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COMMENTARY

Plasmid Segregation: Birds of a Feather Try Not To Flock Together[▽]

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Bacterial plasmids are extrachromosomal genetic elements having copy numbers ranging from one to a few thousand. The maintenance of plasmids in bacterial cells is dependent upon factors and processes that control their replication, selection, copy number, and segregation. Segregation of low-copy-number plasmids is generally dependent upon plasmid-encoded cytoskeletal elements that act as partitioning proteins (4). Inactivation of these proteins results in plasmid instability and loss (4). Past studies of segregation of the low-copy-number P1 plasmid (1 to 2 copies per chromosome) in *Escherichia coli* showed them to preferentially localize to the midcell or cell quarter position, depending upon their copy numbers and the cell division phase of the host (10). However, those studies were carried out on dividing cells with 2 copies of the plasmid, which is ideal for studying segregation—one copy is segregated to each daughter cell when the mother cell undergoes cell division. However, real-life scenarios could be far from ideal. In this issue, Sengupta and colleagues from the Austin group report their recent studies of P1 plasmid partitioning under more natural) conditions (14a). In their paper, they show that multiple copies of the low-copy-number P1 plasmid exist within the same cell in significant numbers, which is contrary to our present understanding. Using powerful cell biology tools and thoughtful studies that followed individual plasmid foci over extended periods, they discovered patterns that did not emerge in earlier time-averaged studies. They observed rampant chaotic behavior of plasmids. Their studies address how P1 plasmids segregate when they exist in more than the ideal copy number. Their data question the notion that sister plasmids are always paired and located at fixed positions within a cell and that segregation simply ensures the movement of sister plasmids to different daughter cells. The random pairings and diffusion of plasmid copies within a cell reported by Sengupta et al. (14a) invoke the possibility of random assortment of daughter plasmids (sisters and nonsisters) during cell division. Quite significantly, they show the existence of a self-correcting mechanism that places the copies of plasmids apart from each other and at more or less equal distances from each other when they exist within the same cell. Further investigations of the precise behavior of the P1 partitioning proteins at different growth rates and identification of hitherto unknown components will be crucial for deciphering the mechanistic basis for the dynamic self-correcting mechanism reported for the P1 plasmid by the Austin group. Due to the similarities in localization patterns of multicopy and high-copy-number plasmids that have been investigated so far (Fig. 1A) (13, 16), it will not be surprising if the self-correcting mechanism for plasmid localization reported here for the P1 plasmid proves to be widespread.

THE P1 PARTITION COMPLEX

In spite of their functional similarity to the eukaryotic spindle, the organization of plasmid segregation complexes is rather simple (8). The partition complex of P1 is a paradigm for understanding plasmid segregation. The P1 partition complex is composed of *parS*, which is a centromere-like element present on the plasmid DNA (7), and two P1-encoded proteins, ParA and ParB. ParB binds specifically to *parS*, and ParA binds to the *parS*-ParB complex (2, 15). Since multiple copies of ParB bind to the plasmid by spreading around the sequences surrounding the *parS* site, cells harboring the P1 plasmid and expressing ParB-fluorescent protein fusions form bright foci that can be used for visualizing the P1 plasmid foci *in vivo* (10). Most of the plasmid segregation complexes (segrosomes) identified so far show similar organizations consisting of three basic elements—a DNA sequence element, a DNA binding protein that binds to this sequence, and a cytoskeletal protein that binds to the complex formed between the two previous components (4, 8). Due to the similarities between plasmid segregation systems, new insights into P1 partitioning are potentially of wide significance in improving our understanding of plasmid segregation mechanisms.

MULTIPLE PLASMID COPIES AND DIFFUSION

Earlier studies describing P1 plasmid localization utilized overexpression of ParB-green fluorescent protein (GFP) fusion for visualization of plasmid foci, which caused plasmid instability (10). In this issue, Sengupta et al. describe plasmid localization studies carried out under more physiological conditions (14a). They immediately noticed that P1 exists in multiple copies within *E. coli* cells. This implies that P1 could actually be a multicopy plasmid under natural conditions. Considering the sensitivity of the quantitative PCR (qPCR)-based plasmid copy number assay used by Sengupta et al., this might indeed be the case. Other multicopy plasmids, such as RK2 and ColE1, associate into clusters that localize to fixed positions within cells (13, 16). For this reason, foci formed from

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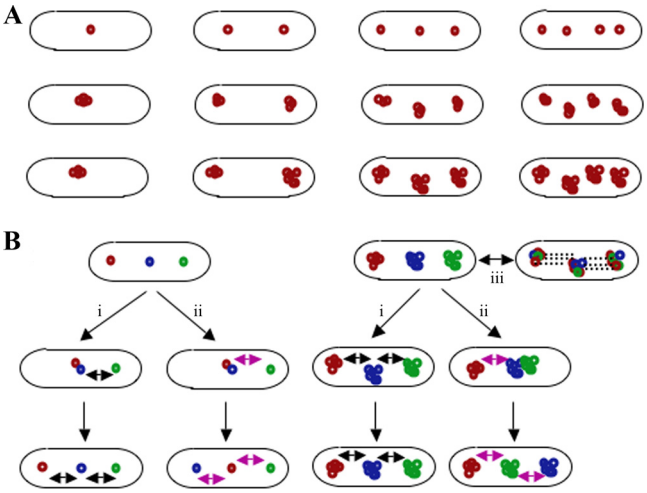

FIG. 1. Focus formation by plasmids. (A) Focus formation is not unique to low-copy-number plasmids. Multicopy and high-copy-number plasmids have been shown to assemble foci consisting of clusters of several plasmid molecules. The foci formed from clusters of RK2 or pUC plasmids localize similarly to those consisting of individual plasmids of P1 in terms of rapid separation from each other. (Top row) Focus distribution of the multicopy (4 to 6 copies per chromosome) P1 plasmid. (Middle row) Focus distribution of the multicopy (5 to 8 copies per chromosome) RK2 plasmid. (Bottom row) Focus distribution of high-copy-number (40 to 250) plasmids. (B) How strict is the conservation of the identity of foci? Strict conservation of focus identity means that plasmids cannot exchange their relative positions within a cell (black double-headed arrow). A more relaxed mechanism allows foci to shift their relative positions (purple double-headed arrow). In the case of foci assembled from clusters of high-copy-number plasmids, it is possible that distance correction occurs only between clusters, i.e., it does not involve individual plasmids of different foci (i or ii). On the other hand, members of individual clusters might also rapidly exchange positions from one cluster to another (iii). All of the mechanisms outlined here imply the existence of a plasmid copy-counting mechanism within individual clusters as a requirement for the splitting of foci when a cell divides. (Left) Distance correction in multicopy P1 plasmid foci. (Right) Distance correction in foci formed by clusters of multicopy or high-copy-number plasmids.

RK2 and ColE1 harbor more than one plasmid. Therefore, it appears that the behavior of P1 as a multicopy plasmid is unique—the copies resulting from replication separate from each other immediately, and the number of plasmids per focus is always one.

The Austin group observed that multiple plasmid foci formed from P1 are not located at fixed positions within the cell. Rather, they are highly mobile and are capable of diffusion over large distances. This is in stark contrast to the results reported for the multicopy RK2 plasmid by Derman et al. (3). In that study, the RK2 plasmid was found to be highly mobile in the absence of its ParA homolog. Expression of ParA resulted in fixed RK2 foci at particular locations within the cell (3). These results suggested that mobility is the default state and that partitioning proteins are required for "fixing" plasmids. In view of the contrasting observations for the P1 and RK2 plasmids, it remains to be seen whether the reverse is true for the P1 plasmid. Localization of the P1 plasmid in the absence of ParA should reveal whether it is less mobile under the natural conditions reported by Sengupta et al. Since P1 is a bacteriophage-derived plasmid (14), it is possible that many of the differences between P1 and other multicopy plasmids result from the unique biological origin of P1. Further investigation of other phage-derived plasmids should answer this question.

PAIRING, CLUSTERING, AND THE ROLES OF PARTITIONING PROTEINS

During segregation, replicated plasmids are initially paired by the action of the protein components of the segrosomes, and subsequently, each copy in a pair is pushed away from the other by the action of the polymerizing or oscillating component of the segrosomal complex (4, 8, 12). As mentioned above, multicopy plasmids, such as RK2 and ColE1, form clusters that localize to fixed positions in a bacterial cell instead of being randomly distributed. Irrespective of their numbers, these clusters distance themselves equally from each other within a cell (13, 16). ColE1 lacks a plasmid-encoded partitioning system. The high-copy-number pAFS52 plasmid (a pUC derivative; 70 copies per cell under the conditions tested) also forms clusters that localize as well-defined foci that position themselves at equal distances from each other in the absence of plasmid-encoded partitioning proteins (13). From these results, it is not clear what actually drives the localization and segregation of plasmids that lack plasmid-encoded partitioning proteins. Since the localization of some plasmids, such as P1, F, and R27, is affected if their partitioning proteins are inactivated (6, 9, 11), the big question is, what is the real advantage of harboring a partitioning system? ColE1 clusters can be disrupted by pBAD33 (a p15A-based plasmid), leading to plasmid loss, but not by RK2 (16). However, ColE1 can be made more stable in the presence of pBAD by incorporating a partitioning system derived from another plasmid, such as F (16). Thus, it appears that partitioning systems probably started off as elements that provided additional fitness to the plasmids that harbored them.

In the studies described by Sengupta et al., copies of the P1 plasmid diffuse randomly and often come in contact with another copy within the same cell (14a). However, upon contact or pairing, as described by the Austin group, the plasmids do not remain together but continue their random diffusion so that over longer periods they correct their positions and space themselves more or less equally distant from each other. The clusters formed by the RK2 plasmid have been reported to vibrate in three-dimensional space (13). Similarly, approximately 30% of cells that formed pAFS52 foci seemed to harbor randomly diffusing plasmids (13). Currently, it is not clear if the vibrational or diffusional behavior reported for plasmid clusters formed by RK2 and pAFS52 is similar to the behavior of the P1 plasmid reported by the Austin group.

Pairing of already segregated plasmids does not appear to serve any useful purpose. Therefore, an important question that arises is whether pairing is noise or a necessity. A striking observation made by the Austin group is that the rate of separation of copies that become paired as a result of random diffusion is much lower than that of replicated copies of the P1 plasmid (14a). Therefore, further investigation is required to verify plasmid pairing by optical sectioning and the use of ParA mutants (Fig. 2). It remains possible that within the three-dimensional space of the cell, plasmid foci could diffuse ran-

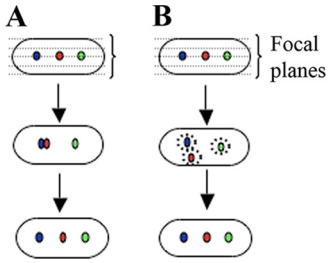

FIG. 2. Is plasmid pairing required for distance correction? Three foci within a single cell are shown in different colors for convenience. (A) Sectional view of a cell that supports pairing as a required step in distance correction. The dotted lines show the focal planes or depth of the cell. In this case, pairing is a step that is required to trigger partitioning of plasmids that are brought close to each other by random diffusion. (B) Sectional view of a cell that supports distance sensing without pairing. In this case, segregated plasmids are never allowed to come within a certain distance of each other (dotted circles).

domly and appear to pair because measurements are done only in the two-dimensional space. Such studies should address whether the dynamic self-correcting behavior of the P1 plasmid is directed toward overcoming the tendency of multicopy plasmids to cluster.

It is tempting to speculate that P1 may behave as an evolutionary intermediate in this respect—neither a true low-copy-number plasmid nor a clustering multicopy plasmid. It appears that newly replicated P1 plasmid copies are immediately separated, kept at maximum distance from each other, and not allowed to cluster. However, it is not clear how this behavior can increase the probability of passing down at least one copy to the next generation. It is possible that the phage-derived P1 plasmid overtly prepares itself for distribution to daughter cells even before the host chromosome has duplicated by replicating and separating its copies. It is also not clear whether there is any obvious advantage to random segregation of plasmids, even if the foci could exchange their locations. Therefore, it will be interesting to test if foci can really exchange places with each other as shown in Fig. 1B. In the case of clusters formed by multicopy and high-copy-number plasmids, it is possible that distance correction could occur either between clusters or between individual plasmids residing within two different clusters. In the latter case, one would expect to see periodic movement of plasmids in and out of individual clusters.

FUTURE QUESTIONS

Among plasmids that encode partitioning proteins, there are components that function by polymerizing, oscillating, or treadmilling (1, 4, 5, 12). Therefore, an interesting question is whether similar distance-correcting mechanisms exist in these plasmid segrosomes or if this behavior is unique to the P1 segrosome. Since multicopy and high-copy-number plasmids that do not encode any partitioning proteins form clusters, localize as foci to specific locations within a cell, and exhibit diffusional behavior in three-dimensional space, the larger question would deal with the precise role of partitioning proteins in plasmid localization and partitioning. Another question that should be addressed is the strength (the maximum

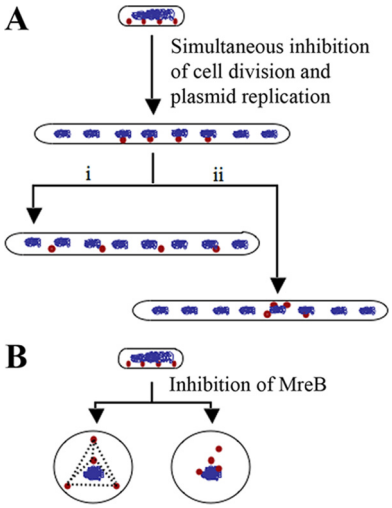

FIG. 3. Can the self-correcting plasmid distribution system sense and measure sudden changes in cell length? (A) How are copies of P1 plasmid distributed when cell division and plasmid replication are blocked simultaneously? (i) In the absence of plasmid replication, the available plasmid copies spread out within the given volume of a long filamentous cell, such as in an *ftsZ* mutant, by a self-correcting mechanism that senses the change in cell length. (ii) Plasmids do not sense the change in cell length. The distribution of available copies is restricted by pairing and inherent limitations of the partitioning apparatus. Only one of the many random possibilities is depicted. (B) How do plasmids localize in a spherical cell by the action of the self-correction mechanism? Will they form a tetrahedron or distribute randomly?

distance over which distance correction can take place) and/or adaptability of the dynamic self-correcting mechanism. It is already known that plasmid foci distribute evenly in the absence of cell division. Do a fixed number of foci redistribute and maintain distances in various *fts* mutants? For example, can plasmid segregation machinery sense and measure longer distances that occur in filamenting *E. coli* (Fig. 3)? Such adaptations might be useful under conditions where cell division is blocked due to DNA damage or slowed down due to nutritional deprivation. Similarly, it will be interesting to test how the P1 plasmid localizes in spherical cells induced by A22 (a compound that inhibits MreB) or by mutations in *mreB* (Fig. 3). The paper by Sengupta et al. sets the stage for testing the strengths and weaknesses of the dynamic self-correcting system. As mentioned above, the advantages of pairing between already segregated plasmids are not clear. Future studies should address whether pairing is noise in the system (a result of random collisions of freely diffusing plasmids) or a necessity (a step that is required for reversing the natural tendency of plasmids to assemble into clusters). It is possible that the partitioning system encoded by P1 represents an evolutionary intermediate between authentic low-copy-number plasmids that exist as foci harboring a single plasmid copy and multicopy plasmids that exist as clusters.

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