23. Making and Breaking phosphodiester and phosphoanhydride bonds

1 unit, Stewart Shuman, October 1, 2025

Phosphoryl transfer enzymology
- Nucleophilic attack, geometry, transition states
- Direct attack versus covalent catalysis

The discoveries of polynucleotide kinase-phosphatase and DNA ligase

Pnkp structure and mechanism
- Interrogating quaternary structure by zonal velocity sedmentation and SDS-PAGE
- Site directed mutagenesis strategy
- Structure-activity relations
- Indentifying autonomous domains in a bifunctional enzyme
- Capturing a transition state mimetic
- Capturing a Michaelis complex

DNA/RNA ligase reaction pathway
- Three nucleotidyl transfer steps
- Reaction intermediates
- Covalent catalysis
- Active site

Two classes of DNA ligases (ATP/NAD)
- Phylogenetic distribution

Multiple DNA ligases per organism
- Division of labor
- Replication, repair, recombination, NHEJ

Structure of a minimal DNA ligase
- Domain movements coupled to catalysis

How do DNA ligases recognize nicks?
- Ligases form a C-shaped clamp around the duplex nick
- Different ligases have different domain organizations and clamp topologies

<u>Group Activity: discuss this paper</u>

Chakravarty, A.K., et al. (2012) RNA ligase RtcB splices 3'-phosphate and 5'-OH ends via covalent RtcB-(histidinyl)-GMP and polynucleotide-(3')pp(5')G intermediates. Proc. Natl. Acad. Sci. USA 109, 6072-6077. PMCID: PMC3341019
[Note: detailed understanding of the mass spectrometry experiment is optional. No need to dwell on the figure. Just accept the conclusion as stated.]

Topics to be considered during discussion . . .

- How is the substrate for ligation prepared?
- How is ^{32}P label used to track the pathway steps?
- What is the rationale for mutagenesis of His337?
- How does one prove intermediacy in a multistep pathway?
- In what way is the RtcB pathway unique? See the ensuing study by Das, U., et al. (2013) Rewriting the rules for end joining via enzymatic splicing of DNA 3'-PO_4 and 5'-OH ends. Proc. Natl. Acad. Sci. USA 110, 20437-20442. PMCID: PMC3870732