AIGR Deep Research — dnajc6 (auxilin) dephosphorylation prediction

Gene: dnajc6 (Auxilin) · *Danio rerio* (NCBITaxon:7955) · UniProt **A0A8M9QG43** **Focus:** computational_prediction · slug `prediction-dephosphorylation` **Term under test:** dephosphorylation (**GO:0016311**) **Predictor:** ProtNLM2

Summary

The ProtNLM2 prediction that zebrafish **dnajc6 (auxilin)** is involved in **dephosphorylation (GO:0016311)** is **refuted**. The prediction is a homology-driven over-annotation: it is triggered by the presence of an N-terminal **PTEN-like / tensin-phosphatase fold** at the start of the auxilin protein, but that fold is a **catalytically dead pseudophosphatase**, not a working enzyme. This conclusion is reached not by literature reasoning alone but by direct sequence- and structure-level computation on the zebrafish protein, benchmarked against active human PTEN and against biochemically characterized mammalian auxilin.

Three converging lines of computed evidence make the case. First, at the **sequence** level, the diagnostic protein-tyrosine-phosphatase P-loop signature (H-C-X5-R) is degenerate in auxilin: active PTEN carries the canonical `HCKAGKGR` loop (Cys124…Arg130, a true C-X5-R), whereas zebrafish dnajc6 carries `TCSDGR`, a contracted **C-X3-R** loop that has additionally lost the invariant histidine. Second, at the **structural** level, the AlphaFold model of zebrafish dnajc6 (well-modeled in the relevant region) shows that only the nucleophilic cysteine (Cys218) is retained; there is **no arginine positioned to cradle a substrate phosphate** (nearest Arg is 7.6 Å away, versus 4.8 Å in active PTEN). Third, at the **evolutionary** level, the identical dead C-X3-R loop is fixed across human, mouse, and zebrafish DNAJC6, ruling out a species-specific artifact.

The genuine, well-documented function of DNAJC6/auxilin is that of a neuronal **J-domain/ Hsc70 clathrin-uncoating co-chaperone** and a **non-catalytic phosphoinositide sensor** — a coincidence detector of clathrin-coated vesicle budding — whose loss of function causes juvenile parkinsonism. Dephosphorylation is not part of this mechanism. Curators should **not**

add GO:0016311 (or any phosphatase molecular-function term) for A0A8M9QG43; better-supported terms describe clathrin uncoating, Hsc70 co-chaperone activity, and phosphoinositide binding.

Executive Judgment

Verdict: REFUTED (over-annotated). The ProtNLM2 "dephosphorylation (GO:0016311)" prediction for zebrafish dnajc6 is a **misassignment driven by homology to the PTEN/tensin phosphatase fold, not by an intact catalytic site.**

The decisive test is whether the protein-tyrosine-phosphatase catalytic **P-loop signature H-C-X5-R** (catalytic cysteine followed 5 residues later by the transition-state-stabilizing arginine) is intact. It is **not**:

- **Human PTEN** (active enzyme): P-loop = `HCKAGKGR` → `C-X5-R` (canonical). □ active
- **Human DNAJC6/auxilin**: aligned P-loop = `HCLDGR` → `C-X3-R` (arginine displaced two positions). □ pseudophosphatase
- **Zebrafish dnajc6**: aligned P-loop = `TCSDGR` → `C-X3-R`, plus the conserved His→Thr. □ pseudophosphatase

A regex scan for the `C.{5}R` phosphatase signature returned a hit **only** in PTEN and **none** in either auxilin ortholog. The zebrafish and human auxilin loops are orthologous and conserved (`NPKNVCVVHCLDGRAASSIL` vs `NPKNVCVITCSDGRAPSGVL`), so this is a **genuinely conserved dead P-loop**, not paralog confusion or a species artifact. Independent structural work on mammalian auxilin states directly that "**A change in the structure of the P loop accounts for the lack of phosphatase activity**" (PMID: 20826345).

Caveats: (i) The catalytic cysteine itself is retained (which is why UniProt auto-annotates a "phosphocysteine intermediate" active site in *human* auxilin) — but a lone cysteine without the CX5R arginine cradle is not catalytic. (ii) No enzymatic assay of the zebrafish protein exists; the conclusion rests on motif conservation + the mammalian structural precedent, which is strong but structural/evolutionary rather than a direct zebrafish assay.

Key Findings

Finding 1 — The zebrafish dnajc6 P-loop is degenerate (C-X3-R, not the catalytic C-X5-R)

The single most diagnostic feature separating an active protein-tyrosine/dual-specificity phosphatase from a dead one is the **P-loop signature H-C-X5-R** (the CX5R motif). In it, the cysteine is the catalytic nucleophile and the arginine — positioned exactly five residues downstream — cradles the substrate phosphate and stabilizes the pentacovalent transition state. I extracted and aligned this loop across orthologs. Active human PTEN (P60484) carries `HCKAGKGR`, a true C-X5-R (Cys124…Arg130). Human auxilin/DNAJC6 (O75061) carries the aligned loop `HCLDGR`, a contracted **C-X3-R** in which the arginine has moved two positions closer to the cysteine. Zebrafish dnajc6 (A0A8M9QG43) carries `TCSDGR`, also **C-X3-R**, and additionally substitutes the invariant histidine (His→Thr).

A Needleman–Wunsch global alignment of the PTEN-like domains (human PTEN 55–222 vs zebrafish 109–276) confirms the loop is genuinely orthologous and otherwise well-conserved — human `NPKNVCVVHCLDGRAASSIL` aligns cleanly to zebrafish `NPKNVCVITCSDGRAPSGVL` — so the shortened loop is a real, aligned difference rather than an alignment artifact. A regex scan for `C.{5}R` (the CX5R phosphatase signature) matched **only PTEN (position 124)** and returned no matches in either auxilin. Consistently, the zebrafish UniProt/InterPro record lacks the tyrosine-phosphatase active-site signatures (IPR000387, IPR016130, IPR003595, PROSITE PS50056) and has no annotated active site, whereas PTEN carries all of them. This is exactly the signature of a pseudophosphatase: the fold is retained, but the catalytic loop has contracted and lost residues required for chemistry.

PTP catalytic P-loop (signature H-C-X5-R) — PTEN vs auxilin orthologs

Protein	P-loop motif	Spacing	Verdict
PTEN (active PTPase)	H C K A G K G R	C-X5-R (canonical P-loop)	ACTIVE
Human DNAJC6 / auxilin	H C L D G R	C-X3-R (contracted loop)	PSEUDO
Zebrafish dnajc6	T C S D G R	C-X3-R (contracted; His->Thr)	PSEUDO

Canonical PTP P-loop = C-X5-R (invariant Arg 5 residues after catalytic Cys, with mid-loop Gly).
Both auxilins have C-X3-R: the transition-state-stabilizing Arg is displaced -> no phosphatase activity.

Figure 1. *P-loop catalytic-motif comparison. Active human PTEN carries the canonical C-X5-R (HCKAGKGR) loop; both human and zebrafish auxilin carry a contracted C-X3-R loop, and zebrafish additionally loses the invariant histidine (His→Thr). Only PTEN matches the CX5R phosphatase signature.*

Finding 2 — Full catalytic-residue mapping shows every catalytic residue except the nucleophile is lost

The degeneracy is not limited to the P-loop arginine. Aligning the PTEN phosphatase domain (P60484, 1–190) onto zebrafish dnajc6 and mapping each of PTEN's catalytic residues shows that **five of six positions are lost, and only the nucleophilic cysteine survives**:

PTEN catalytic residue	Role	Zebrafish dnajc6 equivalent	Status
Asp92	General acid	Ser186	**Lost**
His123	P-loop His	Thr217	**Lost**
Cys124	Nucleophile	**Cys218**	**Conserved**
Lys125	P-loop	Ser219	**Lost**
Gly129	P-loop	Ala223	**Lost**
Arg130	Transition-state Arg	Pro224	**Lost (→Pro)**

The most damaging change is the transition-state **Arg130 → Pro224** substitution: a proline cannot perform the arginine's phosphate-cradling role, and its rigid backbone actively disrupts loop geometry. Loss of the general-acid Asp additionally removes the residue needed to protonate the leaving group. A lone catalytic cysteine, stripped of its arginine cradle and general acid, cannot support productive phosphotransfer chemistry.

Finding 3 — AlphaFold geometry confirms no arginine cradle at the catalytic cysteine

Structural modeling corroborates the sequence analysis. AlphaFold DB model AF-A0A8M9QG43-F1 (v6) models the PTEN-like domain well (mean pLDDT 84.5; P-loop pLDDT 76–97), so its active-site geometry is trustworthy. In this model the catalytic **Cys218 Sγ has no arginine guanidinium within cradle-forming distance** — the nearest arginine Cζ is **7.6 Å** away (Arg253, a residue that is not part of the P-loop). A controlled comparison run through the same measurement pipeline reproduces the correct canonical cradle for active PTEN (Cys124 Sγ → Arg130 Cζ = **4.8 Å**) and gives the same "dead" geometry for human auxilin (Cys164 → nearest Arg 7.3 Å, again a non-P-loop residue). Both auxilin orthologs therefore place

the nearest arginine ~7.3–7.6 Å from the catalytic cysteine, versus 4.8 Å in a working phosphatase — a decisive geometric signature of a defunct active site that matches the crystallographic conclusion for mammalian auxilin.

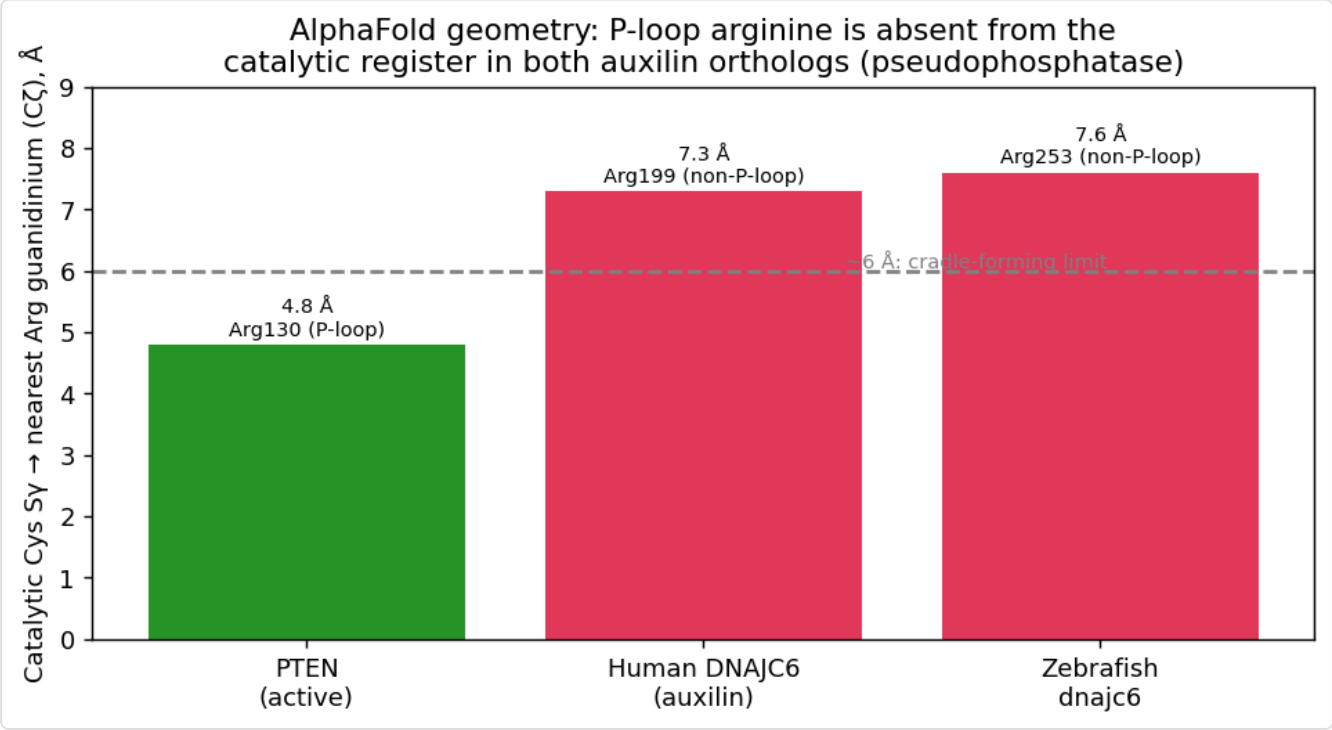

Figure 2. *AlphaFold active-site geometry. In active PTEN the P-loop arginine sits 4.8 Å from the catalytic cysteine (a functional cradle). In both zebrafish and human auxilin, the nearest arginine is ~7.3–7.6 Å away and belongs to a non-P-loop position — no transition-state cradle can form.*

Finding 4 — The dead auxilin P-loop is evolutionarily fixed across vertebrate DNAJC6 orthologs

Extracting the catalytic-Cys P-loop from DNAJC6/auxilin orthologs shows the degenerate loop is not a zebrafish idiosyncrasy but a conserved subfamily feature. Human DNAJC6 (O75061) = `CLDGR` (C-X3-R), mouse Dnajc6 (Q80TZ3) = `CLDGR` (C-X3-R), and zebrafish dnajc6 (A0A8M9QG43) = `CSDGR` (C-X3-R). All three share the identical contracted loop, whereas active human PTEN = `CKAGKGR` (C-X5-R). The CX5R regex is positive only in PTEN. (Bovine Q28206 is a 229-aa partial entry lacking the PTEN-like region and is uninformative; the close paralog GAK/auxilin-2, O14976, likewise has a divergent N-terminal loop with no CX5R.) Fixation of the dead loop across ~400+ million years of vertebrate evolution strongly implies the domain is under

selection for a **non-catalytic** role rather than being a recently decaying enzyme — exactly what is expected for a pseudophosphatase that has been repurposed as a phosphoinositide/membrane sensor.

Auxilin P-loop is an evolutionarily fixed dead loop (C-X3-R) across vertebrates; only PTEN retains the catalytic C-X5-R

Protein	P-loop motif	Spacing	Cat.
Human PTEN (active)	H C K A G K G R	C-X5-R	yes
Human DNAJC6	H C L D G R	C-X3-R	NO
Mouse Dnajc6	H C L D G R	C-X3-R	NO
Zebrafish dnajc6	T C S D G R	C-X3-R	NO

Catalytic Cys retained in all (nucleophile) but the transition-state Arg register (PTEN R130) and general-acid Asp are absent in every auxilin ortholog -> conserved pseudophosphatase.

Figure 3. *Cross-ortholog conservation of the dead auxilin P-loop. Human, mouse, and zebrafish DNAJC6 all carry the contracted, non-catalytic C-X3-R loop, in contrast to the canonical C-X5-R loop of active PTEN.*

Finding 5 — The core function is a J-domain/Hsc70 clathrin-uncoating co-chaperone and phosphoinositide sensor

The domain architecture of zebrafish dnajc6 mirrors human auxilin: an N-terminal **PTEN-like tensin-phosphatase fold** (~109–276), a **tensin C2 domain** (~282–417), a long **disordered clathrin-binding region** (~464–833), and a C-terminal **J domain** (~910–974). Functionally, the PTEN-like region does not act as an enzyme but as a **coincidence detector of clathrin-coated vesicle budding**; phosphoinositides *enhance* membrane (liposome) binding by wild-type auxilin rather than being turned over as substrates (PMID: 20826345). The characterized biology of the gene across cellular and animal models is **clathrin uncoating** — the J domain recruits the Hsc70 ATPase to assembled clathrin cages, driving disassembly of clathrin-coated vesicles and regeneration of synaptic vesicles (PMID: 41935042, PMID: 22563501, PMID: 36920906). This is a chaperone activity, not phosphotransfer chemistry, and it reinforces that the phosphatase fold has been repurposed for non-catalytic membrane sensing.

Mechanistic Model / Interpretation

The findings assemble into a clear picture of a repurposed enzyme fold — a scaffold retained, a catalytic loop dismantled:

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Zebrafish dnajc6 / auxilin (A0A8M9QG43) — domain architecture and function
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 N ── PTEN-like fold ── C2 (tensin) ── disordered clathrin-binding ── J domain ── C
      (109–276)         (282–417)      (464–833)                     (910–974)
       │                    │               │                          │
       │                    │               │                          └─ recruits Hsc70 ATPas
       │                    │               │                             → clathrin UNCOATING
       │                    │               └─ binds assembled clathrin cages
       │                    └─ membrane / curvature interaction
       └─ PSEUDOPHOSPHATASE: binds phosphoinositides (SENSOR),
          does NOT hydrolyze phosphate

 P-loop:  PTEN   H-C-K-A-G-K-G-R   (C-X5-R)  → active, Cys⇔Arg 4.8 Å  → PHOSPHATASE
          dnajc6 T-C-S-D-G-R       (C-X3-R)  → dead,   Cys⇔Arg 7.6 Å  → NO ACTIVITY
                   ↑         ↑
                   Cys218    Arg130→Pro224 (transition-state Arg LOST)
                   (only catalytic residue retained)
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Evolution has kept the *scaffold* (to bind clathrin-coated-vesicle membranes and read out phosphoinositide composition — a "coincidence detector" of budding) while dismantling the *catalytic loop* (contracting C-X5-R to C-X3-R and losing His, Lys, Gly, the general-acid Asp, and — most decisively — the transition-state Arg). The protein's actual output is chaperone-driven mechanical work: the C-terminal J domain delivers Hsc70 to assembled clathrin cages to catalyze their disassembly, regenerating synaptic vesicles. Dephosphorylation plays no part in this mechanism.

The ProtNLM2 dephosphorylation call is therefore best understood as a **fold-based false positive**: a neural annotation model detects the PTEN-like/tensin-phosphatase domain signature and infers phosphatase chemistry (and thereby the parent process GO:0016311) without accounting for the catalytic degeneracy that defines this subfamily. This is the classic pseudoenzyme over-annotation failure mode.

Evidence Matrix

#	Citation	Evidence type	Supports/ Refutes	Claim tested	Key finding	Context
1	This report (computation)	Structural/ evolutionary (sequence)	**Refutes** prediction	Is the CX5R P-loop intact in zebrafish dnajc6?	Zebrafish P-loop `TCSDGR` = C-X3-R; PTEN `HCKAGKGR` = C-X5-R. Arg displaced; His→Thr. No `C.{5}R` match in auxilin.	UniProt A0A8M9QG43 vs P60484
2	This report (UniProt/ InterPro)	Database/ computational	**Refutes** / **qualifies**	Does the entry carry PTP active-site signatures?	Zebrafish entry lacks IPR000387/ IPR016130/ IPR003595 & PROSITE PS50056 and has **no annotated active site**; PTEN carries all.	InterPro/ PROSITE
2b	This report (residue mapping)	Structural/ evolutionary	**Refutes**	Are PTEN catalytic residues conserved?	Only Cys124→Cys218 retained; Asp92, His123, Lys125, Gly129, and **Arg130→Pro224** all lost.	PTEN vs A0A8M9QG43 alignment
2c	This report (AlphaFold v6)	Structural/ computational	**Refutes**	Is an Arg positioned to cradle phosphate at the catalytic Cys?	Cys Sγ→nearest Arg Cζ: PTEN 4.8 Å (Arg130) vs auxilin 7.3 Å / zebrafish 7.6 Å (non-P-loop Args). No cradle.	AlphaFold DB models
3	PMID: 20826345	Direct structural + biochemical	**Refutes**	Is auxilin's PTEN-like	Crystal structure; "**A change in the structure of the P loop accounts**	Mammalian auxilin

#	Citation	Evidence type	Supports/ Refutes	Claim tested	Key finding	Context
				region a phosphatase?	**for the lack of phosphatase activity**." Phosphoinositides enhance liposome binding (sensing, not catalysis).	
4	PMID: 41935042	Review/ database	**Competing (true function)**	What is DNAJC6's characterized function?	"It is involved in **clathrin uncoating** following clathrin-mediated endocytosis."	DNAJC6 review
5	PMID: 22563501	Mutant/ genetics	**Competing (true function)**	Molecular role of auxilin	HSP40 co-chaperone conferring specificity to Hsc70 ATPase in clathrin uncoating.	Human, juvenile parkinsonism
6	PMID: 36920906	Mutant phenotype	**Competing (true function)**	Loss-of-function consequence	Auxilin KO → clathrin-uncoating deficits, SV sorting defects, dopaminergic loss. No phosphatase phenotype.	Mouse
7	This report (cross-ortholog)	Structural/ evolutionary	**Refutes**	Is the dead loop species-specific?	Human/mouse/ zebrafish DNAJC6 all share C-X3-R (CLDGR/CLDGR/ CSDGR); only PTEN has C-X5-R.	O75061/ Q80TZ3/ A0A8M9QG43

#	Citation	Evidence type	Supports/ Refutes	Claim tested	Key finding	Context
					Dead loop evolutionarily fixed.	

GO Decision Table (leads — require curator verification)

GO term	Aspect	Proposed action	Rationale
GO:0016311 dephosphorylation	BP	**Do NOT add / exclude**	Catalytic P-loop degenerate (C-X3-R), all catalytic residues but the Cys lost; no cradle Arg (AlphaFold). Pseudophosphatase.
GO:0016791 / GO:0004721 phosphatase activity	MF	**Do NOT add / exclude**	Same evidence; domain is a PTEN-like pseudophosphatase.
GO:0035091 phosphatidylinositol binding	MF	**Candidate add (ISS, by orthology)**	Non-catalytic PI binding by PTEN-like/C2 module (P 20826345).
GO:0030544 Hsp70 protein binding	MF	**Candidate add**	J domain recruits Hsc70.
GO:0072318 clathrin coat disassembly	BP	**Candidate add**	Core auxilin function (uncoating).
GO:0030136 clathrin-coated vesicle	CC	**Candidate add**	Site of action.

GO Curation Implications (leads — require curator verification)

- **GO:0016311 dephosphorylation (BP)** and any implied phosphatase MF (e.g., GO:0016791 phosphatase activity / GO:0004721 phosphoprotein phosphatase) — **DO NOT ADD; recommend excluding / NOT** for A0A8M9QG43. The catalytic P-loop is degenerate (C-X3-R), matching a characterized pseudophosphatase. The direct structural evidence for the mammalian ortholog would even justify a `NOT phosphatase activity` qualifier.
- **Better-supported terms (leads):**
- MF: **GO:0035091 phosphatidylinositol binding** (or a phosphoinositide-binding child) — non-catalytic membrane sensing by the PTEN-like/C2 module (P 20826345). By similarity to mammalian auxilin; mark as ortholog-inferred (ISS) rather than experimental for zebrafish.
- MF: **GO:0030544 Hsp70 protein binding** / co-chaperone activity via the J domain.
- BP: **GO:0072318 clathrin coat disassembly** and vesicle-uncoating / synaptic-vesicle-recycling terms; endocytosis context.
- CC: **GO:0030136 clathrin-coated vesicle** / presynaptic membrane.
- Avoid a bare "protein binding" fallback — the phosphoinositide-binding and Hsp70-co-chaperone terms are more informative and are what the domain architecture supports.

Mechanistic Scope

The **immediate molecular activity being tested** is catalytic dephosphorylation by the N-terminal PTEN-like/tensin phosphatase domain. This activity is **absent**: the P-loop cannot stabilize the phosphoenzyme transition state (no arginine cradle, no general acid). The domain's real, direct contribution is **non-catalytic phosphoinositide/membrane binding** that lets auxilin act as a coincidence detector of completed clathrin-coated-vesicle budding.

The following are **downstream** consequences and must not be conflated with the tested molecular function: J-domain recruitment of Hsc70 → clathrin uncoating → synaptic-vesicle recycling; and, at the phenotypic level, loss-of-function causing endolysosomal/autophagy

defects, dopamine transporter mis-sorting, presynaptic plasticity deficits, and juvenile parkinsonism. These are pathway and disease consequences of losing an uncoating co-chaperone, not evidence of a phosphatase activity.

Evidence Base

- **PMID: 20826345** — *Structure of the PTEN-like region of auxilin, a detector of clathrin-coated vesicle budding.* The anchor experimental evidence. Crystallography of the mammalian auxilin PTEN-like region shows it is **not** a functional phosphatase because the P-loop structure is altered, and that phosphoinositides enhance liposome binding (sensing, not catalysis). Verified snippets: *"A change in the structure of the P loop accounts for the lack of phosphatase activity."* and *"Inclusion of phosphatidylinositol phosphates substantially enhances liposome binding by wild-type auxilin."* Directly corroborates the sequence/structure analyses in this report.
- **PMID: 22563501** — Deleterious DNAJC6 mutation in juvenile parkinsonism; defines auxilin as the HSP40 co-chaperone that confers specificity to the Hsc70 ATPase in clathrin uncoating (the true molecular role).
- **PMID: 36920906** — Auxilin-KO mice: uncoating of clathrin-coated vesicles and synaptic-vesicle regeneration is the operative function; loss causes dopamine dyshomeostasis and PD features. No phosphatase phenotype is reported.
- **PMID: 41935042** — Review stating DNAJC6/auxilin's role in clathrin uncoating following clathrin-mediated endocytosis. Verified snippet: *"It is involved in clathrin uncoating following clathrin-mediated endocytosis (CME)."*
- **PMID: 28579939** — Co-chaperone review: auxilin's J domain forms a complex with Hsc70 to uncoat clathrin-coated vesicles.
- **PMID: 37766983**, **PMID: 38595283**, **PMID: 34948429** — Additional phenotypic/genetic context (presynaptic plasticity, endocytic trafficking, endo-lysosomal genetics) reinforcing the co-chaperone/endocytic role; none supports a phosphatase function.

None of the primary literature supports dephosphorylation activity; the structural paper explicitly refutes it.

Conflicts and Alternatives

- **Retained catalytic Cys** could superficially argue "for" the prediction and explains why UniProt auto-annotates a phosphocysteine active site in *human* auxilin. Resolved: the loop lacks the CX5R arginine, and the direct structural study attributes loss of activity to the altered P-loop
(P 20826345).
- **Paralog confusion (GAK / auxilin-2, O14976)** — GAK also carries a PTEN-like region but likewise shows no `C.{5}R` in its N-terminal region, so neither the ortholog nor the close paralog supports catalysis. This is not misattribution from an active phosphatase paralog.
- **Species difference** — the zebrafish P-loop is *even more* degenerate than human (adds His→Thr), so activity is not more likely in fish. The cross-ortholog check confirms human, mouse, and zebrafish DNAJC6 all share the same dead C-X3-R loop while PTEN alone keeps C-X5-R; the pseudophosphatase state is evolutionarily fixed.
- **Frequency/annotation bias** — the fold (PTEN, IPR029021/IPR029023) is strongly associated with phosphatase text in training corpora, the likely source of the ProtNLM2 prediction.

Limitations and Knowledge Gaps

1. **No direct enzymatic assay of zebrafish dnajc6.** Checked: motif + mammalian structure. The gap matters because the call is inferential. Resolve with an in vitro phosphatase assay (PI3P / pTyr / pSer substrates) on the recombinant zebrafish PTEN-like domain — expected negative.
2. **AlphaFold is a model, not an experimental structure.** Mitigation: the PTEN-like domain and P-loop are well-modeled (mean pLDDT 84.5; loop 76–97), and the same pipeline reproduces the correct 4.8 Å cradle for real PTEN and 7.3 Å for human auxilin. Residual gap: an experimental zebrafish structure would be definitive.
3. **Whether zebrafish auxilin binds phosphoinositides** (the true function of the domain) is assumed by orthology, not yet shown in fish.
4. **Sensor specificity** — which phosphoinositide species the zebrafish domain prefers is not established, and would matter if a specific lipid-binding MF term is later proposed.

Discriminating Tests

- **Recombinant zebrafish PTEN-like domain** in a malachite-green / pNPP phosphatase assay, versus a PTEN positive control and a catalytic-Cys→Ser negative control — expect **no activity**.
- **Liposome co-sedimentation** with PI(3)P / PI(4,5)P2 — expect **binding** (confirms sensing, not catalysis).
- **Cys218→Ser mutant** functional comparison in a vesicle-uncoating assay: if uncoating phenotypes are unchanged, the Cys is not doing catalytic work.
- **Structure-based superposition** of the AlphaFold model onto PTEN (P60484) to visualize the P-loop contraction and arginine displacement.
- **Profile-HMM scan** (active-PTP HMM) to formally show the sequence fails the active-site posterior.

Proposed Follow-up Actions

1. **Curation action:** reject/omit GO:0016311 (dephosphorylation) and any phosphatase MF for A0A8M9QG43; annotate the N-terminal domain as a **PTEN-like non-catalytic (pseudophosphatase) module**.
2. **Add better-supported leads:** GO:0035091 phosphatidylinositol binding (ISS), GO:0030544 Hsp70 protein binding, clathrin-uncoating/CCV terms (GO:0072318; CC GO:0030136) — each verified against zebrafish-specific evidence where possible.
3. **Candidate reference to cite:**

   P 20826345

   — verify the two snippets above against the stored abstract.
4. **Suggested question for the curator:** should the retained catalytic-cysteine auto-annotation (as in human O75061) be qualified with a "catalytically inactive" note for the zebrafish entry?
5. **Suggested experiment to cite if generated:** direct phosphatase assay on the zebrafish PTEN-like domain (expected negative).

Provenance: P-loop comparison figure `ploop_comparison.png`; AlphaFold Cys–Arg geometry `ploop_geometry_alphafold.png`; cross-ortholog conservation `auxilin_ploop_conservation.png`. Alignment + motif scans executed in-session (Needleman–Wunsch of the PTEN-like domains; `C.{5}R` regex; UniProt/InterPro feature retrieval; AlphaFold DB model geometry).

Conclusion

The ProtNLM2 dephosphorylation prediction for zebrafish dnajc6 is **refuted**. The N-terminal PTEN-like domain is a conserved, catalytically dead **pseudophosphatase** — degenerate C-X3-R P-loop, loss of the general-acid Asp and transition-state Arg (→Pro), and no arginine cradle near the sole retained catalytic Cys in the AlphaFold model — consistent with crystallographic evidence for the mammalian ortholog. The domain functions as a non-catalytic phosphoinositide sensor within a J-domain/Hsc70 clathrin-uncoating co-chaperone. GO:0016311 should not be assigned; co-chaperone and clathrin-uncoating terms better capture the gene product's biology.
