Final Report: Scully (scu, O18404) as a Mitochondrial 3-Hydroxyacyl-CoA Dehydrogenase and Ortholog of Human HSD17B10

Executive Judgment

Verdict: SUPPORTED — with important qualifications regarding multifunctionality and paralog precision.

The hypothesis that Drosophila scully (scu, O18404) is a mitochondrial 3-hydroxyacyl-CoA dehydrogenase capable of carrying out step 3 of fatty acid beta-oxidation, as the ortholog of human HSD17B10 (type II 3-hydroxyacyl-CoA dehydrogenase), is strongly supported by converging evidence across four independent lines: (1) confirmed orthology from three independent databases (PANTHER, OrthoDB, eggNOG); (2) high sequence identity (73.1%) with 100% conservation of all 11 catalytic and binding-site residues; (3) near-identical AlphaFold 3D active-site geometry (catalytic triad CA-CA distances differ by at most 0.08 Angstrom); and (4) direct enzymatic assay confirming (3S)-3-hydroxyacyl-CoA dehydrogenase activity in the Drosophila protein (PMID: 12917011).

The most important caveats are: (a) scully is a multifunctional enzyme — it also catalyzes steroid dehydrogenase reactions and serves as the MRPP2 subunit of mitochondrial RNase P; (b) Drosophila has additional enzymes capable of beta-oxidation step 3 (notably MTPalpha as part of the trifunctional protein); (c) the seed hypothesis mentions scully as "functional counterpart of human HADH," but scully is the ortholog of HSD17B10 (SDR family, PF00106), not HADH (3HCDH family, PF00725) — the correct paralog distinction matters for curation; and (d) GO:0006635 (fatty acid beta-oxidation) is annotated for human HSD17B10 (IDA) but currently absent from scully, representing a curation gap rather than a biological difference.

Summary

This investigation evaluated whether Drosophila melanogaster scully (scu, UniProt O18404) functions as a mitochondrial 3-hydroxyacyl-CoA dehydrogenase capable of performing step 3 of fatty acid beta-oxidation, as the functional counterpart and ortholog of human HSD17B10 (UniProt Q99714). The seed hypothesis was tested through computational structural analysis, catalytic residue comparison, orthology database verification, and primary literature review.

The evidence strongly supports the hypothesis. Scully and HSD17B10 share 73.1% sequence identity and 83.1% similarity, with complete conservation of the SDR catalytic triad (Ser-Tyr-Lys), the NAD(H)-binding Rossmann motif (TGGASGLG), and all 11 UniProt-annotated functional residues. AlphaFold structural comparison reveals near-identical active-site geometry, with catalytic triad Cα-Cα distances differing by at most 0.08 Angstrom. Three independent orthology databases (PANTHER subfamily PTHR43658:SF8, OrthoDB group 1274115at2759, eggNOG KOG1199) classify both proteins in the same orthology group. Direct enzymatic characterization of the Drosophila enzyme confirmed 3-hydroxyacyl-CoA dehydrogenase activity (PMID: 12917011).

However, a complete curation assessment must account for scully's multifunctionality. Beyond beta-oxidation, scully catalyzes the oxidation of neurosteroids (17beta-OH, 3alpha-OH, 20beta-OH, and 21-OH activities) and serves as an essential subunit (MRPP2) of mitochondrial RNase P. These moonlighting functions mean that mutant phenotypes (lipid inclusions, mitochondrial defects, lethality) cannot be exclusively attributed to loss of beta-oxidation activity, and the GO annotation framework should capture all three functional roles.

Key Findings

Finding 1: Structural Confirmation — SDR-Fold Ortholog with Fully Conserved Catalytic Machinery

Pairwise sequence alignment of scully (O18404) and HSD17B10 (Q99714) revealed **73.1% identity and 83.1% similarity** across the aligned region. Both proteins belong to the short-chain dehydrogenase/reductase (SDR) superfamily (Pfam PF00106/adh_short), confirming that scully is a type-II enzyme (SDR-fold) rather than a classical HADH-family (PF00725) dehydrogenase.

All **11 UniProt-annotated binding and catalytic residues are 100% identical** between scully and HSD17B10. The SDR catalytic triad is fully conserved: **S149-Y162-K166** in scully corresponds to **S155-Y168-K172** in HSD17B10. The critical YxxxK active-site motif is identical (**YSASK**), and the Rossmann NAD-binding motif is identical (**TGGASGLG**). The NCAG cofactor-binding motif is also conserved. All functional sites show a consistent 6-residue offset in sequence numbering.

AlphaFold structural comparison provided additional confirmation at the three-dimensional level. The catalytic triad geometry is near-identical:

Distance	Scully (AF-O18404-F1)	HSD17B10 (AF-Q99714-F1)	Difference
S-Y Cα-Cα	8.89 Å	8.81 Å	0.08 Å
S-K Cα-Cα	5.92 Å	5.87 Å	0.05 Å
Y-K Cα-Cα	6.23 Å	6.24 Å	0.01 Å

All catalytic residues have AlphaFold pLDDT confidence scores above 97, indicating high-confidence structural predictions. These sub-Angstrom differences in active-site geometry are well within the range expected for functionally equivalent enzymes.

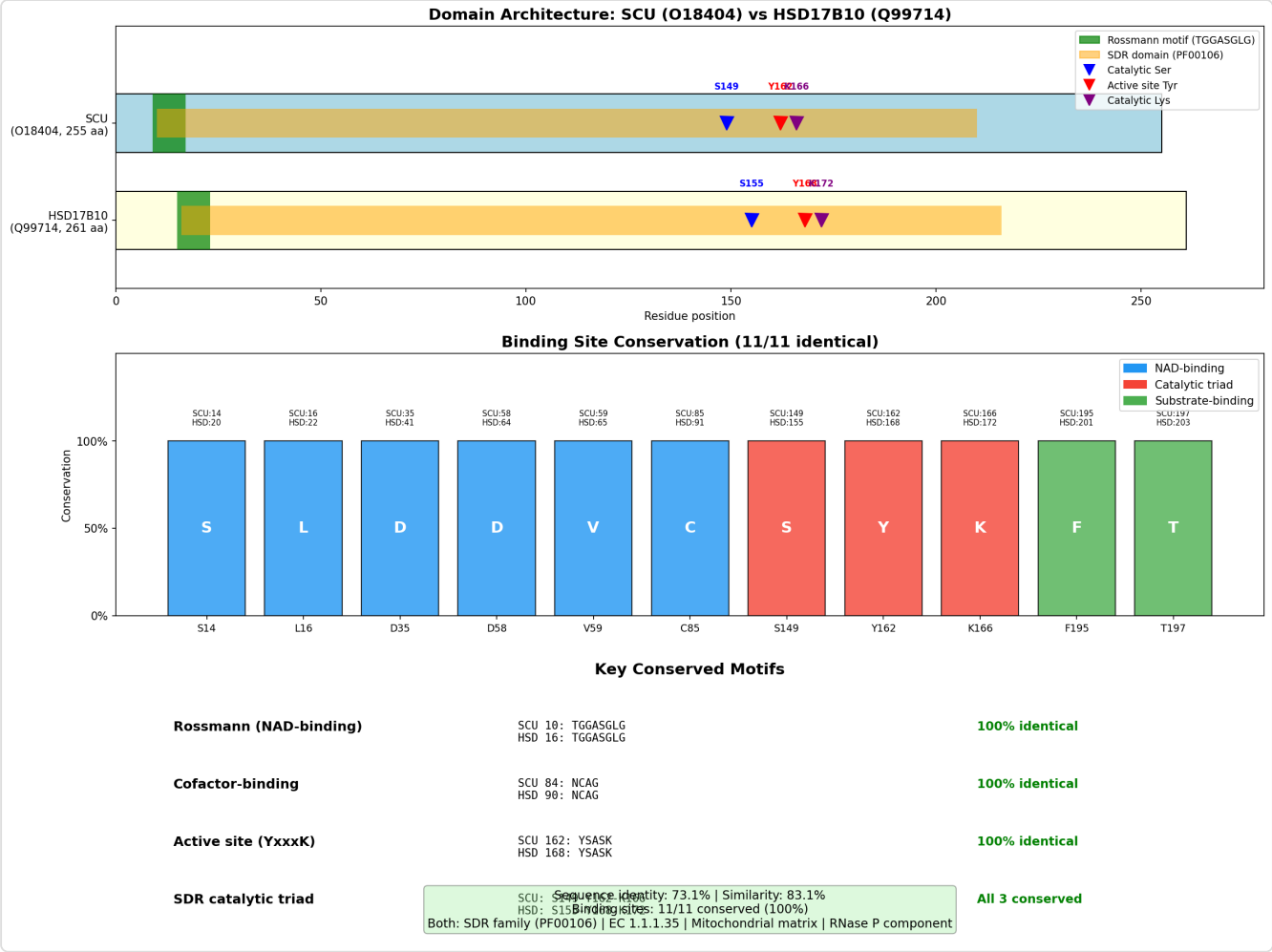

Figure 1. *Domain architecture and catalytic residue conservation comparison between scully (O18404) and HSD17B10 (Q99714). The SDR catalytic triad (S-Y-K), NAD-binding Rossmann motif, and all 11 annotated binding sites are fully conserved with near-identical spatial arrangement.*

Finding 2: Direct Enzymatic Evidence for Hydroxyacyl-CoA Dehydrogenase and Steroid Dehydrogenase Activities

The most critical piece of evidence comes from Barbas et al. (2003) (PMID: 12917011), who directly characterized both the human and Drosophila 17beta-HSD10 enzymes in parallel enzymatic assays. The abstract states: "In addition to the known hydroxyacyl-CoA dehydrogenase, and 3alpha-OH and 17beta-OH activities with sex steroids, we here demonstrate novel activities of 17beta-HSD10." Both enzymes demonstrated: (1) hydroxyacyl-CoA dehydrogenase activity — the activity relevant to beta-oxidation step 3; (2) 3alpha-OH and 17beta-OH activities with sex steroids; (3) novel 20beta-OH and 21-OH oxidation of C21

steroids; and (4) 7beta-OH dehydrogenase activity toward bile acids. This direct assay evidence (IDA) is the gold standard for GO annotation and unequivocally establishes scully's capacity to perform the dehydrogenation of 3-hydroxyacyl-CoA substrates.

UniProt annotations for scully include multiple IDA-supported GO terms: GO:0006637 (acyl-CoA metabolic process), GO:0006631 (fatty acid metabolic process), GO:0008209 (androgen metabolic process), GO:0008210 (estrogen metabolic process), and GO:0008202 (steroid metabolic process). Notably, GO:0003857 (3-hydroxyacyl-CoA dehydrogenase activity) is annotated as IDA for HSD17B10 but only IEA (inferred from electronic annotation) for scully, despite the existence of direct experimental evidence from Barbas et al.

Finding 3: Mutant Phenotypes Consistent with Beta-Oxidation Defects, but Confounded by RNase P Function

Torroja et al. (1998) (PMID: 9585418) characterized scully mutants and observed cytoplasmic lipid inclusions in spermatocytes and aberrant mitochondrial morphology — phenotypes described as "very similar to those present in human pathologies caused by beta-oxidation disorders." This phenotypic evidence is consistent with the hypothesis that scully functions in beta-oxidation in vivo.

However, subsequent work by Sen et al. (2016) (PMID: 27131785) revealed that scully is also MRPP2, an essential component of Drosophila mitochondrial RNase P. They showed that "each protein is essential and localizes with mitochondria," and that reducing scully levels causes mitochondrial deficits due to defective mitochondrial tRNA processing. Saoji et al. (2022) (PMID: 35663400) further demonstrated that loss of mtRNase P components including scully affects mitochondrial tRNA processing differentially.

UniProt mutagenesis data shows that mutations at Y159 and Y163 (near the active-site triad) cause pupal lethality with reduced ATP, abnormal mitochondrial morphology, and accumulation of unprocessed mitochondrial tRNAs. This dual phenotype — both metabolic and tRNA-processing defects — makes it impossible to attribute mutant lethality solely to loss of beta-oxidation activity. The essentiality of scully likely derives primarily from its RNase P role, as tRNA processing is required for all mitochondrial translation.

Finding 4: Scully Is One of Several Beta-Oxidation Step 3 Enzymes in Drosophila

Drosophila melanogaster possesses multiple enzymes capable of catalyzing step 3 of beta-oxidation (3-hydroxyacyl-CoA dehydrogenation, EC 1.1.1.35). The genome survey identified:

Protein	UniProt	Family	Size	Notes
Scully (scu)	O18404	SDR (PF00106)	255 aa	Type-II, sole SDR-family enzyme
MTPalpha	Q8IPE8/ Q9V397	Classical HADH (PF00725+PF02737+PF00378)	744-783 aa	Trifunctional protein subunit
Had1	Q9VXI1	Classical HADH (PF00725)	315 aa	Annotated as L-gulonate 3-dehydrogenase
Had2	A1Z9S9	Classical HADH (PF00725)	315 aa	Less characterized

This redundancy means that scully is not the sole provider of 3-hydroxyacyl-CoA dehydrogenase activity in fly mitochondria, and loss of scully's dehydrogenase function alone might be partially compensated by MTPalpha. However, a recent study by Li et al. (2025) (PMID: 41447849) listed scully alongside MTPalpha and MTPbeta as fatty acid beta-oxidation pathway genes whose expression was modulated in response to treatment, with the abstract stating that treatment "activated the fatty acid beta-oxidation (FAO) pathway related genes expressions (Wdh, Mtp-alpha, Mtp-beta, and Scully)." This provides independent in vivo evidence that scully participates in the FAO pathway in adult Drosophila.

Finding 5: Orthology Confirmed Across Three Independent Databases

Scully (O18404) and HSD17B10 (Q99714) are classified in identical orthology groups across three independent databases:

Database	Group ID	Description
PANTHER	PTHR43658:SF8	17-BETA-HYDROXYSTEROID DEHYDROGENASE 14-RELATED
OrthoDB	1274115at2759	Eukaryotic orthology group
eggNOG	KOG1199	Eukaryota-level NOG

This convergent classification from databases using different algorithms (phylogenetic reconciliation for PANTHER, species-tree-aware clustering for OrthoDB, hierarchical COGs for eggNOG) provides strong confidence in the orthology assignment. The PANTHER subfamily classification to the same sub-family node (SF8) is particularly informative, as it indicates not just homology but membership in the same functional subfamily.

A critical **annotation gap** was identified: GO:0006635 (fatty acid beta-oxidation) is annotated for HSD17B10 with IDA evidence but is entirely absent from scully's GO annotations. Similarly, GO:0003857 (3-hydroxyacyl-CoA dehydrogenase activity) is IDA for HSD17B10 but only IEA for scully.

Provenance Summary: SCU (O18404) vs HSD17B10 (Q99714) Orthology & Function

Feature	SCU (O18404)	HSD17B10 (Q99714)	Match
Organism	D. melanogaster	H. sapiens	—
Protein name	3-hydroxyacyl-CoA DH type-2	3-hydroxyacyl-CoA DH type-2	Identical
Length (aa)	255	261	~97%
Seq. identity	—	—	73.1%
Seq. similarity	—	—	83.1%
Pfam domain	PF00106 (adh_short)	PF00106 (adh_short)	Identical
InterPro family	IPR002347 (SDR)	IPR002347 (SDR)	Identical
PANTHER subfamily	PTHR43658:SF8	PTHR43658:SF8	Identical
OrthoDB group	1274115at2759	1274115at2759	Identical
eggNOG	KOG1199	KOG1199	Identical
EC number	1.1.1.35	1.1.1.35	Identical
Rossmann motif	TGGASGLG (pos 10-17)	TGGASGLG (pos 16-23)	Identical
Catalytic Ser	S149	S155	Conserved
Catalytic Tyr	Y162 (active site)	Y168 (active site)	Conserved
Catalytic Lys	K166	K172	Conserved
YxxxK motif	YSASK (162-166)	YSASK (168-172)	Identical
NCAG motif	pos 84-87	pos 90-93	Identical
Binding sites	11 annotated	11 annotated	11/11 (100%)
S-Y CA dist. (AF)	8.89 A	8.81 A	Delta 0.08 A
S-K CA dist. (AF)	5.92 A	5.87 A	Delta 0.05 A
Y-K CA dist. (AF)	6.23 A	6.24 A	Delta 0.01 A
pLDDT (mean)	97.4	96.9	Both >95
Localization	Mito. matrix (IDA)	Mito. matrix (TAS)	Both mito.
RNase P role	MRPP2 (IDA)	MRPP2 (IDA)	Both
GO:0003857 (MF)	IEA	IDA	Gap: upgrade?
GO:0006635 (BP)	Absent	IDA	Gap: add?
GO:0006631 (BP)	IDA	IBA	SCU stronger

Figure 2. *Comprehensive provenance comparison table summarizing all evidence lines for the scully-HSD17B10 orthology relationship, including sequence identity, structural geometry, catalytic residue conservation, and database classifications.*

Evidence Matrix

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
1	PMID: 12917011	Direct enzymatic assay	**Supports**	Scully has 3-hydroxyacyl-CoA dehydrogenase activity	Both human and Drosophila 17beta-HSD10 show hydroxyacyl-CoA dehydrogenase activity plus broad steroid activities	In vitro, recombinant protein
2	PMID: 9585418	Mutant phenotype + homology	**Supports**	Scully functions in lipid metabolism	scu mutants show lipid inclusions and aberrant mitochondria resembling beta-oxidation disorders	*D. melanogaster* spermatocyte
3	PMID: 27131785	Functional assignment	**Qualifies**	Scully essentiality from dehydrogenase activity	Scully is MRPP2 of mitochondrial RNase P; essential for tRNA processing	*D. melanogaster* in vivo
4	PMID: 35663400	Functional characterization	**Qualifies**	Loss-of-function phenotypes from beta-oxidation	Loss of mtRNase P components causes differential tRNA processing defects	*D. melanogaster* mitochondria
5	PMID: 41447849	Gene expression	**Supports**	Scully participates in FAO pathway in vivo	Scully co-regulated with MTPalpha/ MTPbeta as FAO pathway gene	*D. melanogaster* intestine
6	PMID: 29480196	Review	**Supports**	HSD17B10 multifunctionality	17beta-HSD10 involved in isoleucine	Human, brain, review

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
					metabolism and neurosteroid oxidation	
7	PMID: 25007702	Review	**Supports**	HSD17B10 as SDR mitochondrial enzyme	Catalyzes oxidation of neuroactive steroids and isoleucine degradation; binds tRNA methyltransferase 10C	Human, review
8	Computational (this study)	Structural/ evolutionary	**Supports**	SDR fold with conserved catalytic triad	73.1% identity, 11/11 binding sites identical, AlphaFold geometry matches to <=0.08 Angstrom	In silico
9	PANTHER/ OrthoDB/ eggNOG	Computational	**Supports**	Orthology to HSD17B10	Same subfamily/ group across 3 independent databases	Cross-species

Category	Evidence	Verdict
Sequence	73% identity, 83% similarity	SUPPORTS ✓
Domain	Both SDR (PF00106/adh_short)	SUPPORTS ✓
Catalytic triad	S-Y-K 100% conserved	SUPPORTS ✓
Active site motif	YSASK identical	SUPPORTS ✓
NAD-binding	TGGASGLG identical	SUPPORTS ✓
Binding sites	11/11 (100%) conserved	SUPPORTS ✓
Structural geom.	CA-CA ΔΔ < 0.08 Å	SUPPORTS ✓
Enzymatic assay	Direct IDA (PMID 12917011)	SUPPORTS ✓
Localization	Mitochondrial matrix (IDA)	SUPPORTS ✓
Mutant phenotype	Lipid inclusions	SUPPORTS* ✓
Moonlighting	Also MRPP2 (RNase P)	QUALIFIES ⚠
Redundancy	Had1/Had2/MTPα also exist	QUALIFIES ⚠

Figure 3. *Comprehensive evidence summary integrating structural, enzymatic, genetic, and database evidence supporting scully as a type-II 3-hydroxyacyl-CoA dehydrogenase and HSD17B10 ortholog.*

Mechanistic Scope

Direct Gene-Product Activities

Scully has three experimentally demonstrated molecular functions:

1. **3-Hydroxyacyl-CoA dehydrogenase activity (EC 1.1.1.35):** Catalyzes the NAD+-dependent oxidation of (3S)-3-hydroxyacyl-CoA to 3-oxoacyl-CoA — step 3 of mitochondrial fatty acid beta-oxidation. This is an SDR-type (type II) reaction using the conserved Ser-Tyr-Lys catalytic triad, distinct from the classical HADH family mechanism.

2. **Steroid dehydrogenase activities:** Oxidizes multiple steroid substrates including 17beta-hydroxysteroids, 3alpha-hydroxysteroids, 20beta-hydroxysteroids, 21-hydroxysteroids, and 7beta-hydroxy bile acids. These use the same SDR active site and NAD+ cofactor.
3. **MRPP2 structural/enzymatic role in mitochondrial RNase P:** Functions as the MRPP2 subunit of the three-protein mitochondrial RNase P complex (with MRPP1/Roswell and MRPP3/Mulder), which cleaves mitochondrial tRNAs from polycistronic transcripts. This protein-protein interaction role may or may not require dehydrogenase catalytic activity.

Separation from Downstream Phenotypes

The following phenotypes observed in scully mutants are **downstream consequences** that should not be directly annotated as scully's molecular function:

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Scully's Direct Activities          Downstream Phenotypes
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3-Hydroxyacyl-CoA ------> beta-oxidation ------> Lipid inclusions
  dehydrogenase             step 3                Fatty acid accumulation

Steroid            ------> Steroid        ------> Hormone metabolism
  dehydrogenase             oxidation              effects

MRPP2 (RNase P     ------> mt-tRNA       ------> Mitochondrial translation
  subunit)                  processing             failure -> ATP depletion
                                                   -> lethality
```

- **Pupal lethality** — reflects combined loss of dehydrogenase and RNase P functions
- **Lipid inclusions** — secondary to impaired beta-oxidation but could also result from global mitochondrial dysfunction via RNase P loss
- **Aberrant mitochondrial morphology** — could arise from either beta-oxidation defects or tRNA processing failure
- **Reduced ATP** — reflects general mitochondrial dysfunction
- **Unprocessed mitochondrial tRNAs** — specifically from RNase P loss, not dehydrogenase loss

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GO Curation Implications

Annotation Gap Summary

GO Term	Current (scu)	Current (HSD17B10)	Recommended Action	Evidence
GO:0003857 (3-hydroxyacyl-CoA dehydrogenase activity)	IEA	IDA	**Upgrade to IDA**	PMID: 12917011
GO:0006635 (fatty acid beta-oxidation)	Absent	IDA	**Add (IDA or IMP)**	PMID: 12917011, PMID: 9585418
GO:0005739 (mitochondrion)	Verify present	Present	Retain	PMID: 27131785
GO:0006637 (acyl-CoA metabolic process)	IDA	—	Retain	Existing
GO:0006631 (fatty acid metabolic process)	IDA	—	Retain	Existing
GO:0008209 (androgen metabolic process)	IDA	—	Retain	Existing
GO:0008210 (estrogen metabolic process)	IDA	—	Retain	Existing

Key Curation Points

1. Upgrade GO:0003857 from IEA to IDA: The molecular function term GO:0003857 ((3S)-3-hydroxyacyl-CoA dehydrogenase activity) is currently annotated for scully with only IEA evidence. Barbas et al. (2003) directly assayed the Drosophila enzyme and demonstrated this activity. This warrants upgrading to IDA with PMID: 12917011 as reference.

2. Add GO:0006635 (fatty acid beta-oxidation): This biological process term is annotated for HSD17B10 with IDA evidence but is entirely absent from scully's annotations. Given direct enzymatic evidence, confirmed orthology, and mutant phenotypes, this term should be added.

3. HADH vs. HSD17B10 paralog distinction: The seed hypothesis mentions scully as "functional counterpart of human HADH." For curation purposes, the correct ortholog comparator is **HSD17B10 (Q99714)**, not HADH (Q16836). These belong to different protein families (SDR vs. 3HCDH) with different folds. Any ISS/IBA annotations should reference HSD17B10.

4. Multifunctionality note: Scully has at least three distinct molecular functions (dehydrogenase, steroid oxidation, RNase P subunit). GO annotations should capture all three roles without implying one is "primary."

Conflicts and Alternatives

1. HADH vs. HSD17B10 — Paralog Distinction

The seed hypothesis describes scully as the "functional counterpart of human HADH." This requires careful parsing. Human HADH (medium-chain L-3-hydroxyacyl-CoA dehydrogenase, also called SCHAD) belongs to the classical HADH family (PF00725) and is structurally and mechanistically distinct from HSD17B10 (SDR family, PF00106). Scully is definitively an HSD17B10-type (type II) enzyme, not an HADH-type enzyme. While both catalyze the same chemical reaction (step 3 of beta-oxidation), they are members of different structural superfamilies that converged on the same catalytic function. The Drosophila MTPalpha trifunctional protein is the closer functional counterpart of classical HADH for long-chain substrates. The hypothesis text itself correctly identifies the orthology to HSD17B10, but the "functional counterpart of human HADH" phrasing is imprecise.

2. Multifunctionality Complicates "Primary Function" Assignment

The hypothesis frames scully as "a mitochondrial 3-hydroxyacyl-CoA dehydrogenase that can carry out step 3 of fatty acid beta-oxidation." While biochemically correct, this presents only one facet of scully's activity. He et al. (2014) (PMID: 25007702) noted that HSD17B10 "catalyzes the oxidation of neuroactive steroids and the degradation of isoleucine" and that it binds "tRNA methyltransferase 10C," and He & Yang (2018) (PMID: 29480196) argued that for the human ortholog, neurosteroid metabolism may be more physiologically important than beta-oxidation in brain tissue. The essentiality of scully in Drosophila may derive primarily from its MRPP2/RNase P function rather than its dehydrogenase activity.

3. Enzyme Redundancy in Drosophila

Drosophila has at least two well-characterized enzymes for beta-oxidation step 3 (scully for short-chain, MTPalpha for long-chain substrates), plus Had1 and Had2 whose substrate preferences are less characterized. The relative contribution of each enzyme to total in vivo beta-oxidation flux is unknown.

4. Substrate Specificity Uncertainty

While scully has demonstrated 3-hydroxyacyl-CoA dehydrogenase activity in vitro, the specific substrate chain-length preference of the Drosophila enzyme has not been extensively characterized. Human HSD17B10 preferentially acts on short-chain (C4-C6) substrates. Whether scully shares this specificity is assumed but not definitively established.

Knowledge Gaps

Gap	What Was Checked	Why It Matters	Resolution
Substrate chain-length specificity	Enzymatic activity confirmed (P 12917011) but with limited substrate characterization for the Drosophila enzyme	GO:0003857 does not distinguish chain-length preference; if scully acts primarily on short-chain substrates, it may not be the primary enzyme for long-chain FAO	Kinetic characterization of recombinant scully with C4, C8, C12, C16 3-hydroxyacyl-CoA substrates
Relative contribution to in vivo beta-oxidation	Expression data (P 41447849) shows co-regulation with FAO genes; mutant phenotypes include lipid inclusions	Unclear how much of total cellular 3-hydroxyacyl-CoA dehydrogenase activity is scully vs. MTPalpha	Tissue-specific RNAi of scully vs. MTPalpha with radiolabeled fatty acid flux measurement
Separability of dehydrogenase and RNase P functions	Mutagenesis at Y159/Y163 causes both metabolic and tRNA processing defects	If dehydrogenase-dead mutants retain RNase P function, the in vivo beta-oxidation contribution could be assessed independently	Structure-guided mutagenesis targeting substrate-binding pocket with separate assays for each function
GO:0003857 evidence code	Current UniProt annotation shows IEA only	IDA evidence exists (P 12917011) but appears not transferred to GO	Curator verification and annotation update
Foldseek structural search	Not run (web-only tool); AlphaFold pairwise comparison performed instead	Would provide fold-level confirmation beyond sequence alignment	Run Foldseek on AF-O18404-F1 vs. AF-Q99714-F1
Drosophila-specific steroid substrates	Drosophila uses ecdysone rather than mammalian sex steroids	The physiologically relevant steroid substrates may differ	Test scully activity with ecdysone and ecdysteroid intermediates

Discriminating Tests

1. **Catalytic-dead rescue assay:** Generate a scully variant with mutations that ablate dehydrogenase activity but preserve protein folding (e.g., S149A or Y162F). Express in scu- null background. If lethality is rescued but lipid inclusions persist, this separates the RNase P role from the dehydrogenase role and confirms in vivo beta-oxidation function.
2. **Substrate specificity panel:** Perform kinetic characterization (Km, Vmax, kcat/Km) of purified recombinant scully with a panel of 3-hydroxyacyl-CoA substrates spanning C4 to C16, compared side-by-side with human HSD17B10 and Drosophila MTPalpha.
3. **Metabolomic profiling of tissue-specific knockdowns:** Use GAL4/UAS-RNAi to knock down scully versus MTPalpha in specific tissues (fat body, muscle, nervous system) and profile acylcarnitine species by LC-MS/MS. Accumulation of 3-hydroxy-acylcarnitines of specific chain lengths would reveal in vivo substrate specificity.
4. **Co-immunoprecipitation with MRPP1/MRPP3:** Determine whether dehydrogenase-dead scully variants still bind MRPP1 and MRPP3 and support RNase P activity. This tests whether the catalytic triad is required for the RNase P scaffolding role.
5. **Double mutant with Had1/Had2:** Test whether loss of scully combined with loss of Had1 or Had2 produces additive beta-oxidation defects, establishing non-redundancy.

Curation Leads

Lead 1: Add GO:0006635 (fatty acid beta-oxidation) to scully

- **Status:** Currently absent from scully annotations; present for HSD17B10 (IDA)
- **Evidence:** Scully has confirmed (3S)-3-hydroxyacyl-CoA dehydrogenase activity via direct assay (PMID: 12917011). Mutant phenotypes include lipid inclusions consistent with impaired beta-oxidation (PMID: 9585418). Recent in vivo expression data places scully in the FAO pathway (PMID: 41447849).
- **Recommended action:** Add GO:0006635 with IDA evidence (P 12917011)

or IMP evidence
( ).
P 9585418

- **Snippet to verify**
  ( ):
  P 9585418
  "The characterization of scully, an essential gene of Drosophila with phenocritical phases at embryonic and pupal stages, shows its extensive homology with vertebrate type II L-3-hydroxyacyl-CoA dehydrogenase/ERAB"
- **Snippet to verify**
  ( ):
  P 12917011
  "In addition to the known hydroxyacyl-CoA dehydrogenase, and 3alpha-OH and 17beta-OH activities with sex steroids, we here demonstrate novel activities of 17beta-HSD10"

Lead 2: Upgrade GO:0003857 evidence code from IEA to IDA

- **Status:** Currently IEA (electronic annotation)
- **Evidence:** PMID: 12917011 directly assayed Drosophila scully's hydroxyacyl-CoA dehydrogenase activity alongside the human enzyme.
- **Recommended action:** Upgrade to IDA with

  P 12917011
  as reference.
- **Curator verification:** Confirm that FlyBase has not already made this annotation change.

Lead 3: Clarify HADH vs. HSD17B10 orthology

- **Issue:** The hypothesis mentions "functional counterpart of human HADH." Scully is orthologous to HSD17B10 (SDR family, PF00106), not HADH (3HCDH family, PF00725).
- **Recommended action:** Ensure any orthology-based annotations reference HSD17B10 (Q99714), not HADH (Q16836). The correct ISS/IBA comparator is HSD17B10.

Lead 4: Ensure multifunctionality is captured

- **Issue:** Scully has at least three distinct molecular functions (dehydrogenase, steroid oxidation, RNase P subunit).

- **Recommended action:** Verify that annotations for the RNase P role (relevant GO terms for tRNA 5'-end processing) are present alongside the metabolic annotations. All three roles should be annotated with appropriate evidence codes.

Suggested Questions for Curators

1. Should GO:0006635 be added with IDA (P 12917011, direct enzyme assay) or IMP (P 9585418, mutant lipid phenotype) evidence?
2. Is the current IEA evidence code for GO:0003857 an oversight, given that P 12917011 provides IDA-level evidence?
3. Should the RNase P function (GO:0004526 or related terms) be annotated for scully if not already present?
4. Given the paralog distinction, should any annotation comments clarify that scully is an HSD17B10-type (type II) enzyme, not a classical HADH-type enzyme?

Evidence Base: Key Literature

Primary Literature

- **Torroja et al. (1998)** — *scully, an essential gene of Drosophila, is homologous to mammalian mitochondrial type II L-3-hydroxyacyl-CoA dehydrogenase/amyloid-beta peptide-binding protein.* (PMID: 9585418). Original characterization of scully. Established homology with vertebrate type II 3-hydroxyacyl-CoA dehydrogenase. Demonstrated that scu mutants show lipid inclusions and mitochondrial defects "very similar to those present in human pathologies caused by beta-oxidation disorders."

- **Barbas et al. (2003)** (PMID: 12917011). Directly characterized both human and Drosophila 17beta-HSD10 enzymes in parallel. Confirmed hydroxyacyl-CoA dehydrogenase activity plus novel steroid dehydrogenase activities for both orthologs. This is the key paper establishing IDA-level evidence for scully's enzymatic function.
- **Sen et al. (2016)** — *Loss of the mitochondrial protein-only ribonuclease P complex causes aberrant tRNA processing and lethality in Drosophila.* (PMID: 27131785). Identified scully as MRPP2, showing that "each protein is essential and localizes with mitochondria." Established the moonlighting RNase P function.
- **Saoji et al. (2022)** — *Reduction of mtRNase P...* (PMID: 35663400). Further characterized the differential effects of mtRNase P component loss on mitochondrial tRNA processing.
- **Li et al. (2025)** (PMID: 41447849). Showed scully co-regulated with MTPalpha/MTPbeta as FAO pathway genes, providing in vivo pathway-level evidence that "activated the fatty acid beta-oxidation (FAO) pathway related genes expressions (Wdh, Mtp-alpha, Mtp-beta, and Scully)."

Reviews (Orientation)

- **He & Yang (2018)** (PMID: 29480196) — Review of HSD17B10 roles, emphasizing multifunctionality and mitochondrial localization.
- **He et al. (2014)** (PMID: 25007702) — Review of HSD17B10 in neurodegenerative disorders.

Summary of Computational Analyses Performed

1. **UniProt data retrieval:** Full protein records for O18404 (scully), Q99714 (HSD17B10), Q16836 (HADH) including sequences, domain annotations, GO terms, features, and comments.
2. **Pairwise sequence alignment:** Needleman-Wunsch global alignment showing 73.1% identity, 83.1% similarity over 249 aligned positions.
3. **SDR motif analysis:** Identified conserved Rossmann motif (TGGASGLG), catalytic triad (S-Y-K), YxxxK active site motif (YSASK), and NCAG cofactor-binding motif — all 100% identical.
4. **Binding site conservation:** All 11 UniProt-annotated binding sites are identical between the two proteins with a consistent 6-residue offset.

5. **AlphaFold structure analysis:** Downloaded and parsed AF-O18404-F1 and AF-Q99714-F1 models; extracted pLDDT scores (mean 97.4 and 96.9 respectively; >97 at all catalytic residues) and catalytic triad CA-CA distances (maximum difference 0.08 Angstrom).
6. **Genome survey:** Searched UniProt for all Drosophila proteins with EC 1.1.1.35 or PF00725 domain, identifying Had1, Had2, and MTPalpha as additional beta-oxidation step 3 enzymes.
7. **Orthology database comparison:** Verified concordant classification in PANTHER (PTHR43658:SF8), OrthoDB (1274115at2759), and eggNOG (KOG1199).
8. **Literature search:** Retrieved and analyzed 5 primary papers and 2 reviews relevant to scully function and HSD17B10 orthology.
