Subsystem: Methylcitrate cycle

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Introduction

Fatty acids are important sources of carbon and energy for prokaryotes. While catabolism of medium-chain (C6 C12) and long-chain (>C12) fatty acids is known to proceed by β-oxidation to acetyl coenzyme A, short-chain f acids (<C6) are common fermentation by-products of microorganisms and are catabolized by prokaryotes throu different routes. The most complicated of these oxidation pathways is the breakdown of propionate (reviewed b Textor et al. (6)). One of the many know routes of propionate breakdown, the 2-methylcitric acid cycle, is enco in this Subsystem.

Subsystem Notes, open problems, conjectures

PrpE. An optional component, since propionyl-CoA can be formed by other routes. PrpE is clustered in a num of genomes. In *Shewanella*: no candidates except an Acetyl-CoA synthase ortholog. It appears that Acs c work as well as PrpE (KEGG: also acts on propanoate and propenoate). The pathway to propionyl-CoA i gram-positive bacteria remains to be elucidated. MISSING FLUX. For example, one possibility discusse Corynebacteria includes: "Thus, the activation of propionate to propionyl-CoA in C. glutamicum may be dependent on the activity of the phosphotransacetylase and the acetate kinase, encoded by the *pta-ack* ope since the acetate kinase Ack was shown to be active also with propionate as substrate, and since no acetyl CoA synthetase activity is measurable for *C. glutamicum* " (Ref.5)

PrpC. Annotation problem is an overlapping activity of 2-methylcitrate synthase and citrate synthase. Discuss Ref.5.

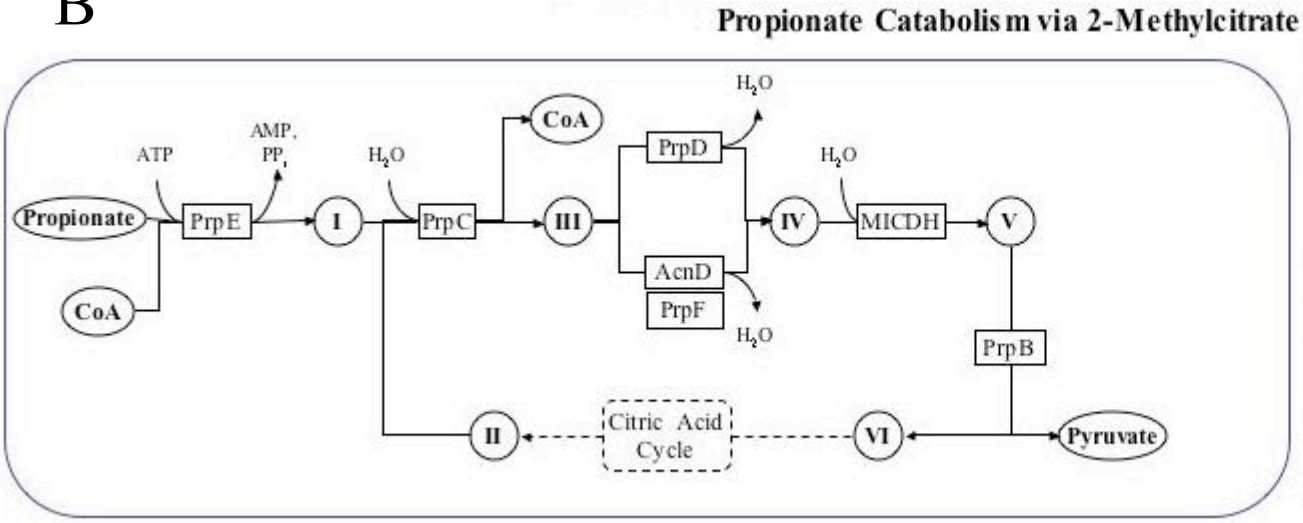
PrpD. A classic form, Fe/S independent. Not present in many bacteria where it is functionally replaced by AcnD/PrpF, see below.

The list of functional roles (A) and subsystem diagram (B)

A

Abbrev	Functional Role
prpC	2-methylcitrate synthase (EC 2.3.3.5)
prpD	2-methylcitrate dehydratase (EC 4.2.1.79)
prpB	Methylisocitrate lyase (EC 4.1.3.30)
prpE	Propionate--CoA ligase (EC 6.2.1.17)
PrpF	PrpF protein involved in 2-methylcitrate cycle
prpR	Propionate catabolism operon regulatory protein Pr
acs	Acetyl-coenzyme A synthetase (EC 6.2.1.1)
acnB	Aconitate hydratase 2 (EC 4.2.1.3)
acnD	2-methylcitrate dehydratase FeS dependent (EC 4.2
MICDH	2-methylisocitrate dehydratase (EC 4.2.1.99)

B



Abbrev	Functional Role
PrpE	Propionate--CoA ligase (EC 6.2.1.17)
PrpC	2-methylcitrate synthase (EC 2.3.3.5)
PrpD	2-methylcitrate dehydratase (EC 4.2.1.79)
AcnD	2-methylcitrate dehydratase Fe/S dependent (EC
PrpF	PrpF protein involved in 2-methylcitrate cycle
MICDH	2-methylisocitrate dehydratase (EC 4.2.1.99)
PrpB	Methylisocitrate lyase (EC 4.1.3.30)

Committed (connecting) intermediates	
Propionate	Propionic acid
I	Propionyl-CoA
II	Oxaloacetate
III	2-Methylcitrate
IV	cis-2-Methylaconitate
V	Methylisocitrate
VI	Succinate
Pyruvate	Pyruvic acid

Functional role abbreviations are as in Panel A. Key intermediates are shown in circles with Roman numerals explained in the inset. Reactions are shown by arrows (directionality shows the flow of biosynthesis without reflecting reversibility).

Subsystem notes continued

AcnD. An alternative form, Fe/S dependent. Experimentally characterized in *V.cholerae* and *S.oneidensis* in R
In these and several other genomes, the corresponding gene occurs in the cluster with *prpB* and prpC, ar
another gene termed *prpF* (homolog of *ybhH* in *E.coli*). PrpF was shown to be required for the AcnD
activity *in vivo*. AcnD/PrpF is a nonorthogous displacement of PrpD, which is critical for *S.oneidensis* a
other genomes lacking PrpD. AcnD is a close homolog of AcnA and mammalian mitochondrial cis-
aconitase. AcnA and AcnD share the Fe/S dependent mechanism and have overlapping activities, The r
differences are:

- preferred substrate (aconitate vs methylcitrate (MCA))
- AcnA is not active with MCA, but has an appreciable activity on methylisocitrate (MIC);
- AcnD is not active with MIC, but has an appreciable aconitase activity.

Surprisingly, *ybhJ*, an *E.coli acnD* homolog, failed in the complementation studies.

PrpF. Although no useful annotations can be currently found in public archives for this protein family (DUF54
homologs of *E.coli ybhH*), the corresponding gene from *Shewanella* and *Vibrio* were recently
experimentally characterized in an insightful study (Ref.1). Genetic complementation in *Salmonella* mo
implicated this gene in the 2-Methylcitrate cycle of propionate catabolism. It can be termed an accessory
protein required for in vivo functioning of the alternative Fe/S-Dependent 2-Methylcitrate Dehydratase
(termed *acnD*). A combination AcnD/PrpF is a nonorthologous functional replacement of the previously
characterized classic form of 2-Methylcitrate Dehydratase (gene *prpD* of *E.coli,* "missing" in *S.oneiden*
and several other bacteria). The precise molecular function of PrpF is still undetermined, and several
possibilities should be explored:

a) ISOMERASE of one of the intermediates in the conversion of Methylcitrate (MCA) to Methylisocitrate (MI
This possibility is discussed in general terms but not favored by Ref.1. Notwithstanding the strength of t
arguments, we believe that another evidence, a long-range sequence similarity between PrpF and severa
thiol-dependent isomerases (left unnoticed so far), calls for revisiting this possibility. Proline racemase
diaminopimelate epimerase were detected as the best hits using FFAS server (http://ffas.ljcrf.edu/ffas-
cgi/cgi/).

Subsystem notes continued

b) ASSEMBLY OF Fe-S CLUSTER required for the activity of AcnD. In spite of certain caveats (see Ref.1), authors seem to favour this interpretation.

The most puzzling are the data in Ref.1 suggesting that ybhH and ybhJ genes, E.coli homologs of prpF and acnI failed to functionally replace their counterparts from *Shewanell*a and *Vibrio*. Notwithstanding these data v retain the respective annotations for E.coli genes. We expect a clarification of this puzzle coming soon in t literature.

MICDH. AcnA and AcnB were shown to play an additional role of "missing" 2-methylisocitrate dehydratase (4.2.1.99) [Ref.4]. Distribution of the exact substrate preferences in this family of paralogs appears to be extremely convoluted.

PrpB. Conserved gene and the most straightforward assignment. Can be used as a pathway signature.

Regulatory factors: **PrpR** was experimentally defined in *Salmonella.*

PREDICTION: Based on chromosomal clustering, we predict another protein of GntR family to be a transripti regulator of the prp operon in P.aeruginosa (PA0797), S.oneidensis (SO0346)and several related species

Exact phylogenetic distribution of the pathway is yet to be described. It appears that there is an indication of its presence in some archaea.

Subsystem spreadsheet (fragment)

Organism	prpC	prpD	prpB	prpE	PrpF	prpR	acs	acnB	acnD	MICDH	
Vibrio cholerae O1 biovar eltor str. N16961 [B]	1322		1321	1325	1324		294	595	1323	595	1
Shewanella oneidensis MR-1 [B]	322		323, 981	2494	320		2494	408	321	408	3
Corynebacterium efficiens YS-314 [B]	718	716	717								
Salmonella enterica subsp. enterica serovar Typhi str. CT18 [B]	354	355	353	356		352	3963	162		162	
Pseudomonas aeruginosa PAO1 [B]	796	793	797	3568	794		255, 4731	1788	795	1788	7
Escherichia coli K12 [B]	330	331	328	332	755	327	3979	118	1263	118	

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