MINIREVIEW

The Asymmetry of Existence: Do We Owe Our Existence to Cold Dark Matter and the Weak Force?

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A common theme throughout biology is homochirality, including its origin and especially implications. Homochirality has also intrigued scientists because of the hypothesis that life, as it currently exists, could not have occurred without it. In this review, we discuss several hypotheses regarding homochirality and their linkage to processes that range from subatomic in scale to processes that help define the structure of the universe. More importantly, this exploration begins with the knowledge that humans inhabit the universe in which there is an excess of normal matter over antimatter. It is a universe characterized by homochirality but is nonetheless contained in what is most easily described as a 3+1 dimensional spacetime wherein most laws of physics are invariant under spacetime transformations. This restriction on spacetime poses significant constraints on the processes that can be invoked to explain homochirality. However, in dealing with such restraints, including the total mass contained in the universe, the concepts of cold dark matter and dark energy can be incorporated into cosmological models with resultant behaviors and predictions very much in accord with the findings of the cosmic background surveys. Indeed, the introduction of cold dark matter and dark energy to solve problems relating to the mass found in the universe may provide a means for generating the needed asymmetry to allow homochirality to arise. Exp Biol Med 229:21–32, 2004

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We live in a 3+1 dimensional spacetime with symmetry principles that, in the case of special relativity, require that the laws of physics be invariant under spacetime transformations. Symmetry and asymmetry have been powerful organizing concepts in a host of disciplines, including art and mathematics. More than a century ago, van't Hoff pointed out a connection between molecular chirality and the fundamental symmetries in physics (1). In the case of biology, Pasteur was the first to recognize "dissymétrie" at the molecular level and concluded from data on (+)- and (–)-tartaric acid that they represented nonsuperimposable mirror images of each other (Fig. 1). Kelvin later introduced the term *chirality* for this type of asymmetry, which comes from the Greek word for hand.

There is a fundamental asymmetry in the distribution of the constituents of the universe. That is, there appears to be an excess of normal matter over antimatter in the most current and compelling models of the universe (cold dark matter [CDM]). The origin of this asymmetry remains unexplained as do the nature of both dark matter and dark energy. Dark matter and dark energy are required by the latest CDM models that have recently been shown to be very much in accord with the findings of the cosmic background surveys (2). However, most intriguingly, this fundamental cosmic asymmetry appears to manifest itself by way of other asymmetries observed in other more complex systems. For example, there has been a much discussed thesis that the left-right asymmetry encountered in simple as well as complex multicellular organisms, including human laterality and cerebral asymmetry, are a consequence of asymmetry at the molecular level (3). This, in turn, is thought to arise from asymmetry at the level of elementary

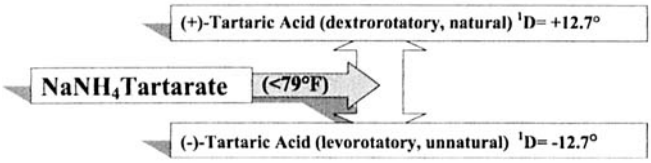

Figure 1. Pasteur's recognition of "dissymetrie" and the recognition that + and – tartaric acid represent nonsuperimposable mirror images.

particles. However, although connecting links between molecular—and subatomic—chirality and macroscopic handedness and asymmetry are not established, the implications of this asymmetry for biologic processes and evolution are profound.

We now know that proteins in life forms consist (almost) exclusively of L-α-amino acids, whereas nucleic acids contain only the D-isomers of ribose or deoxyribose. Although there is considerable controversy concerning the questions of when and how this homochirality arose, there appears to be the fundamental, but incompletely tested, assumption that life as we know it could not have arisen without it. Much less attention seems to have been paid in recent years to the reasons for homochirality and its connection to the origin of life. Older studies have held that the structure-destabilizing effects of "chiral defects" (i.e., the incorporation of D-amino acids or L-nucleotides into their respective polymers would render them incapable or unable to participate in "biology"). However, although newer studies confirm some destabilization, they also indicate that there is more ability to accommodate unnatural enantiomers than was previously appreciated. These findings provide new insights into the constraints imposed on life's origin with respect to chiral purity. We should note, however, that this is a subject that has attracted considerable interest and has been reviewed in the past (4–7). Indeed, one can even use one's nose and establish that stereoisomers can smell different (Fig. 2). Or on a more tragic note, the story of thalidomide where the R isomer is a teratogen while the S isomer is a tranquilizer (Fig. 3).

In this review, we provide a discussion of the biochemical basis of chirality and the implications for life and demonstrate the connections between them and features of the universe at both ends of the scale (i.e., the subatomic and the cosmological level).

The Stereochemistry of Polypeptide Polymerization

The primary amino acid sequence determines the structure and function of a protein. The two most common structural motifs are the α-helix and β-sheet. Although α-helices are now more abundant in proteins than β-sheets, it is thought that the β-sheet occurred earlier during chemical evolution (8). Generally, L-amino acids form a right-handed helix; a right-handed helix exhibits optical rotation of its own. Similarly, β-sheets are not flat but, if made of L-amino acids, exhibit a right-handed twist when viewed along their

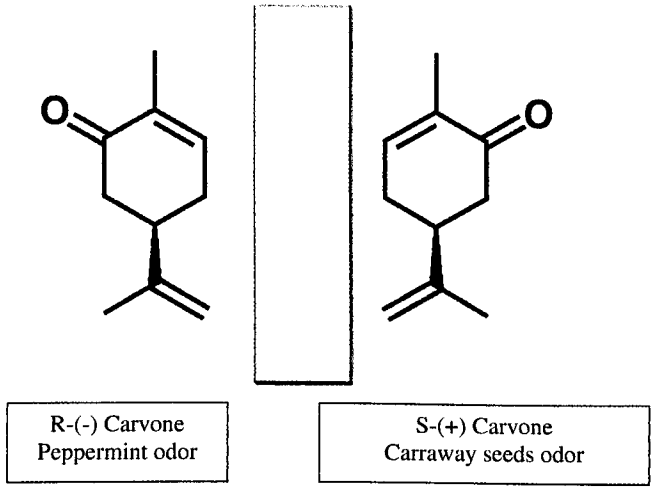

Figure 2. R-(–)Carvone has a peppermint odor, whereas S-(+) has a caraway seed odor.

strands. This right-handedness of turn arises from energetic constraints in the bonding of L-amino acids; a chain consisting of D-amino acids would produce sheets with a left-handed twist (9).

Indeed, the circular dichroism (CD) spectrum produced by the all-D enantiomer of the full-length β-amyloid peptide (42 amino acid residues) was a mirror image of the spectrum obtained with the natural all-L enantiomer, indicating that the two enantiomers had opposite optical rotation (10). Furthermore, there are indications that such mirror image conformation translates into functional stereospecificity. When the D- and the L-enantiomer of the complete enzyme HIV-1 protease were chemically synthesized, they were found to have identical covalent structure and CD spectra of equal, but opposite, optical rotation (11). These data suggest that the folded forms of the D- and L-protease enzymes are mirror images when viewed in three dimension (see Fig. 4). Most notably, the enantiomers exhibited reciprocal chiral specificity, the L-enzyme cleaving only the L-substrate and the D-enzyme showing activity only for the D-substrate.

Steric considerations suggest that the presence of D-amino acids in an α-helix or β-sheet formed of mostly L-isomers would have a distorting and destabilizing effect (12). The question of whether and to what extent such distortion or destabilization affects the nonenzymatic polymerization of amino acids has repeatedly been addressed by using N-carboxyanhydrides (NCA) and a variety of initiators and solvents.

When the polymerization of γ-benzyl-Glu-NCA is initiated with strong bases, the L- and the D-isomer polymerize with similar rate constants and yield polymers with comparable degrees of polymerization (13). Addition of as little as 5 mole % of L-NCA to the D-NCA (or vice versa) results in a considerable reduction in the rate constant and degree of polymerization, compared with the pure isomer. A further substantial rate decrease was observed when equimolar amounts of the D- and L-isomer were used.

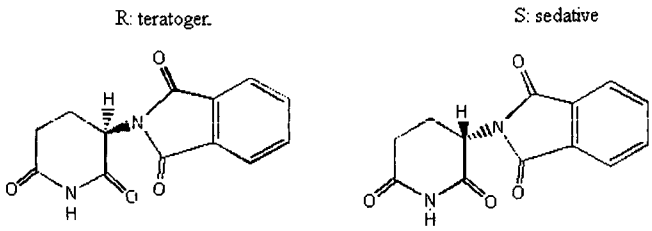

Figure 3. The structures of the R- and S-form of thalidomide present entirely different biologic potentials. The R-isomer is a teratogen, whereas the S-isomer is a tranquilizer.

Similar stereoselectivity in the polymerization of γ-benzyl-Glu NCA has been reported by others (14–16) and described for a variety of other amino acid NCAs, including Ala, Leu, Val, Ile, and Phe (16–19). It is noteworthy, however, that with many of these NCAs, stereoselective polymerization was observed with some initiators but not with others (16). Whether the rate of polymerization was greater for the pure stereoisomers than for their mixture also significantly depended on the type of the polymerization reaction mechanism (19).

The secondary structure of polypeptides initiated by strong bases is generally that of an uninterrupted α-helix. In addition, in the polymerization of γ-benzyl-L-Glu NCA in dioxane and a variety of other solvents, an abrupt transition from an initial slow rate constant to a much faster rate of oligomerization was observed once the chain reached a length of 7–12 monomers (14). At about the same chain length, a transition from a random structure to an α-helix had previously been demonstrated (20). It was, therefore, suggested that this structural transition and the resulting decrease in the entropy of activation accounted for the increase in polymerization rate. Conversely, disruption of the α-helical structure represents a possible mechanism for the reduced rate and degree of polymerization observed when D-amino acids are present in the reaction mixture. Although the interpretation of the rate change has been questioned (15), decreasing helix stability with increasing amounts of D-isomer in copolymers of γ-benzyl-D- and L-Glu was indeed demonstrated by measuring the specific optical rotation in the presence of decreasing ratios of solvent favoring helical conformation and solvent favoring random coil configurations (20). It should be noted, however, that stereoselective polymerization has also been observed for NCAs of amino acids that do not form α-helices (15). Conversely, no stereoselectivity was observed in the polymerization of Phe NCA initiated by dimethylamides, even though poly(L-Phe) adopts an α-helix conformation (17, 18). When poly(N-methyl-L-Ala) diethylamide was used as the initiator, however, the polymerization of L-Phe NCA proceeded considerably faster than that of the DL-NCA. Taken together, these results indicate that mechanisms other than a dependence on the secondary structure of the growing polymer must be responsible for the observed stereoselective polymerization of at least some amino acids. They further demonstrate that the type of amino acid NCA, the nature of the initiator, and the nature of the reaction mechanism for the addition of amino acid residues can all play a role in determining the stereoselectivity of a polymerization process.

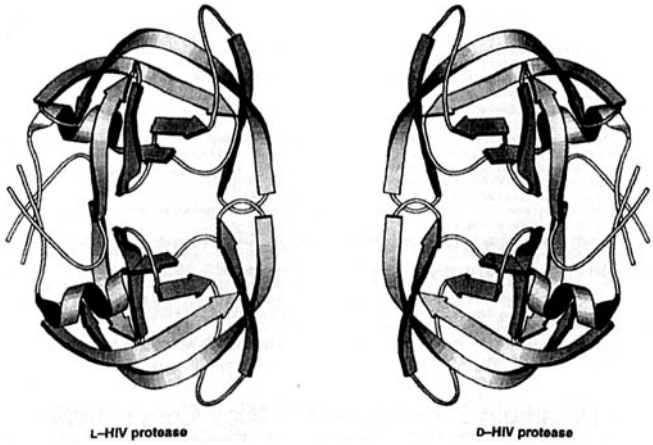

Figure 4. The folded forms ofthe D- and L-protease enzymes are mirror images when viewed in three dimension. Importantly, the enantiomers exhibit reciprocal chiral specificity, the L-enzyme cleaving only the L-substrate and the D-enzyme showing activity only for the D-substrate.

The preceding data show that α-helices can accommodate residues of both configurations. It had been postulated, however, that an enantiomeric excess in the reaction mixture would result in the favoring of one or the other helical sense (8). In other words, with an excess of L-residues, the amount of right-handed helicity (Hr), expressed as the ratio of Hr/(Hr + Hl), was expected to be higher than the ratio of L-enantiomer residues to total residues (L/(L + D).

Experimental data, however, indicated that this was not entirely correct for a small excess of one enantiomer, although correct for a large excess (21). In a reaction system containing 55% L-monomer of γ-benzyl-Glu NCA, helicity was below 50% and consisted of both right-handed and left-handed helical content. Although right-handed helical structures were formed faster early during polymerization, formation of left-handed helical content increased and eventually almost equaled right-handed helical content. In contrast, when the L-fraction was increased to 85%, the helical content was essentially 100%, and the helix was of the same handedness throughout. The same group of researchers reported similar findings with alanine NCA (22). In that study, they additionally demonstrated that the monomer that was in excess in the original reaction mixture was preferentially incorporated in the early stages of polymerization, with addition of monomers of the opposite chirality following later.

The above data agree with observations from the polymerization of leucine NCA (23, 24). When polymerization was allowed to proceed only to ~50% completion, the resulting polymers were enriched in the enantiomer that was present in excess in the original reaction mixture. A corresponding decrease was observed in the monomer

fraction. Quite unexpectedly, the opposite was observed with valine NCA (i.e., the polymer contained a lower fraction of enantiomer excess than had been present in the starting mixture), whereas a simultaneous increase of the dominant enantiomer was detected in the monomer fraction (23). Others had previously reported that polymerization of valine, as well as isoleucine, NCA exhibited no stereoselectivity (16, 25), except when a tertiary amine was used as initiator (16). In one of these studies, various ratios of D- to L-valine NCA monomers were investigated, and their ratio in the polymer was always found to correspond directly to the initial ratio. The discrepancy between these results and those reported by Blair and Bonner (23) might be attributable to the use of different initiators and solvents. The fact that valine NCA exhibits either no stereoselectivity in most systems or selectivity against the dominant enantiomer might be at least partly due to the fact that it forms a β-sheet rather than an α-helix structure (25).

Recently isotope labeling of one of the enantiomers of tryptophan NCA followed by selected ion monitoring mass spectroscopy was used to investigate the stereoisomer distribution in oligomers consisting of up to 10 residues (26). Homochiral oligo-Trp were 8.3 and 40 times more frequent in 7-mers and 10-mers, respectively, than would have been expected for a statistical distribution. Conversely, 7-mers and 10-mers with approximately equal proportions of D- and L-isomers were underrepresented. These results suggest that, once a short homochiral sequence is formed, the addition of another monomer of the same chirality is favored over the incorporation of a residue of the opposite chirality.

Molecular models of poly(Leu-Lys) combined with calculations indicated that mixtures of enantiomers would give rise to "nuclei" of β-sheets surrounded by random coil segments (8, 12). They further suggested that at least seven residues of the same chirality would be necessary for the formation of β-sheets. Both predictions were confirmed experimentally (27). Another prediction was that, if the initial ratio of L- and D-monomers deviated from the racemic mixture, the fraction of the dominant monomer in the β-structures would increase rapidly so that the arising β-sheets should almost attain optical purity once the ratio of L/(L + D) reached 0.75 (12). Under conditions of mild acid hydrolysis, the unordered random coil segments were found to hydrolyze more rapidly than the β-structures, thereby allowing the isolation of a fraction enriched in β-structures and the subsequent determination of whether enantiomer enrichment occurred (27). The results suggested that this was not the case because a starting L:D ratio of 80:20 yielded β-segments containing 84% L-residues (27). Others observed that the ratio of D- to L-valine NCA in the polymers, which typically adopt a pleated β-sheet structure, corresponded to the starting ratio at all stages of polymerization (28). The incorporation of D-residues had little or no effect on the stability of the β-sheet, suggesting that no random-coil structures formed in this model.

D-Amino Acid Substitutions in Peptides

In recent years, the effect of D-amino acid substitutions on structure and function of antimicrobial peptides has been studied extensively. These peptides are usually <40 residues long, and many are largely unstructured when free in solution but adopt an amphipathic α-helical conformation upon partitioning into membranes. The aim of these studies has been to understand the interaction of these antimicrobial peptides with cell membranes to determine the relationship between structure and function and to elucidate the action mechanism(s) but also to find means of reducing the toxicity that some of them exhibit against host cells. In addition, some studies have used D-amino acid substitutions to investigate the ability of amyloid β-peptide to form β-structures and subsequently self-assemble into the types of aggregates that play a role in a variety of neurodegenerative diseases.

Substituting a specific amino acid residue with its D-enantiomer almost invariably results in some destabilization of the α-helix conformation, but the magnitude of the effect is largely position dependent (29–33). Replacements at or near the center of the helix have repeatedly been found to have the greatest helix-destabilizing effect, whereas substitutions closer to the terminals of the peptide commonly result in only slight reduction in the helicity of the peptide (29, 31, 34, 35). Generally, substitutions in two adjacent amino acids disrupt the α-helical conformation to a considerably greater extent than a replacement of a single residue (34).

Recently an amphipathic α-helical model peptide was used to systematically investigate the effect of all 19 chiral amino acid stereoisomer pairs on helix stability in comparison with glycine (36). Individual amino acids differed considerably in their ability to stabilize or destabilize the α-helical conformation. Interestingly, there was little correlation between the helical propensities of the two enantiomers in each pair, and the D-amino acid residues were almost invariably more destabilizing than their L-amino acid counterparts, with D-amino acids with branched side chains the most destabilizing.

The effect of D-amino acid substitution has also been investigated in a triple-helical peptide model of collagen through the use of a host-guest peptide approach (37). This approach is based on the substitution of a single amino acid or a specific small peptide, the guest, in a common peptide framework, the host. In this case, guest triplets consisted of Gly-Xaa-Yaa, where Xaa is D- or L-Asp and Yaa is Ala or Hyp (hydroxyproline) flanked by three and four repeats of Gly-Pro-Hyp, respectively. Asp was chosen because it is the amino acid most susceptible to spontaneous racemization in a variety of proteins. Circular dichroism spectra indicated that both of the peptides containing D-Asp formed little, if any, triple helical structure in phosphate-buffered saline. They were, however, able to form such a structure in a helix-stabilizing buffer, though to a lesser extent than the control containing L-Asp in the same position, as shown by thermal transition data. Although in the case of peptides with D-Asp

flanked by Gly and Ala formation of heterotrimers was observed, no heterotrimers formed from chains containing D-Asp flanked by Gly and Hyp. This suggests that the helix-disturbing effect of a single D-amino acid residue depends not only on the amino acid itself but also on the nature of the neighboring residue, particularly its flexibility and hence its ability to accommodate the distortion introduced by the D-residue.

The structure of collagen is rather unique in the world of proteins (38). With very few exceptions, the helices found in proteins are right handed, but each of the three amino acid chains of collagen is twisted into a left-handed helix with three amino acids per turn as compared with the 3.6 residues per turn in the common α-helical structure of proteins. The three collagen chains, in turn, wind around each other, thereby forming a right-handed superhelix. Pro is rarely found in α-helices because the N-atom in Pro is part of a rigid ring, which makes rotation about the N-C_α bond impossible and thereby disrupts the helix structure. Collagen, on the other hand, consists of repeating tripeptide units, each of which contains either Pro or Hyp (Gly-X-Pro or Gly-X-Hyp, where X can be any amino acid). Thus, in the case of collagen, the triple helical structure does not depend so much on the chirality of its constituent amino acids but rather on the highly repetitive and ordered amino acid sequence and the interaction of specific side chains (38).

Interestingly, a similar situation exists in Z-DNA, a type of DNA structure that is characterized by a striking regularity in its sequence and the adoption of a left-handed helical structure (38, 39). It appears to be the inordinate regularity of the primary sequence that allows the formation of a structure with handedness opposite to the most common one (i.e., the right-handed helix). In other words, a prerequisite for left-handed DNA (as in Z-DNA) and left-handed protein helices (as in the case of individual collagen strands) appears to be substantially greater order in the primary sequence than is commonly found.

Double D-amino acid substitutions in an amyloid β-peptide consisting of 42 residues reportedly resulted not only in the disruption of α-helical structure but a transition to β-sheet structure (40). There are, however, only few data concerning the effects of D-amino acid substitutions on the stability of β-sheets themselves. Some of these studies used a model peptide consisting of a β-sheet–forming domain with increasing numbers of VT repeats between two unstructured octapeptides that promote solubility (41, 42). Using CD and Fourier-transform infrared (IR) spectroscopy, it was shown that substitution of two adjacent residues with their D-enantiomers in the central part of the peptide containing five VT-repeats completely abolished β-sheet formation (41). In an extension of these analyses, double D-amino acid replacement was also found to destroy β-structure in the peptide containing six VT-repeats (42). The effect diminished with increasing length of the VT repeat region, but a difference between the analogues containing D-amino acids persisted in peptides with seven or eight VT repeats, indicating that the β-structure was still destabilized. Similar disturbances were observed in a group of 12-mer peptides containing four D-amino acid residues in various positions (33).

In another study, single residues were systematically replaced by their D-enantiomers in a peptide (GS14) based on gramicidin S, which exists in an amphipathic β-sheet conformation even in aqueous environments (43). Nuclear magnetic resonance (NMR) and CD studies showed that, compared with the all-L-peptide, each of the diastereomers disrupted the β-structure in aqueous and hydrophobic environments. Interestingly, the extent and location of the disruption differed considerably among the diastereomers and, in addition, depended markedly on the environment.

In summary, D- and L-amino acids can copolymerize and form α-helical as well as β-sheet structures. Heterochiral polymers generally seem to form at slower rates and appear to be somewhat less stable than their homochiral counterparts, possibly because of disruption of secondary structure. Substitution experiments confirm the destabilizing effect of D-amino acids both in α-helical– and β-sheet–forming peptides but indicate that the magnitude of the effect strongly depends on a variety of factors, including the nature, number, location, and environment of the substitutions. That destabilization is present in mixed polymers may prove to be the key for generating homochirality. One might argue as Chaisson (44) has argued that life emerged as a subset of orderly dissipative structures, all of which arise via their ability to increase the rate of entropy production. Orderly dissipative structures arise repeatedly in nature (e.g., hurricanes, thermal columns in heated water) driven by gradients. They exist because they offset their high degree of order (i.e., negative entropy) by virtue of their ability to increase overall entropy. Chaisson further argues any changes that occur in evolution are constrained by the second law of thermodynamics. That is, they must lead to the creation of what are called evermore orderly dissipative structures (i.e., those that increase entropy in the most rapid fashion). Using this approach would imply that the existence of biological systems using a mixture of D- and L-isomers would not be favored. This bias would result from the destabilizing effect of these mixed isomers (i.e., their disorderliness) which would hinder the achievement of the an orderly dissipative structures system and fail to increase entropy in the most rapid fashion.

Nucleic Acids

Although protein macromolecules are carriers of function, DNA macromolecules are the transgenerational informational carriers of most contemporary organisms (45). RNA plays the role of an intermediary between DNA and proteins in eukaryotes and can take on both informational as well as functional roles. As in proteins, the monomeric units of DNA and RNA are homochiral, each of the nucleotides containing either D-ribose or D-deoxyribose. Also like

proteins, nucleic acids are able of taking on a variety of secondary structures, most famous among them the double helix.

Both RNA and DNA are matrices for the assembly of a complementary replica, and homochirality has been postulated to be an absolute necessity for complementarity (46). Molecular modeling was interpreted as indicating that incorporation of a single T of the opposite chirality in double-stranded poly(A)/poly(T) would prevent base coupling, thereby destroying the template property and the ability to act as an information carrier (46). This does not, however, appear to be entirely correct. An NMR study with a dodecadeoxynucleotide containing a single nucleotide with a L-deoxyribose (the G4 residue) formed a stable base pair with the natural C-9 residue within a right-handed B-form conformation (47). Similarly, although substitution of a D-nucleotide with an L-nucleotide somewhat destabilized a short DNA duplex, the D-isomer could nonetheless be accommodated via changes in some of the backbone torsion angles around the phosphates and the glycosidic bond (48). Others have confirmed that cooperative binding between mixed L/D-oligodeoxynucleotides and single-stranded DNA and RNA is possible despite the destabilizing effect of L-substitutions (49). The magnitude of this destabilization was found to depend on the position and type of the nucleotide (48, 49). In addition, there appears to be a limit to the number of substitutions that a sequence can tolerate (49).

Whereas oligonucleotide sequences themselves can accommodate at least some L-enantiomers, some problems arise in their stepwise replication, as was demonstrated in seminal experiments conducted by Joyce *et al.* (50) using a homochiral poly(C_D) template to direct the oligomerization of activated guanosine mononucleotides (5′-phospho-2-methylimidazole, 2-MeImpG). A solution of chirally pure D-2-MeImpG resulted in efficient synthesis of oligomers consisting of up to 20 links. When the 2-MeImpG consisted of racemic mixture, however, the L-enantiomer inhibited oligomerization of the D-monomers. This "enantiomeric cross-inhibition" was attributable to the preferential incorporation of L-monomers at the 2′(3′) end, at which they then acted as chain terminators. This was thought to arise from the adoption of a syn conformation in the L-isomer and its great similarity to the anticonformation of the D-isomer (50, 51).

Computer simulations, however, indicated that the conformation of the L-ribose is anti (52), and this has been confirmed in NMR studies of actual heterochiral oligonucleotides (47, 48). The computer simulations did, however, confirm that L-riboses terminate growth of poly-rG_D assisted by a poly-rC_D template (52).

Joyce *et al.* (51) themselves recognized that a system using poly(C) as a template for the synthesis of oligo(G) was probably not historically relevant and emphasized that the reaction system was intended as a general chemical model of RNA-dependent RNA polymerization. One of the reasons for this irrelevance is that cytosine is unlikely to have been used in early information carrier molecules. It is not produced in spark discharge experiments simulating the atmospheric conditions that are thought to have existed on early Earth (53). It has also not been detected in meteorites, even though other purines and pyrimidines have. Although a variety of mechanisms for the prebiotic synthesis of cytosine have been proposed (54), it has been argued that their yield would be insufficient to compensate for the rapid decomposition of cytosine (53, 55). Similar objections have been raised against a possible role of adenine in early replication systems (56). In addition, the very complexity of nucleotides makes their use in pre-RNA/RNA unlikely. This, along with enantiomeric cross-inhibition and several other problems associated with RNA, strongly suggests that RNA was not the original informational carrier and was preceded by some other genetic system (57). There are, however, many arguments for the existence of an RNA world before the emergence of DNA (57, 58).

A variety of nucleic acid analogues have been proposed as an alternative or precursor to RNA in the prebiotic world (59–61 and reviewed in Ref. 58). Among them, peptide nuclei acids (PNAs) do not solve the problem of the prebiotic scarcity of cytosine and adenine but address the issue of whether template chirality is involved in enantiomeric cross-inhibition. Monomers of PNAs are nonchiral and PNA oligomers can adopt left- and right-handed helical conformations. Accordingly, a PNA C10 template was essentially equally effective in directing oligomerization, up to octamer length, of D- or L-2-MeImpG (62). In contrast, reaction mixtures containing equal concentrations of D- and L-2-MeImpG resulted only in dimers and trimers with traces of tetramers. These results strongly suggest that enantiomeric cross-inhibition is independent of whether the template is chiral or achiral and that other molecules and/or mechanisms must be invoked for enzyme-free template-directed replication in the pre-RNA world.

One such mechanism could be the ligation of short oligonucleotides, as was recently suggested by investigations of the properties of pyranosyl-RNA (i.e., RNA consisting of nucleotides that contain ribopyranose rather than ribofuranose [63]). The short oligomers used in these experiments were tetramers with hemi–self-complementary base sequences in which the phosphate group at the 2′ end was activated by conversion to 2′,3′-cyclophosphate. Oligomerization in this system is template directed in that two of the bases in each tetramer are complementary to two bases of another tetramer. When individual purine or pyrimidine nucleotides were replaced with their L-enantiomers, the resulting diastereomeric tetramers did not exhibit cross-inhibition of the oligomerization of their homochiral isomers. Nonetheless, the rate of oligomerization was significantly reduced when only heterochiral tetramers were available for co-oligomerization, indicating that oligomerization in this system was highly chiroselective. The magnitude of the suppression depended on the type of base and the location of the substitution, with

substitutions at the 2′-end resulting in complete suppression of oligomerization.

In summary, unlike what earlier studies suggested, oligonucleotides appear to be able to accommodate an occasional nucleotide containing L-deoxyribose and maintain complementarity. Nonetheless, enantiomeric cross-inhibition may still prevent replication in the presence of L-nucleotides if oligomerization proceeds via stepwise addition of monomers. Ligation of short oligomers constitutes an attractive mechanism to circumvent such cross-inhibition.

Goldanskii and Avetisov (45, 46) postulated that chiral purity was a prerequisite for the emergence of life (i.e., in the chemical [abiotic] stages of evolution). At the same time, and well aware of the conundrum this represented, they argued that chirally pure medium can arise only from the action of highly enantioselective catalytic processes. Enantiospecific functions, however, are encountered only at the biochemical level of complexity.

One of the cornerstones of their arguments seems to arise from the results of their own analysis of molecular models (46) and the findings of Joyce *et al.* (50), suggesting that a single "chiral defect" resulted in the complete destruction of the matrix (template) properties of informational carriers. As discussed above, neither one of these problems appears insurmountable. The introduction of a L-nucleotide into actual oligonucleotide sequences neither requires the breaking of chemical bonds nor results in loss of complementarity, and the ligation of short oligonucleotides presents a way to overcome the enantiomeric cross-inhibition. This seems to suggest that "chiral purity" is not the kind of absolute prerequisite to which it had been perceived. Instead, one can envision a "predominantly homochiral" scenario, as developed by Bolli *et al.* (63). Extrapolation of their findings suggests that ligative oligomerization of pyranosyl-RNA tetramers containing both L- and D-nucleotides would result in the generation of sequence libraries consisting of "predominantly homochiral" sequences. If co-oligomerization proceeded stochastically, a level of complexity would eventually be reached in which the number of possible sequences far exceeded the number of sequences actually formed. At that point, the two libraries would differ in their diastereomer composition and consequently in their chemical properties (45, 63, 64). With the addition of evolutionary pressure, a winner species of either the D- or the L-configuration would emerge.

Asymmetry at the Atomic and Subatomic Level Parity Violation

Of the four fundamental forces observed in nature (i.e., gravitation, electromagnetism, and the strong and weak nuclear forces) only the weak force is not invariant under a parity transformation. *Parity transformation* or operation refers to the replacement of a system of fundamental particles with a type of mirror image in which the spatial coordinates describing the system are inverted through the point at the origin; that is, the coordinates x, y, and z are replaced with $-x$, $-y$, and $-z$. This nonconservation of parity in the weak interaction (the unification of the weak force and electromagnetism) implies that elementary particles are susceptible to mirror reflection and, hence, exhibit some right- or left-handedness. Indeed, the parallel and antiparallel spin and momentum vectors of such particles correspond to right-handed and left-handed helicity (65, 66). The parity-violating character of the weak interaction can be observed not only in subatomic particles but also in whole atoms. This is due to the fact that the atom can experience the weak interaction because of the weak neutral current, which is mediated by the exchange of a neutral vector boson Z° between a nucleon and a valence electron. One of the consequences is that L and D molecules are really diastereoisomers, not enantiomers, the true enantiomer of an L-amino acid being the D-amino acid made of antimatter. Hence, the two natural enantiomers of a chiral molecule differ slightly in many ways, including energetically (66).

Ab initio quantum mechanical calculations that include the electroweak interaction have been performed to determine the parity-violating energy difference, ΔE_{pv}, of the binding energy in the electronic ground state for some major biomolecules. Earlier results indicated that the naturally occurring L-amino acids and D-glyceraldehyde, the presumed precursor of all naturally occurring D sugars, have lower energies than the corresponding natural enantiomer, the difference being $\sim 10^{-14}$ J/mol (67). This corresponds to about 10^6 molecules of the thermodynamically more stable enantiomer per mole (6×10^{23}), or about one part in 10^{17}, at thermodynamic equilibrium at 300 K. For the amplification of this small excess, two main mechanisms have been proposed, a sudden catastrophic one (68) and a gradual evolution (69). Their potential relevance was enhanced by several recent *ab initio* calculations showing that the magnitude of ΔE_{pv} is actually at least one order of magnitude greater than previous results had indicated (70–72).

It is noteworthy that two recent articles containing the highest level *ab initio* calculations conducted on alanine to date reported that the sign of ΔE_{pv} changed repeatedly from negative to positive as a function of the orientation of the conformational angle of the carboxylate group in the gas phase and in solution (73, 74). Both groups concluded that the currently available data neither supported nor refuted a significant role of weak neutral currents in the evolution of biomolecular homochirality.

All other scenarios that have been evoked to explain homochirality may explain how one enantiomer comes to be produced in slight excess over the other, but chance determines which of the enantiomers is favored. Thus, these mechanisms do not provide an explanation for why the L-enantiomer of amino acids and the D-enantiomer of sugars should have formed preferentially. That would, however, be accounted for by the parity violation scenario if the

calculations showing that the parity-violating energy differences favor L-amino acid and D-glyceraldehyde could be confirmed experimentally.

There is another significant difference between the parity violation and all other scenarios. The weak interaction is a universal force. Thus, if it were responsible for the enantiomeric excess that was at the origin of the homochirality observed in terrestrial biomolecules, then life anywhere in the universe would contain molecules of the same handedness as observed on Earth. With all other sources of homochirality, the sign of the handedness is accidental (i.e., the same or the opposite chirality as on Earth would be equally possible on life-supporting planets in other solar systems).

Circularly Polarized Light and Asymmetric Photolysis (Circular Dichroism)

A variety of other mechanisms for the production of an enantiomeric excess and the eventual evolution of homochirality has been proposed (75). The most widely accepted one among them appears to be circularly polarized light (CPL). In this type of light, the electric field vector is rotating around the axis of light propagation. The electric field vector can rotate in either the right or left direction (as viewed in the direction of light propagation), and the light is called right circularly polarized or left circularly polarized, respectively. That means that CPL is itself a chiral phenomenon.

Asymmetric photolysis has become to be considered as the most plausible CPL process for the induction of an enantiomeric excess (75). Chiral molecules differ in their molar absorption coefficients for right-handed and left-handed CPL (circular dichroism) and, hence, in their susceptibility to photolysis. The CD bands in amino acids are generally at wavelengths of 200–250 nm (i.e., in the UV region). Thus, UV radiation is capable of preferentially destroying one enantiomer, leaving an excess of the other, if the reaction is stopped before it has run to completion (75, 76). Over 100 years ago, LeBel and van't Hoff envisioned CPL as a source of enantiomeric excess, and in 1929 Kuhn and Braun were able to prove this hypothesis experimentally (reviewed along with other such experiments in Refs. 77–79). Rubenstein *et al.* (80) were the first to propose an extraterrestrial origin of enantiomeric excess because of CPL, namely supernovae synchroton radiation, with several refinements of their model published since then (75, 78, 81, 82).

Bailey (79), however, presented detailed arguments for why synchroton radiation was not the most likely source of CPL and why reflection nebulae in star formation regions were more plausible candidates. This conclusion was in part based on the detection by Bailey and colleagues (83) of circular polarization in IR wavelengths in a star-forming region (a nebula in Orion OMC-1). In OMC-1, CP is produced by Mie scattering of unpolarized light (i.e., scattering of light by nonspherical grains that are aligned by a magnetic field). Such interstellar dust grains consist of ice and various organic components (84). Calculations of CP using the refractive index of such "dirty ice" indicated that the level of CP at UV wavelengths was comparable with that at IR wavelengths (83). Unfortunately, direct observation of polarized UV radiation is impossible because of the obscuring effect of the very dust particles that would be responsible for this polarization.

The issue has been raised that CPL cannot explain the homochirality of all protein amino acids because their CD spectra differ, making it unlikely that the same spectrum of CPL would induce an enrichment of L-enantiomers in all protein amino acids (85). One obvious reply is that we do not know whether all amino acids used for protein synthesis now were part of the original repertoire of life on Earth. More importantly, however, it is the origin of an enantiomeric excess *per se* that is at issue, not the origin of such an excess in all required biomolecules (76). It needs to be emphasized that any molecule could have served as a chiral initiator, and that molecule does not have to be presently associated with life.

The enantiomeric excess produced by CPL is generally in the order of a few percent. Thus, as in the case of ΔE_{pv}, an amplification mechanism is required. Cycles of partial polymerization and hydrolysis (27, 86, 87), evaporation and crystallization, and kinetic resolution have been proposed (reviewed in Ref. 75). A more promising candidate appears to be asymmetric autocatalysis, which reportedly amplified starting enantiomeric excesses of as little as 2% to a final yield in the range of 88% to >99% (88–90).

Rosen (91) has recently proposed yet another mechanism for the generation of chiral molecules. He suggests that they arise as a result of cold dark matter's effects on normal matter such as that in the galactic halo regions to produce an asymmetrical distribution of electron states. These states have been shown by Hegstrom (92) to produce molecular homochirality in a pronounced manner through electromagnetic interactions. The origin of homochirality has also been studied using UV circular polarization and star formation regions; this has likewise been proposed as the basis of homochirality (93).

The involvement of CDM in generating both chirality and life appears to have been anticipated by Schrödinger (1944) as he indicated that "living matter, while not eluding the laws of physics as established to date, is likely to involve other laws of physics hitherto unknown" to account for its origin.

Extraterrestrial Contributions

An excess of L-amino acids has been detected in Murchison and Murray, two meteorites of the carbonaceous chondrite class (94–96), although some discrepancies in the reported results remain to be resolved. Cronin *et al.* (97) originally discarded the evidence for small excesses of L-enantiomers in Murchison as controversial and possibly

caused by terrestrial contamination. Later, however, they themselves found an enantiomeric excess of various amino acids that have never been reported, or are of limited occurrence, on Earth (95, 96). The detection of a significant ^{15}N enrichment in individual amino acid enantiomers from Murchison, compared with their terrestrial counterparts, further confirms that the source of these amino acids is extraterrestrial and not terrestrial contamination.

Carbonaceous chondrites formed ~4.5 billion years ago (i.e., before the origin of life on Earth). There is still some controversy regarding the actual origin of meteoritic amino acids (i.e., on the meteorite parent body via Strecker synthesis in liquid water [97, 98] or in the interstellar medium followed by incorporation into the parent body [95, 99]). Experiments with interstellar ice analogues have shown that the UV-light–induced synthesis of amino acids is possible under the types of conditions likely to be found in interstellar dust (99, 100). No matter which scenario is the correct one, the finding of an excess of L-amino acids in carbonaceous chondrites strongly suggests that the excess is of extraterrestrial origin and existed in the solar system before the origin of life on Earth.

The experiments further indicate that at least some amino acids do not undergo complete racemization during their residence in space, transit to Earth, atmospheric entry, and surface impact. The α-methyl amino acids found to exhibit considerable excess of the L-enantiomer in the Murchison meteorite are reportedly quite resistant to racemization (95). Racemization half-lives of meteoritic α-amino acids, the ones used for protein synthesis in contemporary terrestrial organisms, were calculated from models, taking into account the various environments that such an amino acid is exposed to in space (101). In the temperature range between 150 and 300K, the racemization half-lives varied between amino acids by approximately 5 orders of magnitude, with glutamic acid and isoleucine predicted to retain an enantiomeric excess much longer than phenylalanine, aspartic acid, and alanine. These calculations suggested that the reported D/L value for glutamic acid in Murchison of 0.3 (94) was close to the original value, whereas that of alanine (D/L = 0.5) could correspond to original values in the range of 0.5 to 0.35 (101). Note, however, that others did not observe any enantiomeric excess in alanine (96). Other experiments suggested that amino acid racemization at high temperatures, as may be encountered during atmospheric entry and surface impacts of space bodies, would be very rapid (102). Incorporation into rocks of a size to prevent their being heating all the way through should, however, overcome this problem.

The presence of a variety of amino acids in meteorites raises the further question of whether not only the source of enantiomeric excess in terrestrial amino acids but also possibly the provenance of prebiotic amino acids themselves was extraterrestrial. Meteorites are actually considered unlikely to have made a significant contribution to the total amount of prebiotic organics (103, 104). In contrast, impacts of carbonaceous asteroids and comets during the period of heavy bombardment 4.5–3.8 billion years ago are thought to have been important sources not just of amino acids but also a variety of prebiotic organic molecules (103, 104). Even greater amounts of organic material are likely to have been accreted from interplanetary dust particles, which are currently contributing $\sim 3.2 \times 10^5$ kg year^{-1} of intact organics.

How large a portion of the total inventory of organics on early Earth came from extraterrestrial sources depends on a variety of factors, foremost among them the actual composition of Earth's early atmosphere and hence the extent of endogenous production. Whereas Miller and Urey assumed a fully reducing early terrestrial atmosphere for their famous experiments, it is now thought that it was nonreducing or slightly reducing (104–106). The efficiency of organic synthesis decreases rapidly as a function of the H_2/CO_2 ratio. It has been calculated that with UV light as the energy source, a yearly production of 2×10^{11} kg organics would have occurred in a reducing atmosphere, whereas only 3×10^8 kg year^{-1} would be produced in a neutral atmosphere ($H_2/CO_2 = 0.1$) (104). Recent experiments suggested that high-energy particles, but not UV light, were able to generate amino acid precursors under mildly reducing conditions (107).

Panspermia

The delivery to Earth of large amounts of extraterrestrial carbonaceous compounds, including many of the building blocks of life, might actually fall under a new expanded definition of panspermia (108). Originally, however, the term *panspermia* referred to the transfer of some form of viable extraterrestrial organism. Theoretically, the transfer of such organisms between planets within our solar system is possible on rocks ejected by large impacts (109). A majority of these ejecta are heated to temperatures that would kill all microbes; however, some remain almost unshocked (110). Further heating during the ascent through the atmosphere of the home planet requires that the ejecta be of a size that prevents heating to 100°C all through, with a diameter of >0.2 m estimated as necessary. Similar heating occurs during the entry into and passage through the atmosphere of the target planet and the landing there. In between, microbes would have to survive thousands of years of travel through space. Space is a very hostile environment in which UV and ionizing radiation, extreme vacuum, and very cold temperatures individually, and even more so in combination, are potentially lethal (111). Theoretical and experimental results indicate, however, that protection from these sterilizing factors may be possible (110, 112, 113).

The ability of some bacteria to form spores makes them attractive candidates for extraterrestrial organisms that might have introduced life to Earth (113). Spores represent a dormant state. This offers the advantage of the absence of

(detectable) metabolism and high resistance to a variety of physical insults, including those imposed by prolonged space travel. Only a small proportion of spores were found to survive space travel of up to 6 years (i.e., a minute fraction of the actual time they may have to spend in space during transfer between planets [112, 113]). A single living organism may be enough to seed life on another planet, however.

Panspermia theories offer the advantage of overcoming the difficulties arising from the shortness of the time interval during which life on Earth must have become established. Life could not have arisen, or would have been destroyed if it did, during the heavy bombardment period that ended about 3.8 Gyr ago. Microfossils and stromatolites indicate that life must have originated more than 3.5 Gyr ago, and evidence of biologically mediated carbon isotope fraction puts the existence of life back even farther, to ~3.8 Gyr ago. This leaves a very narrow window of time for the emergence of terrestrial life and adds some plausibility to scenarios in which a preformed extraterrestrial life form started life on Earth. Ultimately, however, postulating an extraterrestrial origin not just for organic biomolecules but for entire organisms simply shifts the location of the origin of life, without addressing the underlying questions of how life arose and at what point during this process homochirality became established.

Clearly the questions of life's origin and the relationship of its emergence to the phenomenon of homochirality are the subject of active investigation. To conclude this review, we are struck by the "symmetry" of some of the possible mechanisms linking these questions and the expressions of these in aspects of biology. Homochirality, a prerequisite of life's emergence in some scientists' view, might arise as a consequence of the roles played by cosmology (e.g., by cold dark matter and cold dark energy) and occur at the far edge of galaxies. The conjunction of these (the dark) with our increasing understanding of the processes that control nuclear fusion and supernovas in providing both the building blocks and the energy (the light) to drive life's processes leads us to conclude with a quote alluding to the symmetry of light and dark.

> Thus the darkness bear its fruit, and prove itself to be good, no less than the light
>
> —Henry David Thoreau

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