Microorganisms play indispensable ecological roles that benefit all life forms on Earth. Nitrogen fixation converts $\text{N}_2$ and carbon fixation converts $\text{CO}_2$ into substances usable by other organisms. Many of the nutrients needed by complex organisms are the breakdown products of enzymes present only in bacteria. Without recycling of organic matter to valuable raw materials, much of the usable source of carbon, nitrogen, and oxygen would soon be depleted.

After creation, many new ecological challenges and interactions between organisms developed, and a proper design would have to be flexible and adaptable, or require constant direct corrective intervention. Evolutionists often rely on examples of putative suboptimal designs to deny a divine creation and plan. Hence, if over time the biosphere were actually unable to adapt to new circumstances, this would be construed as suboptimal design and proof of lack of divine foresight. Inability to protect the environment, including rendering harmless substances that are poisonous to other organisms, would be consistent with purposeless evolution. However, both atheists and theists would expect God to anticipate these ecological challenges, including those substances that would be manufactured by man. Our worldview also includes the deleterious effects of the Fall and therefore a well-functioning but less-than perfect biological world thousands of years after creation.

The mechanisms of adjustment provide important clues for deciding whether chance or pre-intended adaptability offer a better interpretation. To illustrate, an immune system must react quickly to be of any use, within the lifetime of a challenged organism. Suppose countless lineages went extinct all the time due to viral or bacterial infections and very rarely random mutations in the germ-line permitted some to survive. Such an inefficient, cruel evolutionary mechanism could legitimately be viewed as due to chance.

However, maturation of B-cells, a key immune defence mechanism, is known to be precisely guided, with the necessary components already in place. Only a small 15–22 amino acid portion of an antibody (the paratope) is refined through an iterative mutational algorithm (mutations that can’t harm the germ-line). The optimized paratope has a shape and charge distribution which complements a portion of the invader’s antigen (the epitope), targeting it to be destroyed by the B-cell.

The necessary somatic mutations are mechanistically induced and are not the result of flawed replication of DNA, relying instead on recombination signals in the right kind of cell, at the precise best location of the resulting protein. A single germ-line mutation leading to a novel function could be interpreted in evolutionary terms but design is clearly a better explanation when complex machinery has been set up to guide a process of iterative fine-tuning during an organism’s lifetime. In-depth analysis of the underlying causal factors is often necessary before arguing chance or design, which is why we devote much effort to parts 1–3 of this series.

Various bacteria are known to carry plasmids (small circular DNA elements) with genes that code for enzymes able to break down unnatural substances such as toluene, camphor, salicylate, alkanes, and naphthalene. The degradative effectiveness of these enzymes can be improved with minor fine-tuning through mutations. Everything reported so far on this topic is consistent with foresight in anticipation of future challenges.
‘Nylon-eating’ bacteria

In 1975, a research team from Osaka University in Japan investigated whether bacteria could live off synthetic materials collected from the waste water released during nylon-6 production. Nylon-6 is produced by ring cleavage polymerization of caprolactam (figure 1) and its manufacture releases unnatural materials such as caprolactam, 6-aminohexanoic acid cyclic dimer, and 6-aminohexanoic acid oligomers into the environment.

By selectively subculturing the variants able to grow most rapidly in ε-aminocaproic acid cyclic dimer, a population designated K172 was isolated that could grow on this dimer as its sole source of nitrogen and carbon but could also metabolise several other organic waste materials.

Since nylon production began in the 1930s, it seemed unlikely that highly tuned enzymes that are able to metabolise these unnatural waste substances should already have existed, which led to much discussion as to whether this was evidence for evolution of de novo proteins. In fact, three enzymes analyzed in sp. K172 were able to degrade three synthetic compounds, and seem to be quite different from enzymes present in any of the other Flavobacterium species reported.

Although it is known that other microorganisms can also hydrolyse linear and cyclic oligomers of ε-aminocaproic acid, this is the first report of a bacterial species using synthetic substances as its sole source of carbon and nitrogen nutrient.

We examine next the three new or, more accurately, modified enzymes that are able to degrade waste substances resulting from the manufacture of nylon-6: E-I, E-II, and E-III.

E-I. Hydrolysis of 6-aminohexanoic acid cyclic dimer (Acd)

Flavobacterium sp. K172 (reported as Achromobacter guttatus K172 in the older literature) is a bacterial strain found in damp soils and fresh water. Research led by Negoro and Kinoshita showed that new colonies could grow on a medium containing 6-aminohexanoic acid cyclic dimer (Acd), a side product of nylon-6 manufacture, as the sole source of carbon and nitrogen (first reaction shown in figure 2).

The enzyme E-I displayed considerable substrate specificity and did not measurably process several other aminohexanoic acids and polypeptides (as determined by the laboratory sensitivities available over three decades ago). The low kinetic turnover number (8 s⁻¹) compared to other cyclic amide hydrolases (e.g. 35–260 s⁻¹ for penicillinase) raised the possibility that a variant enzyme had adapted to a new synthetic substrate. Plasmid depletion experiments (i.e. elimination from the host) identified plasmid pOAD2 as supplying the necessary gene.

The ability to degrade Acd was also found in another bacterial species, Pseudomonas sp. NK87, isolated from the waste water of a nylon factory in Unitika, Uji, Japan. This strain was also able to grow on Acd as the sole source of carbon and nitrogen. The wild-type gene already functioned to some extent in free nature and could be optimized under carefully guided strong selection in a laboratory at 30°C for two to three days. Colonies that appeared were further purified on Acd plates.

E-II. Hydrolysis of 6-aminohexanoic acid oligomers (Ald)

Flavobacterium sp. K172 (reclassified later as Arthrobacter sp.) was isolated from the waste water of a nylon factory. An enzyme was isolated that could hydrolyse the second step in the pathway presented in figure 2, using 6-aminohexanoic acid oligomers ranging from dimer to hexamer and icosamer. It did not hydrolyse heptamers, however. The activity decreased with increase of the polymerization number of the oligomer.

The 6-aminohexanoic acid residue was removed successively from the amino terminus. However, the enzyme could not hydrolyse other linear amides, cyclic amides, dipetides, or tripeptides tested, such as 6-aminohexanoic acid cyclic dimer, 6-caparolactam, 5-valerolactam, 2-pyrollidone, 30 kinds of peptides, 8 kinds of tripeptides, tetra-alanine and penta-alanine, casein, 6-N-acetyllysine, N-acetyltrialanine, N-carboxyglycylleucinamide, N-acetyltrialanine methyl ether, glutamine, and asparagine.

High enzyme specificity had also been reported for E-I in the same bacterial strain, and the genes for E-I and E-II were located at distant sites on the same plasmid. E-II hydrolase activity was also observed in another bacterial strain, Corynebacterium aurantiacum, which led the authors to suggest an independent origin for both kinds of genes although they did not propose from which genes. Significantly, they pointed out that adapting to unnatural synthetic compounds, such as nylon oligomers, does not seem to be a very rare phenomenon in nature.

Further insight was gained when Negoro et al. analysed the pOAD2 plasmid by cleaving it with various restriction endonucleases (EcoR1, HindIII, BamHI, Sall, PstI, BglII, MluI, PvuII, XhoI, Smal). Fragments were ligated to a standard research vector (pBR322) and transformed to

![Figure 1. Ring-opening polymerization of caprolactam to form nylon-6.](image)
E. coli recipients, which were able to survive exposure to ampicillin. Transformed E. coli were isolated and shown to possess E-I and E-II enzymatic activity. This confirmed that both enzymes were present on the same plasmid in K172. By systematically analysing shorter deletion fragments the precise locus of the gene that codes for E-II on the plasmid was identified.19

Analysis of hybridisations between a DNA fragment on plasmid pOAD2 that contained the gene that codes for E-II showed no similarities with portions of the other two plasmids in K172, nor chromosomal DNA. However, some hybridisations did occur between fragments that contained the genes that code for E-I and the E-II with other portions of plasmid pOAD2. Without actual DNA sequence data, made possible about two decades later, little could be concluded at that time about the degree of similarity and whether recent gene duplications may have occurred. It was concluded that two fragments containing the gene that codes for E-II were present but only one led to a functional E-II protein.20

The enzyme resulting from gene F-E-II (from Flavobacterium) was reported to degrade 6-aminohexanoate oligomers with chain lengths between two and 20 subunits. The P-E-II enzyme found in Pseudomonas sp. NK87 exhibited similar activities against oligomers of 6-aminohexanoate ranging in chain length between two and five subunits, and no significant activities against 56 different dipeptides tested.21,22 P-E-II and F-E-II were believed to digest the oligomers stepwise starting at the amino-terminus.22

E-I. Hydrolysis of endo-type 6-aminohexanoate (Ahx)

Plasmid pOAD2 in Arthrobacter sp. K172 (formerly called Flavobacterium sp. K172) also contains the gene F-E-III, which codes for a third enzyme (NylC).23

The enzyme NylC could also degrade larger cyclic polymers derived from Ahx24 and larger linear oligomers,25 see figure 3. E-III was also discovered in Pseudomonas sp. NK87.26

In another study,27 degradation of Ahx was developed in the laboratory using a new bacterial strain that is not inherently capable of such activity, Pseudomonas aeruginosa PAO. The wild type could process neither Acd nor Ald. The nutritional source of carbon and nitrogen was limited to Ahx. After 9 days of incubation a mutant strain able to grow on Ahx was transferred to an Ald minimal medium. In the third week some growth was observed in one of the cultures, which was retransferred to an Ald minimal medium. In the third month significant growth rates were obtained. A strain (PAOS002) was isolated and shown to grow rapidly on either Ald or Acd, even after changing the nutrient to glucose. This result demonstrated that the extraordinary lag (ca. 3 months) was not due to very slow growth of the parental line.

The bacteria could remain in a starved condition for a long time and thus accumulate many genetic alterations within the same generation in order to activate a cryptic region. Alternatively, the high frequency of hyper-growing mutants in medium containing Ahx might be a result of a high mutation rate under starvation conditions.11 Environmental stress can cause adaptive mutations by increasing polymerase error rates28 and by recombinations.29 Other researchers have observed up to 10,000-fold increase in Mu element excision due to starvation.30

The authors concluded that two enzymes formerly not found in P. aeruginosa PAO had arisen, able to hydrolyse Acd into Ald and then onto Ahx, although the exact pathway was not determined.31

Widespread existence of enzymes E-I, E-II, and E-III

Other microorganisms, taken from a sewage disposal plant and from waste water from a nylon factory, were also able to metabolise waste products from nylon-6 production after incubation for five to seven days on cyclic-oligomer-enriched...
All strains (two from the sewage disposal and five from the nylon factory) produced enzymes similar to E-II (called nylB in the paper) known from Arthrobacter K172. All but two strains from the nylon factory had similar proteins to the E-I enzyme (called nylA in the paper). Strain KY5R from the nylon factory was identified as Agromyces, and KY2 (from the sewage disposal plant) as Kocuria. Both are alkalophilic bacteria and further experimentation revealed that they possess an additional gene that codes for an enzyme able to hydrolyse larger cyclic oligomers derived from Ahx. These enzymes were labelled NylC_p2 (from Arthrobacter), NylC_A (from Agromyces) and NylC_K (from Kocuria). The polypeptides contain 355 residues in all three cases, the same ATG initiation codon, and the same Shine-Dalgarno sequences (GGAGG). Relative to the NylC_p2 sequence, NylC_A and NylC_K have 5 and 15 amino acid substitutions, respectively, and their genes are hypothesized to reside on the chromosome and not plasmids.

These three enzyme variants are generated after a post-translational proteolytic cleavage, which is a specific feature of the N-terminal nucleophile (N-tn) hydrolase family. This is significant for part 3 of this series.

Once an enzyme’s scaffolding is in place, replacing just a few amino acids at specific locations can often modify the enzyme’s properties. A thermostable variant of NylC_p2 produced in the laboratory by replacing four carefully selected residues was able to hydrolyse mechanically disintegrated nylon-6 powder at 60°C, a discovery of potential industrial applications. The fragments produced tended to remain bound to the nylon polymer through hydrogen bonding but smaller fragments (<10 monomeric units) which separated from the polymer were degraded by a subsequent NylB-based reaction.

**Discussion**

The chemical bonds cleaved by hydrolases are present in many kinds of bio-chemicals, such as proteins (figure 4) and many kinds of enzymes are able to hydrolyse them.

Of particular interest here are amide bonds, a fundamental feature of proteins. Proteins are usually extensively modified biochemically after translation. Furthermore, after being folded in three dimensions, embedded in membranes, or linked to sugars or to other bio-molecules, the protein’s amide bonds are found in a large variety of electronic and steric environments. Nevertheless, the enzymes easily recycle these proteins. With this in view, it is not particularly surprising that existing enzymes could fairly easily be modified to hydrolyse amide bonds of substances such as those shown in figures 2 and 3.

Many potential precursor candidates for E-I, E-II, and E-III, present in many varieties, are already distributed among bacterial strains. In addition, as Batten pointed out, plasmid pOAD2 has the means to enhance rapid mutations. “There are five transposable elements on the pOAD2 plasmid. When activated, transposase enzymes coded therein cause genetic recombination. Externally imposed stress such as high temperature, exposure to poisons, or starvation can activate transposases. The presence of the transposases in such numbers on the plasmids suggests that the plasmid is designed to adapt when the bacterium is under stress.”

Negoro’s group already reported that the five IS6100 elements are identical on the plasmid over 880 base pairs (bp), except that the 420-bp region was duplicated in one case (RS-IB) and two of them have a reversed orientation on the plasmid. Furthermore, the IS6100 sequence was identical to the one found on Mycobacterium fortuitum and seems to be widely distributed among microorganisms. Sequences similar to IS6100 were also found in Pseudomas sp. NK87 on plasmid pNAD2, which contains the NylA gene. In agreement with Batten, Negoro concludes that the IS6100 sequences cannot be very ancient or they would display far more sequence variety.

This is consistent with another key observation. Batten added: “The Japanese researchers demonstrated that nylon degrading ability can be obtained de novo in laboratory cultures of Pseudomonas aeruginosa [strain] POA, which initially had no enzymes capable of degrading nylon oligomers. This was achieved...
in a mere nine days! The rapidity of this adaptation
suggests a special mechanism for such adaptation,
not something as haphazard as random mutations and
selection… . It seems clear that plasmids are designed
features of bacteria that enable adaptation to new food
sources or the degradation of toxins.39

Another example was reported in which enzymatic
activity appeared after only 14 days.42 A variant of
Flavobacterium (K1725) from which the entire E-I gene
sequence had been removed, could not grow on waste which
consisted primarily of 6-aminohexanoate-cyclic oligomers
but did have about 5% of the activity of the unmodified
K172. Plated on a minimal waste medium, cells from strain
K1725 produced colonies spontaneously with activity two –
to six-fold higher.

Again, all this is not very surprising because the system
is very simple. As Behe pointed out,

“Those enzymes are very simple ones which
simply hydrolyse precursors to nylon. That’s a very
simple task, which can be done even by small organic
catalysts.”43

Negoro also reiterated the known fact that environmental
stress can lead to adaptive mutations by generating polymerase
errors and, in addition, recombination can also be involved
in adapting to crisis situations.44 As another example, Lenski
had already shown a 10,000-fold increase in bacterial Mu
element excision induced by starvation, as mentioned above.30

Drake estimated a typical rate of mutation for bacteria
of \(10^{-10}\) to \(10^{-9}\) per nucleotide per generation,45 which is
probably representative of plasmids. Plasmids are generally
dispensable for bacteria, can be present in multiple copies,
are much smaller than bacterial chromosomes, and can
be transferred to other bacteria. Plasmid pOAD2, which
contained genes E-I, E-II, and E-III, was reported to consist
of 44,000 nucleotides.46 Being so much smaller than the
host chromosome, the chances are much better of avoiding
a deleterious mutation somewhere along its sequence.
Proportionally, many mutations should be tolerated, since
plasmids need not be permanent components of the genome
and therefore aren’t as critically important as the main
chromosome. Furthermore, several copies of a plasmid tend
to be present.

For large bacterial populations, it is likely that most or all
non-deleterious single nucleotide alternatives of the genes
coding for E-I, E-II, and E-III precursors were already
present on a plasmid, plus many cases of multiple mutations.
Upon initial contact with side-products of nylon production,
a minimally functional gene was already available or easily
attainable after a few mutations. Strong selection under harsh
mutation-increasing conditions, as created in the laboratory
experiments, could then fine-tune upon a favourable starting
point.

**Adaptation vs true evolution**

Degradation of bio-chemicals or related synthetic materials
by bacteria does not support the notion of macro-evolution.
Creation scientists hold to the view of flexible organisms
designed to cope with their environment. This flexibility
includes continual adjustments, such as temperature
regulation, digestion of different foods, decision making
such as in normal walking, and numerous other forms on
non-deterministic behaviour. Minor adjustments requiring
several generations are also part of the ecological design
plans. Anderson and Purdom point out that “a wide range of
mutations can be shown to provide a beneficial phenotype to
the cell”47 but also that these mutations “frequently eliminate
or reduce pre-existing cellular systems and functions. This
has been referred to as antagonistic pleiotropy”. An example is genomic
truncation,48,49 whereby valuable
energy and material can be saved under
duress by eliminating genetic elements
not needed at that time.

These kinds of fine-tuning, linked
to clear guiding processes, provide no
support for evolutionary claims such as
development of joints or organs from
a single cell predecessor. Naturalistic
mechanism must not be confused with
magic. As Gatlin pointed out astutely
in *Information theory and the living
system*, “The words ‘natural selection’
play a role in the vocabulary of the
evolutionary biologist similar to the
word ‘God’ in ordinary language.”50

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**Figure 5.** Model for front-loaded adaptability. Initially there were separate categories of generalist
life-forms, which specialized to new environments.
Potential for designed adaptation

Nature is full of delightful novelty, as organisms interact and adapt to new conditions. Birds don’t just build a nest; there are many kinds of nests. Spiders don’t just build webs; there are numerous creative variants. Neurons in the brain are not static, nor are gene circuits in the cells. They are dynamic and are refined as the need arises.

The notion of change and adjustment are implicit in man’s God-given authority over nature and duty to manage it wisely.51

Based on these comments, we propose that after the six days of direct creative activity, front-loaded information was made available, able to express itself at a later time in manifold ways in response to relevant triggers. A conceptual model is shown in figure 5. Initially (at creation, then after the Flood) a limited number of members within original kinds were present with considerable potential for variation. The diagram illustrates how new species could arise within the kind, die out or features converge, which would be easy if the necessary variety-enhancing information was present in different species.

Extra robustness is hypothesized to have been designed into proteins52,53 so that a few mutations may be tolerated with no harm. Some variants may be maintained to ensure population robustness under new conditions, or developed by a few random mutations, or by exposure of front-loaded cryptic genetic instructions.

Over time various environmental niches (which include interaction with other adapting organisms) became populated. The genes not needed for a particular circumstance were deactivated, saving energy and material. Eventually, highly specialized organisms will lose the potential for further adaption to new environments and are less fit if made to compete with members in the preceding environment.54

However, this loss of adaptability is slowed down by special features such as the ability of microorganisms to recover genetic material from other species.55–58

Terborg has emphasized the need for rapid change in biology, and proposed that VIGEs (variation-inducing genetic elements) are a designed feature to transfer genetic material from other species.55–58

Behe’s first rule of adaptive evolution

Michael Behe conducted a comprehensive study of reported adaptive mutations in viruses and bacteria64 on the basis of what he called Functional Coded elemenTs (FCTs).

“An FCT is a discrete but not necessarily contiguous region of a gene that, by means of its nucleotide sequence, influences the production, processing, or biological activity of a particular nucleic acid or protein, or its specific binding to another molecule. Examples of FCTs are: promoters; enhancers; insulators; Shine-Dalgarno sequences; tRNA genes; miRNA genes; protein coding sequences; organellar targeting—or localization signals; intron/extron splice sites; codons specifying the binding site of a protein for another molecule (such as its substrate, another protein, or a small allosteric regulator); codons specifying a processing site of a protein (such as a cleavage, myristoylation, or phosphorylation site); polyadenylation signals; and transcription and translation termination signals.”

This is very interesting, since these elements are a combination of parameters and parameter values in cellular logic processing and relevant to Truman’s Coded Information Systems (CIS) Theory discussed in part 4. Behe classified the benefit from a mutation as due to one of three causes: 1) Loss of FCT; 2) Gain of FCT; and 3) Modification of function. The latter is not caused by a loss or gain of a specific FCT. “It includes point mutations as well as other mutations that have a quantitative effect on a pre-existing FCT, increasing or decreasing its strength, for instance, or shifting its activity somewhat (such as allowing a protein to bind a structurally related ligand at the same site as its normal substrate).”64

Behe concluded that virtually all adaptive mutations are due to loss of modification of a pre-existing molecular function. “… snakes have lost legs, cavefish have lost vision, and the parasitic bacterium Mycoplasma genitalium has lost its ability to live independently in the wild, all in an effort to become better adapted to their environments.”65

Outlook

In parts 2 and 3 of this series we will revisit the question of the origin of the three genes for enzymes E-I, E-II, and E-III. In part 3 we show that enzymes E-I to E-III are the result of small changes in the enzymatic region of activity and led to a minor modification of an existing function—simple catalytic hydrolysis of an amide bond. As discussed above, the chemical context around amide bonds is significantly variable among bio-chemical substances. The functional boundaries for E-I to E-III enzymes can be viewed to lie, with easily attained modification, within the range of activity for which they were intended to work.

Given the existence of very large bacterial populations carrying suitable predecessors for these three enzymes, and the built-in adaptability provided to bacteria to serve their environmental recycling duties, degradation of man-made synthetic materials with virtually identical functional groups as the usual target poses no difficulty to a creation scientist. It is statistically very likely that microorganisms will often be available with rearranged genomes able to adapt immediately to a new environmental challenge.
Coded Information Systems (CIS)

In contrast to the code of (micro) organisms, computer code is deterministic (even when elements of randomness are programmed in). In the code of life there is no complete blueprint in DNA, which specifies all possible biological outcomes. Adaptability of organisms can be understood by reflecting on how message-enhanced instructions work in biology. A separate gene was not created to degrade each unique protein. Instead, enzymes exist which are able to identify a single functional group or feature that can appear in thousands of proteins, even though the alternative chemical contexts may be very different (due to their location within folded proteins, attachment of other chemical groups, and so on).

Different enzyme variants are generally needed for a category of problem. Minor changes in existing enzymes could improve performance in special cases.

In part 4 we point out that flexible designs, pre-planned to respond to alternative parameters, offer an alternative to multiple dedicated programs but can be more efficient in usage of matter, energy, and maintenance. No new source of information created enzymes E-I, E-II, and E-III. Producing a variant of a general-purpose enzyme class designed to provide nutrients was facilitated by designing high populations of adaptable bacteria with very slow rates of mutation. In other words, an optimization algorithm had been set up.

Conclusion

Several species of bacteria can degrade waste materials generated during the production of nylon-6. Three kinds of enzymes are used to cleave amide bonds, which are a functional group present in many kinds of bio-chemicals. We pointed out that bacteria must play important roles in ever-changing ecologies, and we view God’s creative design as deliberately incorporating the necessary degree of anticipatory adaptability. It would be an incompetent God indeed who would need to prepare a separate gene for every possible amide variant. Instead, a collection of similar genes perform part of the recycling of substances by focusing of a problem category, hydrolysing amide bonds per se.

New discoveries suggest the ability of bacteria to degrade newly produced synthetic substances should not be so surprising. Resistance to modern man-made antibiotics is a common trait in ancient microorganisms, demonstrating this resistance did not evolve recently. As an example, resistant bacteria have been recovered from Greenland deep ice sheet, Antarctica, and other permafrost environments.

Small fine-tuning of an existing class of enzymatic function is not an example of the kinds of macro-evolutionary innovations required by evolutionists. What would be necessary to provide support for macro-evolution are examples of true novelty, which typically demand multiple new gene families simultaneously, to produce molecular machines and other complex features.

In part 2 we will review Ohno’s popular theory that de novo genes arose via frameshift mutations, and show that there are good statistical reasons to reject this hypothesis. The theory is worth examining in depth, since Ohno popularized the notion of genes having originated from much simpler oligomeric DNA repeat sequences, and his theory is often mistakenly quoted as fact, based on these ‘nylon-eating bacteria’.

In part 3 we review the work by Negoro, who showed that these amide-hydrolysing genes arose easily from existing predecessors, which discredited Ohno’s theory.

In part 4 we use CIS (Coded Information Systems) Theory to interpret the existence of the three modified enzymes.

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