Tidal chain reaction and the origin of replicating biopolymers

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Abstract: Template-directed polymer assembly is a likely feature of prebiotic chemistry, but the product product blocks further synthesis, preventing amplification and Darwinian selection. Nucleic acids are unusual because charge repulsion between opposing phosphates permits salt-dependent association and dissociation. It was postulated (Lathe, R. (2004). Fast tidal cycling and the origin of life. Icarus 168, 18–22) that tides at ocean shores provide the driving force for amplification: evaporative concentration promoted association/assembly on drying, while charge repulsion on tidal dilution drove dissociation. This permits exponential amplification by a process termed here the tidal chain reaction (TCR). The process is not strictly contingent upon tidal ebb and flow: circadian dews and rainfalls can produce identical cycling. Ionic strength-dependent association and dissociation of nucleic acids and possible prebiotic precursors are reviewed. Polymer scavenging, chain assembly by the recruitment of pre-formed fragments, is proposed as the primary mechanism of reiterative chain assembly. Parameters determining prebiotic polymer structure and amplification by TCR are discussed, with the suggestion that Darwinian selection may have operated on families of related polymers rather than on individual molecules.

Key words: DNA, evolution, Moon, nucleic acid, PCR, polymer, tidal chain reaction (TCR).

Introduction

‘If it were given to me to look beyond the abyss of geologically recorded time … I should expect to be a witness of the evolution of living protoplasm from not living matter … I should expect to see it appear under forms of great simplicity, endowed … with the power of determining the formation of new protoplasm from such matters as ammonium carbonates, oxalates and tartrates, alkaline and earthy phosphates, and water, without the aid of light’. In these words Thomas Huxley saw the emergence of life from simple prebiotic materials (Huxley 1870).

Haldane (1929) and Oparin (1953) proposed that the early ocean was a prebiotic soup, a rich diversity of organic and inorganic compounds for assembly into more complex precursors of true lifeforms. Miller and Urey addressed this by subjecting a mixture of ammonia, methane, hydrogen and water to heat and electric discharge, and saw the production of hydrogen cyanide, aldehydes and amino acids (see Miller (1953), Miller & Urey (1959) and Ferris (1987)). These can be further converted to nucleobases (from HCN and methane) and sugars (from HCHO) (Oro 1961; Sanchez et al. 1967; Sanchez & Orgel 1970; Fuller et al. 1972; Ferris & Hagan 1984; Shapiro 1995). In turn, polymerization can generate polypeptides and, notably, polynucleotides (Fox & Harada 1958; Schramm et al. 1962; Naylor & Gilham 1966; von Kiedrowski et al. 1989; Ferris et al. 1996; Luther et al. 1998).

The Watson–Crick alignment of precursors along a template strand, promoting chemical linkage to generate a complementary strand, is a compelling model for the origin of self-replicating systems, but a question arises regarding the driving force for association and dissociation. Template-directed polymerization is a dead-end. ‘Somewhere in this cycle work must be done, which means that free energy must be expended. If the parts assemble themselves on a template spontaneously, work has to be done to take the replica off; or, if the replica comes off the template of its own accord, work must be done to put the parts on in the first place’ (Blum 1957).

Thus, despite ample evidence suggestive of prebiotic polymerization, the evidence argues against cyclic copying in the absence of a further driving force. A previous proposal that rhythmic tidal flooding provided this driving force (Lathe 2004) is put into context. This paper attempts to provide a broad general overview; for in depth discussions of particular aspects the reader is addressed, where possible, to more detailed treatments.

Tidal chain reaction (TCR)

The association of two nucleic acid strands to form a double-helical structure, as in DNA, is determined not only by temperature, but also by ionic strength. This offers the
possibility that rhythmic changes in salt concentration 
brought about by dilution and drying permitted early bio-
polymers to associate (with template-directed polymerization) 
and dissociate (see below). This formally resembles the 
polymerase chain reaction (PCR), cyclic enzyme-mediated 
polymerization where repetitive changes in temperature 
(rather than ionic strength) drive exponential amplification. 
These two processes are contrasted below.

**PCR**

This technique is now widely used for DNA typing in forensic 
and genetic studies. PCR permits repetitive amplification of a 
DNA sample, in the presence of a thermostable polymerase 
enzyme and monomeric precursors, by oscillating the sample 
two temperatures: at 50°C single DNA strands 
direct complementary strand synthesis (doubling the mole-
cule number), then on a shift to 100°C the strands disassociate 
to permit the next round of synthesis. Multiple cycling 
produces exponential amplification, converting a single starting 
DNA molecule, in vitro, into \(10^{15}\) identical molecules over 40 
cycles (Mullis et al. 1986).

On the early Earth there is no evidence for substantial 
marine temperature cycling, although night-time cooling and 
day-time heating will have had some impact on surface 
(ocean) temperatures (discussed further below). Instead, the 
cycling of salt concentration may have provided a driving 
force (Lathe 2004).

**TCR**

Modern nucleic acids are unusual because, unlike most bio-
polymers, the association of the two complementary strands 
is promoted by increasing ionic strength (Schildkraut & 
Lifson 1965). This property reflects the structure of the double 
helix, where strong charge repulsion between phosphate 
groups is neutralized by increasing salt concentration (Fig. 1).

As a consequence, nucleic acids may have replicated 
through tide-driven drying and dilution. Elevated salt 
concentrations produced on drying promote association of single 
strands to form duplex structures; then at low ionic strength 
(e.g. following tidal inflow or rainfall) the duplex is driven to 
dissociate.

By the same mechanism, salinity increase is likely to favour 
association of monomeric precursors with a preformed template strand: tight alignment favours the formation of 
covalent links between adjacent monomers (Fig. 2). Partially 
anhydric conditions also encourage non-enzymatic poly-
ermerization through dehydration reactions (Schramm et al. 

**Two rules for the origin of life**

For primitive biopolymer replication to emerge from tidal 
cycling, two rules are likely to apply. First, because precursor 
concentrations were certainly limiting, polymerization must 
take place during the drying phase when precursors are most 
abundant; partial anhydric conditions also favour chemical 
bond formation. Second, as noted by Blum (1957), if 
assembly takes place on drying, work must be done to remove 
the copy so as to permit amplification: dissociation must 
therefore take place on dilution. It is suggested here that only 
molecules similar to modern nucleic acids fulfil these two 
criteria.

**Reconsideration of parameters**

Emphasis is placed on the rapidity of tidal cycling and the 
ionic conditions of the early ocean. These and other relevant 
parameters are revisited.

**The importance of rapid cycling: inherent instability 
of polymers**

The speed of cycling is important because of product insta-
bility. Nucleobases are generally stable at high temperatures 
(half-lives in the order of weeks or more at 100°C for adenine 
and cytosine), ribose is reported to have a half-life of only 
73 minutes at the same temperature (Larralde et al. 1995; 
Miller & Lazcano 1995; Levy & Miller 1998), although 
deoxyribose (lacking the 2’ hydroxyl of ribose) is more stable.

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**Fig. 1.** Salt-dependent association and dissociation of nucleic acids.

**Fig. 2.** Salt increase on drying promotes both (a) duplex formation 
and (b) monomer alignment for polymerization; salt removal on 
dilution then drives dissociation.
However, the instability of a polymer increases linearly as a function of length. For example, if under Archaean conditions the chemical half-life (leading to chain scission) of a monomer in primitive nucleic acid is 1 week, the half-life of a 100-mer strand is only $\sim 3$ h. Ultraviolet irradiation and radioactivity compound the problem.

Because absolute biopolymer number depends on the rate of assembly by de novo polymerization (or by scavenging, see below) versus the rate of fragmentation/ degradation by thermochemical and other processes, rapid cycling may be critical to permit biopolymer accumulation.

A second perspective concerns the total tidal volume subjected to cyclic drying and dilution. The absolute number of polymers may be critical if a further rare event or events are required to generate bona-fide lifeforms. Candidate rare events include the origin of coding and micellar inclusion to generate cellular life. If these events are rare, the likelihood of occurrence will depend on the absolute number of opportunities for their occurrence. The emergence of true lifeforms is favoured by, but not contingent upon, large and rapid tidal cycling.

There is a downside to rapid cycling: because evaporation is time-dependent, rapid cycling will also limit the extent of water loss from large volumes. Simulations will be required to address the constraints that cycling speed places on evaporative concentration.

**Early oceans**

Oceans first formed when surface temperatures fell below 100 °C. Although there was a period of intense bombardment (Hartmann 1980), leading to an unstable surface and potentially extinguishing prebiotic evolution several times (Maher & Stevenson 1988), by $\sim 4$ Ga it is thought that stable water cover existed at the terrestrial surface. Ocean temperatures in the range 50–100 °C are plausible, stabilized by high atmospheric CO$_2$ content (Kasting & Ackerman 1986).

The early salinity of the ocean remains a matter of debate. Concentrations have remained fairly constant over the last 1 Ga or more (Knauth 1998), but before that they may have been higher. The conventional view is that volatiles outgassed rapidly and, because sequestration could not take place until continent formation ($\sim 2.5$ Ga), the early ocean must have been highly saline (Knauth, Personal communication). A recent review of this topic concluded that early oceans were hot and super-saline, with a NaCl concentration somewhat in excess of present-day levels (Knauth 2005).

Early salinity is an important issue because excess [NaCl] can block TCR amplification (see below), challenging an oceanic origin for replicating biopolymers. Nevertheless, empirical data for (or against) a highly saline ocean are limited. An alternative scenario is that the high chemical reactivity of outgassed chlorines (and other elements) would have led to rapid deposition of solid phase chlorides prior to ocean formation. These salts would only have commenced dissolution on condensation of the oceans. Under this interpretation the salt concentration could have been lower than at present. To speculate: if it took 1 Ga for ocean salt to rise to near present day values (460 mM NaCl) and only 0.1 Ga for life to evolve, the [NaCl] range in the first 100 Ma might have been between 0 and 46 mM (values consistent with the association/dissociation of long polymers, see below). However, given conflicting estimates and the crucial importance for biopolymer replication, the issue of early salinity needs to be resolved with urgency.

Six ions comprise ~99% of salts in the present-day ocean: chloride (Cl$^-$), sodium (Na$^+$), sulfate (SO$_4^{2-}$), magnesium (Mg$^{2+}$), calcium (Ca$^{2+}$) and potassium (K$^+$), with NaCl by far representing the major component. Carbonates and ammonium ions may have been far more abundant, reflecting CO$_2$ and NH$_3$ in the Hadean/Archaean atmosphere, but phosphorus ions (principally phosphates), key ingredients of modern nucleic acids and their precursors, are relatively insoluble (present-day concentrations up to 1 mM), although pyrophosphates appear to be more soluble.

**Early tides**

The frequency of early tidal ebb and flow could have been far faster than at present, but the evidence is contradictory. Fast rotation of the early Earth has been associated with the impact event that produced the Moon, and since that time the Moon is presumed to have receded from the Earth, with concomitant slowing of the rotation rates of both bodies. Darwin (1879) suggested an early terrestrial rotation period of under 6 h (discussed in Brush (1986); see also Hartmann (1999)).

Relevant empirical data have been derived from the accurate measurement of the fast recession rate (3.82 cm yr$^{-1}$) of the Moon over recent decades (Dickey et al. 1994) and analysis of striations in paleobiological residua (fossil corals, bivalves, stromatolites and tidalites) from the last 1–2 Ga (Panella 1975; Scrutton 1978; Lambeck 1978, 1980; Vanyo & Asramik 1982; Sonett et al. 1996; Williams 1997, 2000; Kvale et al. 1999). Regression curves from these empirical data (dependent both on the length of day and lunar orbital rate) suggest steep and steady recession and orbital decay in the last 1.5 Ga, pointing to cataclysmic proximity between $\sim 1.5$ and $\sim 3$ Ga, an event for which there is no evidence (Walker & Zahnle 1986). It is possible that the Earth–Moon pair has decayed in two phases, first slowly to about $\sim 2$ Ga, followed by more rapid decline to present-day values (Krasinsky 2002). With limited and contradictory data it is unsafe to attempt to retropredict relative rotation at $\sim 3.9$ Ga with any accuracy. Despite this, the relative Earth versus Moon rotation frequency was probably faster than at present, although whether 6 or 16 h (with tides every 3–8 h) is not yet known (see Varga et al. (2005) and Lathe (2005) for further debate).

**Early shores and tidal flooding**

At present, 70% of the terrestrial surface is covered by ocean; in the Archaean the Earth is felt to have been far flatter and ocean coverage may have exceeded 90%. The extent of early tidal flooding is therefore subject to great uncertainty. Factors determining tidal height and extent are the hindrance
to the
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**DNA dissociation at low ionic strength**

A core contention of the tidal cycling theory is that the two strands of DNA associate at high ionic strength, but separate at low ionic strength, largely due to charge repulsion between opposing phosphate groups. This association and dissociation is inherent to the Schildkraut–Lifson (1965) relation and extended in other analyses (Thomas & Dancis 1973; Wetmur & Davidson 1986; Wetmur 1991):

$$T_m = 81.5 + 16.6 \log M + (0.41\%G + C) - 820/l$$

where $T_m$ (melting temperature) is the 50% dissociation temperature, $M$ is the monovalent cation ($Na^+$) concentration, $\%G+C$ is the percentage composition of the duplex in guanosine and cytosine and $l$ is the length of the duplex (see Lathe (1985)).

However, it is accepted in the molecular biology laboratory that even small duplexes (e.g. 20-mer complementary oligonucleotides) can remain associated at moderate temperatures and low salt concentrations. This raises the question of whether DNA truly dissociates, at room temperature, on reduction of $[NaCl]$.

The stability of a nucleic acid duplex is commonly expressed in terms of melting temperature or $T_m$, the temperature (in °C) at which 50% of the sample is dissociated. This has two non-obvious consequences.

First, $T_m$ becomes dependent on the length of the duplex. This is because association/dissociation at temperatures close to the $T_m$ is dynamic: subdomains dissociate while other regions remain associated (‘breathing’). Because the complementary strands are physically retained in proximity, the chains do not diffuse apart, favouring reassociation. A negative reciprocal function (e.g. 820/$l$ (Lathe 1985) or 660/$l$ (Hames & Higgins 1985), where $l$ is the length of the duplex) is appended to the Schildkraut–Lifson relation to take account of this.

Second, a rise in strand concentration strongly increases the apparent $T_m$, as expected for a conventional two-order reaction. High concentrations of oligonucleotide primers and linkers are often employed in the laboratory, raising $T_m$ by as much as 50 °C above the value predicted from the Schildkraut–Lifson relation.

Melting temperatures at low strand concentrations, such as those expected in the prebiotic ocean, are much reduced. A standard DNA 60-mer oligonucleotide duplex dissociates, in 10 mM NaCl, at 55–70 °C (Schildkraut & Lifson 1965) or below, depending on base composition (the standard Schildkraut–Lifson equation omits the concentration dependency). Dissociation of a perfectly matching 169-nucleotide DNA duplex in 10 mM NaCl at 61.5 °C has been empirically demonstrated (Rose et al. 2002). At further reduced salt concentrations it was observed (in a study on DNA stretching) that if NaCl was eliminated from the buffer, typically parts of or even the entire molecule dissociate before overstretches the DNA (Clausen-Schaumann et al. 2000). One must conclude that, at 50 °C and low [NaCl], protomeric acids will have dissociated into separate chains, only reassociating on evaporative concentration.

**Divalent cations**

Salt [NaCl] is not the only variable: trace divalent ions including $Mg^{2+}$ can stabilize nucleic acid duplexes to a far greater extent than the same concentration of $Na^+$ (Williams et al. 1989). In an extended study, Nakano et al. (1999) reported that $Mg^{2+}$, $Ca^{2+}$, $Mn^{2+}$ and $Ba^{2+}$ are far more effective stabilizers than $Li^+$, $Cs^+$, $Na^+$ or $K^+$. The melting temperature of a short DNA duplex was disproportionately increased by 4 °C when 10 mM $Mg^{2+}$ was added to a solution of 100 mM NaCl (Nakano et al. 1999). However, $Mg^{2+}$ is not abundant in seawater (presently ~40 mM) and early concentrations may have been very much lower. The trace concentrations inferred for the Archaean ocean (up to 2 mM, paralleling [NaCl]) are probably insufficient to exert a major effect (although, as with NaCl, significant concentrations are expected on drying).
**How do ions stabilize nucleic acids?**

Monovalent cations bind to and neutralize charges on opposing phosphate groups. However, the interactions of ions (including water) with DNA and RNA are complex. Phosphates, sugars and the sides of the base pairs are exposed to and interact with solvents in both the minor and major grooves (Westhof 1988; Auffinger et al. 2004; Schneider et al. 1998). Interactions are not restricted to cations, and include anionic chloride and sulfate (Auffinger et al. 2004). Ion exposure modulates both charge and base stacking interactions—in the Watson–Crick duplex, a major stabilizing force is the juxtaposition of parallel nucleobases (or base pairs), attributed to orbital overlap. Interacting ions modulate this interaction: stabilization and destabilization of the double helix is more complex than simple charge neutralization.

In partly anhydric conditions, brought about either by drying or by organic solvent replacement, the standard B-form duplex undergoes a series of transitions to more compact forms including the A-form helix (Franklin & Gosling 1953; Piskur & Rupprecht 1995) but which conserve base-pairing specificity. If evaporation routinely proceeded to near-dryness and precursor assembly took place under these conditions, the A-form could be as relevant as the B-form.

**Chaotropism**

As we have seen, increasing [NaCl] increases DNA stability according to a log function over the range 0–1 M (Schildkraut & Lifson 1965). At even higher concentrations (>2 M) the converse effect is seen, with a reduction in stability. This is ascribed to the chaotropic effects of high ion concentrations. Chaotropicity is a function of ion size (and concentration), where small ions (Na, Cl, Li) exert minimal effects while large ions (trichloroacetate, guanidium, perchlorate) dissociate rather than stabilize nucleic acid duplexes (Hamaguchi & Geiduschek 1962; Haung 1968; Ott et al. 1975). Less ionic molecules (urea, formamide, imidazole) can also dissociate nucleic acids (Cox & Kanagalingam 1968; McConaughy et al. 1969; Kourilsky et al. 1970, 1971; Casey & Davidson 1976; Eli et al. 1999), but only at high concentrations (e.g. 1–5 M or 50% w/v).

The abundance of chaotropic molecules in the prebiotic ocean has not been assessed. Urea is formed in significant quantities in prebiotic simulations (Schlesinger & Miller 1983) and, if tidal recession permitted evaporation to near-dryness, it is plausible that association of early nucleic acids was followed by a dissociation phase at extremely high solute concentrations. The possibility that such pre-dissociation might influence strand separation on subsequent tidal dilution deserves investigation.

**Condensation versus chaotropicity**

In solution, DNA will spontaneously collapse and aggregate by a process termed condensation. This usually requires cations with a charge +3 or greater (Bloomfield 1991) and ions used in condensation studies include the naturally-occurring polyamines spermine and spermidine (Gosule & Schellman 1976; Burak et al. 2003). Large (potentially chaotropic) ions of positive/mixed charge also cause collapse by simultaneously neutralizing surface phosphates while, at the same time, permitting interactions between adjacent helices. Collapse is inhibited by potent chaotropic ions such as trichloroacetate (Ott et al. 1975).

Whereas divalent metal ions do not normally provoke condensation, they can do so in water–ethanol mixed solution (Arscott et al. 1990b). High ethanol concentrations are unlikely in the prebiotic ocean, but it is plausible to suggest that removal of water by evaporation may have left primitive biopolymers in a milieu enriched in non-volatiles including oils and salts, conducive to condensation, leading to large particulate aggregates. One feature of such a collapse is the formation of toroidal structures (Eickbush & Moudrianakis 1978; Arscott et al. 1990a). These could presage the emergence of circular chromosomes (see also below) and further study is warranted.

**Did triple helixes play a role?**

One peculiar feature of modern day DNA is the ability to form triple- (rather than merely double-) helical structures. This is because the standard double helix contains a lateral gap, the ‘major groove’, into which a further strand can fit (Fig. 4). The sides of the nucleobases (base-pairs) are exposed in the groove, allowing them to form unconventional (non-Watson–Crick) pairs with a third strand.

Triple helices are stabilized by many factors that stabilize the duplex, including polyamines (Musso & Van Dyke 1995; Musso et al. 1997; Saminathan et al. 1999) and divalent cations (Blume et al. 1999; Floris et al. 1999). However, monovalent cations inhibit triple-helix formation (Cheng & Van Dyke 1993; Singleton & Dervan 1993; Plum et al. 1995; Floris et al. 1999), arguing against the possibility that triple-helical structures were a major feature of tide-driven prebiotic replication. This conclusion would need revision if it can be shown that polyamines or equivalent molecules were abundant in the early ocean, a possibility that is not unlikely.
Getting started: minerals

TCR is proposed as a scheme whereby primitive polymers directed their own replication through the ordered assembly of complementary monomers into a complementary strand. The first polymers may have assembled by chance, but assembly can be catalysed by mineral surfaces (Bernal 1949, 1967). Clays and silicates promote alignment and polymerization of precursors; the structure of the earliest nucleic acids may have borne an imprint of the facilitating mineral surface (Cairns-Smith 1982, 2001). Surface association facilitates polymerization in several models (Ferris et al. 1983, 1996; Luther et al. 1998; Ertem & Ferris 1996, 2000; Orgel 1998; Liu & Orgel 1998). TCR-cycling may extend to mineral catalysis, as nucleic acids will adsorb to clays/silicates at high salt concentration, facilitating polymer synthesis, but not at low salt concentration, permitting dissociation on dilution (Vogelstein & Gillespie 1979; Mizutani & Narihara 1982).

Copy number and length increase

Tidal cycling predicts an exponential increase in the number of prebiotic polymers structurally similar to nucleic acids. It also predicts an increase in chain length. Just as two monomers are inferred to align on a template strand during the drying phase, prompting chemical bond formation between them (Fig. 5(a)), two part-formed polymers are likely to juxtapose by base-pairing with a template strand (Figs 5(b) and (c)), also favouring covalent linkage.

Ligation-mediated PCR

This polymer ligation process is formally similar to the PCR but, instead of using an enzyme to direct polymerization, linkage between two abutting oligonucleotides is driven through the addition of the ligase enzyme (Wu & Wallace 1989; Barringer et al. 1990). Combined ligation and polymerase-driven reactions have been performed (Bi & Stambrook 1997). The efficiency of this reaction supports scavenging (below) as a realistic process.

Ligation TCR: polymer scavenging

Ligation of short pre-formed polymers, a process termed ‘polymer scavenging’ here, is an alternative mechanism of complementary strand generation in a prebiotic context (Fig. 5(b)). Non-enzymatic ligation and amplification of oligonucleotides with suitably activated end-groups is efficient (Xu & Kool 1999; Gao & Orgel 2000). Given that the rate and affinity of association of short oligonucleotides with a complementary strand is far higher than for monomers with the same template, the process of complementary strand formation by scavenging (rather than de novo polymerization) is likely to have provided the primary mechanism for reiterative chain assembly. As in Figs 5(b) and (c), scavenging leads to an increase in both copy number and mean chain length. This process also opens the door to Darwinian selection in the sense where ‘successful’ polymers can exploit fragments produced by degradation of ‘unsuccessful’ molecules.

Circularity

A consequence of polymer scavenging is the generation of circular forms. When overall polymer abundance is low, the effective concentration of one end of a molecule versus the other exceeds the concentration (expressed in ends ml⁻¹) of other molecules and circularization becomes more likely than
extension (Jacobson & Stockmayer 1950; Dugaiczyk et al. 1975; Legerski & Robberson 1985). This effect may be enhanced by agents that promote the formation of condensed toroidal structures (see above). The formation of circular molecules is disadvantageous to prebiotic replication because the two strands are intertwined and dissociation on dilution is prevented. However, subsequent thermochemical breakage releases strand fragments for scavenging.

**Caveats: primitive polymers**

Were precursors sufficiently abundant to permit replication of nucleic acids? Although some studies indicated that precursors including phosphate-activated nucleosides (Gulick 1955; Schramm et al. 1962) were present, concentrations may have been low (Bernal 1960; Hull 1960). The relative instabilities of cytosine (Levy & Miller 1998) and ribose (with critically unstable 2’-3’ adjacent hydroxyls) are cases in point. Prebiotic conditions were not favourable for the accumulation of these key ingredients (Shapiro 1988, 1995, 1999).

There are two routes out of this impasse. First, prebiotic conditions may not have been quite so hostile for the production of precursors.

Radioactivity is a significant source of organic molecule formation. Irradiation of methane, CO and CO$_2$ can efficiently generate alcohols and long-chain polymers, while radioactive minerals are particularly susceptible to hydrodynamic sorting and concentration on the margins of oceans (this has been reviewed by Parnell (2004)). These processes would favour the accumulation of complex organics in tidal regions.

Cyclic drying also provides a rather more favourable chemistry for precursor assembly. Anhydric conditions promote assembly by chemical dehydration reactions (Schramm et al. 1962; Calvin 1969; Weber et al. 1977); although challenged (Shapiro 2002), cytosine may be efficiently produced at 100 °C under dry-down conditions (Nelson et al. 2001).

Second, simpler and more abundant components may have preceded both DNA and RNA (Joyce et al. 1987; Shapiro 1995).

**Base structure**

Adenine is commonly generated in prebiotic simulations (Oro 1961; Sanchez et al. 1967); both adenine and guanine are stable at 100 °C (Levy & Miller 1998) while pyrimidines tend to be formed in low amounts and are less stable. Such considerations suggest that an all-purine nucleic acid may have predated the purine–pyrimidine pairing of both DNA and RNA (Wachtershauser 1988); a purine-only nucleic acid does not appear to be inconsistent with the emergence of coding.

**Sugars**

Low yields of ribose and deoxyribose in prebiotic experiments, coupled with the inherent instability of ribose, suggests the possibility of alternative sugars or related molecules as components of protonucleic acids, including glycerol, erythritol and acrolein (Joyce et al. 1987). Acrolein synthesis occurs under simulated prebiotic conditions and chemical reactivity with nucleobases has been demonstrated (Cleaves 2002). Polymers based on the sugar threose have been shown to pair with both RNA and DNA (Schoning et al. 2000).

**Phosphates and activating groups**

Internucleoside phosphate groups provide the strong charge repulsion needed for TCR, but also reflect precursor activation (triposphate in the case of present-day DNA and RNA). The incubation of modern nucleoside (DNA precursor) triphosphates at 74 °C with thermostable enzyme, in the absence of any template, has been shown to give rise to long double-stranded polymers (Ogata & Miura 1997); one must note that enzymes conventionally only catalyse reactions that would otherwise take place (although more slowly) in their absence. Therefore, one may presume that nucleoside triphosphates spontaneously convert to polymeric forms.

Nevertheless, there is a debate regarding the likely availability of phosphate- or polyphosphate-activated nucleosides. Solubility problems have been noted (Gulick 1955) and polyphosphate concentrations may fall short of those required for precursor activation (Keefe & Miller 1995). Nevertheless, volcanic activity is a rich source of P$_2$O$_5$, converting to polyphosphate on solution in water (Yamagata et al. 1991). P$_2$O$_5$ heated in anhydric organic solvent generates a polyphosphate ester with wide reactivity for organic molecules including nucleosides (Schramm et al. 1962). Phosphates in the presence of urea afford a further route to nucleoside activation (Lohrmann & Orgel 1971).

While the extent of phosphate activation is not yet known, *in vitro* studies have employed nucleosides 5′-activated with phosphorimidazolide (Imp); these can polymerize efficiently *in vitro*, a reaction stimulated by divalent cations and mineral surfaces (Lohrmann et al. 1980; Ding et al. 1996; Ertem & Ferris 1996; Ertem 2004). However, the existence of prebiotic Imp-activated nucleosides remains questionable.

If significant polyphosphate concentrations are questionable, one might speculate that other chemistries could have been employed. Unlike phosphate, sulfate is a major ion in the present-day ocean. Early biopolymers could have exploited a diversity of mixed phosphorus/sulphur/oxygen activating groups (and linkages). Phosphorothioates and phosphorodithioates retain the hybridization properties of standard DNA (Jaroszewski et al. 1996), while phosphorothioate linkages retain the charge repulsion property of the phosphodiester linkages in DNA and RNA. Other possible derivatives include sulfonyl (replacing phosphorus by sulphur) and boronophosphate linkages. Mixed sulphur–phosphorus chemistry in PAPS (3′- phosphoadenosine 5′-phosphosulphate) is exploited in present-day organisms. Nucleoside 5′-thiosulfates and 5′-thiophosphates have been described (Golos et al. 2001), but without any evidence that they can participate in polymerization reactions. This area warrants further thought.

Nevertheless, phosphate activation remains the strongest contender. Schramm et al. (1962) reported remarkable synthesis of a phosphate-activated nucleoside by drying sugars.
and adenine with polyphosphate ester. Further incubation of nucleotides with polyphosphate ester and pyridine at 50–60 °C led to polymeric forms (Fig. 6) that were sensitive to nucleases.

**Unconventional backbone structures**

Polyamide or peptide nucleic acids (PNAs) fully eliminate the sugar phosphate moieties, replacing them by pseudo-peptide (polyamide/N-(2-aminoethyl)-glycine) groupings. PNAs have great affinity for natural nucleic acids, contributing to stable double-stranded molecules (Nielsen et al. 1991; Egholm et al. 1993), and PNA has been proposed as an alternative structure for the first replicating biopolymers (Nelson et al. 2000). Although the complementary-strand coding properties of such molecules are intact (Bohler et al. 1995), the lack of a large negative charge replacing the phosphate group prevents dissociation at low ionic strength. PNA-like molecules would seem unable to replicate by tidal cycling.

**Chirality and catalysis**

A major obstacle for all of the theories addressing the origin of replicating biopolymers concerns chirality. Chemical generation of precursors systematically results in a mixture of enantiomeric forms (D versus L), but only one is employed in present-day polymers (L amino acids in polypeptides, D sugars in nucleic acids; see Sandars (2005) for a review).

This problem is so far unresolved. However, it is well known that handedness is copied in crystalline forms: if mineral surfaces directed early polymerization it is likely that polymers were either D or L and rarely a mixture (see Cairns-Smith (1982)). For peptides, it has been argued that mixed L- and D-containing polymers will fail to form stable tertiary structures, favouring the accumulation of homochiral sequences (Brack 1987). By the same token, mixed (D and L) oligonucleotides may fail to adopt a stable double-helical structure. If this is so, strand-directed polymerization and scavenging could generate co-existing but separate homochiral D- and L-sugar forms.

Second, like RNA (Altman 1990), early nucleic acids (as single strands) may have folded to produce enzymatically-active molecules. This is enhanced under elevated temperature and cation concentrations (e.g. Peracchi (1999) and Takagi & Taira (2002)) typical of TCR. A catalytic molecule capable of discriminating between D and L sugars at synthesis, assembly or isomerization could provide a plausible route to ‘only-D’ polymers.

**Sequence motifs persist independently of exact molecular structure**

The information content of a prebiotic polymer is largely only dependent on the base-pairing identity/specificity and position of the nucleobase. Therefore, a sequence motif may be conserved through successive replications even if the exact backbones and base structures are not.

We should consider the possibility that evolution operated on families or populations rather than on individuals. DNA can show catalytic (enzymatic) activity (Breaker 1997), but the more flexible RNA chain appears better adapted. If a parent molecule (e.g. DNA) is stable but catalytically inert, while daughters (e.g. RNA) are unstable but effective catalysts, selective pressures might operate on a mixed family of related polymers rather than on individual molecules. Coexistence might contribute to resolution of the DNA versus RNA debate (Dworkin et al. 2003).

**Cyclicities: further reflections**

Cyclic alteration to the local physicochemical environment affords a solution to Blum’s (1957) conundrum. Tides, proposed here as a major player, are one of several cyclicities, each with potential to drive association and dissociation.

**Macro- versus micro-scale**

Tidal flooding addresses pools on ocean margins, with large volumes of incoming water subsequently evaporating. This implicit assumption may be too restrictive, as parallel processes can easily occur on a micro-scale. The flooding of beaches leads to hydration of particulate sands; these then dry progressively on tidal ebb. Here the volumes of water surrounding each particle are much smaller, but the same processes will operate, although perhaps faster at the micro-scale. The total cycling volume is comparable to the tidal pool scenario.

**Wave action**

Ocean waves, the result of wind action on surface waters, could provide a mechanism for cyclic flooding. However, the cycling speed (seconds to minutes) is likely to be far too fast to allow significant physicochemical changes, for instance drying or heating/cooling. Volcanic and impact-driven (Tsunami) flooding merits consideration.

**Spring and neap tides**

Tidal ebb and flow is a major source of cycling, but tides are not uniform and follow higher-order cyclicities. The alignment of the Sun and Moon generates spring tides (unrelated to season) that are higher than the mean high tide; a week later (when the Sun and Moon are at 90°) their tidal influences partially cancel out, producing neap (lowest) tides (nep, Saxon, lacking or scanty; nipflod, Danish). Although of lower
periodicity, spring tides provide for more extensive flooding and a protracted drying phase.

**Heat cycling: night/day**

Terrestrial rotation provides a mechanism for cyclic heating and cooling: this was suggested as a possible driving force for the emergence of replicative molecules (Blum 1957). However, circadian changes in temperature of the present-day ocean are restricted to shallow areas; the same is likely to be true of the Hadean/Archaean ocean. Moreover, such cycling is not conducive to the inferred replication of prebiotic polymers, as heating (that dissociates duplex structures) counters association driven by an increase in salinity. A stagnant pool (without significant evaporation) cycling between 50 °C (night time) and 100 °C (day time) could afford PCR-type association and dissociation, but the absence of precursor concentration and regeneration on drying affords an unattractive scenario.

**Rains and dews: TCR**

Present-day clouds and periodic intense precipitation are contingent upon particulate matter suspended in the atmosphere. It is possible that the early atmosphere was, compared with the present day, relatively free of such particles, although volcanic activity would clearly contribute. If sporadic rainfall is questionable (and it may not be), a more likely scenario could involve condensation of water (dew) during the night-time phase with day-time re-evaporation. This process, if extensive, produces TCR cycling – condensation results in dilution (promoting the dissociation of prebiotic duplexes, and subsequent evaporation produces an increase in salt and precursor concentration (fostering the assembly of prebiotic duplexes).

**Multivalent cycling**

When the tidal cycle is out of phase with circadian cycling, the combination produces a faster cyclicity than either alone and deserves study. Tidal and circadian rhythmicities could combine to provide multivalent cycling of both temperature and salinity (simultaneous PCR and TCR). The cycle hot/drying, warm/dilute, warm/drying, hot/dilute would permit amplification of polymers with a structure similar to nucleic acids.

**Submarine vents**

Deep-sea hydrothermal vents (Corliss et al. 1979) have been suggested as a possible site for the origin of life. In support, extreme thermophiles are among the earliest cellular organisms (Woese et al. 1990; Woese 2000) and such hot vents provide a honey-pot of chemical energy and complexity (Ferris 1992; Shock 1996; Nisbet & Sleep 2001; Reysenbach & Shock 2002). Vent-driven convective cycles of heating and cooling could simulate PCR conditions and oligomerization of amino acids has been demonstrated in a simulation (Imai et al. 1999). Nevertheless, the hydrothermal hypothesis has been questioned because the extremely high temperatures decompose organic molecules (Miller & Bada 1988); less extreme temperatures at adjacent sites may have been more conducive (MacLeod et al. 1994). A greater problem concerns the dilution factor. Heat dissociation of prebiotic polymers in the locality of the vent is followed by dilution (as hot liquid rises and cools), opposing reassociation and assembly. It is not felt that open hydrothermal vents and macro-scale convection afford a promising candidate for chain amplification reactions driven by either salinity or heat cycling, but other mechanisms may operate.

**PCR by laminar convection**

PCR is performed in the laboratory by imposing rapid temperature shifts; this is argued here to be an unlikely scenario at the origin of life. However, two groups have demonstrated that thermal (PCR) cycling can take place in a steady temperature gradient (Krishnan et al. 2002; Braun et al. 2003). In a typical experiment, a fluid-filled 5 × 1 mm disc was heated in the centre using an infrared lamp, inducing stable laminar (toroidal) convection. DNA dissociates in the hot centre, from where it is transported to the periphery for copying mediated by thermostable polymerase included in the reaction mixture. Further convection transports the DNA back to the centre. The amplification achieved was comparable to conventional heat-cycling PCR (Braun et al. 2003). Krishnan et al. (2002) used a vertical sandwich of similar size heated from below and cooled at the top to achieve a comparable result.

Such closed-chamber PCR devices profit from a phenomenon known as thermophoresis (or thermodiffusion) where molecules are repelled from a heat source and move down a hot–cold gradient. A 50 °C temperature gradient led to a 1000-fold enrichment of a 5 kb DNA in the cold zone (Braun & Libchaber 2002).

Braun & Libchaber (2004) suggested that such mechanisms operated at the origin of life, notably in association with hydrothermal vents. They postulated that porous rocks could have provided the small (<1 cm) chambers required, with temperature gradients generated by the contrast between the hydrothermal vent outflow (>300 °C) and ocean bottom seawater (perhaps as low as 4 °C).

Disadvantages with these scenarios at the origin of life include the need for a closed chamber and a steep temperature gradient, and the absence of any mechanism for concentrating and generating precursors. Thermophoresis could have contributed to this mechanism, but smaller molecules (27 nucleotides) were less than two-fold concentrated under the conditions described by Braun & Libchaber (2002). Nevertheless, thermal cycling remains a viable contender.

**Salinity: tides versus dews**

The salinity of the early ocean is not known, but some estimates have suggested that it could have been higher than present-day values (in excess of 0.5 M NaCl) (Knauth 2005). This affords a major barrier for chain amplification by dilution and concentration – even short duplexes (e.g. 100-mer) only dissociate at elevated temperatures at this NaCl concentration (>80 °C; see Fig. 3) and dissociation by tidal
inflow becomes less plausible. It remains possible that early [NaCl] was far lower, but if high early salinity should be confirmed this would argue against tidal inflow as the drive for dissociation. However, TCR amplification (defined as cyclic dilution and evaporation) could exploit condensation (dews and rains), relocating the origin of replicating biopolymers away from coastal areas to inland lakes and pools.

**Freshwater tides**

Even at high ocean salinity, tidal salt cycling takes place in estuaries. Outflowing freshwater is backed up by tidal inflow, only to be released (with drying sands and pools) on tidal ebb. Flood-ebb cycles can affect river levels for hundreds of kilometres inland (with far inland tidal ranges up to 4 metres being recorded in St. Lawrence). Within the upper reaches of the Bay of Fundy salinities vary from nearly marine to fresh within a single tidal cycle (Kvale, Personal communication). Estuarine tides afford an alternative location for prebiotic TCR cycling.

**Discussion**

The amplification of a primitive biopolymer requires repetitive assembly and dissociation, a process blocked under steady-state conditions (Blum 1957). Tidal cycling allows this process to take place. The importance of coastal areas and evaporative drying has been recognized previously (Blum 1957, 1962; Bernal 1961, 1967). Cyclic drying and dilution (TCR) affords a mechanism for the repetitive assembly and dissociation of primitive biopolymers resembling nucleic acids (Lathe 2004). High early ocean salinity (if confirmed) would inhibit this process, but would point instead to inland lakes and pools (via circadian condensation and precipitation) and estuaries.

Simple considerations suggest two rules. Association and polymerization must take place during the drying/concentration phase, when precursors are most concentrated; the duplex must then dissociate on dilution. Only polymers structurally similar to nucleic acids appear able to exploit this mechanism, highlighting their prevalence as informational macromolecules. The speed and volume of cycling could be critical determinants. Polymers will only accumulate if the rate of assembly (depending on cycling speed) exceeds the rate of fragmentation. The absolute number (rather than concentration) could be important. If the subsequent emergence of true lifeforms (defined as capable of self-replication and metabolism independent of physical cycling) requires one or more evolutionary jumps (e.g. auto-catalytic polymers, coding, metabolism and cellular inclusion) the likelihood of such rare events is in proportion to absolute number of prebiotic polymers. The emergence of life does not strictly require fast and large cycling, but such cycling might increase the likelihood.

Tidal cycling at the early terrestrial surface is favoured by a large and close Moon: the tidal periodicity in the Archaean is not known, but may have been fast. The presence of a large close satellite is not an absolute requirement. In the absence of any moon the terrestrial tidal range (driven by Solar tides) is a significant fraction (~2/5) of the lunar tide. Identical processes can operate with a tidal height range of 5 cm versus 5 m (although wave action at low tidal range would compromise the process).

The fast cycling requirement is further reduced if a mechanism exists to reassemble intact molecules from broken chains. Polymer scavenging (the recruitment of preformed fragments into a longer chain) affords such a mechanism. This possibility could favour fragment ligation as a primary target for nucleic acid (ribozyme-type) catalysis: both RNA and DNA ‘enzymes’ with ligase activity have been described (e.g. Cuenoud & Szostak (1995)).

To conclude, the picture painted here is of a steamy hot flat oceanscape, broken only by volcanic islands. Coastal erosion soon forms beaches, while a fast orbiting Moon, closer than today, drives regular tidal flooding of the shoreline. Rains and dews periodically inundate inland depressions. Evaporation then concentrates precursors in pools and sands, where partial dehydration favours aggregation and chemical linkage. Complex macromolecules are formed. Some are capable of template-directed assembly on concentration, but remain firmly locked together. Only molecules similar to nucleic acids dissociate on subsequent dilution, to allow another round to take place. Over time, with cyclic drying and dilution, these become more abundant.

Assembly was probably disordered at the beginning. The composition of new strands is dictated only by base-pairing efficacy and reactive substrate availability. Diverse structures accumulate, with many different sugars, nucleobases and non-classical linkages. Information content, however, can be conserved even if backbones and bases vary. Some sequences in these drying pools have unusually favourable properties – they are easily copied, relatively stable and perhaps they or their daughters have helpful enzymatic activity – these then accumulate at the expense of others (Darwinian selection), scavenging fragments of less-successful molecules.

If all of these processes operated, amplification of primitive nucleic acids was possibly not so much a limiting factor; indeed, the early seas could have teemed with these molecules. The next steps are perhaps more critical, including the evolution of coding, metabolism and a cellular environment. Tidal cycling could contribute to these evolutionary leaps. Long-chain hydrocarbons may have arisen in the Archaean; with wind, wave and tidal action, one imagines the formation of foams, froths and other micelles that accumulate on ocean margins. Further consideration of the turbulence of the prebiotic soup (itself influenced by tidal ebb and flow) may provide new pointers to prebiotic evolution.

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