Organic Phosphorus

Guest lecturer: Ben Van Mooy

I. P review.

II. Organic P distributions in the sea.

III. Organic P bonds (^{31}P NMR).

IV. Molecules (Lipids, nucleic acids, other).

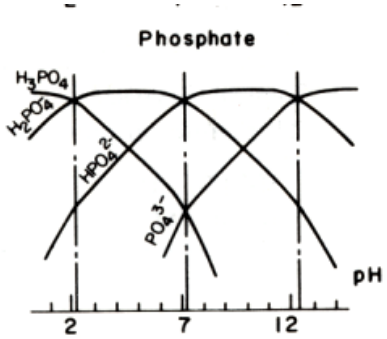
V. Organic P degradation/preservation.

I. P review

- P has 15 protons and there are 17 known isotopes.
- Only three are of any consequence.
  - ^{31}P
    - Only stable isotope
    - "100% abundant"
  - ^{32}P and ^{33}P
    - T_{half} = 14.3 and 25.3 days respectively.
    - Cosmogenic. Delivered to the sea via rain.
    - ^{33}P and ^{32}P are about 10^{14} times less abundant than ^{31}P.
    - Natural abundance ^{33}P/^{32}P is a tracer of the "age" of P reservoirs (Benitez-Nelson).
    - Commercially available and used for spiking incubations and tracing P flow in environments and cells.

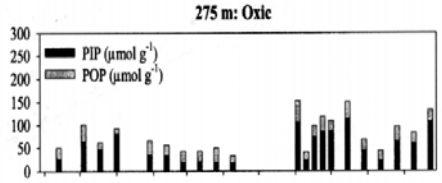
I. P review

- P exhibits no redox chemistry in the sea.
  - Nearly all P is (V).
  - True for biochemicals and dissolved forms.
- Phosphoric acid is the sole inorganic form
  - The dominant inorganic form is HPO_4^{2-}, which contributes to seawater alkalinity.
  - Termed "phosphate", "PO_4^{3-}" or "P*i*"



II. Organic P distributions in the sea.

- Sinking particulate P is more labile
  than OC or ON?
  - C:N:P in traps is much higher than Redfield (1980s).
  - Maybe not?!?!?

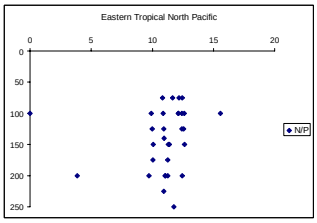


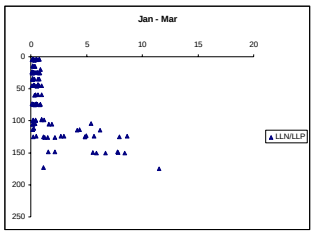
Depth (m)	Fluxes C (mmoles m^{-2} day^{-1})	Fluxes N (mmoles m^{-2} day^{-1})	Fluxes P (mmoles m^{-2} day^{-1})	Fluxes as % Surface Production C (percent)	Fluxes as % Surface Production N (percent)	Fluxes as % Surface Production P (percent)	Atomic Ratios C : N : P	Atomic Ratios C:N
COASTAL, UPWELLING								
50	35 37 (36)*	3.9 4.3 (4.1)	0.20 0.18 (0.19)	53	39	32	190 : 22 : 1.0	8.8
250	20 22 (21)	1.8 1.8 (1.8)	- 0.13 (0.13)	30	18	21	160 : 14 : 1.0	12
700	10 9.1 (9.6)	0.94 0.86 (0.90)	0.055 0.052 (0.053)	14	8.7	8.5	180 : 17 : 1.0	11
COASTAL, NON-UPWELLING								
50	6.4 6.2 8.4 9.4 (7.6)	0.57 0.64 0.90 0.98 (0.77)	0.032 0.026 (0.029)	34	23	13	260 : 27 : 1.0	9.9
250	4.8 4.3 4.3 3.8 (4.3)	0.41 0.38 0.52 0.40 (0.43)	0.013 0.013 (0.013)	19	13	6.3	330 : 33 : 1.0	10
700	3.6 4.3 4.4 (4.1)	0.22 0.41 0.21 (0.28)	0.010 0.012 (0.011)	18	8.1	4.9	370 : 25 : 1.0	15
OPEN OCEAN								
75	8.0 5.2 4.0 (5.7)	0.41 (0.41)	0.013 0.014 (0.014)	50	24	13	410 : 29 : 1.0	14
575	1.2 1.3 (1.2)	0.086 0.093 (0.089)	0.0019 0.0032 (0.0026)	11	5.2	2.4	460 : 34 : 1.0	13
1,050	1.6 0.75 0.77 (1.0)	0.034 0.048 0.021 (0.034)	0.0016 0.0006 (0.0011)	9	2	1.2	910 : 31 : 1.0	29

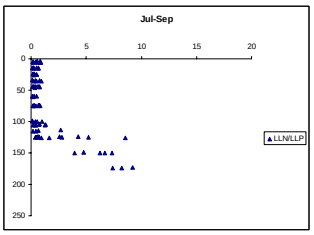
*Numbers in parenthese indicate averages.

II. Organic P distributions in the sea.

- Sinking particulate P: more labile than OC or ON (cont.)?
  - N:P not Refield below mixed layer.
  - May be not?!?!?
    - Denitrification
    - Advent of low-level (LL) nutrient analyses.

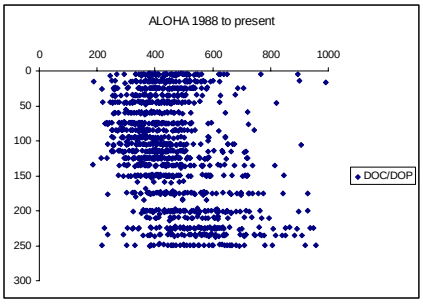






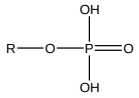
II. Organic P distributions in the sea.

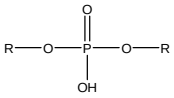
- DOP more labile than DOC?
  - DOP/DOC much higher than Redfield.
  - DOP/DOC increases below mixed layer.
  - Residence times:
    - DOC
      - About 2 years
      - Repeta et al natural abundance ^{14}C.
    - DOP
      - About ½ year
      - Björkman and Karl ^{33}P tracer experiments.
      - Benitez-Nelson and Karl natural abundance $^{33}P/^{32}P$.



III. Organic P bonds (^{31}P NMR).

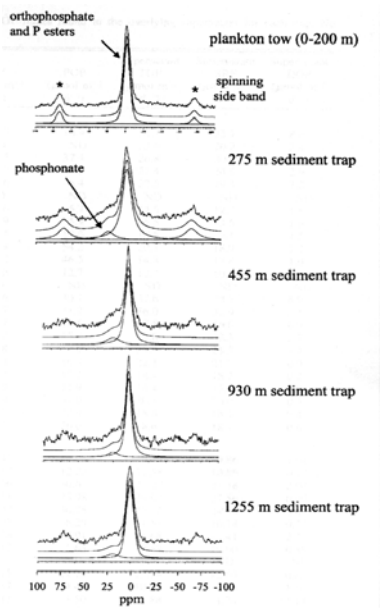
- P in organic matter occurs in three different types of bonding environments
  - Monoesters
    - Nucleotides and5' ends of nucleic acids.
    - Rare phospholipids
    - Rare phosphorylated carbohydrates and proteins.
  - Diesters
    - Nucleic acids
    - Most phospolipids
  - Phosphonates
    - Lipids
    - ???
    - One of great mysteries of organic geochemistry.





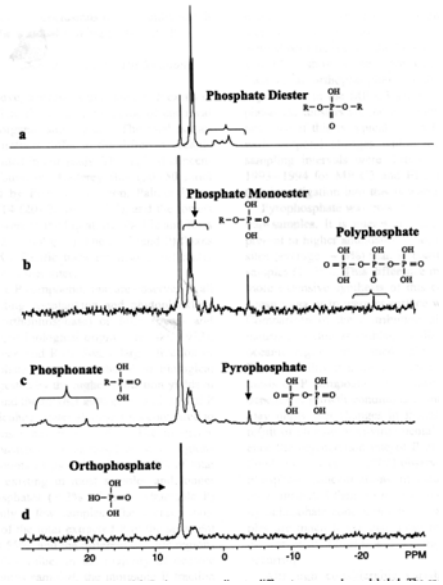
III. Organic P bonds (^{31}P NMR).

- Particulate OP by <u>solid</u> state NMR.
  - Advantages
    - Requires no chemical pre-treatment.
  - Disadvantages
    - Need a lot of material
    - Poor resolution
  - Results:
    - Plankton are all esters, which agrees with wet chemical analyses.
    - Phosphonates enriched on sinking particles?
    - Lots of inorganic P.



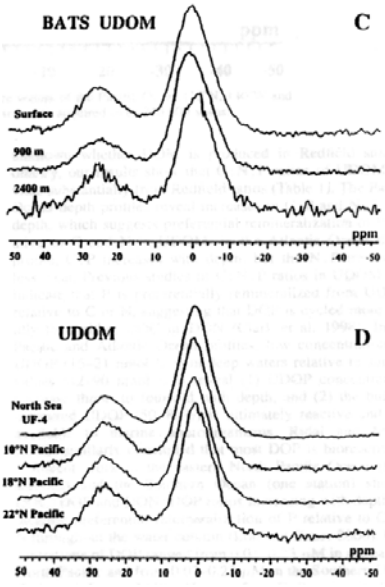
III. Organic P bonds (^{31}P NMR).

- Particulate OP by liquid state NMR.
  - Advantages
    - Need less material
    - Greater resolution
  - Disadvantages
    - Dissolving the OP requires a base hydrolysis that cleaves RNA.
    - Difficult to interpret.
  - Results:
    - Hard to say…
    - Phosphonates enriched on deeper particles?



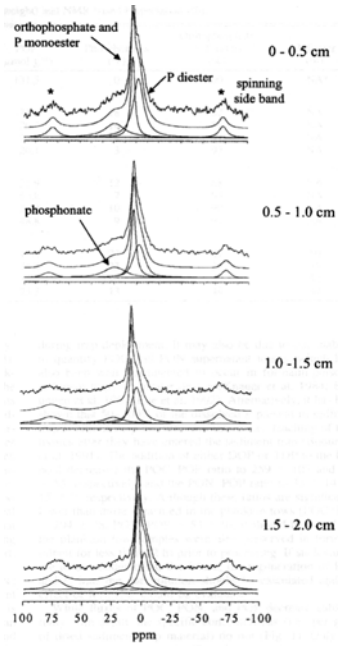
III. Organic P bonds (^{31}P NMR).

- DOP by solid state NMR.
  - DOP is composed of primarily of phosphoesters.
  - Also, phosphonates.
  - The ratio of phosphoesters to phosphonates is fairly constant.
  - Is there a common origin or common structure to refractory DOP?



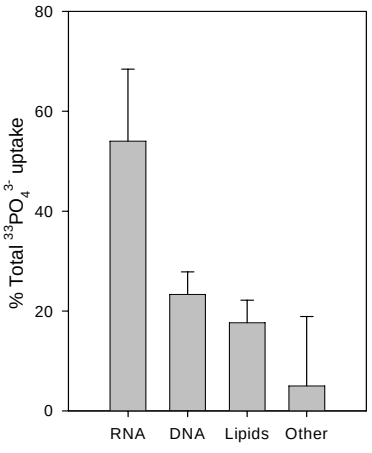
III. Organic P bonds (^{31}P NMR).

- Sedimentary OP by solid state NMR.
  - Looks a lot like particles
  - But, phosphodiesters were able to be resolved.
    - Possibility of intact nucleic acids and phospholipids.
    - Agrees with what few studies have shown using wet chemical techniques.



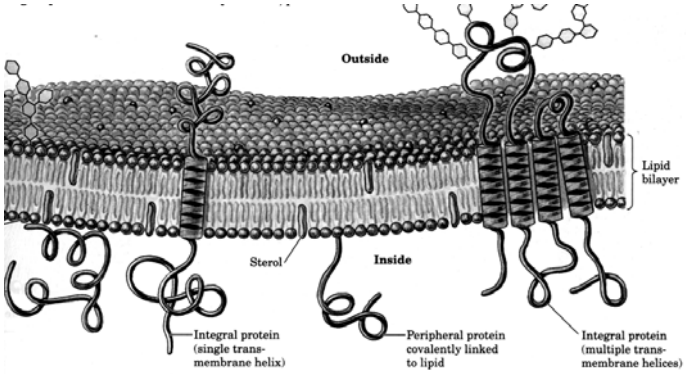
IV. Organic P molecules.

- Extremely labile
  - Found only in living organisms.
  - Source of P for other cells.
  - Source of bad genetic information (e.g. viruses).
- Almost all of the PO_4^{3-} that is taken up by plankton is used to synthesize phopsholipids, DNA, and RNA.
  - Consistent with NMR.
  - Sum of phopsholipids, DNA, and RNA is equal to total particulate P by wet chemical methods.



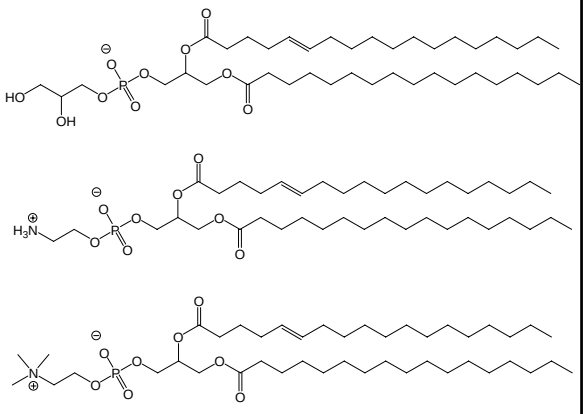
IV. Organic P molecules (phospholipids).

- Phospholipids
  - Are found primarily in cell membranes.
  - Composed of a polar “head” group and a nonpolar “tail” group.



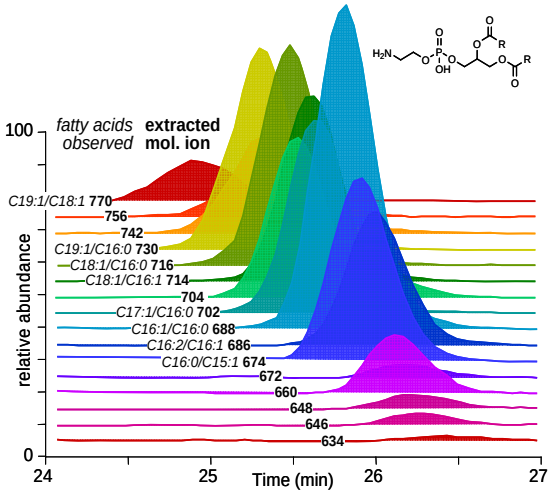
IV. Organic P molecules (phospholipids).

- Three basic types of phospholipids in plankton.
  - Phosphatidylglycerol (PG).
  - Phosphatidyl-ethanolamine (PE).
  - Phosphatidylcholine (PC).



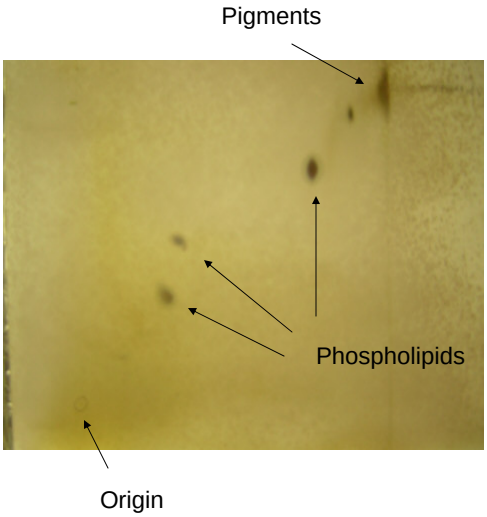
IV. Organic P molecules (phospholipids).

- Phospholipids analyzed by HPLC electrospray ionization ion trap mass spectrometry.
  - Allows phospholipids to be analyzed while they are intact.
  - Significant advance over GC FAME approach.
    - Can resolve different phospholipids.
    - Can resolve glycerol position of each FA.



IV. Organic P molecules (phospholipids).

- Phospholipids still analyzed by 2D TLC.
  - Use two solvents systems.
  - Great for large scale preparative work.
  - Decades old method
    - Phospholipids ID's by various stains.
    - Cheap
    - Easy
  - TLC plate of TLE from marine *Vibrio*.



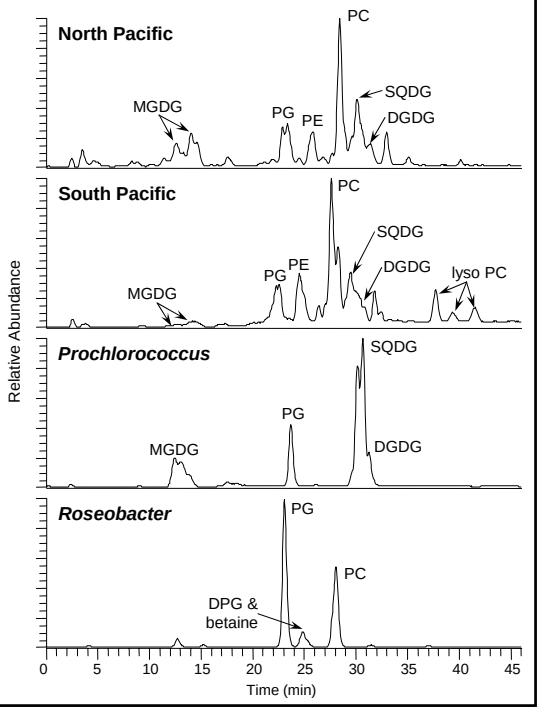
IV. Organic P molecules (phospholipids).

- Which organisms have which kinds of phospholipids?
  - Bacteria are loaded with phospholipids while photosynthetic plankton have glycolipids (MGDG, DGDG, SQDG).

	n	MGDG	SQDG	DGDG	PG	PC	PE	Other P-lipid	Be-taine
Prochloro-coccus	6	21	67	10	2				
Synecho-coccus	5	10	48	37	5				
Roseobacter (BacChl)	1				44	39		9	8
Vibrio	2				10		76	14	
Pseudo-monad	1				13		65	22	
Isochrysis	2	42		31	3	6			18

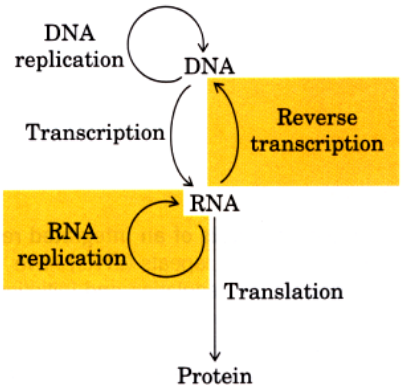
IV. Organic P molecules.

- Unpublished:
  - Phospholipid distribution is the same wherever you look (more or less).
  - Bacteria are the primary source of phospholipids.



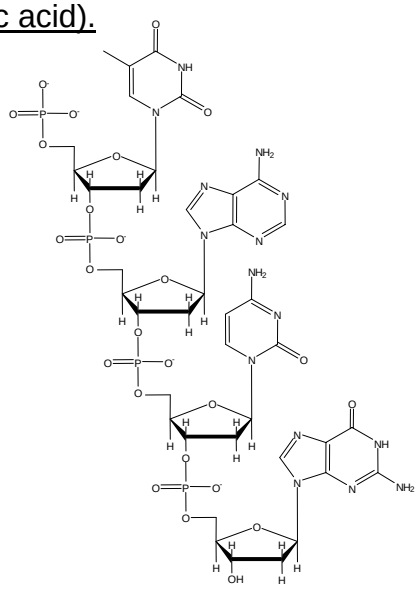
IV. Organic P molecules (nucleic acids).

- Are the information bearing molecules of the cell.
  - DNA
    - Double stranded.
    - Genes.
    - Template for transcription.
  - RNA
    - Single stranded.
    - Messages.
    - Template for translation.
    - Also in ribosomes.
  - "Central dogma".



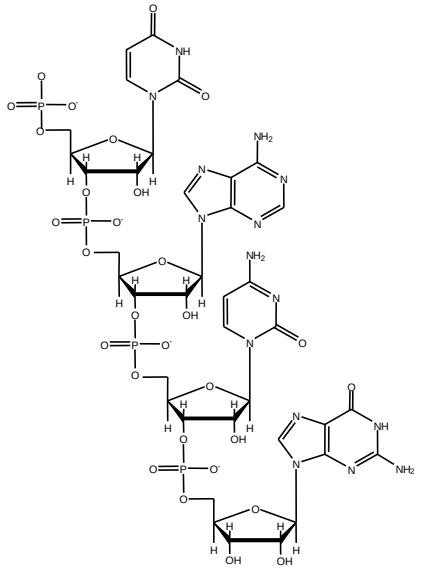
IV. Organic P molecules (nucleic acid).

- DNA
  - Deoxyribonucleic acid.
  - No hydroxyl at 2' carbon.
  - Only phosphodiester bonds, except at the 5' end of the molecule.



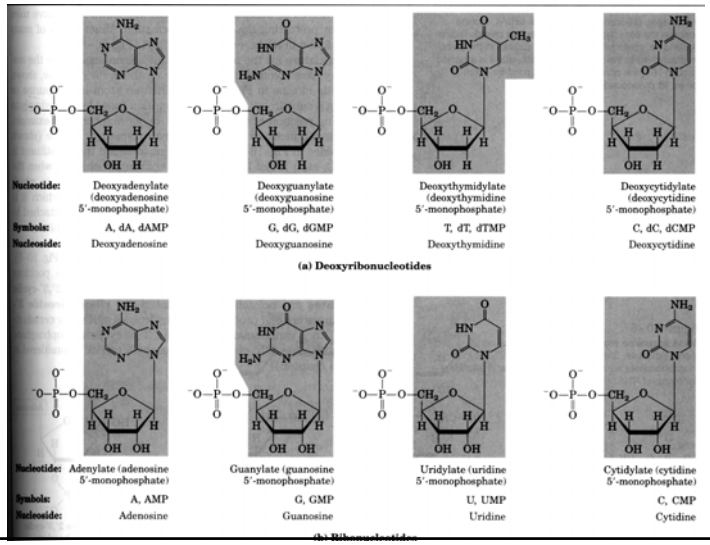
IV. Organic P molecules (nucleic acids).

- RNA
  - Ribonucleic acid.
  - Hydroxyl at 2’ carbon.
  - Also also only phosphodiester bonds, except at the 5’ end of the molecule.



IV. Organic P molecules (nucleic acids).

- Base nomenclature and composition.

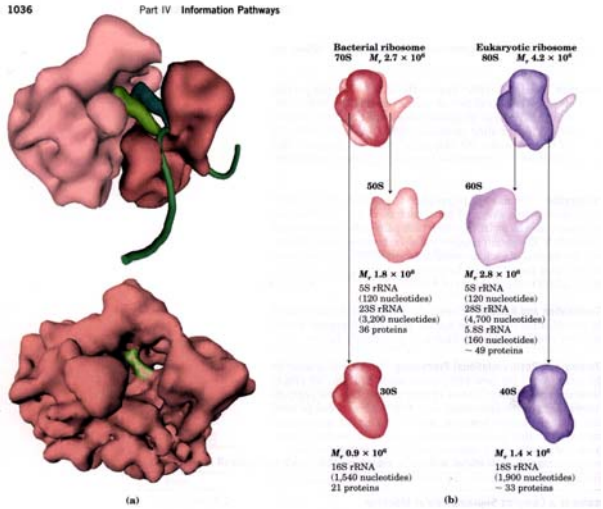


IV. Organic P molecules (nucleic acids).

- Analysis of nucleic acids.
  - Vast, vast variety of ways to analyze nucleic acids.
  - Health sciences provides methods, instruments and reagents.
  - Working with environmental samples presents problems.
    - Amount of sample.
    - Preservation and shipment.
    - Contamination.
- They are the ultimate biomarker
  - Encode all life information.
  - Total marine DNA sequence: several light years in length.
- Types of information marine scientists might gain from nucleic acids.
  - Gene sequences in the environment.
  - Enumeration of genetically defined cells types.
  - Community characterization.
  - Gene expression (potential is there, but few examples).

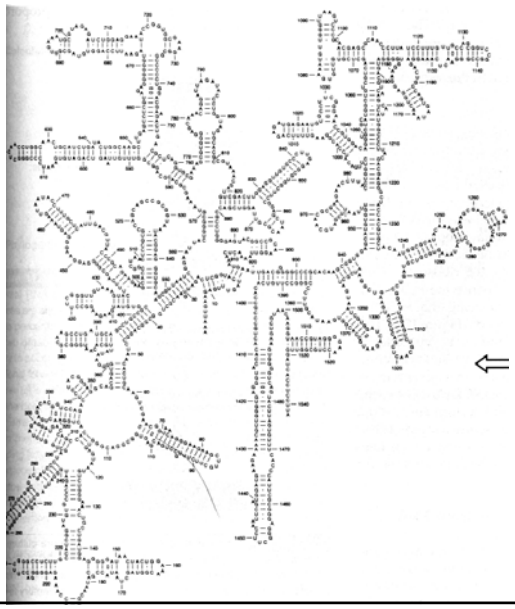
IV. Organic P molecules (nucleic acids).

- 16S and 18S rRNA.



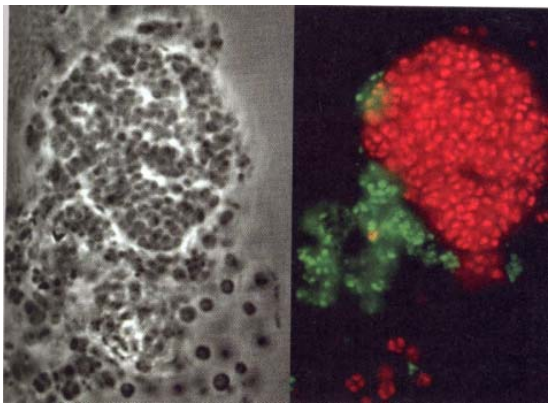
IV. Organic P molecules (nucleic acids).

- 16S rRNA tertiary structure.
  - Affects function in ribosome
  - Some structures are highly conservative.
  - Others are hyper-variable.



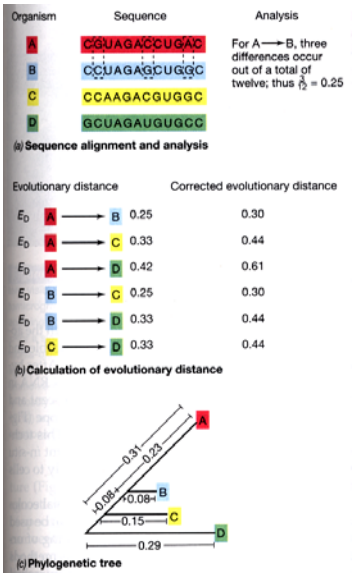
IV. Organic P molecules (nucleic acids).

- Conservative regions of rRNA can be targeted
  - Oligonucleotides (probes) are complementary to rRNA sequence.
  - Used for FISH
    - Probes are fluorescently labeled.
    - Stain the ribosomes inside the cells.
    - Visualized by epifluorescence microscopy.



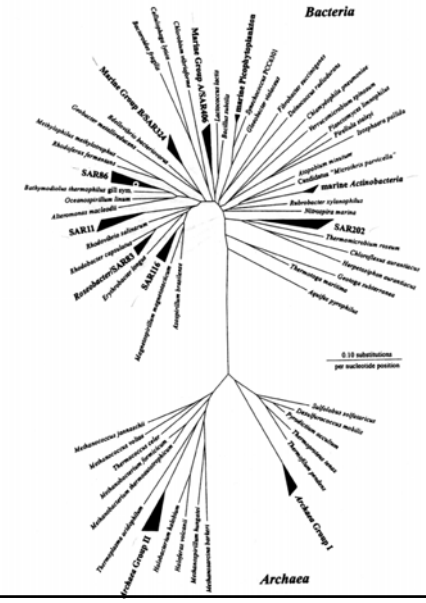
IV. Organic P molecules (nucleic acids).

- 16S and 18S rRNA genes (a.k.a. rDNA).
- Polymerase chain reaction and cloning
  - Used to retrieve individual genes from a complex community.
  - Can be used to construct phylogenetic trees.



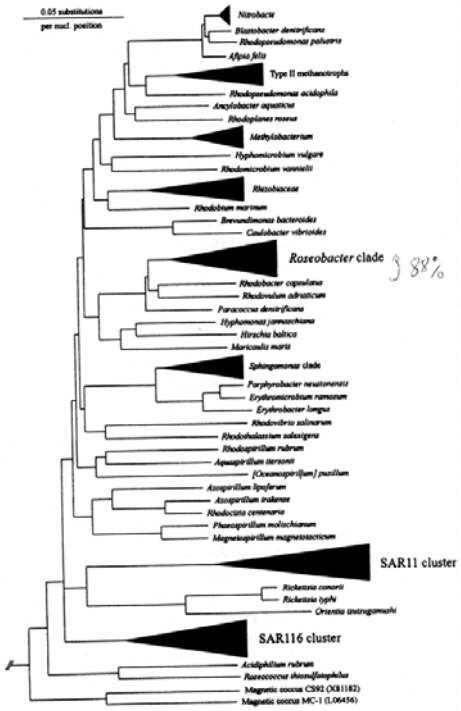
IV. Organic P molecules (nucleic acids).

- 16S and 18S rRNA are the basis for the tree of life.
- Define domains:
  - Bacteria
  - Archaea
  - Eukarya
- Tree of marine prokaryotic life.



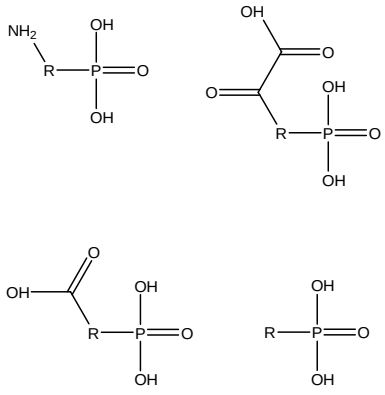
IV. Organic P molecules.

- Rooted trees are most common means to visualize sequence data.
  - Phylogenetic relationships are relative to the root organism.
  - Nodes represent “common ancestor”.
  - Some pitfalls of interpretation
    - Only horizontal lines have meaning.
    - Need to add “forward and backward” to determine distances.
    - Those next to each other may not be the most closely related.



IV. Organic P molecules (phosphonates).

- Very little is known about phosphonates.
  - Biochemical distribution
    - Phosphonolipids?
    - Small intermediates
  - Appear to be four major classes.
    - Aminoalkylphosphonate
    - Phosphonopyruvate
    - Phosphonoalkyl carboxylic acids
    - Alkyl or phenyl phosphonates



V. Organic P degradation/preservation.

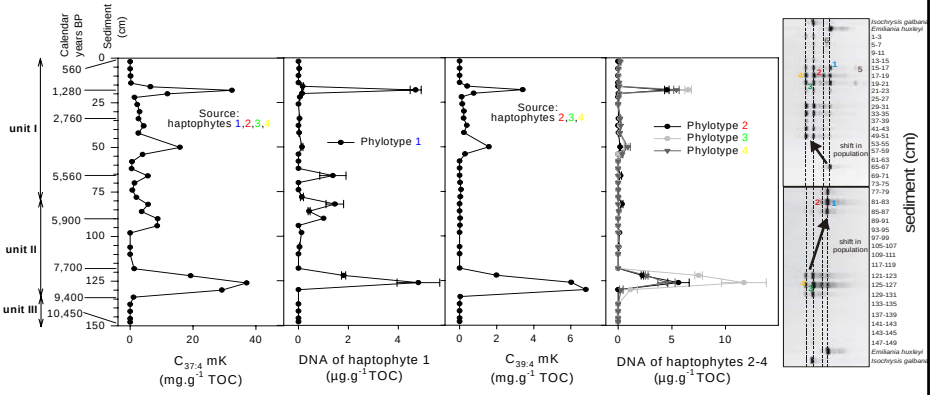
- Phospholipids and nucleic acids are found only in living cells.
  - They are a source of nutrient P.
  - Internal cell cycling of P.
    - Reversible enzymatic processes.
    - All cells routinely destroy nucleic acids (phosphodiesters).
    - All cells routinely manipulate cell membranes (phosphodiesters)
    - All cells routinely phosphorylate and dephosphorylate proteins and carbohydrates (phosphomonoesters).
    - Phosphonates, again, may be the exception (refractory or super labile?).
      - Can't find them in plankton.
      - Genomes encode phosphonatase activity.
  - Nucleic acids can be foreign genetic material.
  - Phospholipids may be another organism on the attack.

V. Organic P degradation/preservation.

- Organic P is not chemically diverse?
  - >75% of the organic P in plankton may be accounted for by five molecules: DNA, rRNA, PG, PE, PC.
- Requires only a limited suite of enzymes?
  - Alkaline phosphatases hydrolyze monoesters.
  - Endonucleases hydrolyze diesters.
    - Restriction endonucleases target by primary structure (i.e. specific sequence).
    - RNAses, DNAses, and S1 nucleases target by tertiary structure.
  - Four major phosphonatases for each major type of phosphonate.

V. Organic P degradation/preservation.

- Paleorecords?



V. Organic P degradation/preservation.

- Paleorecords?
- "Sequencable" DNA may be extracted from "non-modern" muds.

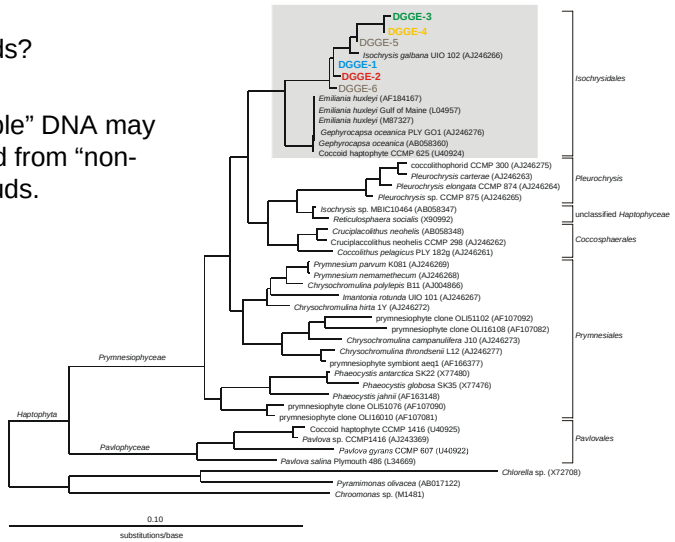


Figure credits and selected reading.

- Benitez-Nelson, C.R. and Karl, D., M., 2002. Phosphorous cycling in the North Pacific Subtropical Gyre using cosmogenic 32P and 33P. Limnol. Oceanogr., 47: 762-770.
- Benitez-Nelson, C.R., O'Neil, L., Kolowith, L.C., Pellechia, P. and Thunell, R., 2004. Phosphonates and particulate organic phosphorus cycling in an anoxic basin. Limnol. Oceanogr., 49: 1593-1604.
- Björkman, K. and Karl, D.M., 2003. Bioavailibility of dissolved organic phosphorus in the euphotic zone at station ALOHA, North Pacific Subtropical Gyre. Limnol. Oceanogr., 48: 1049-1057.
- Björkman, K., Thomson-Bulldis, A.L. and Karl, D.M., 2000. Phosphorus dynamics in the North Pacific subtropical gyre. Aquat. Microb. Ecol., 22: 185-198.
- Giovannoni, S. and Rappé, M. (Editors), 2000. Evolution, diversity, and molecular ecology of marine prokaryotes. Microbial Ecology of the Oceans. Wiley-Liss, New York, 47-84 pp.
- Karl, D.M. and Björkman, K.M., 2002. Dynamics of DOP. In: D.A. Hansell and C.A. Carlson (Editors), Biogeochemistry of marine dissolved organic matter. Academic Press, Amsterdam.
- Kolowith, L.C., Ingall, E.D. and Benner, R., 2001. Composition and cycling of marine organic phosphorus. Limnol. Oceanogr., 46: 309-320.
- Madigan, M.T., Martinko, J.M. and Parker, J., 2002. Brock biology of microorganisms. Prentice Hall, New York.
- Nelson, D.L. and Cox, M.M., 2000. Lehninger principles of biochemistry. Worth, New York.
- Paytan, A., Cade-Menum, B.J., McLaughlin, K. and Faul, K.L., 2003. Selective phosphorus regeneration of sinking marine particles: evidence from 31P-NMR. Mar. Chem., 82: 55-70.
- Van Mooy, B.A.S. et al., submitted. RNA, DNA and the phosphorus cycle of the oligotrophic North Pacific subtropical gyre. Limnol. Oceanogr.