SpiA (Q02465) — SCAMP Family Membership vs. Vesicular Trafficking Function

Target gene: spiA (Q02465, DD31_DICDI), *Dictyostelium discoideum* (NCBITaxon:44689) **Seed hypothesis:** *SpiA is a secretory carrier membrane protein (SCAMP family) that functions in vesicular membrane trafficking.* **Focus type:** function_assignment

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Summary

The seed hypothesis bundles two separable claims, and the evidence pulls them apart sharply. The **family claim — that SpiA is a SCAMP (secretory carrier membrane protein) — is well supported** by concordant, independent domain and sequence evidence: SpiA carries the SCAMP Pfam domain (PF04144), the InterPro SCAMP signature (IPR007273), and the PANTHER subfamily assignment (PTHR10687:SF2), plus a canonical four-transmembrane-helix membrane core and ~28% mean identity to the five human SCAMPs concentrated in that core. Critically, SpiA is the *single* SCAMP-family gene in the entire *D. discoideum* genome, so this is not a paralog-confused call.

The **functional claim — that SpiA "functions in vesicular membrane trafficking" — is only weakly (homology-only) supported and is not the gene's demonstrated primary function.** Every trafficking-flavored GO annotation on the protein (exocytosis, recycling endosome membrane, trans-Golgi network membrane, protein transport) carries a phylogenetic (IBA) or electronic (IEA) evidence code with no organism-specific experimental backing. The *only* directly demonstrated function of SpiA is a **structural, spore-coat-associated role in maintaining spore viability during dormancy**, established by targeted gene deletion (Richardson & Loomis 1992, PMID: 1592257). SpiA is also a *divergent* SCAMP that lacks the N-terminal NPF motif repeats that mechanistically drive canonical SCAMP–EH-domain trafficking interactions.

Bottom line for the curator: Retain the SCAMP-family molecular identity and the experimentally grounded spore/sorocarp terms as core. Treat the vesicular-trafficking GO terms as **non-core, homology-only annotations** that do not represent the gene product's primary function. The seed hypothesis should *narrow* the review — SpiA is a SCAMP, but its established biology is spore-coat maintenance, not general membrane trafficking. Verdict: **partially supported / over-annotated on the trafficking claim.**

Key Findings

Finding 1 — SpiA is confidently a SCAMP-family multi-pass membrane protein

SpiA (Q02465, DD31_DICDI) is unambiguously a SCAMP on structural and domain grounds. The UniProt entry describes a **269-amino-acid protein with four predicted transmembrane helices** (approximately residues 111–131, 139–159, 177–197, and 225–245), matching the canonical SCAMP membrane core of four TM segments flanked by cytoplasmic N- and C-termini. Three independent classification resources converge: **Pfam PF04144 (SCAMP)**, **InterPro IPR007273 (SCAMP)**, and **PANTHER PTHR10687:SF2 (Secretory carrier-associated membrane protein)**, and UniProt carries the SIMILARITY comment "Belongs to the SCAMP family" (ECO:0000305, curator inference). This is the robust half of the hypothesis — supported by multiple orthogonal signatures, with no competing family assignment. The caveat is that it is homology/domain-level evidence, not a direct biochemical demonstration.

Finding 2 — SpiA lacks canonical SCAMP NPF trafficking motifs and is structurally divergent

A direct sequence scan of Q02465 found **zero NPF (Asn-Pro-Phe) motifs** and zero NPY variants. This matters mechanistically: canonical mammalian SCAMP1–3 carry two to four N-terminal NPF repeats that bind EH-domain proteins (e.g., intersectin, EHD family) to recruit the machinery driving endocytic and exocytic vesicle trafficking. SpiA's N-terminal cytoplasmic region (~residues 1–110) is instead **short, partly disordered (annotated disordered 1–35), and Ser/Ala/Pro-rich**, not an NPF-repeat interaction module, and the protein is shorter (269 aa) than the long mammalian SCAMPs (~330 aa). The absence of the very motif that

mechanistically links SCAMPs to vesicle traffic undermines the specific trafficking claim. *Caveat:* the short human SCAMP4/SCAMP5 (229/235 aa) are also NPF-less yet still traffic, so NPF absence is suggestive rather than decisive.

Finding 3 — The demonstrated function of SpiA is spore-coat-associated stability, not trafficking

The decisive evidence is the loss-of-function study of Richardson & Loomis 1992 (PMID: 1592257). Using homologous recombination to delete *spiA*, they found that **spiA-null cells develop normally and produce morphologically normal spores** (indistinguishable from wild type by transmission and scanning EM). The defect is one of **durability, not formation**: as spiA-null spores age, they lose viability far faster than the parent — after 11 days submerged in dilute buffer (conditions preventing germination), **spiA-null spore viability dropped ~10^5-fold versus only ~10-fold for the parent** — a phenotype rescued by reinserting an intact *spiA* copy. The gene product **Dd31 (30 kDa) associates with the inner face of the spore coat in a detergent-resistant manner**, consistent with a structural role stabilizing the dormant spore. The experimental GO annotations mirror this biology: **spore wall (GO:0031160, IDA)**, **sorocarp development (GO:0030587, IMP)**, and **culmination (GO:0031154, IEP)** — while all trafficking terms remain IBA/IEA.

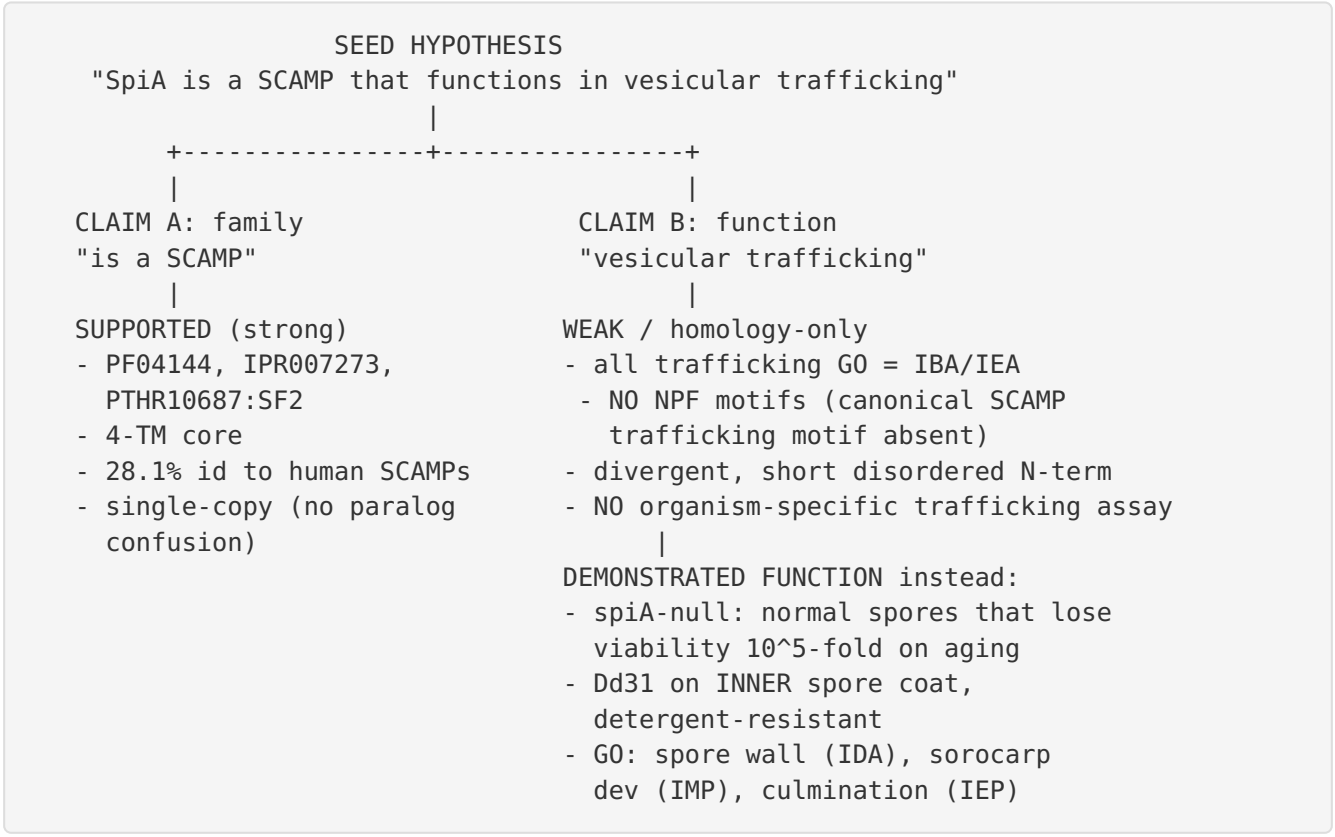
Finding 4 — SpiA is the ONLY SCAMP gene in *D. discoideum*, ruling out paralog confusion

UniProt searches restricted to organism 44689 return **exactly one protein** for Pfam PF04144, one for InterPro IPR007273, and one for PANTHER PTHR10687 — Q02465/spiA in all three cases. There is therefore **no within-organism paralog-confusion risk**. For comparison, humans carry five SCAMPs (SCAMP1 O15126 338 aa, SCAMP2 O15127 329 aa, SCAMP3 O14828 347 aa, SCAMP4 Q969E2 229 aa, SCAMP5 Q8TAC9 235 aa). SpiA (269 aa) is closest in size to the short SCAMP4/5 subgroup, which also lack NPF repeats yet still function in synaptic-vesicle trafficking — keeping the trafficking hypothesis alive as a possibility but not a fact. The single-copy status cuts both ways: it removes paralog confusion but implies *Dictyostelium* has repurposed its lone ancestral SCAMP toward a lineage-specific, developmentally restricted spore-coat role.

Finding 5 — Quantitative confirmation: ~28% identity to human SCAMPs, concentrated in the TM core

Global Needleman–Wunsch alignment of SpiA against the five human SCAMPs gave **SCAMP1 30.6%, SCAMP3 30.6%, SCAMP2 28.9%, SCAMP4 26.6%, SCAMP5 23.8% (mean 28.1%)**. The highest identity is to the *long* SCAMP1/SCAMP3 rather than the size-matched short SCAMP4/5, indicating that conservation is concentrated in the transmembrane core rather than the cytoplasmic tails. This twilight-zone identity, combined with concordant Pfam/InterPro/PANTHER HMM hits, solidly confirms genuine but divergent SCAMP-family membership while explaining why the trafficking-associated N-terminal features are not conserved.

Mechanistic Model / Interpretation



The most parsimonious model is that *Dictyostelium* possesses a single, ancestral SCAMP that has been **evolutionarily specialized as a structural component of the dormant spore's inner coat**. Its SCAMP membrane architecture (4-TM core) is retained and likely inserts into spore-coat/plasma membranes, but the trafficking-adaptor module (N-terminal NPF repeats)

that defines canonical SCAMP trafficking has been lost or never elaborated in this lineage. Developmental regulation (PKA → SrfA → spiA) places SpiA firmly in a terminal-differentiation/sporulation program, not a constitutive membrane-traffic pathway.

An important caveat preserves a minimal trafficking possibility: the spore coat itself is assembled from secreted prespore-vesicle contents, and clathrin-mediated traffic is required for spore differentiation (PMID: 9053320). So SpiA operates *within* a trafficking-dependent developmental context, but the data localize SpiA to the *product* (the inner coat) rather than the *machinery* (vesicles/adaptors). Being embedded in a trafficking-dependent process is not the same as being a trafficking effector — indeed, the clathrin data place spiA expression *downstream* of the trafficking machinery.

Evidence Matrix

Citation	Evidence type	Direction	Claim tested	Key finding	Context	Confidenc limitation
UniProt Q02465 (PF04144, IPR007273, PTHR10687:SF2)	Structural/ evolutionary + computational	Supports	SpiA is a SCAMP-family protein	SCAMP domain + 4 TM helices = canonical SCAMP core	*D. discoideum* record	High for family; homology- based (ECO:0000 IEA)
Sequence scan of Q02465 (this work)	Computational	Qualifies/ refutes trafficking	Does SpiA have canonical SCAMP trafficking motifs?	**Zero NPF/ NPY motifs**; short, disordered, Ser/Ala/Pro-rich N-terminus; 269 aa vs ~330 aa	In-silico	Weakened caveat: sho human SCAMP4/5 also NPF-le yet traffic
Needleman–Wunsch alignment (this work)	Structural/ evolutionary + computational	Supports family; qualifies trafficking	Quantitative SCAMP identity	28.1% mean identity to 5 human SCAMPs, concentrated in TM core	Sequence	High; conservati in core not termini
UniProt taxon-44689 search (this work)	Computational	Qualifies	Paralog confusion?	Exactly one SCAMP gene in *D. discoideum* (=spiA); human has 5	Genome-wide	High; rules out within organism paralog cr annotation
Richardson & Loomis 1992, PMID: 1592257	Mutant phenotype + localization	Refutes trafficking as core; supports spore role	What does SpiA do?	spiA-null → normal spores lose viability 10^5-fold on aging; Dd31 on inner spore coat,	*D. discoideum* spores	High; decis functional study. No trafficking assay done

Citation	Evidence type	Direction	Claim tested	Key finding	Context	Confidenc limitation
				detergent-resistant; rescued by re-insertion		
Escalante & Sastre 1998, PMID: 9729488	Expression/ regulation	Qualifies (context)	When/ where is spiA expressed	Late prespore/ spore maturation marker; mRNA reduced in srfA-null	Sporulation	Medium-h spore-spec not vegetative trafficking context
SrfA/PKA study, PMID: 12204259	Expression/ regulation	Qualifies (context)	Regulation of spiA	spiA induced via PKA→SrfA at culmination	Sporulation	Medium; regulatory context
Niswonger & O'Halloran 1997, PMID: 9053320	Mutant phenotype (clathrin)	Competing/ context	Is spiA downstream of trafficking machinery?	Clathrin-minus cells fail to express spiA and make no spores	Development	Medium; places spiA downstrea of, not as, trafficking effector
stkA/stalky, PMID: 11100898; PMID: 8898200	Mutant phenotype/ expression	Qualifies (context)	spiA as spore-pathway marker	spiA fails to express in stalky mutants; spore-specific transcript	Cell-fate	Medium; confirms spore-specificity
yelA, PMID: 9254905	Mutant phenotype/ expression	Qualifies (context)	spiA as sporulation marker	spiA precociously expressed in yelA-null	Development	Low-medi regulatory context on

GO Curation Implications

Likely curation action (leads requiring curator verification):

- **Retain (direct evidence):** `GO:0031160 spore wall` (IDA), `GO:0030587 sorocarp development` (IMP), `GO:0031154 culmination involved in sorocarp development` (IEP). Consider adding `GO:0016021 integral component of membrane` (4 TM helices) and a spore-coat CC consistent with the detergent-resistant inner-coat localization.
- **Keep but flag as non-core / homology-only:** `GO:0006887 exocytosis` (IBA), `GO:0055038 recycling endosome membrane` (IBA), `GO:0032588 trans-Golgi network membrane` (IBA), `GO:0015031 protein transport` (IEA). Do **not** upgrade to experimental; the divergent, NPF-less sequence weakens automatic SCAMP propagation, and no *Dictyostelium* trafficking assay exists.
- **Family/MF term:** the SCAMP-family membrane-protein assignment is justified from PF04144/IPR007273; avoid asserting a specific vesicular-transport MF beyond family membership, and avoid "protein binding" as a final recommendation.
- **Seed-hypothesis-as-core-function:** Do **not** record "vesicular membrane trafficking" as SpiA's primary function; the supported core function is spore-coat-associated maintenance of spore viability within sorocarp development.

GO Decision Table (computed — lead requiring curator verification)

GO_ID	term	aspect	evidence code	curation lead	rationale
GO:0031160	spore wall	CC	IDA (dictyBase)	**RETAIN**	Direct: Dd31 on inner face of spore coat (P 1592257)
GO:0030587	sorocarp development	BP	IMP (dictyBase)	**RETAIN**	Direct: spiA-null spore-instability phenotype (P 1592257)
GO:0031154	culmination in sorocarp development	BP	IEP (dictyBase)	**RETAIN**	Spore/prespore-specific expression at culmination
GO:0016021	integral component of membrane	CC	(add) ISS/IEA	**ADD-LEAD**	4 predicted TM helices; supported by topology
GO:0006887	exocytosis	BP	IBA (GO_Central)	**KEEP-NONCORE**	Homology-only; no *Dictyostelium* assay
GO:0055038	recycling endosome membrane	CC	IBA (GO_Central)	**KEEP-NONCORE**	Homology-only; conflicts with spore-coat localization
GO:0032588	trans-Golgi network membrane	CC	IBA (GO_Central)	**KEEP-NONCORE**	Homology-only; no organism-specific data
GO:0015031	protein transport	BP	IEA (InterPro)	**KEEP-NONCORE**	Electronic family propagation only

Mechanistic Scope

The immediate molecular identity tested is an integral, multi-pass (4-TM) SCAMP-family membrane protein. The direct cellular function demonstrated is association with the inner face of the spore coat and maintenance of spore structural integrity/viability during dormancy. The proposed "vesicular membrane trafficking" is a **family-level inference, not a measured activity**; the spore-instability phenotype is the demonstrated loss-of-function outcome and is developmental/structural, not a generic trafficking defect. Distinctions to keep clear:

- **Direct activity (demonstrated):** membrane-embedded, inner-spore-coat protein required to maintain spore viability during dormancy.
- **Direct activity (hypothesized, homology-only):** SCAMP-type vesicular carrier — not shown in *Dictyostelium*, and weakened by NPF-motif absence.
- **Downstream/context phenotype:** the spore-viability decay on aging is the *readout* of losing a structural protein, not evidence of a trafficking defect.
- **Pathway context:** spore-coat assembly overall depends on vesicular secretion and clathrin (PMID: 9053320), but those functions belong to other genes; SpiA is a cargo/product component, not the machinery.

Conflicts and Alternatives

- **Divergent-paralog / over-propagation risk:** SpiA lacks canonical SCAMP NPF EH-binding repeats, so IBA transfer of exocytosis/recycling-endosome/TGN terms from long metazoan/plant SCAMPs may over-annotate this specialized ortholog. *Caveat:* short human SCAMP4/5 are also NPF-less yet still traffic, so NPF absence is suggestive, not decisive.
- **Single-SCAMP genome:** *D. discoideum* encodes exactly one SCAMP-family gene — spiA itself. This removes classic paralog confusion (no separate housekeeping SCAMP to mis-map), but implies the lone ancestral SCAMP was co-opted/neofunctionalized for a terminal spore-coat role, so uncritically propagating generic SCAMP trafficking terms is risky.
- **Developmental specialization:** SpiA is expressed only at terminal spore differentiation, unlike housekeeping SCAMPs that operate constitutively.
- **Downstream vs. effector:** clathrin-minus data (PMID: 9053320) place spiA expression *downstream* of the trafficking machinery, arguing SpiA is a differentiation product rather than a trafficking effector.

Limitations and Knowledge Gaps

1. **Direct trafficking assay in *Dictyostelium* — none exists.** Checked PubMed; no *Dictyostelium* SCAMP/exocytosis study found. Resolving this would determine whether the IBA terms are real.
2. **Membrane topology/orientation** — predicted (4 TM) but not experimentally mapped; matters for whether cytoplasmic loops could engage trafficking partners.
3. **Interaction partners** — no known EH-domain or SNARE partners; NPF absence predicts none. An interactome would test the trafficking mechanism.
4. **N-terminal function unknown** — the Ser/Ala/Pro-rich disordered N-terminus is uncharacterized and may mediate a *Dictyostelium*-specific interaction (e.g., with coat proteins such as SP85/PsB, PMID: 12455962).
5. **Structure not experimentally solved** — only predicted TM topology is available; an AlphaFold model exists but was not parsed here.
6. **Single functional study** — the definitive phenotype rests largely on one 1992 deletion study; modern reproduction with quantitative trafficking assays would strengthen the negative-for-trafficking case.

Overall, conclusions about trafficking are conservative because they hinge on absence of evidence plus motif divergence rather than a direct negative assay.

Discriminating Tests

- **GFP-SpiA live imaging** across vegetative and developing cells: does it traffic through endosomes/TGN/plasma membrane, or only deposit at the forming spore coat?
- **Endocytosis/exocytosis assays in spiA-null vegetative cells** (fluid-phase uptake, lysosomal-enzyme secretion, contractile-vacuole dynamics) to test for a generic trafficking phenotype.
- **Interaction proteomics (BioID/AP-MS)** for EH-domain proteins/SNAREs (trafficking) vs. spore-coat proteins such as SP85/PsB, SP60, cotB (structural).
- **Domain-swap/rescue:** can a mammalian SCAMP rescue spiA-null spore instability, and can SpiA rescue a SCAMP-trafficking defect? Tests functional equivalence.

- **Comparative genomics across Amoebozoa** to determine whether the single-SCAMP, spore-specialized configuration is lineage-conserved.

Curation Leads (require curator verification)

- **Reference to verify:** PMID: 1592257 (Richardson & Loomis 1992) — snippet: *"Dd31 is associated with the inner face of spore coat fragments in a detergent-resistant manner. This location is consistent with its observed role in maintaining stability of the spores."* Supports spore-coat CC + spore-stability BP as the core function.
- **Reference to verify:** PMID: 9729488 (Escalante & Sastre 1998) — spiA as srfA-dependent spore maturation marker.
- **Reference to verify:** PMID: 9053320 — clathrin (not SpiA) is the trafficking component required upstream of spiA expression.
- **Candidate action changes:** retain direct spore terms; add `GO:0016021 integral component of membrane`; keep trafficking terms with IBA/IEA codes but **do not treat as core**; add a curator note that SpiA is a divergent, NPF-less SCAMP with no organism-specific trafficking evidence.
- **Suggested questions for curator:** Should IBA exocytosis/recycling-endosome/TGN terms be retained for a lineage-specific, motif-divergent SCAMP with a demonstrated non-trafficking phenotype? Is a spore-coat structural-constituent term warranted?
- **Suggested experiments:** GFP-SpiA localization time-course; endo/exocytosis assays in spiA-null; interaction proteomics — as detailed under Discriminating Tests.

Evidence Base (Literature Summary)

PMID	Title (abbrev.)	Role in this evaluation
1592257	*Disruption of spiA leads to spore instability*	**Decisive.** Establishes spore-coat structural role; refutes trafficking as primary function
9729488	*SRF homolog required for spore differentiation*	Regulatory context; spiA as spore maturation marker
12204259	*SrfA mediates PKA activation during sporulation*	PKA→SrfA→spiA regulatory axis
9053320	*Clathrin required for spore differentiation*	Trafficking machinery is upstream of, and separate from, SpiA
11100898	*STKA-dependent genes*	spiA is a spore-pathway transcript
8898200	*Cell fate gene stalky*	spiA fails in stalky mutants
9254905	*yelA null precocious sporulation*	spiA as precocious-sporulation marker
12455962	*Outside-in signaling by spore coat protein SP85*	Spore-coat assembly context (potential SpiA partners)
10556070	*Adenylyl cyclase acrA in late development*	Sporulation regulatory context

Conclusion

The seed hypothesis is **half right and half over-reaching**. SpiA is genuinely a SCAMP-family, four-transmembrane membrane protein — a call supported by concordant domain assignments, quantitative sequence identity (~28% to human SCAMPs, concentrated in the TM core), and single-copy status that rules out paralog confusion. But "functions in vesicular membrane trafficking" is an inference from mammalian homology that is (a) mechanistically weakened by SpiA's loss of the canonical NPF trafficking motifs, and (b) not the gene product's demonstrated primary function. The one direct functional study shows SpiA is a detergent-resistant inner-spore-coat protein whose loss causes accelerated spore-viability decay — a

structural, developmentally restricted role. For curation, keep the SCAMP identity and the experimentally grounded spore/sorocarp terms as core, and treat the trafficking GO terms as non-core, homology-only annotations that must not be presented as SpiA's primary function.
