Versatile persistence pathways for pathogens of animals and plants

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The glyoxylate cycle and the glyoxylate cleavage system are part of conserved metabolic pathways involved in the chronic persistence of microorganisms in animal hosts. In the chromosome of the plant pathogen *Rhodococcus fascians*, the $vic$ locus has been identified as a region containing genes essential for persistence inside induced leafy galls. Sequence analysis showed that this 18-kb locus is syntenic with chromosomal regions of *Mycobacterium* species that encompass the ‘persistence’ loci of these mammalian pathogens. Hence, the ability to switch diet inside the host appears to be governed by ‘persistence’ enzymes that are conserved between pathogens of animals and plants.

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An important feature of the lifestyle of inter- and intracellular pathogens is the acquisition of nutrients from their host. For intracellular pathogens of mammals, a two-carbon diet through the glyoxylate cycle is crucial for virulence and persistence. The persistence of the bacterium *Mycobacterium tuberculosis* and the yeast *Candida albicans* inside macrophages depends on the activity of the glyoxylate shunt of the tricarboxylic acid (TCA) cycle to allow growth in an environment where fatty acids are probably the main carbon source [1–3]. Here, we show that the phytopathogenic bacterium *Rhodococcus fascians* has similarly adopted the glyoxylate shunt as a persistence pathway in infected plant tissues.

Persistence of pathogens of mammals

The glyoxylate shunt is a bypass of the TCA cycle that permits gluconeogenesis starting from acetyl-coenzyme A (acetyl-CoA), which is generated following fatty acid catabolism [4]. The shunt is composed of isocitrate lyase, which cleaves isocitrate to succinate and glyoxylate, and malate synthase, which condenses glyoxylate and acetyl-CoA to malate. As such, it circumvents the $CO_2$-generating steps of the TCA cycle and converts one molecule each of acetyl-CoA and isocitrate to two C4 compounds that can be fed into biosynthetic processes.

Mycobacterial persistence occurs after the emergence of the adaptive immune response. The intracellular environment of activated macrophages becomes more hostile and bacterial growth is restrained [3]. The pathogen adapts to the nutrient deprivation by shifting its metabolism towards the degradation of fatty acids. Expression of the isocitrate lyase gene is induced after phagocytosis in activated macrophages and its deletion results in reduced virulence, coinciding with a loss of persistence during lung infection of mice [1]. The reliance on a fatty acid diet is further illustrated by the relative abundance of *M. tuberculosis* genes encoding proteins involved in fatty acid oxidation [5].

Using whole-genome microarray analysis, the genes of the glyoxylate cycle have been shown to be induced in phagocytosed *Saccharomyces cerevisiae* cells [2]. In the related fungal pathogen *C. albicans*, both the malate synthase and isocitrate lyase genes are induced upon contact with macrophages [2]. When the isocitrate lyase gene is deleted, the virulence of *C. albicans* is reduced, suggesting that the interior of a macrophage is a glucose-deficient environment.

These observations show that for these bacterial and fungal pathogens of mammals persistence and virulence rely on the ability to switch diet within host cells. Furthermore, they suggest that an active glyoxylate cycle has widespread significance in the strategies used by these pathogens.

Persistence of the plant pathogen *Rhodococcus fascians*

The genus *Rhodococcus* is closely related to *Mycobacterium* and includes the species *Rhodococcus equi*, a facultative intracellular pathogen of macrophages in different animals [6]. The phytopathogenic species *R. fascians* infects a wide range of plants, provoking the formation of leafy galls consisting of masses of shoot buds that are suppressed for further growth [7]. Pathogenesis relies on a linear plasmid carrying virulence genes involved in synthesizing signal compounds that initiate cell division in plant cortical tissues, leading to the formation of shoot meristems [8,9]. Upon epiphytic colonization, endophytic forms are found in the intercellular spaces of gall tissues and, sometimes, inside plant cells.
The expression of the malate synthase gene was induced by extracts of plant and gall tissues. Growth of the mutant ceased rapidly when confronted with gall extracts, but not with extracts from uninfected plants. In symptomatic plant tissues the number of vic mutant bacteria was significantly lower than that of wild-type bacteria.

These results suggest that there is a shift in the diet of R. fascians during plant infection that requires specific metabolic reactions involving vic-encoded biochemistry. The absence of an active malate synthase in the vic mutant results in the accumulation of glyoxylate when bacteria are grown on gall extracts. Glyoxylate accumulation interferes with the metabolism of the bacteria and, ultimately, their viability, with a reduced virulence phenotype as a consequence [12].

Syntenic regions comprise ‘persistence’ loci
Interestingly, the malate synthase gene is part of a locus carrying 14 genes that are highly conserved both in sequence and organization with the corresponding genomic regions of M. tuberculosis and Rhodococcus fascians (Fig. 1). Also comparable to M. tuberculosis, the malate synthase gene of R. fascians is not linked to the previously cloned isocitrate lyase gene; its gene product has high sequence identity (55%) with malate synthase G, which is involved in glycolate utilization, and a lower identity (20%) with malate synthase A of Escherichia coli [5,12–14].

In addition to genes encoding hypothetical and membrane-targeted proteins, the syntenic region also carries the genes comprising the glyoxylate cleavage system (Table 1), which is responsible for the rapid production of glyoxylate when bacteria are grown on gall extracts.
What are the nutrient sources for *R. fascians* in leafy galls?

In congruence with *Mycobacterium*, the *vic* locus together with isocitrate lyase can be involved in the specific metabolism of fatty acids in leafy gall tissues. However, the physiology of the niche that is occupied by *R. fascians* – arial plant parts that are active in photosynthesis – could suggest other gall-derived nutrients. From our data, it is conceivable that the *vic* locus functions in the metabolism of glycine, glycolate and/or glyoxylate, in a pathway that does not require isocitrate lyase.

Interestingly, these compounds are shuttled between several cellular organelles of C3 plants during a process known as photorespiration [19,20]. Assuming that photorespiration is high in leafy galls, these intermediates could be produced in sufficiently large amounts to be scavenged by *R. fascians* and metabolized by the *vic*-derived enzymes.

Future research

Several questions remain to be answered to demonstrate further the analogy between the strategies used by *R. fascians* and *M. tuberculosis* and their respective hosts. What is the relationship between the metabolic adaptation and the intra- and intercellular forms of *R. fascians*? Are there leafy gall-abundant fatty acids that can sustain growth of *R. fascians*? Is the photorespiration level higher in leafy galls? Are the photorespiration intermediates used by *R. fascians*? If so, how are they obtained by the intercellular population? What is the role of isocitrate lyase in these processes, and hence in virulence?

In conclusion, it is apparent that *R. fascians* and its close relative *M. tuberculosis* have adopted similar metabolic pathways that are linked to persistence in animal hosts or particular plant tissues. The integration of the corresponding biochemistry into the host’s metabolism might differ between pathogens of plants and animals, but relies on common genetic grounds, thus illustrating the versatility of these persistence pathways in adaptation to different host environments.

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