Targets for drug action – part 2

Target	*examples*
2.1. Receptors for physiological ligands	
Transmembrane receptors	
2.1.1. G-protein-coupled receptors	*- adrenergic receptors* *- opioid receptors*
2.1.2. ligand-gated ion channels	*- $GABA_A$ receptors*
2.1.3. kinase-linked receptors	***- insulin receptor***
Intracellular receptors	
2.1.4. nuclear receptors	***- PPARγ (peroxisome proliferator-activated receptor γ*** ***- pregnane X receptor***
2.2. Other targets/approaches	
2.2.1. enzymes	***- cyclo-oxygenase (in pain chapter)*** ***- dihydrofolate reductase*** ***- HIV protease*** ***- tyrosine kinases*** ***- angiotensin-converting enzyme***
2.2.2. ion channels and transporters	*- voltage-gated Na channels*
2.2.3. protein therapeutics	*- GLP-1 receptor agonists* *-TNF-α monoclonal antibodies (e.g. infliximab)*
2.2.4. gene therapy	*- Nusinersen* *- Tisagenlecleucel /Axicabtagene ciloleucel*

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2.1.3. kinase-linked receptors

→ mediate action of a wide variety of protein mediators (growth factors, cytokines, hormones)

→ effect mainly via changes in gene transcription

→ > 100 receptor clones

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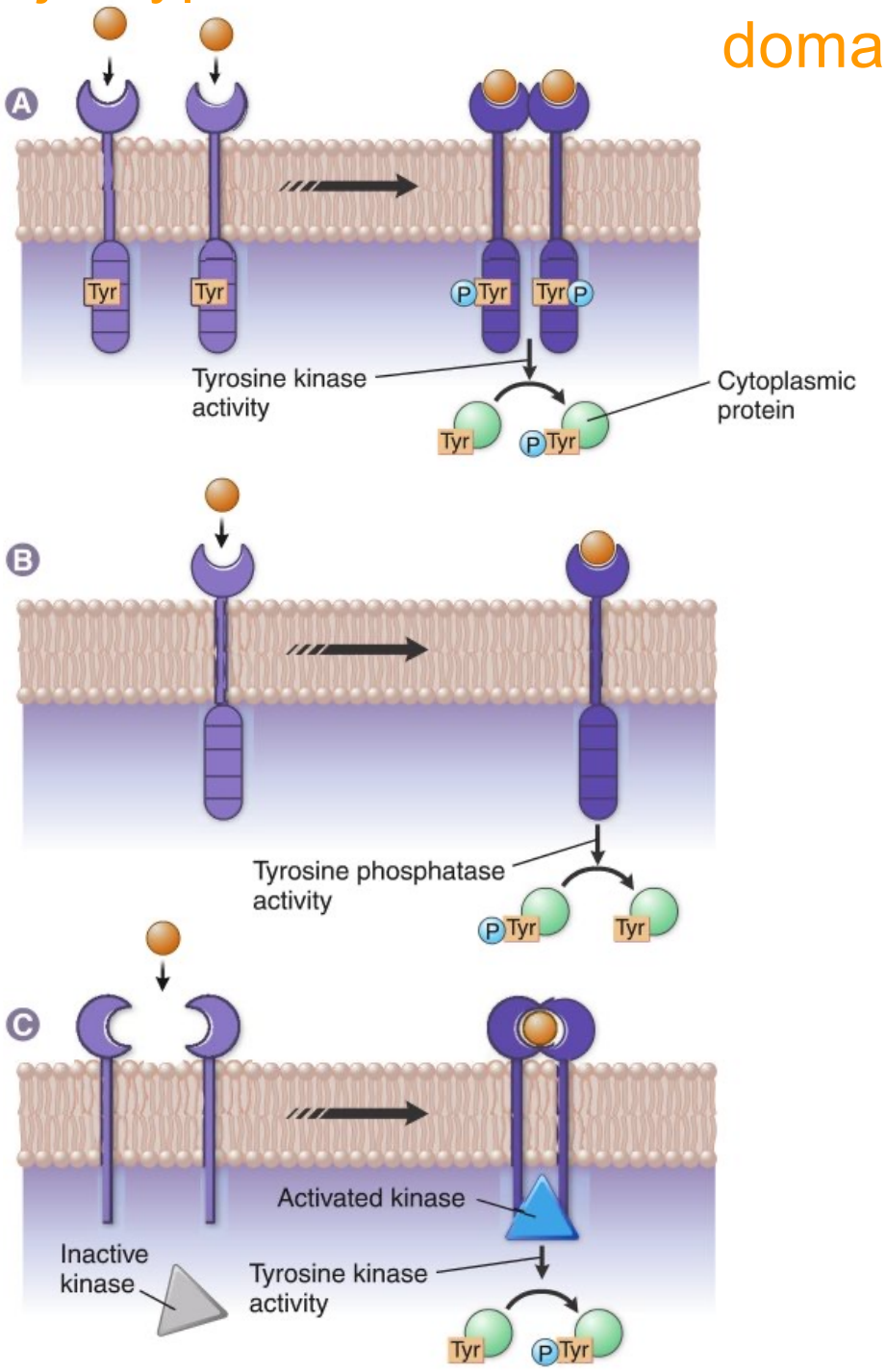
Major types of transmembrane receptors with enzymatic cytosolic domains (1)



receptor tyrosine kinases: after ligand-induced activation, these receptors dimerize and transphosphorylate tyrosine residues in the receptor and then, often on target cytosolic proteins.
examples: insulin receptor, PDGF (platelet-derived growth factor)-β receptor, EGF receptor

Tyrosine phosphatase-associated receptors: These receptors dephosphorylate tyrosine residues either on other transmembrane receptors or on cytosolic proteins
examples: many receptors of the immune system

tyrosine kinase-associated receptors: binding of ligand triggers activation of receptor-associated protein kinases that then phosphorylate target cytosolic proteins.
examples: many cytokine receptors

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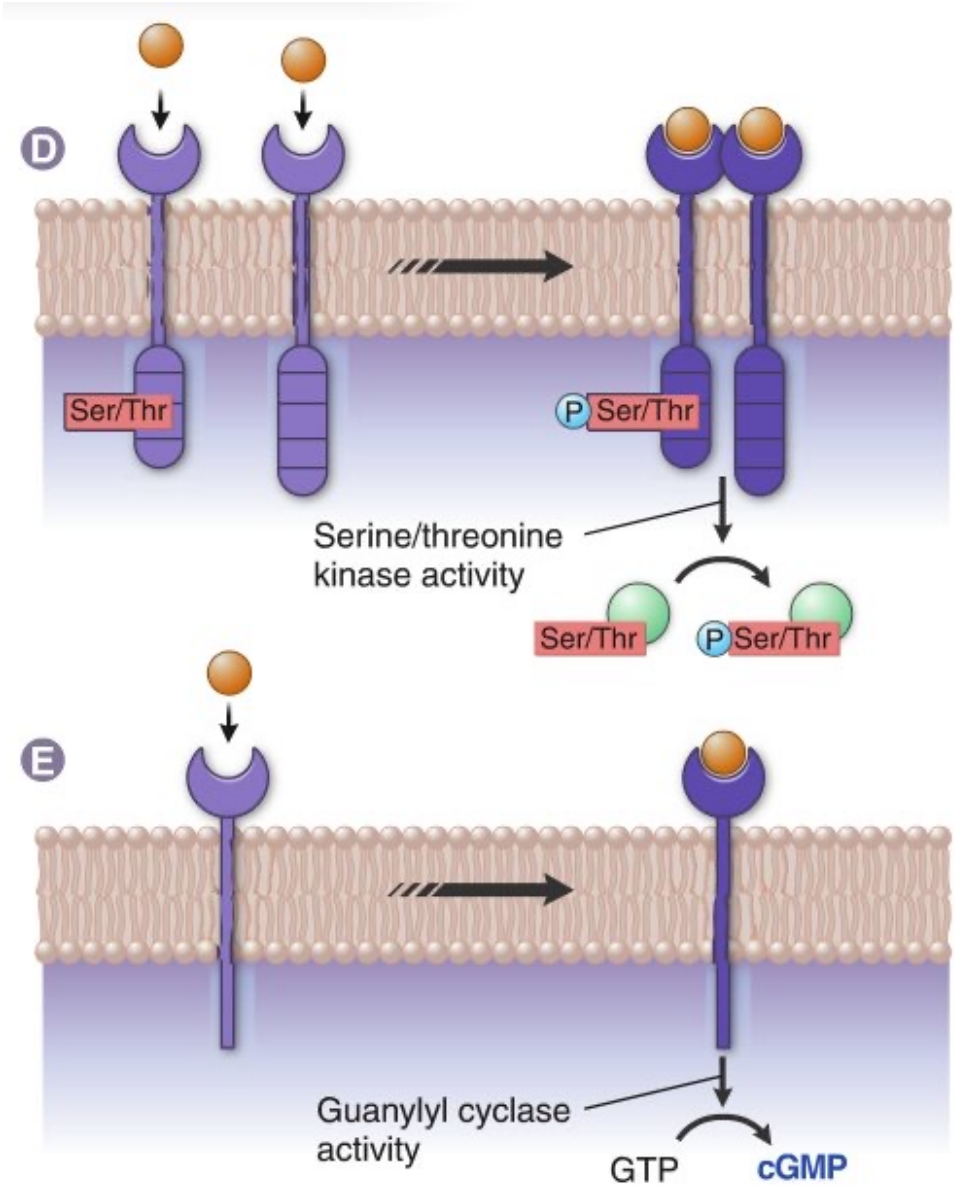
Major types of transmembrane receptors with enzymatic cytosolic domains (2)



receptor serine/threonine kinases: phosphorylate ser and thr residues on target cytosolic proteins.
examples: receptors of the transforming growth factor β (TGF-β) family, involved in cell growth and differentiation

receptor guanylyl cyclases: contain a cytosolic domain that catalyzes the formation of cGMP from GTP
examples: B type natriuretic peptide receptor (*secreted by ventricles in response to volume overload; synthetic peptide, nesiritide, used for treatment of decompensated heart failure)*

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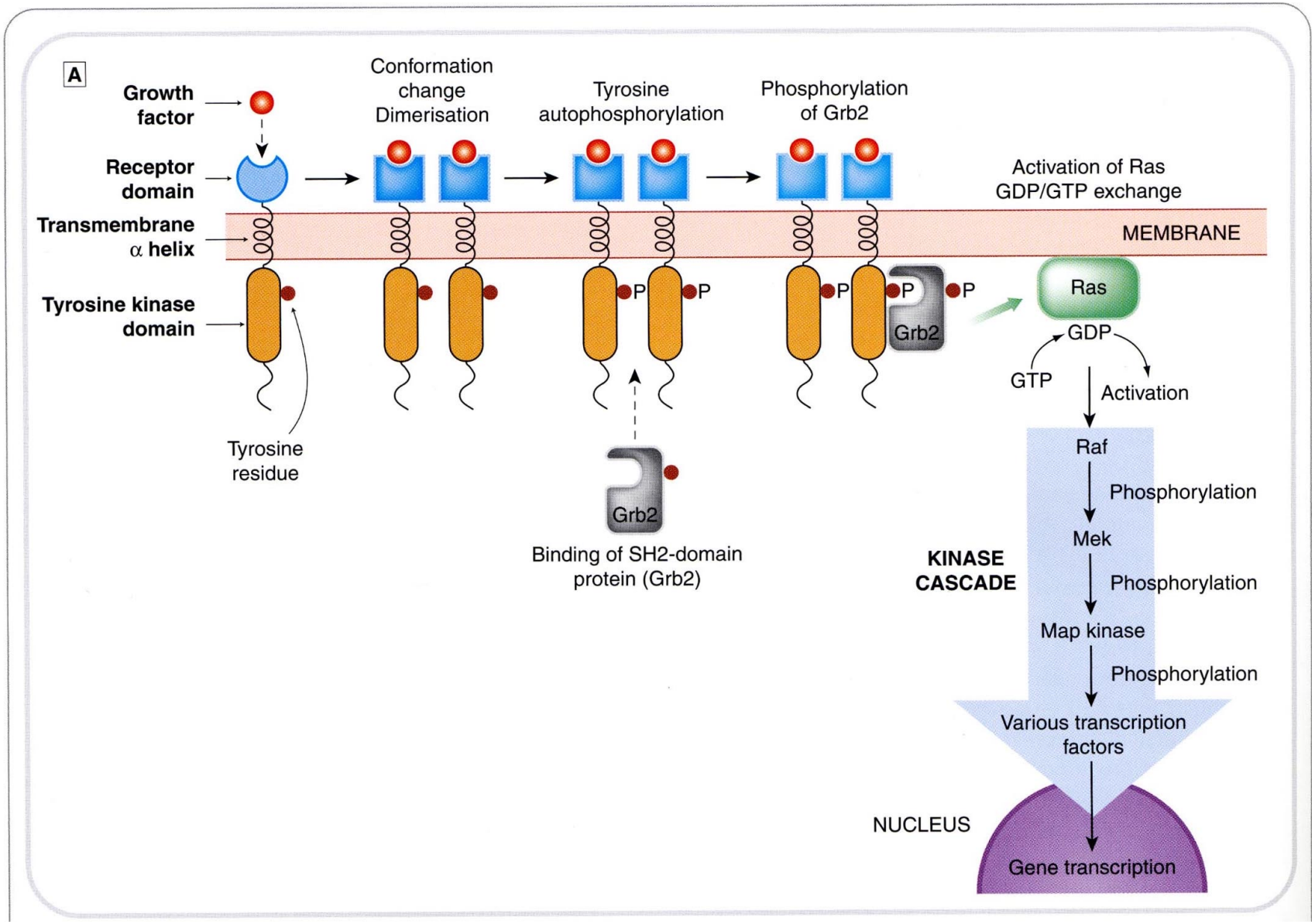
Transduction mechanisms of tyrosine-kinase-linked receptors (ex)



The first step following agonist binding is dimerisation, which leads to autophosphorylation of the intracellular domain of each receptor. SH2 domain proteins then bind to the phosphorylated receptor and are themselves phosphorylated. One well-characterised pathway is shown: The growth factor (Ras/Raf/mitogen-activated protein [MAP] kinase) pathway. Several other pathways exist, and these phosphorylation cascades interact with components of G-protein systems.

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Case: diabetes

At her annual checkup, 55-year-old Mrs. S. complains of fatigue and frequent urination (polyuria), even at night. She also reports drinking large volumes of fluids (polydipsia) to quench her thirst. Although these symptoms have been “going on for a while” and are getting worse, Mrs. S. has difficulty pinpointing their exact onset. She denies other urinary symptoms such as pain on urination, blood in her urine, dribbling, and incontinence. Her medical history is remarkable for hyperlipidemia of 10 years' duration. Both of her parents died of coronary heart disease in their early 60s.
On physical examination, Mrs. S. is moderately obese but otherwise appears well. Glucose is detected in her urine, but proteins and ketones are not. Her blood tests are significant for elevated glucose (240 mg/dL); elevated total cholesterol (340 mg/dL); and HbA1c, a measure of glucose covalently bound to hemoglobin, of 9.2%. The physician explains to Mrs. S. that she has Type II diabetes mellitus. In this disease, the body fails to respond normally to insulin (insulin resistance) and cannot produce a sufficient amount of insulin to overcome this resistance.

what are the cellular and molecular actions of insulin ?

what are the possible treatments ?

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example for kinase-linked receptors: the insulin receptor

Normal glucose concentrations in plasma:

after a 24h fast:	3.3-4.4 mM (60-80 mg/dl)
2h after mixed meal:	5.6-7.8 mM (100-140 mg/dl)

Insulin is a peptide hormone. It consists of two peptide chains linked to each other by disulfide bonds. It is produced and secreted in the pancreas, and it acts in the tissues on kinase-linked receptors

- Islets of Langerhans of the endocrine pancreas secrete insulin from B (or β) cells, glucagon from A cells and somatostatin from D cells.
- Many factors stimulate insulin secretion, but the main one is blood glucose.
  - Insulin has essential metabolic actions as a fuel storage hormone and also affects cell growth and differentiation.

Glucagon is a fuel-mobilising hormone, stimulating gluconeogenesis and glycogenolysis, also lipolysis and proteolysis. It increases blood sugar and also increases the force of contraction of the heart.

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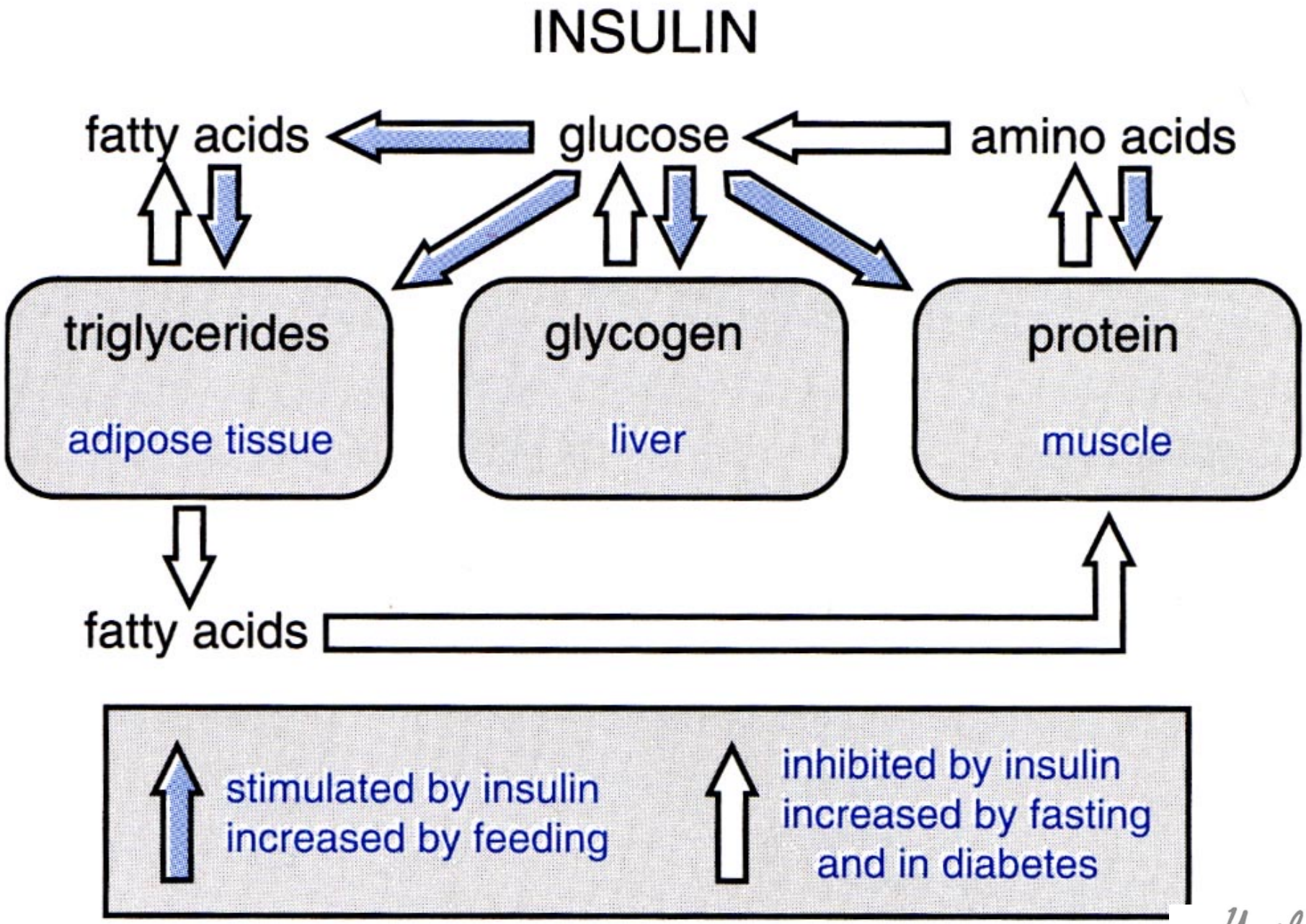
Insulin: physiological context



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Diabetes mellitus

Diabetes mellitus is a chronic metabolic disorder in which there is hyperglycaemia. There are two main types:

type 1 (insulin-dependent, 5-10%) diabetes, with an absolute deficiency of insulin.

- results from autoimmune destruction of pancreatic β cells.
- insulin-dependent tissues fail to take up and store glucose, amino acids and lipids
- starvation-like response of the cells and tissues
- diabetic keto-acidosis, a serious, potentially life-threatening medical emergency, if diabetes is not treated

type 2 (non-insulin-dependent, >90%) diabetes, with a relative deficiency of insulin associated with reduced sensitivity to its action (insulin resistance).

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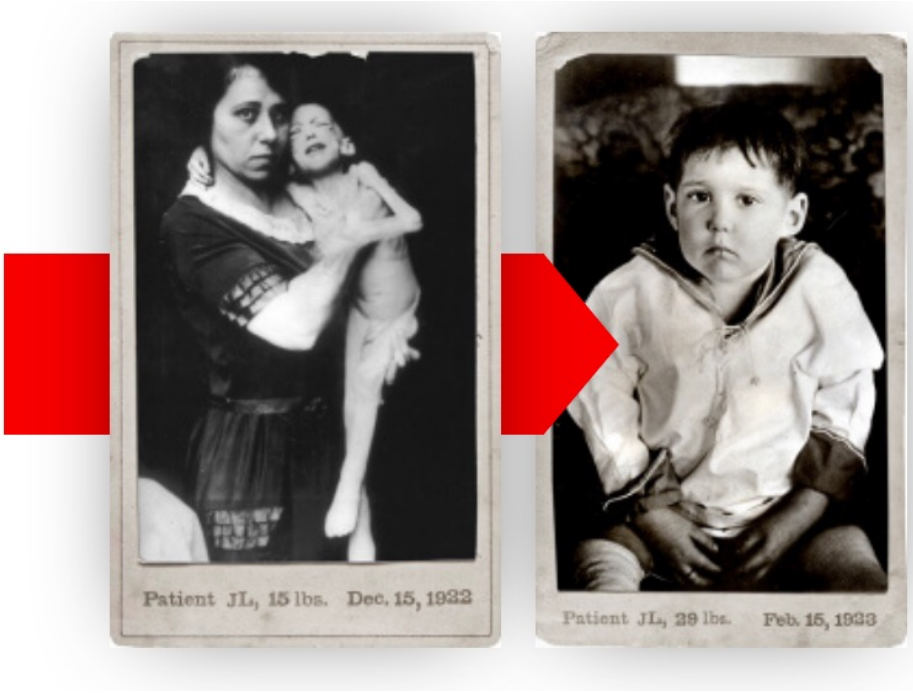

1923

Lilly introduced **Iletin** ® (animal-source insulin, Lilly), the world's first commercially available insulin product, for the treatment of diabetes – then a fatal disease with no effective treatment options.

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Insulin release

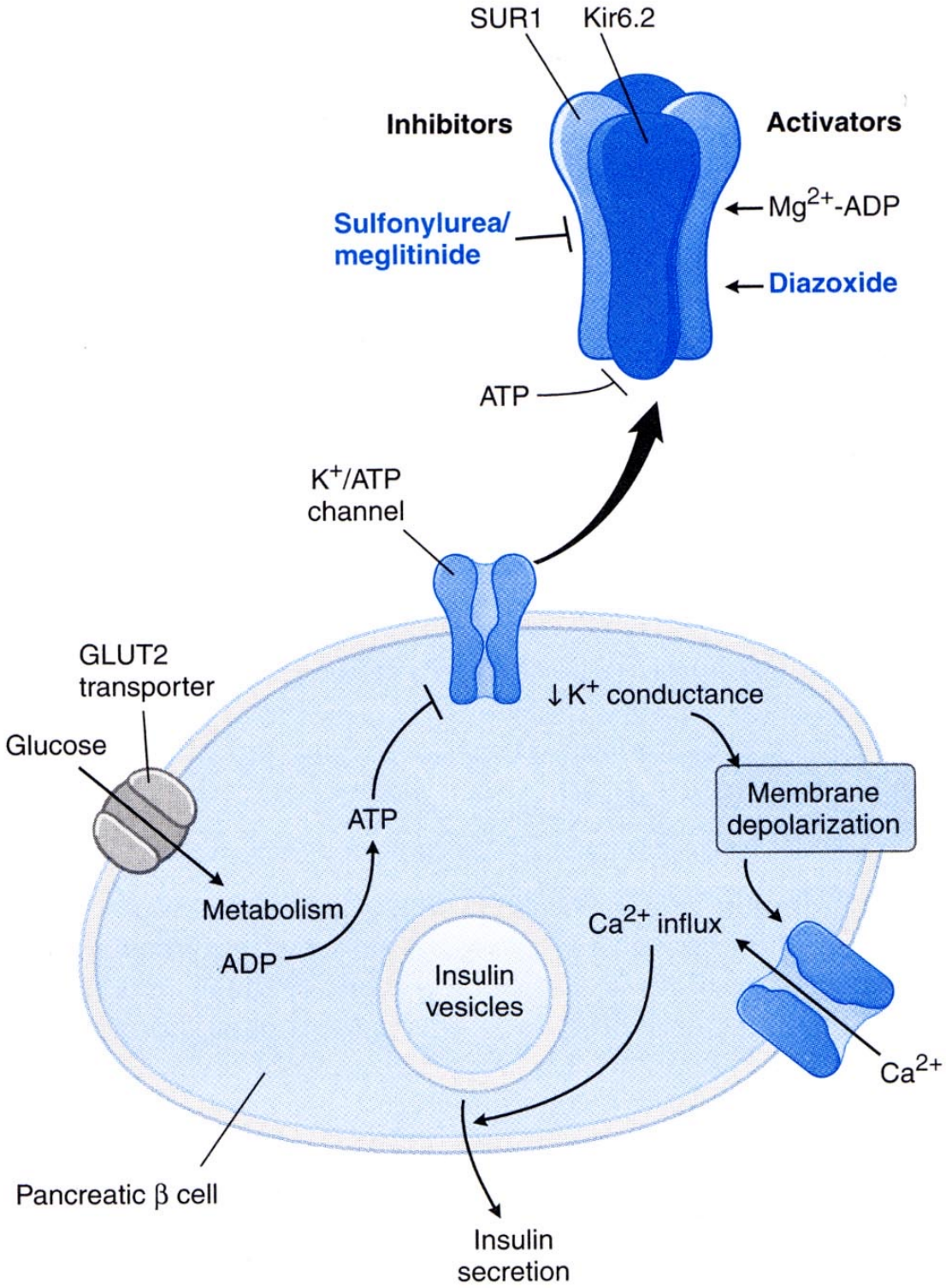

Figure 28-3. Physiologic and Pharmacologic Regulation of Insulin Release from Pancreatic β cells. In the basal state, the plasma membrane of the β cell is hyperpolarized, and the rate of insulin secretion from the cell is low. When glucose is available, it enters the cell via GLUT2 transporters in the plasma membrane and is metabolized to generate intracellular ATP. ATP binds to and inhibits the plasma membrane K^+/ATP channel. Inhibition of the K^+/ATP channel decreases plasma membrane K^+ conductance; the resulting depolarization of the membrane activates voltage-gated Ca^{2+} channels and, thereby, stimulates an influx of Ca^{2+}. Ca^{2+} mediates fusion of insulin-containing secretory vesicles with the plasma membrane, leading to insulin secretion. The K^+/ATP channel, an octamer composed of Kir6.2 and SUR1 subunits, is the target of several physiologic and pharmacologic regulators. ATP binds to and inhibits Kir6.2, while sulfonylureas and meglitinides bind to and inhibit SUR1; all tl
mote insulin secretion. Mg^{2+}-ADP and
activate SUR1, thereby inhibiting insu
only four of the eight K^+/ATP channel

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signaling of the insulin receptor

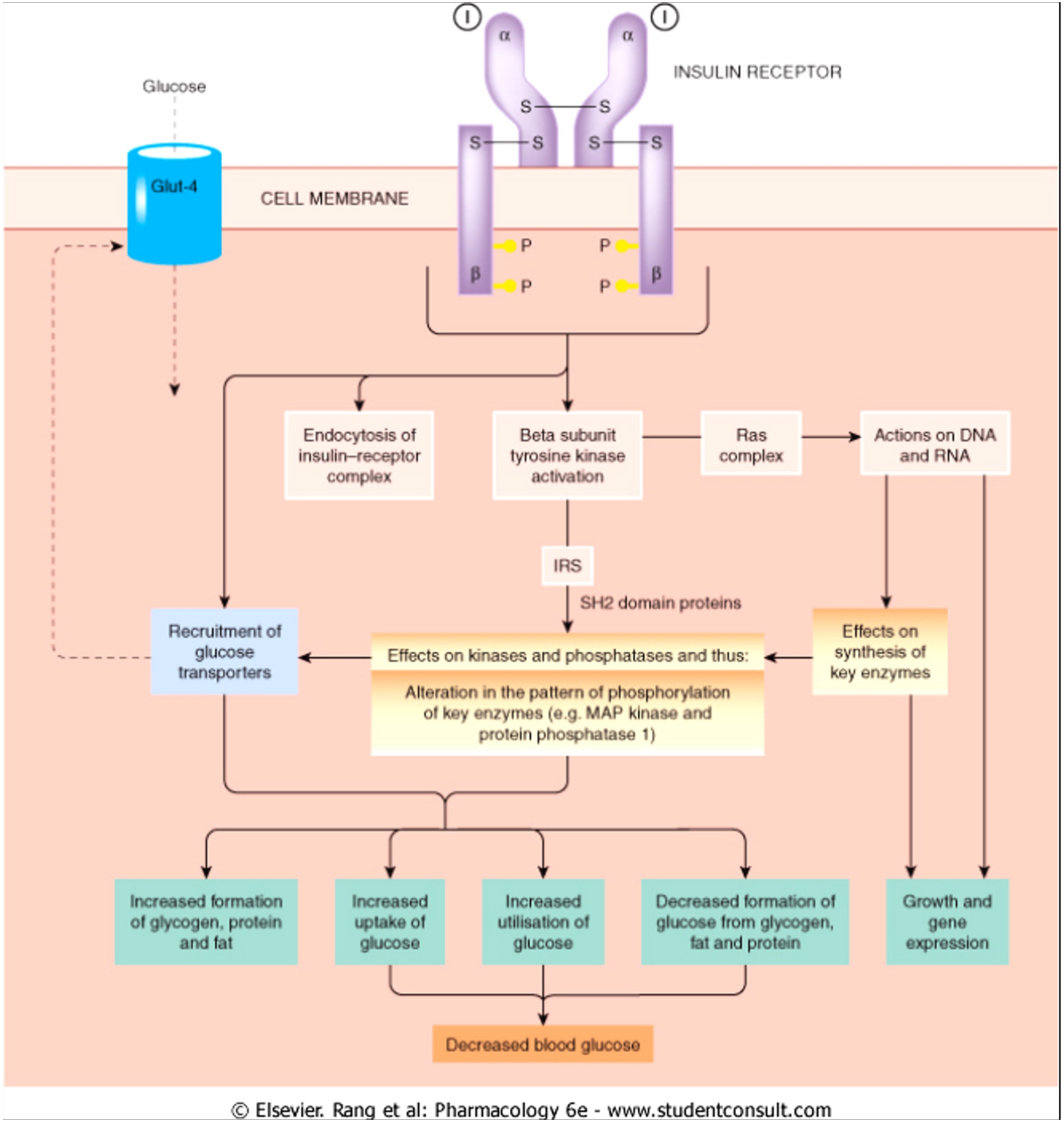

Figure 26-3 Insulin signalling pathways. I, insulin; Glut-4, an insulin-sensitive glucose transporter present in muscle and fat cells; IRS, insulin receptor substrate (several forms: 1-4); central role of the phosphatidylinositol 3-kinase in rapid effects (phosphorylation).

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Therapy for diabetes: aims and strategies

Aim: normalize metabolic parameters such as blood sugar, to reduce the risk of long term complications (long-term vascular pathology: premature atherosclerosis, retinopathy, nephropathy, neuropathy)

Strategies:

Type I diabetes: administer exogenous insulin to achieve normoglycemia without inducing hypoglycemia

Type II diabetes: reduce weight, change diet; drugs that slow glucose absorption from the gut, increase insulin secretion by β-cells or increase insulin sensitivity in target cells

Marker:

Glucose levels → acute concentrations

Glycohemoglobin (HbA1c) → reflects average blood glucose over the last several months (< 6% in non-diabetics, aim in diabetics: < 7%)

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Insulin

- Human insulin is made by recombinant DNA technology. For routine use, it is given subcutaneously (by intravenous infusion in emergencies).

- Different formulations of insulin differ in their duration of action:
  - fast- and short-acting soluble insulin: peak action after subcutaneous dose 2-4 hours and duration 6-8 hours; it is the only formulation that can be given intravenously
  - intermediate-acting insulin (e.g. isophane insulin)
  - long-acting forms (e.g. insulin zinc suspension).
  - recombinant human insulin preparations with different durations of action have been engineered by changing the amino acid sequence or modifying amino acid residues.

- The main unwanted effect is hypoglycaemia.

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Several pharmacological strategies for the treatment of diabetes mellitus type 2!

- Insulin secretagogues
  - Sulfonylureas (tolbutamide, glibenclamide) and meglitinides (nateglinide, repaglinide) stimulate insulin secretion by binding to and inhibiting the SUR1 K^+ channel in the beta cells. The properties of these two classes are similar: they
    - are effective only if β cells are functional
    - are well tolerated but can cause hypoglycaemia, which stimulates appetite and leads to weight gain
    -

- **Reduction of hepatic glucose production:**
  - Biguanides (e.g. metformin):
  - have complex peripheral actions in the presence of residual insulin, increasing glucose uptake in striated muscle and inhibiting hepatic glucose output and intestinal glucose absorption
  - cause anorexia and encourage weight loss
  - can be combined with sulfonylureas.

- **GLP-1-based "Incretin" therapies**
  - GLP-1 Analogues (Exenatide, Semaglutide and others = long-acting analogs), injected. GLP-1 (glucagon-like peptide-1) is produced primarily in enteroendocrine cells of the small bowel; increasing in β-cells the secretion of insulin. Short half-life (1-2 min), due to enzymatic degradation by dipeptidyl peptidase-4 (DPP-4)
  - DPP-4 inhibitors (sitagliptin, saxagliptin) → prolong half-life of endogenous GLP-1.

- **Insulin sensitizers**
  - Thiazolidinediones (=TZDs) (e.g. rosiglitazone, pioglitazone)
    - increase insulin sensitivity and lower blood glucose in type 2 diabetes
    - can cause weight gain and oedema
    - are peroxisome proliferator-activated receptor-γ (PPAR γ, a nuclear receptor) agonists.
    - a possible unwanted effect is an increased risk of cardiovascular events *(heart failure).* For this reason, Rosiglitazone can not be used any more in CH (since October 2010).

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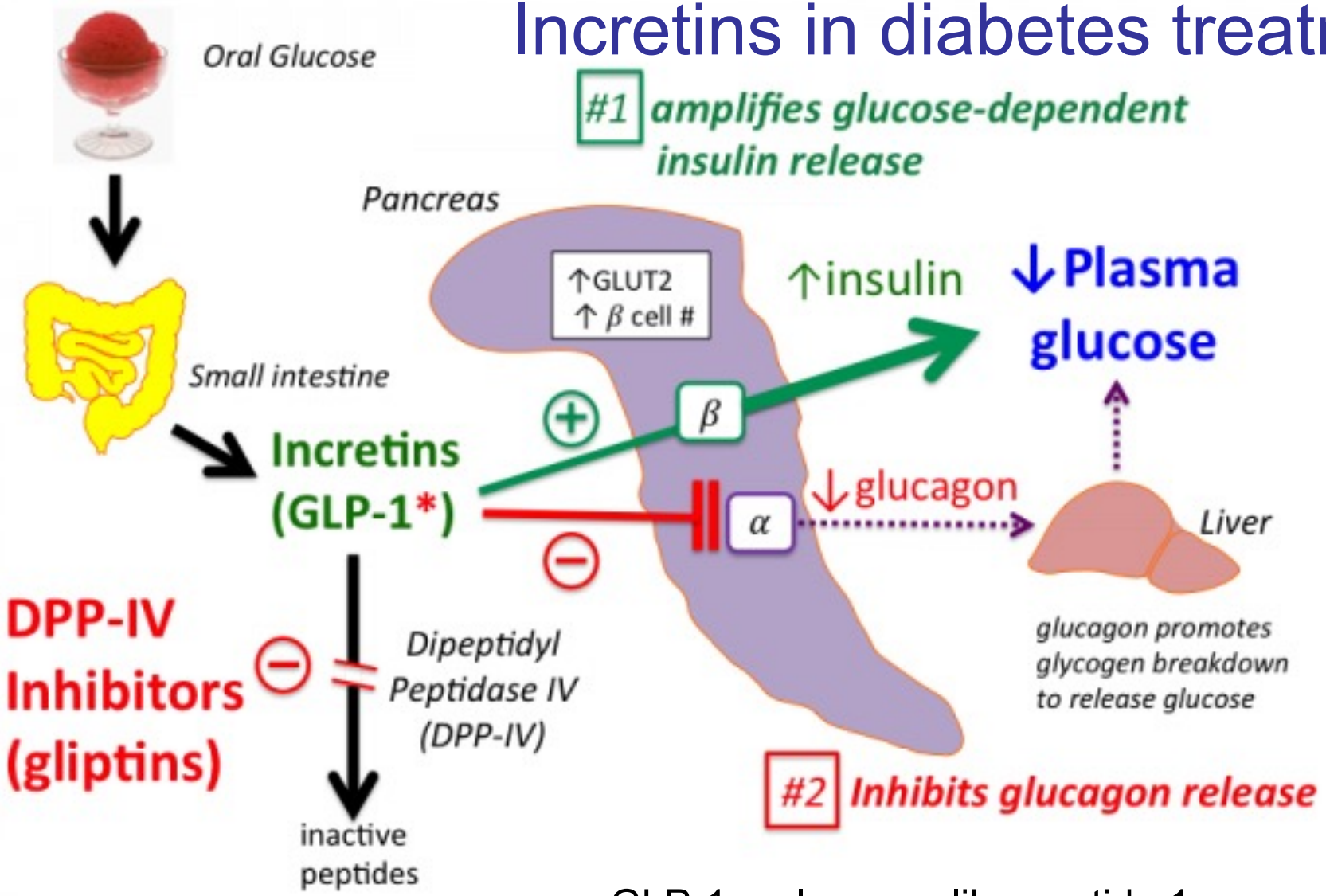
Incretins in diabetes treatment



GLP-1 = glucagon-like peptide 1

* Physiological $t_{1/2}$ ≈2 mins due to rapid inactivation by DPP-IV

GLP-1 receptor agonists with longer $t_{1/2}$ than GLP-1 have been developed and are clinically used. These drugs are administered subcutaneously.

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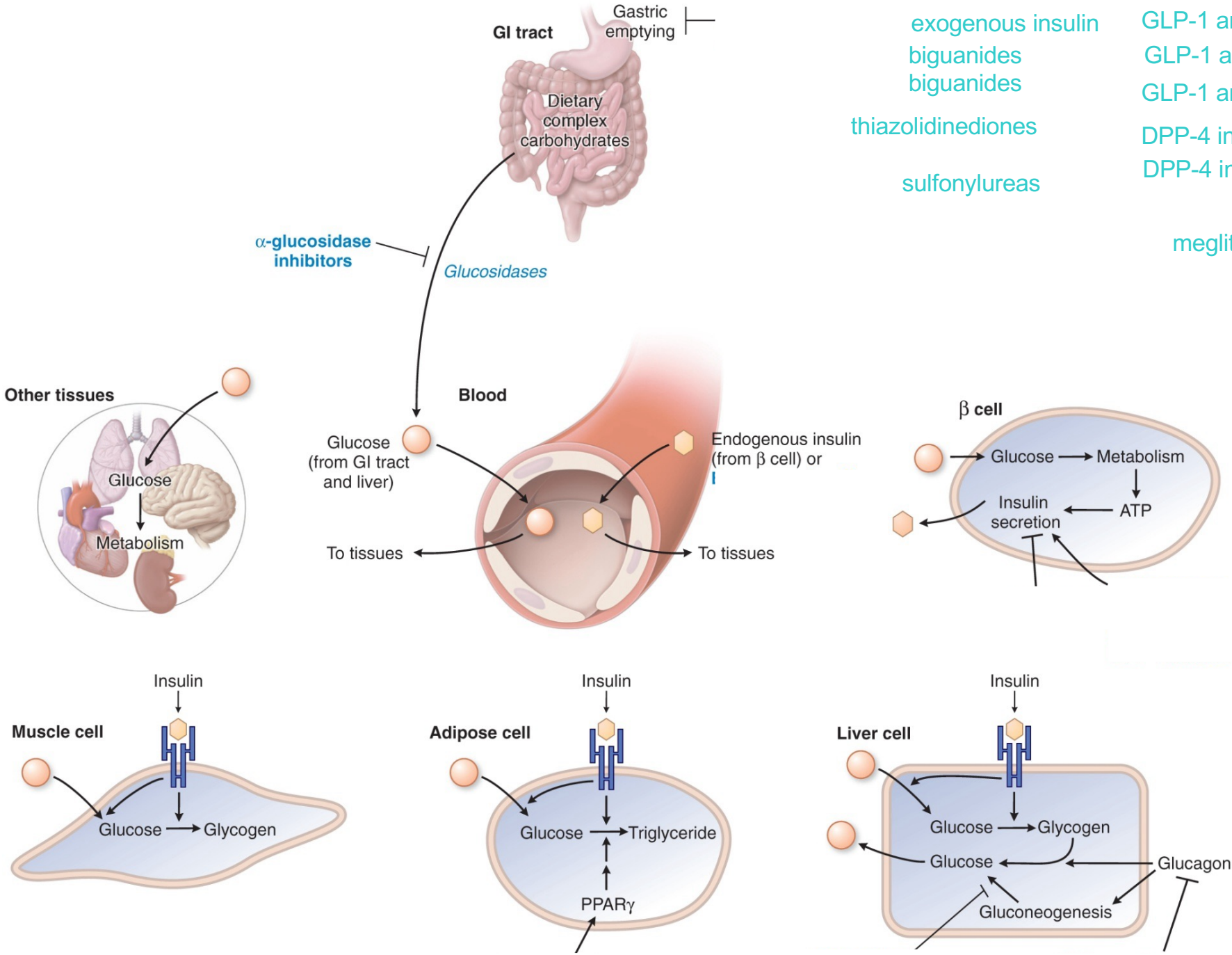
exogenous insulin
biguanides
biguanides
thiazolidinediones
sulfonylureas

GLP-1 analogue
GLP-1 analogue
GLP-1 analogue
DPP-4 inhibitor
DPP-4 inhibitor
meglitinides



Management of hyperglycemia in type 2 diabetes

Table 1—*Summary of antidiabetic interventions as monotherapy*

Interventions	Expected decrease in A1C (%)	Advantages	Disadvantages
Step 1: initial			
Lifestyle to decrease weight and increase activity	1–2	Low cost, many benefits	Fails for most in 1st year
Metformin	1.5	Weight neutral, inexpensive	GI side effects, rare lactic acidosis
Step 2: additional therapy			
Insulin	1.5–2.5	No dose limit, inexpensive, improved lipid profile	Injections, monitoring, hypoglycemia, weight gain
Sulfonylureas	1.5	Inexpensive	Weight gain, hypoglycemia*
TZDs	0.5–1.4	Improved lipid profile	Fluid retention, weight gain, expensive
Other drugs			
α-Glucosidase inhibitors	0.5–0.8	Weight neutral	Frequent GI side effects, three times/day dosing, expensive
Exenatide	0.5–1.0	Weight loss	Injections, frequent GI side effects, expensive, little experience

Exenatide: activates the glucaton-like peptide-1 (GLP-1) receptor: stimulates insulin secretion, induces Glucose uptake and glycogen synthesis in periphery.
TZDs = Thiazolidinediones

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Case: questions

1. What are the cellular and molecular actions of insulin?

2. Mrs. S. has both blood glucose and HbA1c levels measured during her annual appointment. What do these test results indicate about blood glucose concentrations?

3. Which of the following agents increases insulin secretion?

A. α-glucosidase inhibitors
B. neutral protamine Hagedorn (NPH) insulin
C. Sulfonylureas
D. Rosiglitazone
E. Pioglitazone

4. Why did Mrs. S have to go so often to the toilet ?

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Target	*examples*
2.1. Receptors for physiological ligands	
Transmembrane receptors	
2.1.1. G-protein-coupled receptors	- *adrenergic receptors* - *opioid receptors*
2.1.2. ligand-gated ion channels	- *$GABA_A$ receptors*
2.1.3. kinase-linked receptors	- insulin receptor
Intracellular receptors	
2.1.4. nuclear receptors	- PPARγ (peroxisome proliferator-activated receptor γ - pregnane X receptor
2.2. Other targets/approaches	
2.2.1. enzymes	***- cyclo-oxygenase (in pain chapter)*** ***- dihydrofolate reductase*** ***- HIV protease*** ***- tyrosine kinases*** ***- angiotensin-converting enzyme***
2.2.2. ion channels and transporters	- *voltage-gated Na channels*
2.2.3. protein therapeutics	- *GLP-1 receptor agonists* *-TNF-α monoclonal antibodies (e.g. infliximab)*
2.2.4. gene therapy	- *Nusinersen* - *Tisagenlecleucel /Axicabtagene ciloleucel*

2.1.4. nuclear receptors

examples of drug targets:

- mineralocorticoid receptor
- glucocorticoid receptor
- Vitamin D receptor
- Peroxisome proliferator-activated receptor
- thyroid hormone receptors
- **pregnane X receptor**

The human genome contains at least 48 members of the nuclear receptor family, many of which are important drug targets and play a vital role in endocrine signaling as well as metabolic regulation

responsible for pharmacology of 10% and pharmacokinetics of 60% of all prescription drugs

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Nuclear receptors

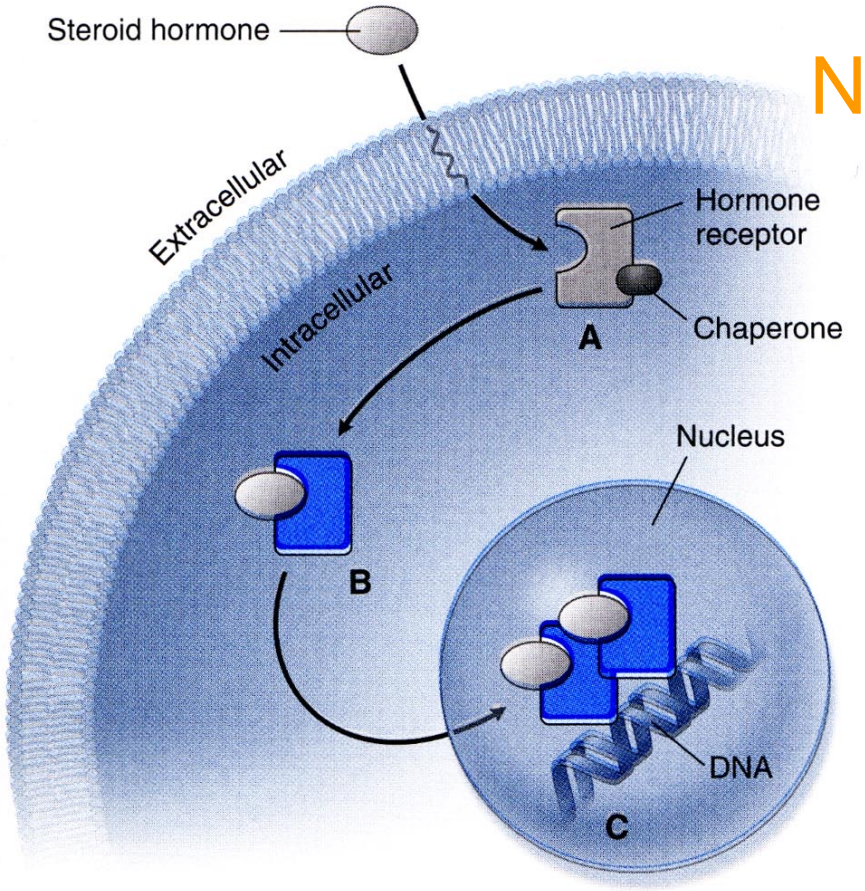

Figure 1-8. Lipophilic Molecule Binding to an Intracellular Transcription Factor. **A.** Small lipophilic molecules can diffuse through the plasma membrane and bind to intracellular transcription factors. In this example, steroid hormone binding to a cytosolic hormone receptor is shown, although some receptors of this class may be located in the nucleus before ligand binding. **B.** Ligand binding triggers a conformational change in the receptor (and often, as shown here, dissociation of a chaperone repressor protein) that leads to transport of the ligand-receptor complex into the nucleus. In the nucleus, the ligand-receptor complex typically dimerizes. In the example shown, the active form of the receptor is a homodimer (two identical receptors binding to one another), but heterodimers (such as the thyroid hormone receptor and the retinoid X receptor) may also form. **C.** The dimerized ligand-receptor complex binds to DNA, and may then recruit coactivators or corepressors (not shown). These complexes alter the rate of gene transcription, leading to a change (either up or down) in cellular protein expression.

The **class I nuclear receptors** include receptors for:

- steroid hormones (estrogen, androgens progesterone, glucocorticoids, mineralocorticoids)
- thyroid hormone
- vitamins D and A and derivatives

class II nuclear receptors bind mostly lipids that are already present to some extent in the cell. They include:

-peroxisome proliferator activated receptor (PPAR)
- liver oxysterol receptor (LXR)
- farnesoid receptor (FXR)

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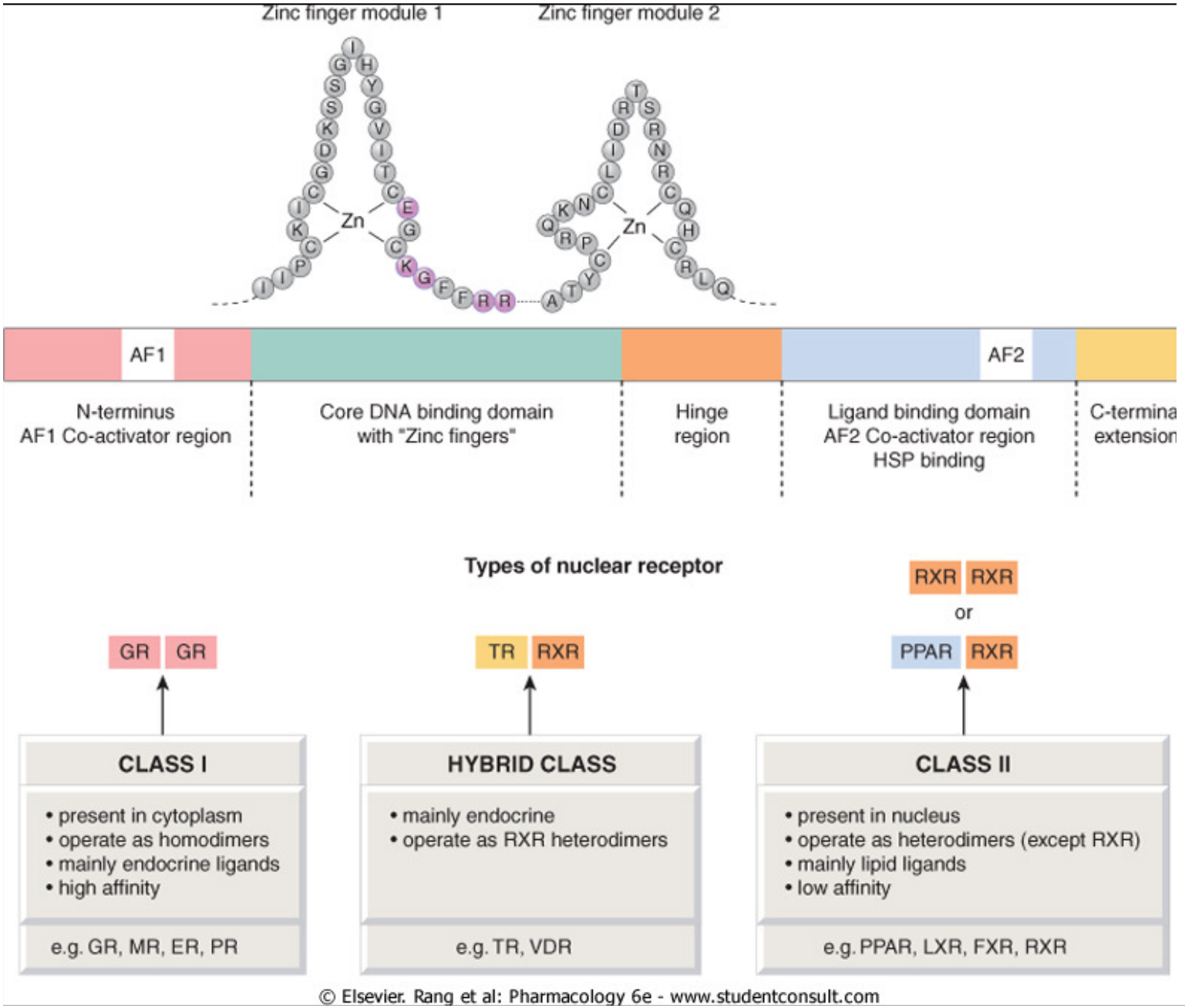
Nuclear receptors



TOP: Illustration of the different domains of a nuclear receptor. The heterogeneous N-terminal domain harbors the AF1 (activation function 1) site. This binds transcription factors that modify the properties of the receptor. The highly conserved core domain comprises two “zinc fingers”, cys- (or cys/his-) rich loops in the amino acid chain that are held in a particular conformation by zinc ions and which are responsible for DNA recognition and binding. The hinge region allows receptor dimerization, and the C-terminal domain contains the ligand-binding domain. Lower panel. The two main classes of nuclear receptors.ER, oestrogen receptor; FXR, farnesoid receptor; GR, glucocorticoid receptor; LXR, liver oxysterol receptor; MR, mineralocorticoid receptor; PPAR, peroxisome proliferator receptor; PR, prolactin receptor; RXR, retinoid receptor; TR, thyroid receptor; VDR, vitamin D receptor.

Downstream signaling

- Class I receptors: Binding of ligand → homodimerization, exposure of DNA-binding domains → translocation to nucleus → transactivate or transrepress genes by binding to positive or negative hormone response elements
- Regulation of a large number of genes by a single ligand: Glucocorticoid receptor: ~1% of the genome
- Hormone response elements are sequences of 4-5 base pairs
- Co-activators and co-repressors are, among others, enzymes involved in chromatin remodeling (ex. Co-activator: *histone acetylase, regulating unraveling of DNA, facilitating transcription*)

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Molecular mechanism of basic transactivation and transrepression by glucocorticoids

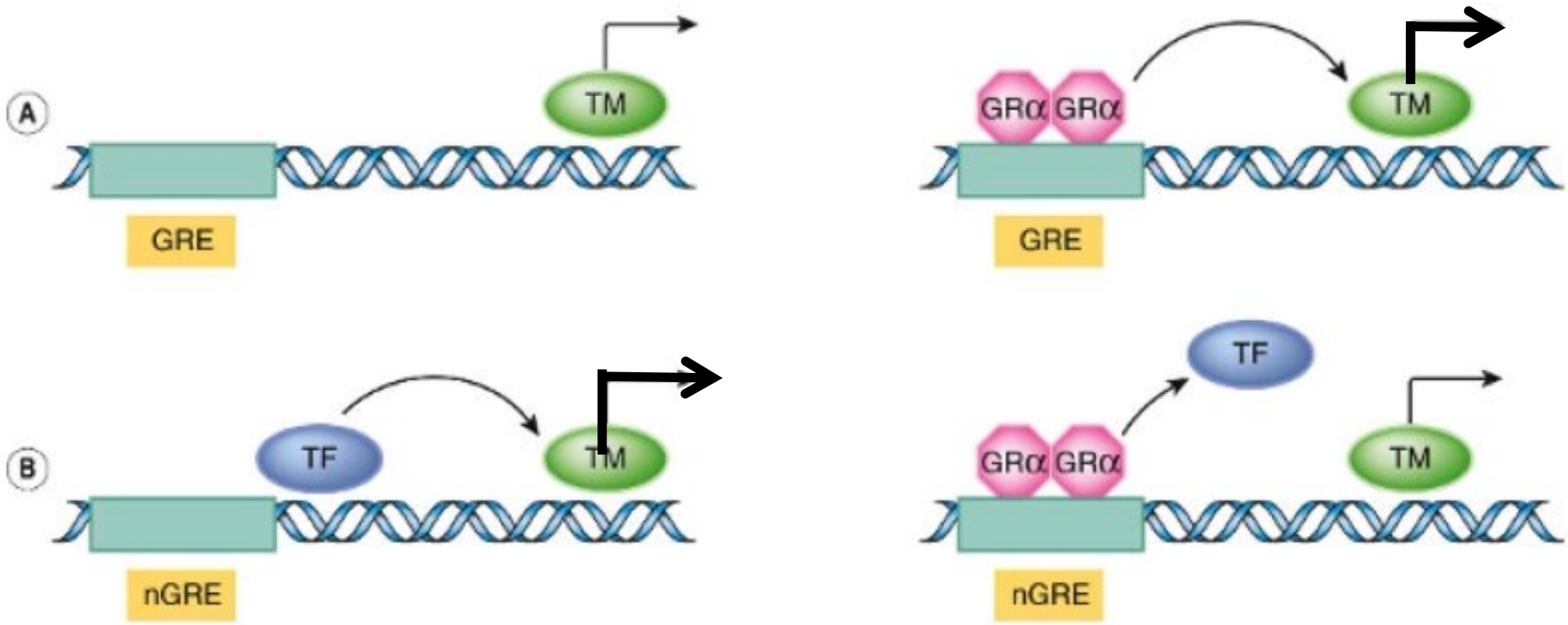
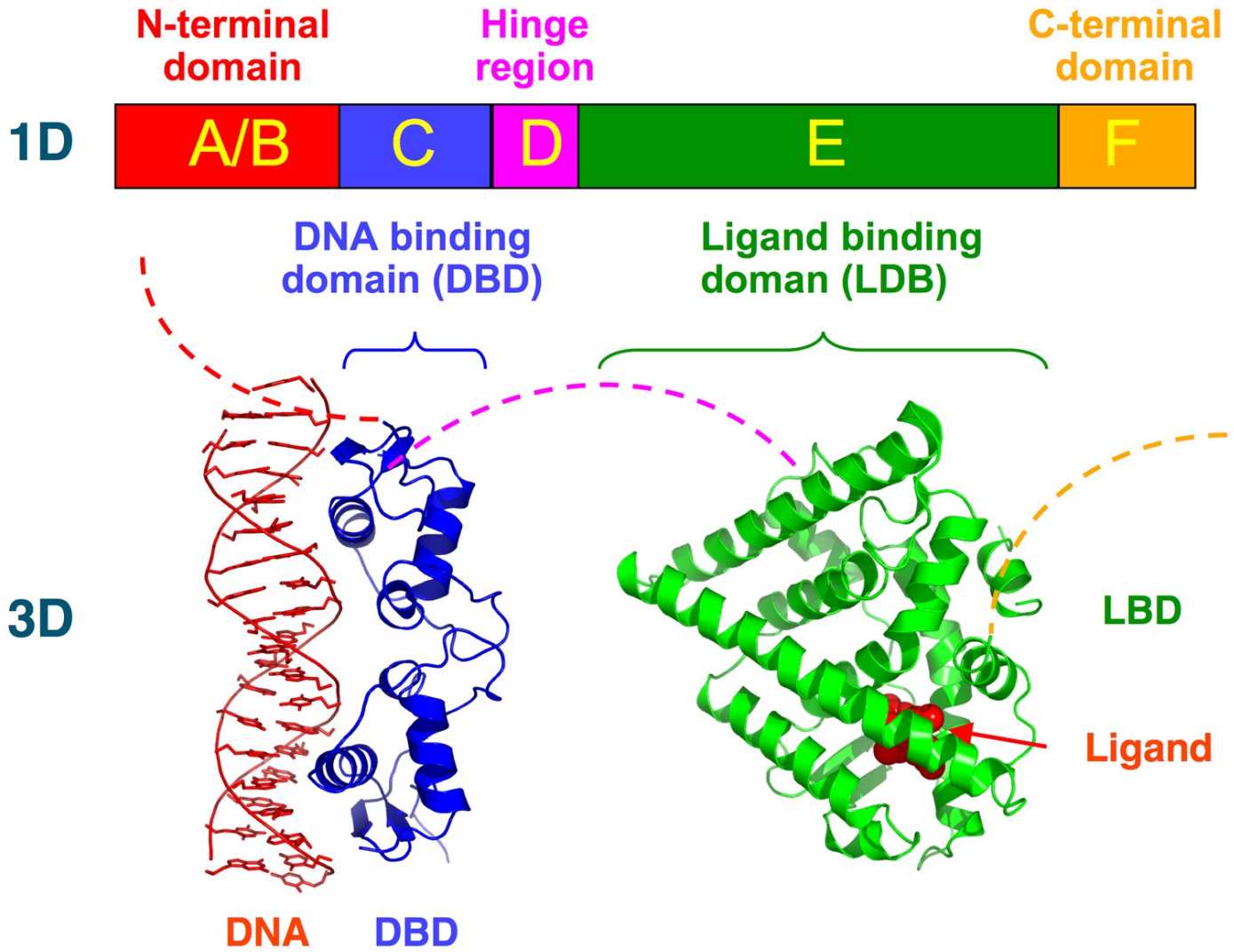


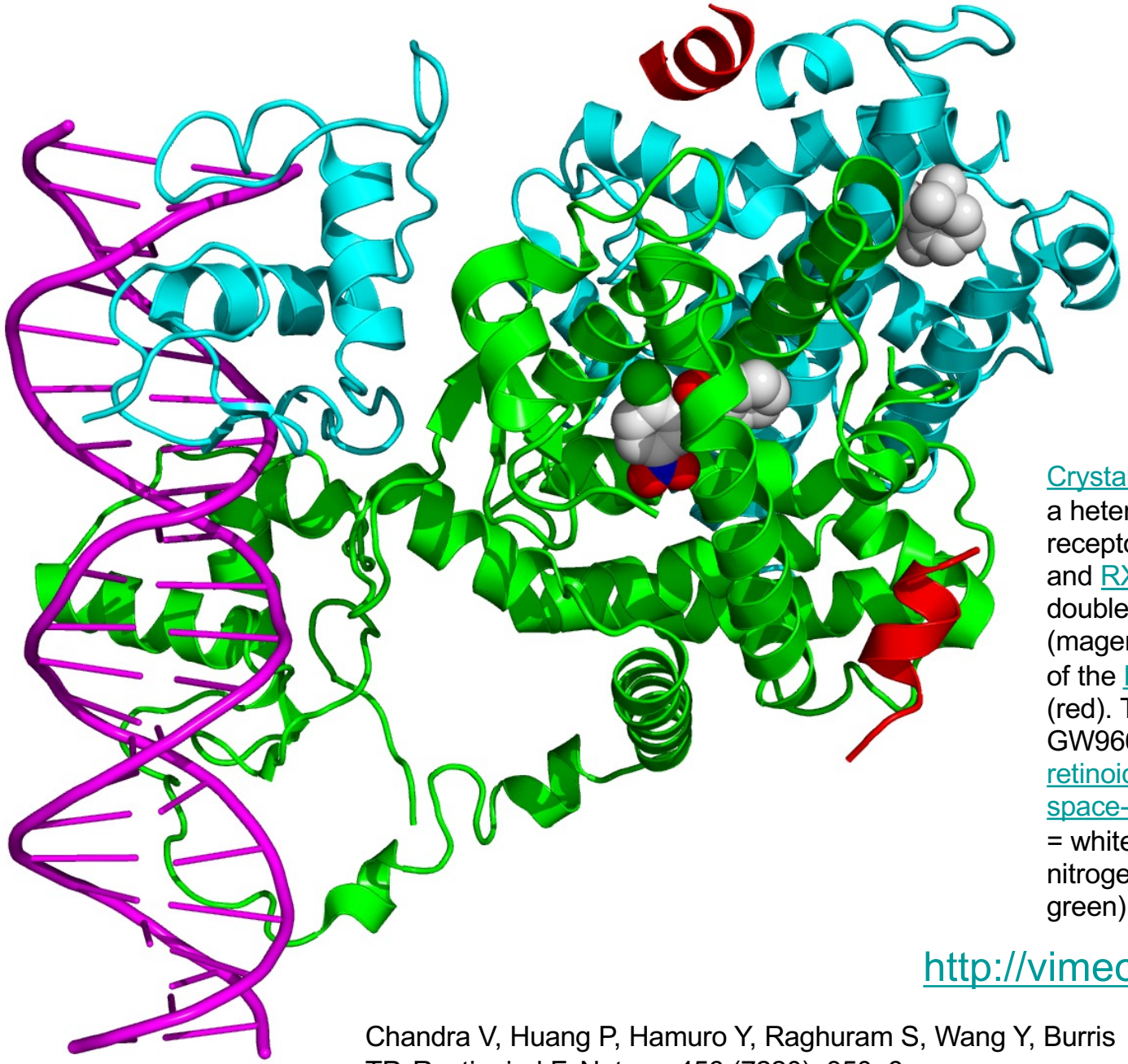
Figure 33.6: Molecular mechanism of action of glucocorticoids. Possible ways by which the liganded glucocorticoid receptor can control gene expression following translocation to the nucleus. [A] Basic transactivation mechanism. Here, the transcriptional machinery (TM) is presumed to be operating at a low level. The liganded glucocorticoid receptor (GR) dimer binds to one or more 'positive' glucocorticoid response elements (GREs) within the promoter sequence (shaded zone) and upregulates transcription. [B] Basic transrepression mechanism. The transcriptional machinery is constitutively driven by transcription factors (TF). In binding to the 'negative' GRE (nGRE), the receptor complex displaces these factors and expression falls.

Structural Organization of Nuclear Receptors



Structural Organization of Nuclear Receptors Top – Schematic 1D amino acid sequence of a nuclear receptor. Bottom – 3D structures of the DBD (bound to DNA) and LBD (bound to hormone) regions of the nuclear receptor. The structures shown are of the estrogen receptor. (By Boghog2 - Own work, Public Domain, https://commons.wikimedia.org/w/index.php?curid=10577727)

example: peroxisome proliferator-activated receptor γ (PPARγ)



Crystallographic structure of a heterodimer of the nuclear receptors PPAR-γ (green) and RXR-α (cyan) bound to double stranded DNA (magenta) and two molecules of the NCOA2 coactivator (red). The PPAR-γ antagonist GW9662 and RXR-α agonist retinoic acid are depicted as space-filling models (carbon = white, oxygen = red, nitrogen = blue, chlorine = green).

http://vimeo.com/8320279

Chandra V, Huang P, Hamuro Y, Raghuram S, Wang Y, Burris TP, Rastinejad F. Nature. 456 (7220): 350–6.

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PPARγ receptors

- Expressed in adipose tissue, skeletal muscle, heart, lung, ovary
- Physiological roles in adipocyte differentiation and glucose homeostasis
- Disease relevance: Diabetes, psoriasis, cancer, inflammation
- Drugs: thiazolidinediones (agonists) as antidiabetic agents of second choice. Great clinical interest for further applications

PPARγ agonists induce

- Decrease of free fatty acid levels in serum
- Increase of lipogenesis in adipose tissue

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Dominant negative mutations in human PPARγ associated with severe insulin resistance, diabetes mellitus and hypertension

I. Barroso*†, M. Gurnell†‡, V. E. F. Crowley†‡§, M. Agostini‡, J. W. Schwabe‖, M. A. Soos‡§, G. Ll Maslen*, T. D. M. Williams¶, H. Lewis#, A. J. Schafer*, V. K. K. Chatterjee‡ & S. O'Rahilly‡§

(Nature (1999), 402:880)

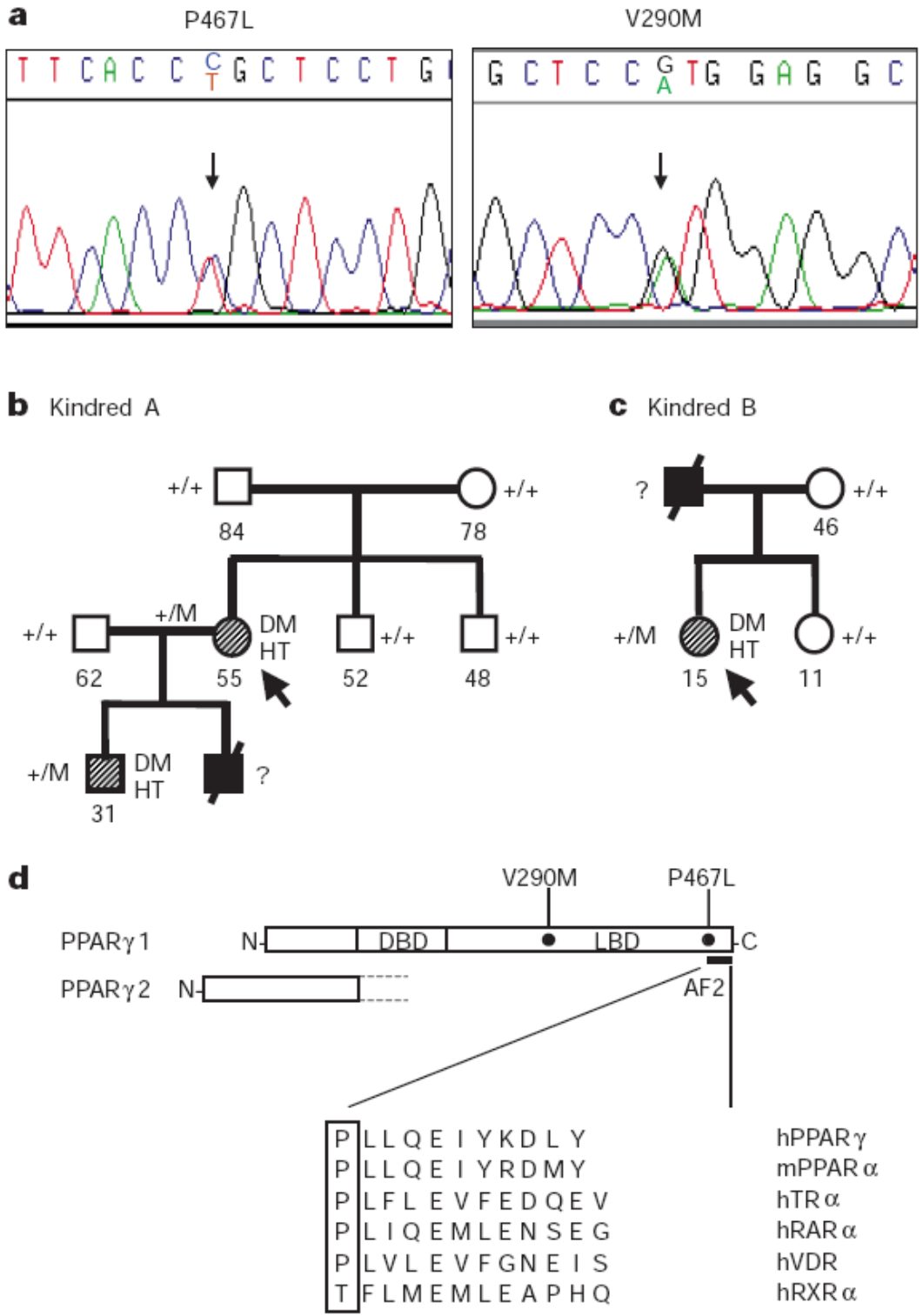
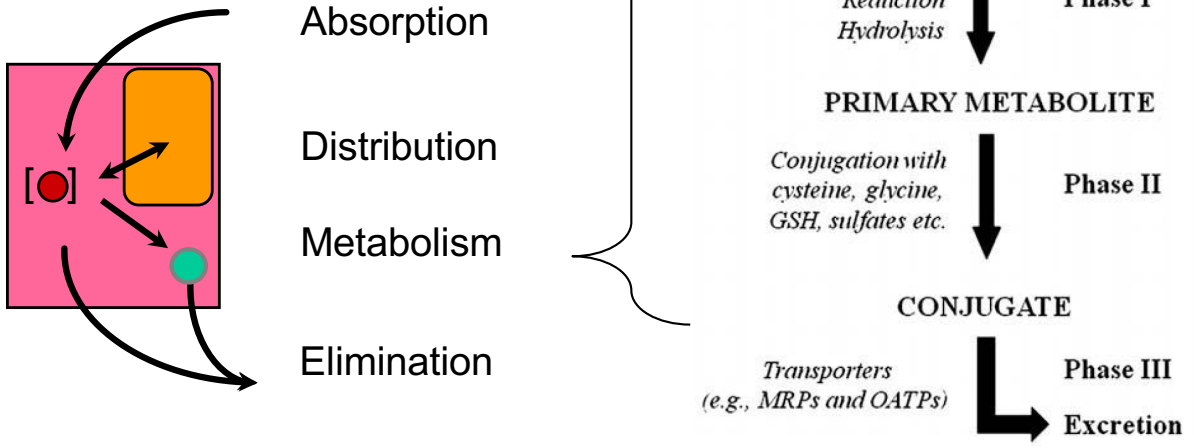


Figure 1 Two new mutations, P467L and V290M, in human PPARγ. **a**, Heterozygous nucleotide substitutions. Left, CCG to CTG corresponding to a proline to leucine mutation at codon 467; right, GTG to ATG corresponding to a valine to methionine mutation at codon 290. **b**, **c**, Family pedigrees confirm complete concordance between the clinical phenotype and the presence of the heterozygous P467L (**b**) and V290M (**c**) receptor mutations. The age and genotype (+, wild type; M, mutant) of members is indicated. The affected individuals (striped symbols) were diabetic (DM) and hypertensive (HT), with no known diabetes or hypertension in other family members (empty symbols). Arrows indicate the probands and filled symbols denote deceased individuals. **d**, Location of the P467L and V290M mutations in PPARγ. The γ1 and γ2 receptor isoforms share common DNA-binding (DBD) and ligand-binding (LBD) domains linked to divergent N-terminal regions. Val 290 is located in the centre of the LBD and Pro 467 at the origin of a C-terminal amphipathic α-helix. An alignment of nuclear receptor sequences indicates that this residue and the helical motif are highly conserved. Studies have shown that this region is important for ligand-dependent transcription activation (AF-2) and coactivator recruitment[11].

example nuclear receptors: The nuclear receptor family regulates pharmacokinetics of about 60% of all prescription drugs

- Mainly two nuclear receptors, the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR), are regulators of drug metabolizing enzymes (see Pharmacokinetics class)
- CAR and PXR are xenosensors that mediate drug-induced changes by increasing transcription of genes that are involved in drug clearance and disposition
- genetic variability in these receptors affects drug pharmacokinetics and drug-drug interactions



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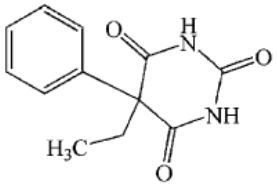
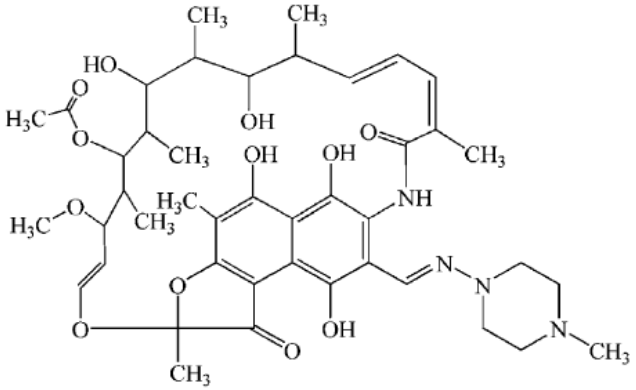
Chemical structure of some CAR or PXR ligands

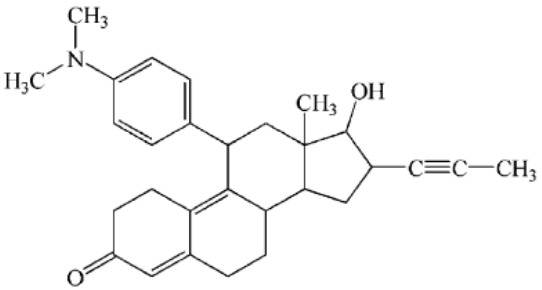
DRUGS



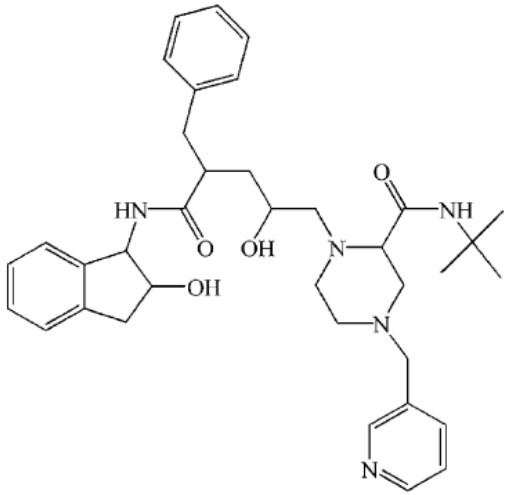
Phenobarbital



Rifampicin

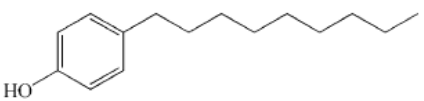


RU486

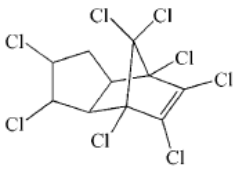


Indinavir

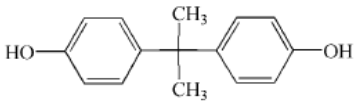
CHEMICAL POLLUTANTS



Nonylphenol



Chlordane



Bisphenol A

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Potency of some CAR and PXR ligands

Table 6
Ligand affinity for CAR and PXR.

Ligand	Receptor	EC_{50}–IC_{50}–K_i (nM)	Notes	References
16,17-Androstane-3-ol	mCAR	EC_{50} = 500		Shan et al. (2004)
5α-Androstan-3α-ol	hCAR	EC_{50} = 1000–>10,000	Inverse agonist	Moore et al. (2000a)
	mCAR	EC_{50} = 250–1500	Inverse agonist	Moore et al. (2000a)
				Tzameli et al. (2000)
				Forman et al. (1998)
5α-Androst-16-en-3α-ol	mCAR	EC_{50} = 400–~5000	Inverse agonist	Forman et al. (1998)
				Kawamoto et al. (2000)
CITCO	hCAR	EC_{50} = 25–304		Maglich et al. (2003)
				Huang et al. (2003)
Clotrimazole	hCAR	EC_{50} = 50–~1000	Inverse agonist	Maglich et al. (2003)
				Huang et al. (2003)
				Moore et al. (2000a)
Di(2-ethylhexyl) phthalate	hCAR	EC_{50} = 211		DeKeyser et al. (2009)
17β-Estradiol	mCAR	EC_{50} = 1000		Kawamoto et al. (2000)
Estrone	mCAR	EC_{50} = 1000		Kawamoto et al. (2000)
Meclizine	hCAR	EC_{50} = ~500–1000	Inverse agonist	Huang et al. (2003)
	mCAR	EC_{50} = 25		Huang et al. (2003)
(5β)-Pregnane-3,20-dione	hCAR	EC_{50} = 670–3000		Moore et al. (2000a)
				Maglich et al. (2003)
	mCAR	EC_{50} = >10,000	Weak agonist	Kawamoto et al. (2000)
		≫10,000	Inverse agonist	
Progesterone	mCAR	EC_{50} = ~3000	Inverse agonist	Kawamoto et al. (2000)
TCPOBOP	mCAR	EC_{50} = 20–100		Suino et al. (2004)
				Moore et al. (2000a)
				Tzameli et al. (2000)
Testosterone	mCAR	EC_{50} = ~7000	Inverse agonist	Kawamoto et al. (2000)
Artemisinin	hPXR	EC_{50} = 5000–34,000		Burk et al. (2005)
				Persson et al. (2006)
Betamethasone	hPXR	EC_{50} = 20,000		Persson et al. (2006)
Carbamazepine	hPXR	EC_{50} = 15,600		Persson et al. (2006)
5β-Cholestan-3α,7α,12α-triol	hPXR	EC_{50} = 5000		Goodwin et al. (2003)
	mPXR	EC_{50} = 2500		Goodwin et al. (2003)
CITCO	hPXR	EC_{50} = ~3000		Maglich et al. (2003)
Clotrimazole	hPXR	EC_{50} = 800–5000		Moore et al. (2000a)
				Lehmann et al. (1998)
				Bertilsson et al. (1998)
	mPXR	EC_{50} = 1000		Moore et al. (2000a)
Colupulone	hPXR	EC_{50} = 10		Teotico et al. (2008)
Corticosterone	hPXR	EC_{50} = 30,000		Blumberg et al. (1998)
				Kliewer et al. (1998)
Coumestrol	hPXR	EC_{50} = 25,000		Blumberg et al. (1998)
Dexamethasone	hPXR	EC_{50} = 5000–~10,000		Lehmann et al. (1998)
				Persson et al. (2006)

some CAR and PXR target genes

Table 8
Some of the CAR and PXR target genes involved in the phases I, II and III metabolism.

Nuclar receptor	Target gene	Organism	Effect on target gene
Phase I			
CAR, PXR	*Aldh1A1*	Mouse	↑
CAR, PXR	*Aldh1A7*	Mouse	↑
CAR	*CYP1A1*	Mouse	↑
PXR	*CYP1A1*	Mouse	↓
PXR	*CYP1A1*	Human	↑
PXR	*CYP1A2*	Human	↑
PXR	*CYP1A6*	Human	↑
CAR	*CYP2A4*	Mouse	↑
CAR	*CYP2A6*	Human	↑
CAR, PXR	*CYP2B1*	Rat	↑
CAR, PXR	*CYP2B2*	Rat	↑
CAR, PXR	*CYP2B6*	Human	↑
CAR, PXR	*CYP2B10*	Mouse, human	↑
CAR	*CYP2C6*	Rat	↑
CAR	*CYP2C7*	Rat	↑
PXR	*CYP2C8*	Human	↑
CAR, PXR	*CYP2C9*	Human	↑
CAR, PXR	*CYP2C19*	Human	↑
CAR, PXR	*CYP3A1*	Rat	↑
PXR	*CYP3A2*	Rat	↑
CAR, PXR	*CYP3A4*	Human	↑
CAR, PXR	*CYP3A11*	Mouse, human	↑
PXR	*CYP3A13*	Mouse	↑
PXR	*CYP3A23*	Rat	↑
PXR	*CYP3A44*	Mouse	↑
PXR	*CYP7A1*	Human	↓
PXR	*CYP11A1*	Human	↑
PXR	*CYP11B1*	Human	↑
PXR	*CYP11B2*	Human	↑
CAR, PXR	*Por*	Mouse	↑
Phase II			
CAR, PXR	*GSTA1*	Mouse, rat	↑
CAR, PXR	*GSTA2*	Mouse, rat	↑
CAR	*GSTA3*	Mouse, rat	↑
PXR	*GSTA4*	Mouse	↑
CAR, PXR	*Gstm1*	Mouse, rat	↑
CAR, PXR	*Gstm2*	Mouse	↑
PXR	*Sult2A1*	Mouse, human	↑
CAR, PXR	*UGT1A1*	Mouse, human	↑
PXR	*UGT1A3*	Human	↑
PXR	*UGT1A4*	Human	↑
CAR	*UGT2B1*	Rat	↑
Phase III			
CAR, PXR	*MDR1A*	Mouse, human	↑
PXR	*MDR1B*	Mouse	↑
CAR	*MRP1*	Mouse	↑
CAR, PXR	*MRP2*	Mouse, rat, human	↑
CAR, PXR	*MRP3*	Mouse, human	↑
CAR	*MRP4*	Mouse	↑
CAR, PXR	*OATP2*	Mouse, rat	↑

Phase 1

Phase 2

Phase 3

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Target	*examples*
2.1. Receptors for physiological ligands	
Transmembrane receptors	
2.1.1. G-protein-coupled receptors	*- adrenergic receptors* *- opioid receptors*
2.1.2. ligand-gated ion channels	*- $GABA_A$ receptors*
2.1.3. kinase-linked receptors	***- insulin receptor***
Intracellular receptors	
2.1.4. nuclear receptors	***- PPARγ (peroxisome proliferator-activated receptor γ*** ***- pregnane X receptor***
2.2. Other targets/approaches	
2.2.1. enzymes	***- cyclo-oxygenase (in pain chapter)*** ***- dihydrofolate reductase*** ***- HIV protease*** ***- tyrosine kinases*** ***- angiotensin-converting enzyme***
2.2.2. ion channels and transporters	*- voltage-gated Na channels*
2.2.3. protein therapeutics	*- GLP-1 receptor agonists* *-TNF-α monoclonal antibodies (e.g. infliximab)*
2.2.4. gene therapy	*- Nusinersen* *- Tisagenlecleucel /Axicabtagene ciloleucel*

2.2.1. Enzymes

examples of targets:	aspect of interest
- Oxidoreductases	
- cyclooxygenases (COXs)	much used → pain
- dihydrofolate reductase	selective targeting
- Transferases	
- protein kinase C	
- tyrosine kinases	*inhibitors as important new drug class*
- GABA transaminase	
- Proteases	
- HIV aspartyl protease	development of molecule
- angiotensin-converting enzyme	several targets in 1 pathway
- Ligases (=synthases)	
- dihydropteroate synthase	selective targeting

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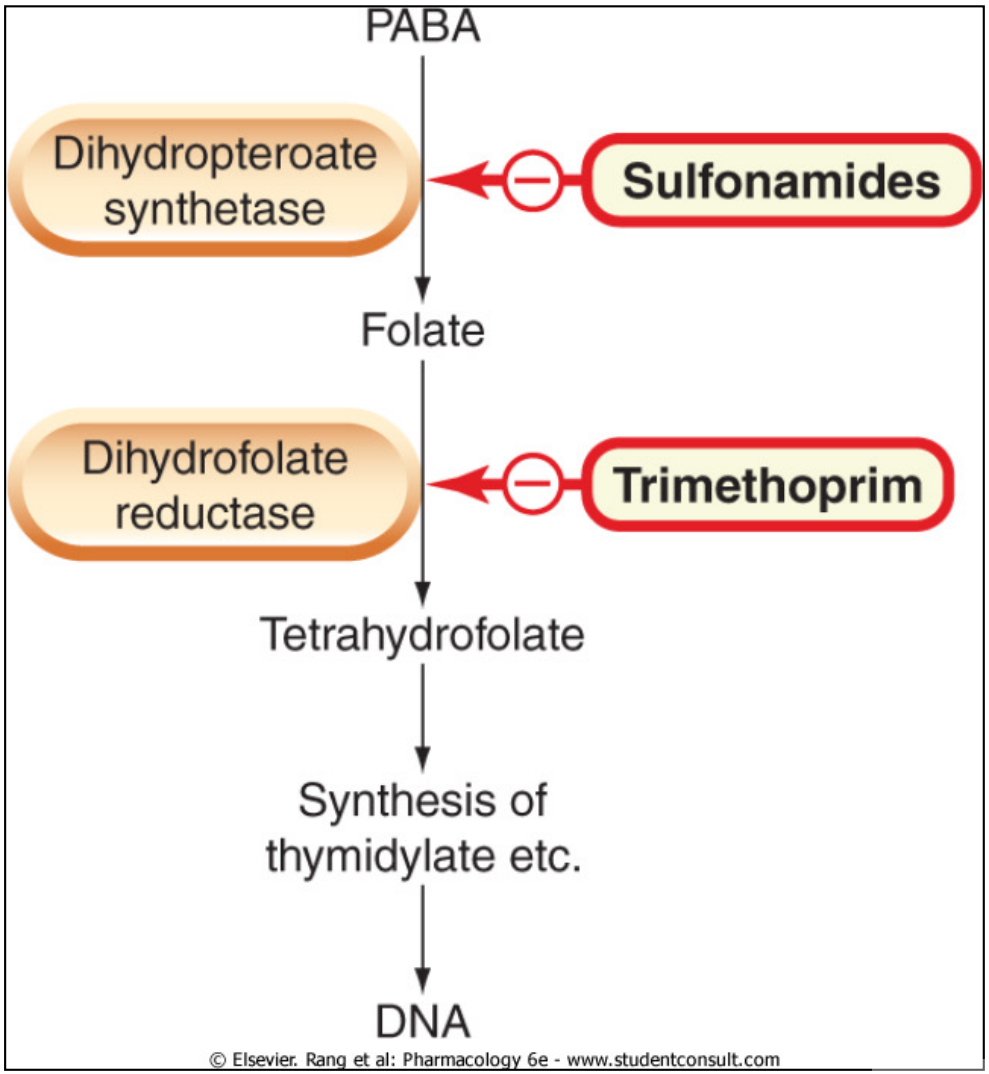
example oxidoreductases, 2: dihydrofolate reductase

the synthesis of bacterial folate



- Folate is required for DNA synthesis in both bacteria and humans
- Humans cannot synthesize folate and must obtain it from the diet, while most species of bacteria, as well as the asexual forms of malarial protozoa, lack the necessary transport mechanisms and must synthesize their own folate de novo. → sulfonamides take advantage of this difference between the bacterial and human organisms.
- the pathway for transforming folate to tetrahydrofolate, which is required for thymidilate synthesis, is identical in humans and bacteria, however, the sensitivity of the human and bacterial isoform of the dihydrofolate reductase for inhibitors such as Trimetoprim is different (→ see table on following slide)

Figure 46-2 The action of sulfonamides and trimethoprim on bacterial folate synthesis. See Figure 22.2 for more detail of tetrahydrofolate synthesis, and Table 45.1 for comparisons of antifolate drugs. PABA, p-aminobenzoic acid.

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structure of sulfonamides and trimethoprim, and specificity of dihydrofolate reductase for inhibitors

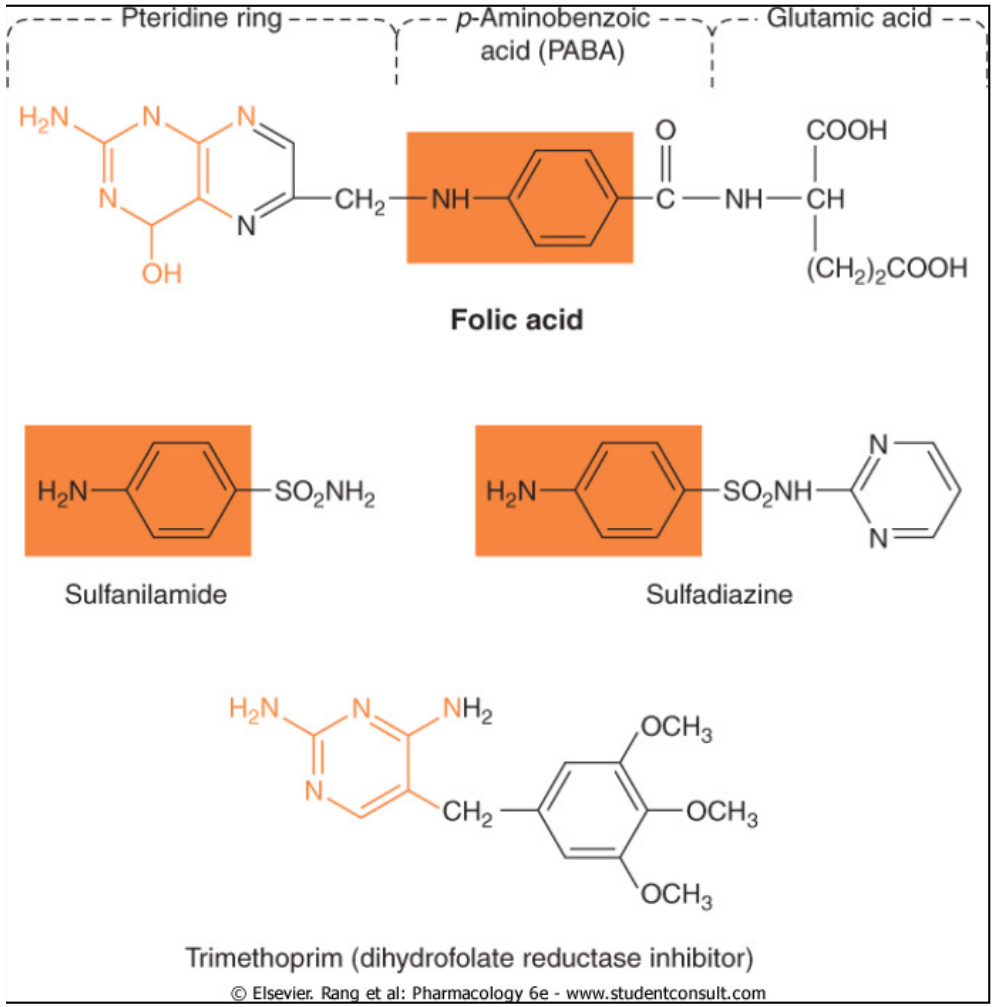


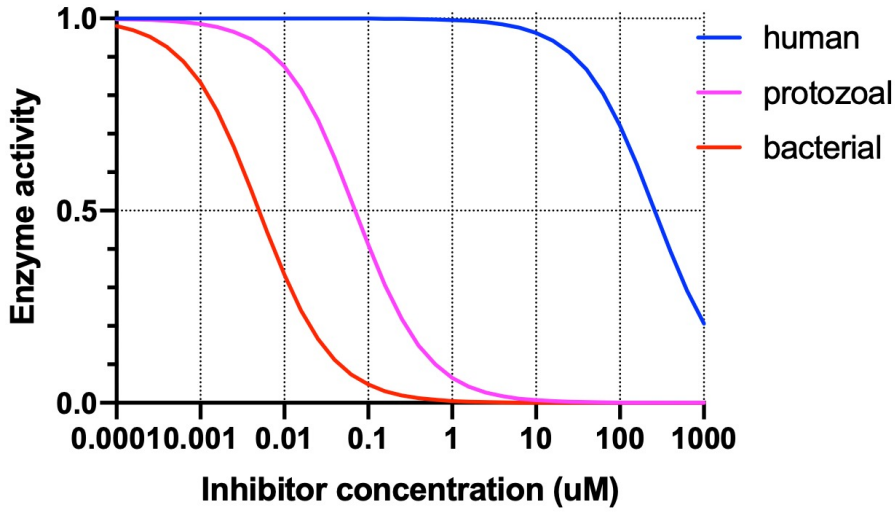
Figure 46-1 Structures of two representative sulfonamides and trimethoprim. The structures illustrate the relationship between the sulfonamides and the p-aminobenzoic acid moiety in folic acid (orange box), as well as the possible relationship between the antifolate drugs and the pteridine moiety (orange). Co-trimoxazole is a mixture of sulfamethoxazole and trimethoprim.

Table 45-1. Specificity of inhibitors of dihydrofolate reductase

Inhibitor	IC_{50}(µmol/l) for dihydrofolate reductase		
	Human	***Protozoal***	***Bacterial***
Trimethoprim	260	0.07	0.005
Pyrimethamine	0.7	0.0005	2.5
Methotrexate	0.001	~0.1[a]	Inactive

[a]Tested on *Plasmodium berghei*, a rodent malaria.

Trimethoprim is an antibiotic, used in combination with sulfonamides, pyrimethamine is used in combination to treat malaria infections, methotrexate is an anticancer drug.



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tyrosine kinase inhibitors

- tyrosine kinases (tk) regulate cellular processes that – if deregulated - contribute to tumor development and progression, including cell growth, differentiation, migration, and apoptosis
- the human genome encodes 90 proteins with tyrosine kinase domains
  - Receptor tyrosine kinases (extracellular ligand-binding domain)
  - Non-receptor tyrosine kinases (in cytoplasm)
- many human tumors display aberrant activation (e.g. constitutive activation) of tyrosine kinases, caused by genetic alterations

  → for tumors whose growth is driven by these activated kinases, targeted drugs can potentially inhibit or reverse malignant progression
  - tyrosine kinase inhibitors are relatively new drugs, they are a type of anticancer drugs, called “targeted cancer drugs” (together with monoclonal antibodies and cytokines). The prototype drug is imatinib (Gleevec©, Novartis)

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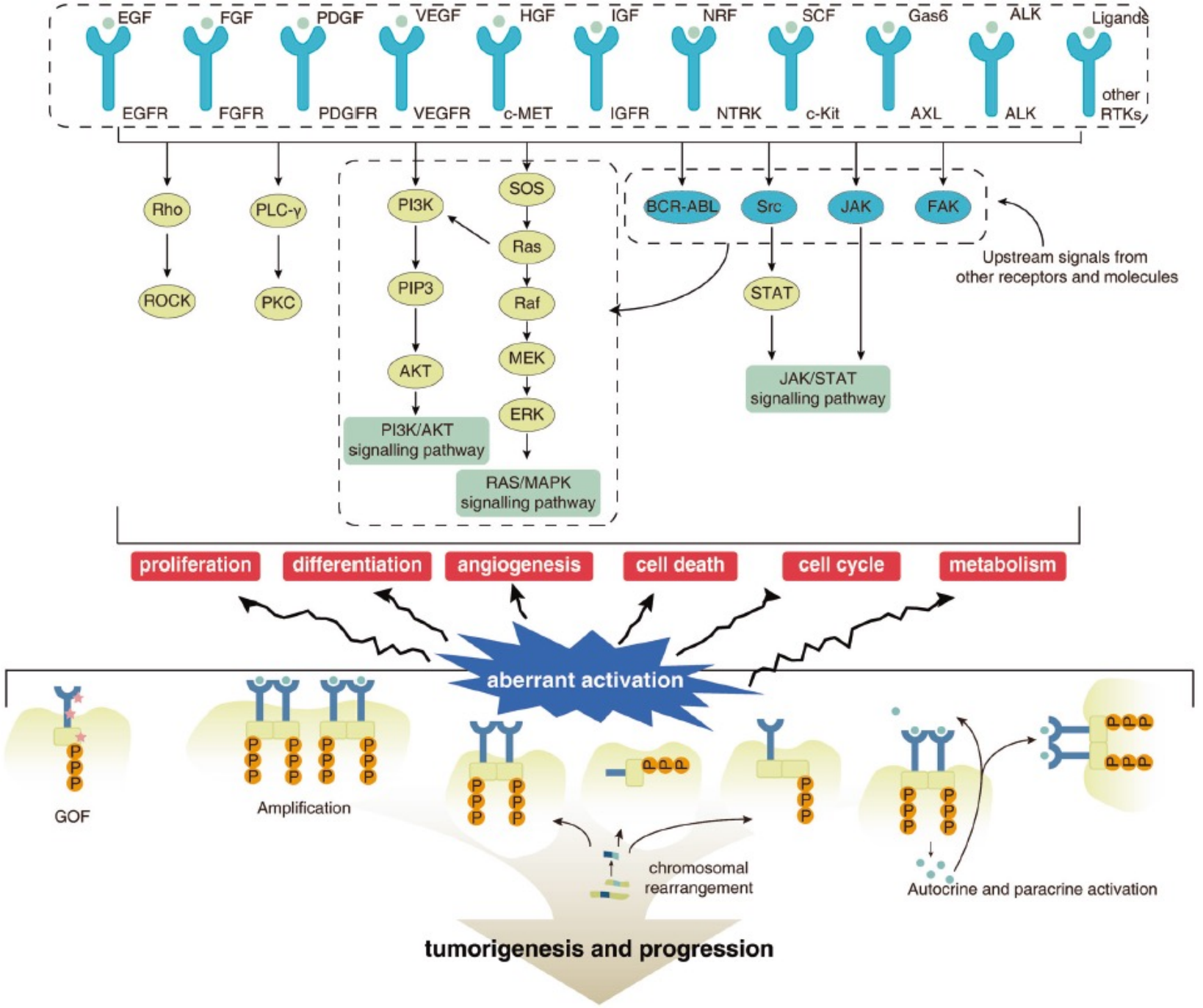


Fig. 1 The relationship between PTK and tumors. The normal activation and inactivation of PTK is essential to maintain normal cellular function. PTK activation mutations include gain-of-function mutations, genomic amplification and overexpression, chromosomal rearrangements (gene fusions), and ligand autocrine/paracrine loops

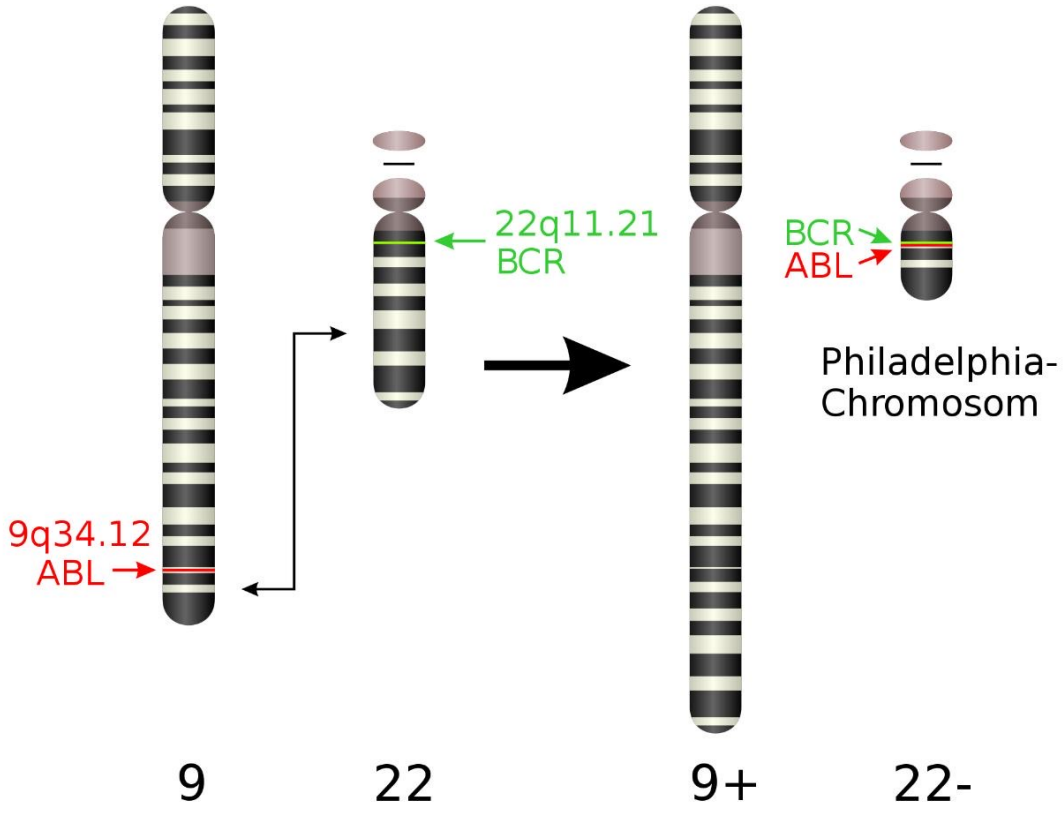
https://doi.org/10.1038/s41392-022-01168-8

Chronic myeloid leukemia

15% of leukemias

Fusion of Abelson murine leukemia (ABL) gene (chromosome 9, a tyrosine kinase) with breakpoint cluster region (BCR) gene (22)

→ deregulated, constitutively active tyrosine kinase



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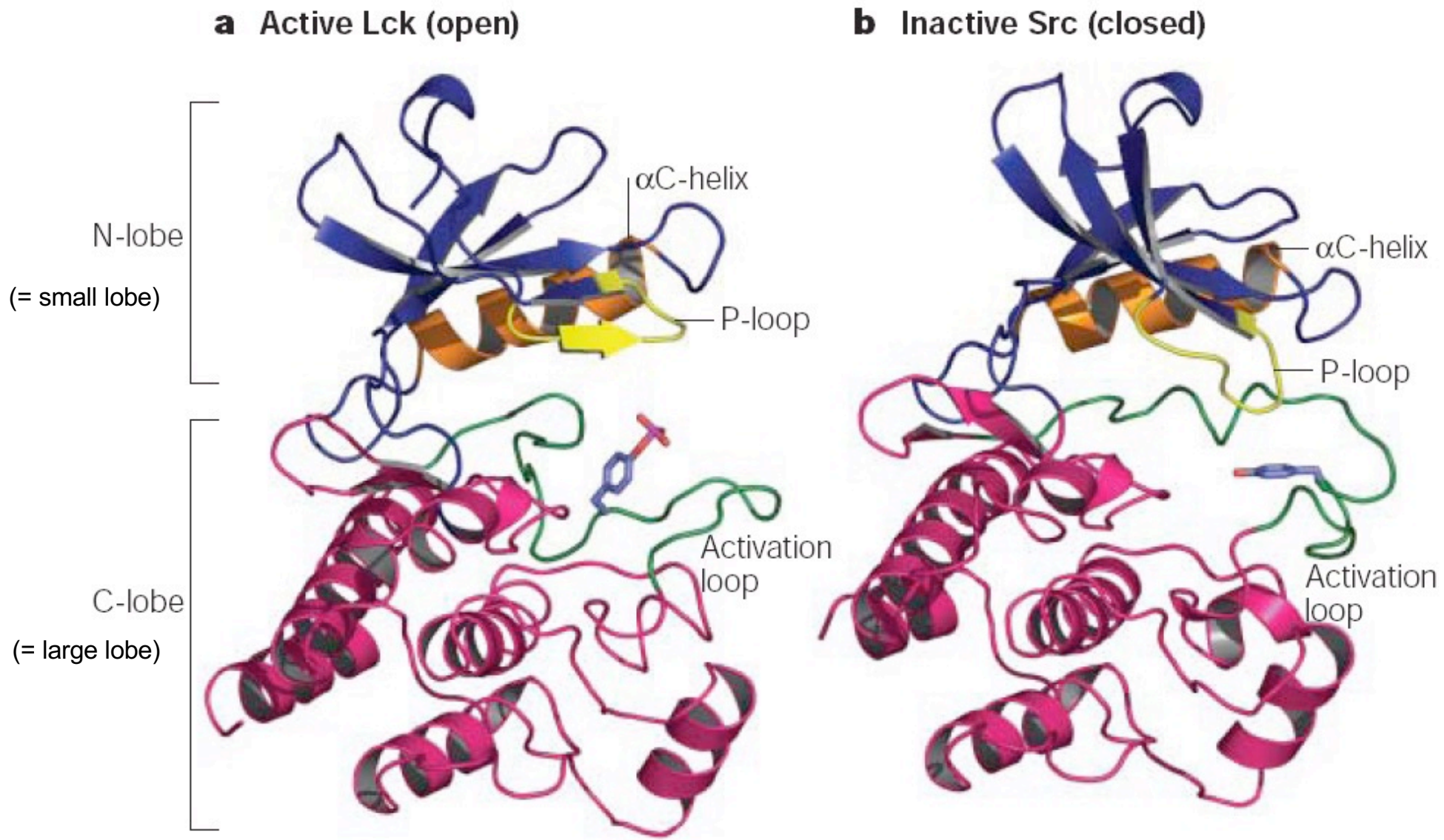
Box 2 | **Structure and catalysis by protein kinase domains**



-P-Loop: forms the roof of the active site, contributes to coordination of γ-phosphate group
-changes in the relative orientation of the two lobes are required for binding of substrates, catalysis and release of products
-the conformation of the αC helix and of the activation loop are associated with the activated and the inhibited state of the TK
-the activation loop adapts an extended conformation in the active state, stabilized by phosphorylation of one or more residues, thereby acting as binding platform for the substrate

Chronic myelogenous leukemia

Table 3. Responses to imatinib versus IFN plus cytarabine in newly diagnosed patients with CML in chronic phase

	CHR, %	MCR, %	CCR, %	Progression-free survival, 14 mo
Imatinib, n = 553	95.3	85.2	73.8	92.1
IFN + cytarabine, n = 553	55.5	22.1	8.5	73.5
P	.001	.001	.001	.001

Median duration of follow-up equaled 19 months. CHR indicates complete hematologic response; MCR, major cytogenetic response; and CCR, complete cytogenetic response.

Hematologic response: blood cell counts go back to normal
Cytogenetic response: no cells with Philadelphia chromosome can be found in the blood or the bone marrow

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development of resistance

- imatinib inhibits several types of permanently active TKs and is an extremely important drug in anticancer therapy
- however, despite this success, the majority of responding patients will with time develop resistance to the drugs
  - due to amplification of the tk gene or other mechanisms
  - in many cases due to secondary mutations in the tk gene

    → in many cases, these mutations are located in the catalytic domain and prevent or weaken interaction with the drug

→ second generation TK inhibitors with different site of action or conformational requirements can be used

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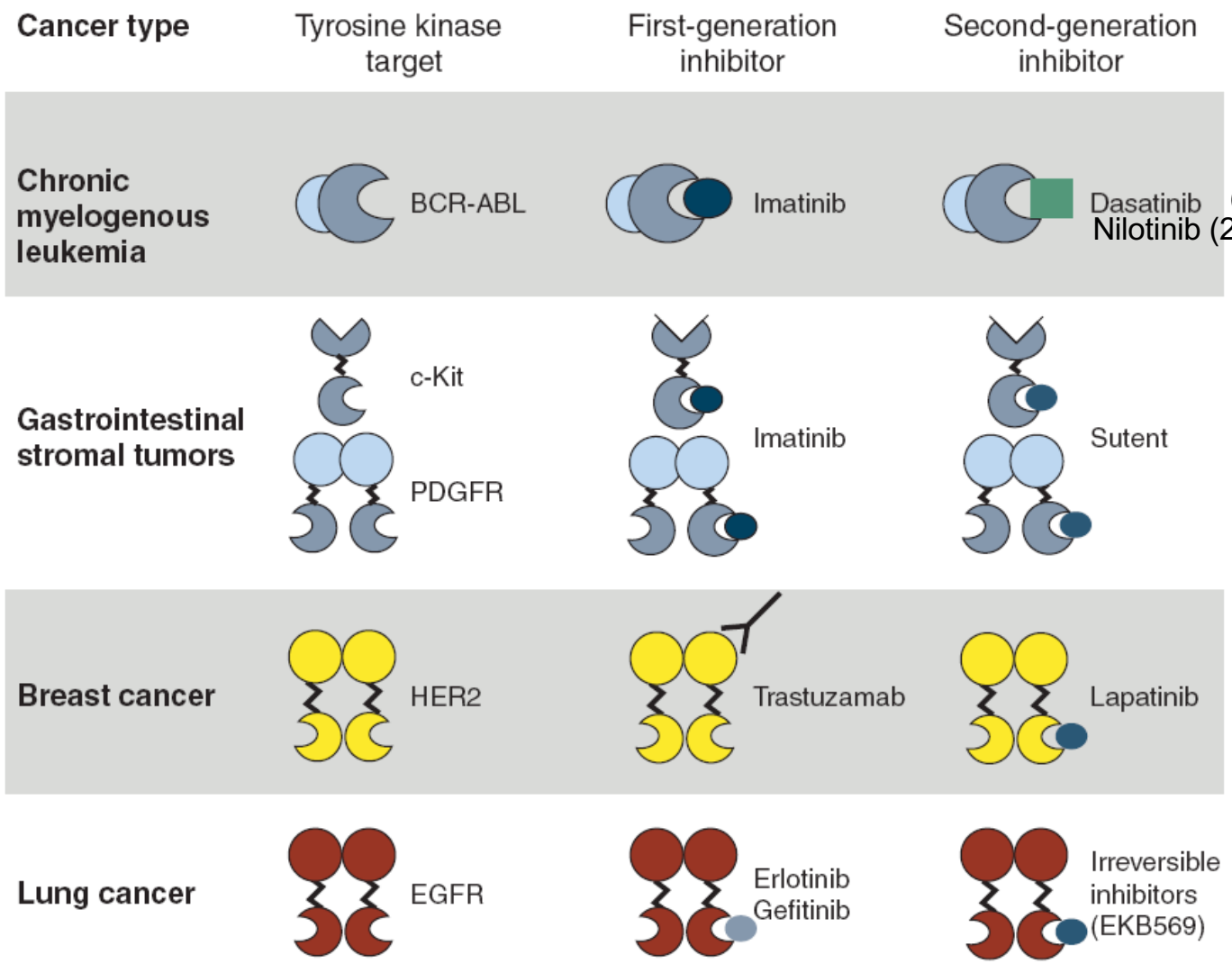
examples of first and second generation kinase inhibitors



(2006)
Nilotinib (2007)

More recent:
Bosutinib
Ponatinib (3rd gen)

The question of the development of resistance will also be discussed later.

for cancer treatment. Over time, evelop resistance to the drugs, in in the gene encoding the targeted tyrosine kinase. Second-generation inhibitors have been developed to address this problem. The first-and second-generation drugs can be administered sequentially or as a combination therapy.

(until 2013, a total of 25 small molecule kinase inhibitors have been approved by the FDA)

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Questions

- how does imatinib interrupt the activity of the BCR-Abl tyrosine kinase fusion protein?
- Why is the use of imatinib in this context called a “targeted cancer therapy”?

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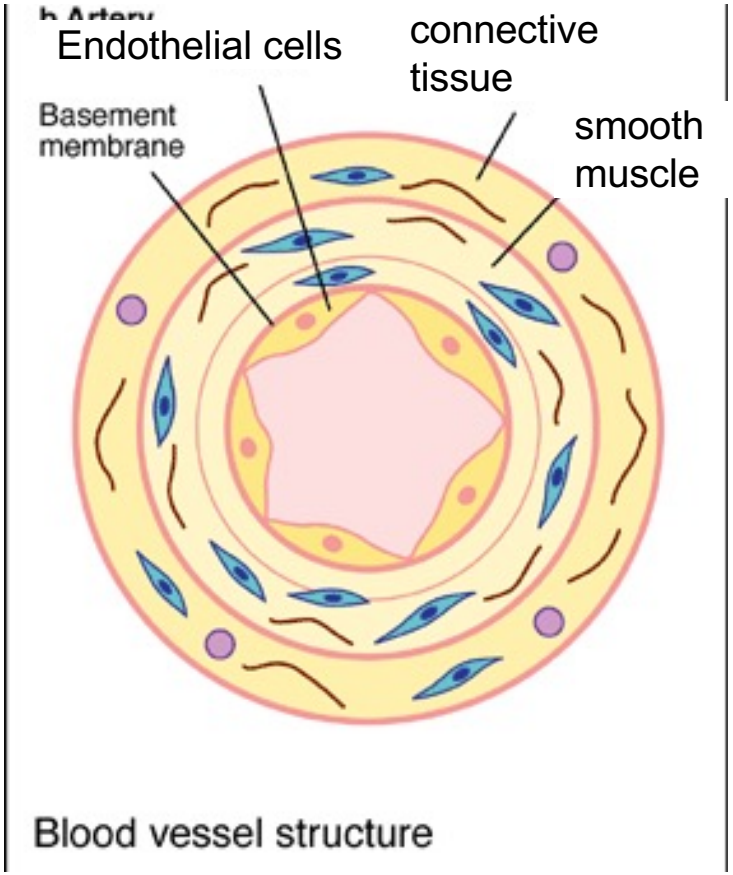
example proteases: angiotensin-converting enzyme context: control of vascular smooth muscle tone



Blood vessel structure

-The vascular system includes the vessels and tissue that carry or circulate fluids such as blood or lymph through the body

-The walls of arteries, arterioles, venules and veins contain smooth muscle whose contractile state is controlled by circulating hormones and by mediators released locally from sympathetic nerve terminals

-actions of drugs on the vascular system can be general or selective for certain organs or tissues

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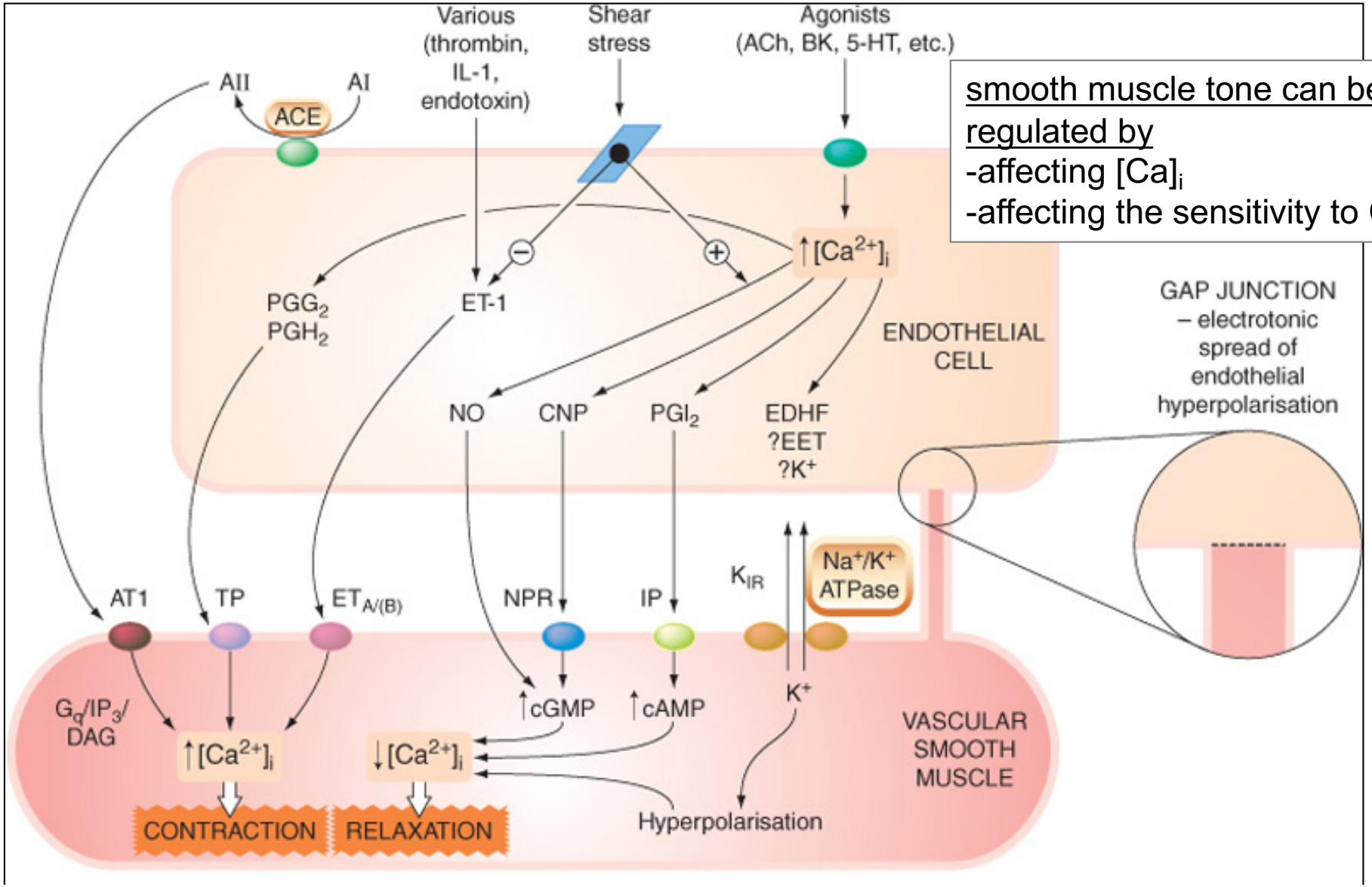
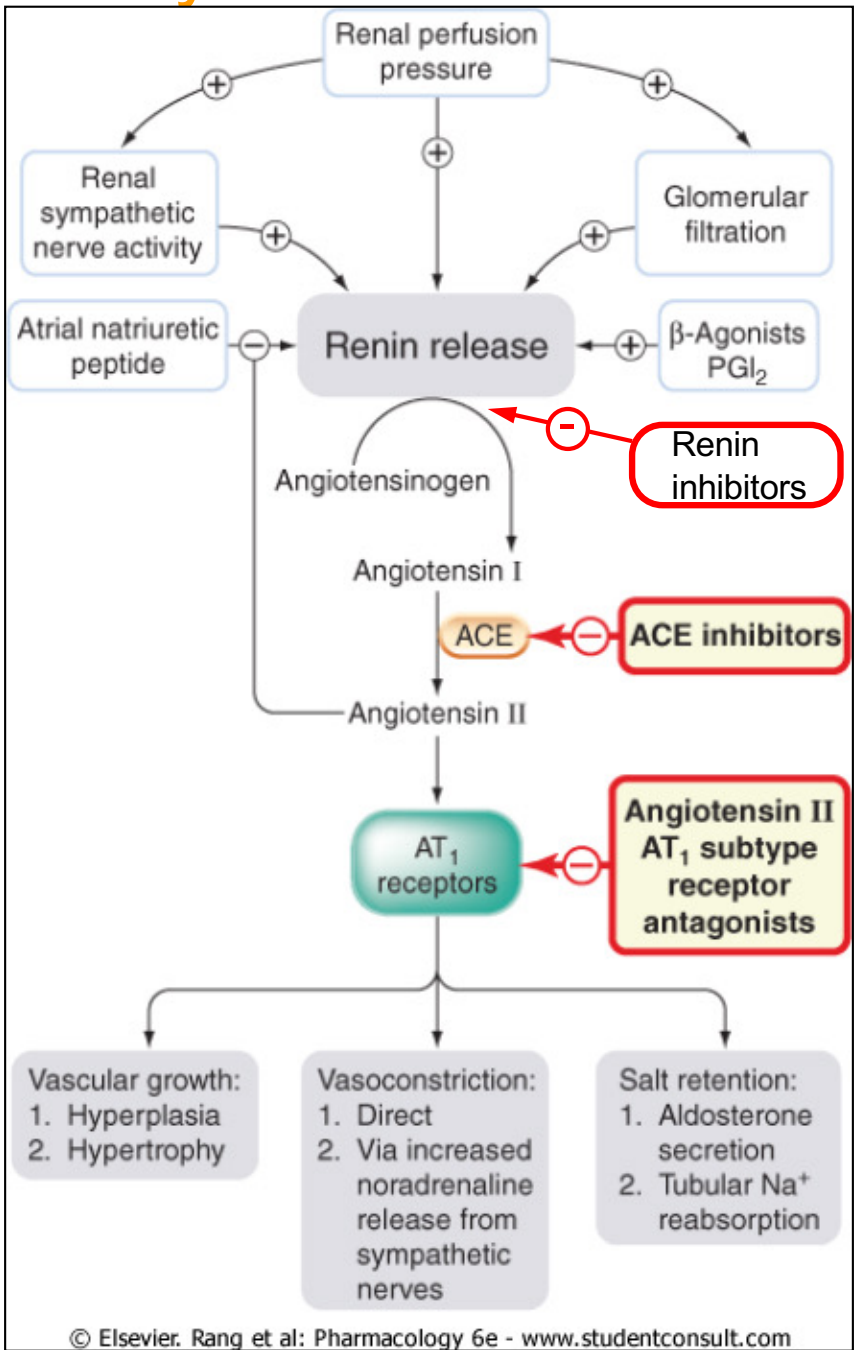
control of vascular smooth muscle tone



smooth muscle tone can be regulated by
-affecting $[Ca]_i$
-affecting the sensitivity to Ca

5-HT, 5-hydroxytryptamine; A, angiotensin; ACE, angiotensin-converting enzyme; ACh, acetylcholine; AT1, angiotensin AT1 receptor; BK, bradykinin; CNP, C-natriuretic peptide; DAG, diacylglycerol; EDHF, endothelium-derived hyperpolarising factor; EET, epoxyeicosatetraenoic acid; ET-1, endothelin-1; ETA/(B), endothelium A (and B) receptors; Gq, G-protein; IL-1, interleukin-1; IP, I prostanoid receptor; IP3, inosinol 1,4,5-trisphosphate; KIR, inward rectifying potassium channel; Na+/K+ ATPase, electrogenic pump; NPR, natriuretic peptide receptor; PG, prostaglandin; TP, T prostanoid receptor.

the renin-angiotensin system: synthesis of the vasoconstrictor Angiotensin II



The renin-angiotensin system
→ aldosterone secretion ↑
→ control of Na excretion, fluid volume
→ control of vascular tone

renin is secreted in the kidney by the juxtaglomerular apparatus in response to physiological stimuli, as a fall in Na concentration in the distal tubule and a fall in renal perfusion pressure

Two Angiotensin receptor types, AT1 and AT2, both GPCRs, exist. Angiotensin II does not distinguish between the two. However, Angiotensin II actions are predominantly mediated by AT1 receptors. The functional roles of AT2 receptors are currently incompletely understood.

Aliskiren, a direct renin inhibitor, on the market since 2007; has many unwanted effects

Figure 19.4 Control of renin release and formation, and action of angiotensin II. Sites of action of drugs that inhibit the cascade are shown. ACE, angiotensin-converting enzyme; AT1, angiotensin II receptor subtype 1.

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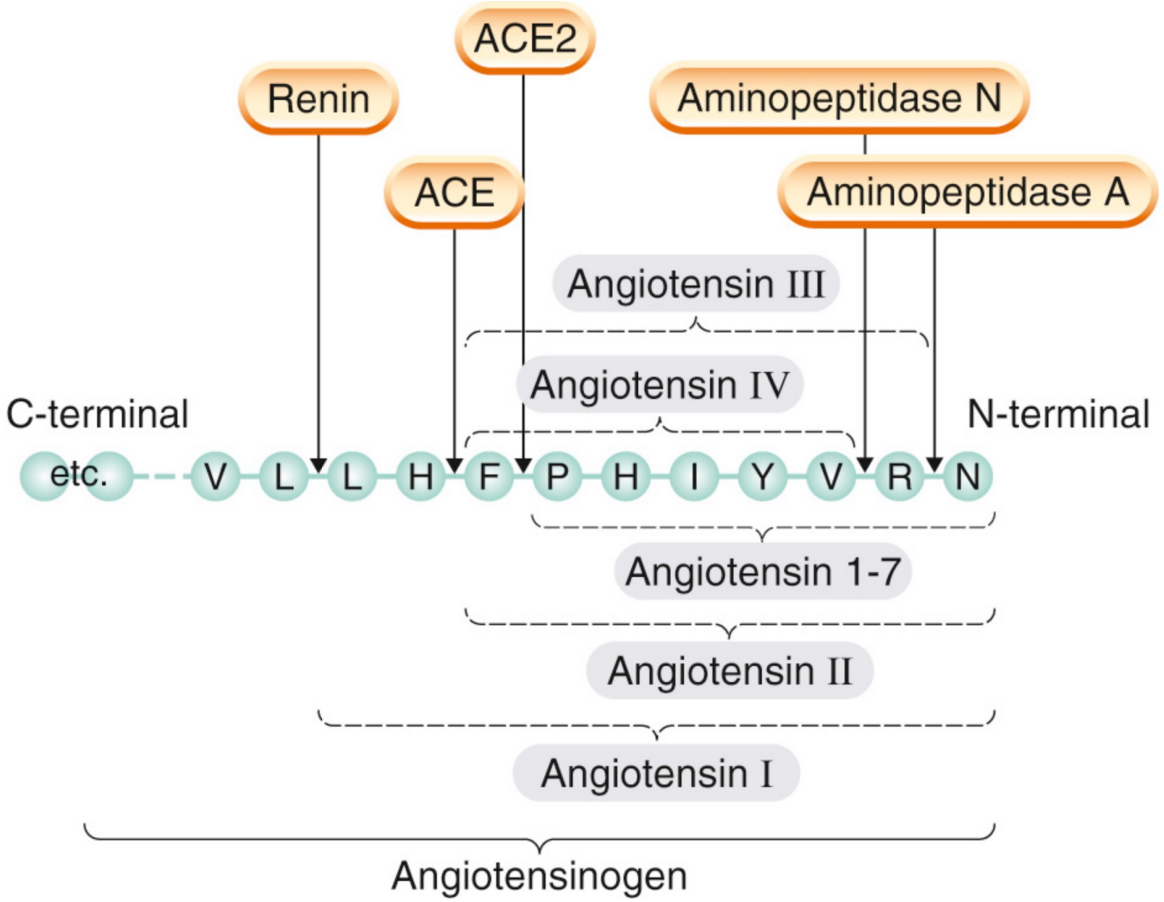
cleavage steps in Angiotensin II synthesis



peptide name	function, origin	enzyme leading to its formation
Angiotensinogen	a plasma globuline made in the liver	
Angiotensin I	-	renin
Angiotensin II	**vasoconstriction, aldosterone secretion ↑, Na reabsorption ↑ (acting on AT1 receptors)**	angiotensin-converting enzyme
Angiotensin III (probably less important)	aldosterone secretion ↑, thirst	aminopeptidase A
Angiotensin IV (probably less important)		aminopeptidase N
Angiotensin 1-7	opposes effects of Ang II	ACE2

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design of captopril

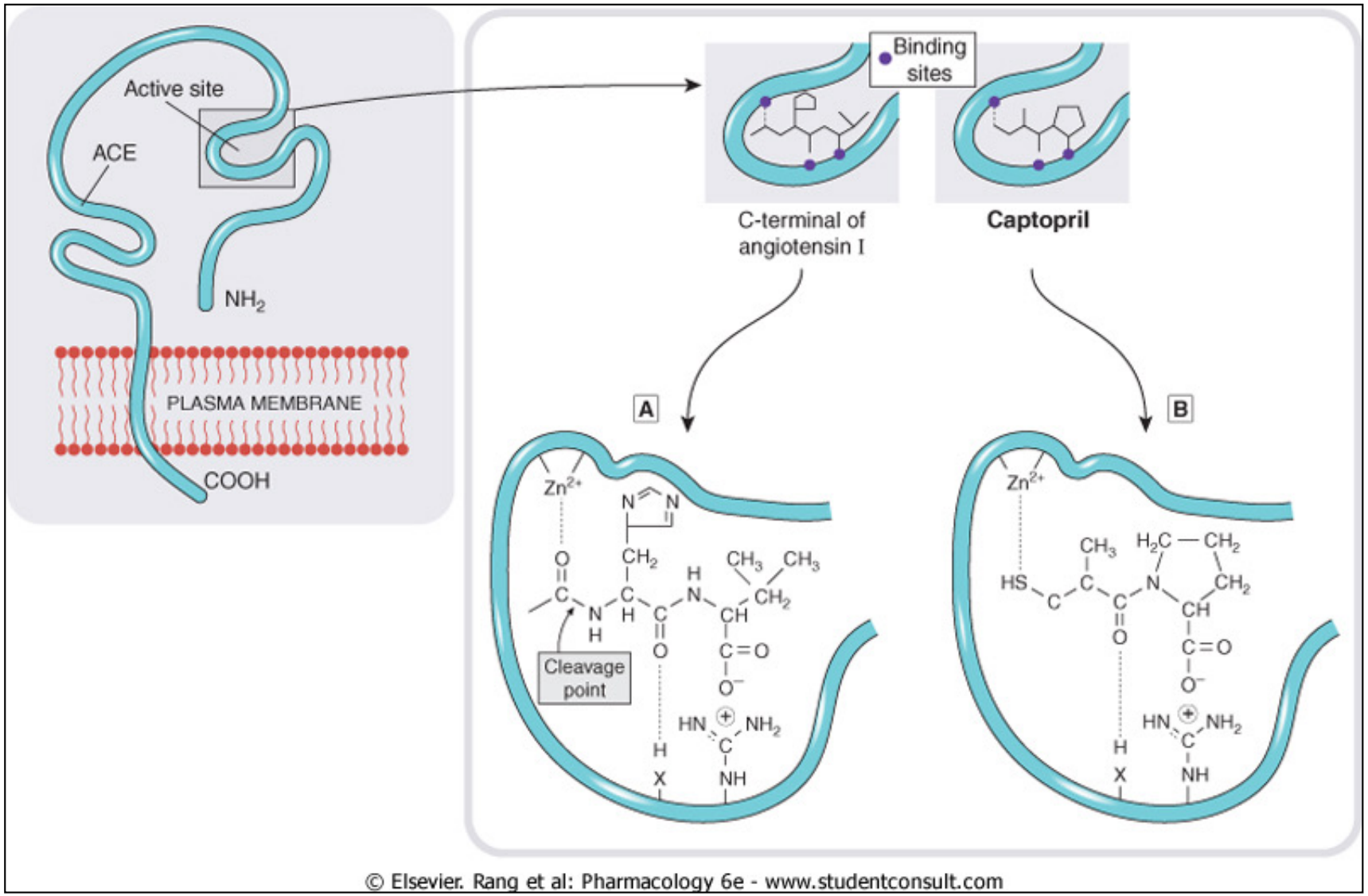

Figure 19.7 The active site of angiotensin-converting enzyme. Binding of angiotensin I. Binding of the inhibitor captopril, which is an analogue of the terminal dipeptide of angiotensin I.

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ACE inhibitors

Several specific ACE inhibitors have been developed, the first of which was captopril. Several ACE inhibitors, differing in duration of action and tissue distribution, are used clinically, including enalapril, lisinopril, ramipril, perindopril and trandolapril.

Action: inhibits the conversion of angiotensin I to angiotensin II, → lowers cardiac load as well as arterial pressure. *In part the effect on pressure is also due to the inhibition of the degradation of bradykinin (a vasodilator) by inhibition of Kininase II.*

Most important clinical uses of angiotensin-converting enzyme inhibitors

- Hypertension
- Cardiac failure
- Following myocardial infarction
- In people at high risk of ischaemic heart disease

Angiotensin II receptor subtype 1 antagonists (=sartans) have a different pharmacological profile but behave rather similarly to ACE inhibitors in clinical practice.

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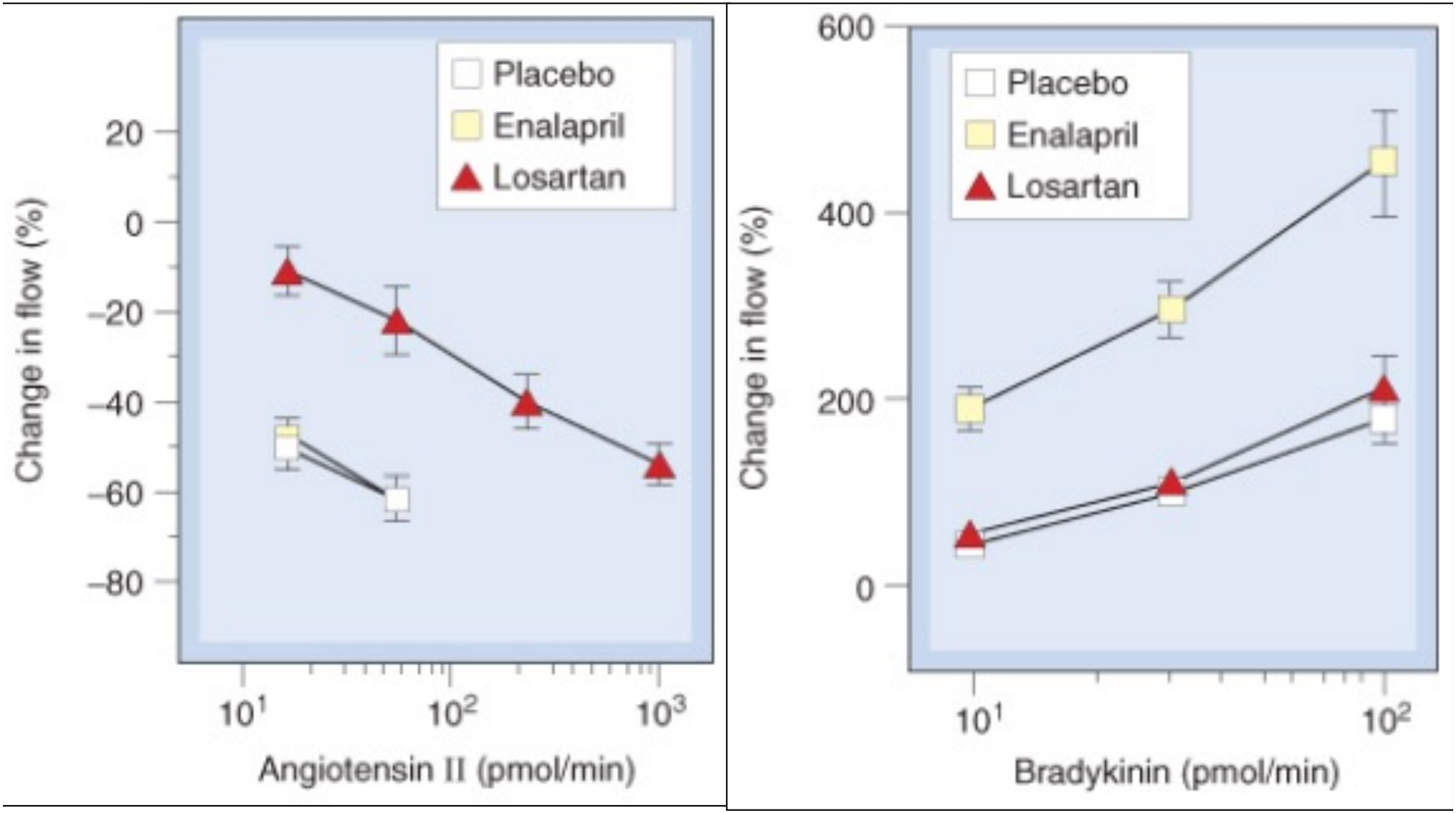

Figure 19.8 Comparison of effects of angiotensin-converting enzyme inhibition and angiotensin receptor blockade in the human forearm vasculature. Effect of brachial artery infusion of angiotensin II (left) or bradykinin (right) on forearm blood flow after oral administration of placebo, enalapril (10 mg) or losartan (100 mg). (From Cockcroft J R et al. 1993 J Cardiovasc Pharmacol 22: 579-584.)

example HIV protease: the viral life cycle and pharmacological intervention

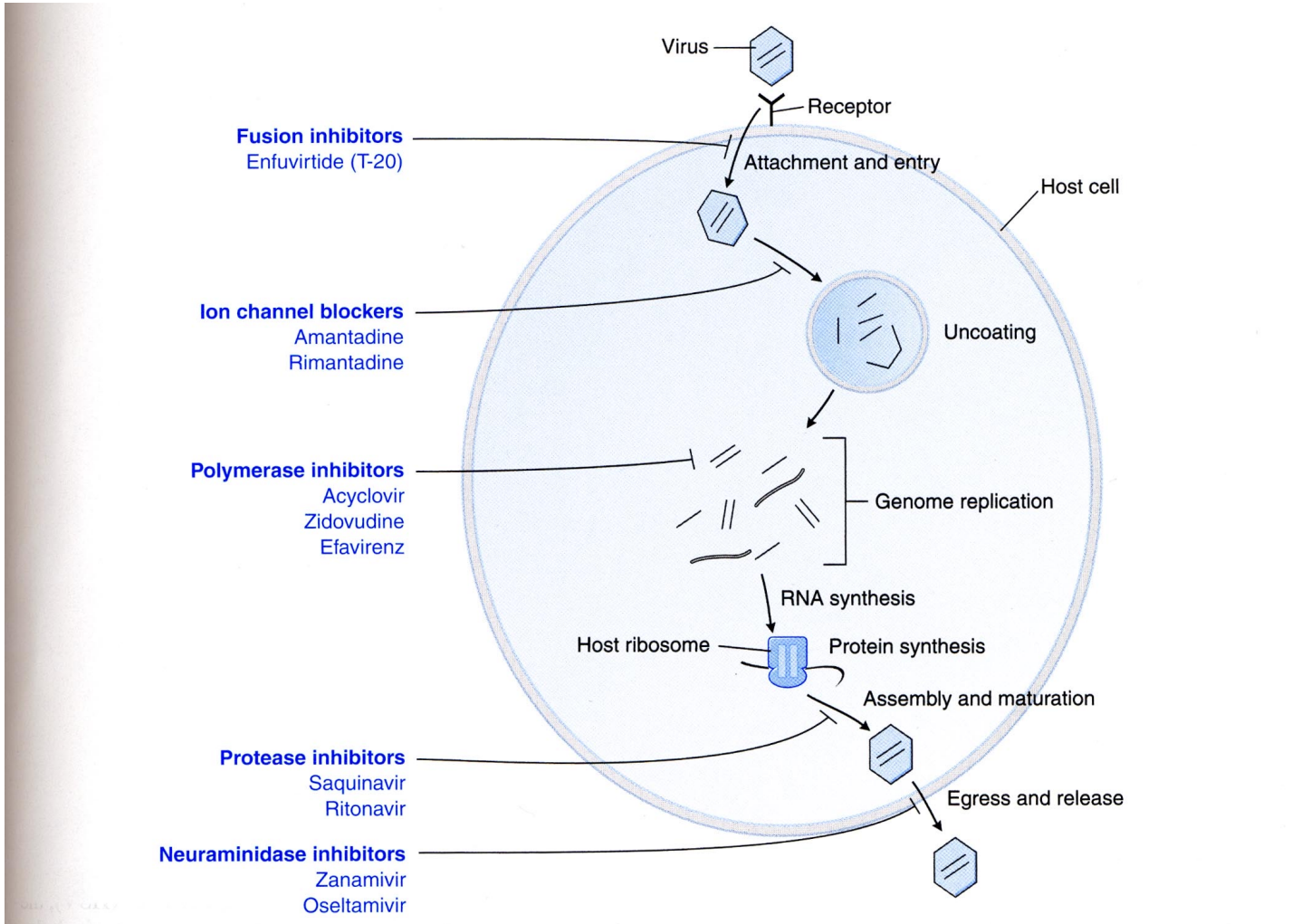

Figure 34-1. Viral Life Cycle and Pharmacologic Intervention. The viral life cycle can be divided into a sequence of individual steps, each of which is a potential site for pharmacologic intervention. Shown are drug classes and examples of individual agents that block each step. The majority of the currently approved antiviral agents are nucleoside analogues that target genome replication, typically by inhibiting viral DNA polymerase or reverse transcriptase. A number of other drug classes target other steps in the viral life cycle, including attachment and entry, uncoating, assembly and maturation, and egress and release. It should be noted that the details of viral replication differ for each type of virus, often presenting unique targets for pharmacologic intervention and drug development. For example, the life cycle of HIV (and other retroviruses) includes such additional steps as integration (see Fig. 34-2).

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development of ritonavir

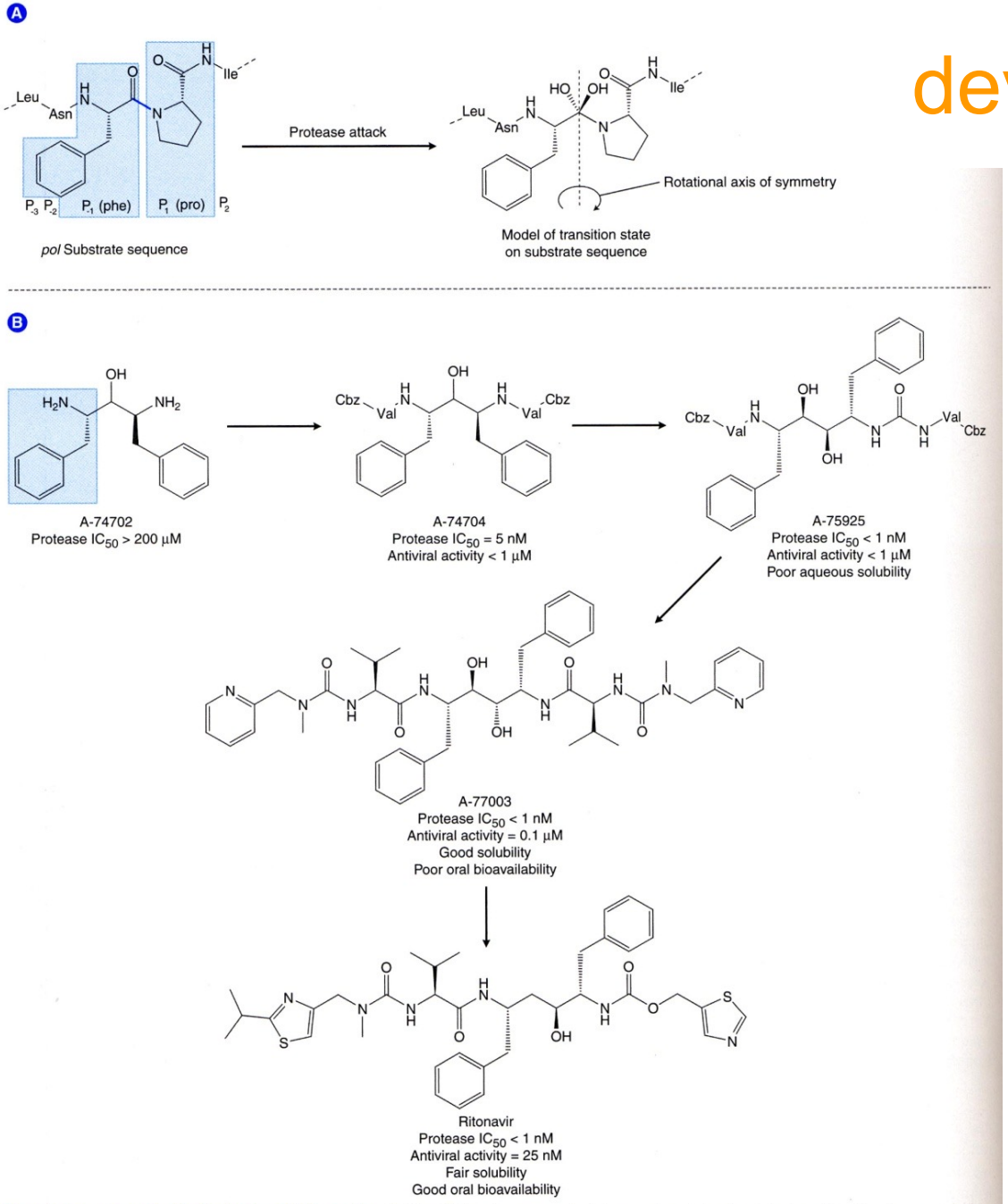

Figure 34-8. Steps in the Evolution of Ritonavir. **A.** The HIV *pol* gene product has a phenylalanine (phe)-proline (pro) sequence that is unusual as a cleavage site for human proteases. HIV protease cleaves the phe–pro bond. The transition state of this protease reaction includes a rotational axis of symmetry. **B.** Structure-based development of a selective HIV protease inhibitor began with a compound (A-74702) that contained two phenylalanine analogues with a CHOH moiety between them. This compound was then modified to maximize antiprotease activity (measured as IC_{50}, the concentration required to cause 50% inhibition of the enzyme), while also maximizing antiviral activity, aqueous solubility, and oral bioavailability. See Box 34-3 for details.

BOX 34-3. Development of Ritonavir

The development of ritonavir is an example of structure-based ("rational") drug design. Scientists began with a model of the transition state that forms during the cleavage of a substrate by HIV protease (Fig. 34-8). An analogue of the transition state was designed, using just one residue on each side of the cleavage site. Knowing that HIV protease is a symmetric dimer, the scientists chose to use the same residue—phenylalanine—on both sides of the cleavage site, with a CHOH group that mimics the transition state as the center of symmetry. This molecule, A-74702, was a very weak inhibitor of HIV protease, but adding symmetrical groups at both ends to form A-74704 (Fig. 34-8, where Val is valine and CBZ is carbobenzyloxy) resulted in a > 40,000-fold increase in potency (IC_{50} = 5 nM). Attempts to modify A-74704 to improve aqueous solubility reduced potency, however, so a related potent inhibitor, A-75925, in which the center of symmetry was a C-C bond between two CHOH groups, became the scaffold for further modifications. Symmetric changes to both ends of the molecule resulted in a soluble, highly potent inhibitor, A-77003. This compound was not orally bioavailable, however. Further modifications, which removed a central OH group and altered other moieties at each end, resulted in a compound—ritonavir—that was less soluble but had improved antiviral activity and good oral bioavailability. Therapeutically achievable plasma concentrations of ritonavir greatly exceed the concentration required for antiviral activity. In the process of structure-based drug design, successive modifications to these molecules took advantage of X-ray structures of HIV protease complexed to each inhibitor. By examining these structures, scientists were able to make informed guesses about what chemical groups to add or subtract. The result was a therapeutically useful HIV protease inhibitor, ritonavir.