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ORIGIN OF LIFE

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Abstract The environment of protocells might have been crowded with small molecules and functional and non-specific polymers. In addition to altering conformational equilibria, affecting reaction rates and changing the structure and activity of water, crowding might have enhanced the capabilities of protocells for evolutionary innovation through the creation of extended neutral networks in the fitness landscape.

Keywords Crowding \cdot Excluded volume effect \cdot Protocell \cdot Vesicle \cdot Evolution \cdot Fitness landscape \cdot RNA \cdot Neutral network \cdot Evolutionary optimization

The Crowded Environment

The environment within biological cells is vastly different from the simple buffered solutions traditionally used in most biological studies. In fact, 30 % of the total cellular volume is essentially occupied by proteins, nucleic acids (Zimmerman and Trach 1991) and a complex mixtures of metabolites, ions, and polyamines (Bennett et al. 2009; Tabor and Tabor 1985). Concentrations of biomolecules in a modern eukaryotic cell exceeds those used in most in vitro experiments by a factor of 10–100 (Ellis 2001). A similarly crowded situation might have existed in the earliest cells, such as with non-specific polymers synthesized prebiotically. Therefore, possible effects of crowding on the origins of cellular life should be carefully considered.

The concept of crowding encompasses a number of effects, such as enhanced concentrations and altered conformational equilibria of solutes, and changes in water structure and activity. Although these effects are related, their physical underpinnings are somewhat different. The highly crowded intracellular environment has a major influence on at least two types

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of intermolecular interactions: hard-core physical repulsions and chemical ('soft') interactions (Wang et al. 2012). Hard-core repulsions in a crowded environment reduce the number of available conformational states and favor compact molecular states (Minton 1981). The space excluded by macromolecules and cellular organelles is effectively inaccessible to other molecules, creating an excluded volume effect due to steric hindrance (Minton 1998). This entropic effect is thought to cause the observed improved native-state folding of biopolymers (Cheung et al. 2005), although enthalpic effects might also play a role (Senske et al. 2014). The excluded volume effect not only increases chemical activities of dissolved solutes but also alters the kinetics of biological processes, possibly by several orders of magnitude (Lareu et al. 2007; Munishkina et al. 2004). There are two opposing effects of excluded volume that might influence the reaction rates (Minton 1998). If the rate of decay of the transition state complex controls the overall reaction rate, then crowding favors the forward reaction through enhancing the relative abundance of the transition state complex. However, if the overall reaction rate depends on collisional frequency of the reacting molecules, then crowding, which retards diffusional motion (Muramatsu and Minton 1988), reduces the forward reaction rate (Minton 2001).

Chemical interactions can be attractive or repulsive. In the crowded environment, repulsion between like charges reinforces the effect of hard-core repulsion. In contrast, attractive interactions between macromolecules of interest and other components of cells destabilize the folded states by exposing more surface to the crowding molecules (Sarkar et al. 2013). Thus, the effect of crowding depends on the details of the system at hand.

The structure and dynamics of water, the universal solvent for life (Pohorille and Pratt 2012), are also significantly perturbed in crowded, confined environments. Numerous studies show that hydrophobic interactions in these environments markedly differ from those in bulk aqueous solution. Similarly, the dynamics of water exhibit significant modifications. For example, the solvation relaxation time of a water molecule is significantly retarded in the presence of amphiphilic assemblies, such as vesicles (Saha et al. 2011) and lamellae (Verma et al. 2010).

These considerations illustrate that crowding and confinement influence the structure and function of biomolecules (Nakano et al. 2013; Saha et al. 2013). These conditions might have also affected rates of protocellular reactions, thus shaping the primordial metabolism. Here, we hypothesize that the crowded environments of protocells (Fig. 1) not only influenced biomolecular structures and chemical kinetics, but also promoted Darwinian evolution.

Evolution in the RNA World

Evolution of an informational polymer can be conceptualized as a random walk through the space of all of its possible sequences. Variations in fitness bias this walk, yielding a 'fitness landscape' in sequence space in which peaks represent sequence families that are fit (Maynard Smith 1970; Wright 1931). It has been proposed that this landscape contains large, near-neutral networks, which allow biological systems to discover innovations by traversing long distances in sequence space without appreciable loss of fitness (Ellington et al. 2009; Gavrilets 2004; Rendel 2011; Schuster 2011). In fact, it was suggested that such networks provided the main evolutionary paths for primordial life (Maynard Smith 1970). RNA is a particularly elegant genomic material, because it can exhibit both templating and catalytic activity. An



Fig. 1 Illustration of the crowded environment of a protocell. The ribozymes (*red*) are encapsulated in a fatty acid vesicle (diameter~100 nm) that is crowded by various prebiotic polymers and subcellular structures. Elements of this illustration are not drawn to scale

RNA fitness landscape for 'shape' was studied by *in silico* RNA folding, showing a vast, interconnected network of neutral mutations (Schuster et al. 1994).

Recently, we studied whether neutral networks can be found experimentally during in vitro evolution of RNA (Jiménez et al. 2013). A random library of 24-mers that saturated sequence space was subjected to in vitro selection for interaction with guanosine triphosphate-agarose resin. The analysis revealed several fitness peaks and identified a few evolutionary pathways among them. However, a major finding from this work was that the fitness peaks were largely isolated from one another. In contrast to the neutral networks of RNA shapes (Schuster et al. 1994), this landscape topology suggests that historical accidents essentially determine evolutionary outcomes. Yet neutral networks are desirable for evolutionary optimization. We hypothesize that certain environments might reveal neutral evolutionary networks.



Fig. 2 A schematic of disconnected fitness landscape, as found by Jiménez et al. (2013) (*left panel*) and a landscape forming a near-neutral network, as anticipated for a crowded environment (*right panel*)

RNA Evolution in Crowded Environments

Evolution is inextricably linked to environmental conditions. Fitness and heritability can only be defined in the context of a specific environment. In the realm of the RNA World hypothesis, a biochemical environment of interest for early evolution is a membranous compartment that contains RNA and presumably other specific or non-specific polymers and small molecules (Szostak et al. 2001). These components would create a crowded environment for RNA. Crowding can stabilize structures of functional RNA and increase their activity (Desai et al. 2014; Nakano et al. 2013; Strulson et al. 2012). It has been proposed that crowding reduces configurational entropy and widens the free energy gap between folded and unfolded states (Kilburn et al. 2013). Crowding can also increase catalytic turnover and activate catalytically less active ribozyme sequences (Nakano et al. 2009). For a hairpin ribozyme, the cellular environment shifts the equilibrium between cleavage and ligation markedly towards cleavage (Donahue et al. 2000). The effect of crowding appears to be more pronounced at low ionic strength (Desai et al. 2014; Strulson et al. 2013), a feature of interest for fatty acid protocells that are sensitive to ionic strength (Adamala and Szostak 2013; Chen et al. 2005). Indeed, the cellular environment may stabilize active folds better than Mg⁺² alone (Tyrrell et al. 2013).

On the basis of the experimental findings outlined above, we hypothesize that the protocellular environment provides crowded conditions that might enhance evolutionary connectivity on the fitness landscape (see Fig. 2). A possible mechanism would be simply enhancing RNA folding and reaction rates, thus raising many sequences that are essentially inactive in dilute aqueous solution to a high level of activity. If this enhancement reaches a critical threshold it could potentially reveal a giant neutral network in sequence space (Gavrilets 2004). This means that crowded protocells might enable evolutionary optimization of functional RNAs that would not be possible in dilute aqueous solution. Furthermore, by increasing the frequency of functional sequences, crowding might make the evolution of novel functions substantially more probable. Thus, in addition to creating competition among individuals in heterogeneous populations of protocells (Szostak et al. 2001), compartmentalization in protocells might enhance Darwinian evolution through biophysical effects on encapsulated RNA.

An experimental test of our hypothesis is possible in principle, by comparison of fitness landscapes in the absence and presence of crowding agents (e.g., in the method of Jimenez et al.). Analogous in vitro evolution experiments carried out on longer RNA molecules, though unlikely to detect neutral networks, might provide valuable information about how the probability of discovering a functional RNA in sequence space depends on RNA length.

In summary, modern life is characterized by biochemical reactions in highly crowded environments. We hypothesize that crowding was also present in protocells and might have enhanced evolutionary optimization and innovation.

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Molecular Crowding and Early Evolution

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