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Impact of catabolic plasmids on host cell physiology

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It is difficult to know the exact extent to which catabolic plasmids influence the metabolism of different hosts, but this information is crucial for improving the use of xenobiotic degraders possessing conjugative catabolic plasmids. To determine the molecular mechanisms by which catabolic plasmids affect host-cell physiology and host responses, comprehensive molecular surveys have examined host responses to plasmid carriage. These studies have clarified the various interactions between catabolic plasmids and host cells and the importance of the effects on host-cell physiology and metabolic pathways. It has been suggested that catabolic plasmid-borne nucleoid-associated proteins play key roles in the adaptation of catabolic plasmids to the host-cell regulatory network.

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Introduction

Among the various mobile genetic elements, plasmids occupy a distinct position because they can carry large genetic regions with a variety of auxiliary functions and can induce self-transfer into new host cells at a relatively high frequency [1–3]. This feature is advantageous to increase the genetic diversity of genomes. Moreover, many xenobiotic degradative plasmids (catabolic plasmids) have been discovered [4,5], and the number of catabolic plasmids reported is increasing.

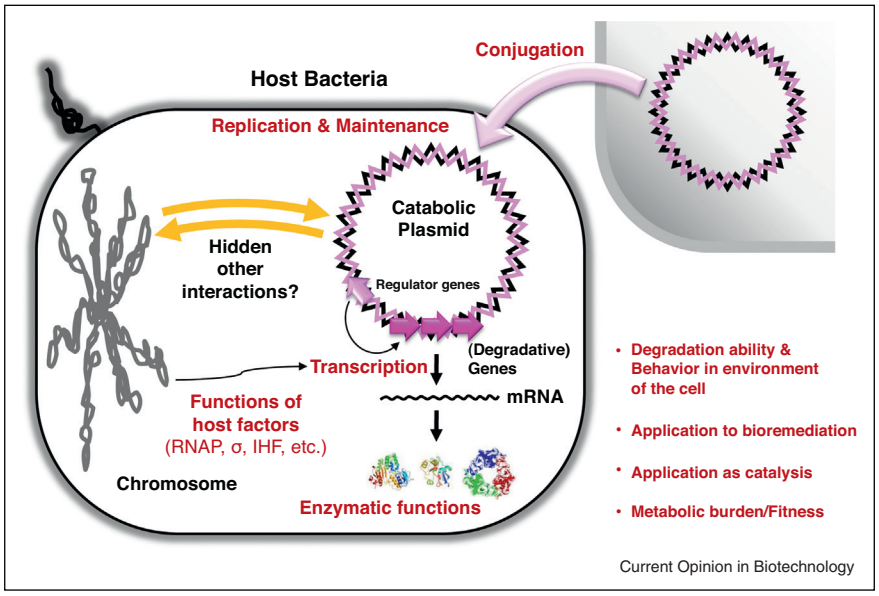
Many previous studies of catabolic plasmids aimed to clarify their degradation potential, their control of the expression of catabolic pathways, and their functions as vehicles with novel metabolic capacities (Figure 1). This knowledge is indispensable, but our understanding of how catabolic plasmids transform a host into a degrader is inadequate. It is presumed that there are hidden mechanisms determining the functional interaction between catabolic plasmids and host chromosomes. In addition, many catabolic plasmids are known to be conjugative, and accurately predicting the effect of the same catabolic plasmid on the degrading capacity of different hosts is problematic. Chromosome-encoded host factors are important for regulating the transcription of toluene/xylene degradative operons on the TOL plasmid pWW0 in *Pseudomonas putida* mt-2 [6,7]. However, their regulation via host factors may differ in other host strains. Plasmids replicate within their hosts at the expense of cell metabolic energy and act as a kind of parasite, with hosts bearing an unavoidable metabolic burden. Although the survival of a 'degrader' in a contaminated site is one of the key issues for successful bioremediation, we do not know exactly how the behavior, survival, and fates of both plasmids and hosts change among various hosts carrying the same plasmid. This review summarizes pioneering work that explores the impact of catabolic plasmids on host-cell physiology and responses, and discusses future directions in this research area.

Impact of the extrachromosomal elements on host cell physiology

IncP-7 carbazole-degradative plasmid pCAR1

The incompatibility (Inc) P-7 group 200-kb plasmid pCAR1 was originally isolated from *Pseudomonas resinovorans* CA10 and plays an important role by dispersing carbazole-degradative *car* and *ant* operons, at least in the genus *Pseudomonas* [8•]. The full nucleotide sequence of pCAR1 has been determined [9,10], and pCAR1 was proven to be conjugative experimentally [11]. To determine how pCAR1 alters the host transcriptional network, pCAR1-carrying and pCAR1-free host chromosomal transcriptomes were compared under succinate growth conditions [12]. pCAR1 had only subtle effects on *P. putida* KT2440 [13], except for the significant induction of *parI*, which encodes an orphan homologue of the ParA family of plasmid partitioning proteins from a cryptic genomic island (Figure 2). pCAR1 is maintained stably in KT2440 cells, whereas mini-replicons of IncP-7 plasmids, including pCAR1, pDK1, and pWW53, are unstable in KT2440 cells [14•]. This instability was recently shown to be attributable to interference with the IncP-7 plasmid partitioning system by ParI [14•]. Considering this fact, *parI* induction by pCAR1 carriage is noteworthy in relation to the fate of IncP-7 plasmids, although the reason for the stable maintenance of pCAR1 itself in KT2440 cells is unclear. To further understand the host chromosomal response to pCAR1 carriage, chromosomal RNA maps of the pCAR1-carrying and pCAR1-free hosts *P. putida* KT2440, *Pseudomonas aeruginosa* PAO1 [15], and *Pseudomonas fluorescens* Pf0-1 [16] were compared at the early-log phase using high-density tiling arrays [17••].

Figure 1



Research trends in the studies of catabolic plasmids. Research targets related to the functionalities of catabolic plasmids investigated during the past three decades are shown in red. As many of the studies examined a single combination of plasmid and host cell, it is presumed that there are hidden interactions between catabolic plasmids and host chromosomes.

Transcription of 121 (KT2440), 73 (PAO1) and 125 (Pf0-1) genes was found to be altered by pCAR1 carriage. For each strain, the number of genes upregulated was greater than those that were downregulated. Based on Clusters of Orthologous Groups of Proteins (COG) analysis, 'amino acid transport and metabolism' and 'inorganic ion transport and metabolism' are common categories among differentially transcribed genes in each strain. Remarkably, pCAR1 carriage induced the transcription of iron acquisition system genes in each host, for example, the production of the major siderophore pyoverdine was greater in pCAR1-carrying KT2440 and PAO1 strains than in pCAR1-free strains (Figure 2). Some carbazole-degradative Car enzymes contain iron at the active site and/or in the electron transport chain, and the *car* gene cluster is expressed constitutively in succinate-grown KT2440 cells [8•]. The iron requirement for the expression of *car* genes carried by pCAR1 is part the reason for the induction of iron acquisition machinery genes. The *mexEF-oprN* operon, which encodes an RND-family efflux pump, was specifically upregulated by pCAR1 carriage in KT2440 cells, resulting in hyper-resistance to chloramphenicol (Figure 2).

Alteration of the RNA maps of pCAR1 itself was assessed in various host *Pseudomonas* bacteria [18,19]. Genes for partition were differentially transcribed according to the host, whereas *repA*, which encodes replication initiation protein, was transcribed at comparable levels in all hosts. 'Accessory' genes encoding proteins involved in carbazole degradation, putative transporters, and transposases were also differentially transcribed in different host strains. These differences may partly explain the host-specific responses to pCAR1 carriage.

Integrative and conjugative element, ICE*clc*

Integrative and conjugative elements (ICEs) are self-transmissible mobile genetic elements [20,21]. Similar to plasmids, ICEs transfer via conjugation and like many phages they integrate into and replicate with the host chromosome. Several ICEs contain genes related to aromatic compound degradation, and a well-investigated example is ICE*clc*, isolated from *Pseudomonas* sp. B13. This 103-kb element contains genes involved in the degradation of 3-chlorobenzoate and 2-aminophenol [22,23]. The impact of ICE*clc* on the host *P. aeruginosa* PAO1 was investigated by transcriptome profiling, phenotype array analysis, competition experiments, and biofilm formation tests [24••]. Unexpectedly, few changes were observed. These included reduced biofilm growth and changes in the expression of 0.7% of chromosomal genes (42 of 5900 genes). The largest expression differences occurred in three potential operons on the PAO1 chromosome, which are believed to be involved in the transport of small molecules (PA3232 to PA3235), acetoin catabolism (*aco* genes), and glycolate catabolism (*glcDEF*), although none of these operons has known links to the degradation pathways encoded by ICE*clc*. PAO1 cells carrying ICE*clc* acquired the capacity to grow on 3-chlorobenzoate and 2-aminophenol, but no fitness