



Research Article

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Emergence of Autonomous Genomic DNA by Gas-Exposure

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Abstract

Artificial cell-like structures can emerge from minimal molecular components, providing a platform for exploring autonomous biological behaviors. In a previous study, a DNA fraction (SO-DNA) from artificial cells (termed G-Sph crown cells) was generated from sphingosine (G-Sph), DNA, and adenosine, producing autonomous colony-like structure (SD-cells) during cell culture. The findings suggested that gas is transmitted to DNA from gasses produced by G-Sph. The present study examined whether gas-exposed DNA (hereafter referred to as Auto-DNA) formed SD-like cells. The findings showed that Auto-DNA emerge by gas exposure, resulting in the formation of SD-like cells with Auto-DNA. These findings can be used to define a new research area characterized by unique, autonomous, synthetic DNA systems. In these systems, autonomous DNA not only acts as genetic information, but also as a driver of autonomous assembly and the emergence of biological behavior.

Keywords: DNA crown cells, Sphingosine-DNA, G-crown cell seeds, G-crown cells, Gas powder, Gas exposed DNA, Auto-DNA, AD crown cells

Introduction

Previous studies have examined the construction of self-replicating cells (artificial cells) from defined molecular components. Specifically, the author has previously reported methods for preparing self-replicating artificial cells, termed DNA crown cells, by combining sphingosine, DNA, and adenosine [1-5]. As a result, several kinds of artificial cells based on DNA crown cells, such as antibiotic-producing crown cells or crown cells nema, have been prepared [6-17]. Recently, G-Sph crown cells were prepared using gas-exposed sphingosine instead of sphingosine [18]. G-Sph crown cells, which can be cultivated over generations, could only be prepared with cultures containing mixtures of G-Sph, DNA, and adenosine. In previous experiments [19], when a DNA fraction (SO-DNA) was extracted from G-Sph crown cells and cultured, SD-cells, which develop into self-developing crown cells, emerge during culture with SO-DNA. This behavior suggests that SO-DNA acquires functional units capable of self-organization, and that the gasses

within G-Sph crown cells may be transmitted into DNA within G-Sph crown cells, generating Auto-DNA. Here, Auto-DNA (*E. coli*) refers to gas-exposed genomic DNA derived from *E. coli* which autonomously forms Auto-Developing (AD)-crown cells. AD-crown cells (crown-like cells) are autonomous crown-like cellular structures that are generated from Auto-DNA prepared using gas-exposed *E. coli* genomic DNA which displays enzyme-independent auto-development activity. Therefore, in this study, it was examined whether gas-exposed Auto-DNA and AD-crown cells could be formed from Auto-DNA, which could potentially play the role of DNA in non-genetic biological functions.

Materials and Methods

Materials

E. coli DNA (Sigma-Aldrich, USA) were dissolved in distilled water (5mg/3 mL). Gas-exposed DNA was prepared by gas-powder

treatment [18]. And agar plates and agar medium were prepared using SMA (AS ONE, Japan).

Methods

Preparation of Gas-Exposed DNA: The gas-exposed DNA was prepared using the method used for the preparation of G-Sph, which was prepared using cultures of gas powder (antibiotic-producing crown cells) [18]. Briefly, egg white powder-enclosed bovine crown cells with blueberry extract were cultured. When monolaurin was added to the cultures, gases (e.g., dimethyl di-sulfide and others) were produced [20]. Then, a dish containing monolaurin was placed on the cultures and 0.5 mL of DNA solution was added to the dish. After 2 days of culture at 37°C, the DNA within each dish was collected by adding 0.5mL of distilled water. This DNA, which was used as gas-exposed DNA (Auto DNA (*E. coli*)), was stored in a freezer at approximately 4°C until use Cultures using Auto DNA.

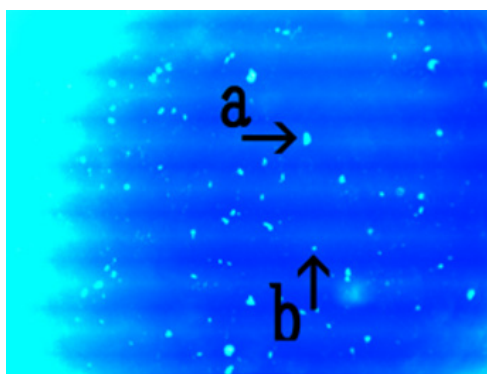
A total of 50μL of Auto-DNA (approximately 45.00μg) was poured onto agar plates and spread using a culture spreader. The plates were then incubated at 37°C for 1 day.

Secondary Culture: Objects which developed after 1 day of culture were collected by adding 6 mL of distilled water. Then, 200μL of this mixture was poured onto a new agar plate, spread using a culture spreader, and incubated at 37°C for 1 and 2 days.

General Observations: Objects on the plates could be observed directly with the naked eye and under a light microscope.

Results and Discussion

Figure 1 shows a microscopic image of Auto-DNA. Direct gas exposure induced the spontaneous emergence of round microscopic objects within 2 days. These objects comprised assemblies consisting of several DNA particles and auto-DNA.



Note*: Objects like round and dot were observed (Fig1a and b). Approximate size of (Fig.1b) was 7μm.

Figure1: Microscopic image of Auto DNA.

Figure 2 shows a colony-like structure formed during primary culture with Auto-DNA, which produced microscopic colony-like spots on agar medium within 1 day. The colonies exhibited circular

or irregular shapes and expanded gradually, demonstrating proliferative behavior triggered solely gas exposure.

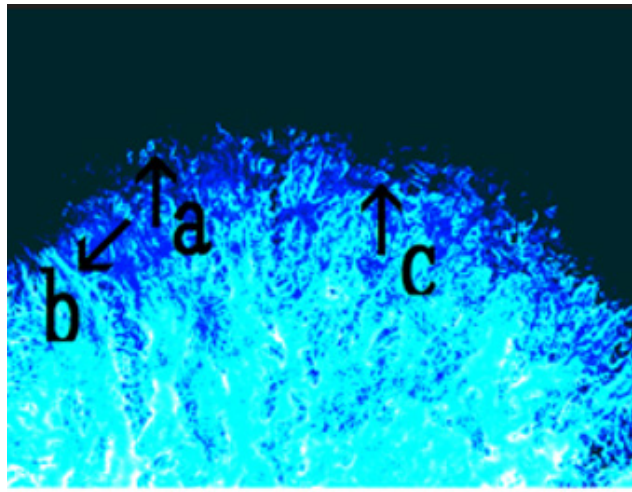


Note*: Circular or irregular shaped objects were observed.

Figure 2: Colony formation on agar medium Photograph of primary cultures of Auto DNA.

Figure 3 shows the microscopic appearance of primary colonies derived from Auto-DNA. Microscopic observation of primary colonies revealed dense aggregates of autonomous DNA-derived parti-

cles. Various particles of differing morphology proliferated from the Auto-DNA, indicating continuous culture and the emergence of macroscopic structures.



Note*: Image shows microscopic appearance of edge of objects like colony after 1 day of primary culture. Round (Fig. 3a) or bar (Fig.3b) like objects in various sizes were observed. Approximate size of Fig. c is 30~40µm.

Figure 3: Growth behavior of AD-crown cells on agar medium.

Figure 4 shows secondary colony formation with sustained autonomous proliferation. Material transferred from primary colonies generated secondary colonies, which were more uniform in

size and morphology, suggesting stabilization of the autonomous proliferation system across the culture cycle.

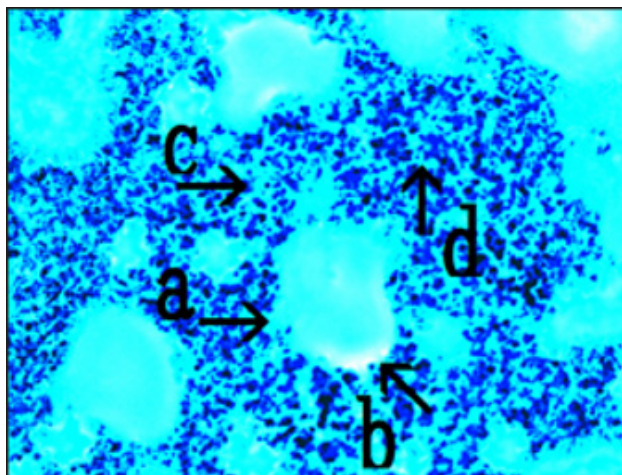


Note*: Microorganism like objects were observed across the entire dish.

Figure 4: Colony formation of secondary cultures.

Figure 5 shows the microscopic appearance of secondary colonies, which contained abundant particles that differed in shape and size from those in primary culture. The persistence of these

structure confirmed that Auto-DNA maintained autonomous proliferative capacity through multiple generations of culture.



Note*: Bar like objects were observed (Fig.5c). Large objects on the bar like object (Fig.5 c) were observed (Fig.5a). The objects contained a colored structure (Fig.5b). Approximate size of the objects shown in Fig.5d, Round object is 8-10 μ m.

Figure 5: Microscopic appearance of a secondary colony.

A previous study showed that G-crown cells constructed from G-Sph, DNA, and adenosine, give rises to a unique DNA-derived fraction (SO-DNA) which exhibited autonomous behavior [19]. Given that G-crown cells can be serially cultured [17], envelope-forming particles may be contained within the original G-crown cells. These particles were therefore contained within the DNA fraction (SO-DNA). So how does SO-DNA acquire the autonomous ability to generate a colony like structure (SD-cells). Sph-associated gas components within G-Sph crown cells were transmitted to DNA within the cells and gas-associated DNA (Auto-DNA) to form autonomous units. Thus, a notable finding of this study is that gas-exposed DNA exhibits autonomous properties. In order, first, Auto-DNA emerges and that AD-crown cells are then formed. Further, it is considered that there may be some interactions with G-Sph, DNA, and adenosine during cell construction of G-Sph crown cells and SO-DNA, resulting in the formation of development-inducing particles [21,22].

On the other hand, it is unclear whether Sph is contained in SO-DNA. However, it is clear that sphingosine was not contained in Auto DNA. Therefore, Sph may not be directly associated with the emergence of autonomous DNA. The challenge therefore remains as to what role sphingosine plays in autonomous behavior. It is possible that Sph facilitates gas adsorption, resulting the formation of G-Sph-DNA particles. If so, then the gas of G-Sph may have been transmitted into DNA. Then, Gas-associated DNA which represents autonomous ability emerge. Thus, sphingosine may play a role in gas transmission. Such mechanisms could be used to explore how gas-exposed DNA exhibits autonomous behavior and produces AD-crown cells. In other words, the autonomous behavior observed in auto DNA may be attributed to gas-induced modifications that initially occur in G-Sph, which are then subsequently transmitted to the DNA fraction.

On the other hand, SD-cells, or AD-crown cells generated from

SO-DNA and Auto-DNA may exhibit distinct morphological or developmental characteristics reflecting differences in the underlying autonomous properties of their SO-DNA and Auto-DNA. Thus, the emergence of SD-cells from SO-DNA [23] and AD-crown cells from Auto-DNA suggests that multiple types of autonomous DNA may exist. Further, the concept of SO-DNA and Auto-DNA potentially extends the function of DNA beyond its conventional role as a store of genetic information. In this system, DNA acts as a driver of autonomous assembly, contributing to emergent biological behavior without requiring intact cellular machinery. This positions SO-DNA and Auto-DNA within the emerging field of systematic autonomous DNA systems, which explore DNA-based self-organization and minimal autonomous units. Overall, SO-DNA and Auto-DNA potentially provide new platforms for investigating autonomous molecular systems. Although Auto-DNA provides a valuable model for pre-biotic molecular systems, the present findings also suggest the possibility that Auto-DNA behavior may occur under present-day environmental conditions and this will be an important question for future studies.

Acknowledgement

None.

Conflict of Interest

None.

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