Evolution Finds Shelter in Small Spaces

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When RNA is replicated in cell-free systems, a ubiquitous problem is the hijacking of the system by short parasitic RNA sequences. In this issue of *Chemistry & Biology*, Bansho et al. show that compartmentalization into water-in-oil droplets can ameliorate this problem, but only if the droplets are small. This result helps to both recapitulate abiogenesis and optimize synthetic biology.

Pre-cellular evolution has been a tough nut to crack. Cells provide contemporary life with benefits too numerous to list, and it is hard to imagine a time in Earth's biotic history when they were not around. Nevertheless, the very origins of life required something simpler; full-blown cellular life, even bacterial, could not have spontaneously appeared on the Earth some four billion years ago. Hence prebiotic chemists and evolutionary biochemists have been busy developing acellular systems that have the properties of life but that do not include complex cellular structures. These systems include self-replicating RNAs or other polymers such as polypeptides or peptide nucleic acids. In both mathematical models and in test tubes, these macromolecules, especially RNA, have great potential to reveal evolutionary patterns that inform abiogenesis.

As anyone who has actually tried to evolve RNA in the lab knows, a persistent problem to coaxing a population toward any sort of evolutionary goal is the spontaneous, and usually devastating, emergence of parasites. These are short RNA sequences that can act as replication templates, but themselves do not participate in either their own replication or in the propagation of other species. Sol Spiegelman discovered these in the 1960's in the world's first extracellular Darwinian experiments using RNA from the coliphage Qβ (Mills et al., 1967). Short RNAs that later became known as ''Spiegelman's Monsters'' or simply ''minimonsters'' quickly took over the population. Being replicated but not replicases, they exhaust supplies of resources such as nucleotides, running the molecular ecosystem into the ground unless the resources are constantly replenished. And even if they are, in a serial dilution experimental format, these parasites will out-compete replicator species simply by having a higher reproductive rate. This is not simply a laboratory artifact. Spiegelman's data inspired decades of theoretical work on the dynamics of self-replicating molecular systems, starting with Eigen's (1971) treatise on the matter. The results are striking: parasites are inevitable, and innovations such as hypercycles, spatial heterogeneity, DNA, and importantly, compartmentalization are all possible means to keep the main RNA population resistant to invasion by short, nonproductive species.

It is this last phenomenon that may ultimately be the most effective (Szathmáry, 2006). Compartmentalization of the environment into protocell structures can promote a kind of group selection that ensures the survival of replicator lineages. Groups (i.e., protocells—these need not be real cells, just pliable and bounded compartments such as oil droplets in a sea of water) that contain replicators but no parasites will eventually outcompete those that are infected with parasites. This in fact may have explained the original advent of cells, although there is much discussion about this point.

Many researchers have exploited the protocell concept to create little bags of replicators either as models for the origins of life or as attempts to perform synthetic biology. The Yomo group has turned to encapsulation to solve the problem of parasites in their powerful in vitro translation-coupled replication system (the PURE system). This system encodes for a variant of the Qβ RNA and its replicase protein, along with a complete transcription-translation repertoire (Kita et al., 2008). Although the production of RNA and proteins works smoothly for about an hour, it then grinds to a halt. In this issue of *Chemistry & Biology*, Bansho et al. (2012) demonstrate how encapsulation into water-in-oil emersions can keep the system productive for much longer by protecting it from parasitic RNA species (Figure 1). Of particular interest is the fact that smaller compartments significantly out-perform larger ones, a finding that has implications for both prebiotic chemistry and practical synthetic biology: more smaller cells are better than fewer large ones.

The (originally) cell-free coupled transcription-translation system allows for the concomitant replication of both plus and minus strands of the Qβ RNA genome along with the RNA-dependent RNA polymerase protein that makes more RNA. This is a complex system with hundreds of components, such as genomic RNA, the replicase, ribosomes and other translation proteins, tRNAs, all four NTPs, all 20 amino acids, etc. Such complexity is a natural breeding ground for parasites, and Bansho et al. (2012) discovered that an RNA variant around 220 nucleotides in size was arising spontaneously in the mixture and responsible for the drain of resources from the replication of the Qβ RNA genome, which by comparison is 10-fold longer. The genesis of this parasitic species was intra-genomic RNA recombination, and the authors identified a likely recombination hot-spot in the plus-strand Qβ RNA that leads to a class of sequences related to the first Spiegelman's Monster, known as MDV-1. More work needs to be done to pin down the exact sequence of molecular events that leads to these parasites, but in the PURE System, it may be the non-homologous type of

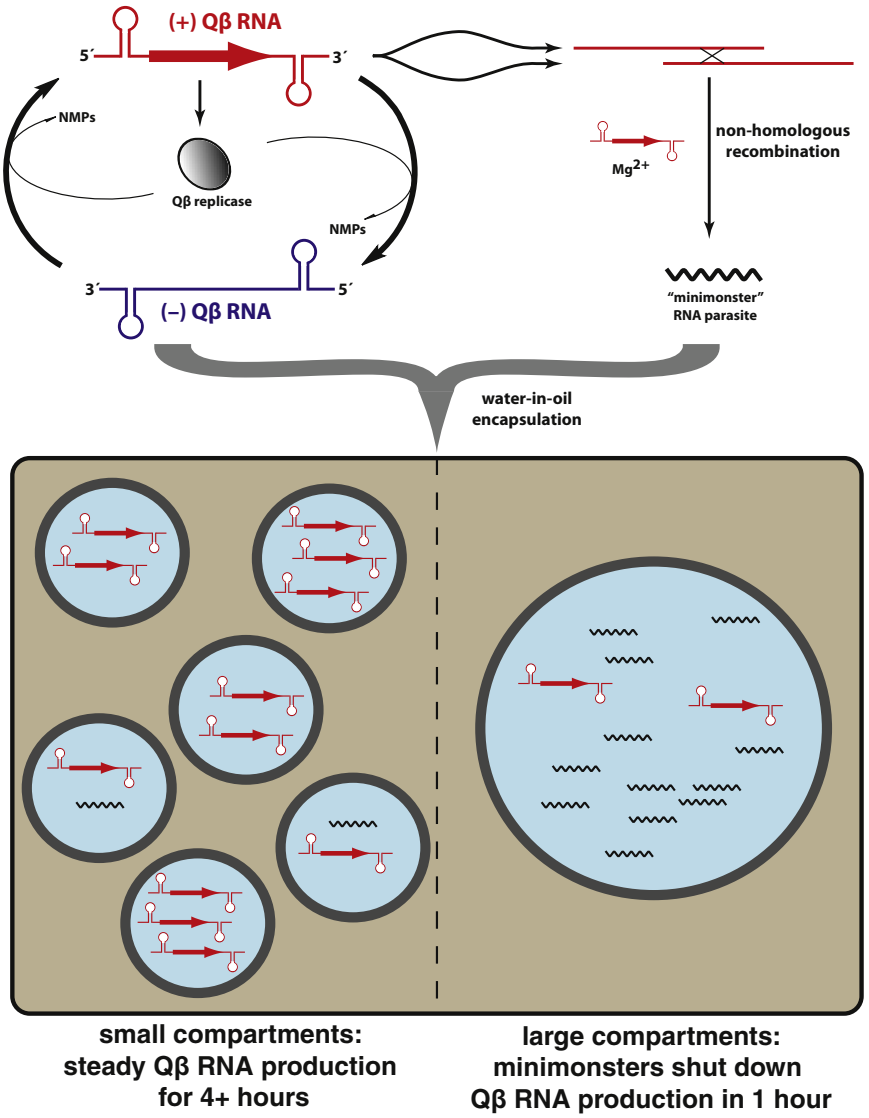

Figure 1. Small Compartmentalization Provides a Coupled Transcription-Translation System from Takeover by Small Selfish RNA Parasites or "Minimonsters"
Bansho et al. (2012) show that the PURE system of Qβ RNA replication (Kita et al., 2008), depicted in the upper left, spontaneously generates short 220 nt species via Mg^{2+}-catalyzed non-homologous RNA-RNA recombination. In larger water-in-oil droplets (e.g., 100 μm in diameter), these parasites quickly shut down full-length Qβ RNA production, but in smaller compartments (e.g., 100 μm in diameter), the parasite load is minimized and the system can remain productive for several hours.

recombination described by Chetverin et al. (1997). Bansho et al. (2012) propose that spontaneous Mg^{2+}-ion-catalyzed strand exchange at the hot-spot is the most likely explanation for the production of the minimonsters, but it is also possible that the Qβ polymerase itself plays a role or that the 3′ end of the RNA is involved in a *trans*-esterification reaction (Lutay et al., 2007). However, given that the Qβ-directed replication is rather sloppy, odds are that producing an RNA species that has some recombinase activity itself, aided by Mg^{2+} (Lehman, 2008), is indeed the more likely explanation.

Encapsulation in water-in-oil microdroplets (Tawfik and Griffiths, 1998) solves this problem. This turned out to be because in smaller droplets, the Qβ genomic RNA can win the selection numbers game (Figure 1). The authors compared the parasite loads in droplets of 10 μm and 100 μm in diameter and found that after an hour there were on average 1,000 times more parasites in the larger compartments. In the smaller droplets then, the Qβ RNA can replicate for several hours without a decline in yield.

As Bansho et al. (2012) point out, this result can guide our thinking on how life got started and made the transition to cells. The notion is that compartmentalization must have been a critically important evolutionary discovery and probably happened quite early in the history of our planet. The numerical consequences of "smallness" are not restricted to spherical structures that we normally associate with cells, however. One can easily imagine more shelter from the parasite plague driving life into the smaller interstices in rocks in a deep-sea hydrothermal vent (e.g., Baaske et al., 2007) or even into riding the more fragmented of traveling waves propagating through a semi-viscous solution (e.g., Boerlijst and Hogeweg, 1991). And lastly, when modern-day synthetic biologists are searching for optimal compartments in which to churn out desired polymeric products, they will find that bigger is not always better.

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