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A Model for the Modular Evolution of RNA Addressing Open Questions on the Origin of Life

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A main unsolved problem in the RNA world scenario for the origin of life is how a template-dependent RNA polymerase ribozyme emerged from short RNA oligomers generated by random polymerization of ribonucleotides (Joyce and Orgel 2006). Current estimates establish a minimum size about 165 nt long for such a ribozyme (Johnston et al. 2001), a length three to four times that of the longest RNA oligomers obtained by random polymerization on clay mineral surfaces (Huang and Ferris 2003, 2006). To overcome this gap, we have developed a stepwise model of ligation-based, modular evolution of RNA (Briones et al. 2009) whose main conceptual steps are summarized in Figure 1. This scenario has two main advantages with respect to previous hypotheses put forward for the origin of the RNA world: i) short RNA

modules resulting from template-independent polymerization on different microenvironments might suffice to produce the first functional RNAs in the absence of template replication; ii) modular evolution shortens adaptation times and generates complex structures that could not be directly selected. Therefore, ligation-based modular evolution might have bridged the gap between the last stages of the pre-RNA world and a fully established RNA world. The emergent information-based molecular machinery could subsequently evolve and be inherited by DNA-based precellular systems leading to the progenote. Although mainly focused on the origin of the RNA world, our model addresses in different ways several questions posed in the OQOL'09 workshop, as we discuss in the following.

Contingency vs. determinism—Our stepwise model describes levels of increasing biochemical complexity. Experimental and computational data support that the appearance of an RNA world may be the plausible outcome of molecular evolution even in the absence of template replication, and hence not a completely contingent event. The existence of many less RNA structural families than possible sequences points to a principle of 'canalized contingency'. This concept is analogous to the existence of attraction basins of sequences, where the initial randomness is strongly suppressed by the convergence to a limited set of structures. Determinism is related to the partial independence of the functional level from the microscopic level. We have shown in silico that the fraction of short, random molecules displaying catalytic activity is large enough to trigger the processes that lead to a modular origin of the RNA world.

Plausibility of the RNA world—One main problem stated in this question is the difficulty of obtaining many identical copies of a specific macromolecular sequence, apparently a requirement for the appearance of effective chemical function. However, different RNA sequences fold into identical structures, which group themselves into a reduced number of structural families. Since simple biochemical functions can be performed by slightly different structures of a given family, a pool of random, short oligomers may be a viable starting point for the RNA world. Assuming that there is a unique sequence able to perform a given function is misleading, since this premise overlooks the huge—and convenient—degeneration of the sequence-structure-function map in RNA.

Life as a unity or confederacy—Our scenario illustrates a possibly general principle for attaining functional complexity from short and non-informative molecular modules, ready to be combined in a constructive way. It suggests that the emergence of complex replicative molecules—the substrate of life—is due to a confederacy of subsystems which might have previously undergone partly independent evolution and selection. Once ligated into multi-modular molecules, the initial setting, characterized by the competition among modules, turned into a cooperative framework where the joint modules constituted selectable units. Analogous changes from confederacy to unity could have been further produced, for example by the eventual encapsulation of two polymerase ribozymes into a common compartment. From those transitions onwards, the evolution of the joined systems could erase the features of their prior, independent evolution, thus raising the question of whether, even if life started as a confederacy of modular units, this origin could be traced in modern cells.

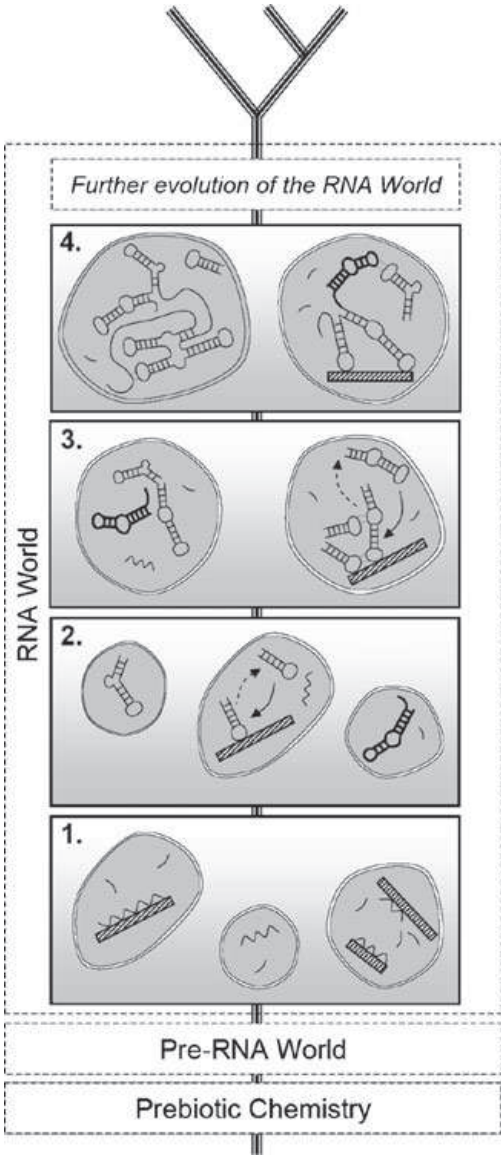

Fig. 1. Stepwise model for the modular evolution of RNA in the origin of life. The proposed evolutionary process can be divided into four conceptual steps, triggered once prebiotic chemistry had provided the required monomers and oligomerization was possible in the pre-RNA world. **1.** The abiotic polymerization of RNA from activated nucleotides could have occurred on mineral surfaces exposed to bulk solution (Huang and Ferris 2003, 2006) or within vesicles (Hanczyc et al. 2003), yielding up to 30–50mer random RNA oligomers. **2.** Every sequence folded into its minimum free energy structure. Computational analyses of the structural repertoire present in large populations (10^8 molecules) of random RNA sequences of length 12 to 40 nt reveal that topologically simple modules are the most abundant ones, especially hairpin structures and stem-loops (Stich et al. 2008). **3.** A fraction of hairpin modules could have displayed RNA ligase activity (in bold line), as certain ribozymes currently do, and thus catalyzed the assembly of larger, eventually functional molecules. Ligation processes allow a fraction of the combined molecules to retain their previous modular structure, such that structural and functional complexity can progressively increase even in the absence of template replication (Manrubia and Briones 2007). **4.** The iteration of that process could have assembled RNA molecules endowed with novel functionalities, paving the way to the emergence of a—relatively long and complex—ribozyme (Johnston et al. 2001) with

template-dependent polymerase activity. At this step, information-driven evolution would be triggered. More details on the model can be found in Briones et al. (2009).

Defining the very origin of life—A question indirectly addressed in the previous paragraph is the possibility that the first complex molecule required to establish a robust RNA world (i.e., an RNA polymerase) could have been the result of the interaction among a collection of modular subsystems, each with its own dynamics and proto-metabolic network of interactions. If simple modules are seen as functional and relatively stable entities, the scenario devised could also act as a meeting point for two traditionally opposite views: metabolism-first and information-first scenarios. This is, indeed, one of the most productive controversies behind the definition of the origin of life.

Is life an emergent property?—Once a functional ligase ribozyme opened the possibility of combining modules with different functionalities, new molecules endowed with unexpected chemical properties and activities could emerge. The resulting products are larger molecules whose functionality cannot be known a priori given the properties of the constituting modules. This fact has been experimentally demonstrated by in vitro evolution of RNA, where ligation and exchange of structural domains can be used to engineer new functional RNAs (reviewed in Joyce 2004). In our model, the appearance of a ligase molecule in a random pool of oligomers, the acquisition of new functionalities through modular evolution, and the origin of an RNA replicase, all represent emergent properties that could, in turn, be seen as pre-requisites for the emergence of life.

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