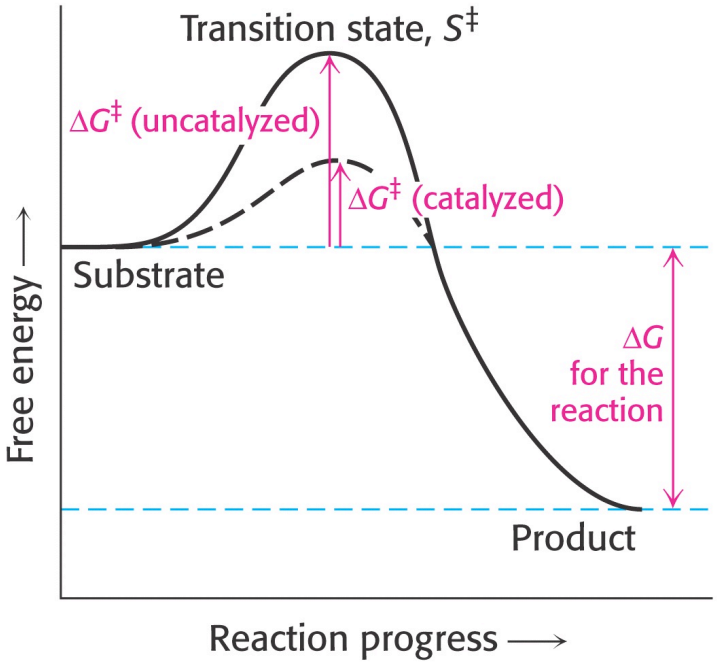
Biomolecular chemistry

5. Enzymes and their roles in intracellular signaling circuits

Suggested reading: **Sections 2.1 to 2.4** of
Mikkelsen and Cortón, *Bioanalytical Chemistry*

- Many figures and the descriptions for the figures are from the educational resources provided at the Protein Data Bank (http://www.pdb.org/)
- Most of these figures and accompanying legends have been written by David S. Goodsell of the Scripps Research Institute and are being used with permission. I highly recommend browsing the Molecule of the Month series at the PDB (http://www.pdb.org/pdb/101/motm_archive.do)

An enzyme is a protein that is also a catalyst.



We have seen many examples already including:
DNA polymerase,
RNA polymerase,
uracil-DNA glycosylase,
methyltransferase,
reverse transcriptase,
tRNA synthetase,
ribonuclease.

- Enzymes, the catalysts of biological systems, are remarkable molecular devices that catalyze all of the essential chemical transformations necessary for life as we know (except protein synthesis of course). They also mediate the transformation of one form of energy into another. The most striking characteristics of enzymes are their catalytic power and specificity. Catalysis takes place at a particular site on the enzyme called the active site. Proteins do not have an absolute monopoly on catalysis; the discovery of catalytically active RNA molecules provides compelling evidence that RNA was an early biocatalyst.
- Proteins as a class of macromolecules are highly effective catalysts for an enormous diversity of chemical reactions because of their capacity to specifically bind a very wide range of molecules. By utilizing the full repertoire of intermolecular forces, enzymes bring substrates together in an optimal orientation, the prelude to making and breaking chemical bonds. They catalyze reactions by stabilizing transition states, the highest-energy species in reaction pathways. By selectively stabilizing a transition state, an enzyme determines which one of several potential chemical reactions actually takes place.
- A chemical reaction of substrate S to form product P goes through a transition state S‡ that has a higher free energy than does either S or P. The double dagger denotes a thermodynamic property of the transition state. The transition state is the most seldom occupied species along the reaction pathway because it is the one with the highest free energy. The difference in free energy between the transition state and the substrate is called the Gibbs free energy of activation or simply the activation energy, symbolized by ΔG‡.
- The activation-energy barrier immediately suggests how enzymes enhance reaction rate without altering ΔG of the reaction: enzymes function to lower the activation energy, or, in other words, enzymes facilitate the formation of the transition state.
- Because enzymes are such superb catalysts, it is tempting to ascribe to them powers that they do not have. An enzyme cannot alter the laws of thermodynamics and consequently cannot alter the equilibrium of a chemical reaction. This inability means that an enzyme accelerates the forward and reverse reactions by precisely the same factor.

OMP-decarboxylase accelerates a reaction by a factor of greater than 10^{17}!!

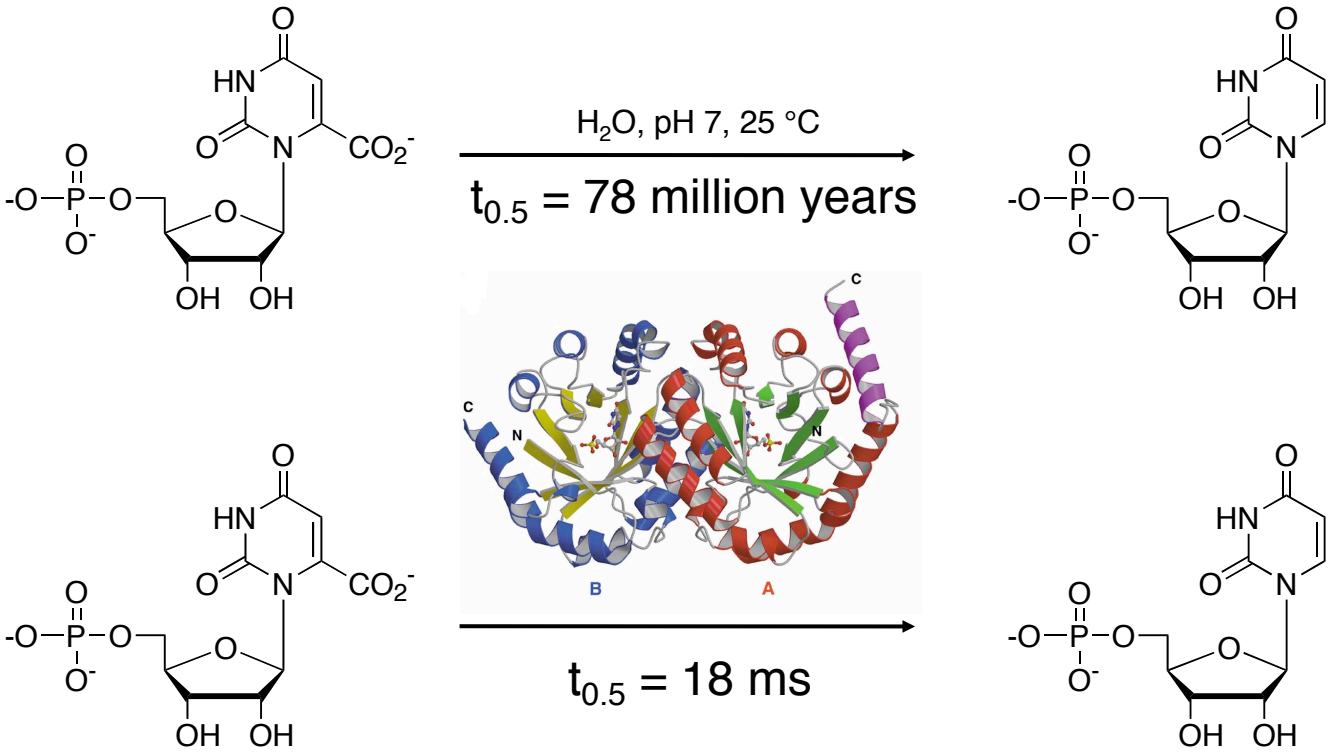


Image source: http://www.chem.umn.edu/groups/gao/enzyme.htm
More information: http://arjournals.annualreviews.org/doi/full/10.1146/annurev.biochem.71.110601.135446

- OMP-decarboxylase turns its substrate over with a half-time of 18 ms, in a reaction that proceeds in its absence with a half-time of 78 million years in neutral solution.
- This means that the enzyme accelerates the rate of this reaction by a factor of >10^{17}!!

Depending on the difficulty of the task that they need to perform, enzymes provide different rate enhancements

TABLE 8.1 Rate enhancement by selected enzymes

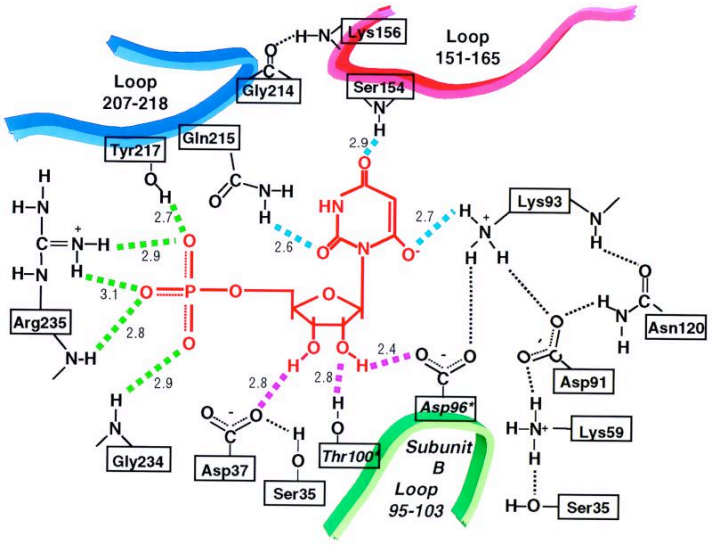
Enzyme	Nonenzymatic half-life	Uncatalyzed rate (k_{un} s^{-1})	Catalyzed rate (k_{cat} s^{-1})	Rate enhancement (k_{cat}/k_{un})
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000 years	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9 days	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.
Source: After A. Radzicka and R. Wofenden. *Science* 267 (1995):90–93.

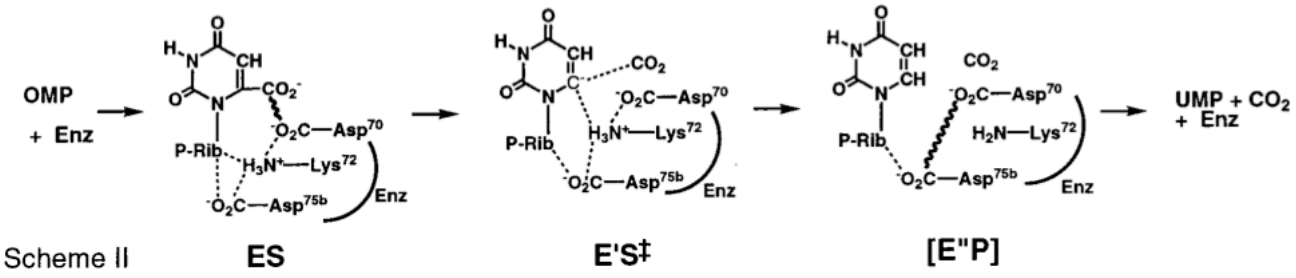
Notice that the uncatalyzed rates span ~14 to 15 orders of magnitude but the catalyzed rates span only 3 to 4 orders of magnitude.

- It has long been recognized that biological reactions vary in their spontaneous rates in neutral solution, so that efficient enzymes differ considerably in the severity of the tasks that they perform. The hydration of CO_2, for example, occurs spontaneously within a matter of seconds in neutral solution, whereas the phosphodiester bonds of DNA must be able to withstand spontaneous hydrolysis for long periods of time in the absence of a nuclease if DNA is to serve its purpose in conserving genetic information.
- To obtain a quantitative measure of the degree of difficulty of that task for any enzyme, it is necessary to know the rate constant (k_{non}) of the corresponding reaction proceeding spontaneously in dilute aqueous solution in the absence of a catalyst. Only then can one truly appreciate how effective proteins are at performing their jobs!!
- By comparing the rate constant of an uncatalyzed reaction (k_{non}) with the turnover number of the corresponding enzyme reaction (k_{cat}), it is possible to appreciate the increase in reaction rate that an enzyme produces.
- The resulting rate enhancement (the dimensionless ratio of two first-order rate constants) indicates the factor by which an enzyme's affinity for the transition state is greater than the enzyme's affinity for the ground state substrate.

Enzymes stabilize the transition state through specific binding interactions



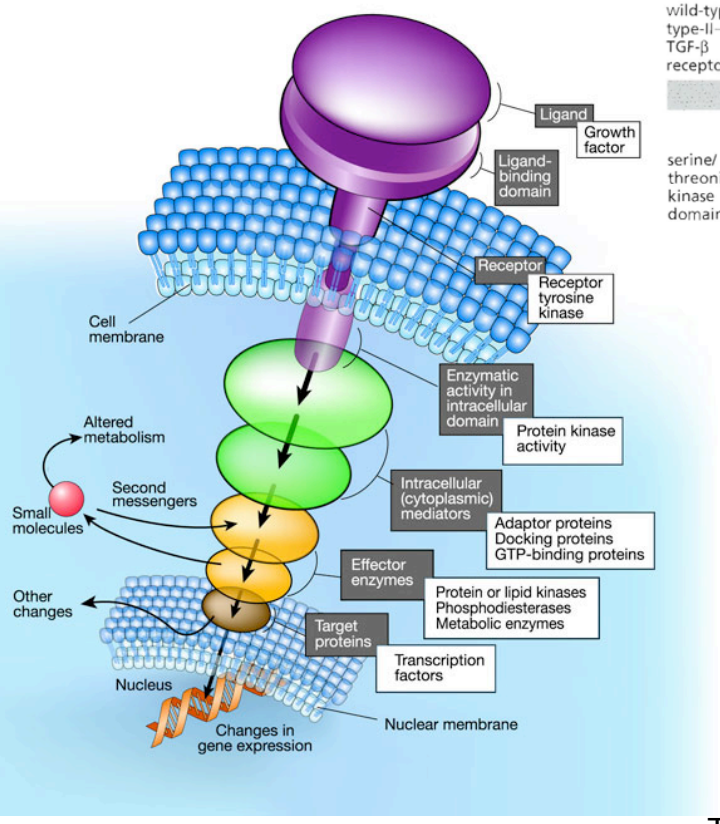
Contacts between ODCase and a proposed transition state analog

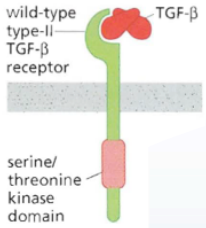


Scheme II **ES** **E'S‡** **[E"P]**

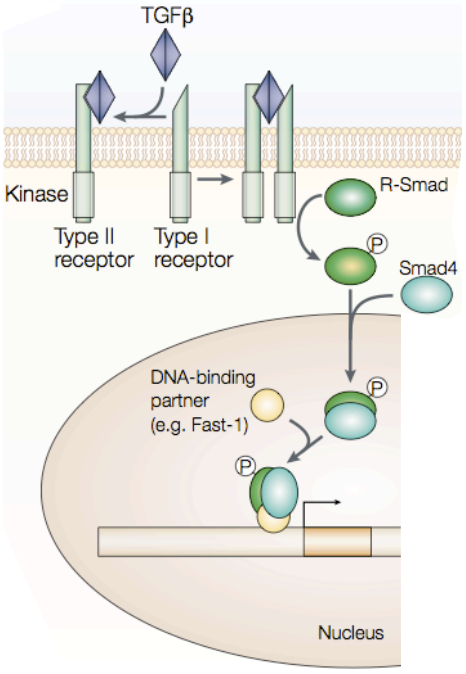
- Shown are contacts between ODCase and a proposed transition state analog. These contacts were observed in the x-ray crystal structure of the complex.
- The interaction of Lys-93 with the O- group of the transition state analog is relatively favourable, foreshadowing the very great affinity that the enzyme evidently develops for the carbanion generated at C-6 in the transition state
- *Proc Natl Acad Sci U S A*. 2000 February 29; 97 (5): 2011–2016
- *Proc Natl Acad Sci U S A*. 2000 February 29; 97 (5): 2017-2022

The role of enzymes in intracellular signaling





We saw TGF-beta earlier. This protein is inactivated due to a frame-shift mutation in some prostate cancers

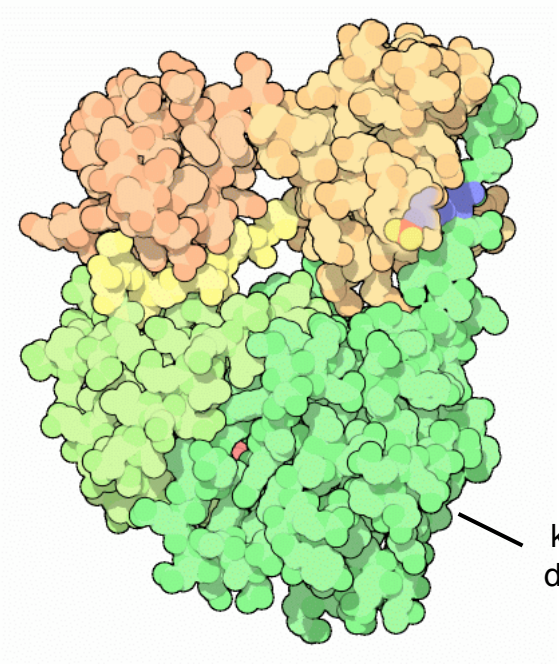


This is a very simple intracellular signaling pathway. They are often much more complex.

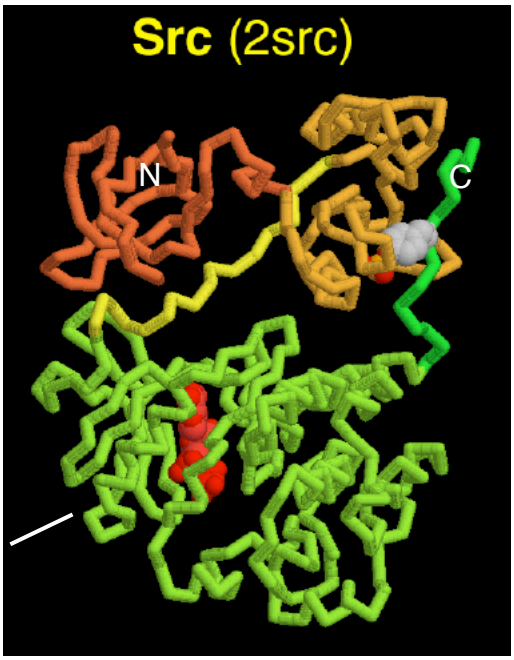
Downward, *Nature* **411**, 759-762(14 June 2001)
Packard et al. *Nature Reviews Neuroscience* **4**, 113-120 (2003)

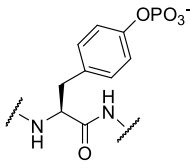
- On the left is shown a general structure of a signalling pathway. The grey boxes give the general description and the white boxes are specific examples. Signalling generally starts with the binding of a protein or ligand to a surface receptor. This binding even is communicated to the interior of the cell through the transmembrane portion of a protein.
- This leads to a change in the activity of kinase domain which phosphorylates other enzymes (typically kinases themselves).
- Through a series of phosphorylations, and phosphorylation-dependent protein-protein interactions, a transcription factor becomes activated.
- This activated transcription factor binds to DNA, recruits RNA polymerase, and starts to transcribe certain genes.
- The R-SMAD/SMAD4 complex can bind weakly to DNA at GTCT nucleotide sequences. It needs to bind with another transcription factor in order to turn on gene expression. Once bound to the DNA, this complex recruits RNA polymerase and transcription of adjacent genes can begin. These genes tend to be ones involved in cell growth, differentiation, and programmed cell death (apoptosis).

The crystal structure of Src



kinase domain



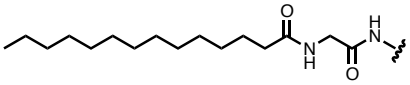


Src phosphorylates tyrosines in different proteins in the cell. It seems to be not very specific for the surrounding sequence. One example of a peptide that is a substrate is KVEKIGEGT**Y**GVVYK (amino acids 6-20 of p34$^{cdc2)}$

http://www.rcsb.org/pdb/101/motm.do?momID=43

- Using the scheme on the previous page, Src would be an example of an example of an effector enzyme.

Src is activated by a conformational change

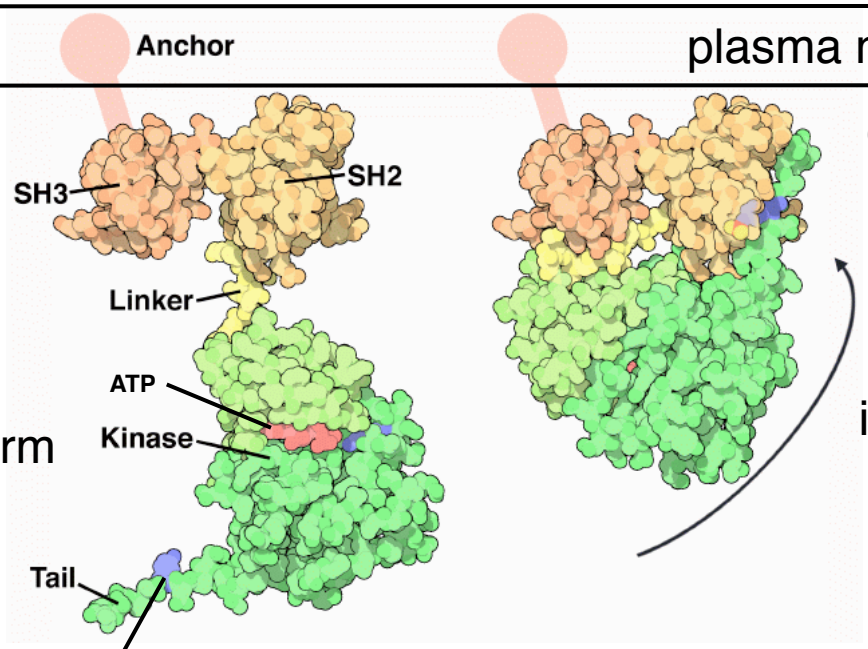


myristolyation

outside

plasma membrane

cytoplasm



active form

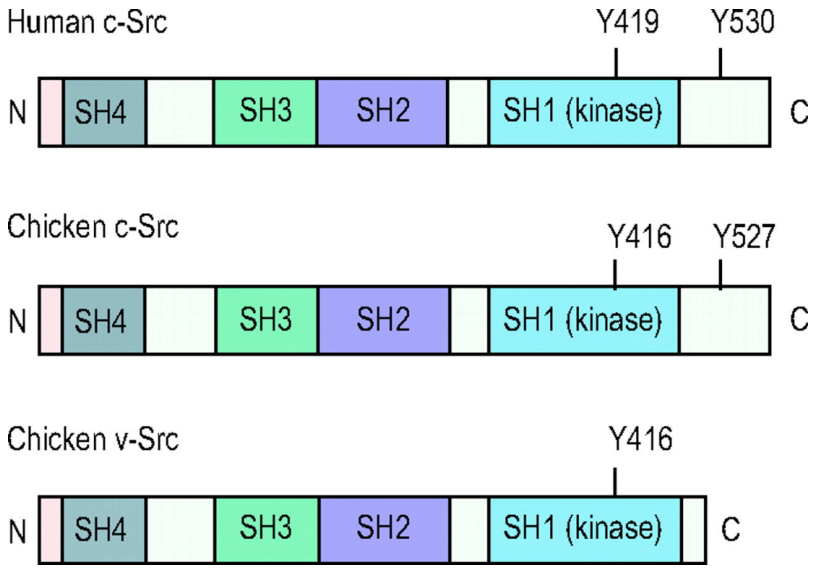
inactive form

When this tyrosine is phosphorylated, it binds to the SH2 domain and folds up the protein into its inactive form. Accordingly, the activity of Src is regulated by phosphorylation of the tyrosine in its C-terminal tail.

http://www.rcsb.org/pdb/101/motm.do?momID=43

- Anchor – a myristoylation site on the protein that attaches a very hydrophobic group that inserts into the plasma membrane
- Src can undergo a dramatic conformational change to go between an active and inactive form.
- Src activity can be regulated through interactions of both the SH3 domain and SH2 domain.

v-Src vs c-Src kinase



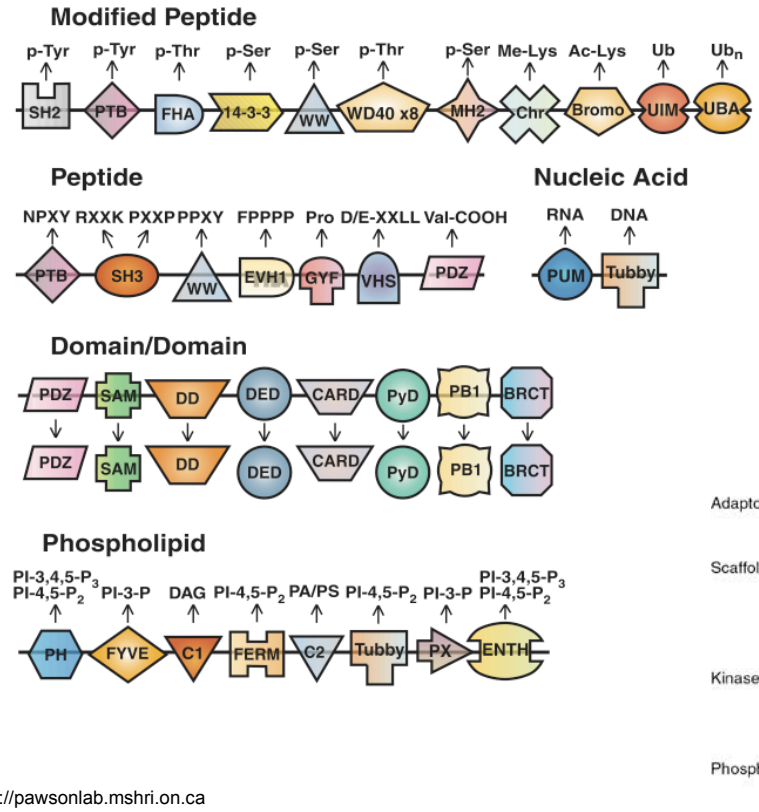
The key difference between Chicken c-Src and v-Src, is the deletion of the C-terminal tail. This means that v-Src is 'always on' since it has lost the ability to be regulated by phosphorylation on the C-terminal tyrosine.

Src is such a 'classic' example of a kinase, that the domains of the protein (which are also found in many other proteins) are named after Src. That, they are named Src homology 1,2,3,4 (SH1,2,3,4).

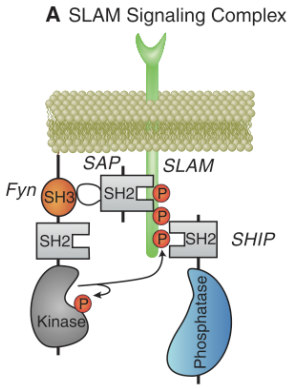
Wheeler et al., *The Oncologist* 2009; **14**: 667–678 www.TheOncologist.com

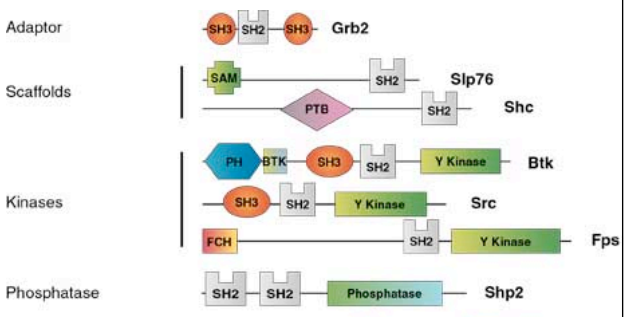
- The same mutation of Src (truncation of the C-terminal portion) has now been found in some advanced colon cancers.

Many proteins are assembled from interaction domains with predictable functions



Examples:

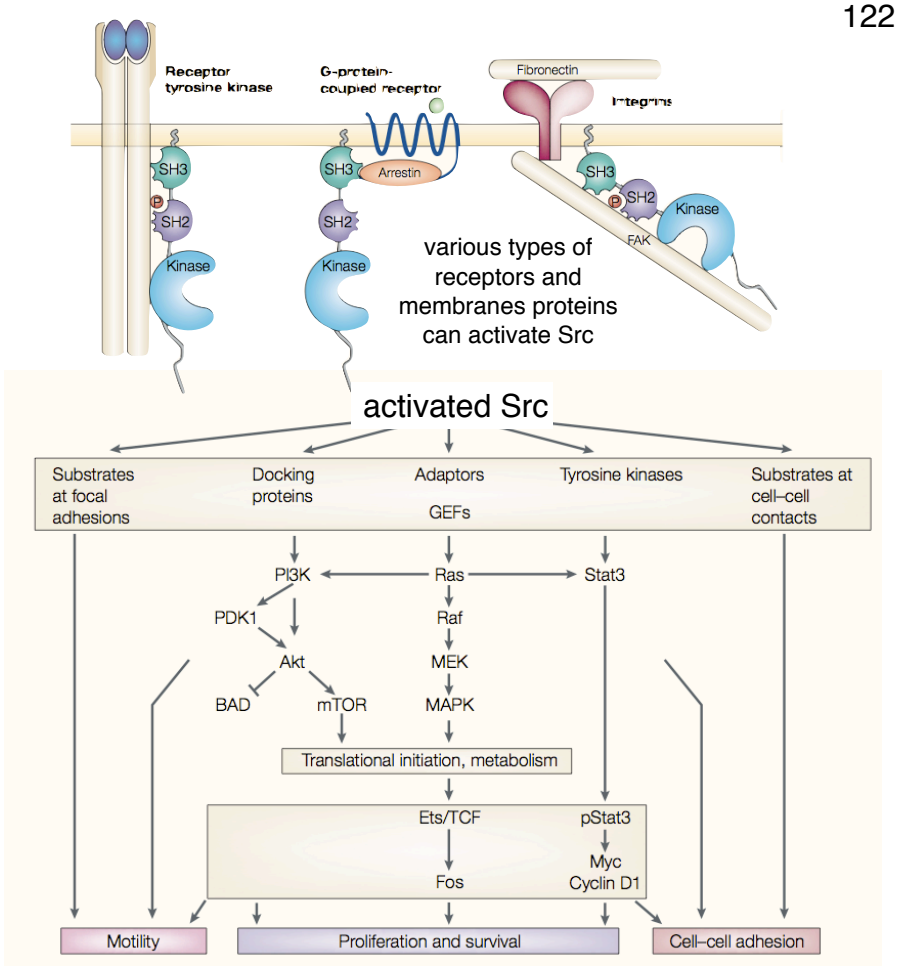




http://pawsonlab.mshri.on.ca
Pawson and Nash, *Science* **300**, 445 (2003)

Src regulates many processes in the cell

We now know that there are many ways to activate Src, and that Src has many substrates in the cell. **Researchers can not yet point to any one effect of Src and say 'this is the reason it is an oncogene'.** This is still an area of active investigation.



Martin, G.S., *Nat Rev Mol Cell Biol.* 2001 Jun;**2**(6):467-75.

- Given it's many roles, you would expect Src to be an important protein in the cell. You might be surprised to learn that a $Src^{-/-}$ mouse is viable. That is, even if the mouse has both copies of its Src gene knocked out, it can still survive (Soriano, P., Montgomery, C., Geske, R. & Bradley, A. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. *Cell* 64, 693–702 (1991)).
- This leads to another lesson about intracellular signalling - it is highly redundant. The reason the mouse could survive is probably that other very similar kinases play almost identical roles to Src. There are at least 2 other kinases (yes and fyn) that are very similar to Src in terms of structure, function, and expression patterns.
- Double knockouts of Src/yes or Src/fyn die before birth and triple knockouts die at an early stage of embryonic development.