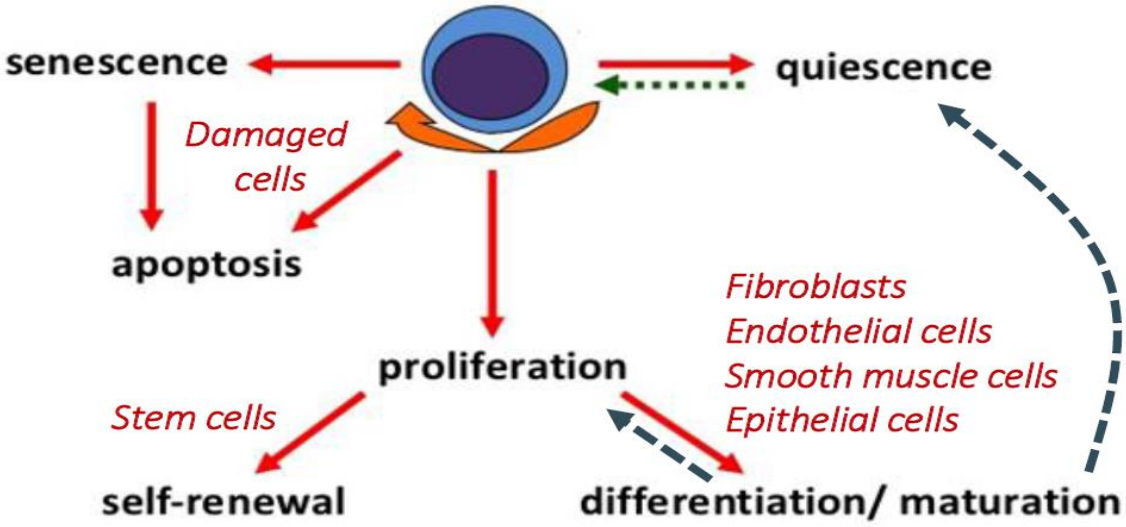
Now what determines the cell's fate? It is **the environment where the cell is found**; so, it may undergo 1- proliferation, and if it is a stem cell, the proliferation aims to

A- cell's renewal (to maintain stem cell population) and
B- To give rise to cells for **differentiation** to give rise for all cell types.

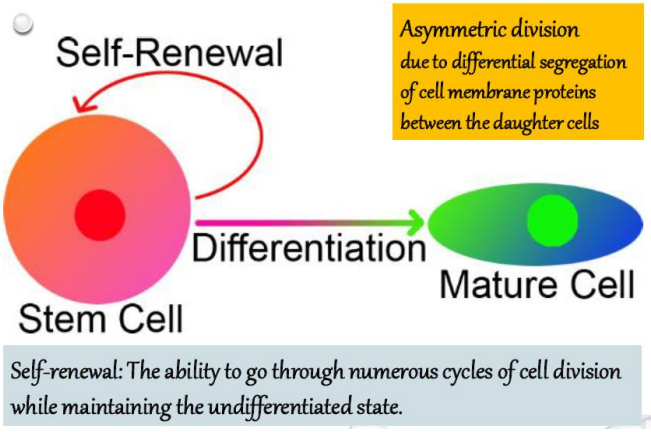
Or in other environments (assume there is no growth factors- even it has the ability for proliferation-) it shift to a state known as 2- **quiescence** (سكون) here the cell is "quite", it is a **reversible state** (the cell cycle stops in the G0 phase) and it maybe undergo proliferation again (remember it is a reversible state).For example; the fully maturated cell or fully differentiated (osteocyte, chondrocyte), or in another word terminally differentiated; there is no state after it, it keeps functioning until acquiring any abnormality or mutation; here it undergoes **apoptosis**. So, when a cell is in quiescence it can't give rise for cells "mainly" and it does its own function; **so, the main source for the population of the cells is the stem cells.** After a period of time; in conjunction with cell aging, it reaches a state of 3- **senescence** (الهرم) which has a special characteristics in a specific age (this doesn't mean that it will lose its function) it keeps functioning until it acquire any mutation or abnormality that affects its function negatively and damages other tissues it'll undergoes 4- **apoptosis**.

Apoptosis (programmed cell death); is not limited for senescent cells. As we said before, any abnormality or mutation can damage the cell irreversibly makes it undergoes apoptosis (it decides to death).

So **different environments** or **the age of the cell** determine the state of the cell as summarized in the pic.



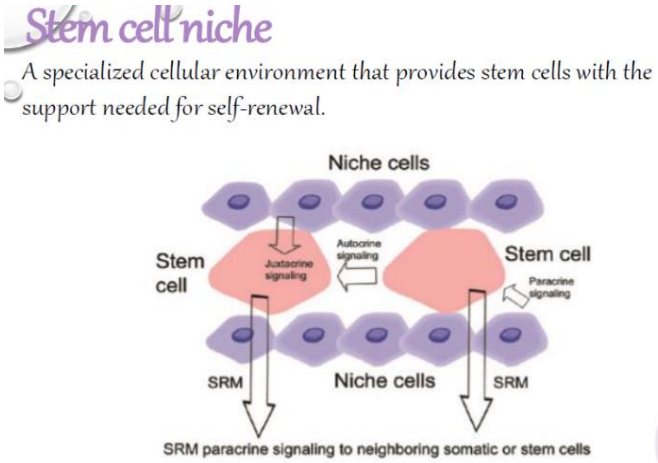
As we said the main source for cell proliferation or differentiation is the stem cells. So, what are the stem cells? They are **undifferentiated, unspecialized cells that have the ability to divide asymmetrically**; meaning that it won't give rise for identical cells (one of them is stem cell for **self-renewal** and the other one it will go for **differentiation pathway**).



What makes or what determines this asymmetrical division? Or how this process is going to be made?

When stem cells divide asymmetrically the proteins are **not equally segregated** –particularly the **membrane proteins**- so we aggregate a number of proteins in a place or specific side that are important for keeping the **stemness of the cell** (keep it or make it a stem cell) and the other proteins that are **responsible for differentiation** aggregated in another side of the cell making another type of cells (that will go to be differentiated).

What makes the stem cells to stay stem cell? As we said there is a group of factors (one of them is the **segregation process**), and the **microenvironment** that surround the stem cell which is known as **stem cell niche**; this keep the stemness of the stem cells. The stem cell niche for one type of stem cells is **different** from the stem cell niche of another type of stem cells by **the composition or the standards** of it.

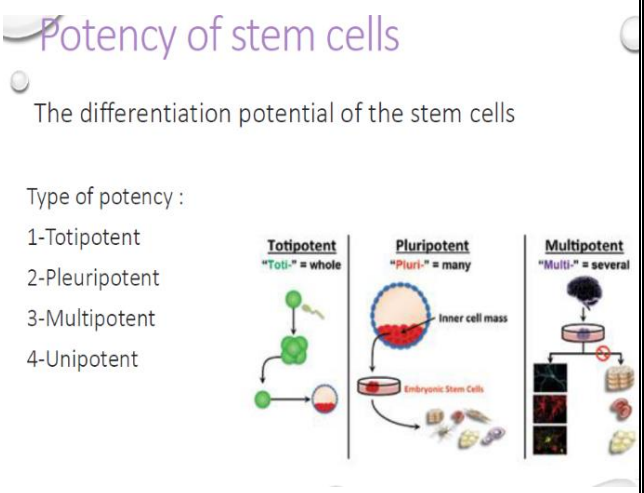


What is the composition of stem cell niche that is probably found for specific type of stem cells? It may be **a type of cells** that surround the stem cells to keep it stem cell, or **specific amount or composition of ECM** (we studied before that different ECM share some components or compositions, but sometimes they differ in some compositions; maybe differ in the amounts, or the GAGs has more or less branching, or the presence and the absence of some compositions). Sometimes it is **a combination** of the cells and the ECM. Sometimes there is a **soluble factor** that is released by cells that affect the stem cells and keep it stem cell.

The stem cells are very sensitive; they are sensitive for temperature, forces that may affect them. Why? Because they tend to differentiate. So in the lab we are very careful when we grow them in dishes; when we move the dish from place to another, or when we put them in the table or in the microscope, so we must or tend to keep the standard niche for a specific type of stem cells.

We categorize the stem cells depending on their potency (their potential to differentiate), they have different potency; some of them differentiate to always 5 cells and some differentiate into 4 or 5 cells:

1) TOTIpotent: the **highest potent**; stem cell population formed in the **embryonic development**, it gives rise to all body cells of the embryo (**embryonic tissue**) as well as **extra embryonic tissue** (not part of the embryo but it is important for at such as; placenta and amniotic sac).
2) PLEURIpotent: gives rise for **the embryonic tissue only** (they don't give rise for the extra embryonic tissue).

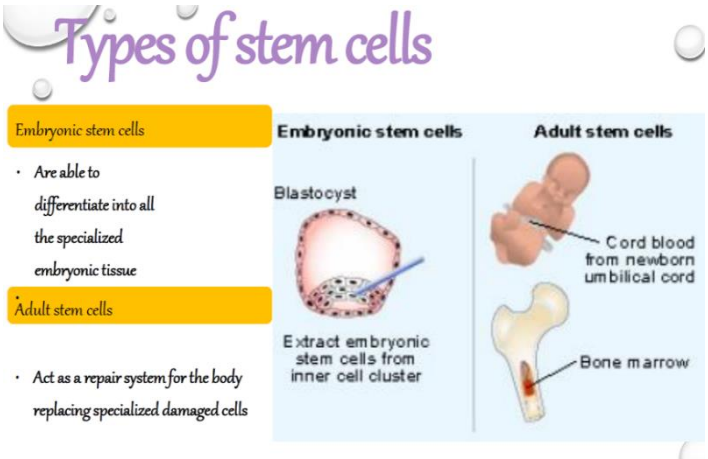


<u>These two types found just in the embryo and not found in us as adults,</u> and those found in adults tend to be less potent which are (and are important for regeneration).

3) MULTIpotent: gives rise for **several cell types** (5/6/10 cells).
4) UNIpotent: gives rise for **one cell type**.

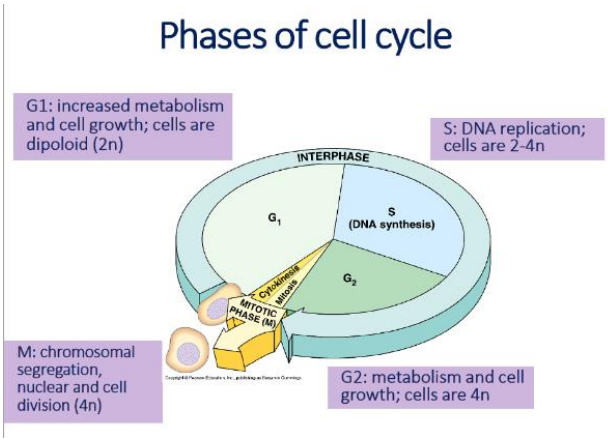
Other categorization **depending** on their **presence (stage):**

1) Embryonic stem cells: after fertilization (as we took in the last year); the fertilized egg starts to divide; the 2 become 4 and the 4 become 8 and so on. Firstly it is like a closed ball of embryonic tissue and then the process of forming a lumen or cavity inside it, and the cells accumulate in the surface which is the blastocyst, and the cavity is filled with liquid except one region where there is an aggregation of cells we call them inner cell mass (where the stem cells are present which are **pluripotent** stem cells)
2) Adult stem cells: they are found in **different sites** (adipose tissue, bone marrow, in the eyes, umbilical cord) which is responsible for **regeneration**.



That is all for stem cells, now we will talk about cell division.

We'll talk about mitosis which gives two identical cells. Any cell must enter a preparation phase before it divides; this preparation known as interphase (G1, S, G2) and then it goes to the M phases or mitotic phase. In these phases, specific changes and specific metabolic processes in preparation for mitosis happen. Now the cell that is not ready for division will go to the quiescence "as we said before". Now we'll discuss these changes one by one.



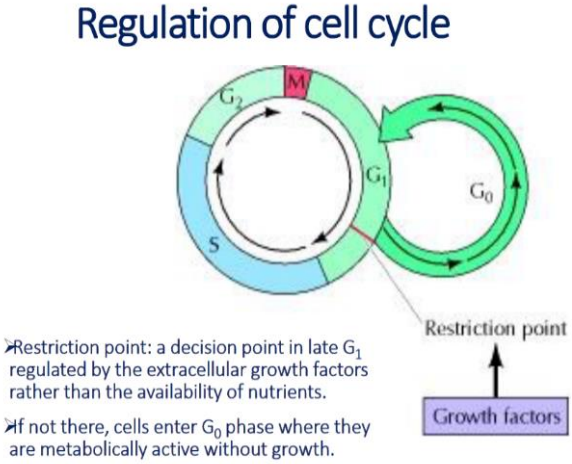
G1: firstly; there is no changes in the genetic material **2n**. Then it should prepare for division; so, the metabolism is increased, and it is influenced by growth factors and signalling pathways underneath growth factors.

S: DNA synthesis, so **all the enzymes that are needed for replication are synthesized** in the cell in this phase. Here in this phase; the genetic material **in the beginning is 2n then we are finishing it by 4n.**

G2: beginning of other metabolic processes, more cylinder growth in preparation of mitosis and cell division.

Then it followed by **mitosis and cytokinesis**; where the daughter cells divided into 2 mature cells.

Now, to ensure that there are no errors happening as we proceed in phases of cell division; we must have some regulation for these phases. So, how can we regulate them?

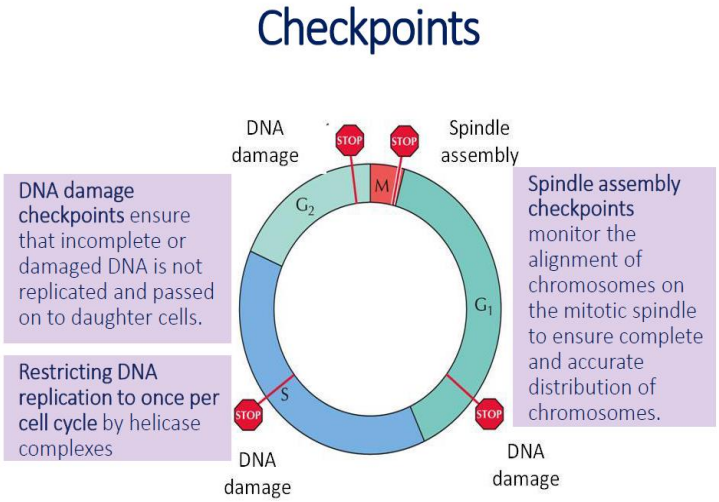


The main aim for regulation is to **prevent errors that give rise for abnormal cells, and prevent these abnormal cells from division**, so we prevent some disease that may happen as a consequence of it. So, we have **restriction points** and **check points**.

Let's start with restriction points. Restriction points is affected by the abundance or the presence of **growth factors**: if there is an enough growth factors surrounding the cell; the cell will proceed in the cell cycle then cell division. So, if there are **no growth factors** and there is **not enough energy** for cell division and **the environment is not encouraging** enough for cell division, why will it divide (it is not a question it is just a logic conclusion)???!

Therefore; we have the restriction point in an early station before **the end of G1 phase** (to preserve the energy); if we have the suitable environment and we detect the growth factors this will lead to the activation of the **signalling pathway** which is responsible for the activation of the cell proliferation process and therefore cell division.

Check points are needed during cell cycle as regulators, and they are divided into 2 major types. One of them is called **DNA damage check point** (repeated several times in G1, S, G2), and the other is **spindle assembly check point** (in M phase or mitosis).



G1 check point: there is no replication process; so here we check the **row material** (genetic material) whether it is normal or abnormal; so, we check if we have the **correct sequence of the DNA** to be copied in the next phase and transferred to the daughter cells.

NOTE: if there is an inherited genetic mutation it is already considered **to be a normal part of the cell** and will not to be detected, and if there is an acquired mutation that didn't do a great amount of damage and didn't affect the cell gravely, it will also be considered as a **component of the normal structure of the DNA**. And they'll not be checked for in the check points.

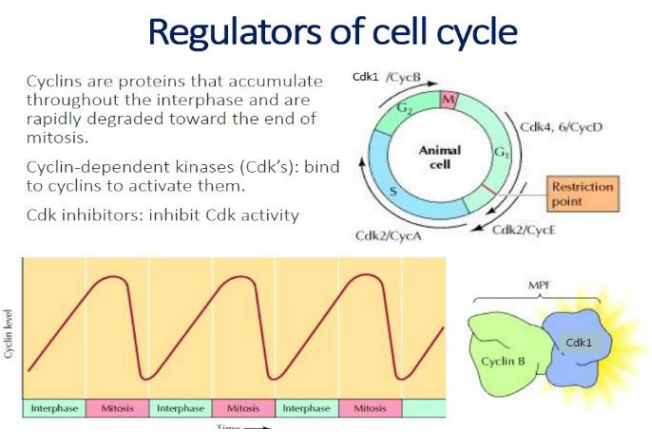
S check point: the process of replication of DNA is considered as a source of mistakes and we have the polymerases as we took in molecular biology which has high fidelity-some have low fidelity- that

check and proofread the sequence of the DNA, so here we check if there are **no mistakes in the replication process**.

G2 check point: here we check the **quality of the DNA**; we check **the whole DNA** after the replication is over, suppose that there is one region is replicated 2 times and another region isn't replicated at all; here we have the same amount of the which are 100% identical. So, we check if it is **a complete replication process of the whole DNA sequence**.

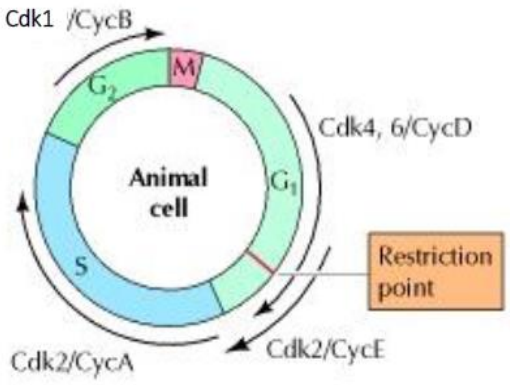
M check point (spindle assembly check point): **In metaphase** when the chromosomes are aligned; if the process of aligning isn't correct for example; the two pairs of chromosome no. 5 will go to one daughter cell, and another daughter cell won't have this chromosome at all (non-disjunction). So, we need to check **the alignment** and **the distribution of the chromosomes** in this check point to make sure that the replication gives rise for 2 identical normal cells.

In the molecular level how can we regulate the cell cycle? The molecules that regulate the cell cycle is a group of proteins called cyclins, and these cyclins is associated with cyclin dependent kinases CDKs (each cyclin has its own CDK). Note in the pic. that its level is increased in the interphase and go down in mitosis, go up and go down, and so on.



Also note that each phase of the interphase has its own cyclin, and each cyclin has its own CDK.

- Cyc D in G1 with CDK4, 6
- Cyc E in the late G1 with CDK 2
- Cyc A in S with CDK 2
- Cyc B in G2 with CDK 1

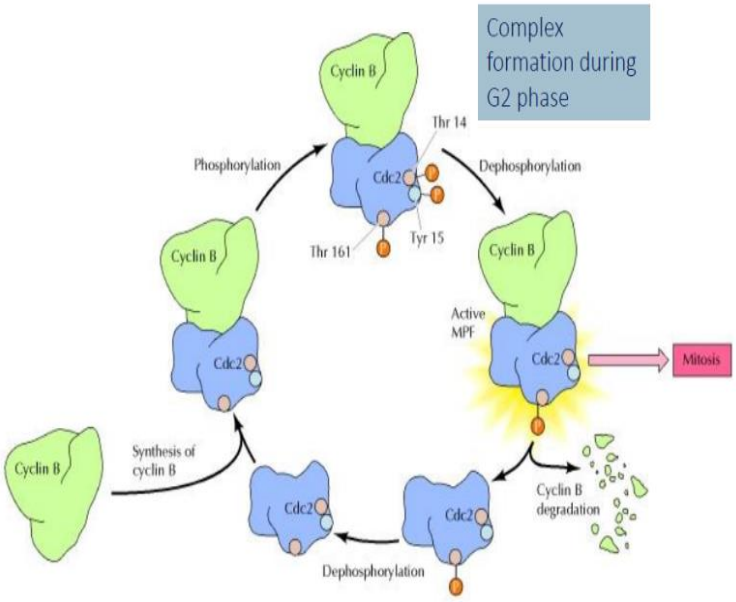


The general principle of the cyclins' regulation of the cell cycle:

Firstly; cyclins are synthesized by the ribosomes and get modified. Then they get **associated with CDKs**, for example; Cyc B with CDK 1; they **form a complex** and this complex **is still inactive**. ***Secondly;*** this complex is get **phosphorylated in the CDK molecule**; two of them is adjacent (Thr 14, Tyr 15) and one far away from them (Thr 161), and again the complex **is still inactive** (not all phosphate group means activation). Note here that when these sites all of them is dephosphorylated the molecule is inactive, and when all these sites are phosphorylated the molecule is also inactive (indicating that some of them is inhibitory phosphates). So here; the two adjacent phosphates are **inhibitory phosphates** and now we should dephosphorylate them. After de-phosphorylation the molecule (complex) now it **is active** and can do its function in his phase (here in G2 phase), and the Cyclin is **degraded** (after it finished its function-here in the G2 phase-), and the CDK is going to be **recycled** by dephosphorylating it, and now it is ready to combine with another Cyc B.

NOTE 1: CDC 2 is the same of CDK 1, but it is in another organism, so you must be familial with CDK 1 only.

NOTE 2: in the pic you found that the complex is going to be function in mitosis, but in true it is active in G2 phase which lead terminally to mitosis.

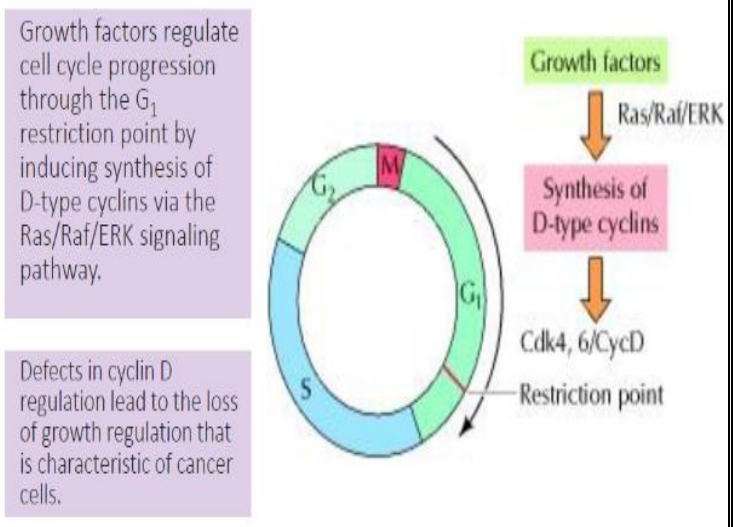


NOTE 3: don't memorize the numbers; just know that there is 2 adjacent inhibiting and one far away activating.

NOTE 4: there is another regulatory molecule which is CKI that inhibit the whole complex and we will take some examples soon (its site is found in the complex).

Now; what is the relationship between these complexes (cyclins and its CDKs) with the signalling pathways?

Let's take the Cyc D which is active during G1 phase (in the beginning of the cell cycle) and its CDKs, and as we said before there is a restriction point in the G1 that sense the growth factors. Now assume that there is an abundant growth factors, these growth factors bind to its receptor (receptor tyrosine kinase RTK). RTK activates several pathways; one of these pathways is RAS/RAF/MEC/ERK signalling pathway. ERK enters the nucleus and activates target genes, one of them is **Cyc D and CDK 4, 6**. So now we can **connect** the **restriction point's** sensation of growth factors and the signalling pathway with the **activation of the Cyc D** in the **G1 phase**.

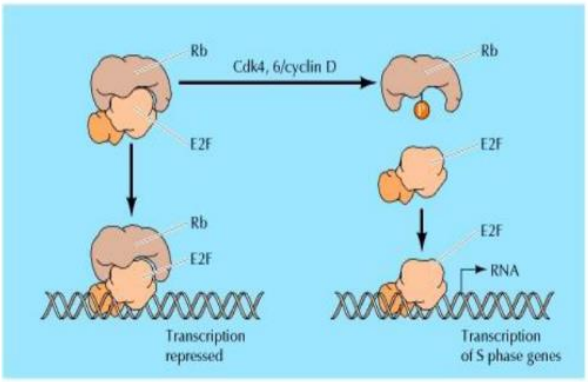


Now what happens if there is a problem in regulation of Cyc D? Assume that it is always active what happens? The cell cycle now passes the restriction point and shifts toward the S phase. So, the possible scenario that will lead to this situation is that there is a mutation in the **signalling pathway** RAS/RAF/MEC/ERK (the commonest is the RAS); so, it is always active; sensing like that there is an abundant GFs even if there is not. Also, **the receptor** may be acquiring a mutation making a conformational change on it keeping it sensing like there is a GF binds to it. And this happens in the cancer cells where the cells have the potency to proliferate.

Another pathway that affects the cell cycle and interacts with the Cyclins is the **retinoblastoma** RB. RB is a gene that when it is active **deactivate** the cell cycle acting like a tumour suppressor gene. RB make a complex with **E2F factor** and the complex bind to DNA and **suppress** gene expression. Once the RB is phosphorylated by **the CDK 4, 6** of the **Cyc D** (in the beginning of the cell cycle) the RB and E2F complex disassembles; so the E2F is activated and can bind to DNA and **activate gene expression**, and the RB is **inhibited** as a tumour suppressor gene.

When **unphosphorylated**, **Rb** binds to **E2F** proteins and **represses** transcription of E2F-regulated genes.

E2F is freed when **Rb** is **phosphorylated** by **Cdk4, 6/cyclin D stimulating** cell cycle progression through restriction point.



What are the target genes of E2F factor? They are a group of genes and proteins that is involved in the **S phase**, so the process of inhibition of the RB by the CDK 4, 6 of the Cyc D/CDK 4, 6 complexes in G1 phase aims to **prepare** the cell for the S phase.

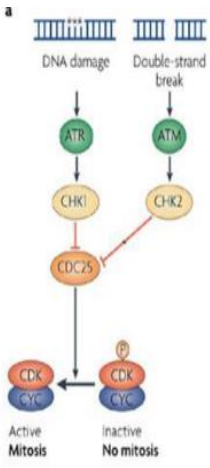
As we said before, there is many check points through the cell cycle, and once they detect a DNA damage; they try to fix it. If it does; it is okay and going to progress in the cell cycle. And if the damage isn't t fixed or the damage is irreversible; cell cycle arrest happens.

How could this happen? Firstly, we must **detect** the damage; and detect if it is a single stranded damage or double stranded break. **Single stranded DNA damage** is going to be detected by **ATR** (a kinase), then ATR phosphorylate and **activate CHK 1** (check 1 kinase), and once it is active it **inhibit CDC 25** (phosphatase) and as a consequence **inhibition of the de-phosphorylation** of the inhibitory

- ATM and ATR are protein kinases
- ATR is activated by ss DNA damage.
- ATM is activated by ds DNA damage.

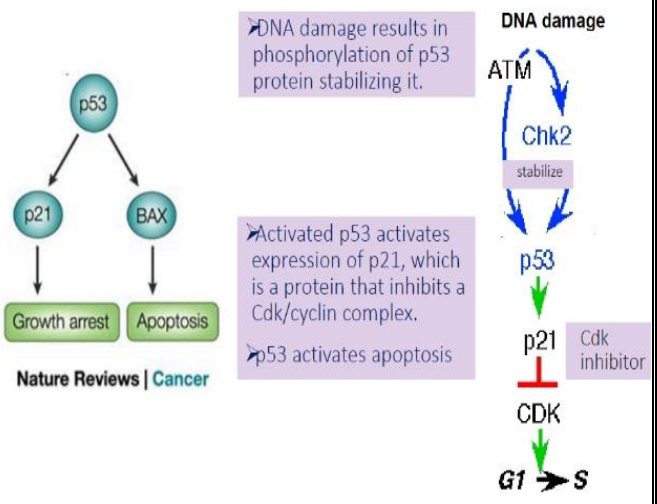
ATR and ATM activate the checkpoint kinases, Chk1 and Chk2, respectively, which inhibit Cdc25 phosphatase.

Phosphatases cannot activate Cdk's causing cell arrest.



phosphates in the CDK; so **inhibiting cell cycle**. Now the **double stranded DNA damage** is detected by another kinase called **ATM** and ATM **phosphorylate CHK 2**, then the CHK 2 **inhibit CDC 25** and then **inhibiting of the cell cycle**.

Now CHK 2 (ATM) in the double stranded damage, **activates p 53** (tumour suppressor gene). Then p 53 is going to **activate p 21** (CKI; which is a CDK inhibitor), so once it is activated it **inhibit the transition to the S phase** (cell cycle arrest) through inhibiting cyclins and CDK required to go to the S phase specifically. Another target of p 53 is a protein called **BAX** (involved in apoptosis); so, once **BAX is active**, the **apoptosis process is active** (note that the damage here is **irreversible**).



The End

If you have any question on the sheet don't hesitate to ask.