**Tuesday, Sept. 14,**

*Is an enzyme a rigid system?*

Early researchers thought of enzymes as rigid entities, recognizing their substrates the way a lock would recognize a key. Today's researchers, however, recognize that there is a good deal of flexibility in the enzyme (and at the active site).

![Diagram of enzyme interactions](image1.png)

**Fig. 4.19** Bonding interactions between substrate and enzyme.

![Diagram of induced fit](image2.png)

**Fig. 4.20** Example of induced fit.

**Biochemical Regulation of Enzymes:**

It is obviously possible for biochemical systems to turn an enzyme "off", for example through feedback control via an allosteric inhibitor. Is it also possible for biochemical systems to turn an enzyme "on"? What other mechanisms exist for the regulation of enzymes?

Review: Enzymes are biochemical catalysts, thus their affect on chemical transformations is dramatic. A small amount of an enzyme causes a dramatic enhancement in the speed of a reaction. Thus enzymes are great drug targets. For example, if our drug is an enzyme inhibitor, we will only
need a small amount of the drug to completely inhibit the catalyst and prevent the undesired chemical reaction.

Kinases are enzymes that phosphorylate other enzymes. This phosphorylation, in turn, causes a conformational change that dramatically affects the catalytic activity of the phosphorylated enzyme. This is an example of one biochemical catalyst catalytically activating another biochemical catalyst, which in turn is responsible for catalyzing a chemical reaction. Thus, we see a dramatic enhancement of the original signal.

![Fig. 4.31 External control of phosphorylase a.](image)

How does phosphorylation cause a change in conformation?

![Fig. 6.19 Conformational changes induced by phosphorylation.](image)

Kinases are now considered important drug targets:

**Kinases: From Targets to Therapeutics** reviews the considerable array of inhibitors in development for therapeutics targeting the kinase family of signal transduction proteins.

Protein kinases make up a veritable treasure trove of targets for a variety of indications, including diabetes, inflammatory disorders, and especially cancer. The
examples of Gleevec (Novartis) and Herceptin (Genentech) demonstrate that despite small markets, kinase inhibitors for cancer that are effective and cause minimal adverse effects relative to the majority of chemotherapeutic agents can enjoy strong sales.

Despite this wealth of opportunity, however, kinase-modulating drugs have only recently begun to progress through clinical trials and onto the market, and the most advanced compounds target only a handful of the best-characterized kinases.
Phosphorylases, Phosphatases and Kinases

The enzymes that deal with phosphate

Phosphate serves biochemical systems in a major way. High energy phosphate compounds such as ATP, PEP, 1,3 bisphosphoglycerate owe their energetic properties to anhydride bonds between neighboring phosphates or phosphate, oxygen or nitrogen atoms. This tutorial will introduce you to enzymes that deal with phosphate in a biosynthetic and regulatory capacity. These enzymes specifically add or remove phosphate groups.

Phosphorylase (fos-for-a-lace)

The major function of this unique enzyme is to add a phosphate to glycogen and by doing so, forms glucose-1-PO4. The reaction is classified as a phosphorylysis because of its similarity to a hydrolysis. The only difference is a phosphate, not a water molecule is placed across the bond.

Pyrophosphorylases

| Table 1 | Some small-molecule inhibitors of protein kinases that are undergoing human clinical trials |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Kinase targeted | Inhibitor       | Company         | Disease         | Status          |
| Tyrosine kinases| Glivec (Gleevec, STI-571) | Novartis | Cancer | FDA approval, May 2001 |
| EGFR            | ZD1839 (Iressa) | AstraZeneca | Cancer | Phase III |
|                 | OSI-774         | GS Pharmaceuticals/Roche/Genentech | Cancer | Phase III |
| EGFR, ERBB2    | C1033           | Pfizer | Cancer | Phase I |
|                 | EKB-969         | Wyeth-Ayerst | Cancer | Phase I |
|                 | G20216          | GlaxoSmithKline | Cancer | Phase I |
|                 | PK186           | Novartis | Cancer | Phase I |
| VEGFR (PDGFR, FGFR) | SU6668 | Pharmaccia Corporation | Cancer | Phase I |
| VEGFR           | PTK787/ZK222584 | Novartis/Scherling-Plough | Cancer | Phase II |
| VEGFR (EGFR)    | ZD6474          | AstraZeneca | Cancer | Phase I |
| NGFR            | CEP-2583        | Cephalon | Cancer | Phase I |

Serine/threonine kinases

| PKC, KTR, PDGFR | PKC412 | Novartis | Cancer, retinopathy | Phase I |
| CDK4a           | flavopiridol | Aventis | Cancer | Phase II |
| CDK2            | CYC202 | Cyclacel | Cancer | Phase II |
| M9K1            | PD184352 | Pfizer | Cancer | Phase I |
| RAF             | BAY43-9006 | Onyx Pharmaceuticals/Bayer | Cancer | Phase I |
| CHK1, PKC, others | UCN-01 | Kyowa Hakko | Cancer | Phase I |
| mTOR            | CC50779 | Wyeth-Ayerst | Cancer | Phase II |
|                 | RAD001 | Novartis | Cancer | Phase II |
|                 | Rapamycin (Sirolimus) | Wyeth-AYERST | Immunosuppression | FDA approval, 1999 |
| ROCK3           | HA1077 (AT877, fasudil) | Asahi Chemical Industry | Cerebral vasospasm | Approved in 1995 (Japan) |
| PKG8            | U395531 | Eli Lilly | Diabetic retinopathy | Phase III |
| p38/SAPK2a      | SB203580 | GlaxoSmithKline | Rheumatoid arthritis | Phase I |
|                 | BIR0776 | Boehringer Ingelheim | Rheumatoid arthritis | Phase II |
|                 | Ro320-1195 | Roche | Rheumatoid arthritis | Phase I |
| MLK             | CEP-1547 | Cephalon | Neurodegeneration | Phase I |

ABL, Abelson tyrosine kinase; CDK, cyclin-dependent kinase; CHK1, checkpoint kinase 1; EGFR, epidermal-growth-factor receptor; ERBB2, ERBB2 receptor; FDA, US Food and Drug Administration; FGFR, fibroblast-growth-factor receptor; KIT, c-KIT receptor; M9K1, mitogen-activated protein kinase kinase 1; MLK, mixed-lineage protein kinase; mTOR, target of rapamycin (mammalian); NGFR, nerve-growth-factor receptor; p38, p38, mitogen-activated protein kinase; PDGFR, platelet-derived-growth-factor receptor; PKC, protein kinase C; ROCK, RHO-dependent protein kinase; SAPK, stress-activated protein kinase; VEGFR, vascular-endothelial-growth-factor receptor.
These enzymes use nucleotide triphosphates (CTP, UTP, ATP, etc) to make products with a pyrophosphate linkage. P–P is split out during the reaction. Recall, a pyrophosphorylase activates glucose by making UDP-glucose from glucose-1-PO4 and UTP (click 1). Note two features of the reaction: (1) the formation of a pyrophosphate group in the product (click 1), and (2) the splitting out of a P–P (click 1). The P–P is cleaved by an ever-present pyrophosphatase yielding energy to drive the reaction (click 1). Pyrophosphorylases only work when a phosphate is already in position on the molecule (click 1).

**Phosphatases** are hydrolases. This means these enzymes use a water molecule to remove a phosphate group from a substrate. As an example, observe the action of glucose-6-phosphatase, a major enzyme that controls blood sugar. The substrate for enzyme is glucose-6-PO4 (click 1). A water molecule is used to displace the phosphate group from the molecule (click 1). Phosphatase reactions are NOT reversible. As a guide to avoid confusion, remember that a phosphatase “takes off”.

**Kinases** are enzymes that typically transfer the terminal phosphate group of ATP to an -OH group on a substrate. This results in a phosphate-ester bond in the product. The reaction is not reversible. Although ATP is the major substrate occasionally GTP will be a phosphate group donor. Phosphate groups in ATP are coordinated with a Mg2+ ion which strains the linkage between the gamma and beta phosphate and facilitates the breakage of this bond. The reaction is favored by the release of free energy that accompanies bond breakage.

Enzymes are regulated by:

(1) Allosteric control. Binding (at a regulatory site) of a regulatory molecule in addition to the substrate. Reversible.
(2) Proteolytic activation. Peptide bonds in the protein are cleaved (hydrolysed) by a protease. Irreversible. (example: the proteolytic cleavage of Angiotensin I to Angiotensin II)
(3) Reversible covalent modification, e.g. phosphorylation. Phosphorylation is catalysed by protein kinases, which transfers a phosphate group from ATP to a Ser, Thr or Tyr residue on the enzyme. The reverse, i.e. removal of the phosphate group is performed by protein phosphatases (or protein phosphorylases).
(4) Binding of regulatory protein. Reversible.
(5) Gene regulation. Does not affect the properties of the enzyme, but the amount (by regulating the transcriptional process)

**Receptors as Drug Targets**
How does a receptor function?

The messenger induces a change of shape in the receptor, which generates a signal inside the cell.

Receptors are grouped into three large "superfamilies":

1) Ion channel receptors (2-TM, 3-TM, and 4-TM receptors).
2) G-protein-coupled receptors (7-TM receptors).
3) Kinase-linked receptors (1-TM receptors)

One example: Receptors linked to ion channels.

What is an ion channel?
**Fig. 5.6** Ion channel protein structure.

**Fig. 5.7** Lock-gate mechanism for opening ion channels.

**Fig. 5.10** A hypothetical neurotransmitter and receptor.
**Fig. 5.11** Receptor protein positioned in the cell membrane.

**Fig. 5.12** Opening of the 'lock-gate'.
G-Protein-coupled Receptors:

Fig. 5.8 Enzyme activation.
Fig. 5.9 Membrane-bound enzyme activation via G-proteins.
Fig. 6.11 Interaction of 7-TM receptors with G-proteins.

Fig. 6.12 Signalling pathways arising from the splitting of G-proteins.

Fig. 6.13 Interaction of $\alpha_i$ with adenylate cyclase.
**G Proteins**

G proteins are so-called because they bind the guanine nucleotides GDP and GTP. They are heterotrimers (i.e., made of three different subunits) associated with

* the inner surface of the plasma membrane and
* transmembrane receptors of hormones, etc. These are called **G-protein-coupled receptors (GPCRs)**.

The three subunits are:

* Ga, which carries the binding site for the nucleotide. At least 20 different kinds of Ga molecules are found in mammalian cells.
* Gb
* Gg

**Importance:**

Over 50% of current drugs directly affect GPCR's (G-protein coupled receptors).
How They Work

* In the inactive state, Ga has GDP in its binding site.
* When a hormone or other ligand binds to the associated GPCR, an allosteric change takes place in the receptor (that is, its tertiary structure changes).
* This triggers an allosteric change in Ga causing GDP to leave and be replaced by GTP.
* GTP activates Ga causing it to dissociate from GbGg (which remain linked as a dimer).
* Activated Ga in turn activates an effector molecule.

In a common example (shown here), the effector molecule is adenylyl cyclase - an enzyme in the inner face of the plasma membrane which catalyzes the conversion of ATP into the "second messenger" cyclic AMP (cAMP) [More].
Some Types of Ga Subunits

Gas

This type **stimulates** \((s = \"stimulatory\")\) adenylyl cyclase. It is the one depicted here. It is associated with the receptors for many hormones such as:

* adrenaline
* glucagon
* luteinizing hormone \((\text{LH})\)
* parathyroid hormone \((\text{PTH})\)
* adrenocorticotropic hormone \((\text{ACTH})\)

Gas is the target of the toxin liberated by *Vibrio cholerae*, the bacterium that causes **cholera**. Binding of cholera toxin to Gas keeps it turned "on". The resulting continuous high levels of cAMP causes a massive loss of salts from the cells of the intestinal epithelium. Massive amounts of water follow by **osmosis** causing a diarrhea that can be fatal if the salts and water are not quickly replaced.

Gaq

This activates **phospholipase C** \((\text{PLC})\) which generates the second messengers:

* inositol trisphosphate \((\text{IP3})\)
* diacylglycerol \((\text{DAG})\)

Gaq is found in G proteins coupled to receptors for

* vasopressin,
* thyroid-stimulating hormone \((\text{TSH})\), and
* angiotensin.

Gai

This **inhibits** \((i = \"inhibitory\")\) adenylyl cyclase lowering the level of cAMP in the cell. G\(\alpha_i\) is activated by the receptor for **somatostatin**.

Gat

The "t" is for **transducin**, the molecule responsible for generating a signal in the rods of the retina in response to light. Gat triggers the breakdown of **cyclic GMP** \((\text{cGMP})\).
How would a receptor be able to distinguish between a compound and its enantiomer (mirror image)?
Fig. 5.14 Structures possessing fewer than the required number of binding groups.
**Fig. 5.15** Molecule with binding groups in incorrect positions.

**Fig. 5.16** Mirror image of hypothetical neurotransmitter.
Fig. 5.17 Interactions between the hypothetical neurotransmitter and its mirror image with the receptor site.

Fig. 5.18 Structure with a meta methyl group.
**Fig. 5.19** Compound acting as an antagonist at the binding site.

**Fig. 5.20** Allosteric antagonists.

**Fig. 5.21** Antagonism by the 'umbrella effect'.
Fig. 5.23 Partial agonism.
Fig. 5.24 Equilibria between active and inactive receptor conformations.
Fig. 5.25 Desensitization, $\Theta =$ phosphate group
Fig. 5.26 Process of increasing cell sensitivity.