AIGR Gene Hypothesis Deep Research — Final Report

Target: Drosophila melanogaster rdgBbeta (UniProt Q9U9P7)

Hypothesis: Phosphatidylcholine transporter activity (GO:0008525)

Focus type: computational_prediction (BioReason-Pro SFT)

Summary

The BioReason-Pro SFT model predicts **phosphatidylcholine transporter activity (GO:0008525)** for the Drosophila melanogaster protein `rdgBbeta` (Q9U9P7). After three iterations of literature review, sequence/orthology analysis, and a family-wide GO annotation audit, this prediction is **REFUTED**. `rdgBbeta` is a **single-domain Class II phosphatidylinositol transfer protein (PITP)** — the Drosophila ortholog of human **PITPNC1** (the "vibrator"/RdgBβ clade) — and the direct biochemistry of this clade shows it binds and transfers **phosphatidylinositol (PI)** and **phosphatidic acid (PA)** but **"hardly binds phosphatidylcholine"** (PMID: 22822086).

Phosphatidylcholine transfer is instead a defining property of a *different* branch of the family: the **Class I PITPs (PITPα/PITPβ)** with dual PI/PC specificity, and the **multidomain/Sec14-type** transfer proteins. A systematic audit of GO:0008525 across human, mouse, rat, fly, and yeast PITPs found direct experimental (IDA) support only in those PC-competent branches (PITPNA, PITPNB, Drosophila multidomain `rdgB`, yeast SEC14) — and **no experimental support anywhere in the Class II clade**. `rdgBbeta` itself carries **no GO:0008525 annotation**; its curated molecular functions are PI binding (GO:0035091, IBA) and PI transfer activity (GO:0008526, ISS), which are the accurate terms.

The prediction is best explained as **paralog / substrate misassignment**: a PC-transfer trait that is genuine for Class I and multidomain paralogs has been incorrectly propagated onto the Class II member. The main caveat is that the decisive "no PC" assay was performed on the mammalian ortholog PITPNC1, not on the Drosophila protein directly, so the refutation rests on strong orthology (61.6% identity to PITPNC1 vs 42.7% to Class I PITPNA) plus concordant clade-level biochemistry rather than a fly-specific PC-transfer assay. Even so, no evidence supports PC transport and direct evidence argues against it. **Curator lead: reject GO:0008525; retain GO:0008526 (PI transfer) as the core molecular function.**

Key Findings

Finding 1 — RdgBβ/PITPNC1 binds and transfers PI and phosphatidic acid, but hardly binds phosphatidylcholine

Direct in vitro lipid binding and transfer assays on the Class II PITP **RdgBβ (PITPNC1)** demonstrate that, besides phosphatidylinositol, the protein binds and transfers **phosphatidic acid (PA)** and is recovered pre-loaded with PA / phosphatidylglycerol when expressed in *E. coli* — but it **"hardly binds phosphatidylcholine"** (PMID: 22822086, Garner et al. 2012). This is the single most decisive piece of evidence against the seed hypothesis, because it assays exactly the activity in question (lipid transfer specificity) on exactly the clade to which `rdgBbeta` belongs.

The result is corroborated by an **independent Class II ortholog**: recombinant mouse **mM-rdgBβ1** "shows the specific binding activity to phosphatidylinositol but not to other phospholipids" (PMID: 12562526). Two independent Class II proteins, characterized in two laboratories, converge on the same conclusion: PI (and, for PITPNC1, PA) is the physiological cargo, and PC is not a bound/transferred ligand.

For contrast, PC binding *is* a real property of the **Class I PITPs**: in Class I PITPα/β "the preferred lipid that can occupy the site can be either phosphatidylinositol (PI) or phosphatidylcholine (PC)" (PMID: 10358925). This dual PI/PC specificity — quantified elsewhere as an ~16-fold preference for PI over PC with measurable PC transfer (PMID: 3651458) — is precisely the trait a PC-transporter GO term captures, and it is a Class I / Sec14-type feature, **not** a Class II feature. The prediction thus maps a real biochemical activity onto the wrong branch of the family.

Finding 2 — Drosophila rdgBbeta (Q9U9P7) is a single-domain Class II PITP orthologous to human PITPNC1

UniProt Q9U9P7 is annotated **PITC1_DROME, "Cytoplasmic phosphatidylinositol transfer protein 1"**, a 273-residue protein consisting of a **single PITP domain** (Pfam PF02121 IP_trans; InterPro IPR001666 PI_transfer; PANTHER PTHR10658:SF54). It lacks the additional regulatory/membrane-targeting domains (FFAT, DDHD, LNS2, transmembrane region) that characterize the large multidomain RdgBα/Nir-type proteins.

Global pairwise Needleman–Wunsch alignments computed in this investigation place `rdgBbeta` firmly in the **Class II** clade: **61.6% identity to human PITPNC1 (Class II)** versus only **42.7% identity to human PITPNA (Class I)**. This ~19-point identity gap is a clear orthology signal — `rdgBbeta` is the fly ortholog of the mammalian "vibrator"/RdgBβ protein PITPNC1, not of the PC-competent Class I PITPs.

The single-domain Class II identity is confirmed in the review literature: "Three members of the PITP family (PITPα, PITPβ, and RdgBβ (retinal degeneration type B) alt. name PITPNC1) are present as single domain proteins" (PMID: 23086419). Critically, the current UniProt GO annotation for Q9U9P7 already reflects the correct biochemistry: **phosphatidylinositol binding (GO:0035091, IBA)** and **phosphatidylinositol transfer activity (GO:0008526, ISS)** — and **no GO:0008525** is present. The predicted PC-transporter term would therefore be an *addition* that contradicts the existing, more accurate PI-transfer annotation.

Finding 3 — GO:0008525 has direct evidence only for Class I / multidomain PITP paralogs, never for the Class II PITPNC1/rdgBbeta clade

A systematic QuickGO/GOA enumeration of **GO:0008525 (phosphatidylcholine transporter activity)** across human, mouse, rat, fly, and yeast PITP-family proteins reveals a sharp phylogenetic pattern. Direct experimental (**IDA**) support for the PC-transporter term exists only for the PC-competent branches:

- **Class I PITPs**: human PITPNA (Q00169) and PITPNB (P48739) and their rodent orthologs;
- **Drosophila multidomain rdgB** (P43125, IDA);
- **Yeast SEC14** (P24280, IDA) — the founding dual PI/PC transfer protein, whose in vitro exchange of PI *or* PC is textbook (PMID: 15299870).

By contrast, the **entire Class II clade** carries **a single** GO:0008525 annotation — rat Pitpnc1 (A0A8I6A182), and that annotation is **ISO only** (inferred electronically from an ortholog), with **no IDA/EXP support anywhere** in the clade. Drosophila `rdgBbeta` (Q9U9P7) carries **no GO:0008525 annotation at all**. Within *Drosophila* specifically, PC-transporter activity is annotated to the paralogs **rdgB (IDA)** and **vib/vibrator (IBA)** — not to `rdgBbeta`.

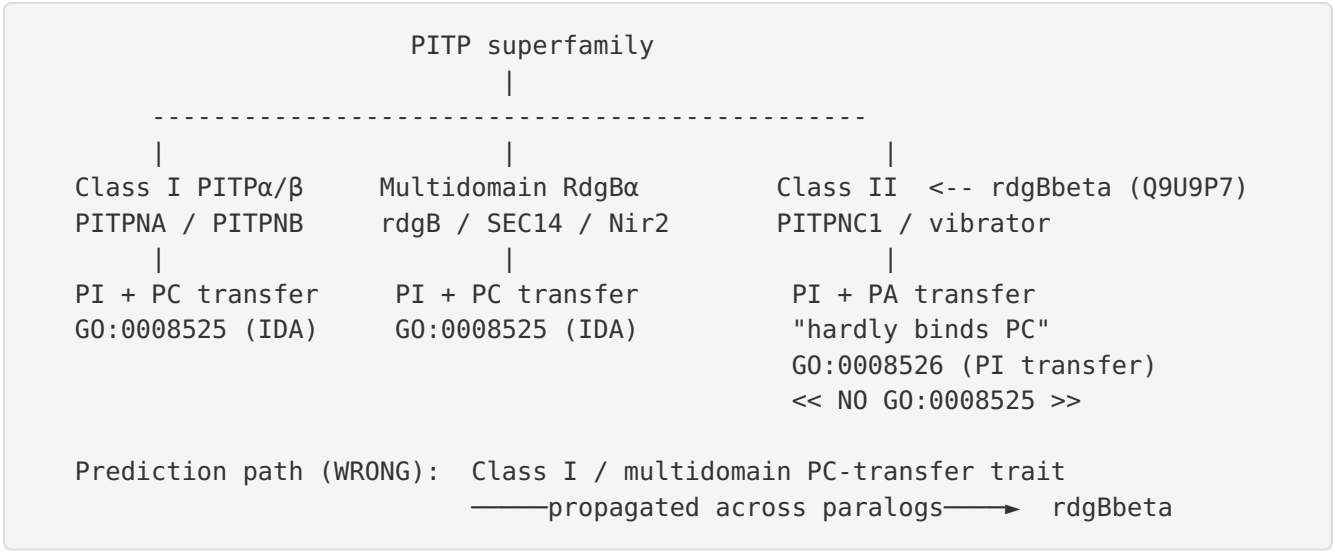
This is the fingerprint of paralog over-annotation. The one Class II GO:0008525 record in existence is itself a non-experimental electronic inference that most plausibly originated by carry-over from a Class I / multidomain paralog. A model trained on such annotation landscapes can readily reproduce the same misassignment for `rdgBbeta`. *(Provenance: computed enumeration saved to `/tmp/go0008525_pitp_family.csv`.)*

Mechanistic Model / Interpretation

The PITP family splits into functionally distinct branches, and the seed hypothesis fails because it ignores that split. The molecular function under test is monomeric, headgroup-specific lipid transfer by a soluble PITP-domain protein: extract a lipid, shield its headgroup inside a hydrophobic cavity, and deposit it at an acceptor membrane. The discriminating variable is **headgroup specificity (PC vs PI/PA)**.

PITP family map and PC-transfer competence

Branch	Representative proteins	Domain architecture	PI transfer	PC transfer	GO:0008525 evidence
Class I	PITPα/PITPNA, PITPβ/PITPNB	Single PITP domain	Yes	**Yes** (dual specificity)	**IDA** (direct)
Multidomain RdgBα / Sec14	Drosophila rdgB, yeast SEC14, Nir2	PITP + FFAT/ DDHD/LNS2	Yes	**Yes**	**IDA** (direct)
Class II (target clade)	**rdgBbeta/ PITPNC1**, vibrator	Single PITP domain	Yes	**No / "hardly"**	none direct; 1 ISO record only



Immediate molecular function. The evidence indicates the direct molecular activity of Q9U9P7 is **PI transfer** (GO:0008526) and, by clade analogy with PITPNC1, **PA binding/transfer** — with PC essentially excluded from the binding pocket. PC transfer is therefore not this gene product's activity; it is the activity of sister paralogs. Downstream roles of PITPs (phosphoinositide signaling, photoreceptor/neural signal transduction) are pathway consequences, not the primary molecular activity, and do not implicate PC transport.

Why the model likely erred. Three compounding factors: (1) *frequency / paralog bias* — most experimentally characterized PITPs (Class I, SEC14) are PC-competent, so "PC transporter" is the family-common label; (2) *shared PITP domain* — the single conserved fold (PF02121) is common to PC-competent and PC-incompetent members, so domain-level features do not discriminate; (3) *annotation carry-over* — the one existing Class II GO:0008525 record is an ISO electronic inference, providing a spurious training signal. None of these reflect the measured biochemistry of the Class II clade.

Evidence Base / Evidence Matrix

Citation	Evidence type	Direction	Claim tested	Key finding	Context
PMID: 22822086	Direct assay (in vitro binding/ transfer)	**Refutes**	Does RdgBβ/ PITPNC1 transfer PC?	Binds/transfers PI and PA; "hardly binds phosphatidylcholine"; preloaded with PA/PG	Recombinant mammalian PITPNC1
PMID: 12562526	Direct assay (recombinant binding)	**Refutes**	PC binding by a Class II RdgBβ ortholog	mM-rdgBβ1 binds PI "but not to other phospholipids"	Recombinant mouse Class II PITP
PMID: 10358925	Review of direct assays	**Qualifies / competing**	Which PITPs bind PC?	PI *or* PC can occupy the Class I PITP site	Mammalian Class I PITPα/ β
PMID: 3651458	Direct assay (affinity/ kinetics)	**Qualifies / competing**	Magnitude of PC vs PI transfer	~16-fold PI-over-PC preference; measurable PC transfer	Bovine brain PI-TP (Class I-like)
PMID: 15299870	Structural/ biochemical	**Competing**	Dual PI/PC specificity origin	SEC14 exchanges PI *or* PC in vitro	Yeast Sec14p
PMID: 23086419	Structural/ evolutionary review	**Supports (classification)**	Is RdgBβ/ PITPNC1 a single-domain Class II PITP?	Confirms RdgBβ = PITPNC1, single-domain, distinct branch	Family-wide
UniProt Q9U9P7 + Pfam/	Computational (sequence/	**Supports (classification)**	Is fly rdgBbeta a Class II	Single PITP domain; closest to PITPNC1 by identity	Bioinformatic

Citation	Evidence type	Direction	Claim tested	Key finding	Context
InterPro/ PANTHER; NW identity (61.6% vs PITPNC1; 42.7% vs PITPNA)	domain/ orthology)		PITPNC1 ortholog?		
QuickGO/ GOA enumeration of GO:0008525	Database landscape (computed)	**Refutes (over-annotation pattern)**	Who has direct PC-transporter evidence?	IDA only in Class I / multidomain / SEC14; Class II has 1 ISO record; rdgBbeta has none	Human/ mouse/rat/fly/ yeast
PMID: 17543578	Review	Orientation	RdgB family function	RdgB proteins are PITP-domain lipid-transfer proteins	Family review
PMID: 9216063	Molecular characterization	Orientation	rdgB family homology	Human rdgB N-terminus homologous to PITPα/β	Rat/human retina

GO Curation Implications

Leads (require curator verification):

1. **Do NOT add GO:0008525 (phosphatidylcholine transporter activity)** to `rdgBbeta` (Q9U9P7). The computational prediction is contradicted by direct assays on the clade ("hardly binds phosphatidylcholine", PMID: 22822086) and by the absence of any experimental PC-transporter evidence in the entire Class II clade. Recommend **reject** the prediction.
2. **Retain the existing, more accurate MF annotations**:
3. **GO:0008526 — phosphatidylinositol transfer activity** (currently ISS): the correct, clade-consistent molecular function and core MF.

4. **GO:0035091 — phosphatidylinositol binding** (currently IBA): consistent with clade biochemistry.
5. **Consider (lead) a phosphatidic-acid–related MF** as a more informative activity than PC transfer, given that PITPNC1 binds/transfers PA (PMID: 22822086). Any such term should be applied only with an ISS/ISO evidence code from PITPNC1 and flagged, since the direct PA assay was on the mammalian ortholog; Drosophila-specific evidence is lacking, so treat as non-core for DROME for now.
6. **Treat GO:0008525 as non-core** for this gene and flag the single existing Class II ISO GO:0008525 record (rat Pitpnc1) as a candidate paralog-carry-over error.

The evidence supports an **MF** decision (lipid-transfer specificity), not a BP or CC change. "Protein binding" is explicitly **not** recommended — the informative term (PI transfer, GO:0008526) is already supported.

Mechanistic Scope

The immediate function tested is **headgroup-specific monomeric lipid transfer**. The seed prediction (PC transporter) conflates the Class II RdgBβ pocket specificity (PI/PA) with the Class I PITPα/β dual PI/PC specificity. Everything downstream — phosphoinositide signaling, secretory/Golgi function attributed to some RdgB proteins, photoreceptor/neural signal transduction — is a pathway or developmental consequence, not the primary molecular activity, and none of it implicates PC transport by `rdgBbeta`. Importantly, `rdgBbeta` (small, cytosolic, PITPNC1-type) must not be confused with `rdgB`-alpha (large multidomain PITPNM/Nir-type); they are distinct paralogs with distinct biology, and it is the alpha/multidomain and Class I paralogs — not `rdgBbeta` — that carry the direct PC-transfer evidence.

Conflicts and Alternatives

- **Paralog confusion (primary alternative and likely source of the prediction).** PC-transporter activity is genuine for Class I PITPα/β (PMID: 10358925), for multidomain Drosophila `rdgB` (IDA), and for yeast SEC14 (PMID: 15299870). These are *paralogs* of `rdgBbeta`, not `rdgBbeta` itself. The model most plausibly generalized a family-common PC-transfer trait onto a Class II member that lacks it.

- **Isoform / organism-specific gaps.** The strongest "no PC" assay (PMID: 22822086) used mammalian PITPNC1; the mouse Class II assay (PMID: 12562526) used mM-rdgBβ1. No published in vitro PC-transfer assay on the Drosophila Q9U9P7 protein itself was located. The refutation rests on strong orthology plus concordant clade biochemistry rather than a fly-specific assay.
- **Quantitative "hardly" vs absolute exclusion.** "Hardly binds PC" is a low but non-zero statement; under artificial vesicle conditions trace PC association is conceivable. This does not justify a core PC-**transporter** MF annotation, which implies a physiological transport function.
- **Database carry-over.** The single Class II GO:0008525 record (rat Pitpnc1, ISO) is electronic and unverified; it should not be treated as independent support and may share the same error origin as the prediction.

Limitations and Knowledge Gaps

1. **No Drosophila-protein-specific PC-transfer assay or functional phenotype paper.** *Checked:* PubMed for CG17818/rdgBbeta biochemistry and photoreceptor/PLC function; GOA. *Why it matters:* specificity is inferred from the mammalian ortholog. *Resolver:* in vitro PI/PC/PA transfer assay with purified recombinant Drosophila `rdgBbeta`. (No experimental PDB exists for Q9U9P7, but a high-confidence AlphaFold model is available, global pLDDT ≈ 91.5, so structural pocket analysis is immediately feasible.)
2. **PA-transfer relevance for the fly protein is inferred, not measured.** *Checked:*

   P 22822086

   (PITPNC1). *Why it matters:* a PA-related MF would be the most informative substitute for the rejected PC term. *Resolver:* PA binding/transfer assay on Q9U9P7 or structural pocket analysis.
3. **Provenance of the single Class II ISO GO:0008525 record.** *Checked:* QuickGO/GOA enumeration. *Why it matters:* if it seeded the prediction, it is a correctable pipeline artifact. *Resolver:* trace the ISO "with/from" source protein.
4. **Provenance of the BioReason-Pro prediction** (whether it used Class I features) is unverified; matters for judging systematic paralog bias.

Discriminating Tests / Proposed Follow-up Experiments

1. **In vitro fluorescent-lipid transfer assay** (pyrene-PI vs pyrene-PC vs NBD-PA) with purified recombinant DROME `rdgBbeta` — the single most decisive experiment. Expectation if refuted: robust PI (and PA) transfer, negligible PC transfer, mirroring PMID: 22822086.
2. **Mass-spectrometric lipidomics of the co-purified ligand** (as done for PITPNC1, which co-purified PA/PG). Absence of PC in the bound pool directly counters the prediction.
3. **Structural pocket comparison** (AlphaFold model of Q9U9P7 vs Class I PITPα holo-structures vs PITPNC1): identify headgroup-coordinating residues and test whether the pocket can accommodate the PC choline headgroup.
4. **Rescue/complementation:** does `rdgBbeta` substitute for Class I PITP PC-dependent functions? A negative result supports non-PC specificity.
5. **Annotation-provenance audit:** trace the rat Pitpnc1 ISO GO:0008525 record's source and any BioReason-Pro training exposure to Class I / multidomain PITP PC-transporter annotations, to confirm the paralog-carry-over mechanism.

Curation Leads (require curator verification)

- **Action change:** Reject/omit **GO:0008525** for `rdgBbeta`; do not import the BioReason-Pro SFT prediction.
- **Retain: GO:0008526** (phosphatidylinositol transfer activity, ISS) and **GO:0035091** (phosphatidylinositol binding, IBA) as the accurate MF core.
- **Candidate new/replacement term (lead):** a **phosphatidic-acid binding/transfer** MF term supported by ISS/ISO from PITPNC1 (PMID: 22822086), clearly flagged as ortholog-inferred and non-core for DROME.
- **Candidate reference + snippet to verify:** PMID: 22822086 — *"besides PI, RdgBβ binds and transfers phosphatidic acid (PA) but hardly binds phosphatidylcholine."*
- **Candidate reference + snippet to verify:** PMID: 12562526 — *"The recombinant mM-rdgBbeta1 protein shows the specific binding activity to phosphatidylinositol but not to other phospholipids."*
- **Paralog-source reference:** PMID: 10358925 — *"The preferred lipid that can occupy the site can be either phosphatidylinositol (PI) or phosphatidylcholine (PC)"* (establishes PC transfer as a Class I trait).

- **Suggested curator question:** Was GO:0008525 propagated from Class I / multidomain PITP paralogs (rdgB, vib, PITPNA/B), and should the single Class II ISO record (rat Pitpnc1) also be flagged?
- **Suggested experiment:** direct DROME `rdgBbeta` PI/PC/PA transfer assay.

Conclusion

The prediction of **phosphatidylcholine transporter activity (GO:0008525)** for Drosophila `rdgBbeta` (Q9U9P7) is **REFUTED**. `rdgBbeta` is a single-domain **Class II PITP** orthologous to human PITPNC1 (61.6% identity vs 42.7% to Class I PITPNA). Direct biochemical characterization of this clade shows it binds and transfers **phosphatidylinositol and phosphatidic acid** but **"hardly binds phosphatidylcholine"** (PMID: 22822086); PC transfer is a Class I / multidomain (Sec14-type) property. The prediction is a **paralog / substrate misassignment**, reinforced by the fact that GO:0008525 has direct (IDA) support only in Class I and multidomain paralogs and none in the Class II clade. Curators should reject GO:0008525 and retain the more accurate **phosphatidylinositol transfer activity (GO:0008526)**.

Provenance: UniProt Q9U9P7 JSON parse and Needleman–Wunsch identity computation were executed in this investigation (rdgBbeta vs human PITPNC1 = 61.6%; vs PITPNA = 42.7%). GO:0008525 family enumeration saved to `/tmp/go0008525_pitp_family.csv`. Literature retrieved via PubMed.
