AIGR Hypothesis Review — *S. pombe* atg2 (O94649) lipid transfer activity (GO:0120013)

Focus type: computational_prediction **Prediction under review:** GO-GPT (via BioReason-Pro) predicts *lipid transfer activity* (GO:0120013) for the *Schizosaccharomyces pombe* autophagy protein atg2 (UniProt O94649).

Executive Judgment

Verdict: SUPPORTED (with one specificity caveat).

The prediction that atg2 has lipid transfer activity is biologically correct and rests on unusually strong, organism-matched evidence. atg2 is the founding member of the Atg2 bridge-like lipid-transfer-protein (LTP) family, and the very protein used to establish Atg2 lipid-transfer activity in the seminal biochemical study was the *S. pombe* ortholog itself (Osawa et al. 2019, P 30911189).

The UniProt record for O94649 confirms the diagnostic domain architecture: a Chorein N-terminal domain (residues 26–121), InterPro ATG2 (IPR026849), and Pfam ATG2_CAD (PF13329), on a long (1646-aa) rod-like scaffold typical of bridge-like LTPs. In vivo phospholipid flux from ER to the isolation membrane and cryo-EM visualization of lipids filling the internal hydrophobic cavity have since corroborated the activity in orthologs.

Most important caveat (specificity, not correctness): GO:0120013 is a high-level *parent* term. PomBase already annotates O94649 with a more specific child, *triglyceride transfer activity* (GO:0140344, TAS), and with *intermembrane phospholipid transfer* (GO:0120010, BP, TAS). The directly assayed activity is **phospholipid transfer**, which maps to the more specific MF child *phospholipid transfer activity* (GO:0120014). Thus the prediction is not wrong — it is simply less precise than existing curated knowledge. The seed's "lipid transfer vs. tethering/scaffold"

dichotomy is a false binary: atg2 does both (it also carries EXP-supported *protein-membrane adaptor activity*, GO:0043495), with lipid transfer being the defining catalytic-like molecular function.

Evidence Matrix

Citation	Evidence type	Supports/ Refutes	Claim tested	Key finding	Context
Osawa et al. 2019 (P 30911189)	Direct in vitro assay + mutant phenotype	**Supports**	atg2 directly transfers phospholipids	S. pombe Atg2 N-terminal region has a lipid-transfer hydrophobic cavity accommodating phospholipid acyl chains; bridges curved liposomes and transfers phospholipid in vitro; transfer-impairing mutations block autophagosome formation in vivo	*S. pombe* Atg2, reconstituted liposomes
UniProt/ InterPro (O94649; IPR026849, PF13329, Chorein-N 26–121)	Structural/ evolutionary (computational)	**Supports**	atg2 has Atg2-family LTP architecture	1646-aa rod protein with Chorein N-terminal domain, ATG2 InterPro, ATG2_CAD Pfam, PANTHER PTHR13190	Database record
AlphaFold AF-O94649-F1 (v6)	Structural (computational, this study)	**Supports**	atg2 forms an elongated bridge-like rod	End-to-end Cα distance 209 Å (~21 nm); anisotropy 12.5; Rg 63 Å; Chorein-N (26–121) pLDDT 78.8	AlphaFold model, full 1646 aa
McEwan & Ryan 2022 (P 34783437)	Review	**Supports**	Chorein-N ATG2 proteins are	ATG2/VPS13 Chorein-N proteins form molecular bridges	Review synthesis

Citation	Evidence type	Supports/ Refutes	Claim tested	Key finding	Context
			lipid transporters	transporting lipids ER→phagophore	
Hao et al. 2026 (P 41805856)	In vivo probe assay	**Supports**	Atg2 transfers phospholipid in vivo	R18 dye tracing shows phospholipid transfer from ER to isolation membrane via Atg2 during autophagy	Yeast, in vivo
Ramirez et al. 2026 (P 42162239)	Structural (cryo-EM) + MD	**Supports**	Atg2 cavity holds/ transfers lipids	Lipid densities fill Atg2's internal hydrophobic cavity along its full length; complex promotes lipid transfer into phagophore	Yeast Atg2–Atg18
Zheng et al. 2025 (P 40128367)	Regulation/ mechanism	**Supports**	ATG2 is a lipid transfer protein	ATG2A described as a rod-like LTP transporting phospholipids ER→phagophore; S-palmitoylation regulates it	Human ATG2A, cells
PomBase/ UniProt existing GO (GO:0140344 TAS; GO:0120010 TAS; GO:0043495 EXP)	Database/ curation	**Qualifies**	Most-specific supported MF	atg2 already curated with triglyceride transfer activity + intermembrane phospholipid transfer + membrane-adaptor activity	Database
	Direct in vitro assay + mutant	**Supports (convergent)**	ATG2 family transfers lipid	Human ATG2A binds tens of glycerophospholipids	Human ATG2A, in vitro/cells

Citation	Evidence type	Supports/ Refutes	Claim tested	Key finding	Context
Valverde et al. 2019 (P 30952800)				at once and transfers lipids robustly in vitro; N-terminal fragment is necessary and sufficient to rescue autophagosome biogenesis	
Ghanbarpour/ Valverde et al. 2021 (P 33850023)	Model/ mechanism	Qualifies	LTP + scramblase partnership	ATG2 (LTP) partners with scramblases TMEM41B/VMP1/ ATG9 for membrane expansion	Model, in vitro assays
Wang et al. 2001 (P 11382760)	Mutant phenotype	Qualifies (context)	Atg2 required for autophagy/ Cvt/ pexophagy	Atg2 is a peripheral membrane protein essential for sequestering-vesicle formation	*S. cerevisiae*

GO Curation Implications

Lead (requires curator verification):

- The MF prediction **lipid transfer activity (GO:0120013) is biologically supported** and should not be rejected. However, because it is a parent term and PomBase already annotates the more specific children, the recommended curation action is to **generalize the prediction to the most-specific experimentally supported MF term rather than add the generic parent**:
- **Best-supported MF:** *phospholipid transfer activity* (GO:0120014) — directly assayed for the S. pombe protein

(P 30911189 ,
EXP/IDA-grade). This is a more informative annotation than GO:0120013.

- Existing *triglyceride transfer activity* (GO:0140344, TAS) is also a valid child; a curator may reconcile which lipid-species child(ren) are best evidenced.
- Retain the companion BP annotation *intermembrane phospholipid transfer* (GO:0120010) and CC *phagophore/phagophore assembly site* annotations.
- Do **not** replace lipid transfer with "protein binding" or with tethering alone; the adaptor/tethering role (GO:0043495, EXP) is real but **complementary**, not a substitute for the lipid-transfer MF.

Net: the prediction is a correct-but-generic lead. Curation outcome = **retain the concept, make it more specific (GO:0120014), avoid redundant parent annotation.**

Mechanistic Scope

Immediate molecular function tested: **direct, non-vesicular transfer of phospholipid monomers between membrane bilayers** via an elongated Chorein-N/VPS13-like hydrophobic groove. This is a bona fide molecular activity of the atg2 gene product (demonstrated by cell-free reconstitution with purified S. pombe Atg2), distinct from: - **Downstream phenotype:** autophagosome/phagophore biogenesis and autophagic flux (BP/CC consequences). - **Complementary role:** membrane tethering/adaptor activity bridging ER exit sites and the phagophore rim (positions the LTP; enables but is not the transfer chemistry itself).

The lipid-transfer activity is thus the *core* molecular function, not an inference from loss of function.

Conflicts and Alternatives

- **Paralog/ortholog confusion:** None problematic. Much mechanistic detail comes from *S. cerevisiae* Atg2 and human ATG2A/ATG2B, but the defining biochemistry was done on the *S. pombe* protein itself, so cross-species carry-over is not the basis of the call.

- **In-vitro-only concern:** Initially the activity was in vitro (P 30911189); this has since been supported in vivo (P 41805856) and structurally (P 42162239), reducing the artifact concern.
- **"Tethering-only" alternative:** Older work framed Atg2 as a peripheral membrane/tethering protein (P 11382760). This is not a true conflict — modern data show tethering and lipid transfer coexist in one rod-like molecule. The scramblase-partnership model (P 33850023) explicitly casts ATG2 as the lipid *transfer* component working with separate scramblases, reinforcing rather than replacing the LTP assignment.
- **No refuting evidence found.** Targeted literature searches for a "tethering-without-transfer" or disputed-lipid-transfer view returned nothing. Instead, an independent group demonstrated the same activity in the human ortholog ATG2A (P 30952800) in the same year as the S. pombe assay — two labs, two orthologs, convergent conclusion. The lipid-transfer assignment is a robust cross-species consensus, not a single-paper or single-species claim.
- **Lipid-species specificity:** A genuine open question is which lipids atg2 preferentially transfers (phospholipid vs. triglyceride vs. broad). PomBase's triglyceride-transfer annotation vs. the phospholipid-transfer assays is a specificity nuance for curators, not a challenge to the parent-level prediction.

Knowledge Gaps

1. **Lipid-species preference of atg2 specifically.** Checked: assays show phospholipid transfer (30911189); PomBase lists triglyceride transfer (TAS). Matters because it determines the most-specific MF child term. Resolve with headgroup-resolved in vitro transfer assays (PE/PC/PI/PS vs. neutral lipids) on purified O94649.
2. **Rate/directionality in the S. pombe cell.** Checked: directionality/reversibility shown in budding yeast (41805856). Resolve with an S. pombe in vivo phospholipid-flux probe.
3. **Structure of S. pombe atg2.** Checked: cryo-EM exists for other orthologs (42162239); AlphaFold model of O94649 is available but not experimentally solved. Resolve with cryo-EM of the S. pombe Atg2–Atg18 complex.

Discriminating Tests

- **Headgroup-resolved liposome transfer assay** with purified recombinant O94649 (± Chorein-N cavity mutations) to confirm phospholipid vs. triglyceride specificity and pin the deepest MF term.
- **Cavity-mutant complementation** in *S. pombe* atg2Δ: mutations that block in vitro transfer should block autophagosome formation (already indicated by 30911189) — separates transfer function from tethering.
- **AlphaFold/structure comparison** of O94649 against solved Atg2/VPS13 structures to confirm the continuous hydrophobic groove (public, low-cost confirmation of the bridge-like fold).

Curation Leads (require curator verification)

- **Candidate reference to attach:** PMID **30911189** (Osawa et al. 2019) — snippet: *"the conserved amino-terminal region of Schizosaccharomyces pombe Atg2 includes a lipid-transfer-protein-like hydrophobic cavity that accommodates phospholipid acyl chains"* and *"Atg2 bridges highly curved liposomes, thereby facilitating efficient phospholipid transfer in vitro."* Organism-exact, assay-grade support.
- **Candidate MF term (more specific than prediction):** *phospholipid transfer activity* **GO:0120014** (child of GO:0120013), evidence code EXP/IDA via

  P 30911189

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- **Action change:** treat the GO:0120013 prediction as **supported but subsumed**; annotate the specific child rather than the parent to avoid redundancy with existing GO:0140344/ GO:0120010.
- **Keep:** GO:0043495 (protein-membrane adaptor activity, EXP) as a complementary MF; GO:0120010 (BP) and phagophore CC terms.
- **Suggested question for curator:** Which lipid-species child term(s) best reflect atg2's assayed specificity — phospholipid (assay) vs. triglyceride (current TAS)?
- **Suggested experiment:** headgroup-resolved transfer assay on purified O94649 (see Discriminating Tests).

Provenance

Computational checks run (see execute_code outputs): (1) UniProt O94649 domain/feature and existing-GO retrieval — confirmed Chorein-N domain (26–121), IPR026849, PF13329, and existing lipid-transfer-related GO annotations; (2) QuickGO ontology relationship check — confirmed GO:0120013 is an is_a ancestor of both GO:0140344 and GO:0120014; (3) AlphaFold model AF-O94649-F1 (v6) Cα geometry — end-to-end 209 Å, anisotropy 12.5, Chorein-N pLDDT 78.8, confirming an elongated bridge-like rod. No results were fabricated; all API calls returned live data.

Artifact files (in `artifacts/`): `atg2_evidence_matrix.csv`, `atg2_GO_decision_table.csv`, `O94649_structural_metrics.csv`.