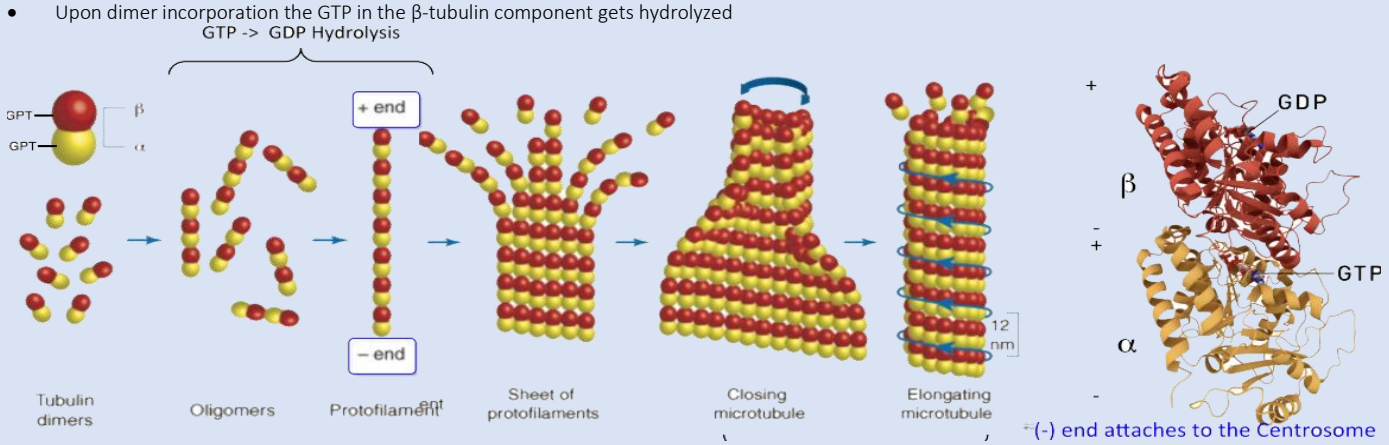
MICROTUBULES:

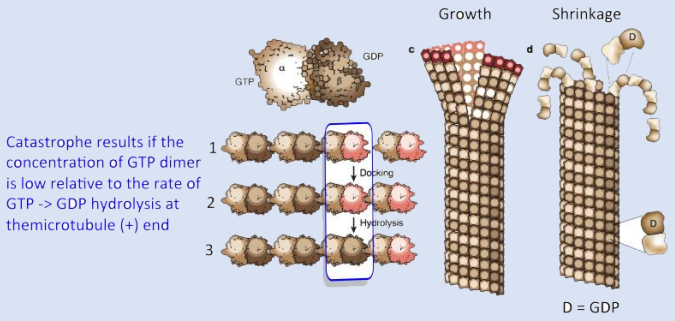
MICROTUBULE FORMATION: TUBULIN DIMER → PROTOFILAMENT

- Tubulin heterodimers made up of α- and β-tubulin (50 kDa each in size)
- α-and β-tubulin bind GTP (GTP α-tubulin is stable/GTP β-tubulin is primed for hydrolysis)
- Microtubules grow through "end-to-end" polymerization of α/β-tubulin-GTP dimers
- First steps lead to formation of a linear "protofilament" (approx. 13 tubulin units)
- Upon dimer incorporation the GTP in the β-tubulin component gets hydrolyzed



MICROTUBULE STRUCTURATION IS HIGHLY DYNAMIC:

- During polymerization to the tubulin (+)-ends switch between slow growth and rapid shrinkage = **dynamic instability**
  - Switch from growth to shrinking = **catastrophe**
  - Addition of new GTP-tubulin dimers to increase length = **rescue**



Catastrophe results if the concentration of GTP dimer is low relative to the rate of GTP -> GDP hydrolysis at themicrotubule (+) end

MICROTUBULE BINDING AGENTS TARGET 3 MAIN SITES:

1. **Colchicine domain:** interface or αTub-βTub junction
   - Important feature in binding: **angle of the A- and C- rings**
2. **Vinca alkaloid domain**:
   - **Vinblastine (R = Me) and Vincristine (R = CHO)**
     - Both compounds are **"vinca"-type alkaloids** (natural products/secondary metabolites)
     - Isolated from the Periwinkle plant (Vinca rosea/C. roseus)
   - **SAR (structural requirements for activities) ****
     - **Peripheral modifications**
     - **Angle** between the two indole-type rings
     - **Configuration** at the center joining the two components
     - **Conformation** of the 9-membered ring
       - "Ring Flip" = higher barrier to conformation shift
3. **Taxol domain:**
   - R = Ph, R' = Ac (**Paclitaxel**)
   - R = t-Bu, R' = H (**Docetaxel**)

CONSEQUENCES OF TUBULIN BINDING:

- At low concentrations both stabilizers and destabilizers suppress microtubule dynamics w/o changing polymer mass (i.e. **slows things down**)
- At higher concentrations:

Vinca alkaloids	Taxols
VLB and cogeners are classified as destabilizing agents that cause: • Microtubule depolymerization • **Suppress dynamic instability** • Inhibit mitotic progression	Taxol binding stabilizes microtubules, resulting in: • Increased polymerization • **Suppression of microtubule dynamics** • Increase polymer mass by inhibiting the dissociation of tubulin (yet allowing addition at both (-) and (+) ends of the microtubule
This ultimately results in cell death by apoptosis.	This leads to cell cycle arrest in the G2/M phase, and ultimately, apoptosis.

- Note: the GTPase activity of β-tubulin requires association with α-tubulin (dimeric state)
- Conjecture: the tubulin binding agents probably interfere with the GTPase activity within the dimer motifs

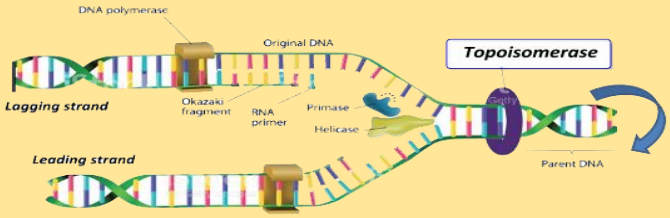
RESISTANCE (IN ADDITION TO P-GP EFFLUX PUMP):

- The structure of tubulin has been highly conserved throughout evolution and tubulin isotypes share a high degree of amino acid homology, except at their unique carboxy-terminal tails (20-27 AAs)
- Abnormal and high levels of expression **of βIII-tubulin**, an isotype that is normally expressed at high levels in neuronal cells and testicular Sertoli cells, associated with more aggressive and resistant cancers
- **Why?**
  - Paclitaxel is thought to diffuse through nanopores in the microtubule to reach its binding site on the interior-facing lumen of the microtubule – mediated by the formation of a hydrogen bond that involves serine 275 in all β-tubulin isotypes, except **βIII-tubulin and βVI-tubulin**

TOPOISOMERASE I/II INHIBITORS AS ANTICANCER DRUGS:

DNA REPLICATION: WHY TOPOISOMERASES

- In all cells, completely replicated chromosomes must be untangled by DNA topoisomerases before partitioning and cell division can occur
- The issue is DNA "unwinding" (release of strain)
  - How: temporarily make single/double strand cleavages, then rezip
  - For a replication fork moving at 500 nucleotides/sec, the parental DNA must rotate 50 revolutions/sec

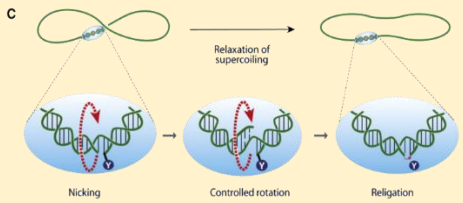


- A topoisomerase is a "reversible nuclease"
  1. **Reacts covalently via a Tyr to form a transient 3'-phosphotyrosine** intermediate from cleavage of the phosphodiester bond(s)
  2. **(Reverse) step**: **phosphodiester bonds are remade**

h-TOPOISOMERASE I

h-TOPOISOMERASE IB: single strand cleavage/relegation in ds-DNA

- Monomeric (isoforms: **Topo 1B** Eukarypte/**Topo 1A** mainly prokaryote)
- Only **tyrosine** residue involved in catalysis (no metal Zn^{2+}/Mg^{2+} ion involvement)
  - One strand is bound via Tyr during rotation
- Reversible enzyme – breaks bond to same phosphate
  - No ATP energy requirement (supplied by release of torsional strain)



1. Top1 binds DNA cross-overs (supercoils) and nicks DNA by transesterification
2. Allows DNA to swivel by controlled rotation
3. Upon DNA realignment by base pairing & stacking across the nick, the DNA 5'-hydroxyl end removes tyrosyl linkage by reverse transesterification

TOPOISOMERASE I INHIBITORS AS ANTICANCER DRUGS:

- Ironetecan (prodrug) → SN-38 (active component
- Camptothecin
- Topotecan
  - Topotecan **mimics a DNA base pair** and binds at the site of DNA cleavage by **intercalating** between upstream (-1) and downstream (+1) base pairs
  - Intercalation displaces downstream DNA = **preventing relegation** of the cleaved strand
  - By specifically binding to the enzyme-substrate complex, Topotecan = **uncompetitive inhibitor**
  - Topo residues R364, n-363 & F361 = mutations lead to drug resistance

h-TOPOISOMERASE II:

h-TOPOISOMERASE IIA/B: double strand cleavage/relegation

- Humans express 2 paralogs/isoforms: TopoIIA (Iiα) & IIB (Iiβ) – 78% identity in Cat. Site
- Cleaves the opposing DNA strands with a **four-base stagger** via Tyr cleavage to produce 5'phosphotyrsoine → relegation emulates DNA synthesis
- Requires Mg^{2+} and ATP (hydrolysis required for enzyme turnover/rapid kinetics)

TOPOISOMERASE II INHIBITORS AS ANTICANCER DRUGS:

- Destabilizes microtubule polymerization (binds to colchicine site, note **orientation of Ar groups**)
  - Etoposide
    - Binding of 2 molecules of etoposide in "cleavage complex" → increase in distance between 5'phosphotyrosine (+1) and 3'OH in residue (-1) obstructs ligation = hinders bond formation, leading back to ds-DNA → lesions in DNA → cell death
    - Mutations in residues involved in drug binding, DNA binding or catalytic functions can confer resistance
  - Tenipose = increase H-bonding
  - Podophyllotoxin
- Doxorubicin
  - Intercalation of doxorubicin interferes with the progression of Typo II along the DNA chain
  - Doxorubicin stabilizes the Topo II complex of ds-cleavage, preventing the relegation step