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Salmonella enterica Requires ApbC Function for Growth on Tricarballylate: Evidence of Functional Redundancy between ApbC and IscU[▽]

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Mutants of *Salmonella enterica* lacking *apbC* have nutritional and biochemical properties indicative of defects in [Fe-S] cluster metabolism. Here we show that *apbC* is required for *S. enterica* to use tricarballylate as a carbon and energy source. Tricarballylate catabolism requires three gene products, TcuA, TcuB, and TcuC. Of relevance to this work is the TcuB protein, which has two [4Fe-4S] clusters required for function, making it a logical target for the *apbC* effect. TcuB activity was 100-fold lower in an *apbC* mutant than in the isogenic *apbC*$^+$ strain. Genetic data show that derepression of the *iscRSUA-hscAB-fdx-orf3* operon or overexpression of *iscU* from a plasmid compensates for the lack of ApbC during growth on tricarballylate. The studies described herein provide evidence that the scaffold protein IscU has a functional overlap with ApbC and that ApbC function is involved in the synthesis of active TcuB.

Three systems for iron-sulfur ([Fe-S]) cluster biosynthesis have been identified. The first system is encoded by the *nif* (*ni*trogen *f*ixation) operon in *Azotobacter vinelandii* and is required for the biosynthesis of nitrogenase (reviewed in reference 15). The second system is encoded by the *iscSUA-hscAB-fdx-orf3* (*i*ron *s*ulfur *c*luster) operon of *Azotobacter vinelandii*. In *Escherichia coli*, the *iscSUA-hscAB-fdx-orf3* operon encodes housekeeping [Fe-S] cluster biosynthetic functions (20, 35, 55) and is regulated by the IscR repressor (49). A third system for the biosynthesis/repair of the [Fe-S] cluster has been described for *E. coli*. In this bacterium, the *sufABCDSE* (*s*ulfur *u*tilization *f*actor) operon is induced during times of limited Fe availability and oxidative stress (20, 28, 35, 37, 44, 54, 63). As in *E. coli*, the genome of *Salmonella enterica* carries both the *isc* and *suf* operons, and cellular viability requires the presence of one of the two (36).

The [Fe-S] cluster biosynthetic systems mentioned above have two general functional components. The cysteine desulfurase enzymes NifS, SufS, and IscS catalyze the removal of inorganic sulfide from L-cysteine (23, 33, 34, 43, 62), while the scaffolding proteins NifU, IscU, IscA, and SufA appear to bind and transfer labile [Fe-S] clusters to apoproteins (12, 27, 41, 56). Additional components can be specific to each system.

It was recently shown that cluster transfer from IscU to apoferredoxin is stimulated by the addition of HscA, HscB, and Mg · ATP (10). These data emphasized a role for ATP-hydrolyzing proteins, e.g., HscA and SufC, in the process of cluster maturation (10, 38, 50).

Work with *Salmonella enterica* serovar Typhimurium LT2 identified several loci outside the above-mentioned operons that impact [Fe-S] cluster metabolism. These loci include *apbC*, *apbE*, *rseC*, and *yggX*, all of which encode proteins with no characterized biochemical function (3, 4, 18, 45). Initially isolated as conditional thiamine auxotrophs, strains with lesions in these loci displayed phenotypic behavior similar to that of strains lacking *isc* operon functions (51–53). The *apbC* locus was the most common location of conditional thiamine auxotrophs identified in general screens (45). ApbC is a 40-kDa cytoplasmic protein that contains two conserved C-terminal cysteine residues separated by two amino acids (CXXC) and a Walker A box used for ATP binding and hydrolysis (25, 51).

Strains with lesions in *apbC* were independently isolated as mutants unable to use tricarballylate as the sole carbon and energy source for growth (A. R. Horswill and J. C. Escalante-Semerena, unpublished data). The tricarballylate catabolic genes *tcuRABC* (*tri*carballylate *u*tilization) have been previously described (31), and a mechanism for this catabolism has been proposed (29). In this model, TcuC transports tricarballylate across the inner membrane, where it is oxidized by the flavoprotein TcuA to *cis*-aconitate, which can then enter the Krebs cycle (29). During growth on tricarballylate, the recycling of the reduced flavin adenine dinucleotide of TcuA is achieved by TcuB, a membrane-bound protein that contains two [4Fe-4S] clusters and heme (30).

The demonstration that TcuB contains iron-sulfur clusters, in combination with work on ApbC and homologs, led to the hypothesis that the [Fe-S] clusters of TcuB were compromised in an *apbC* strain, preventing growth on tricarballylate. Consistent with this hypothesis, we show herein that TcuB activity is 100-fold lower in a strain lacking ApbC. The data further show that derepression of the *isc* operon or overexpression of *iscU* compensated for the lack of ApbC during growth of an *apbC* strain on tricarballylate.

MATERIALS AND METHODS

Bacterial strains, media, and chemicals. All strains used in this study are derived from *S. enterica* serovar Typhimurium LT2, and their genotypes are given in Table 1. MudJ refers to the Mu*d*I1734 insertion element (9), and Tn*10*d(Tc) refers to the transposition-defective mini-Tn*10* described by Way et al. (59).

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TABLE 1. Strains and plasmids[a]

Strain or plasmid[b]	Description[c]	Source
Strains		
S. enterica		
TR6583	*metE205 ara-9*	J. Roth
DM10310	*ara-9* (wild type)	
DM9678	*apbE42*::Tn*10*d(*tet*)	
DM9682	*rseC*::*kan*	
DM9684	*cyaY*::*cat*	
DM10780	*ara-9 apbC55*::Tn*10*d(*tet*) *rseC*::*kan*	
DM10779	*ara-9 apbC::MudJ* (*kan*) *apbE42*::Tn*10*d(*tet*)	
DM10296	*ara-9 yggX*::Gm	
DM10300	*ara-9 apbC55*::Tn*10*d(*tet*)	
DM10302	*ara-9 apbC55*:Tn*10*d(*tet*) *yggX*::Gm	
DM10696	*ara-9 iscR2*::MudJ (*kan*)	
DM10697	*ara-9 apbC55*:Tn*10*d(*tet*) *iscR2*::MudJ (*kan*)	
DM10325	*ara-9* Δ*iscRSUA::cat*	
DM10681	*ara-9 apbC55*::Tn*10*d(*tet*) Δ*isc5*::*cat*	
DM10326	*ara-9 sufS*::*cat*	
DM10682	*ara-9 apbC55*::Tn*10*d(*tet*) *sufS*::*cat*	
DM10667	*ara-9 iscA1*::MudJ (*kan*)	
DM10683	*ara-9 apbC55*::Tn*10*d(*tet*) *iscA1*::MudJ (*kan*)	
DM10604	*ara-9 apbC::MudJ* (*kan*)	
DM10608	*ara-9 apbC*::MudJ (*kan*) *iscR11*	
JE10432	*ara-9 iscR*::*kan*	J. C. Escalante-Semerena
DM10698	*ara-9* Δ*iscR11*	
JE10435	*ara-9 apbC55*::Tn*10*d(*tet*) Δ*iscR11*	J. C. Escalante-Semerena
DM10685	*ara-9 apbC55*::Tn*10*d(*tet*) *yggX*::Gm *iscR11*	
DM10474	*ara-9 apbC55*::Tn*10*d(*tet*) *stm2545*::Tn*10*d(*cat*) *iscR6*	
DM10476	*ara-9 apbC55*::Tn*10*d(*tet*) *stm2545*::Tn*10*d(*cat*) *iscR7*	
DM10480	*ara-9 apbC55*::Tn*10*d(*tet*) *stm2545*::Tn*10*d(*cat*) *iscR8*	
DM10482	*ara-9 apbC55*::Tn*10*d(*tet*) *stm2545*::Tn*10*d(*cat*) *iscR9*	
DM10484	*ara-9 apbC55*::Tn*10*d(*tet*) *stm2545*::Tn*10*d(*cat*) *iscR10*	
DM10771	*ara-9 staC*::Tn*10*d(*tet*) *stm2545*::Tn*10*d(*cat*) *yggX*::Gm	
DM10772	*ara-9 apbC::MudJ* (*kan*) *staC*::Tn*10*d(*tet*) *stm2545*::Tn*10*d(*cat*) *yggX*::Gm	
DM10770	*ara-9 apbC::MudJ* (*kan*) *stm2545*::Tn*10*d(Cm) *yggX*::Gm *iscR7*	
DM10769	*ara-9 staC*::Tn*10*d(tet^+) *stm2545*::Tn*10*d(cat^+) *yggX*::Gm *iscR7*	
E. coli		
C43(λDE3)	F^- *ompT gal* $hsdS_B(r_B^-\ m_B^-)$ *dcm lon*	J. C. Escalante-Semerena
JE10465	F^- *ompT gal* $hsdS_B(r_B^-\ m_B^-)$ *dcm lon apbC*::kan^+	J. C. Escalante-Semerena
DH5α/F′	F′/*endA1 hsdR17*$(r_K^-\ m_K^+)$ *supE44 thi-1 recA1 gyrA* (Nar1r) *relA1* Δ(*lacZYA-argF*)*U169 deoR*[80d*lac* Δ(*lacZ*)M15]	New England Biolabs
Plasmids (vectors)		
pSU19 (pSU19)	None	2
pIscR1 (pSU19)	*iscR* (*S. enterica*)	52
pIscS1 (pSU19)	*iscS* (*S. enterica*)	52
pIscA1 (pSU19)	*iscA* (*S. enterica*)	52
pIscA-orf3 (pSU19)	*iscA-hscB-hscA-fdx-orf3* (*S. enterica*)	52
pIscU1 (pSU19)	*iscU* (*S. enterica*)	
pTCU21 (pBAD30)	*tcuABC* (*S. enterica*)	31
pCTH-ApbC (pET20b)	*apbC* (*S. enterica*)	51
pDB1282 (pAra13)	*iscSUA-hscB-hscA-fdx-orf3* (*A. vinelandii*)	Dennis Dean
pHscB-orf3 (pSU19)	*hscB-hscA-fdx-orf3* (*S. enterica*)	
pGSO164 (pBAD)	*sufABCDSE* (*E. coli*)	43
pIsc2 (pFZY1)	*iscR* operator	26[d]
pTCU55 (pET15b)	*tcuB*	30
pApbC (pSU19)	*apbC*	

[a] Unless indicated otherwise in the description, all strains are *S. enterica* serovar Typhimurium LT2 constructed for this study.
[b] All *S. enterica* strains constructed for this study are derivatives of TR6583 that were transduced to $metE^+$. The TR6583 strain was from the Escalante-Semerena laboratory.
[c] Genotypes are given for strains, and inserts (hosts) are given for plasmids. The following alleles have been previously described: *apbC55*::Tn*10*d (46), *cyaY*::Cm (57), *rseC*::*Kan* (3, 53), *apbE42*::Tn*10*d(Tc) (4), *yggX*::Gm (19), *iscR2*::MudJ (52), Δ*isc5*::Cm (53), and *iscA2*::MudJ (52). Tc, tetracycline; Cm, chloramphenicol; Gm, gentamicin; Kan, kanamycin.
[d] Source also includes Lewis et al. (submitted).

No-carbon essential (NCE) medium of Berkowitz et al. (5) was made with Milli-Q filtered water and supplemented with 1 mM $MgSO_4$ and trace minerals (1, 11, 58). Glucose and tricarballylate were added to NCE medium at 11 mM and 20 mM, respectively. Difco nutrient broth (8 g/liter) with NaCl (5 g/liter) or lysogenic broth (6, 7) was used as rich medium. Difco BiTek agar was added (15 g/liter) for solid medium. When present in the medium, supplements were provided at the following final concentrations: thiamine, 10 nM or 100 nM; adenine, 0.4 mM; and nicotinic acid, 20 μM. When needed, antibiotics were

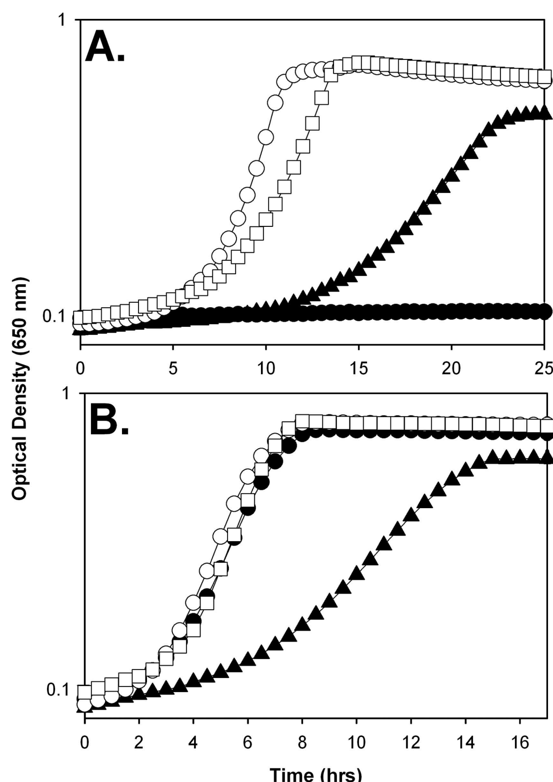

FIG. 1. *apbC* mutants fail to grow on tricarballylate. Strains were grown at 37°C in NCE medium supplemented with thiamine and nicotinic acid and with a sole carbon and energy source. Growth of strains DM10310 (wild type) (○), DM10300 (*apbC*) (●), DM10325 (*iscSUA-hscAB-fdx-orf3*) (▲), and DM10667 (*iscA-hscAB-fdx-orf3*) (□) was monitored on tricarballylate (A) and glucose (B).

added to the following concentrations in rich and minimal medium, respectively: tetracycline, 20 and 10 μg/ml; kanamycin, 50 and 125 μg/ml; chloramphenicol, 20 and 4 μg/ml; ampicillin, 50 and 15 μg/ml; and gentamicin, 6 and 6 μg/ml. All chemicals were purchased from Sigma-Aldrich.

Genetic methods. (i) Mutant isolation. Nine independent cultures of DM10300 (*apbC*) were grown to full density in nutrient broth medium. One hundred microliters of each culture was spread onto individual minimal tricarballylate thiamine plates. Colonies spontaneously arose after 48 h of incubation at 37°C. One colony derived from each culture was saved.

(ii) Isolation of linked insertions. The methods for transduction and the purification of transductants have been previously described (13, 47, 48).

Transposons [Tn*10*d(*cat*)] (14) genetically linked to the suppressor mutations were isolated by standard genetic techniques (24). In each case, mutant strains were reconstructed and verified phenotypically prior to characterization. The relevant insertions were mapped by sequencing using a PCR-based protocol (University of Wisconsin Biotechnology DNA Sequence Facility) (8, 60).

(iii) Phenotypic analysis. Nutritional requirements were assessed on solid medium and by the quantification of growth in liquid medium using either 5-ml cultures in 25-ml shake tubes or 200-μl cultures in a 96-well plate. Protocols for each have been previously described (3, 4). The starting A_{650} was routinely between 0.03 and 0.08, with a final A_{650} between 0.5 and 1.1. Each culture had at least three replicates. Growth on solid medium was scored after replica printing to the relevant medium and after incubation at 37°C for 48 to 60 h.

Molecular biology. Restriction enzymes and DNA ligase were purchased from Promega, and *Pfu* DNA polymerase was purchased from Stratagene. The *iscU* and the *hscA-orf3* genes were amplified from wild-type *S. enterica* by using genomic DNA as the template. The primers were as follows: for *iscU*, the forward primer was 5′-CCGAAGCTTATGGCTTACAGCGAAAAAG-3′ and the reverse primer was 5′-CGGGGATCCTTATTTCGCTTCGCGTTTG-3′; for *hscA-orf3*, the forward primer was 5′-GGGCAAGCTTTGGATTACTTCACCC TCTT-3′ and the reverse primer was 5′-CCTCGGATCCTTACTCTGCTTCAT CCAACC-3′.

The PCR product of *iscU* was purified and digested with BamHI and HindIII. The resulting products were purified and ligated into similarly digested pSU19 (2), creating pIscU. The PCR product containing *hscA-orf3* was blunt end ligated into HincII-digested pSU19, resulting in pHscA-orf3. Plasmids were moved into the appropriate strains via electroporation (42), and their identities were confirmed by restriction digestion and/or sequencing. The plasmids used are given in Table 1.

Enzyme assays. (i) β-Galactosidase. β-Galactosidase assays were performed according to the method of Winston et al. (61).

(ii) TcuB activity. His_6-TcuB was overproduced from plasmid pTCU55 as previously described (29). Cells from either strain JE6664 [C43(λDE3)] or strain JE10465 [C43(λDE3) *apbC*::*kan*] containing pTCU55 were grown at 18°C on Terrific broth to an optical density at 600 nm of ~0.4 and then induced with 300 μM isopropyl-β-D-thiogalactopyranoside (IPTG) overnight. His_6-TcuB-enriched extracts were obtained and assayed as previously described (29). Briefly, 200-μl reaction mixtures contained 2-(*N*-morpholino)ethanesulfonic acid (MES) (100 mM, pH 6.5, at 30°C), dithiothreitol (1 mM), 1 μg TcuA, and 20 μg His_6-TcuB-enriched extract. Reaction mixtures were incubated at 30°C for 10 min following the addition of tricarballylate (10 mM). Reactions were stopped by the addition of 40-μl samples to 60 μl of 166.7 mM H_2SO_4. Fifty microliters of each sample was used to quantify *cis*-aconitate production using a high-performance liquid chromatography protocol (29).

RESULTS

ApbC, but not Isc or Suf protein, is required for growth on tricarballylate. An *apbC* mutant does not grow on tricarballylate as a carbon and energy source, although it is proficient for growth on glucose (Fig. 1) and other carbon sources (e.g., succinate and gluconate) (data not shown). The expression of the *tcuABC* operon in *trans* from a nonnative promoter did not restore growth on tricarballylate, consistent with a posttranscriptional effect. A strain carrying a deletion of the *iscRSUA-hscAB-fdx-orf3* operon or a polar insertion in *sufS* grew well on tricarballylate (Fig. 1; Table 2). The growth of the Δ*iscRSUA-hscAB-fdx-orf3* mutant was reduced compared to that of the wild type on both tricarballylate and glucose, consistent with a previously reported global defect (52). The growth of strains lacking other genes involved in [Fe-S] cluster metabolism was also assessed on tricarballylate; no defect was found for mutants lacking *apbE*, *rseC*, *cyaY*, or *yggX* (data not shown). These growth data suggested a specific role for ApbC function during tricarballylate catabolism.

TABLE 2. An *apbC* mutant is unable to grow on tricarballylate medium

Strain	Relevant genotype[b]	Doubling time (h) on medium containing[a]:		Ratio of doubling times[d]
		Tricarballylate[c]	Glucose[c]	
DM10310	Wild type	1.7 ± 0.1	2.1 ± 0.1	0.81
DM10667	*iscA-hscAB-fdx-orf3*	1.9 ± 0.1	2.1 ± 0.1	0.90
DM10325	*iscSUA-hscAB-fdx-orf3*	4.5 ± 0.2	4.3 ± 0.1	1.1
DM10326	*sufS*	1.8 ± 0.1	2.2 ± 0.0	0.82
DM10300	*apbC*	NG	2.1 ± 0.0	

[a] Doubling times were calculated using the formula $\mu = \ln(X/X_o)/T$, where μ is the growth rate, X and X_o are optical density measurements at 650 nm, T is the time between the absorbance readings X and X_o, and the doubling time (g) was (ln 2)/μ (39). Values are averages of three independent cultures. NG, no growth.

[b] Relevant genotypes indicate the genes that are defective due to the presence of a polar mutation or deletion.

[c] The defined medium included the indicated carbon source and was supplemented with thiamine and nicotinic acid.

[d] Ratio of the doubling time on tricarballylate to the doubling time on glucose.

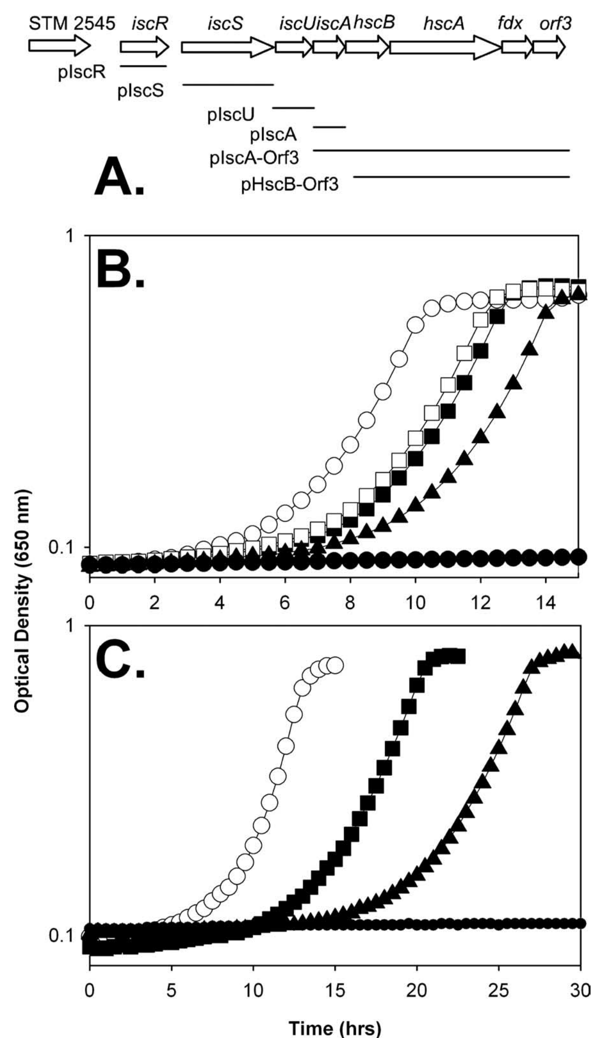

FIG. 2. The overexpression of *isc* genes allows growth of an *apbC* mutant on tricarballylate. Strains were grown at 37°C in NCE medium supplemented with thiamine and nicotinic acid and with tricarballylate as a carbon and energy source. (A) A schematic shows the genetic organization of the *S. enterica isc* operon. The borders of inserts used to generate plasmids are diagrammed below the operon. (B) Growth of strains DM10310 (wild type) (○), JE10435 (*iscR11 apbC*) (■), DM10698 (*iscR11*) (□), DM10474 (*apbC iscR7*) (▲), and DM10300 (*apbC*) (●) on tricarballylate NCE medium. (C) Growth of strains DM10310 (wild type) with pSU19 (○), DM10300 (*apbC*) with pSU19 (●), DM10300 (*apbC*) with pIscU (▲), and DM10474 (*apbC iscR7*) with pSU19 (■) on tricarballylate NCE medium.

TcuB is less active in *apbC* mutants. To determine if ApbC function was involved in the synthesis of active TcuB, we measured the activity of this protein in vitro in a strain lacking ApbC. *E. coli* strain C43(λDE3) and an *apbC* mutant derivative of that strain were used to overproduce TcuB from plasmid pTCU55 (30). The *apbC* mutant extract produced 100-fold less *cis*-aconitate than that of the wild-type strain (220 ± 20 and 25,300 ± 700 pmol *cis*-aconitate produced after 10 min, respectively). In contrast, the activities of aconitase, succinate dehydrogenase, and the non-[Fe-S] cluster protein malate dehydrogenase were indistinguishable in the *apbC* mutant and wild-type extracts (data not shown).

Conditional growth of an *apbC* mutant. An *apbC* strain did not grow in liquid medium with tricarballylate as a carbon source but grew on solid tricarballylate medium after ~48 h. This observation suggested a functionally redundant system working at low efficiency. Insertions in *yggX*, *iscA*, or *iscR* eliminated residual growth of the *apbC* mutants on tricarballylate medium, while *apbC* strains with insertions in *sufS*, *apbE*, or *rseC* retained growth. None of these above-mentioned loci were required for growth on tricarballylate in an otherwise wild-type strain.

Suppressor analysis provides insight into ApbC function. Nine independent spontaneous mutations that allowed the growth of strain DM10300 (*apbC*) on tricarballylate were identified. Genetic analysis determined that a Tn*10*d(*cat*) insertion in open reading frame STM2545 was ~85% cotransducible by phage P22 with five of the nine suppressor mutations, placing the mutations near the *isc* operon (Fig. 2A). Sequence analysis determined that each of the five mutations was in *iscR*, encoding the repressor of the *isc* operon (49). The suppressor mutations resulted in variant IscR proteins with the following amino acid changes: L109Q (*iscR6*), S38F (*iscR7*), Q94Z (stop) (*iscR8*), Y41S (*iscR9*), and G64A (*iscR10*). The four substitutions were located in the predicted helix-turn-helix DNA binding domain of IscR (49) and were expected to generate inactive proteins and result in the constitutive expression of the *isc* operon.

Derepression of the *isc* operon restores the growth of an *apbC* mutant on tricarballylate. Three results confirmed that the suppressor mutations disrupted IscR function and that the resulting derepression of the *isc* operon was sufficient to allow an *apbC* mutant to grow on tricarballylate. First, the introduction of an in-frame deletion of *iscR* (*iscR*Δ*11*) restored the growth of an *apbC* mutant on tricarballylate (Fig. 2B). Second, the expression of the wild-type allele of *iscR* in *trans* eliminated growth on tricarballylate of strain JE10435 (*apbC iscR*Δ*11*) and strains containing the other *iscR* alleles (data not shown).

Third, transcription was monitored using a *lacZ* reporter under the control of the *iscR* promoter (p_{iscR}-*lacZ* transcriptional fusion [plasmid pIsc2]) (J. A. Lewis, J. M. Boyd, D. M. Downs, and J. C. Escalante-Semerena, submitted for publication). Data given in Table 3 show that the chromosomal *iscR* alleles increased the expression of the reporter as efficiently as the chromosomal deletion of *iscR*. Together, these results confirmed that the IscR variants encoded by mutant *iscR* alleles

TABLE 3. Inactivation of IscR derepresses the *isc* operon[a]

Strain	Relevant genotype	β-Galactosidase activity (Miller units)
DM10310	Wild type	70 ± 4
DM10300	*apbC*	70 ± 2
JE10432	*iscR*::Kan	390 ± 72
DM10474	*apbC iscR6*	270 ± 28
DM10476	*apbC iscR7*	430 ± 47
DM10480	*apbC iscR8*	350 ± 31
DM10482	*apbC iscR9*	340 ± 28
DM10484	*apbC iscR10*	290 ± 22

[a] All strains contained a plasmid that carried the *isc* reporter-*lacZ* fusion as described in the text. Strains were grown in LB-ampicillin medium to the mid-exponential phase of cell growth. β-Galactosidase assays were performed as described in Materials and Methods.

failed to repress *iscRSUA-hscAB-fdx-orf3* transcription and that derepression of the *iscRSUA-hscAB-fdx* operon was sufficient to restore growth on tricarballylate in an *apbC* strain.

IscU has functional redundancy with ApbC. The above-mentioned results strongly suggested that one or more proteins encoded by the *isc* operon had a functional overlap with ApbC. Plasmids encoding one or more *isc* genes (Fig. 2A) were introduced into strain DM10300 (*apbC*), and growth on tricarballylate was assessed. Of the plasmids tested, only pIscU affected the growth of the *apbC* mutant strain on tricarballylate (Fig. 2C). Several points can be taken from the data in Fig. 2C. First, pIscU restored the growth of the *apbC* mutant compared to the growth of the same strain with the vector-only control. The uniformly increased lag did not alter the conclusion that the growth of an *apbC* mutant on tricarballylate was allowed by either the derepression of the *isc* operon or the overexpression of *iscU*. Each of the strains retained the pattern of growth upon reinoculation, indicating that the growth was not due to a mutant overpopulating the culture. The doubling times for the strains were 1.5 ± 0.0, 1.7 ± 0.0, and 2.3 ± 0.1 h for the wild type with pSU19, the *apbC iscR7* mutant with pSU19, and the *apbC* mutant with pIscU, respectively.

IscU is not sufficient to compensate for ApbC. Plasmid pIscU failed to restore growth to an *apbC* mutant that was also defective in the *isc* operon (data not shown). This result was particularly significant for the *apbC iscA* genetic background, since the *iscA* mutation alone did not affect growth on tricarballylate (Table 2). These data suggested that the ability of IscU overproduction to allow the growth of an *apbC* mutant on tricarballylate required *iscA* and/or at least one gene downstream in the operon.

DISCUSSION

This study was initiated to understand the inability of *S. enterica apbC* mutants to grow with tricarballylate as a carbon and energy source. Previous studies implicating ApbC in [Fe-S] cluster metabolism (51, 53) and the report that TcuB contained [Fe-S] clusters (30) generated a hypothesis for the growth defect. The result that strains lacking *apbC* displayed 100-fold less TcuB activity than the wild type supported this hypothesis and has provided an additional system that can be exploited to dissect the role of ApbC in *Salmonella*. The physiological studies described herein provide data that IscU has a functional overlap with ApbC and, furthermore, that ApbC has a specific role in tricarballylate utilization that distinguishes it from the general [Fe-S] cluster biosynthesis systems encoded by the *isc* and *suf* operons.

Mutants lacking either the complete *isc* operon or the *suf* operon had no growth defect specific to tricarballylate medium. Thus, the lack of growth on tricarballylate was the first defect described for an *apbC* mutant that was not shared by strains lacking the major [Fe-S] cluster biosynthetic system *isc*. Recently, ErpA, a protein essential for isoprenoid biosynthesis in *E. coli*, was shown to specifically transfer [Fe-S] clusters to IspG (32). Similarly, Iba57 was shown to be essential for mitochondrial aconitase maturation in yeast (16). The results with ErpA and Iba57 were parallel to those with ApbC, since in all cases neither the *isc* system nor the *suf* system expressed at physiological levels could functionally replace these proteins in isoprenoid biosynthesis, aconitase maturation, or tricarballylate catabolism, respectively.

Null alleles of *iscR* restored the growth of the *apbC* mutant on tricarballylate and suggested that a gene(s) in the *isc* operon had a functional overlap with *apbC*. This result was confirmed in a study by Lewis et al. that showed that growth of an *apbC* mutant on tricarballylate could be restored by physiological conditions that increased the expression of the *isc* operon (Lewis et al., submitted). When provided in *trans*, *iscU* restored growth on tricarballylate to an *apbC* mutant. The expression of *iscU* in *trans* failed to restore growth on tricarballylate to an *apbC isc* double mutant, showing that IscU required at least one of the *iscA*, *hscAB*, or *orf3* genes to restore growth. These data were reminiscent of studies on the functional redundancy of the U-type scaffolds in *Azotobacter vinelandii*. Johnson and coworkers found that target specificity distinguished the *isc* and *nif* systems when these operons were expressed at chromosomal levels, which was altered if the relevant genes were overexpressed (21, 22). Transcriptional studies found no effect of IscR on *apbC* expression or vice versa (reference 17 and data not shown).

Eukaryotic ApbC homologues Npb35 and Cfd1 can independently bind [Fe-S] clusters and rapidly and efficiently transfer these clusters to the Leu1 apoenzyme (40). Recent data show that ApbC can similarly transfer an [Fe-S] cluster to Leu1 in vitro (J. M. Boyd, A. J. Pierik, D. J. Aguilar-Netz, R. Lill, and D. M. Downs, submitted for publication). The data presented herein suggest that the TcuB protein provides a physiologically relevant system to further explore the biochemical function of ApbC and to address its specificity in vitro.

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