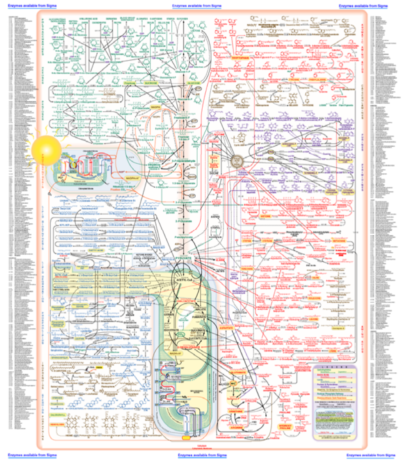
Four categories of enzymes

1. Group transfer reactions
2. Oxidations/reductions
3. Eliminations/isomerizations/ rearrangements
4. Reactions that make or break C-C bonds

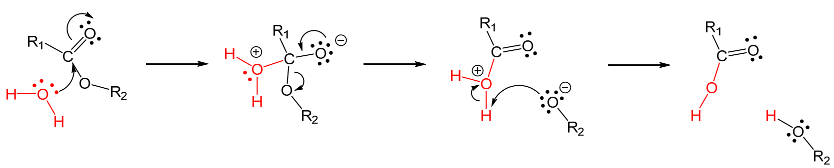


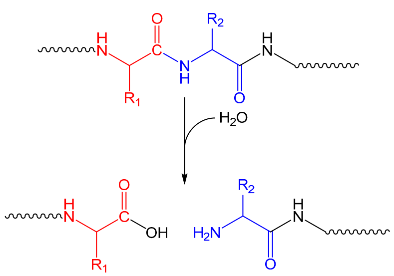
Enzyme-catalyzed group transfers

Transfer of an electrophilic group from one nucleophile to another

- Acyl group transfer
- Phosphoryl group transfer

Acyl group transfer to water: hydrolases





Scope of reactions/physiological functions of proteases

Digestive proteases- best characterized. Classes:

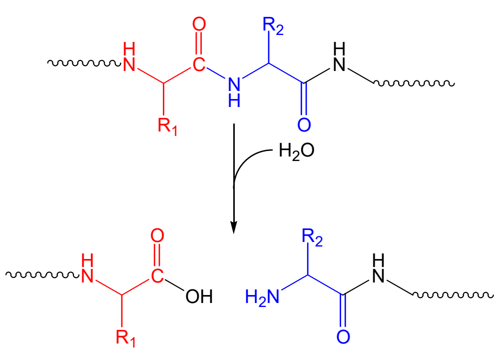
Chymotrypsin-catalyzed hydrolysis of peptide bonds: a case study that illustrates principles of enzyme catalysis

- 25,000 Mr protease
- zymogen → active form

- Cleaves peptide bonds adjacent to aromatic AAs
- A serine protease
- Serine proteases are the most thoroughly studied and understood class of enzymes

Chymotrypsin

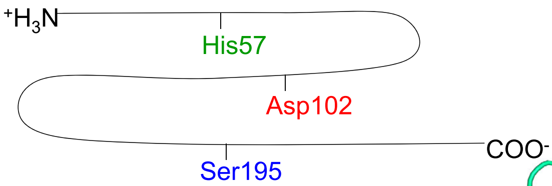
- Cleaves (hydrolyzes) peptide bonds adjacent to aromatic AAs

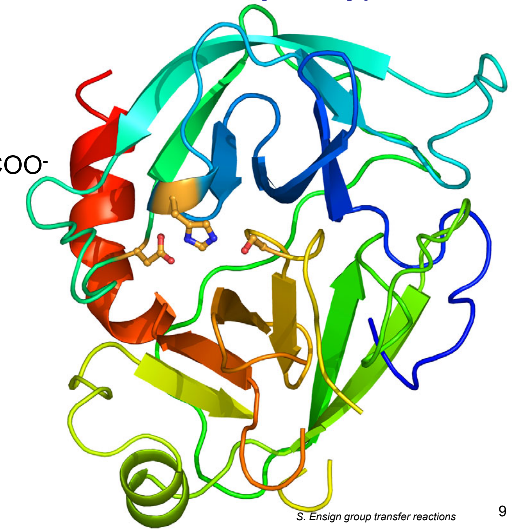


R_1 = Phe, Tyr, Trp

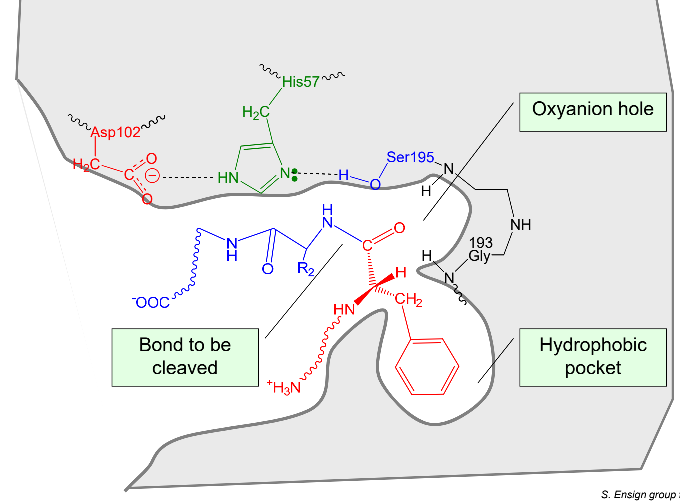
Serine proteases: serine is a nucleophile. Attack of serine on peptide bond generates a covalent acyl-enzyme intermediate

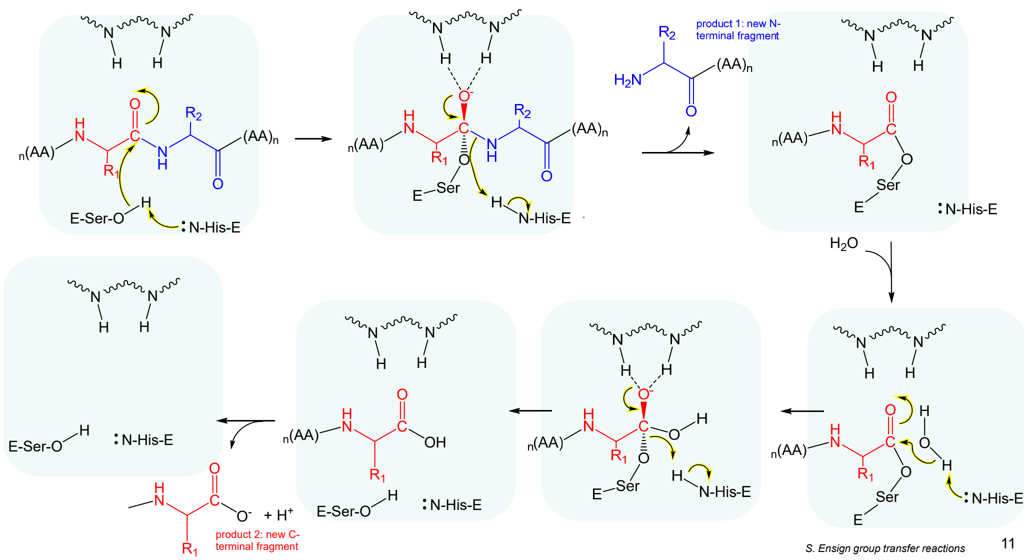
Critical amino acid residues at the active site of chymotrypsin





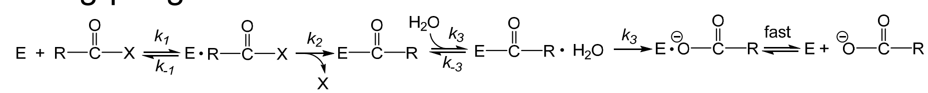
The active site of chymotrypsin



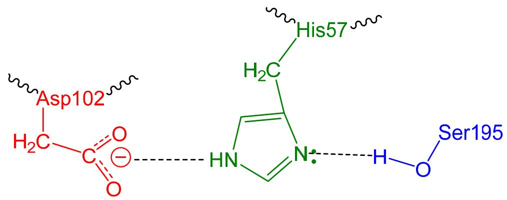


Catalytic principles observed in chymotrypsin

- Ping-pong kinetic mechanism:

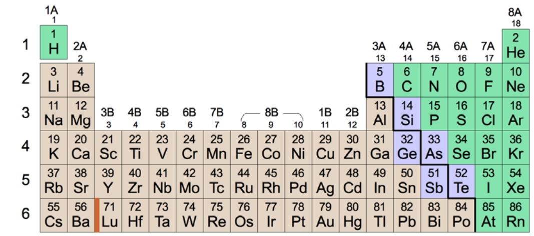


- His57: 2x general base, 2x general acid
- Two tetrahedral intermediates (transition states)
- Oxyanion hole stabilizes tetrahedral intermediates (TS)
- The pKa of “free” serine is 13.6! (6.6 units above phys.): how can it serve as a nucleophile?



Enzyme-catalyzed group transfers

- Acyl group transfer
- Phosphoryl group transfer

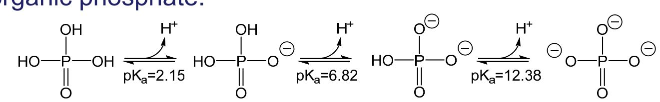


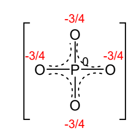
Purposes of phosphorylated compounds

- Convert a poor leaving group to a good one
- Stabilizing structural element
- Increase H_2O solubility of hydrophobic molecules
- Offer reactive handle to biological catalysts
- Enzyme regulation

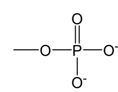
Phosphoryl group transfer: nomenclature

Inorganic phosphate:

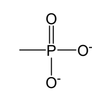




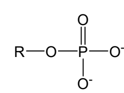
A phosphate group:



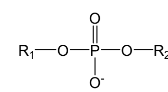
A phosphoryl group:



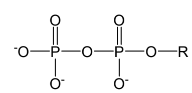
A phosphomonoester:



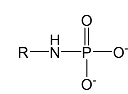
A phosphodiester:



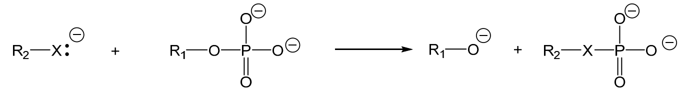
A phosphoanhydride:

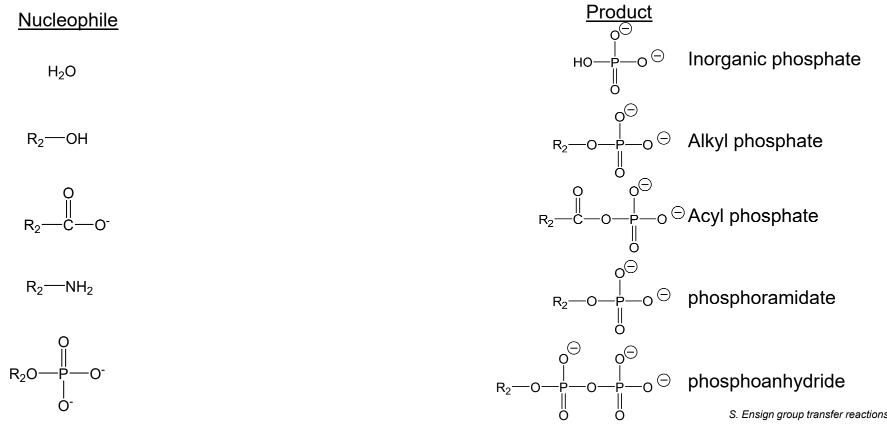


A phosphoramidate:



Phosphoryl group transfer





Scope of “phosphoryl” transfer reactions

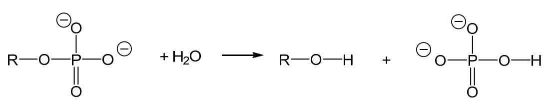
Phosphoryl transfer
- Phosphatase – Water is the acceptor
- Kinase
- Mutase – A nucleophile other than water is the acceptor

Phosphoester transfer
- Phosphodiesterase – Water is the acceptor
- Nucleotidyl transferase – A nucleophile other than water is the acceptor

Phosphorolysis
- Phosphorylase

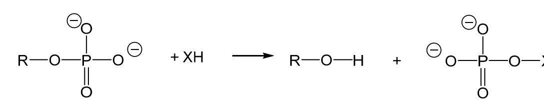
Scope of phosphoryl transfer reactions

- Phosphatase
  – S/T; Y; dual specific
  – Acid and alkaline (small molecules)



Scope of phosphoryl transfer reactions

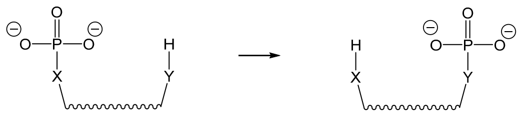
- Kinase
  – S/T, H, Y, D, C, small molecules



Important examples from central metabolism: hexokinase, phosphofructokinase, pyruvate kinase, creatine kinase, protein kinase a, phosphorylase kinase

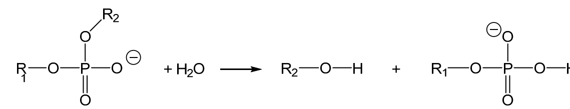
Scope of phosphoryl transfer reactions

- Mutase



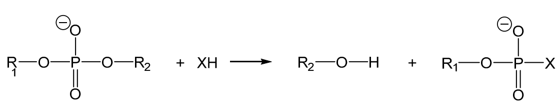
Alkylphosphoryl group (phosphoester) transfer reactions

- Phosphodiesterase
  - DNAse, RNAse, phospholipase
- An alkylphosphoryl group is transferred



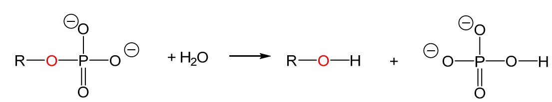
Alkylphosphoryl group (phosphoester) transfer reactions

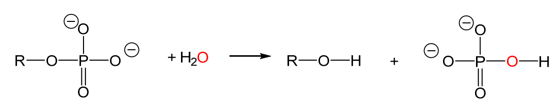
- Nucleotidyl transferase
  - Example: ADP ribosyl transferase



Chemistry of phosphoryl group transfer

- Which bond is broken? Isotopic labelling:

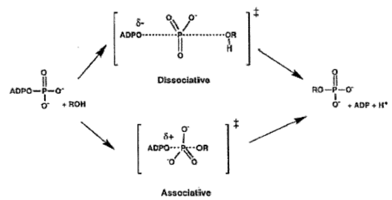




- A phosphoryl group is transferred, NOT a phosphate group

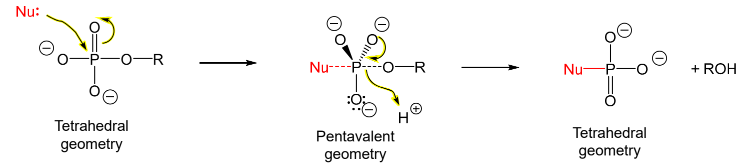
Chemistry of phosphoryl group transfer

- Possible mechanisms for phosphoryl group displacement
  - Dissociative (S_N1)
  - Associative (S_N2)

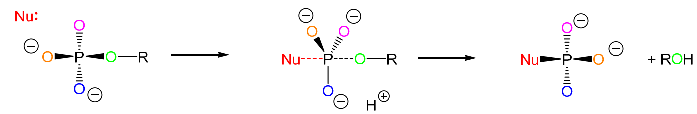


Associative mechanism (S_N2)

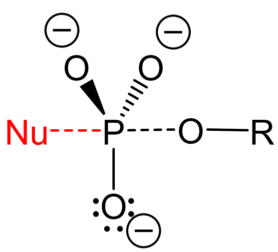
- Pentavalent *intermediate* or *transition state*?



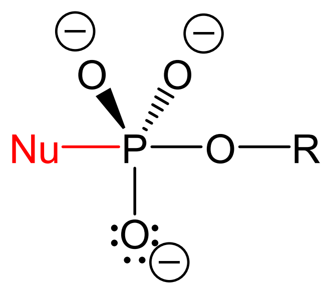
- Stereochemical consequences?



Phosphoryl transfer reactions occur with inversion. This can be proven using “chiral” substrates with three isotopes of O present

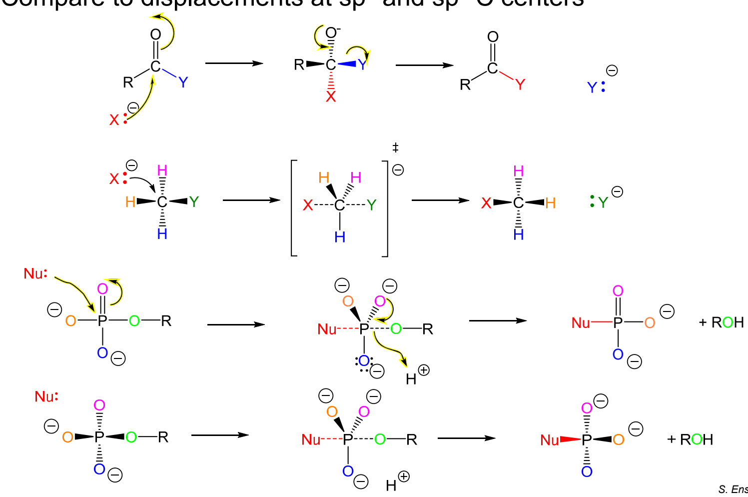


vs.



Associative mechanism (S_N2)

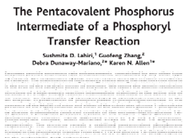
- Compare to displacements at sp^2 and sp^3 C centers



Rules for formation and decomposition of pentacoordinate intermediates of relevance to enzyme mechanisms

- No pentavalent “intermediate” has ever been directly observed or isolated

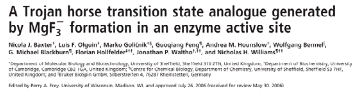
Science 2003: 299, 2067-2071



The Pentacovalent Phosphorus Intermediate of a Phosphoryl Transfer Reaction

Sushmita D. Lahiri, Guofeng Zhang, Debra Dunaway-Mariano, Karen N. Allen

PNAS 2006: 103, 14732-14737

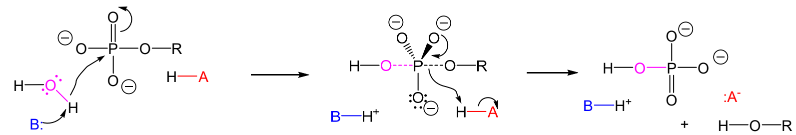


A Trojan horse transition state analogue generated by MgF_3^- formation in an enzyme active site

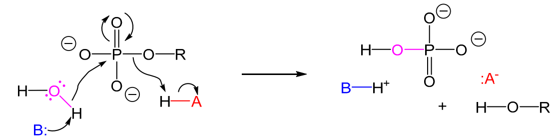
- Nucleophiles approach and leaving groups depart from axial positions
- 4-6 member rings will span axial-equatorial positions
- Electronegative ligands prefer axial positions, electropositive ligands prefer equatorial positions

Phosphoryl group transfer to water: phosphatase

Mechanism showing the pentavalent transition state:

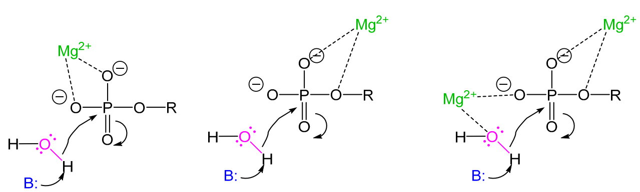


Mechanism “shortcutting” the pentavalent transition state



Mechanistic considerations: identity of general acids/bases? Charge shielding and stabilization?

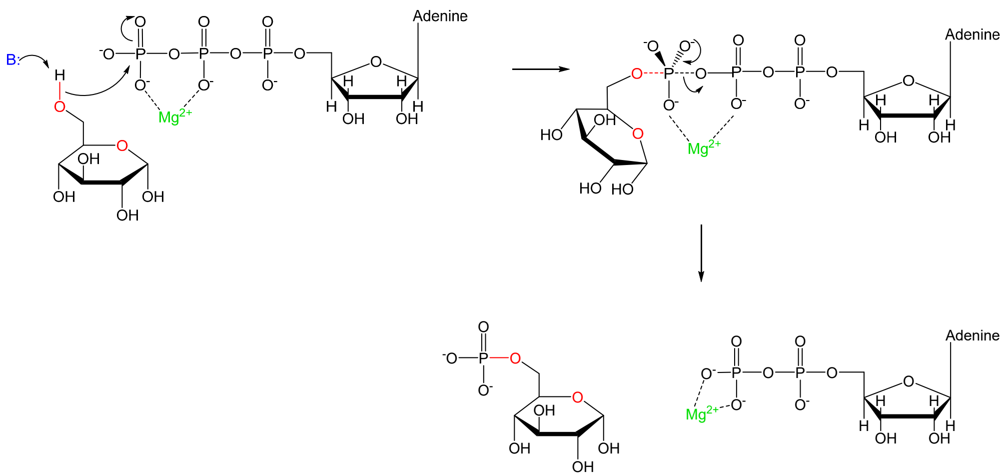
Possible roles for a metal ion in charge shielding/stabilization



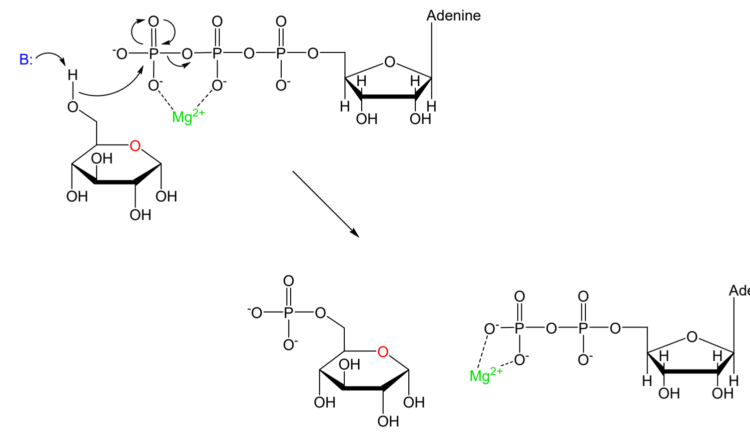
Examples of phosphoryl group transfer

- Transfer of a phosphoryl group from ATP to an alcohol (hexokinase)
- Transfer of a phosphoryl group from one position to another in a molecule (phosphoglyceromutase)
- Substrate level phosphorylation (phosphoglycerate kinase, pyruvate kinase)
- Converting a poor leaving group to a good leaving group (glutamine synthetase)

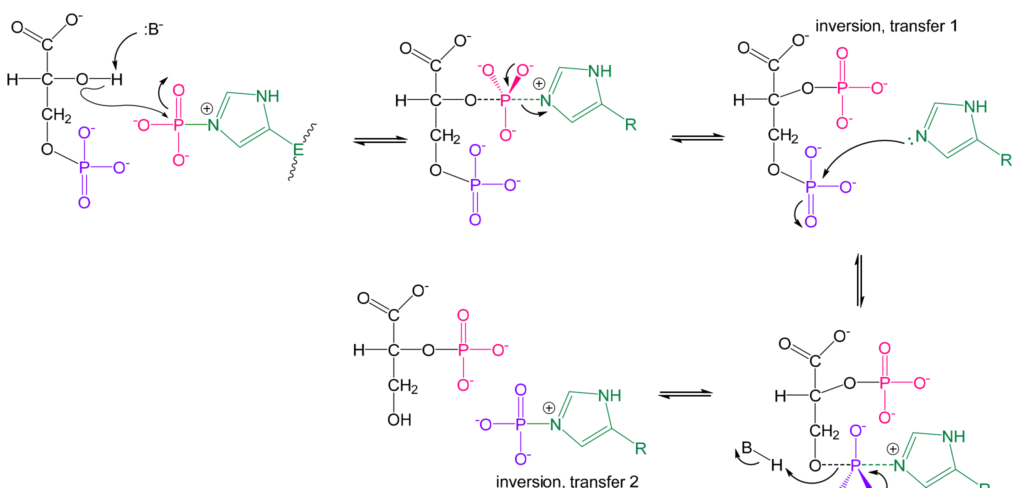
Hexokinase mechanism showing pentavalent TS



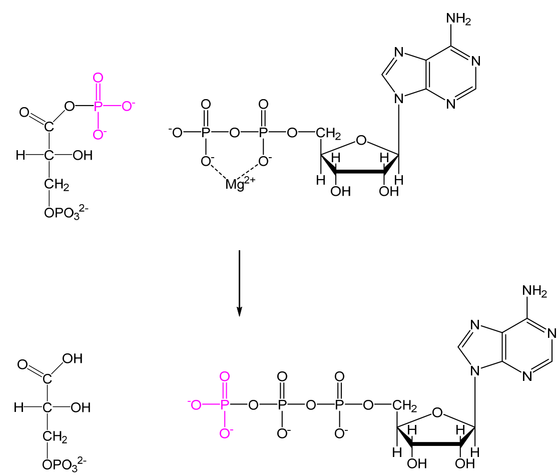
Hexokinase “shortcut” mechanism



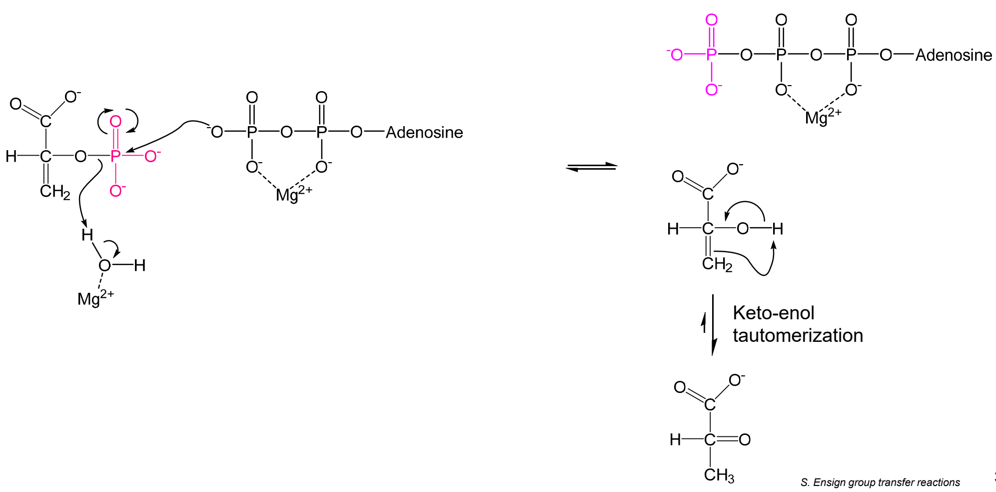
Phosphoglyceromutase



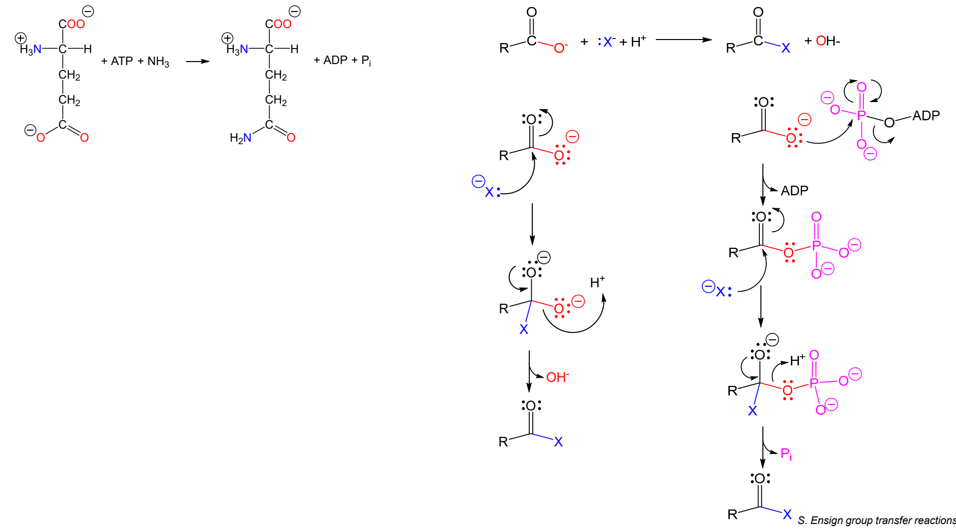
Substrate level phosphorylation: phosphoglycerate kinase



Substrate level phosphorylation: pyruvate kinase



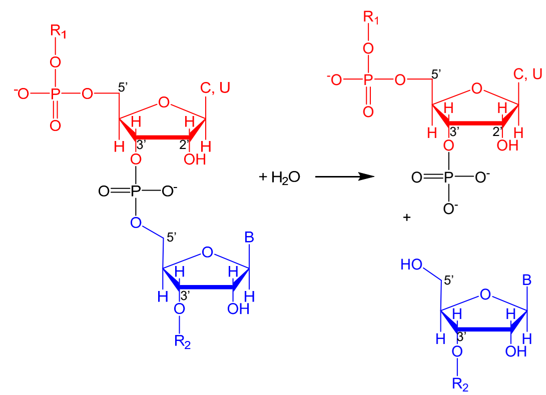
Converting a poor leaving group to a good leaving group: glutamine synthetase



Phosphester group transfer: ribonuclease A

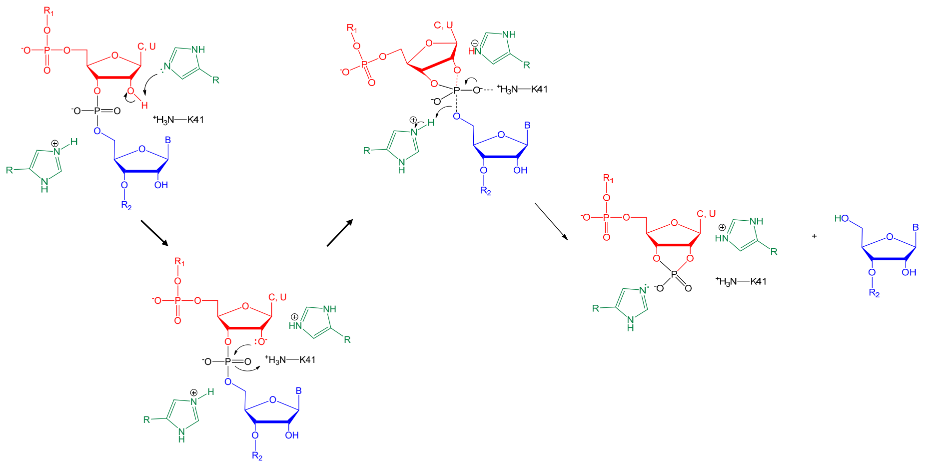
- 2',3'-cyclic phosphate intermediate
- Two pentavalent transition states
  - cyclization, hydrolysis
  - 5-member ring spans axial, equatorial positions
- Two base mechanism
- In-line associative mechanism proposed for both the cyclization and hydrolysis steps

Ribonuclease A



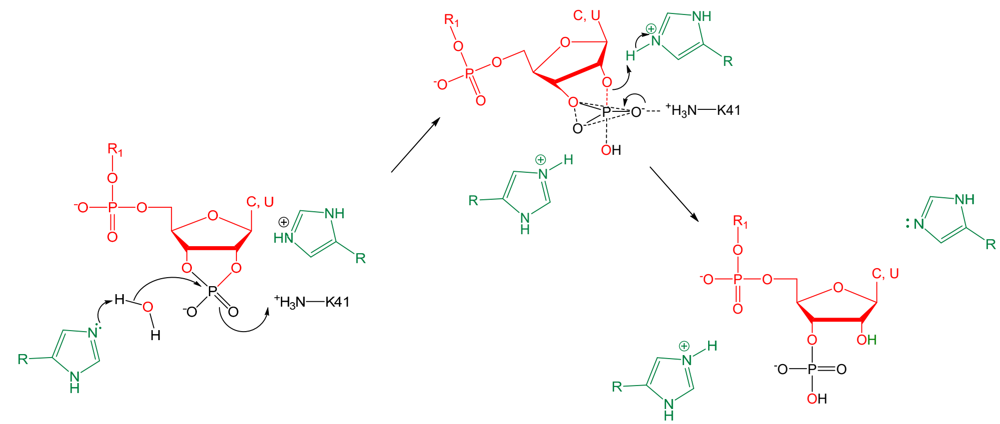
Ribonuclease A

- Formation of a 2'3'-cyclic phosphate intermediate



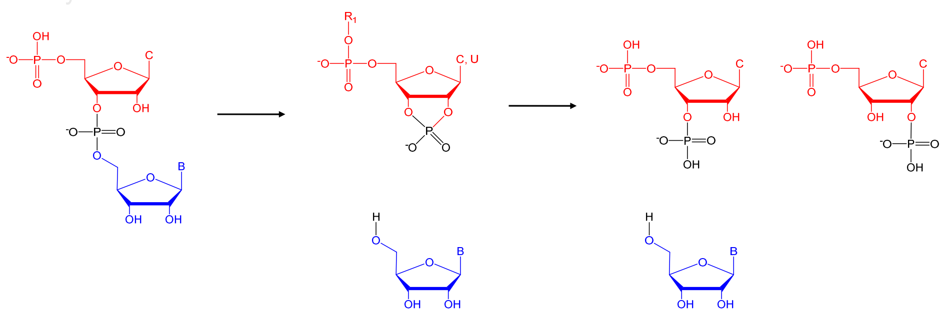
Ribonuclease A

- Hydrolysis of the 2'3'-cyclic phosphate intermediate



Evidences for the mechanism

- model (nonenzymatic) systems
  - Polynucleotides with KOH: mix of 2' and 3' phosphates. No 5' phosphate.
  - Deoxypolynucleotides with KOH
  - Cyclic intermediate with KOH



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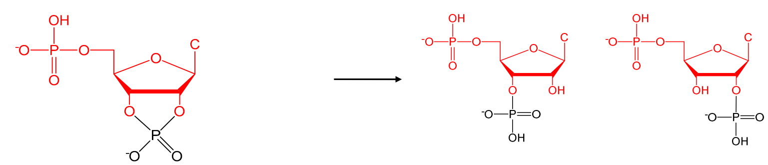

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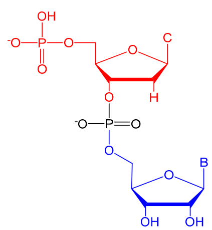
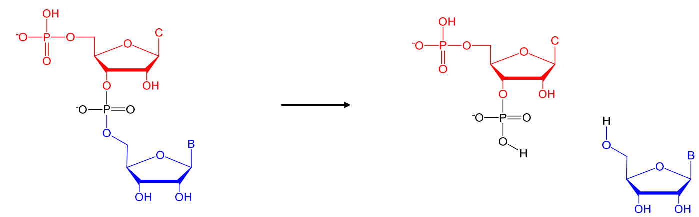
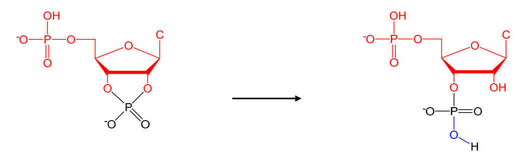


Evidences for the mechanism

- Enymatic activity with:
  - pCpN (poor substrate)



  - 5'-phosphocytidine 2'-3'cyclic monophosphate



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