Molecular War and Peace: the Concurrent Path to Antibiosis and Antibiotic Resistance

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Life did not stop, and one had to live (Leo Tolstoy, War and Peace, 1863)

ABSTRACT

The possibility of the emergence of proto-antibiotic and proto-resistance molecules in the prebiotic world, as primary elements involved in "molecular wars," is examined in this conceptual review. Throughout the Earth's early history, prebiotic chemical processes produced molecules that associated both randomly and persistently. Over time, those configurations that achieved greater stability were favored, their longevity effectively serving as a mechanism for prebiotic selection. Some of these molecular aggregates could result in conformations capable of disrupting the assembly or stability of rival structures, thereby acting as proto-antibiotics in a precellular scenario. Concurrently, some other molecular aggregates may deactivate such proto-antibiotics, acting as primitive mechanisms of resistance. However, both production and protection mechanisms tended to coalesce as proto-antibiotic production began to act on the producers' own multimolecular assemblies. During a prolonged time, the chemical Thioester World, RNA World, and the biological Proto-Cellular World coexisted, and proto-organelles started to be influenced and protected by proto-antibiotics and proto-resistances. Antibiotic production and resistance remained associated, even at the stage of antibiotic polyketides, emerging under a more oxygenated landscape, resulting in early biosynthetic pathways giving rise to contemporary ones, mainly in Actinomycetota. This scenario of integrated action and reaction provided an ecological equilibrium where antibiotics were not necessarily killer agents, but just regulatory signals within the microbiosphere, ensuring healthy bacterial interactions. The massive anthropogenic antibiotic production altered such an equilibrium, favoring an unbalanced resistance reaction through the

massive diffusion of antibiotic resistance genes, now decoupled from antibiotic production and spreading across the microbial world, mostly carried in mobile genetic elements.

INTRODUCTION: ANTIBIOTICS AND ANTIBIOTIC RESISTANCE MOLECULES AS SECONDARY METABOLITES

In cellular life, both antibiotics and antibiotic-resistance biomolecules originate from secondary metabolism. According to the classic view, bacterial secondary metabolites generally comprise small compounds, often including unusual chemical structures, which, unlike those arising from primary metabolism, are not strictly essential for the immediate growth or survival of an organism (Demain, 2007). However, secondary metabolites may be critically involved in microorganisms' interactions with their natural environment, including bacterial interactions. The vast repertoire of secondary metabolites varies significantly across bacterial species, likely reflecting the evolutionary pressures and diversity of their environments. Notably, the basic chemical structures of most clinically relevant natural antibiotics, including beta-lactams, glyco- and lipopeptides, macrolides, tetracyclines, or polymyxins, are non-ribosomally produced, but are synthesized by chromosomally encoded enzymatic complexes, the non-ribosomal peptide synthetases (NRPS) (Miller and Gulick, 2016). These synthetases are located in secondary metabolite biosynthetic gene clusters (BGCs), as first described in *Streptomyces*. Within a single module, multiple catalytic domains are responsible for the incorporation of single molecules. The NRPS enzymes are coordinated by communication (COM) domains, assuring the appropriate assembly of these biosynthetic molecules. In addition, the NRPS enzymes require a prior post-translational modification by a 4'-phosphopantetheinyl transferase, which acts on the thiolation domain of each module, to enable sequential amino acid binding.

These multi-modular ribosomally-produced enzymes catalyze, sometimes in combination with non-modular enzymes, the synthesis of antibiotics, frequently using a variety of standard and non-proteinogenic amino acid substrates (Wang et al., 2014). Interestingly, along the antibiotic biosynthetic pathway, BGCs may contain antibiotic-detoxifying (resistance) enzymes involved in the self-protection of the producer strain. However, another highly effective mechanism, involving transcriptional regulatory cascade regulators of BGCs expression, is frequently present in Actinomycetota (Bibb, 2005). Such regulators activate or repress BGCs' biosynthetic activity, depending on environmental or cell-cycle conditions in the antibiotic-producing strain. That might explain why many antibiotic-producing strains lack resistance mechanisms; in fact, antibiotic production via secondary metabolism often occurs when primary metabolism is poorly active, thereby reducing self-damage. A particular type of these regulators comprises the *Streptomyces* Regulatory Antibiotic Proteins (SARPs) (Yan and Xia, 2024). Production of antibiotics can be modulated by quorum-sensing signals, such as butyrolactones, or by antibiotics themselves. Hypothetically, if the antibiotics cause structural or metabolic damage in the cell, the molecules released from this damage should be able to negatively regulate antibiotic biosynthesis. This may recall that cell wall bioactive molecules resulting from beta-lactam damage can induce beta-lactamases (Baquero et al., 2026). A stimulatory effect of BCG's antibiotic biosynthetic activity might be beneficial if the population is involved in a competition with rival microorganisms. Finally, within a single protein molecule, combining antibiotic and antibiotic-resistance domains, resistance could serve as an intramolecular regulator, altering the molecular conformation and, consequently, the activity in response to environmental cues. Similar intramolecular regulatory effects have been detected in alarmones (Turnbull et al., 2019). The question we are examining in this work is the prebiotic origin of the molecular assemblies that gave rise to the ancestors of

secondary metabolites involved in antibiotic production and antibiotic resistance. Prebiotic molecules involved in these action-reaction molecular interactions imply "molecular wars", favoring the more stable molecular associations. The primordial-chemistry molecular weapons and defenses were evolving over billions of years to become biosynthetically produced and functionally improved. However, this evolutionary process occurred non-linearly, with periods of stagnation or acceleration during certain geological and biogeological periods.

THE COMMON REMOTE ORIGIN AND EVOLUTION OF ANTIBIOTICS AND ANTIBIOTIC RESISTANCE MOLECULES

Comparative genomics and environmental studies suggest that antibiotic biosynthetic genes and resistance determinants have been associated for very long evolutionary periods, billions of years before the anthropogenic era of chemotherapy, in line with the view that antibiotics and resistance are ancient features of microbial life rather than recent inventions (Sengupta et al., 2013). Expanding upon this framework, we hypothesize that primitive antibiotic-like and resistance-like molecules could have evolved in ancient prebiotic times through a sprawling, vast network of stochastic, predominantly futile, pre-metabolic pathways. These ancestral processes likely generated an enormous diversity of small bioactive compounds, probably exceeding the current bacterial population of the biosphere by an order of magnitude (Davies et al., 2012). A minute fraction of this "parvome" may have incidentally produced molecular combinations that acquired properties similar to those of modern antibiotics and resistance factors. They might have been co-selected whenever they contributed to the persistence or robustness of particular molecular assemblies. Perhaps primitive antibiotics and pre-antibiotic resistance molecules evolved in ancient prebiotic times through a mesh of chemical diversification and coalescence of multiple stochastic pre-metabolic synthetic pathways, most of which were futile. As noted earlier, a very

small number of these molecules may have served as precursors to antibiotics and related ancestral antibiotic-resistance molecules that were eventually co-selected. In both cases, the selection of these precursors was probably based on the replication of a specifically ordered molecular sequence. Among these, further selective events acted on the more robust, primarily based on their greater stability and thus their longer persistence over time.

Inorganic synthesis was ultimately replaced by biochemical reactions (Wang and Du, 2025). The origin of these ancient fixed and replicative sequences has been attributed to the prebiotic availability of auto-replicative RNA molecules, the ribozymes, which serve as backbones for substrate attachment (Chen et al., 2007). That was the birth of the biological information. Primitive metabolites could have emerged in such a way without preexisting protein machinery (Coggins and Powner, 2017). Proto-peptides contributed to the stabilization of RNA molecules, and vice versa (Frenkel-Pinter et al., 2020). The strict requirement of ribonucleotidyl coenzymes in contemporary metabolic pathways suggests early intertwining of RNA with biochemical catalytic functions (Negrón-Mendoza et al., 2025). A quintessential example is found in protein synthesis: the critical enzyme peptidyl transferase is catalyzed not by another protein, but by the major 23S rRNA component of the large ribosomal subunit, a true ribozyme, recalling the ancient and modern association between ordering and catalyzing ribozymes and elements from metabolic pathways (Lilley 2015; Lazcano and Miller, 1996). Such ancestral yet conserved pathways include those that may have given rise to precursors and to molecules involved later in antibiotics and antibiotic resistance. Many of their key structural monomer components were available in the ancient chemosphere. That is supported by the detection of non-proteinogenic amino acids and purine nucleobases in asteroids and meteorites (Parker et al., 2022; Johnson et al., 2008). Julian Davies proposed years ago that antibiotics may be among the oldest biomolecules (Amabile-Cuevas,

2003) and could have played a role as effectors of RNA catalytic reactions (Davies et al., 2007). In parallel, primordial antibiotic-resistance biomolecules likely date back billions of years and could have evolved from prebiotic molecules, altering the effects of these primitive antibiotics, which act not on cells but on molecular assemblies. However, such an evolutionary process was discontinuous in time, depending on the changing environmental conditions on the Planet.

NON-LINEARITY PERIODS IN EVOLUTION OF ANTIBIOTIC PRODUCTION AND RESISTANCE

Evolutionary rates of antibiotic production and antibiotic resistance were discontinuous in time and probably spatially heterogeneous. This non-linear progression can be illustrated by the shifting influence of inorganic molecules during Earth's early and later history, including metal ions and oxygen. A critical environmental factor was the dramatic shift in oxygen availability. In prebiotic times, oxygen levels were less than 0.001% of present atmospheric levels (21%) (Carver, 1981). Due to oxygen's high redox potential, its presence can facilitate the removal of electrons, breaking chemical bonds between primordial molecules. Consequently, its *absence* was probably a precondition for the synthesis and aggregation of molecules that were ancestors of proto-antibiotics via prebiotic chemistry, likely operative ca. 4 billion years ago. When the “great oxygenation event” occurred in Earth’s history (Olejarz et al, 2021) occurred ca. 2.4 billion years ago, a quite different evolutionary landscape took place.

In contrast to the anoxic phase, oxygen reactivity was now instrumental in the condensation of the more stable among prebiotic molecular associations. Evolutionary forces started to act, facilitating stability, diversification, and finally replication of cooperative molecular interactions, resulting in proto-enzymes and proto-metabolism. The acceleration of interactive prebiotic chemistry was suddenly paving the way for proto-biochemical processes, such as the formation of polycyclic

hydrocarbons. Oxygen initiated the process that underlies the bedrock of all metabolisms: inorganic carbon fixation into organic matter (Shih, 2015). Simultaneously, novel molecular oligomers became accessible by oxygen degradation of prebiotic aggregates, such as prebiotic oligosaccharides. Oxygen availability is a powerful factor in generating diversity in polyketide metabolites. Oxidation could further introduce functional moieties and even rearrange the existing chemical structure. Flavoprotein monooxygenases were possibly proto-enzymes already present in the first cells, in the last universal common ancestor of all cells (LUCA) (Mascotti et al., 2015) and deeply involved in polyketide biosynthesis (Zhang and Ge, 2024). Monooxygenases are involved in methylations, glycosylations, and reductions, allowing the biosynthesis of polyaromatic antibiotic structures (Grocholski et al., 2021). Of course, the major contributor to the "Great Oxidation Event" that accelerated the building-up of polyketides, and thus antibiotics and antibiotic resistance molecules, was the widespread of oxygenic photosynthesis, using water to produce oxygen, dated 2.4-2.1 billion years ago (Sessions et al., 2009). However, the process of oxygenation was not a brief, rapid, punctuated event. Cyanobacteria, the more successful oxygenic bacteria, had slowly evolved under the influence of abiotic oxidants (Wu et al., 2023; Wang et al, 2016). Phylogenetic analysis traced the first organisms to use oxygen to 3.1 billion years ago, probably first confined in small spatial sources, where oxygenases and oxydoreductases were already present (Jablonska and Tawfik, 2021). These sources may have been located at hydrothermal vents, fissure-like hot springs on the seafloor, where suitable conditions of geothermal light, could have early bacteria allowed to use light for electron donor extraction, fostering the origin of the first, primitive enzymes, with structural pockets recognizing substrates, but much less specialized that later evolved enzymes (Chisholm et

al., 2024), including those involved in antibiotic production and resistance. Most likely, the ancient antibiotic-resistance mechanisms had other unrelated functions in primitive organisms.

In summary, two different periods, basically characterized by anoxic and oxygenated landscapes, the first produced the conditions for multimolecular aggregation and evolution of proto-antibiotics and proto-antibiotic-resistance molecules. (molecular wars). The second, based on the selected molecules, facilitated the formation of polyketides and more mature molecules (proto-organelles, proto-cells, and cellular wars). Both periods overlapped for an extensive period of time, increasing the molecular differentiation and evolution of antibiotics and antibiotic-resistance molecules. In the following sections, these processes, mainly the less-known "molecular wars", are considered to understand the origin of these molecules.

MOLECULAR WAR AND PEACE

In a strictly prebiotic world, antibiotic-like molecules cannot be defined by modern criteria, that is, as agents that act by altering the structure or metabolism of cellular entities. Consequently, antibiotic resistance molecules conferred no protective function on any -non existing- organisms. Similarly, the primary function, mainly self-protection against spontaneous entropic instability, of multimolecular proto-organs and proto-organelles that may have preceded organisms in the origin of life are not necessarily related with the function in an integrated organism (Baquero et al., 2024) Stability and persistence in time of mono- and multi-molecular chemical structures, that is, the features that allow Darwinian selection as a mechanism of evolution, are contingent upon specific primary and secondary folding conformations, as well as the stability of molecular associations, resulting in a lesser degree of degradation by physical and chemical challenges faced in the ancient

environment. Variation, a condition for selection, occurs in these ancestral molecules as a consequence of environmental inputs (Aguanell et al., 2026). The assembly of more stable structures necessitated the recruitment of specific stabilizing molecules, which may be conceptualized as molecular nutrients. This process facilitated the emergence of more stable conformations through mechanisms analogous to non-enzymatic, spontaneous posttranslational modifications. Finally, during the RNA world period, a sequential, information-rich, replicative scaffold was being organized as structured assemblies of molecules. Such structures may give rise to predecessors of ordered metabolic pathways. Hairpin or stem-loop stable RNA structures likely coevolved alongside highly stable amino acid sequences arranged in alpha-helix or beta-folding conformations, providing a kind of stabilizing cooperation (Wang and Du, 2025). Given the already-mentioned large number and diversity of molecules available in the primordial world, it can be expected that a multitude of nearly equivalent successful, stable molecular arrangements may have been environmentally selected in different, occasionally neighboring places that offer the same array of simple components. In that case, the fitness differences among the emerging molecular complexes, frequently phylogenetically related, and/or among proto-organs are likely insufficient to ensure the dominance of a few by independent selection based solely on stability or more efficient production reactions. Instead, the most effective selective events may depend on direct competition: debilitation or destruction of competitors, or interference with their "synthetic" or associative dynamics. That is, from molecular wars. However, war is compensated for by multiple molecular cooperations and individual defense. That is the essence of life.

THE MOLECULAR WARS: WEAPONS AND DEFENSES

As stated above, Julian Davies proposed that antibiotics may be among the oldest biomolecules. However, conventional wisdom holds that the biosynthesis of antibiotics requires enzymatic

functions (i.e., see below for beta-lactams), but this does not preclude the possibility that non-enzymatic molecules can interfere with the formation of prebiotic stable molecules, molecular building block assemblies, and proto-organs. For instance, amino acid condensation processes occur spontaneously under prebiotic conditions; an example concerns depsipeptides, primordial peptide backbones containing both peptide and ester bonds, probably selected by their stability (Fisher et al., 2025). These primitive, self-assembled peptides, predominantly composed of proteinaceous amino acids (Frenkel-Pinter et al., 2019), may bind to specific molecular building blocks, which may act as anti-proto-cell antibiotics. Proto-ribosomes may have contributed to the formation of depsipeptides, as ribosomes catalyzed ester bond formation (Fahnestock et al., 1970). However, prebiotic assembly of monomers may produce lactones and lactams (Chandru et al., 2020). In all cases, we should insist on the difficulty of distinguishing antibiotic actions from regulatory functions (Linares et al., 2006). In either case, these actions paved the way for the evolutionary processes.

Although we cannot have direct evidence that identifiable ancestors of modern antibiotic or resistance systems already operated in this era, the later tight association between secondary-metabolite biosynthesis and self-protection mechanisms suggests that early enzymatic networks were already navigating, exploring a chemical space in which “action” and “reaction” were intrinsically intertwined at the level of molecular assemblies. For millions of years, prebiotic random chemistry and selection for molecular stability likely coexisted with the formation of the first accelerating biochemical processes driven by the emergence of enzymatic functions. These first enzymes were simple proteins endowed with particular motifs such as phosphate-binding loops (P-loops), the seeds of NTPase enzymes, which hydrolyze nucleoside triphosphates, such as ATP. This critical step in the history of life, perhaps preceded by catalysis mediated by solid-

state transition metals, which provided high-energy for the construction of novel molecules by metabolic or polymerization reactions (Romero Romero et al., 2018; Coggins and Powner, 2017). We hypothesize that among the myriad of novel molecules resulting from the action of the earliest evolving enzymes were those related to the early biosynthesis of antibiotics, antibiotic-resistance molecules, and proto-organelles of the bacterial cell, all involving prebiotic molecules and biopolymers.

MOLECULAR WEAPONS AND DEFENSES IN PREBIOTIC WORLD

As previously stated, the possibility of the emergence of proto-antibiotic and proto-resistance molecules in the prebiotic world, as elements involved in "molecular wars," is considered in the present work. Due to brevity constraints, we restrict our examples to five major classes of antimicrobial agents. In each case, we briefly recapitulate the molecular structure of the antibiotic, the possibility of its genesis in abiotic times, the mode of action and presumed prebiotic targets , as well as the possibility of the emergence of concurrent primordial resistance molecules that protect their prebiotic targets.

Beta-lactams, PBPs, and beta-lactamases

The ancient, highly reactive beta-lactam ring is a four-atom structure, the key precursor of penicillins, cephalosporins, carbapenems, and monobactams. The ring mimics the D-Ala-D-Ala peptidoglycan dipeptide, so that the penicillin-binding-proteins (PBPs) in charge of cell wall construction are inhibited by the analogous wrong target. The beta-lactam ring is the result of a non-spontaneous (as far as we know) condensation of three amino acids (δ-(l-α-aminoadipyl)-l-cysteinyl-d-valine), and is non-ribosomally synthesized by the ACV multimodular synthetase (ACVS), a complex of ribosomally-produced enzymes (Byford et al., 1997; Tahlan et al., 2017).

Prebiotic beta-lactam ring may have emerged in the "Thioester World", roughly 4 billion years ago, as we discuss in the next paragraph. The final formation of the β-lactam ring involves an oxidoreduction step mediated by isopenicillin N synthase (IPNS). Some peptide precursors to antibiotics are biosynthesized this way, and later post-translational modifications produce the active antibiotic structure.

The beta-lactam ring contains a cyclic amide bond (C1-N)**,** the substrate of beta-lactamases. Counterintuitively, no other biochemical structures containing beta-lactam rings have been detected -have they existed?- so far in nature, except for beta-lactam antibiotics and related compounds (nocardicin, tabtoxin). Thus, the possibility of a prebiotic beta-lactamase function remains obscure. In the "Thioester World", esterases may certainly have played a role, and such a function has been retained in some beta-lactamases (Cea-Rama et al., 2022), suggesting that earlier beta-lactams would have been formed through spontaneous ester bonds. Also, phylogenetic molecular reconstructions might cast some light on this question. In fact, the widespread serine-beta-lactamases are phylogenetically related to the cell wall DD-transpeptidases, the high molecular weight PBPs (Fröhlich et al., 2021). With only three substitutions in the active site, PBP2X acquires beta-lactamase activity (Peimbert and Segovia, 2003). On the contrary, low-molecular-mass PBPs may exhibit some β-lactamase activity (Henderson et al., 1997; Gonzalez-Leiza et al., 2011). Both proteins probably arose in multiple, independent evolutionary events and compete to be stabilized and become pervasive during evolution by their association with ancestral muropeptide chains, thereby diverging functionally into PBPs and beta-lactamases. When the enzymatic action of ACVS was established, and beta-lactam antibiotics with significant activity emerged, this divergence widened but led to a cooperative outcome. Beta-lactamases preserved the function of PBPs in the presence of beta-lactams, thereby allowing cell wall construction.

Glycopeptides

D-ala–D-ala residues from lipid II are incorporated into the cell wall to form the disaccharide-pentapeptide, assuring the cross-linking of the muropeptide structure. This dipeptide is the target of glycopeptide antibiotics (e.g., vancomycin or teicoplanin), which inhibit polymerization by steric hindrance. As with ACVS in beta-lactams, glycopeptides are non-ribosomally synthesized by biosynthetic gene clusters (BGCs) that contain non-ribosomal peptide synthetases (NRPs). The primordial BGCs evolved into more recent enzymes producing a glycopeptide precursor, paleomycin (Hansen et al., 2023). The essential peptide scaffold of glycopeptides, forming the core aglycone structure, is composed of proteinogenic (Asn and Leu) and non-proteinogenic amino acids (dihydroxyphenylglycine, hydroxyphenylglycine, and β-hydroxytyrosine), possibly present in the prebiotic Earth environment (Frenkel-Pinter et al., 2020). We cannot exclude the possibility of prebiotic precursors of the mature glycopeptide, as the core peptidic aglycone can bind D-ala-D-ala, and thus could have some antibiotic activity. BCGs will, in any case, optimize their formation. The resulting peptide undergoes intramolecular cyclizations and enzymatic modifications, both mediated by BGCs, which are also involved in the synthesis of specialized amino sugars from glucose, arabinose, and mannose. The modified peptide, now associated with the aminosugars, can form five hydrogen bonds with the terminal D-ala-D-ala residues of the peptidoglycan precursor, inhibiting polymerization by steric hindrance and preventing muropeptide cross-linking (Waglechner et al., 2019). Resistance to glycopeptides is considered contemporary to the cellular antibiotic synthesis, both dating to 100–400 Ma. In fact, most glycopeptide BGCs include the glycopeptide-resistance *vanHAX* genes to ensure self-resistance of the producer strains. As in the case of beta-lactams, downregulators of the antibiotic synthesis by BCGs could have preceded, or were contemporary to, the evolution of specific mechanisms of

313 resistance, such as Van determinants (Waglechner et al., 2019). However, it cannot be ruled out
314 that some antibiotic activity precedes the sugar decoration of the aglycone (that allows the binding
315 to D-ala-D-ala) (Malabarba and Ciabatti, 2001). This could promote the evolution of resistance
316 genes through multiple gene-acquisition events. Antibiotic resistance is mainly mediated by
317 alterations in the pentapeptide target by Van determinants, disrupting the hydrogen bonds involved
318 in drug binding using ligases to convert D-ala-D-ala into D-Ala-D-serine, or, with higher efficacy,
319 into D-ala-D-lactate (Arthur and Courvalin, 1993; Stochios and Savchenko, 2020). The operon
320 with the core genes involved (*vanA/B/D/F/M* ligases*, vanH* deshydrogenase *and vanX* dipeptidase)
321 originates most probably from lineages of glycopeptide-producing actinomycetes, but the
322 regulatory genes (*vanRS*) probably emerged in *Bacillus* and *Paenibacillus* (Kardos et al., 2024;
323 Yushchuk et al., 2020). As suggested above, the aglycone may exert an antibiotic effect through
324 D-ala-D-ala binding, as in natural abiotic depsipeptides (D-ala-D-lac) (Frenkel-Pinter et al., 2019).
325 Such may have been involved in some primordial resistance effect.

326 **Macrolides**

327 Macrolide antibiotics are secondary metabolites derived from polyketide compounds with a
328 macrocyclic lactone ring. There is no information about related compounds or components of the
329 macromolecule in pre-cellular, abiotic conditions. Most of what we know about macrolide
330 biosynthesis relates to their production in Actinomycota. The synthesis of polyketides involves a
331 group of enzyme activities called polyketide synthases (PKSs) of different types (Risdian et al.,
332 2019). In *Streptomyces*, the Type I PKS, integrating three modules and 15 domains, and involving
333 components such as acyl carrier protein, acyltransferase, ketosynthase, ketoreductase, dehydratase,
334 and enoylreductase, is responsible for the production of the macrolactone. PKS constructs the
335 macrolactone (as 6-deoxyerythronolide B) through the condensation and reduction of the

precursors propionyl CoA and methylmalonyl CoA. Whether such construction could have emerged in the prebiotic world is unknown, but certainly PKSs accelerated this early synthesis. Most importantly, the polyketide is decorated with a group of deoxy sugars, mostly amino sugars. In the classic view, macrolides inhibit protein synthesis by targeting the nascent peptide (typically 3-10 amino acids in length) as it traverses the exit tunnel of the large ribosomal subunit. However, protein synthesis is not universally abolished; rather, inhibition occurs particularly for a subset of proteins, the so-called macrolide-sensitive proteins. Probably, the synthesis stops because macrolides prevent the ribosome from catalyzing peptide bond formation in proteins that contain macrolide-arrest-motifs (MAMs), which are frequently Arg/Lys-X-Arg/Lys sequences. In that case, macrolides form macrolide-stalled ribosomal complexes, followed by consistent nucleotide rearrangement near the peptidyl-transferase active site (Vazquez-Laslop and Mankin, 2018). It is unclear if macrolides or their biosynthetic ancestors can interact with protoribosomes (Bose et al., 2022). The pepdydil-transferase center is essentially a ribozyme that originated in the prebiotic RNA-world, when RNA must have gained the ability to facilitate the synthesis of, initially, small peptides (Müller et al., 2022). The macrolide precursor molecules that could bind to RNA in pre-cellular times are most likely C5-linked 4,6-dideoxyaminosugars, such as desoxamine, which could attach via the 2′-hydroxyl group to the N1 atom of the nucleotide A2958. Possibly later, hydrophobic interactions of the macrocyclic lactone ring could have contributed to consolidate binding to 23S rRNA, involving the base-paired nucleotides 2611 and 2057. In summary, it cannot be discarded that in prebiotic times the function of protoribosomes could have been affected by these macrolide ancestor components, and thus the emergence of mechanisms of molecular resistance could have started to evolve.

The most relevant mechanism of macrolide resistance is the methylation or dimethylation (using S-adenosylmethionine) of the target nucleotide A2058 in the 23S rRNA, which interacts with the C5 position of the macrolactone ring. The expression of the methylase, encoded by the resistance gene *ermB* (among other *erm genes),* is regulated by a post-transcriptional attenuation mechanism. This occurs as a result of macrolide-ribosome stalling, which influences the translation of the leader peptides ErmBL or ErmCL preceding the co-transcribed methylase sequence in the *erm*-containing mRNA. Ribosome stalling destabilizes the inhibitory stem-loop mRNA structure, which is folded in secondary structures (hairpins) and exposes the Shine-Dalgarno (SD) sequence, allowing the translational expression of the methylase (Wang et al., 2021). A similar attenuation mechanism provides a weak macrolide resistance mediated by HflXR, a homolog of a ribosome splitting factor (Duval et al., 2018). How this ubiquitous mechanism in ribosomal functioning has evolved remains unknown. The alteration of the protoribosome structure might have protected the building up of stable peptide-RNA interactions in the prebiotic world.

Tetracyclines

Tetracyclines and fatty acid biosynthesis are highly related. Tetracycline's polyketide core is modularly assembled from acetate-derived malonyl-CoA (carboxylated acetyl-CoA) and eight two-carbon fragments. This structure is thus based on an acetate-derived sequence rather than an amino acid sequence, synthesized by enzymes not distantly related to the fatty acid synthase (FAS). It iteratively inserts two-carbon units, resulting in a molecule more akin to a lipid than a peptide. The poliketone chain cycles, ultimately forming four fused benzene rings, is then submitted to post-assembly modifications. In any case, there are analogies between biotic and prebiotic synthesis of fatty acids, but the prebiotic synthesis relies on the use of formic and oxalic acids (Clardy et al., 2009). Advances in prebiotic lipidomics, including lipotranscriptomics (Fiore et al.,

2022), might shed light on potential prebiotic tetracycline ancestors. These prebiotic tetracycline ancestral components, particularly their lipid-like components, could have contributed to the stabilization of RNA conformations, leading to changes in ribozyme activity (Czerniak et al., 2022). It is well-established that tetracyclines inhibit translation by binding to nucleotides in helix 34 and helix 31 of the 16S rRNA, thereby preventing the binding of aminoacyl-tRNA to the mRNA-ribosome complex, which probably is a prebiotic ribozyme (Chukwudi, 2016; Suga et al, 2011). Therefore, tetracycline ancestors may have contributed to the dynamics of competitive stability between proto-ribosomes.

In this prebiotic scenario, the main mechanism of tetracycline resistance, efflux pumps, as we understand by now the function of these structures (necessarily associated with cell membranes), was probably irrelevant. It has recently been suggested that translation factors likely emerged early, providing a bridge between pre-cellular chemistry and the last universal common ancestor (LUCA) (Fer et al., 2025). Primordial translation factors are likely ancestors of ribosomal protection proteins (RPPs), producing conformational changes in the ribosomal RNA and, mediating tetracycline resistance. Indeed, these RPPs share structural similarity with translation factors EF-G/EF-2 and EF-Tu/EF-1α (Kobayashi et al., 2007).

Aminoglycosides

Aminoglycosides are composed of two or three amino sugars, linked by glycosidic bonds to a core amino-substituted cyclohexane amino cyclitol (a cyclic alcohol), as streptidine in the case of streptomycin, or 2-deoxystreptamine, for other aminoglycosides. The amino cyclitols are substituted by aminosugars, such as neomycin (4,5-disubstituted) or kanamycin, tobramycin, and gentamicin (4,6-disubstituted). Prebiotic synthesis of monosaccharides originates in the formose network in basic aqueous solution, and also in nonaqueous conditions (Lamour et al., 2019; Yi et

al., 2023). Aminocyclitol molecules can be formed in prebiotic conditions from glycolaldehyde, 2-aminooxazole, and aminonitrile (Whitaker and Powner, 2022). Therefore, the formation of precursors of highly positively-charged aminocyclitols-aminoglycosides in the pre-cellular world cannot be ruled out, as well as their possible interactions with negatively-charged RNA in proto-ribosomes, particularly their ribozyme cores. In the cellular world, Actinomycetes optimized the biosynthetic production of aminocyclitols from simple sugar units (as D-glucose-6-phosphate), using sugar phosphate cyclases (SPCs) (Huang et al. 2022; Kudo et al., 2009) including the 1L-*myo*-inositol 1-phosphate (MIP) synthases and the 2-deoxy-*scyllo*-inosose synthases involved in the biosynthesis of pseudo trisaccharide intermediates, and finally aminoglycoside antibiotics (Mahmud, 2009; Chen et al., 2025; Wehmeier and Piepersberg, 2009). The biosynthesis of the different aminoglycosides follows parallel pathways, likely reflecting a rooted evolutionary tree (Yu et al., 1990; Kirby, 1990). That might explain why aminoglycoside-producing strains frequently biosynthesize several aminoglycoside molecular variants. In contrast to the multistep enzymology presented above for the biosynthesis of polyketides (or non–ribosomal peptides) in other antibiotic groups, here the biosynthetic pathways are largely based on monofunctional enzymes catalyzing single steps.

Aminoglycosides bind the decoding region at the A site of the 30S ribosomal subunit, disrupting bacterial translation. These antibiotics are multiple positively charged compounds. The positive charges are attracted to the negatively charged phosphate RNA backbone. The flexibility of aminoglycosides facilitates accommodation in binding pockets within internal loops of RNA helices or within ribozyme cores to form specific contacts (Schroeder et al., 2000). Binding involves the amino and hydroxyl functional groups of aminoglycosides and RNA bases. The presence of 2-deoxystreptamine (2-DOS) in almost all aminoglycosides indicates a key role in

RNA recognition and biological activity (Chittapragada et al, 2009). The structural modification of the antibiotic by the aminoglycoside-modifying enzymes *N*-acetyltransferases (AACs), *O*-phosphotransferases (APHs), and *O*-nucleotidyltransferases (ANTs), as well as the blocking-binding methylases of the antibiotic decoding center on the 16S rRNA (Arm/Rmt and Kam/Npm methylases**),** comprise the major mechanisms of aminoglycoside resistance present in the antibiotic-producing organisms. The origin of aminoglycoside resistance genes in the cellular biosynthetic machinery of these compounds has been known for a long time (Distler et al., 1985). (The question is whether prebiotic aminoglycoside-resistance ancestors existed before the emergence of cellular life. The sugar acetyltransferase function may have emerged in the prebiotic world, using high-energy compounds such as acetyl phosphate or thioesters as acetyl-CoA precursors in the "Thioester World", a key period in the origin of life (Singh et al., 2025). Prebiotic phosphotransferases are also possible: for instance, sugar-1-phosphates can be produced spontaneously in mixtures of sugars and phosphoric acid (Nam et al., 2017). Prebiotic sugar nucleotidyltransferases facilitate the transfer of a nucleotide from a nucleotide triphosphate to a sugar, and may have originated in promiscuous ribozymes, in the mixed transitional period of the Thioester World and the later RNA world, and could be related to the formation of sugar nucleotides (Biscans, 2018). rRNA methylation was also available in the prebiotic world (Schneider et al., 2018). If the molecules associated with these RNA-modifying structures have any effect on the interactions of aminocyclitol or aminoglycoside ancestors and could constitute the basis for the later evolution of antibiotic resistance enzymes, it remains to be explored. However, the early prebiotic Earth was endowed with the basic constituents of these protective functions.

JUNCTION AND DISJUNCTION BETWEEN ANTIBIOTIC PRODUCTION AND RESISTANCE

According to the hypothesis of molecular wars discussed in the previous paragraphs, an extensive prebiotic period likely existed during which molecules capable of destabilizing rival molecular assemblies emerged. Concurrently, compensatory molecules appeared, functioning to shield these structures from such proto-antibiotics. The evolution of molecular ensembles and pathways responsible for generating these inhibitory agents may have been intrinsically linked to the partial self-destruction of their own synthetic pathway. Consequently, the evolutionary association with protective molecules would have conferred a significant selective advantage, as non-protected target molecules were systematically eliminated. In subsequent evolutionary stages, the synthesis of proto-antibiotics may have been integrated as a requisite component of a comprehensive self-protection strategy. Such an association persisted in later cellular secondary metabolism biosynthetic processes. As previously stated, in Actinomycetota (also in Bacillota), resistance molecules inactivating the produced antibiotic are frequently present, and the chemical structure of these detoxifying mechanisms usually derives from the biosynthetic pathways of antibiotic secondary metabolism. However, some strains harbor antibiotic-resistance mechanisms against antibiotics they do not produce. This is similar to many other non-antibiotic-producing bacteria that harbor resistance mechanisms to antibiotics used in antimicrobial therapy. Note that beyond specific mechanisms inactivating the antibiotic, resistance can be due to a refractory structure of the antibiotic target, or a negative regulation of antibiotic biosynthesis if the bacteria are damaged. However, this strongly suggests that resistance genes have been disjoined from the biosynthetic ones, probably captured by mobile genetic elements as integrons, transposons or integrative-conjugative elements, and finally eventually harbored in transmissible plasmids. This may give

rise to horizontal gene transfer of these resistance genes, which may eventually be acquired by organisms, taxonomically related or unrelated to antibiotic producers, as long ago predicted by Julian Davies and Arnold Demain. However, natural constraints to the mobility of mobile genetic elements impede a pervasive introduction of antibiotic resistance genes in the microbial world (Waglechner and Weight, 2017).

Our modern anthropocentric intuition tends to equate antibiotics with drugs and, consequently, to assume that they act primarily as lethal weapons in "microbial wars". However, for any released bioactive molecules, concentration and context are critical: in natural environments, the amount of antibiotic produced by individual cells or small populations is rarely sufficient to ensure the killing of potential competitors, but can profoundly modulate transcriptional programs and community dynamics at sub-inhibitory levels. In this ecological setting, antibiotics are better understood as signaling or regulatory molecules, whose coordinated production and corresponding resistance mechanisms shape interaction networks and niche occupancy, rather than as simple bactericidal agents (Linares et al., 2006; Davies, 2006; Aminov, 2009; Sengupta et al., 2013). Large-scale anthropogenic production and dissemination of antibiotics have disrupted this long-standing balance between production and resistance, creating pervasive, strong selection for resistance determinants that can spread widely by horizontal gene transfer. In evolutionary terms, this massive and sustained selection does not "aim" to preserve an ancient equilibrium but, blindly, favors mobile genetic elements and bacterial lineages in which resistance functions maintain cellular viability under antibiotic exposure. We can suggest that resistance, as a reaction mechanism, contributes to the maintenance of some kind of equilibrium, ensuring a healthy diversity of the microbiosphere, avoiding the extinction of ecological nodal lineages. In our view, this process was rooted in prebiotic molecular war and peace dynamics.

Conclusion

Our understanding of the origins of antibiotics and antibiotic-resistance genes remains nascent. However, we propose that both cases may be traced back to the prebiotic times, arising from a primordial field of action and reaction dynamics. In this environment, ancestral molecules competed through processes of disaggregation and cooperation to sustain their structural persistence over time (Figure 1). Such molecular competition ("molecular wars") could have exerted a powerful effect on the evolution of life during a prolonged, fuzzy transition period, when pre-cellular and post-cellular worlds coexisted. The mechanisms governing these molecular wars could have been instrumental in the foundational stages of life, for instance, providing weapons and defenses in the hypothetical proto-organs/organelles wars before the emergence of organisms (Baquero et al., 2024). Within the framework of the "virus-first" theory, which posits the existence of proto-viruses in a ribonucleoprotein world (Prosdocimi and Farias, 2025), some of these early antibiotics and defenses could have played a role in the hypothetical wars opposing pre-cellular and post-cellular entities. In the end, a kind of armistice, characterized by cooperation without completely losing the confrontation, could have evolved, and this situation does not contradict the structure of the current natural microbial world. This arrangement was also possible because the antibiotics and antibiotic-resistance genes were mainly confined to bacterial lineages whose lifestyles (mostly sporogenic organisms) required the production of antibiotics in a strictly necessary way. Only the anthropogenic intervention of industrial production of antimicrobial agents, providing a massive selective force for resistance, has altered this armistice, facilitating the spread of antibiotic resistance genes via mobile genetic elements.

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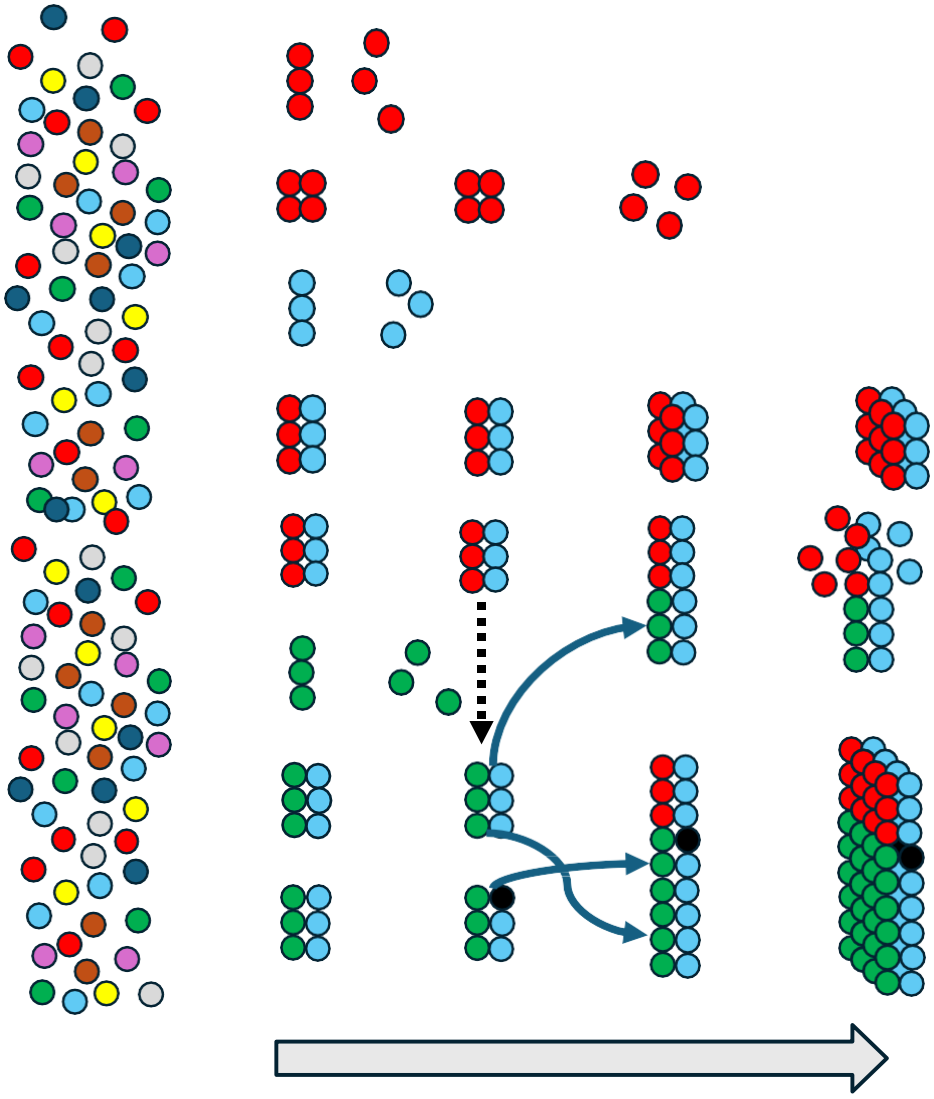

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521 **Figure 1**. At the left, the disordered probiotic molecular soup. Eventually, some of these
522 molecules may associate**. a)** a triplet (red) is formed, but it is unstable; **b)** a 4-membered
523 structure is formed, which is more stable in time, but after a period the stability is lost ; **c)**
524 another triplet is formed, slightly more stable than a), but also transiently; **d)** the triplet in a)
525 associates with the blue triplet in c), and such structure becomes stable in time, and even is able
526 to replicate; **e)** such successful process is interfered by a further association with another
527 molecular association, an antimolecule –or proto-antibiotic- which disassociate the d) structure;
528 note that (dashed arrow) this association might facilitate the antimolecule formation; **f)** again, a
529 (green) triplet is formed, but it is unstable; **g)** stability is gained by association with the blue
530 triplet, so that both triplets in association becomes a stable structure; this is the antimolecule
531 acting in e); **h)** a variation of the antimolecule, now with a black member that does not influence
532 stability, integrates in the successful structure d), and now the antimolecule cannot disturb the
533 successful replication of d) as antimolecule is not active any more (dashed white lines).

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