**Lec. 7:**

**Intracellular acting toxins**

**Diphtheria toxin :**

**Diphtheria is aserious respiratory disease characterized by the formation of pseudomembrane in the throat consisting of necrotic of necrotic tissue and bacteria .**

**Corynebacterium diphtheriae , visualized in 18883 by Klebs in stained sample from pseudomembrane , ( Diphtheria toxin was discovered in late 1800 s ) .**

**C. diphtheriae colonizes the mucous membrane of the upper respiratory and secrets diphtheria toxin , but the toxin is produced only by strain of C. diphtheria infected with lysoyenic bacteriophages which carry the ( tox) gene with in their genome .**

**Diphtheria is easily transmitted from person to another .**

**The minimum lethal dose of diphtheris toxin for human is below 0.1 Mg/ Kg of body weight ( Delivery of single molecule of toxin fragment A to cytosol is sufficient to kill a eukanyotic cell .**

**Diphtheria toxin was the first ( A-B ) TYPE TOXIN TO BE characterized .**

**A-B type toxins have two functionally components :**

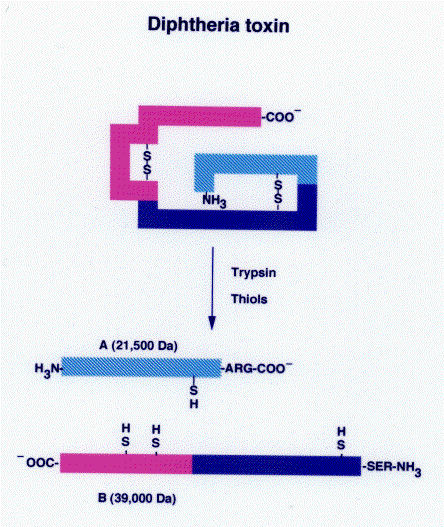
**A, the moiety represcuting the cvatalytic ( toxic ) function**

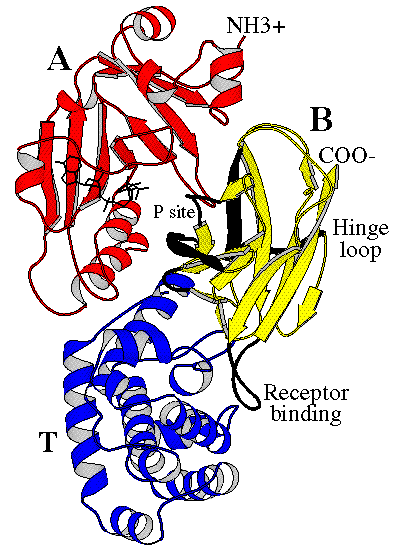
**The structure of Diphtheria Toxin**

**The mature secreted toxin is 535 amino acid residues in lenghth and has a MW of = 58 KDa .**

**The holotoxin contain contains 4 cysteines that participate in two disulfide bonds .**

**The aminoterminal fragment ( A ) MW = 21 KDa is the catalytic , the carboxy terminal fragment ( B ) MW = 37 KDa contains the receptor binding ( R ) and Translocation portion of the toxin ( T )**





**Most mammalian species are sensitive to the effects of diphtheria toxin , but mice and rats and cell lines derived from these animals are highly resistant to this toxin . The resistance is due to lacking of functional receptor on cell surface .**

**Mechanism of entry :**

**After binding to the specific receptor ( pro Heparin – Binding – Epidermal growth factor )**

**( pro – HB – EGF ) on the surface of susceptible cell , diphtheria toxin internalized to the cell by two mechanisms :**

**The first one is the direct entry :**

**The B subunit of the native ( A + B ) toxin binds to a specific receptor on the target cell and induces the formation of opore in the membrane through which the A subunit is transferred ino cell cytoplasm .**

**The second mechanism , the native toxin binds to the target cell and A+B structure is taken into the cell by the process of receptor mediated endocytosis ( RME ). The toxin is internalized in cell in a membrane – enclosed vecicle called endosome .**

**+**

**H ions enter the endosome lower the internal PH which causes the A+B subunik to separate . The B- subunit affects the release of A- subunit from the endosome so that it will reach it target in the cytoplasm , where as the B- subunit remains in the endosome and recycled to the surface .**

**The intoxication of a single eukaryotic cell by diphthtoxin involves :**

**1- the binding of the toxin to its cell surtace receptor .**

**2- clustering of charged receptors into coated pits and internaligation of the toxin by ( RME ) : following acidifications of the endosome by ATP – driver +proton pump .**

**3- insertion of transmenbram domaia into the membrane and facilitated delivery of the catalytic domain to the cytocol .**

**4- the ADP – ribosylation of EF-2 which results in the irreversible inhibition of protein synthesis .**

**It has been shown that a single molecule of the catalytic domain delivered to the cytosol is sufficient to be lethal for the cell .**

**EF2 + NAD+  → ADPR – EF-2 + nicotinamide + H +**

**The reaction forms a covalent bond between ADPR and EF2 leading to blocking of the protein synthesis .**

