Genetics & molecular biology

◉ Sheet ○ Slide

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Microtubules

-Second principal component of cytoskeleton They are rigid hollow rods They are dynamic structures that undergo continual assembly and disassembly within the cell.

-The cytoskeletal element with the largest diameter, which gives them the most rigid structure.

-Functions:

◦ Cell shape

◦ Cell movements (some forms of cell locomotion, lesser extent than actin)

◦ **Intracellular transport of vesicles and organelles**. This is the most important function

◦ Separation of chromosomes during mitosis. Formation of spindle fibres. Actin filaments complete the splitting of the cell by forming a contractile ring.

Structure of microtubules

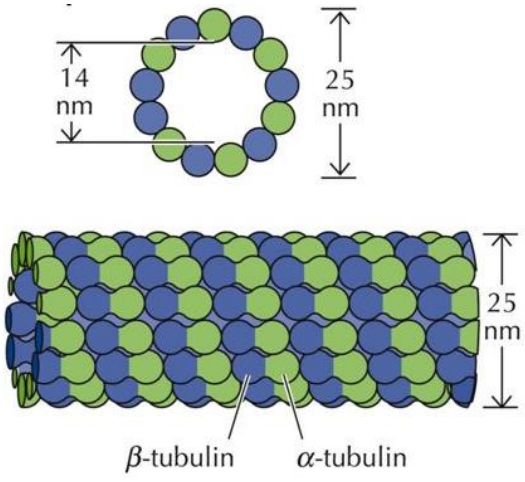
-Microtubules are composed of a single type of globular protein, called tubulin.

-Tubulin is a dimer consisting of two closely related polypeptides (monomers), **α-tubulin and β-tubulin**, which alternate along the chain.

- **γ-tubulin** only exists in the **centrosome.** It initiates microtubule assembly.

-It is a coiled structure. In a cross section there are 13 tubulin monomers.

-Internal diameter is 14 nm. External diameter is 25 nm.

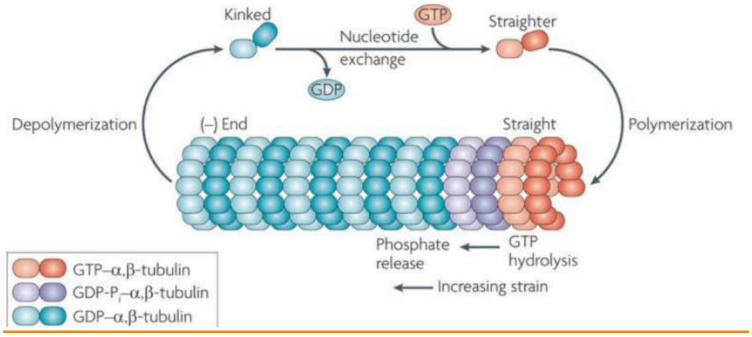


Polymerization of tubulin

-Tubulin dimers polymerize to form protofilaments (head to tail arrays of tubulin dimers). 13 linear protofilaments assemble around a hollow core.

-The dimers are dynamic structures. They are constantly assembling and disassembling.

-They use GTP and GDP to change their conformation. Both **α-tubulin and β-tubulin can bind GTP.**



-A dimer with GTP has a straight structure and it favours polymerization

-A dimer with GDP has a kinked structure, which is less stable, and it will favour disassembly.

- Microtubules are polar structures that have a fast-growing plus end and a slow-growing minus end.

- Polarity determines the direction of movement along microtubules. Plus ends have a greater concentration of GTP bound tubulin. Minus ends have a greater concentration of GDP bound tubulin.

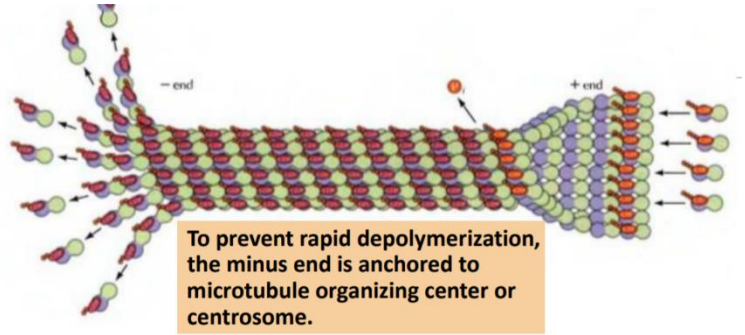
Treadmilling

-It is rapid cycles of assembly and disassembly of microtubules.

-Tubulin molecules are continually lost from the minus end and replaced by the addition of tubulin molecules bound to GTP to the plus end.

-Treadmilling depends on the rates of hydrolysis of GTP to GDP.

-The GTP bonded to β-tubulin is hydrolyzed to GDP during or shortly after polymerization weakening the binding affinity and favoring depolymerization.



To prevent rapid depolymerization, the minus end is anchored to microtubule organizing center or centrosome.

Dynamic instability

-Determined by the rate of polymerization and depolymerization.

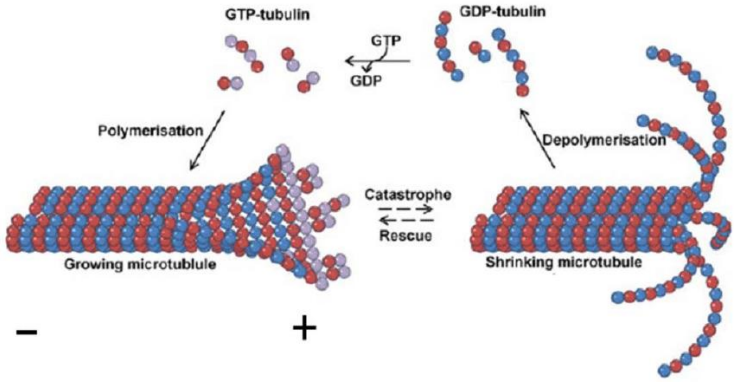
-Alternating cycles of growth (rescue) and shrinkage (catastrophe).

-Growth or shrinkage is determined by the **rate of tubulin addition relative to the rate of GTP hydrolysis.**

-**Catastrophe** occurs when GTP is hydrolyzed at the plus end before new GTP-tubulin is added.

-**Rescue** occurs when GTP hydrolysis is slower than the addition of GTP-tubulin dimers.

-Both processes are important for cell function.



Application

-Drugs that affect microtubule assembly

-**Colchicine and colcemid** bind tubulin, inhibit polymerization, and block mitosis. Used experimentally.

-**Vinblastine and vincristine** bind specifically to tubulin and prevent their polymerization to form microtubules resulting in inhibition of rapidly dividing cells. They stop the formation of spindle fibres. Used in cancer treatment.

-**Taxol** stabilizes microtubule and blocks cell division. It pauses the cell at its current state of assembly. Used in cancer treatment.

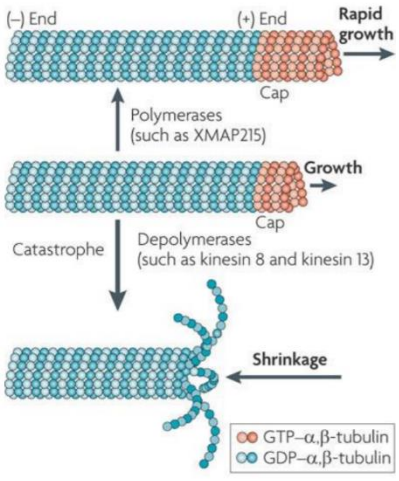
Regulatory proteins

-Microtubule-associated proteins (MAPs) such as:

-**Polymerases** that accelerate growth at the **plus end.**

-**Depolymerases** stimulate shrinkage by accelerating the dissociation of GTP-tubulin from the **plus end.**

-**CLASP** has two actions. It is a MAP that can prevent disassembly (catastrophe) and can promote restarting growth (rescue).



Organization of microtubules within neurons

-Generally, minus ends point towards the nucleus (centre of the cell). The plus end extends toward the periphery of the cell.

-Neurons have two types of processes extend from the cell body:

1-Dendrites: short; receive stimuli from other nerve cells

2-Axon: long; carries impulses from the cell body to other cells. It can be several meters long. Neurotransmitters and many proteins are made by ribosomes in the cell body. These are transported along axons in vesicles via microtubules to the end terminals.

-In dendrites, microtubules are oriented in both directions.

-Microtubules in axons are oriented with their plus ends pointing toward the tip of the axon.

Vesicular transport

-Microtubules-motor proteins such as **kinesin and dynein** move along microtubules in **opposite directions.**

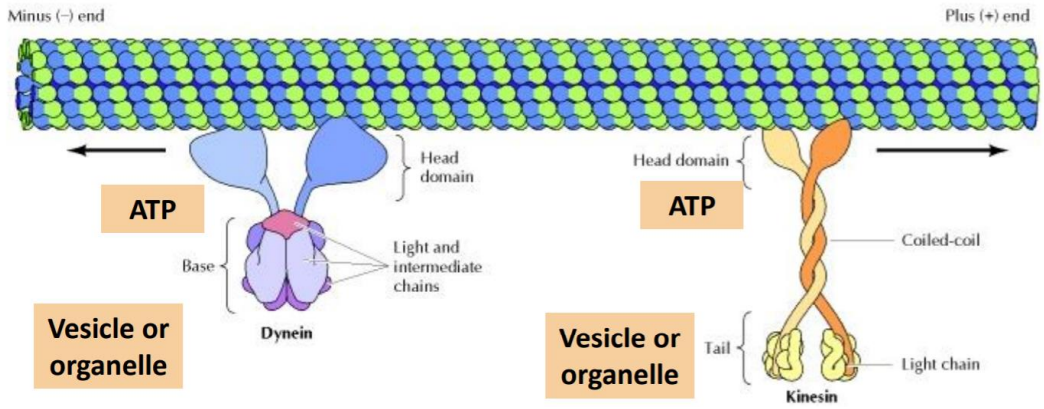
-**Kinesins** move toward the **plus end** (from cell body to periphery of the cell).

-**Dyneins** move toward the **minus end** (from periphery to cell body).

-Both proteins have two heads that interact with microtubules.

-Dynein has a **base** that carries the vesicle or organelle. Kinesin has a **tail** that does the same function.

-They use energy in the form of **ATP**.



Vesicular transport

Kinesin

-In neurons, kinesin assists in transporting vesicles and organelles toward the end of the axon.

-It gets its energy from the hydrolysis of ATP that is bound to the head domain that also binds to microtubule.

-The tail portion binds to cell components, e.g. membrane vesicles and organelles.

Dynein

-The head domain of dynein forms the ATP binding motor domains that are responsible for movement along microtubules.

-The basal portion of dynein is thought to bind to other subcellular structures, such as organelles and vesicles.

Organelle organizations

-Vesicles can be transported in both direction by kinesin and dynein.

-On the other hand, organelles can only move in one direction.

-Kinesin pulls the **endoplasmic** reticulum toward the cell periphery for UPRs (unfolded protein response).

-Kinesin positions **lysosomes** away from the center of the cell towards receptor mediated endocytic vessels.

-Members of the kinesin family control the movements of mitochondria to the periphery after its fission (to distribute energy to all parts of the cell)

-Cytoplasmic dynein positions the **Golgi apparatus** in the center of the cell.

-Both kinesin and dynein transport selective **mRNA molecules** in cell.

Stimulated movement

-Organelles often have **both types of motors** (dynein and kinesin) on their surface, allowing cells to adjust their position.

-Melanocytes position the pigmented organelles, melanosomes, in response to the amount of light.

-In the presence of **light**, **kinesin** moves melanosomes to the periphery of cells.

-In the **dark**, **dynein** returns the melanosomes to the center of the cell.

Kinesins and diseases

-Mutations in certain kinesin proteins reduce the ability of neurons to move essential organelles **from their cell bodies to their axons** leading to neurodegeneration such as **amyotrophic lateral sclerosis (ALS).**

-Mutations in kinesins lead to peripheral nervous system neuropathies such as **Charcot-Marie-Tooth disease.**

-Kinesin has many subtypes. There are more than one gene to be mutated, so different disease arise.

Changing horses in midstream

-Cell cortex is made **of actin filaments**. Kinesin cannot move on actin to deliver organelles to plasma membrane. Organelles are thus moved to myosin.

-Myosins transport organelles over **shorter distances** compared to kinesins and dyneins.

-Kinesins and myosins transport organelles from the center of the cell towards the periphery, where myosins take over moving organelles **near the plasma membrane.**

Intermediate filaments (IFs)

-Their diameter **is intermediate** between those of actin filaments and microtubules.

-They **connect** actin filaments with microtubules.

-They are **non polar** but still have a dynamic structure.

-They provide **mechanical strength** to cells and tissues.

-Sites for cellular processes.

-**Not involved directly** in cell movement.

-They are the most diverse group, which are classified into 5 groups based on similarities between their amino acid sequences.

Types of IFs Proteins

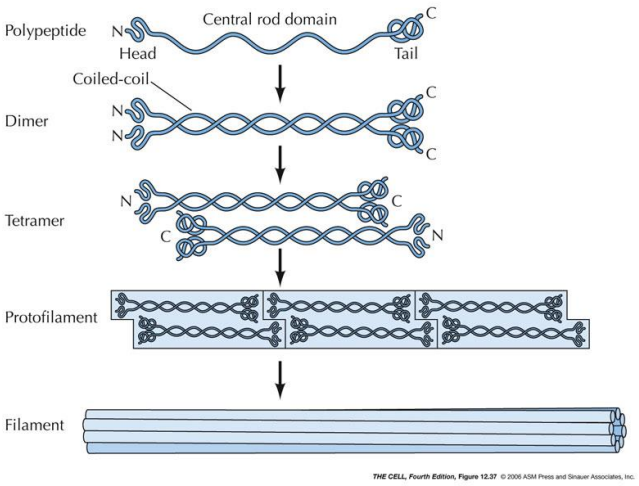
Type	Site	Monomers		
I	**Epithelial cells**	**Acidic keratin**		Each cell type makes at least one type I and one type II keratin. -Hard keratins are used for production of structures such as hair, nails, and horns. -Soft keratins are abundant in the cytoplasm of epithelial cells.
II	**Epithelial cells**	**Basic/neutral keratin**		
III	**Muscle cells, fibroblasts, WBC**	**Desmin**	**Vimentin**	-Vimentin is found in fibroblasts, smooth muscle cells, and white blood cells. -Desmin is specifically expressed in muscle cells.
IV	**Mature neurons, axons of motor neurons**	**Neurofilament proteins**	**Nestin**	-Nestin in stem cells
V	**Nuclear envelope**	**Lamins**		-Components of the nuclear envelope.

Structure of Ifs and assembly

-A monomer has a **head (N terminus), tail (C terminus),** and an α-helical central rod.

-Different monomers have variable sizes of heads and tails.

-Assembly starts with the formation of dimers. With two tails facing each other and two heads facing each other.



-The dimer can be a homodimer (same monomers), or a heterodimer (different monomers).

-Then a tetramer is formed by two dimers**. Now two heads face two tails at each end making the structure non polar.**

-A protofilament is formed by repeating tetramers.

-**8 protofilaments** form an intermediate filament.

Interaction of IF types

-Keratin filaments are always assembled from heterodimers **containing one type I and one type II polypeptide.**

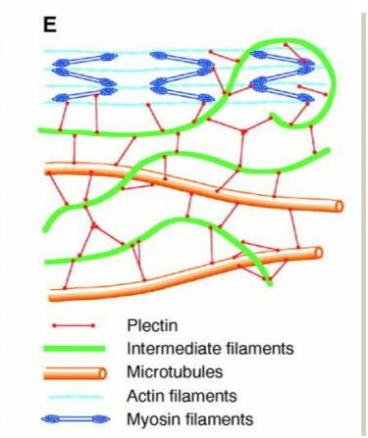
-The type III proteins can assemble into filaments containing only a single polypeptide (e.g., vimentin) or consisting of two different type III proteins (e.g., vimentin plus desmin).

-**The type III** proteins **do not** form copolymers with **the keratins**.

-**α-internexin, a type IV protein**, can assemble into filaments **by itself** (dimers and tetramers are homo), but the **neurofilament proteins copolymerize to form heteropolymers**.

-Phosphate groups are negative. If heads and tails are phosphorylated, they will repel. This causes disassembly. Disassembly is needed at cellular division to disrupt nuclear lamina.

Intracellular Organization of IFs



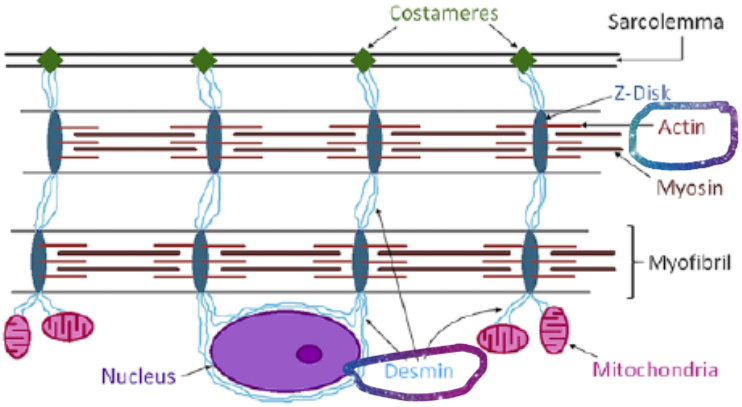
-IFs form an elaborate network in the cytoplasm extending from a ring surrounding the nucleus to plasma membrane

-Both keratin and vimentin filaments attach to the nuclear envelope to position and anchor the nucleus within the cell.

-IFs can associate not only with the plasma membrane but also with the **actin filaments and microtubules** (connect them indirectly via proteins).

-IFs provide a scaffold that integrates the components of the cytoskeleton and **organizes the internal structure of the cell**.

Desmin IFs in muscles



-Desmin connects the actin filaments in muscle cells to one another and to the plasma membrane, thereby **linking the actions of individual contractile elements**.

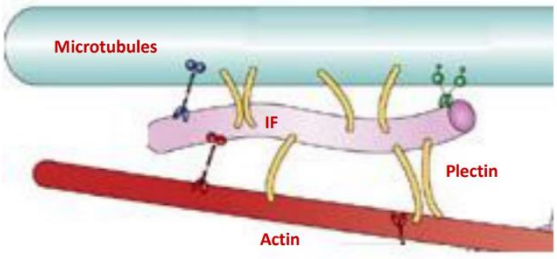
-Desmin mutations cause muscle defects such as early onset **cardiomyopathy.**

Neurofilaments in neurons

-Neurofilaments in mature neurons are **anchored to actin** filaments and **microtubules** by neuronal members of the **plakin family**.

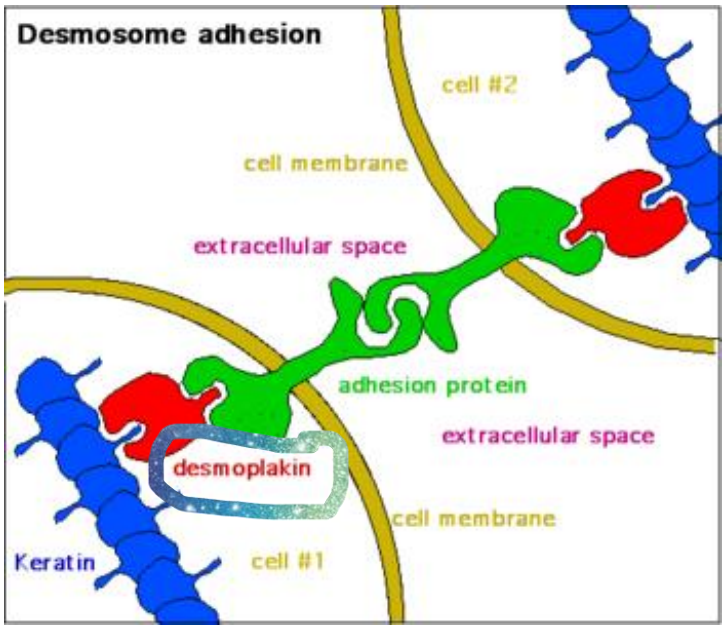
-Neurofilaments provide mechanical support and stabilize the cytoskeleton in the long, thin axons of nerve cells.

Plectin connects IFs to other cytoskeletal elements



-Plectin **bridges microtubules to actin filaments** and **stabilizing** them and increasing the mechanical stability of the cell.

Cellular Junctions: Desmosomes

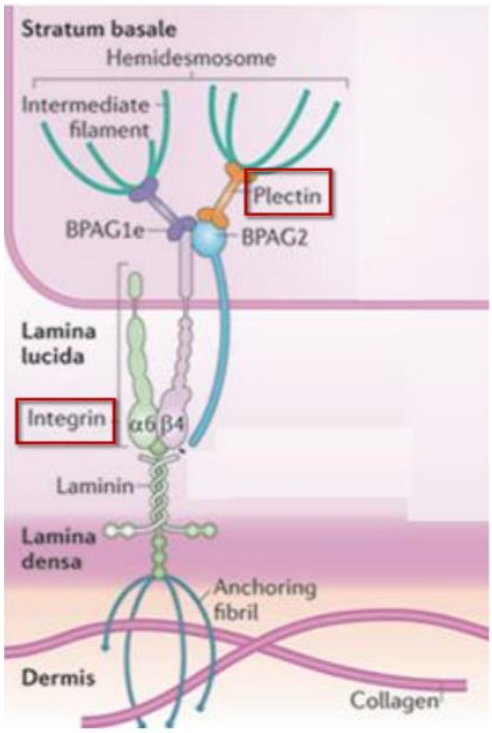


-The **keratin filaments** of epithelial cells are tightly anchored to the plasma membrane at two areas of specialized cell contacts, desmosomes and hemidesmosomes **by the desmoplankin family of proteins.**

-Desmosomes anchor IFs to **regions of cell-cell contacts.**

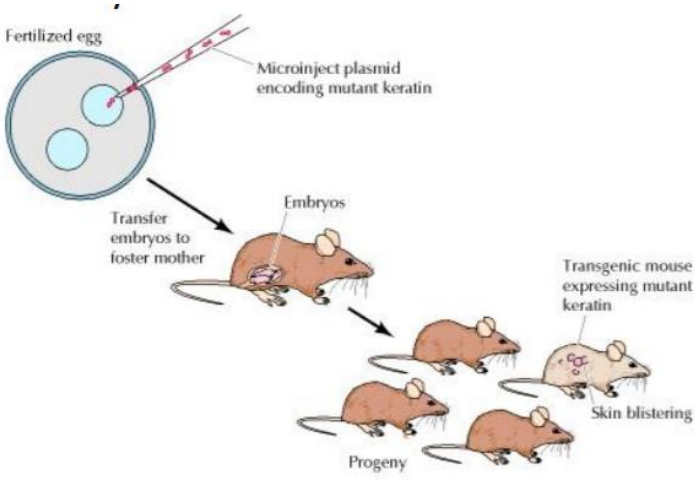
-Keratin filaments are connected to **integrins** (plasma proteins). The integrins interact with another integrin from the neighbouring cell.

-Keratin filaments anchored to both sides of desmosomes serve as a mechanical link, thereby providing **mechanical stability to the entire tissue.**



-**Hemidesmosomes**: they have half the structure of a desmosome. The consist of the IF, a connector protein, and an integrin. The integrin is connected to the **extracellular matrix.**

Application: IFs and diseases



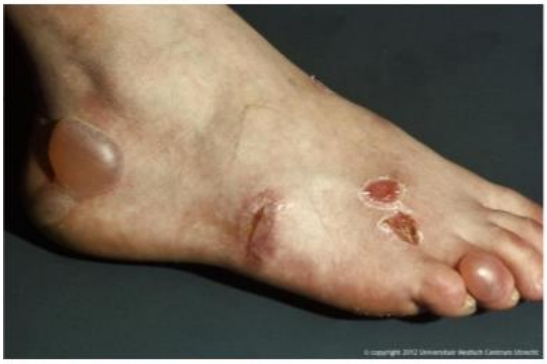
-Previously, disruption of vimentin in fibroblast cells did not affect cell growth or movement.

-Hypothesis: IFs are most needed to strengthen the cytoskeleton of cells in the tissues of multicellular organisms.

-A plasmid with a mutant keratin type I or II was injected into a fertilized mouse egg, then inserted to a mouse's uterus.

-Transgenic mice expressing mutated keratins resulted in mice with severe skin abnormalities due to defect in desmosomes (**blisters** due to epidermal cell lysis following **mild mechanical trauma**).

IFs and Human diseases



-**Human epidermolysis bullosa simplex** is caused by keratin gene mutations that interfere with the normal assembly of keratin filaments causing skin blisters after minor trauma.

-**Amyotrophic lateral sclerosis** (ALS), also known as Lou Gehrig's disease is characterized by the accumulation and abnormal assembly of neurofilaments.

-It is also caused by a mutation in **kinesin** as mentioned earlier.

-The famous astrophysicist Stephen Hawking was diagnosed with ALS when he was young.

Good Luck