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Review

# Spatial distribution of high copy number plasmids in bacteria

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Plasmids play essential roles in bacterial metabolism, evolution, and pathogenesis. The maintenance of plasmids is of great importance both scientifically and practically. In this mini-review, I look at the problem from a slightly different point of view and focus on the spatial distribution of high copy number plasmids, for which no active segregation mechanism has been identified. I review several distribution models and summarize the direct and indirect evidence in the literature, including the most recent progress on measuring the spatial distribution of high copy number plasmids using emerging super-resolution fluorescence microscopy. It is concluded that many open questions remain in the field and that in-depth studies on the spatial distribution of plasmids could shed light on the understanding of the maintenance of plasmids in bacteria.

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## 1. Introduction

Plasmids, which are extrachromosomal genetic elements, play important roles in essential activities in bacteria such as metabolism, virulence, resistance to drugs, and evolution (Coplin, 1989; Diaz Ricci and Hernández, 2000; Silva et al., 2012; Thomas and Nielsen, 2005; Vivian et al., 2001). In addition, due to their small size, typically 1–100 kb (Thomas and Summers, 2008), and easy manipulation, plasmids have been widely used in fundamental research and industrial fermentation, as well as agricultural and medical applications (Grabherr and Bayer, 2002; Tolmasky and Alonso, 2015). Therefore, it is both scientifically and practically important to understand plasmids and their maintenance.

Since their discovery six decades ago, with the name—"plasmid"—coined by Joshua Lederberg in 1952 (Lederberg, 1952), plasmids have been extensively studied in many aspects, including physical structures and biochemistry properties, replication systems, conjugative machinery, genetic traits, partition, as well as the mechanism and regulation of these properties and processes (Kado, 2014; Tolmasky and Alonso, 2015). Exciting progress has been made in plasmid biology but many fundamental questions, such as how plasmids are maintained in bacteria over generations and why they persist in natural and artificial environments, are still not completely answered (Kado, 2014). As antibiotic resistance of bacteria has become a severe public threat (Marchaim et al., 2011; Papp-Wallace et al., 2011; Perez and Van Duin, 2013; Sievert et al., 2013), it is particularly worthwhile

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to understand the fundamentals of the maintenance of plasmids in bacteria.

Low copy number plasmids have developed a range of active partitioning systems for their inheritance in daughter cells (Dmowski and Jagura-Burdzy, 2013; Gerdes et al., 2010; Nordström, 2006; Salje et al., 2010; Thomas, 2002; Watanabe et al., 1992). However, no such active partitioning system has been observed for high copy number plasmids (typically ≥10 copies per cell) (Summers, 1996). Although speculations of the existence of active partitioning mechanisms for high copy number plasmids have been made, such hypothetical genes have not been identified (Million-Weaver and Camps, 2014). In addition, if these genes existed, due to the high variety of high copy number plasmids, it is likely that these genes would have to be encoded on the chromosomal DNA of all kinds of bacteria (Wang et al., 2016). An alternative solution could be that bacteria maintain their plasmids over generations by organizing them in effective and efficient ways; the topic of spatial organization of high copy number plasmids has gained relatively less attention in the field of plasmid biology.

In this mini-review, I focus on the spatial distribution of high copy number plasmids by reviewing several distribution models and summarizing the direct and indirect evidence in the literature. First, the random distribution model of plasmids (Fig. 1A) is presented with evidence that initiated and supported this model. Then, experimental results that challenged the random distribution model but pointed to the clustering model (Fig. 1B) are revisited in detail. Finally, a hybrid distribution model (Fig. 1C) is reviewed and the most recent progress on the spatial distribution of plasmids using emerging super-resolution fluorescence microscopy is summarized. I conclude that many open questions remain in the field and that in-depth studies on the spatial distribution of plasmids might provide a different angle to understand the maintenance of plasmids in bacteria.

## 2. Random distribution model

A random distribution model was proposed a long time ago by Durkacz and Sherratt based on their observation about the segregation kinetics of a naturally occurring plasmid—ColE1 (Durkacz and Sherratt, 1973). With *E. coli* bacterial cells that were temperature sensitive for DNA polymerase I, Durkacz and Sherratt measured both the copy number of ColE1 per cell (10–17 copies) and the kinetics of plasmid loss (Durkacz and Sherratt, 1973). Their results suggested that ColE1 plasmids are well inherited in daughter cells during division by a random process (Durkacz and Sherratt, 1973).

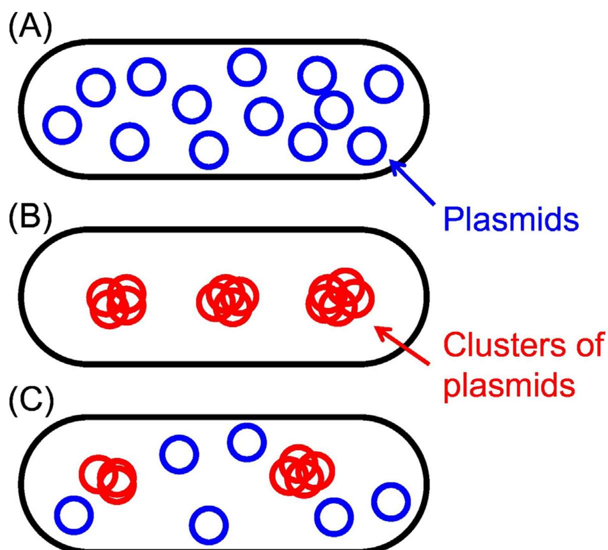


**Fig. 1.** Spatial distribution of high copy number plasmids in bacteria. (A) Random distribution model. (B) Clustering of plasmids. (C) Hybrid distribution of plasmids.

In the random distribution model, the plasmids diffuse freely throughout the cytoplasm before cell division and randomly segregate during cell division (Durkacz and Sherratt, 1973; Nordstrom and Austin, 1989; Summers, 1998, 1991). For completely random segregation between cells, the probability of generating a daughter cell without a plasmid is $p_0 = 2^{1-2n}$ where 2n is the number of plasmids in a bacterium immediately before cell division (Nordstrom and Austin, 1989; Summers, 1998, 1991). The loss rate, or the probability of formation of a plasmid-less cell per cell per generation, is then $L = 2^{-2n}$ (Nordstrom and Austin, 1989; Nordström and Gerdes, 2003; Summers, 1991). If the copy number of plasmids is high enough (>10 copies per cell), the theoretical loss rate is vanishingly small ($<10^{-6}$; Nordstrom and Austin, 1989; Summers, 1998, 1991). The loss rate of ColE1 plasmids was estimated at least $<10^{-5}$ and thus consistent with the random distribution model, although the exact loss rate of ColE1 was difficult to measure experimentally (Durkacz and Sherratt, 1973; Summers and Sherratt, 1984).

Furthermore, the discovery of "helper elements" for multimer resolution of plasmids indirectly supported the random distribution model of plasmids (Austin et al., 1981; Summers and Sherratt, 1984). It was observed that ColE1 plasmids were much more stable (loss rate $< 10^{-5}$) than many reconstructed ColE1 derivative plasmids (loss rate is between $10^{-2}$ and $10^{-5}$) (Summers and Sherratt, 1984). Summers and Sherratt investigated this issue and found that ColE1 and related plasmids were partitioned randomly at cell division; they concluded that the instability of certain ColE1-derivatives was due to multimerization of the plasmids (Summers and Sherratt, 1984). In this beautiful work, it was found that ColE1 plasmids encode a determinant, *cer*, facilitating the efficient resolution of multimers to monomers (Summers and Sherratt, 1984). The *cer* region cloned into other plasmids that lacked dimer resolution systems (e.g. pAT153 and pUC8, both were derived from pBR322) greatly increased their stability (Summers and Sherratt, 1984).

## 3. Clustering of plasmids

The random distribution model of plasmids had also been challenged for a long time. Co-location of high copy number plasmids was first reported by Summers and Sherratt (Summers and Sherratt, 1984). In this study, plasmids extracted via alkaline extraction (Birnboim and Doly, 1979) were run on agarose gels and heavy bands were observed, showing multimerization of the plasmids (Summers and Sherratt, 1984). Multimerization is the process for plasmids to form dimers and higher order oligomers due to, for example, recombination (Field and Summers, 2011; Smith, 1988). It is conceptually different from clustering of plasmids, which simply means that multiple plasmids are spatially close. Although clustering of plasmids is not necessarily due to multimerization of plasmids, multimerization will result in clustering of plasmids. A caveat to note is that high-molecular-weight DNA formed in cells, such as head-to-tail concatemers (Viret et al., 1991), are degraded in the Birnboim and Doly method (Birnboim and Doly, 1979). Therefore, possible higher order of plasmid-oligomers might have been invisible in this study (Summers and Sherratt, 1984). The indirect observation of plasmid-clustering (Summers and Sherratt, 1984) was supported by the first direct visualization of plasmids in bacteria (Eliasson et al., 1992). In this elegant work, plasmids (and inevitably the chromosome) in *E. coli* bacteria were stained with DAPI (4′,6-diamidino-2-phenylindole) and imaged directly under fluorescence microscopy (Eliasson et al., 1992). To distinguish the plasmids from the chromosomal DNA of the bacteria, elongated bacterial cells were obtained using non-permissive temperature (Eliasson et al., 1992). The distribution of two plasmids (pMN90—derivative of R100, and pOU420—derivative of pBR322) were investigated in this study and foci of plasmids were readily observed (Eliasson et al., 1992). It was found that the foci of pMN90 plasmids were regularly distributed along the interior of some of the cells, while foci of pOU420 plasmids

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