Why nature chose phosphates
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Why Nature Chose Phosphates

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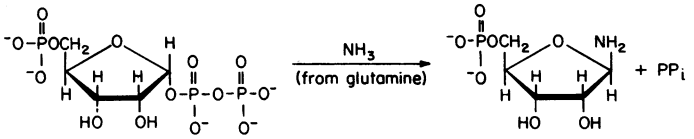
Phosphate esters and anhydrides dominate the living world but are seldom used as intermediates by organic chemists. Phosphoric acid is specially adapted for its role in nucleic acids because it can link two nucleotides and still ionize; the resulting negative charge serves both to stabilize the diesters against hydrolysis and to retain the molecules within a lipid membrane. A similar explanation for stability and retention also holds for phosphates that are intermediary metabolites and for phosphates that serve as energy sources. Phosphates with multiple negative charges can react by way of the monomeric metaphosphate ion PO_3^- as an intermediate. No other residue appears to fulfill the multiple roles of phosphate in biochemistry. Stable, negatively charged phosphates react under catalysis by enzymes; organic chemists, who can only rarely use enzymatic catalysis for their reactions, need more highly reactive intermediates than phosphates.

PHOSPHATE ESTERS AND ANHYDRIDES DOMINATE THE LIVING world. The genetic materials DNA and RNA are phosphodiesters. Most of the coenzymes are esters of phosphoric or pyrophosphoric acid. The principal reservoirs of biochemical energy [adenosine triphosphate (ATP), creatine phosphate, and phosphoenolpyruvate] are phosphates. Many intermediary metabolites are phosphate esters, and phosphates or pyrophosphates are essential intermediates in biochemical syntheses and degradations. Synthetic organic chemists, however, preferentially use other groups for linking hydroxyl, carboxyl, and amino groups, and for activating them for reaction. Why were phosphates, and almost no other groups, selected by evolution for biochemical transformations? Why did nature choose phosphates? In this article, an attempt is made to answer those questions.

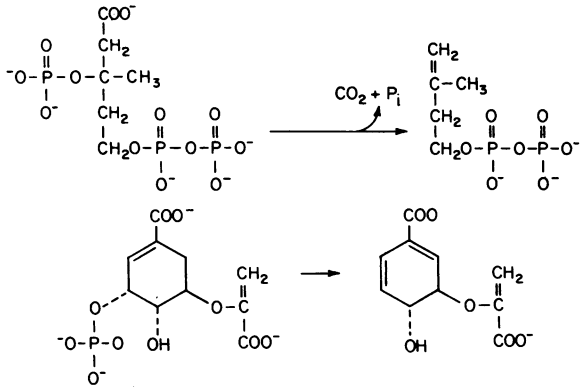
The Role of Phosphates

A few of the biologically important phosphates are listed in Table 1. Granted that phosphates are ubiquitous in biochemistry, what do they do? The answer is that they can do almost everything. In synthetic organic chemistry, nucleophilic displacement reactions at a carbon atom generally require a good "leaving group" [the anion of a strong acid or the conjugate base (for example, water) of a cationic acid (for example, hydronium ion)]. A large number of such leaving groups, such as chlorides, bromides, iodides, tosylates, and triflates, is in use, but phosphates are almost never used. In metabolic reactions, the leaving group is usually phosphate or pyrophosphate (*1*), although sulfonium salts (such as *S*-methyl methionine) are also used. A typical example of the displacement of a pyrophosphate residue is shown below.

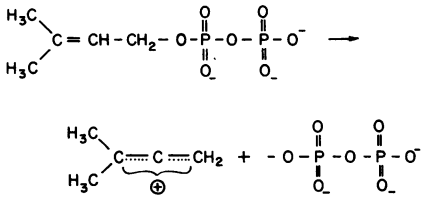


(In this article, inorganic phosphate ion is sometimes abbreviated as P_i and inorganic pyrophosphate as PP_i.)

Elimination reactions in synthetic organic chemistry also require good leaving groups, and here also chemists use groups such as chlorides and tosylates. They also use trialkylamines (which are released from quaternary ammonium salts), sulfoxides and selenoxides, and many other groups, but seldom do they use phosphates. In contrast, although biochemical eliminations of water from hydroxyl compounds often occur without isolable intermediates, when such intermediates are found, they are usually phosphates or pyrophosphates (*2*). Two examples, the first of which is a decarboxylative elimination, are shown below.



Although some biochemical processes proceed as S_N2 reactions, others follow the S_N1 pathway. The formation of terpenes and steroids depends on generating an allylic carbonium ion from dimethylallyl pyrophosphate (*3*):



The corresponding processes in synthetic organic chemistry also involve familiar leaving groups such as bromides and tosylates; the variety is large but seldom includes phosphates or pyrophosphates. A summary of the contrasts between the practices of organic chemists and the pathways in biochemistry is shown in Table 2.

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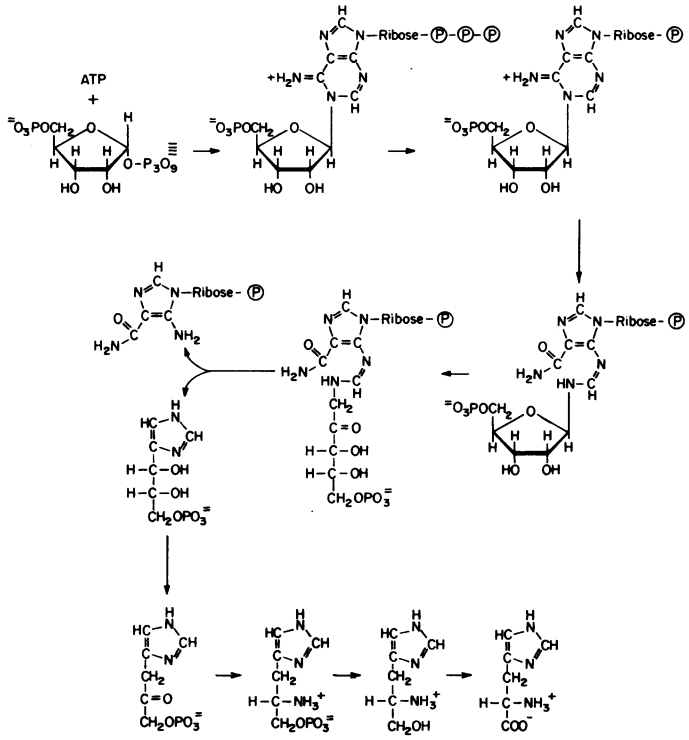
The Importance of Being Ionized

A possible explanation for the role of phosphates begins with a paper published in 1958 by Davis entitled "The importance of being ionized" (*4*). Davis's thesis was that living organisms must conserve their metabolites within the cell membrane. If these compounds had diffused through the membranes of evolutionarily primitive organisms, they would have been lost by dilution in the water outside the cell. Most electrically neutral molecules will have some solubility in lipid and will pass through a membrane; most ionized molecules, that is, salts, are insoluble in lipids. More precisely, the *p*K of an acid should be less than 4 and that of a base greater than 10 to ensure that only a small fraction of the compound remains in the un-ionized form at physiological *p*H. This general rule is not absolute. Polyhydroxylated compounds such as sugars may be lipophobic without being ionized, and compounds that are extremely insoluble in water, such as steroids, either become part of the membranes (*5*) or are conserved within cells in special ways or both (*6*). But certainly molecules can be kept within membranes if they remain ionized. The first *p*K's of phosphoric acid and of phosphate mono- and diesters are about 2, so that phosphates are ionized at physiological *p*H and therefore are trapped within cells.

An interesting example of Davis's principle in action concerns the biosynthesis of histidine (*7*). The details of the transformations are irrelevant to this discussion; the important point is that all the molecules are ionized. The intermediates that occur early in the sequence are all monoesters of phosphoric or pyrophosphoric acid, and, although all phosphate groups are removed from the intermediates that occur late in the sequence, the last phosphate is not removed until other ionized groups have been added. Some of the charged groups carry a negative charge, and some a positive charge, but at no place in this synthesis of histidine are any of the intermediates uncharged. This is not an isolated example; Davis's generalization can be illustrated with a number of different reaction sequences (*8*). [Adapted with permission from (*7*).]



(Ribose-Ⓟ represents 5-phosphoribose; Ribose-Ⓟ-Ⓟ-Ⓟ represents the corresponding triphosphate)

Table 1. Examples of phosphates in biochemistry.

Phosphate	Acid derivative
DNA	Diester of phosphoric acid
RNA	Diester of phosphoric acid
ATP	Anhydride of phosphoric acid
Creatine phosphate	Amide of phosphoric acid
Phosphoenolpyruvate	Enol ester of phosphoric acid
Pyridoxal phosphate	Phenol ester of phosphoric acid
Nicotine adenine dinucleotide	Ester and anhydride of phosphoric acid
Fructose 1,6-diphosphate	Ester of phosphoric acid
Glucose-6-phosphate	Ester of phosphoric acid
Isopentenyl pyrophosphate	Ester of pyrophosphoric acid
Ribose-6-phosphate-1-pyrophosphate	Ester of phosphoric and pyrophosphoric acids

Moreover, the electrostatic interaction of positive and negative charges constitutes the simplest and possibly the most primitive mode of interaction among molecules. The negative charges on phosphates are important in the binding of coenzymes to enzymes and in the "packaging" of nucleic acids.

A linking group for nucleic acids. In addition to the chemistry of intermediary metabolites, the linking of the nucleosides of DNA and RNA by phosphates must be explained. An argument can be advanced on the basis of the twin assumptions that the nucleotides must be connected so as to form a tape that contains a genetic code, and that the tape can later be taken apart, so as to reuse the nucleotides. The conservation of structures as complicated as nucleotides is certainly desirable for the economy of any organism. The best chemistry for taking a tape apart will probably be hydrolytic; if so, then the best connection will be an esterification reaction, or something similar. (A discussion of alternatives to esters occurs later in this article.) To make a tape from small molecules, the connecting agent must be at least bivalent in order to supply one connection for each of the nucleotides. Further, if the resulting molecules must also be ionized, the connecting agent must be at least trivalent. Phosphoric acid comes immediately to mind; sulfuric acid would not do, as it is only a dibasic acid. (Furthermore, sulfate esters are too reactive for a stable genetic material.) Of course, there are numerous molecules that can make two covalent bonds and remain charged; citric acid, glutamic acid, ethylene diamine monoacetic acid, arsenic acid, and silicic acid are examples. Nothing so far indicates that phosphoric acid is unique.

Rates of hydrolysis. Then why phosphoric acid? The answer depends on some basic principles of physical-organic chemistry and on an additional biological constraint on living systems. Living systems must be reasonably stable. Biomolecules not only must be confined within a bag defined by a lipid membrane; these molecules must survive in water for an appreciable time, preferably a long time. Metabolites, or at least some metabolites, can be short-lived, but not the genetic material.

What are the rates of hydrolysis of different kinds of compounds, and in particular what is the effect of structure on the rates of hydrolysis of esters? How can these rates be controlled? In the nearly neutral *p*H of the biological environment, esters such as ethyl acetate (*9*) can survive at ambient temperature for many months. But even a simple gene will have a thousand ester bonds, and most will have several thousand; further, even a simple organism must have a thousand genes or more. If a single ester bond in the genetic material is cleaved during the lifetime of the organism, it may fail to reproduce. Of course, with bacteria, a small sample may contain many billions of organisms, and only a few of them need to reproduce to maintain the species. Still, natural selection will favor a genetic material that, in the majority of the cells, will last for times

comparable to the lifetime of the organism. Certainly higher organisms, whose numbers are limited, would have special difficulties with labile genetic material. If that material is to survive, no more than one in a million ester bonds should cleave during the lifetime of the organisms. At room temperature, and at neutral *p*H, one molecule of ethyl acetate in a million is hydrolyzed every hour (*9*), and at higher acidities or basicities the time required for hydrolysis is less. In the acidic environment of fruits (*10*), one out of a million molecules of a carboxylic acid ester will hydrolyze every few seconds. The *p*H during the early stages of evolution is uncertain, but it may have been far from neutrality. At best, esters of carboxylic acids would be marginal as a genetic material for rapidly dividing bacteria and totally inadequate for longer lived animals or plants. Some sterically hindered esters that are more stable might serve, but the system would become unnecessarily complicated.

The rate of hydrolysis of triesters of phosphoric acid is somewhat less than that for electrically neutral carboxylic acids such as ethyl acetate (*11*), and, although phosphate triesters might be somewhat better as a genetic material, they would still be inadequate. As explained above, however, metabolites should be charged to keep them within the cell membrane, and this charge must be negative, since a negative charge has an important second effect: it sharply diminishes the rate of nucleophilic attack on the ester. Nucleophiles, such as hydroxide ion, are repelled by negative charges and therefore react less rapidly with anions than with neutral substrates. Furthermore, the same effect is qualitatively present with respect to electrically neutral nucleophiles such as water. In this case, attack involves the pushing of a lone pair of electrons from the nucleophile into the electrophilic site of reaction. Because the phosphate ester groups in DNA are negatively charged, they are relatively resistant to hydrolysis and are therefore preferable to esters of carboxylic acids as a genetic material. On the other hand, a residual positive charge near the site of hydrolysis on the genetic tape would not work; such a charge would increase rather than decrease the rate of nucleophilic attack.

The effect of negative charge. Two questions immediately arise. First, how much slower is nucleophilic attack on a negatively charged substrate as compared with attack on a neutral species, and second, would not this effect also occur with diesters of other tribasic acids such as citric acid? The answers to these questions come from standard physical-organic chemistry and show the advantage of phosphates.

The effect of charge on ionization constants and on reaction rates was noted and explained by Bjerrum (*12*) in 1923; a crude model that gives moderately good quantitative results was published (*13*) in 1938. To what extent will a negative charge retard the approach of a nucleophile to a phosphorus or carbon atom, or indeed to the atom of any element, in an ester? Qualitatively, the effect will be comparable to the effect of a negative electric charge on the ionization of the corresponding polybasic acid. When a polybasic acid such as phosphoric acid or citric acid ionizes, the covalent bond between a proton and the oxygen atom to which it is attached must be broken, and the electrostatic work required to take the proton to infinity in the field of the negative charge of the incipient anion must be overcome. The work required to remove a second proton from a dibasic acid should be similar to that to remove the first proton, except that the second proton must be removed not only in the field of the incipient charge on the oxygen atom to which the proton was attached but also in the additional electrostatic field of the negative charge produced by the first ionization. This additional electrostatic work is reflected in the ratio of the first to the second ionization constants of the acid. (A statistical factor must also be taken into account in calculating the ratio of the first to the second ionization constants.)

Table 2. Contrasts between synthetic organic chemistry and biochemistry. The leaving groups shown are typical examples for the indicated type of reaction. The symbols Ar, R, and Ac stand for aryl, alkyl, and acyl groups, respectively.

Reaction type	Synthetic chemistry	Biochemistry
Leaving group in S_N2	Cl^-, Br^-, I^-, $ArSO_3^-$	OPO_3^{3-}, $P_2O_7^{4-}$
Leaving group in S_N1	Cl^-, Br^-, I^-, $ArSO_3^-$	OPO_3^{3-}, $P_2O_7^{4-}$
Leaving group in E2	Cl^-, Br^-, I^-, $ArSO_3^-$, NR_3, –S–Ar, H_2O	OPO_3^{3-}, H_2O
	O ‖	
Driving force	Ac_2O, H_2SO_4, KOH, carbodiimides	ATP

The electrostatic work of ionization decreases with increasing distance between a proton that is undergoing ionization and the residual negative charge or charges in a molecule. The work decreases with increasing distance more than is predicted for the interactions of charges in a uniform dielectric medium because the medium is not uniform; the lines of force between the charges flow through both the molecule and the external dielectric. The result is that the electrostatic effect is astonishingly greater for small distances than for larger ones. This result is in accord with theory (*13*), and is apparent from the ratios of the successive ionization constants of phosphoric and citric acids, as shown in Table 3 (*14*). The successive ionization constants of phosphoric acid differ by factors of more than 10^5, whereas those for citric acid differ by factors of less than 50, even when the statistical factors are included.

The attack of hydroxide ion on esters will be similarly affected by charge. Specifically, the rate constants for the attack of hydroxide on dimethyl phosphate anion should be and are less than that for the attack of hydroxide ion on trimethyl phosphate by a factor of more than 10^5 (*15*). The experimental data, which have been extrapolated to 35°C, are shown in Table 4.

The rate for attack by hydroxide ion on trimethyl phosphate is less than that for attack on ethyl acetate by a factor of 25; the rate for attack of hydroxide on the anion of dimethyl phosphate is less than that for attack on ethyl acetate by a factor that approaches 5 million. (The statistical factors are sufficiently small by comparison with these numbers that they can be neglected.) The half-time for the hydrolysis of dimethyl phosphate in a solution of 1*N* alkali at 110°C is about a day (*15*). Such stability guarantees that genetic material will survive. Further questions related to hydrolysis are discussed later.

Possible Alternatives to Phosphate Esters

Citric acid. In this case the negative charge generated by the ionization of the first proton is distant from the second proton, and the electrostatic effect is correspondingly much less. The effect is reflected in the ionization constants of Table 3. The negative charge will then also cause only a relatively small change (about a factor of 20) in the rate of saponification of the unsymmetrical diester, as compared to that of the triester of citric acid (*16*). (In both cases, the central carboxyl residue is attacked.) The change is real but insufficient to stabilize genetic material. The negative charges on the diesters of phosphoric acid in DNA help retain the molecules within the cell membrane and impart to the nucleic acid the stability required for reproducible genetics.

Arsenic acid and silicic acid. Another compound that must be considered as a basis for a possible genetic material is arsenic acid, which is also tribasic. The poisonous effects, however, of compounds of arsenic probably cannot be avoided, since these effects are

Table 3. Ionization constants for orthophosphoric and citric acids.

Acid	K_1 (*M*)	K_2 (*M*)	K_3 (*M*)	K_1/K_2	K_2/K_3
Phosphoric	7.5×10^{-3}	6.2×10^{-8}	2.2×10^{-13}	1.2×10^{5}	2.8×10^{5}
Citric	6.0×10^{-4}	1.5×10^{-5}	4.0×10^{-7}	40	38

centered in the lower valence states of arsenic (*17*), and the reduction of pentavalent arsenic is much easier than that of pentavalent phosphorus. In any case, arsenic esters are totally unsuitable; the hydrolysis of esters of arsenic acid is remarkably fast. The triisopropyl ester in neutral water at room temperature is completely hydrolyzed in a couple of minutes (*18*). Apparently the hydrolysis of the diesters is even faster than that of the triesters. [Compare with the hydrolysis of phosphites (*19*).]

Silicic acid is more abundant in nature than phosphoric acid and is tetravalent, but it is also unsuitable. Its esters, like those of arsenic acid, hydrolyze far too rapidly (*20*) to survive, and furthermore silicic acid is too weak an acid; its first *p*K (*21*) is about 9.5. Its diesters would not ionize in neutral solution and would not carry a negative charge.

Amides. If esters hydrolyze too easily, why not use amides, which are much more resistant to hydrolysis? Proteins are obviously quite stable in aqueous media. If the system had been designed with amide bonds, presumably natural selection would have led to the synthesis of appropriate amines and of enzymes with sufficient specificity to avoid confusion between structural and enzymatic proteins on the one hand and the postulated proteinlike genetic tape on the other. There must be (and are) other compelling reasons that account for the choice of phosphates.

In general, a driving force is required for chemical and biochemical reactions, such as the formation of a genetic tape. The transfer of nucleotides from molecules such as ATP or guanosine triphosphate (GTP) in the synthesis of ribonucleic acids, or the transfer of nucleotides from molecules such as deoxyadenosine triphosphate or deoxyguanosine triphosphate in the synthesis of deoxyribonucleic acids, or the transfer of the PO_3^- residue from ATP in many phosphorylations all make use of the favorable properties of phosphoric anhydrides. In sharp contrast to carboxylic anhydrides, the phosphoric anhydrides are protected by their negative charges from rapid attack by water and other nucleophiles so that they can persist in an aqueous environment (*22*) even though they are thermodynamically unstable, and thus can drive chemical processes to completion in the presence of a suitable catalyst (enzyme). This remarkable combination of thermodynamic instability and kinetic stability was noted many years ago by Lippmann, who correctly ascribed the kinetic stability to the negative charges in ATP (*23*). A citric acid anhydride would not survive long in water and could not serve as a convenient source of chemical energy.

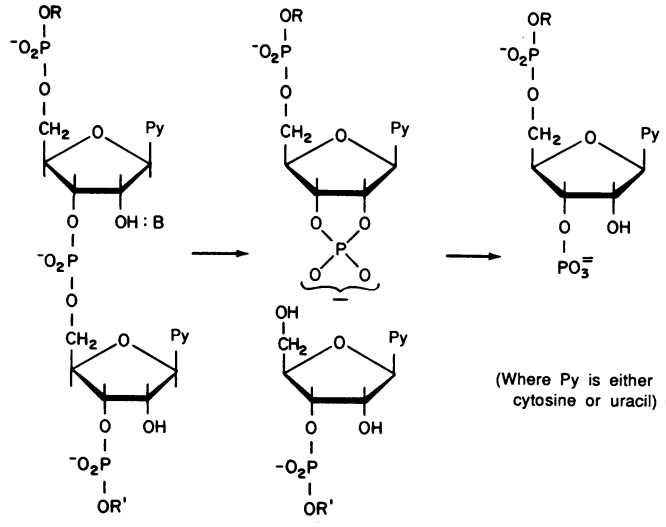
Prebiotic Chemistry

Despite the argument given above, the choice of phosphate esters rather than proteins for the genetic tape may lie in chemical history rather than in chemical kinetics. Recently, both Cech and co-workers (*24*) and Altman and co-workers (*25*) have found that RNA exhibits catalytic properties. Prior to these discoveries, several investigators (*26*) had speculated that RNA was both the original genetic and the original catalytic material; now Cech and co-workers (*27*) have strongly reinforced these early suggestions. This hypothesis is strengthened by the finding (*25, 28*) by Guerrier-Takada, Pace, Altman, and co-workers that ribonuclease-P consists of two subunits, one of which is a protein and the other RNA. Although the catalytic subunit is surprisingly the RNA, the fact that a protein is associated with the nucleic acid and increases its catalytic efficiency suggests that ribonuclease-P is a biochemical fossil (*29*).

Perhaps the original enzymes were made of RNA and then later adsorbed peptides from the prebiotic soup, peptides that stabilized the RNA or enhanced its catalytic efficiency. Eventually the protein component, because of greater variety of catalytic groups, may have taken over the catalytic function and in all but a few cases shed the RNA. If this scenario is correct, then the reason why the genetic material consists of phosphate esters instead of amides is a historical one. Phosphate esters came first and worked well. Natural selection operated on the system and added deoxyribosides, but phosphates, which served well, were not changed. The question might be turned around to ask why proteins were necessary at all. The answer is implied in the discussion above; the greater structural variety of amino acids allowed better catalytic properties in protein enzymes than in those composed of RNA; further, a reasonable prebiotic pathway leads from one to the other.

Catalysis by RNA (*30*) was discovered in a study of the self-splicing of the nucleic acid; the reactions require an ester exchange between phosphate esters. Similar ester exchange is also of great biological importance in genetic recombination. Comparable reactions are rare with amides; the necessity for such ester exchange provides an additional reason to prefer phosphate esters over proteins for the genetic material.

Hydrolysis of RNA. Although RNA is a phosphodiester and carries a negative charge, it is relatively susceptible to hydrolysis; the rate of its spontaneous reaction with water, extrapolated to room temperature, is about 100 times greater than that of DNA (*31*). This occurs because the 2′-hydroxyl group of RNA acts as an internal nucleophile and leads to the formation of a 2′,3′-cyclic phosphate with cleavage of the RNA chain (below) (*32*). Intramolecular reactions



are generally enormously more rapid than the corresponding external ones (*33*). In this case, the cyclic phosphate that is formed is highly strained (*34*), but the cyclization proceeds nonetheless, presumably because of the large positive entropy of the cleavage reaction. The cyclic phosphate in turn readily undergoes hydrolysis. The thermodynamics of the hydrolysis of the cyclic ester depends on the strain energy that is released during cleavage of the ring, and the kinetics depend in part on that strain (*35*).

Since RNA is hydrolyzed relatively easily, it is not as well adapted as is DNA as a storage material for genetic material. The evolutionary advantage of DNA is apparent, although the evolutionary pathway to the difficult reduction process (*36*) is obscure. Some viruses contain RNA and not DNA. However, in the tight viral

package, water is excluded, so that the hydrolytic instability of RNA is not relevant. Subsequently, the time for viral reproduction in a cell during which the viral RNA is exposed to water is only a few minutes. In addition, not all of the enormous number of viral particles need reproduce to preserve the organism.

Enzymatic hydrolysis. A reason then why nature prefers phosphate esters is that they are quite stable. Nevertheless, a biochemical system must not be so stable that it cannot be taken apart. DNA must be metabolized. In fact, a phosphate diester that undergoes spontaneous hydrolysis at a negligible rate can readily be cleaved by enzymatic catalysis, since enzymes can increase reaction rates by factors of 10^9 to 10^{12}. Understanding the mechanisms of enzyme action is among the important challenges to physical organic chemistry today, but from the point of view of this article the fact that enzymes exist that can hydrolyze the diesters of phosphoric acid is sufficient.

Dianions of monoesters of phosphoric acid. But another problem arises in connection with the chemistry of phosphates. The third ionization constant of phosphoric acid is smaller than the second by a factor of 10^5. Should not then the hydrolysis of the monoester dianions of phosphoric acid be slower than that of diesters by a similar factor? If dimethyl phosphate monoanion is hydrolyzed in 1*N* base at 110°C with a half-time of a day, will the hydrolysis of the dianion under the same conditions require 3000 years? Since, however, the second *p*K of phosphoric acid is about equal to physiological *p*H, a considerable quantity of monoanion will be present, and subject to nucleophilic attack.

Monomeric metaphosphates. Actually, hydrolysis and ester exchange for monoester dianions of phosphoric acid occur by way of a different mechanism. Monoester dianions can decompose in analogy to the S_N1 reaction of carbon chemistry to yield the monomeric metaphosphate ion PO_3^- (*37*). (Monomeric metaphosphoric acid, HPO_3, is an unstable chemical intermediate. The stable phosphoric acid, H_3PO_4, is called orthophosphoric acid.) In contrast to nitrate ion, which is its analog in the first row of the periodic table, PO_3^- is a powerful electrophile (*38*) and is unstable in water relative to dihydrogen orthophosphate by approximately 32 kcal/mol (*39*).

The maximum rate of hydrolysis of monoesters of phosphoric acid occurs at *p*H 4 (*37, 40*), where the major species present is the monoprotonated monoanion; the pathway postulated for its decomposition by way of monomeric metaphosphate is shown in Eq. 7.

$$CH_3OPO_3H^- \rightleftharpoons CH_3\overset{+}{\underset{H}{O}}PO_3^{2-} \rightarrow CH_3O^- + PO_3^-$$

$$PO_3^- + H_2O \rightarrow H_2PO_4^- \qquad (7)$$

This mechanism was first postulated (*37*) in the early 1950s, and much research has been performed in the three subsequent decades to test this idea (*41*). Despite some argument about details, the need for monomeric metaphosphate has been firmly established. The species has been identified in the gas phase (*42*) and in tertiary butyl alcohol (*43*), but a vigorous (if friendly) debate (*44*) has centered on whether the monomeric metaphosphate ion is ever truly free in water.

In phosphorylations, PO_3^- is transferred, for example, from ATP to various substrates. Nucleophilic attack has been inferred because many such reactions result in stereochemical inversion (*45*) at phosphorus; at present, only the solvolysis of *p*-nitrophenyl phosphate, made chiral at the phosphorus atom with ^{16}O, ^{17}O, and ^{18}O (*43*), is known to proceed with racemization. Paradoxically, however, the kinetics for some reactions that proceed with inversion at phosphorus resemble those of S_N1 processes (*46*). In the transition state for phosphorylations, metaphosphate is essentially completely formed, while the bond to the monomeric metaphosphate residue from an incoming nucleophile has barely begun to form (*44*). In such a loose transition state, the monomeric metaphosphate ion is nearly, even if not completely, free. Protons are known only in the gas phase and are never free in water, yet mechanistic chemistry requires the concept of protons. The concept of monomeric metaphosphates is needed to explain how monoesters of phosphoric acid and ATP, for example, react in the absence of enzymes at measurable rates despite their negative charges, and react by enzymatic catalysis sufficiently rapidly to participate in metabolism.

Table 4. Rates of saponification at 35°C.

Ester	$k(M^{-1}\ sec^{-1})$	*k*, relative*
$(CH_3O)_2PO_2^-$	2.0×10^{-9}	1.0
$(CH_3O)_3P{=}O$	3.4×10^{-4}	2×10^5
$CH_3CO_2C_2H_5$	1.0×10^{-2}	5×10^6

*Rate relative to that of $(CH_3O)_2PO_2^-$.

Conclusions

In summary, the existence of a genetic material such as DNA requires a compound for a connecting link that is at least divalent. In order that the resulting material remain within a membrane, it should always be charged, and therefore the linking unit should have a third, ionizable group. The linkage is conveniently made by ester bonds, but, in order that the ester be hydrolytically stable, that charge should be negative and should be physically close to the ester groups. All of these conditions are met by phosphoric acid, and no alternative is obvious. Furthermore, phosphoric acid can form monoesters of organic compounds that can decompose by a mechanism other than normal nucleophilic attack, a mechanism that allows them sufficient reactivity to function in intermediary metabolism.

Finally, we can answer the question concerning the choices made by chemists and by natural selection. Chemists need to use reactive intermediates, or at least intermediates that will react at only moderately elevated temperatures. They cannot afford to use compounds as stable as the phosphate anions; phosphate dianions are poor leaving groups in S_N1 or S_N2 reactions, or in eliminations. On the other hand, biochemistry could not tolerate compounds as reactive as alkyl bromides or dialkyl sulfates; the metabolites would spontaneously hydrolyze much too rapidly. Furthermore, the reactivity of alkyl halides and sulfates is directly related to their toxicity; they alkylate and destroy essential metabolites and enzymes. Biochemistry uses phosphate ester anions that undergo slow hydrolysis in the absence of enzymes, and rapid hydrolysis in the presence of enzymes, and that are readily contained within lipid membranes. We can understand the choices made both by chemists and by the process of natural selection. They are both correct.

REFERENCES AND NOTES

1. J. Preiss and P. Handler, *J. Biol. Chem.* **233**, 493 (1958); J. G. Flaks, M. J. Erwin, J. M. Buchanan, *ibid.* **228**, 201 (1957); A. Kornberg, I. Lieberman, E. S. Simms, *ibid.* **215**, 417 (1955); S. C. Hartman and J. M. Buchanan, *ibid.* **233**, 451 (1958).
2. H. Morell, M. J. Clark, P. F. Knowles, D. B. Sprinson, *ibid.* **242**, 82 (1967).
3. K. Bloch, in *Ciba Foundation Symposium on the Biosynthesis of Isoprenoid Compounds*, G. E. W. Wolstenholm and M. O'Connor, Eds., (Churchill, London, 1959), p. 4; C. D. Poulter and H. C. Rilling, in *Biosynthesis of Isoprenoid Compounds*, J. W. Porter and S. L. Spurgeon, Eds. (Wiley, New York, 1981), vol. 1, p. 161.
4. B. D. Davis, *Arch. Biochem. Biophys.* **78**, 497 (1958).
5. K. Bloch, *Curr. Top. Cell Regul.* **18**, 289 (1981).
6. J. L. Goldstein and M. S. Brown, *J. Lipid Res.* **25**, 1450 (1984).
7. See, L. Stryer, *Biochemistry* (Freeman, San Francisco, ed. 2, 1981), p. 499, for example.
8. *Biochemical Pathways* (Boehringer, Mannheim, West Germany, ed. 3, 1978).
9. A. J. Kirby, in *Comprehensive Chemical Kinetics*, C. H. Banford and C. F. H. Tipper, Eds. (Elsevier, Amsterdam, 1972), vol. 10, p. 153.
10. The *p*H of the sap of a Macintosh apple is about 4.0.

11. J. R. Cox and O. B. Ramsay, *Chem. Rev.* **64**, 317 (1964).
12. N. Bjerrum, *Z. Phys. Chem.* **106**, 219 (1923).
13. J. G. Kirkwood and F. H. Westheimer, *J. Chem. Phys.* **6**, 506 (1938); F. H. Westheimer and J. G. Kirkwood, *ibid.*, p. 513; F. H. Westheimer and M. W. Shookhoff, *J. Am. Chem. Soc.* **61**, 555 (1939).
14. M. Kutake, Ed., *Constants of Organic Compounds* (Asakura, Tokyo, 1963).
15. J. Kumamoto and F. H. Westheimer, *J. Am. Chem. Soc.* **77**, 2515 (1955).
16. J. Pinnow, *Z. Electrochem.* **24**, 21 (1918).
17. F. C. Knowles and A. W. Benson, *Trends Biochem. Sci.* **8**, 178 (1983).
18. J.-M. Crafts, *Bull. Soc. Chim. France Ser. 2* **14**, 99 (1870); C. D. Baer, J. O. Edwards, P. H. Rieger, *Inorg. Chem.* **20**, 905 (1981).
19. V. E. Bel'skii and G. Z. Motygullin, *Bull. Acad. Sci. USSR* **1967**, 2427 (1967); E. T. Kaiser, M. Panar, F. H. Westheimer, *J. Am. Chem. Soc.* **85**, 602 (1963).
20. Ebelman, *Ann. Chem.* **57**, 334 (1846); R. Aelion, A. Loebel, F. Eirich, *Recl. Trav. Chim. Pays-Bas* **69**, 61 (1950); *J. Am. Chem. Soc.* **72**, 5705 (1950).
21. D. Barby, T. Griffiths, A. R. Jacques, D. Pawson, *Spec. Publ. Chem. Soc.* **31**, 320 (1977).
22. D. L. Miller and F. H. Westheimer, *J. Am. Chem. Soc.* **88**, 1507 (1966).
23. F. Lippmann, in *Phosphorus Metabolism*, W. D. McElroy and H. B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1951), vol. 1, p. 521.
24. A. J. Zaug and T. R. Cech, *Science* **231**, 470 (1986).
25. C. Guerrier-Takada, K. Gardiner, T. Marsh, N. Pace, S. Altman, *Cell* **35**, 849 (1983).
26. H. B. White III, *J. Mol. Evol.* **7**, 101 (1976); C. M. Visser and R. M. Kellog, *ibid.* **11**, 163, (1978); *ibid.*, p. 171; see also L. E. Orgel, *J. Mol. Biol.* **38**, 381 (1968).
27. B. L. Bass and T. R. Cech, *Nature (London)* **308**, 820 (1984).
28. C. Guerrier-Takada and S. Altman, *Science* **223**, 285 (1984); S. Altman, *Cell* **36**, 237 (1984).
29. F. H. Westheimer, *Nature (London)* **319**, 534 (1986).
30. A. J. Zaug and T. R. Cech, *Cell* **19**, 331 (1980); *Biochemistry* **25**, 4478 (1986); T. R. Cech, *Sci. Am.* **255**, 64 (November 1986).
31. J. Eigner, H. Boedtker, G. Michaels, *Biochim. Biophys. Acta* **51**, 165 (1961).
32. F. M. Richards and H. W. Wyckoff, in *The Enzymes*, P. D. Boyer, Ed. (Academic Press, New York, ed. 3, 1971), vol. 4, p. 647.
33. A. J. Kirby, *Adv. Phys. Org. Chem.* **17**, 183 (1980).
34. J. M. Sturtevant, J. A. Gerlt, F. H. Westheimer, *J. Am. Chem. Soc.* **95**, 8168 (1973).
35. F. H. Westheimer, *Acc. Chem. Res.* **1**, 70 (1968); K. Taira, T. Fanni, D. Gorenstein, *J. Am. Chem. Soc.* **106**, 1521 (1984).
36. L. Thelander and P. Reichard, *Annu. Rev. Biochem.* **48**, 133 (1979); M. A. Ator and J.-A. Stubbe, *Biochemistry* **24**, 7214 (1985).
37. W. Butcher and F. H. Westheimer, *J. Am. Chem. Soc.* **77**, 2420 (1955); P. W. C. Barnard *et al.*, *Chem. Ind. (London)* **1955**, 760 (1955).
38. F. H. Westheimer, *Chem. Rev.* **81**, 313 (1981).
39. J. P. Guthrie, *J. Am. Chem. Soc.* **99**, 3991 (1977).
40. M. C. Bailly, *Bull. Soc. Chim. France Ser. 5* **9**, 314 (1942).
41. J. Rebek and F. Gavina, *J. Am. Chem. Soc.* **97**, 1591 (1975); D. Samuel and B. Silver, *J. Chem. Soc.* **1961**, 4321 (1961); A. J. Kirby and A. G. Varvoglis, *ibid.* **1968B**, 135 (1968); F. Ramirez and J. F. Maracek, *Tetrahedron* **35**, 1581 (1979); A. C. Satterthwait and F. H. Westheimer, *J. Am. Chem. Soc.* **103**, 1177 (1981); K. C. Calvo and F. H. Westheimer, *ibid.* **105**, 2827 (1983).
42. D. J. Harven *et al.*, *J. Am. Chem. Soc.* **101**, 7409 (1979); M. Henchman, A. A. Viggiano, J. F. Paulson, A. Freedman, J. Wormhoudt, *ibid.* **107**, 1453 (1985).
43. S. L. Buchwald, J. M. Friedman, J. R. Knowles, *ibid.* **106**, 4911 (1984); J. Friedman and J. R. Knowles, *ibid.* **107**, 6126 (1985); J. Knowles, personal communication.
44. W. P. Jencks, *Acc. Chem. Res.* **13**, 161 (1980).
45. J. R. Knowles, *Annu. Rev. Biochem.* **49**, 877 (1980); S. L. Buchwald, D. E. Hansen, A. Hassett, J. R. Knowles, *Methods Enzymol.* **87**, 279 (1982); K. C. Calvo, *J. Am. Chem. Soc.* **107**, 3690 (1985).
46. A. J. Kirby and A. G. Varvoglis, *J. Am. Chem. Soc.* **89**, 415 (1967); K. C. Calvo and F. H. Westheimer, *ibid.* **106**, 4205 (1984); K. C. Calvo and J. M. Berg, *ibid.*, p. 4202.
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Research Articles

A Mammalian Mitochondrial RNA Processing Activity Contains Nucleus-Encoded RNA

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Ribonuclease mitochondrial RNA processing, a site-specific endoribonuclease involved in primer RNA metabolism in mammalian mitochondria, requires an RNA component for its activity. On the basis of copurification and selective inactivation with complementary oligonucleotides, a 135-nucleotide RNA species, not encoded in the mitochondrial genome, is identified as the RNA moiety of the endoribonuclease. This finding implies transport of a nucleus-encoded RNA, essential for organelle DNA replication, to the mitochondrial matrix.

ALTHOUGH MITOCHONDRIA CONTAIN THEIR OWN GENETIC information, distinct from nuclear DNA (*1–3*), most proteins required for mitochondrial biogenesis are encoded in the nucleus and transported into mitochondria (*4–6*). Although present in all eukaryotes, mitochondria of metazoan species and higher plants show great variation in morphology and cellular distribution depending on particular developmental stages (*7, 8*) and tissue types (*9, 10*), suggesting a dynamic interaction between the nucleus and mitochondria. Understanding this coordinated interplay between the two distinct intracellular genetic compartments, nuclear DNA and mitochondrial DNA (mtDNA), is a central issue in cell biology. Since the enzymes involved in replication and transcription of mtDNA (*11, 12*), which control genomic expression, are also nucleus-encoded, their identities and modes of regulation may provide some insights into nuclear-mitochondrial interactions. We recently characterized a site-specific endoribonuclease that cleaves RNA near one of the transition sites of primer RNA synthesis to DNA synthesis at the leading-strand origin of mtDNA replication (*13*). We now show that this endoribonuclease, termed MRP (for mitochondrial RNA processing), contains an RNA component that is essential for its enzymatic activity. A partial sequence analysis of this endogenous RNA indicates that it is a nuclear gene product, which necessitates a translocation of a highly charged nucleic acid through the hydrophobic phospholipid bilayer into the mitochondrial matrix.

Micrococcal nuclease sensitivity of the endonuclease. Most biological catalyses are mediated by enzymes composed of polypep-

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