Cancer Biology I :

Topics covered

Week 5:
Lecture 5/Exercises-paper: Telomeres and cellular senescence
(Chapters 10 (Weinberg))

Weeks 6:
Lecture 6/Exercises-Q&A: Telomeres: length and cancer, aging, mouse models;
CDKs and G1/S control
Wednesday: CDKs as drug target, protein-protein interactions; Q&A session
(Chapter 8 (Weinberg book): pRb and control of the cell cycle clock)

1 week break

Week 7, Monday: Q & A session: discussion of your questions
(to be submitted via email to me in advance during week 6!!!)
Wednesday October 30st 2024: exam in **CM 1 121**(contrôle continu)

From Monday:

Pairing of Cyclins with Cyclin-Dependent Kinases

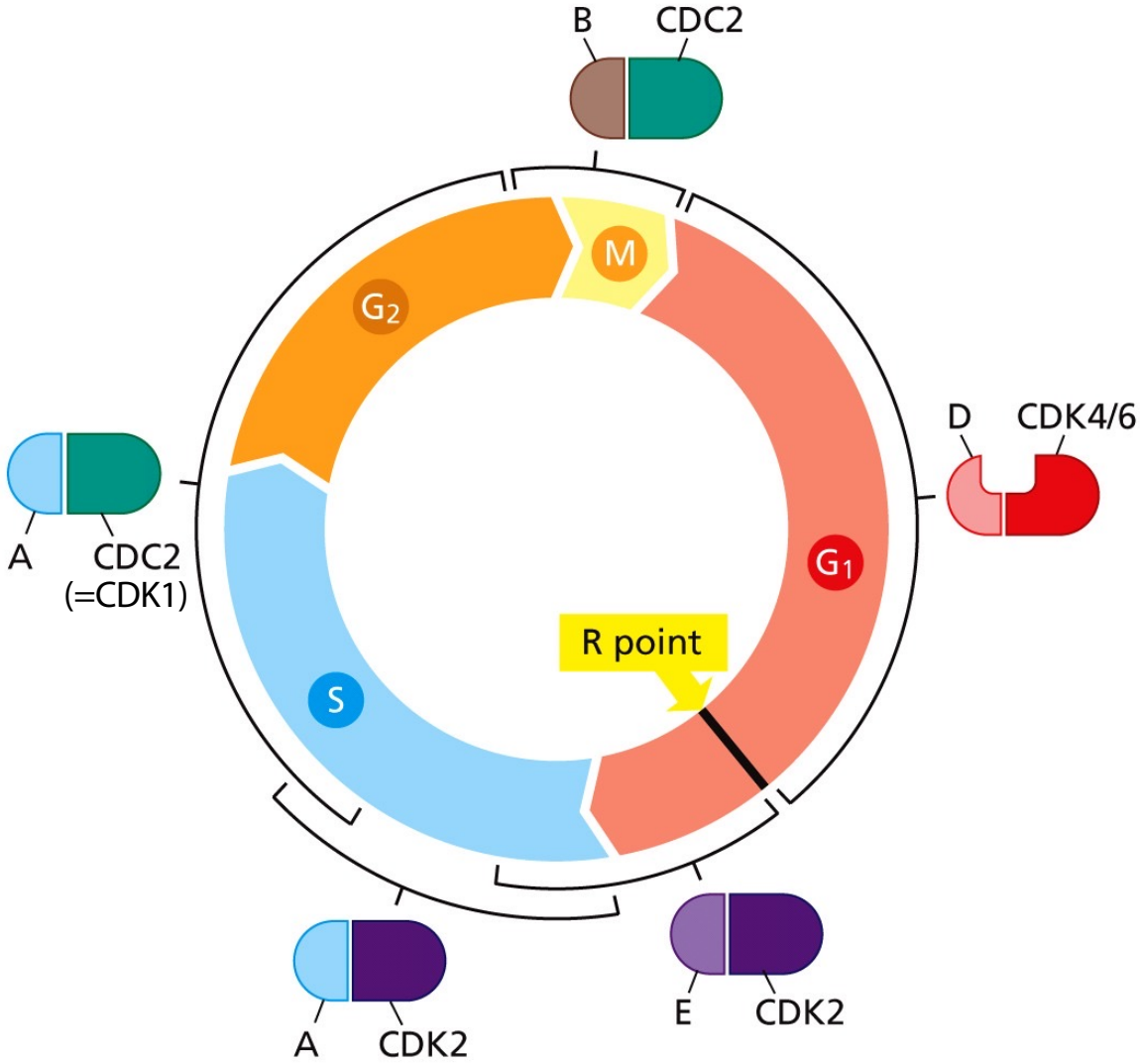


Figure 8.6. Weinberg, The Biology of Cancer

From Monday:

Pairing of Cyclins with Cyclin-Dependent Kinases

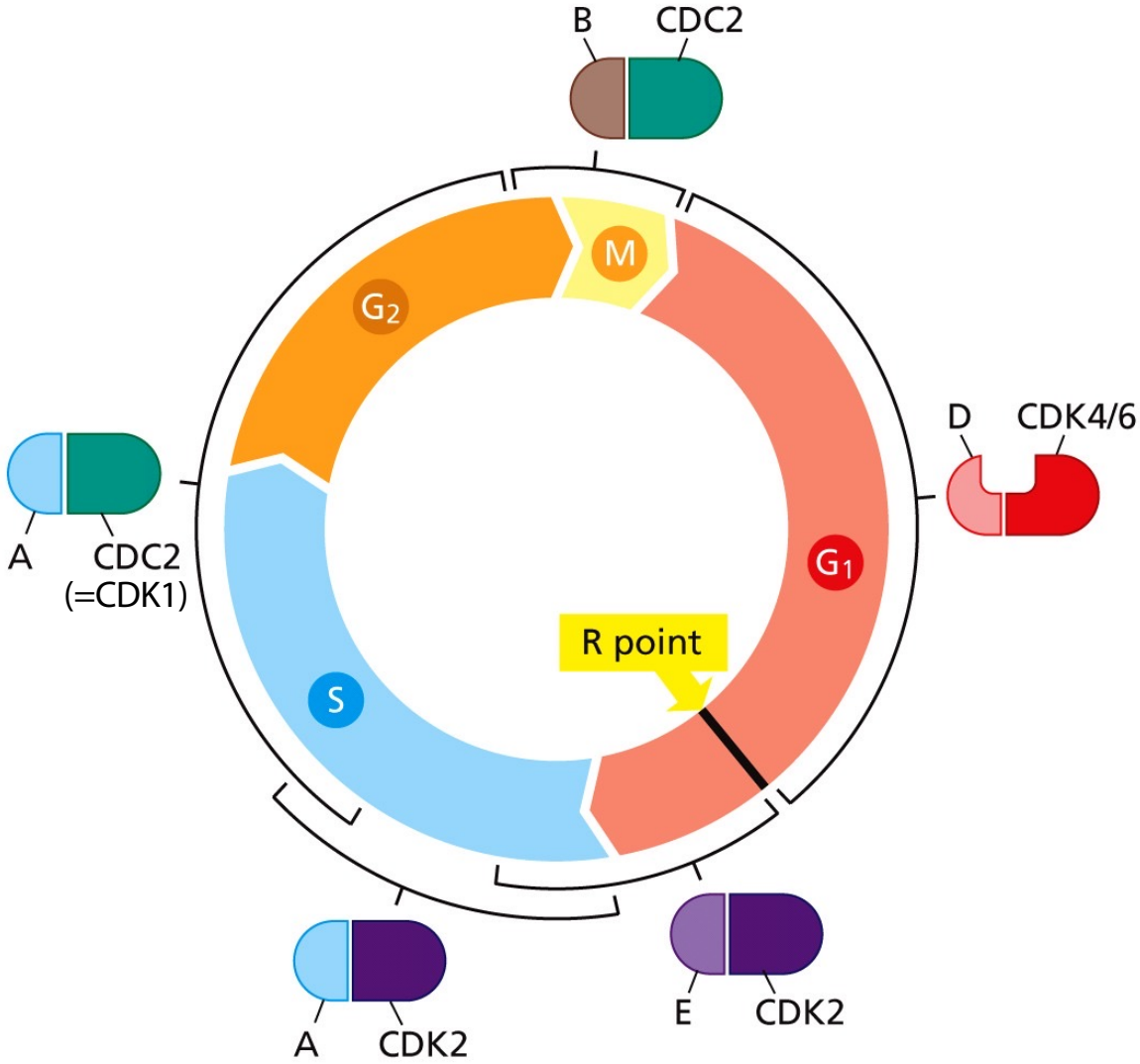


Figure 8.6. Weinberg, The Biology of Cancer

- D-type cyclins: convey signals from the extracellular environment.
- D-type cyclins assemble with CDK4 and CDK6 both of which have similar enzymatic activities.
- Cyclin D1, D2, D3: induced by different transcription factors.

From Monday:

Cyclin-CDK complexes: regulation by 7 CDK-inhibitors (CKIs)

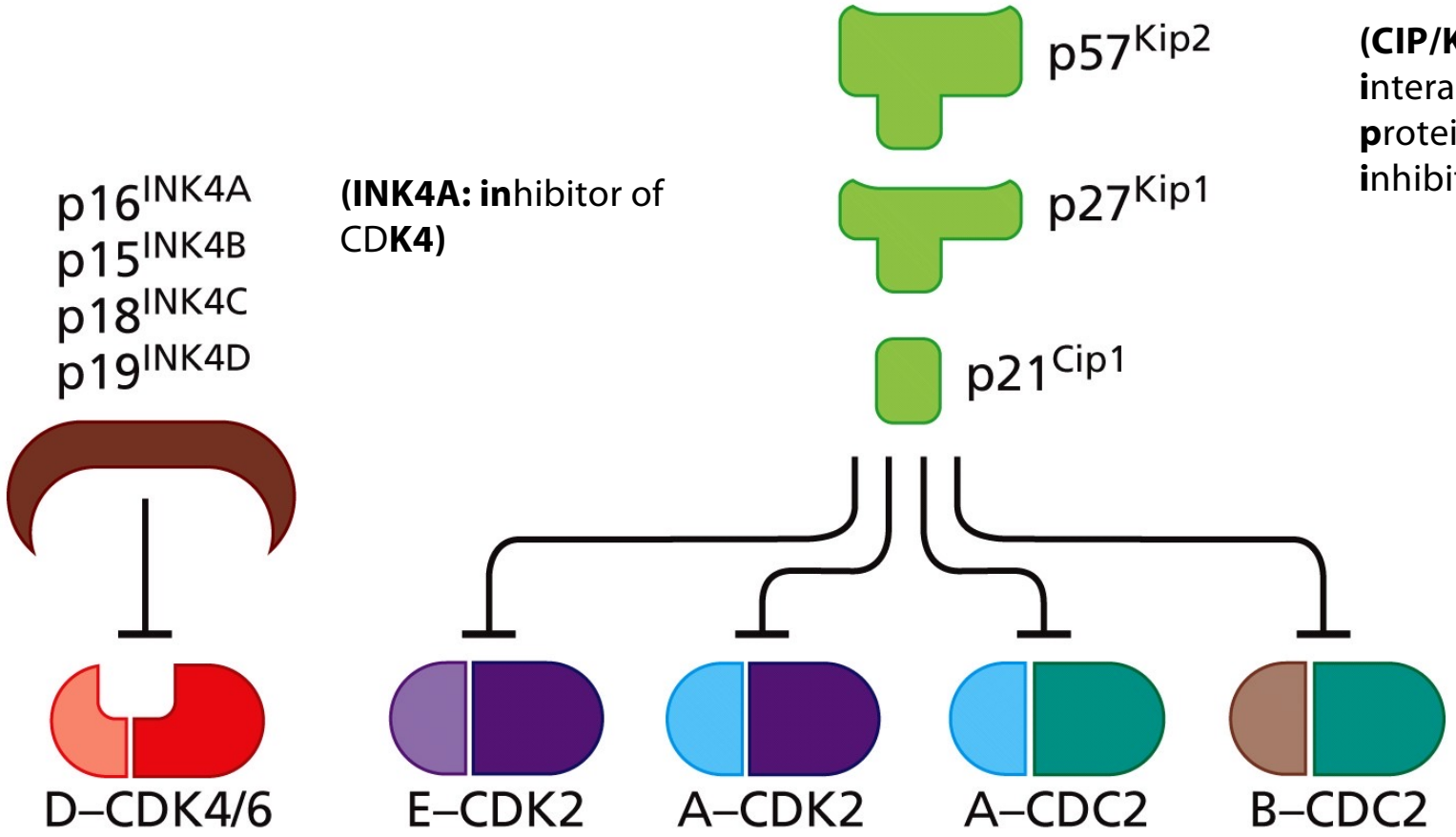


Figure 8.13a The Biology of Cancer (© Garland Science 2014)

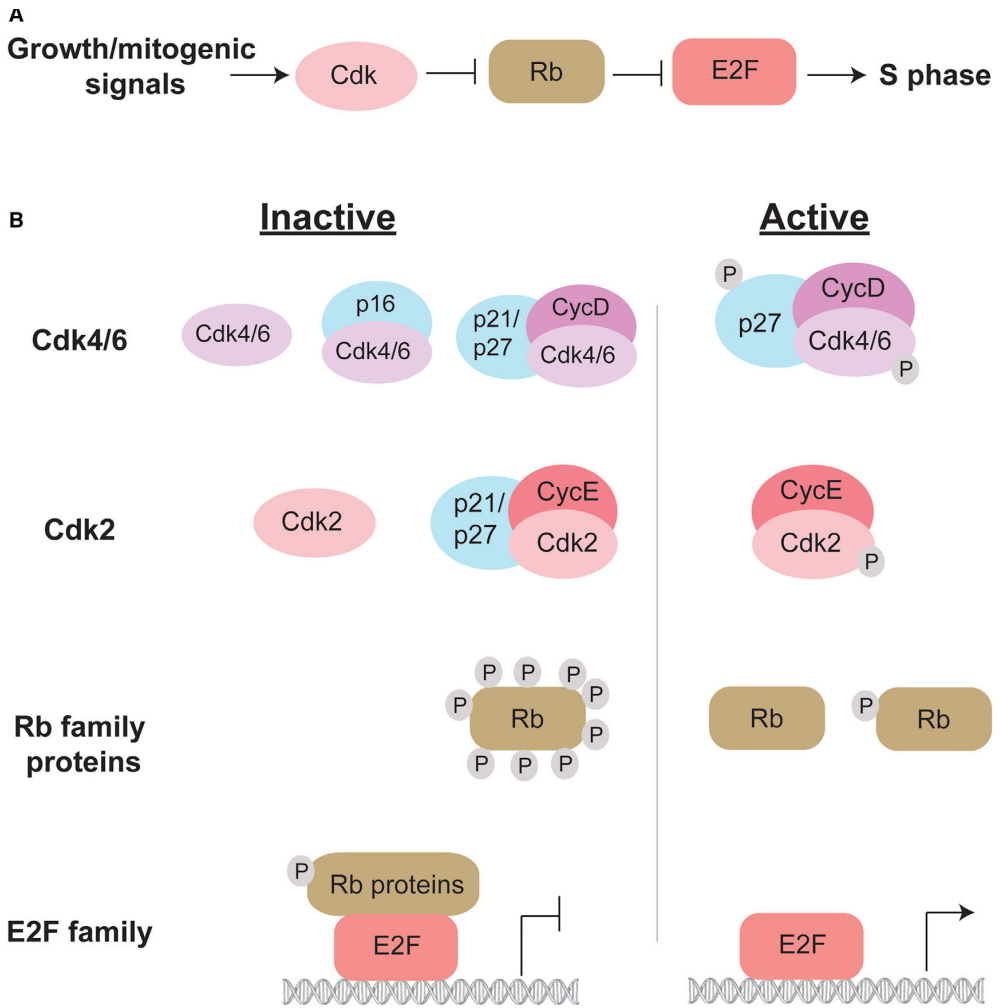


Figure 1. Components of the Cdk-Rb-E2F Pathway Controlling the G1/S Transition

(A) Simplified linear model for the pathway.

(B) Inactive and active states of the key players in the Rb pathway. Cdk4/6 have relatively high sequence homology among Cdks. They are inactive as monomers, bound to p16 family proteins, or bound by unphosphorylated p21/p27 proteins. Cdk4/6 are activated by association with CycD family proteins, but full activity also requires a phosphorylated form of p27 in the complex and phosphorylation on the kinase activation loop. Cdk2 is inactive as a monomer or in complex with p21/p27 family proteins, and it is activated by CycE binding in G1 (or by CycA later in the cell cycle) and activation loop phosphorylation. Rb is considered active when hypophosphorylated or monophosphorylated; in this state, it binds and inhibits E2F. Hyperphosphorylation of Rb leads to its inactivation, dissociation from E2F, and subsequent E2F activation.

Why should CDKs be valuable drug targets?

- Canonical CDK genes were thought to be essential; however, viable mice develop from knockout of CDK2, CDK4, or CDK6 and only CDK1 knockout mice are embryonic lethal.
- Studies in mice describing compound knockout of CDKs 2, 3, 4, and 6 illustrate the ability of CDK1 to support cellular proliferation by binding various cyclins.

…but some tumor subsets depend on CDK4/6 or CDK2 for their proliferation.

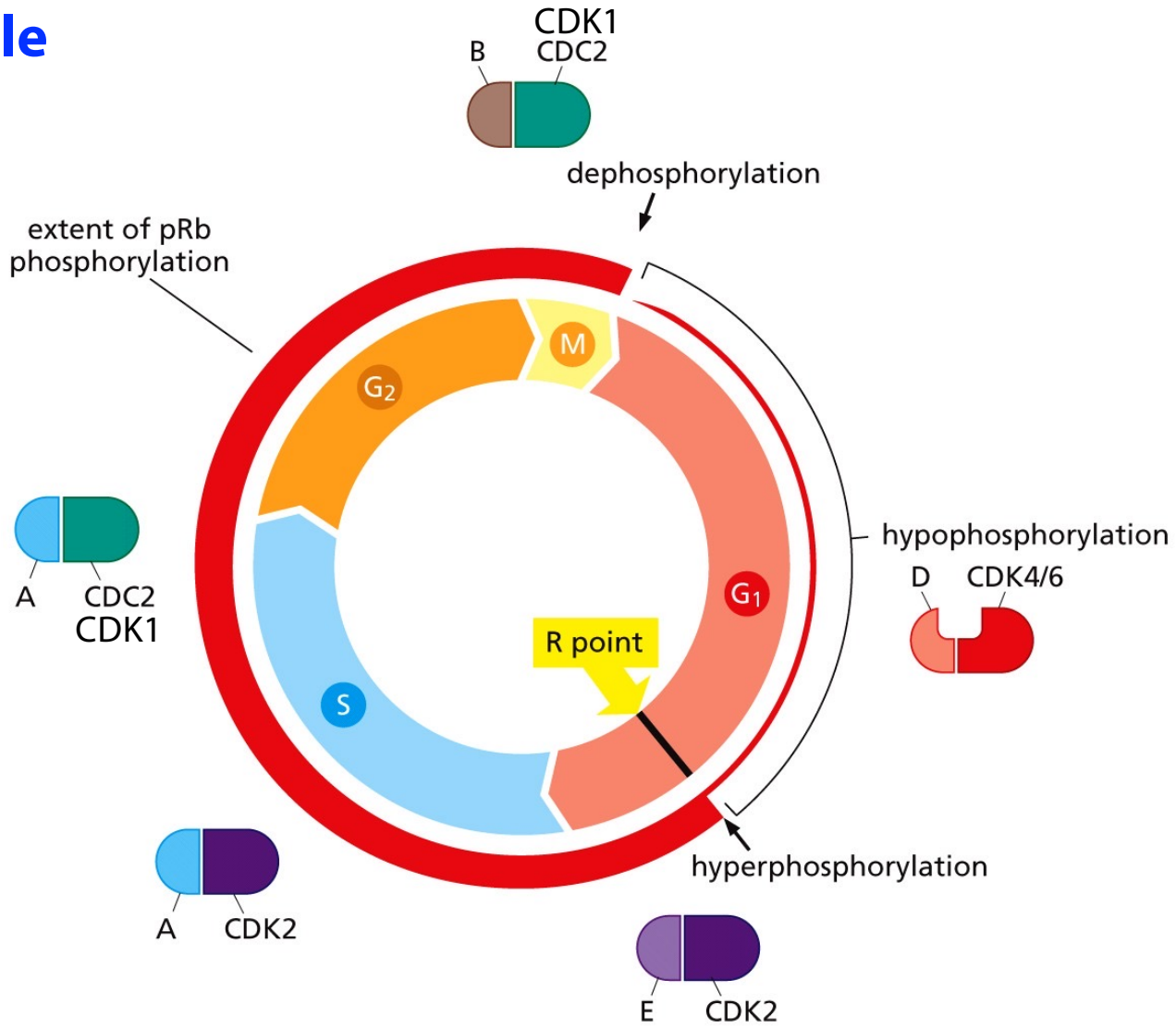
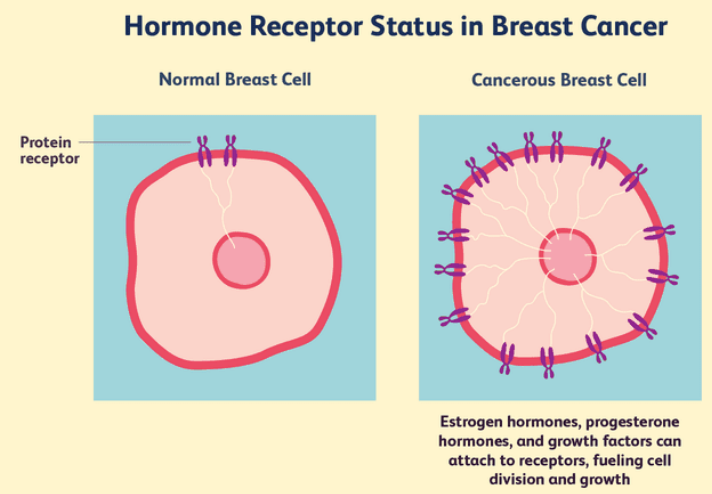
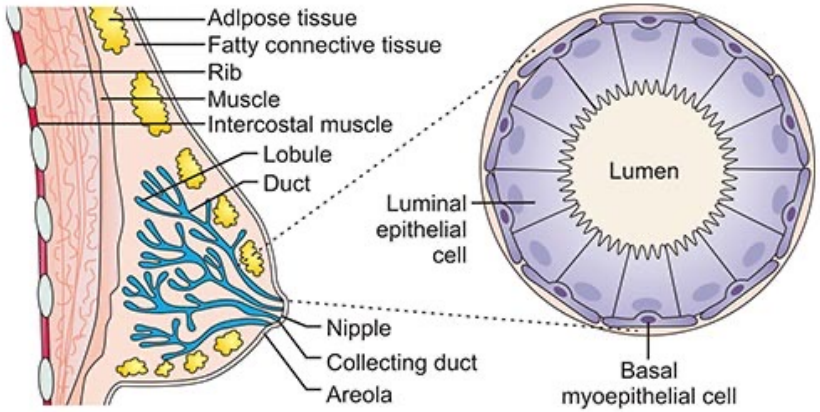


Figure 8.19 The Biology of Cancer (© Garland Science 2014)

The CDK4/6 inhibitor palbociclib (PAL) significantly improves progression-free survival in hormone receptor positive breast cancer (combined with anti-hormonals).





In **luminal estrogen receptor (ER) positive breast cancer**, representing approximately 75% of breast cancer, **ER signaling activates the cyclin D1 promoter**.
ER-positive, luminal breast cancer presents the archetypal model for **CDK4/6 inhibitors**, reflecting the particular dependence of luminal breast cancer on cyclin D1 to initiate G1-S phase transition.

In contrast to luminal breast cancer, **basal-like triple negative breast cancer** is characterized by **loss of RB1** and by **high expression of cyclin E**. Consequently basal-like breast cancer cell lines are resistant to CDK4/6 inhibition (triple negative: breast cancers that are not fueled by estrogens, progesterone, or growth factors binding to HER2 receptors)

Hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative breast cancer is the most common breast cancer subtype, and endocrine therapy (ET) remains its therapeutic backbone. Although **anti-estrogen therapies** (e.g. Tamoxifen) are usually effective initially, approximately 50% of HR+ patients develop resistance to ET within their lifetime, ultimately leading to disease recurrence and limited clinical benefit. The recent addition of **cyclin-dependent kinase 4 (CDK4) and CDK6 inhibitors** (palbociclib, ribociclib, abemaciclib) to ET have remarkably improved the outcome of patients with HR+ advanced breast cancer (ABC) compared with anti-estrogens alone, by targeting the cell-cycle machinery and overcoming some aspects of endocrine resistance.

(Classification:
Breast cancers that are estrogen receptor-positive (ER+) and/or progesterone receptor-positive (PR+) are "fueled" by hormones. They are different from breast cancers that are HER2-positive (human epidermal growth factor receptor 2), in which tumor growth is driven by growth factors that bind to HER2 receptors on the cancer cells (see week 1: Herceptin monoclonal antibodies for the treatment of HER+ breast cancer).

Breast cancers that don't have any of these receptors are called triple-negative.

Some breast cancers are both hormone receptor-positive and HER2-positive, meaning that estrogen, progesterone, ***and*** growth factors can stimulate cell growth. These cancers are often referred to as triple-positive breast cancers.**)**

Conclusions from review in Cancer Cell. 2020 37:514-529

- Among the sensitive cancers, tumor cells that initially require CDK4/6 activity may become insensitive to their specific inhibitors simply by upregulating CDK6, suggesting that developing more specific or stronger inhibitors of CDK6 may have a special value in cancer.

- In other cases, tumor cells become resistant by alternative mechanisms to inhibit RB1, e.g., using additional RB1-inhibitory kinases, such as CDK2, CDK3, or CDK1. These cases will require multi-potent CDK inhibitors.

→ Indeed, a CDK4/6 and CDK2 inhibitor has been shown to suppress the proliferation of multiple tumor models that exhibit resistance to CDK4/6 inhibitors. (Cancer Cell 39, 1-18 (2021); https://doi.org/10.1016/j.ccell.2021.08.009)

- Finally, some tumors escape cell-cycle entry control by eliminating RB1 itself, and these cases may benefit from inhibition of checkpoint kinases, such as CHK1 required for dealing with the replicative or mitotic stress generated in the absence of RB1.

Types of Protein Interactions

- Stable or transient
- Strong or weak

Stable: purified as multi-subunit complexes (e.g. hemoglobin, core RNA polymerase)
-->studied by gel filtration, sedimentation, co-immunoprecipitation.
Transient: require a set of conditions to become detectable
-->captured e.g. by cross-linking, label transfer methods but also 2-hybrid.

Coimmunoprecipitation

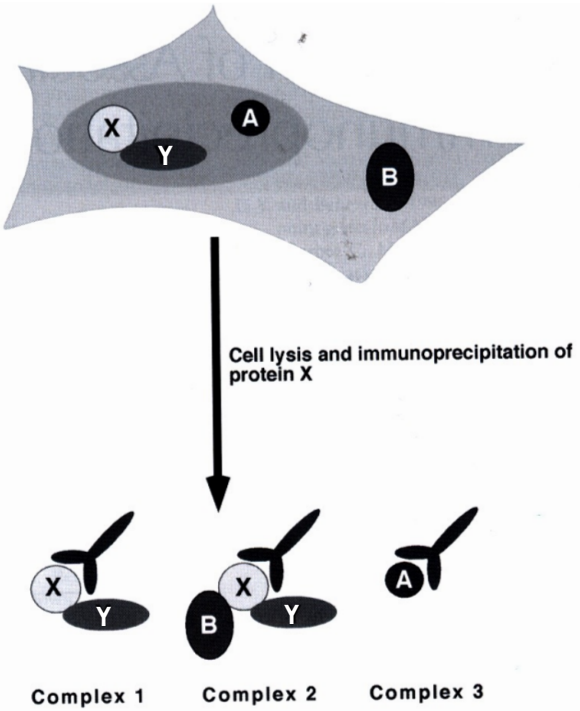


FIGURE 1. Principle and pitfalls of detection of proteins by coimmunoprecipitation. In the intact cell, protein X is present in a complex with protein Y. This complex is preserved after cell lysis and allows protein Y to be coimmunoprecipitated with protein X (complex 1). However, the disruption of subcellular compartmentalization allows artifactual interactions to occur between some proteins, e.g., protein X and protein B (complex 2). Furthermore, the antibody that is used for the immunoprecipitation will nonspecifically cross-react with other proteins, e.g., protein A (complex 3). The key to identification of protein–protein interactions by coimmunoprecipitation is to perform the proper controls so as to identify protein Y but not proteins A and B. (Adapted from Sambrook and Russell 2001.)

Already discussed: **Epitope tags**

Table 1. Common Epitope Tags

Name	Sequence	Detection	Purification	Reference
FLAG	DYKDDDDK	M1,M2, M5	Immunoaffinity	1
6 × His	HHHHHH	Anti-His	Metal affinity	2
HA	YPYDVPDYA	12CA5	Immunoaffinity	4
c-myc	EQKLISEEDL	9E10	Immunoaffinity	5
GST	220 aa GST	Anti-GST	Glutathione	3
Protein A	IgG-binding domain	IgG	IgG	10
CD	18 aa exon	12CA5	Immunoaffinity	19
Strep-tag	WSAPQFEK	Strep-Tactin	Strep-Tactin	11
MBP	Maltose-binding protein	Anti-MBP	Maltose	13
CBD	Chitin-binding domain	Anti-CBD	Chitin	14
S-tag	S-peptide	Anti-S peptide	S-peptide	16
Avitag	GLNDIFEAQKIEWHE	Avidin	Avidin	12
CBP	CBP peptide	Anti-CBP	Calmodulin	15
TAP	Calmodulin- and IgG-binding domains	Anti-CBP	Calmodulin and IgG	15
SF-TAP	Strep Tag II and FLAG	Anti-FLAG	Strep-Tactin	28

GST, glutathione-S-transferase; CBP, calmodulin-binding peptide.

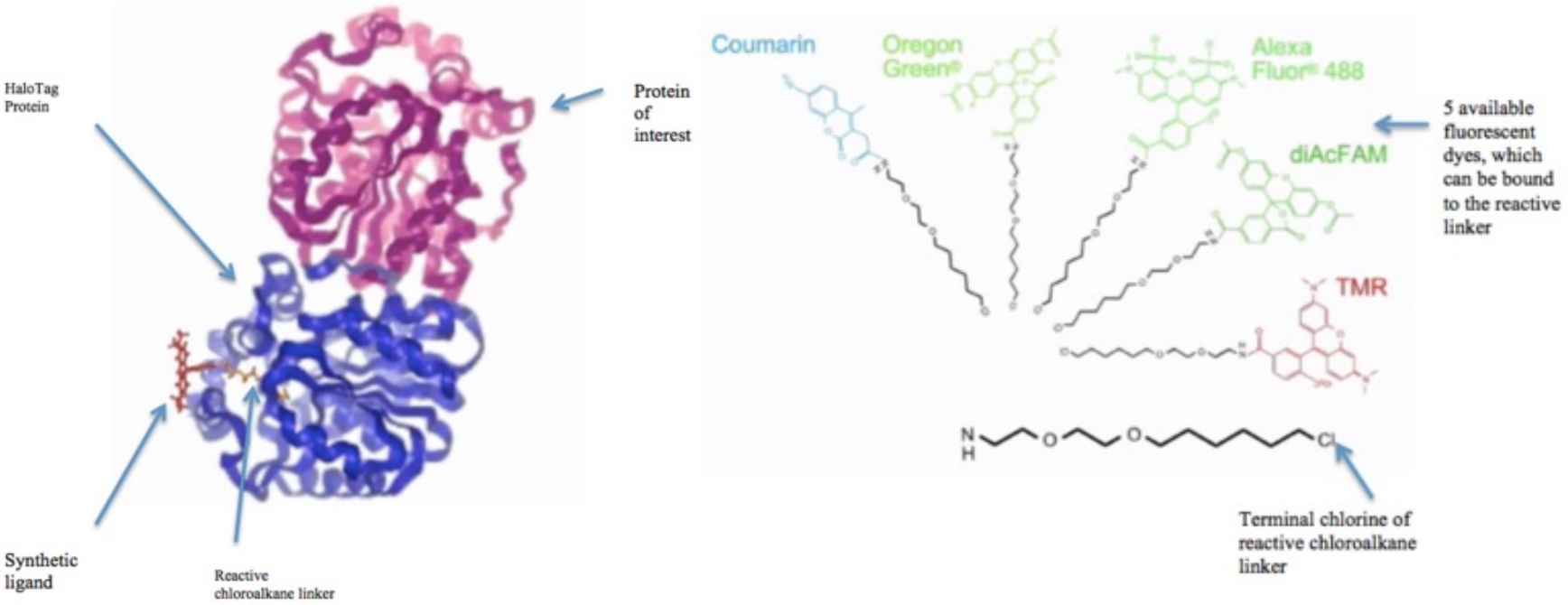
For **Halo-tag**: see next slide.

GFP and derivatives: https://en.wikipedia.org/wiki/Green_fluorescent_protein

Epitope tagging is a technique in which a known epitope is fused to a recombinant protein by means of genetic engineering. By choosing an epitope for which an antibody is available, the technique makes it possible to detect proteins for which no antibody is available.

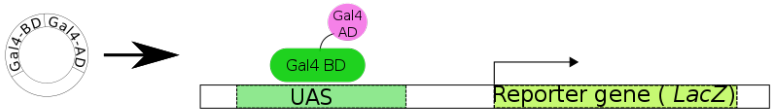
https://en.wikipedia.org/wiki/HaloTag

HaloTag is a self-labeling protein tag. It is a 297 residue peptide (33 kDa) derived from a bacterial enzyme, designed to covalently bind to a synthetic ligand. The bacterial enzyme can be fused to various proteins of interest.

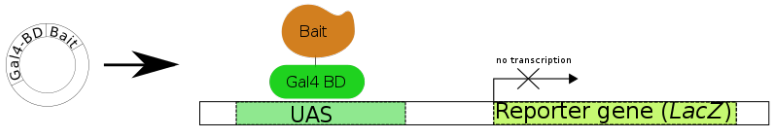


https://www.youtube.com/watch?v=dDtY2iO41cU

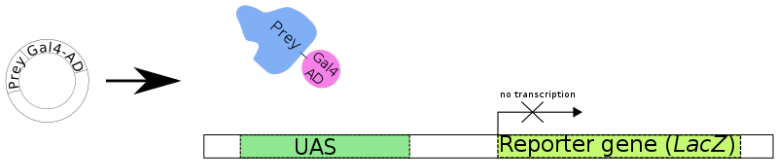
Yeast 2-hybrid System



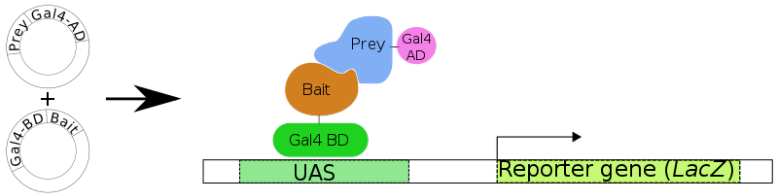
A. Regular transcription of the reporter gene



B. One fusion protein only (Gal4-BD + Bait) - no transcription



C. One fusion protein only (Gal4-AD + Prey) - no transcription

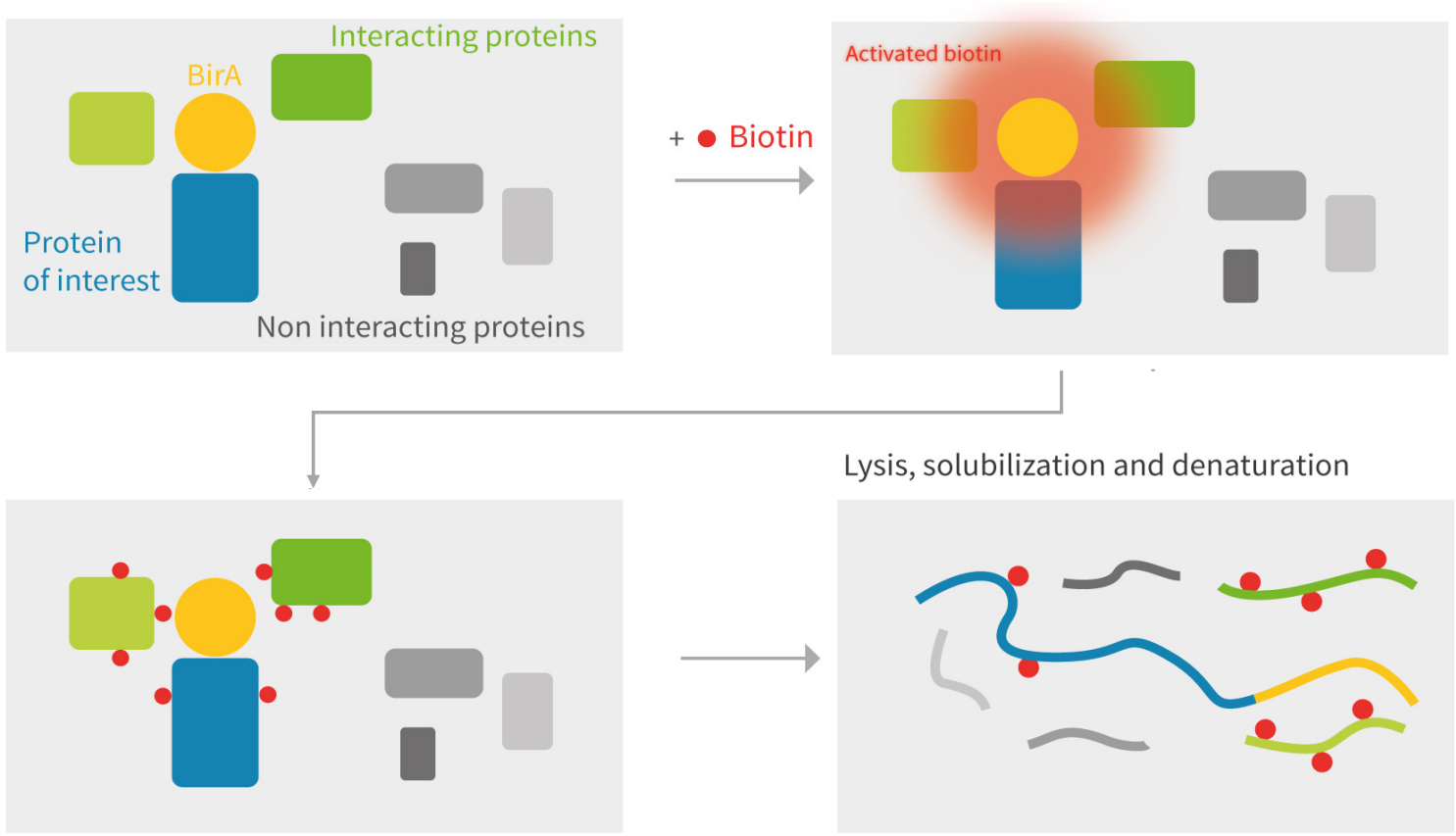


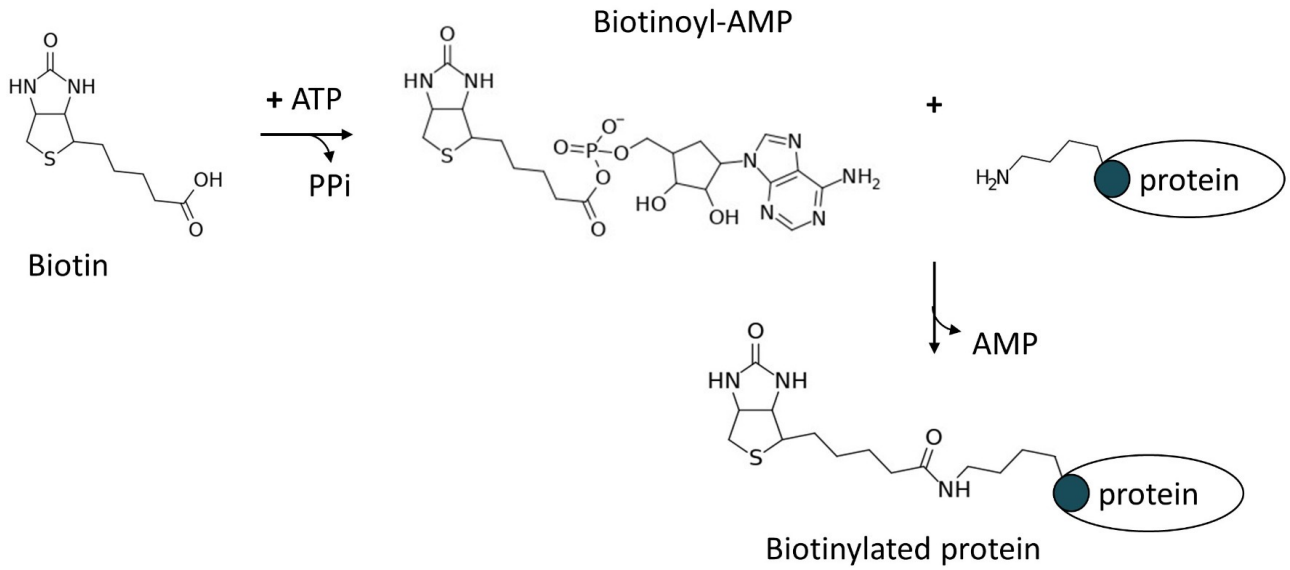
D. Two fusion proteins with interacting Bait and Prey

First developed: Fields S. Song O.
Nature. 1989 Jul 20;340(6230):245-6

Reporter gene can also be a dug-selectable marker etc.

BioID Method: express protein of interest as fusion protein with a biotin ligase (BirA)





BirA catalyzes an ATP-dependent two-step reaction in which **biotin** is used to form biotinyl-5'-adenylate and is transferred to a specific **lysine residue of the accepting protein** via an amide linker.

BioID- Advantages

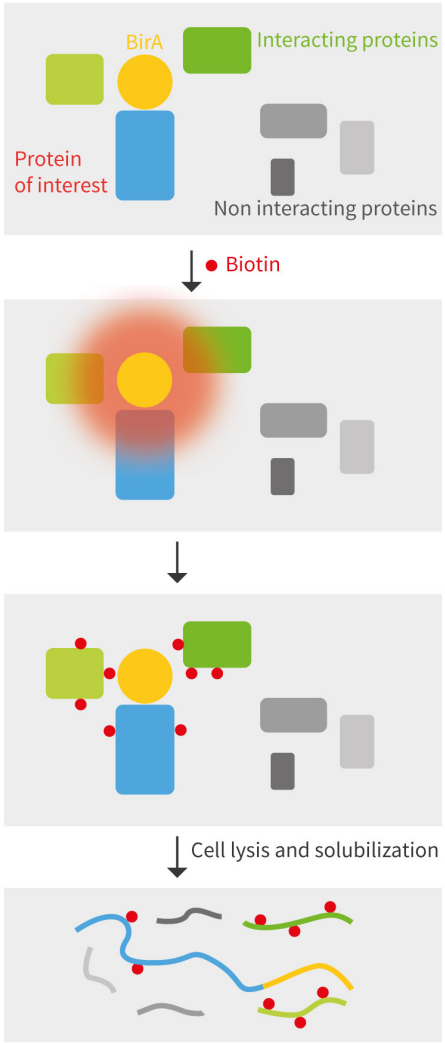
Interacting proteins are covalently labeled → no need to maintain the native interaction through the purification process

→ Application of harsh lysis and solubilization conditions

→allows identification of week and transient interactions

→ Detects not only direct interactions but also the environment of protein

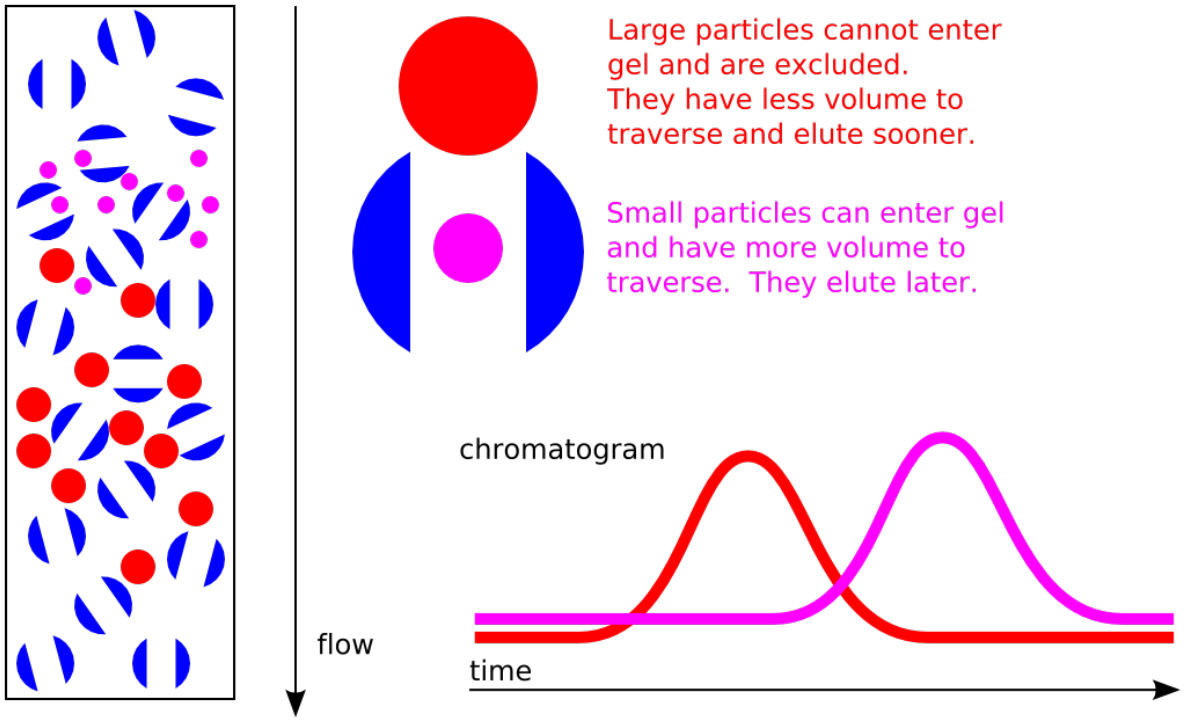
Purification of biotinylated proteins using streptavidin beads



Native size of proteins and protein complexes

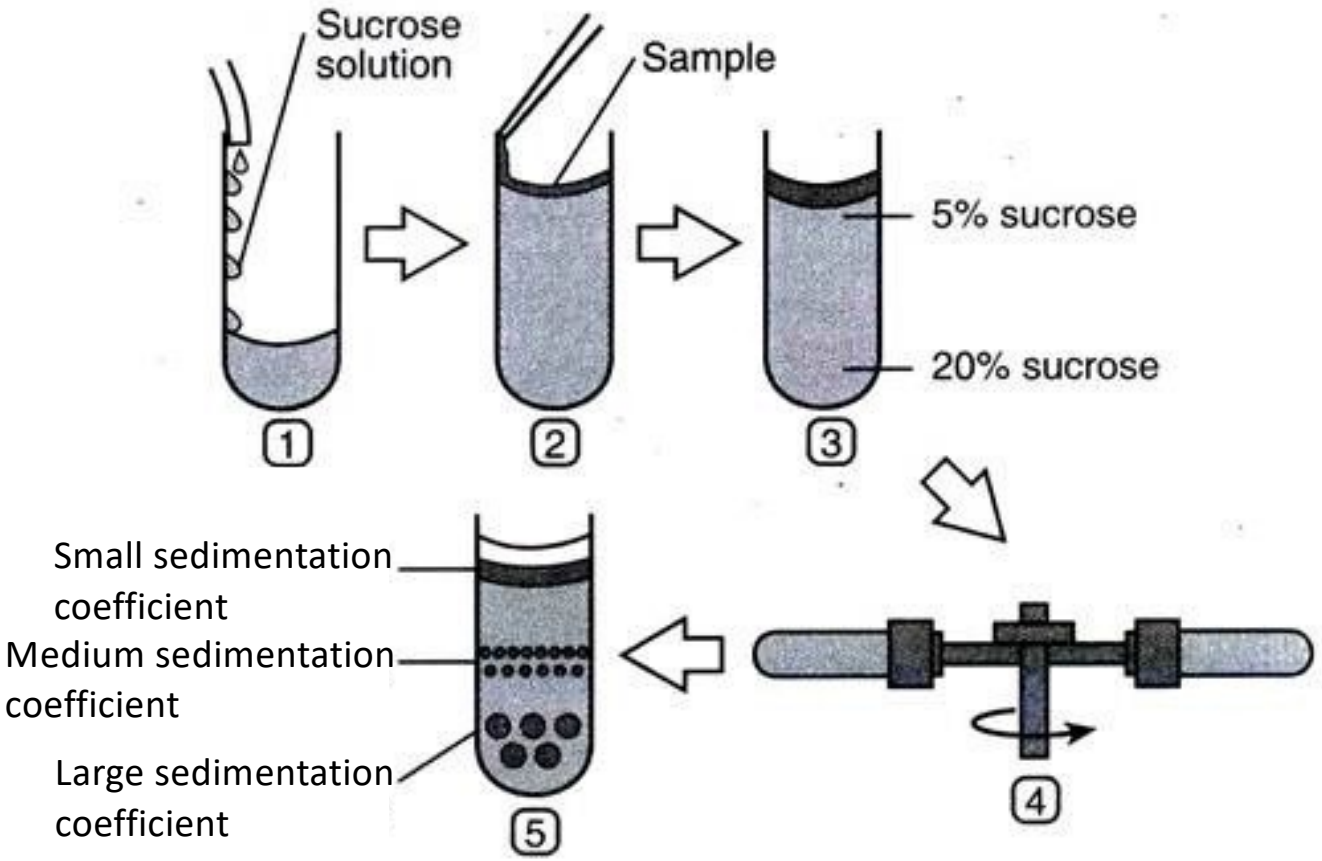
Already discussed:

Size exclusion chromatography / Gel filtration



…Fractionation according to Stokes' radius

Sucrose gradient ultracentrifugation



…Fractionation according to sedimentation coefficient