WHY IS THE MINIMUM UNIT OF LIFE A CELL? : BUILDING AN "RNA WORLD" MODEL PROTOCELL USING DROPLET-BASED MICROFLUIDICS

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ABSTRACT

This paper reports building an experimental model of an "RNA world" protocell using droplet-based microfluidics. The model protocell is much closer to a prebiotic form of life rather than previously reported synthetic cell models [1-3], since it does not contain a complicated cell-free protein expression system. Experimental evolution of this simple model protocell is a powerful model to investigate the role of compartmentalization in early biology. This study experimentally demonstrates that proto-cellular compartments are essential for early life-system evolution.

KEYWORDS

Model protocell, RNA world, Evolution, Compartmentalization, Droplet-based microfluidics.

INTRODUCTION

Why is the minimal unit of life a cell? This is one of the most fundamental questions in biology. Several theoretical approaches proposed that the cellular compartment was essential for reproduction and evolution of early life. A primitive form of life supposes to be composed of several replicating functional polymers (e.g. RNA) which have catalytic activities in metabolism. If the molecules are not compartmentalized, Darwinian selection works only based on replicability of each molecule. Consequently, parasitic molecules dominate the environment and finally other less-replicable molecules will be exterminated. In other words, selfish molecules exploit other functional altruistic molecules, and the system will collapse. However, if the molecules are compartmentalized in a protocell, the level of selection is shifted from a molecule to a protocell, as a result the protocells dominated by parasites are driven out and functional cooperation of molecules is maintained.

To investigate the role of compartmentalization and verify this hypothesis experimentally, we built an "RNA world" model protocell with water-in-oil (w/o) emulsion, using droplet-based microfluidic technology (Figure 1). In this model protocell, a ribozyme (catalytic RNA) replicates as a genetic molecule, which concurrently generates fluorescence as a result of its catalytic activity (phenotype). After the ribozyme replication, the phenotypically active protocells can be selected using a droplet sorting microfluidic device.

EXPERIMENTAL

Development of the biological system inside droplets is mentioned in the main text. The substrate RNA of the ribozyme is conjugated to Alexa Fluor 594 fluorophore and BHQ-2 quencher. Microfluidic chips were fabricated by patterning channels in poly(dimethylsiloxane) (PDMS) using conventional soft lithography methods [4] as described previously [5] to produce droplet size of 12 pL. The oil phase consisted of HFE-7500 (3M) fluorinated oil containing 2% (w/w) of EA surfactant (RainDance Technologies, Lexington, MA), a PEG-PFPE amphiphilic block copolymer [6]. Droplets were generated by flow-focusing [7]. The produced emulsions were collected in a home-made glass capillary [8] and incubated at 37°C. The incubated droplets were reinjected into a sorting device [9] and sorted depending on fluorescence measured using a previously described optical set-up [5].

RESULTS AND DISCUSSION

First, we developed a "replicator" ribozyme that is composed of two RNA domains, the replication domain and the catalytic domain (Figure 1A). It can replicate *in vitro* in the presence of a viral RNA replicase (Qβ replicase) due to the replication domain, but also has a catalytic activity (RNA cleavage activity) which is unrelated to the replication ability. The catalytic activity generates a fluorescent product. Next, the ribozyme, its substrate, and replicase were put into highly monodisperse water-in-oil emulsion droplets that function as analogues of proto-cellular compartments using a microfluidic flow-focusing droplet maker device (Figure 1B). In the model protocell, the ribozyme replicates as a genetic molecule (the replication can be monitored with green fluorescence by an intercalating SYBR Green dye), which concurrently generates orange fluorescence as a result of its catalytic activity (phenotype).

We performed iterative rounds of the ribozyme replication to simulate the protocell life cycle (Figure 1B). The procedure consists of four steps. First, the ribozyme and replicase are compartmentalized in emulsions on the droplet maker device (protocell generation). Second, the produced protocells are incubated off-chip for replication of the ribozyme and expression of the catalytic activity. Third, the protocells are selected using a dielectrophoresis-based droplet sorting device in a phenotype-dependent manner. Fourth, the selected droplets are broken and its RNA contents are extracted, following dilution of the RNA. Then, the diluted RNA is subjected to the next generation of life cycle.

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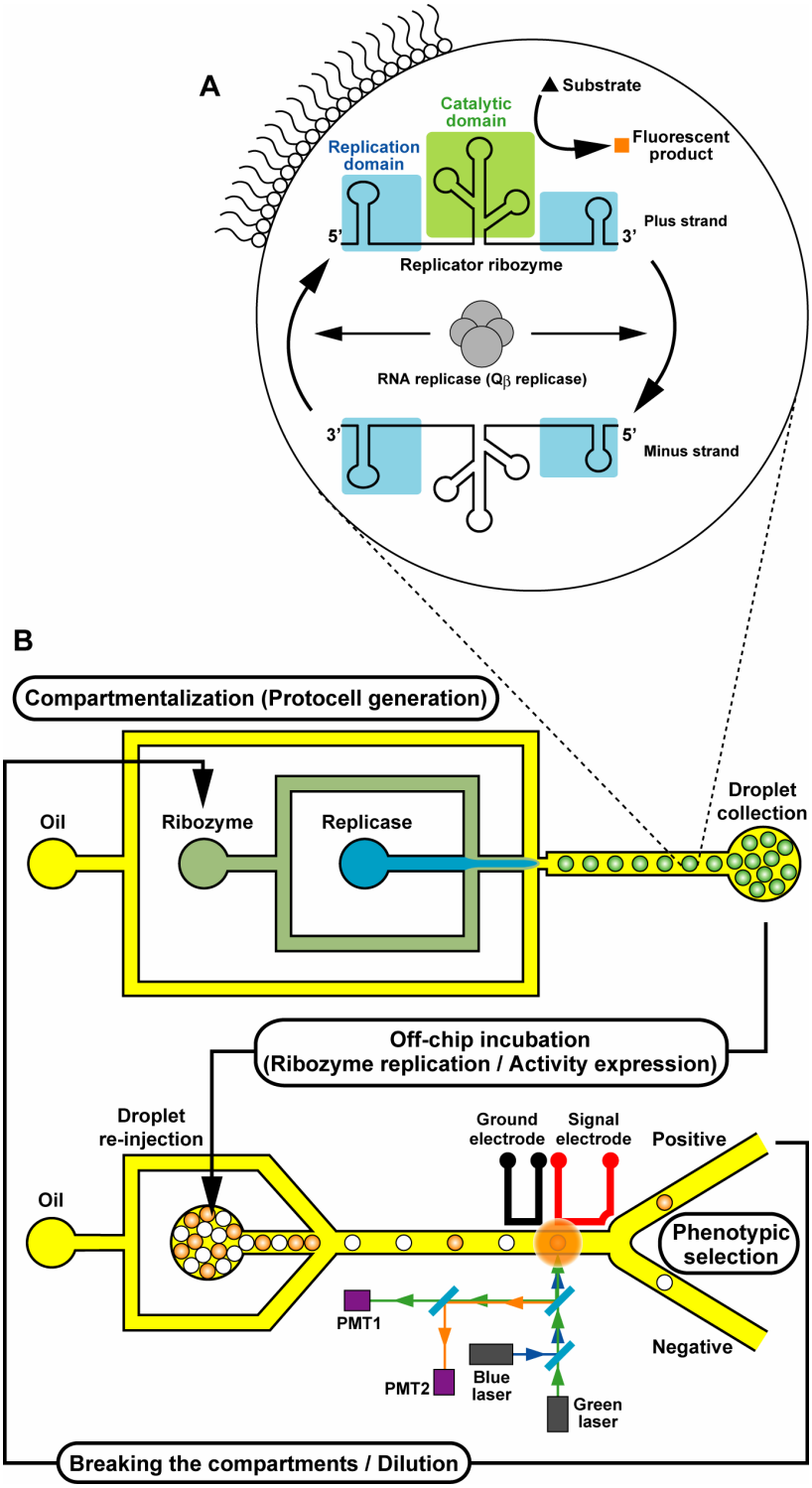

Figure 1. *Experimental system of an "RNA world" model protocell. (A) Schematic representation of the "RNA world" model protocell. The protocell contains a replicator ribozyme, its substrate, and the RNA replicase. In the protocell, the ribozyme replicates autonomously, and also generates fluorescent products. (B) Workflow of the protocell simulation. It consists of four steps (see the text) including two microfluidic divices, flow-focusing droplet maker (upper) and dielectrophoresis-based droplet sorter (lower).*

We carried out three types of experiment. First, the ribozyme replication was performed in the absence of compartmentalization (bulk solution). In this situation, the catalytic activity of the ribozyme disappeared at the 4th round (Figure 2A, Non-compartmentalized). It is due to takeover by catalytically inactive but rapidly replicated parasitic (selfish) RNAs (Figure 2B, Non-compartmentalized). The appeared parasites dominated the environment and consumed resources in the absence of physical barriers. Second, the ribozyme replication was done in the presence of compartmentalization (in droplets), but with no phenotypic selection. The catalytic activity was preserved until the 6th round, which was longer than the non-compartmentalized situation, but it disappeared at the 8th round (Figure 2, Compartmentalized, No phenotypic selection). The penetration of the parasitic RNAs is slowed, but parasites still take over after several generations. In this case, the compartments act as physical barrier to prevent expansion of the parasites, but lack of the phenotypic selection causes domination of the parasites at later phase. Third, we performed the protocell life cycles with phenotypic selection. The catalytic activity and the full length ribozyme were preserved until the 9th round (Figure 2, Compartmentalized, Phenotypic selection). This

demonstrates experimentally that the protocell-based "group" selection effectively prevents takeover by parasitic molecules in line with theoretical predictions, implying the importance of cellular compartments for the evolution of catalytic activity and molecular altruism in the RNA world.

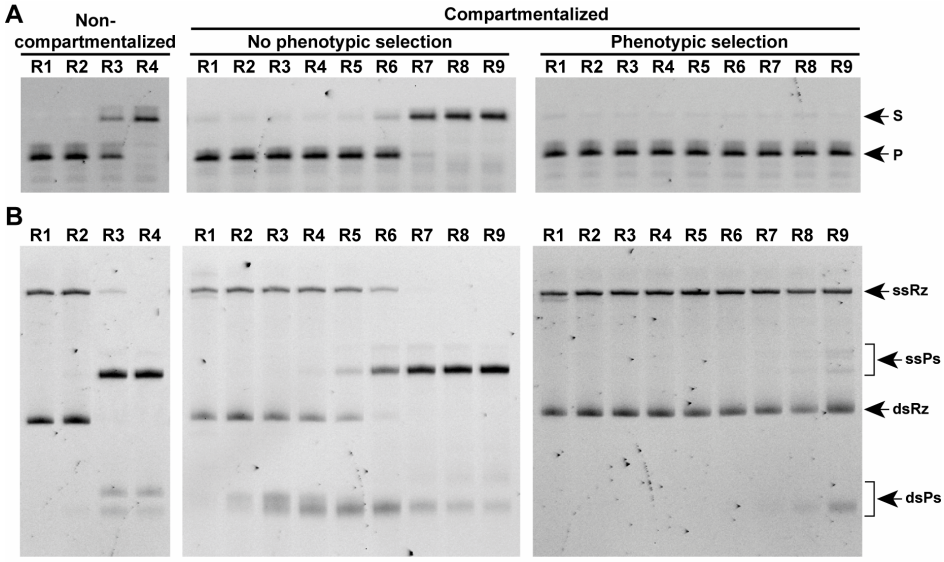

Figure 2. *Gel electrophoresis analyses of the catalytic activity (A) and RNA species (B) at each round of protocell life cycle. (A) The upper and lower bands indicated by arrows correspond to substrate (S) and product (P) RNAs, respectively. (B) The bands indicated by arrows correspond to single-stranded ribozyme (ssRz), single-stranded parasite (ssPs), double-stranded ribozyme (dsRz) and double-stranded parasite (dsPs), respectively.*

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