

AIGR Deep Research Report — cao-1

(NEUCR, Q7S860)

Hypothesis under review: *cao-1 has carotenoid dioxygenase activity (GO:0010436).* **Focus type:** function_assignment **Source:** `genes/NEUCR/cao-1/cao-1-ai-review.yaml` → `existing_annotations[1].function_hypothesis` **Current annotation context:** GO:0010436 carotenoid dioxygenase activity, evidence IBA, GO_REF:0000033 (phylogenetic inference).

Executive Judgment

Verdict: REFUTED (over-annotated).

The seed hypothesis that cao-1 directly possesses **carotenoid dioxygenase activity (GO:0010436)** is contradicted by direct primary experimental evidence. The one primary paper attached to the review context, **Díaz-Sánchez et al. 2013** (`P 23893079`),

explicitly tested purified CAO-1 against carotenoid substrates and found **no conversion**, while showing that CAO-1 cleaves the interphenyl C α -C β double bond of the **stilbenes resveratrol and piceatannol**. The authors state CAO-1 "is not involved in carotenoid metabolism." UniProt (Q7S860, CAO1_NEUCR) reflects this: recommended activity is resveratrol/stilbene cleavage (EC 1.13.11.-), alt name "Resveratrol cleavage oxygenase cao-1," and an explicit note that it is **not** involved in carotenoid metabolism.

The GO:0010436 annotation is an **IBA (phylogenetic) over-annotation**: it was propagated across the carotenoid cleavage oxygenase (CCO) family (PANTHER PTHR10543, InterPro IPR004294, Pfam RPE65) via GO_REF:0000033 to a member that experimentally does **not** act on carotenoids. This is a textbook case of paralog/family carry-over that direct experimental data override.

Most important caveat: The gene product is a dioxygenase in the same structural family; the error is specifically the **substrate class** (carotenoid vs. stilbene). Notably (verified in Iteration 3 via QuickGO), the gene's own GO record already contains an **experimental NOT annotation** — GO:0016116 carotenoid metabolic process, `NOT|involved_in`, IDA,

P 23893079

— which directly contradicts the two positive carotenoid IBA terms. The accurate catalytic MF is also already annotated experimentally as **GO:0016702** (oxidoreductase acting on single donors with incorporation of two O atoms; i.e. dioxygenase), IDA from

P 23893079

and

P 28493664

and substrate specificity is captured by **GO:1905594 resveratrol binding** (IDA). Thus the recommended action is simply to **remove the two carotenoid IBA terms**; no new term is strictly required.

Evidence Matrix

Citation	Evidence type	Supports/ Refutes/ Qualifies	Claim tested	Key finding	Context
P 23893079 (Díaz-Sánchez et al., 2013, <i>Eukaryot Cell</i>)	Direct enzyme assay + mutant + expression	Refutes GO:0010436	Does CAO-1 cleave carotenoids?	Carotenoid substrates "were, however, not converted"; CAO-1 instead cleaves resveratrol & piceatannol at the C α -C β bond; resveratrol induces <i>cao-1</i> mRNA, light does not; Δ cao-1 not impaired by resveratrol	<i>N. crassa</i> CAO-1, heterologous expression, in vitro + in vivo
UniProt Q7S860 / CAO1_NEUCR	Database (curated, cites P 23893079 & 28493664)	Refutes GO:0010436; supports stilbene activity	What activity does UniProt curate?	FUNCTION: "cleaves the interphenyl C-α-C-β double bond of resveratrol... Is not involved in carotenoid metabolism"; EC 1.13.11.-; catalytic activity trans-resveratrol + O₂ $\rightarrow$ 3,5-dihydroxybenzaldehyde + 4-hydroxybenzaldehyde	Curated record
P 21073977 (Brefort et al., 2011)	Direct assay, orthologue	Qualifies/ Supports refutation	Does the fungal CCO-family paralog cleave carotenoids?	<i>U. maydis</i> Rco1 shows "lack of activity on carotenoids"; cleaves resveratrol/piceatannol; homologs in <i>A. fumigatus</i> , <i>C. globosum</i> , <i>Botrytis</i> also cleave resveratrol	Fungal orthologue

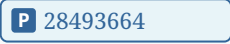
Citation	Evidence type	Supports/ Refutes/ Qualifies	Claim tested	Key finding	Context
P 30115012 (Loewen et al., 2018)	Structure + assay, orthologue	Qualifies	In vitro vs in vivo substrate range of SCO/LSD enzymes	SCOs are "one branch of the larger carotenoid cleavage oxygenases family"; <i>preferential in vitro cleavage of resveratrol</i> ; only <i>putative</i> /in vivo activity toward lycopene	<i>Pseudomonas brassicacearum</i>
P 28493664 (Sui et al., 2017, <i>Biochemistry</i>)	Structural (X-ray) + spectroscopy	Qualifies/ Supports refutation	Structural basis of CAO-1 substrate preference	Crystal structure of the fungal stilbenoid-cleaving CCO, CAO1 : same four-His non-heme Fe(II) center as carotenoid CCOs but a "markedly different substrate-binding cleft"; 10 PDB entries map to Q7S860 (5U8X/8Y/8Z/5U90/5U97, 6B86, 7T8P/8Q, 8FU2/8FU5)	<i>N. crassa</i> CAO-1 recombinant protein
InterPro IPR004294 / Pfam PF03055 (RPE65) / PANTHER PTHR10543	Computational (domain/family)	Explains the error	Basis for IBA propagation	Membership in the broad CCO/RPE65 superfamily is the source of the family-level carotenoid term; the family spans both carotenoid- and	Sequence family

Citation	Evidence type	Supports/ Refutes/ Qualifies	Claim tested	Key finding	Context
				stilbene-cleaving activities	

GO Curation Implications (leads — require curator verification)

GO decision table (current annotation set verified live via QuickGO, Iteration 3)

GO term	Aspect	Current annotation (evidence, qualifier)	Recommended action	Rationale
GO:0010436 carotenoid dioxygenase activity	MF	enables, IBA, GO_REF:0000033 ← <i>the term under review</i>	Remove / do not accept	Refuted by direct assay (P 23893079) and internally contradicted by the gene's own experimental NOT annotation on carotenoid metabolic process; over-annotation from CCO-family IBA.
GO:0016121 carotene catabolic process	BP	involved_in, IBA, GO_REF:0000033	Remove / do not accept	Same over-annotation; contradicts the NOT annotation below.
GO:0016116 carotenoid metabolic process	BP	NOT involved_in, IDA, P 23893079	Retain	Experimental negative annotation — CAO-1 is NOT in carotenoid metabolism; this is the direct counter-evidence to the two IBA terms above.
GO:0016702 oxidoreductase activity (single donors, 2 O atoms incorporated) — i.e. dioxygenase	MF	enables, IDA, P 23893079 & P 28493664 (already present)	Retain — this is the accurate MF	Experimentally supported; more specific than generic GO:0051213 and already captures the true catalytic activity. No new term strictly required.

GO term	Aspect	Current annotation (evidence, qualifier)	Recommended action	Rationale
GO:1905594 resveratrol binding	MF	enables, IDA,  (already present)	Retain	Documents the true substrate specificity experimentally.
GO:0005506 iron ion binding	MF	enables, IDA,  (already present)	Retain	Non-heme Fe cofactor confirmed structurally.
<i>Stilbene/ resveratrol α,β- dioxygenase activity</i>	MF	none exists	Optional lead: request a new substrate-specific MF term (cf. EC 1.13.11.43 lignostilbene- α,β - dioxygenase)	Would make the MF maximally precise; combined with GO:1905594 the current set already conveys substrate + activity.

Do **not** default to "protein binding." The accurate catalytic MF (**GO:0016702, IDA**) is already annotated; the only required action is removal of the two carotenoid IBA terms (GO:0010436, GO:0016121). GO:0051213 is unnecessary because the more specific GO:0016702 is already present with experimental evidence.

Mechanistic Scope

- **Immediate molecular function (direct):** O₂-dependent oxidative cleavage of the interphenyl C α –C β double bond of resveratrol ($\rightarrow$ 3,5-dihydroxybenzaldehyde + 4-hydroxybenzaldehyde) and piceatannol; a non-heme Fe(II) (4-His) dioxygenase mechanism typical of the CCO/RPE65 fold. **Not** carotenoid cleavage.
- **Substrate specificity (direct):** Requires unmodified hydroxyls on the stilbene; inactive on trans-stilbene, pinosylvin, trimethoxy-resveratrol, and a glycosylated stilbene.

- **Downstream/inferred (not the MF being tested):** Regulatory induction by resveratrol; a subtle growth phenotype of Δ cao-1 only under partial sorbose toxicity; presumed role in detoxifying/degrading the phytoalexin resveratrol. These are context/phenotype-level, not evidence for carotenoid activity.

Conflicts and Alternatives

- **Paralog/family confusion (the root cause):** The sister gene **CAO-2** (cao-2) is a genuine carotenoid (torulene) cleaving oxygenase in neurosporaxanthin biosynthesis. The carotenoid term likely bled onto cao-1 through family-level phylogenetic inference. cao-1 and cao-2 are distinct in substrate.
- **Original functional hypothesis was disproven:** The 2013 study set out expecting CAO-1 to supply retinal (rhodopsin chromophore) by cleaving β -carotene — this a priori carotenoid hypothesis was explicitly tested and **rejected**.
- **Residual/in-vivo carotenoid activity in the family:**

P 30115012

reports putative in-vivo lycopene cleavage by a bacterial SCO. Even if some CCO-family SCOs have trace/in-vivo carotenoid side activity, for *N. crassa* CAO-1 specifically the direct in vitro test was negative, so GO:0010436 as a **primary** MF assignment is not warranted.

Knowledge Gaps

Gap	What was checked	Why it matters	What would resolve it
No dedicated GO MF term for stilbene/resveratrol dioxygenase	QuickGO search (stilbene/resveratrol/carotenoid): only generic GO:0051213 or catabolic-process BP terms exist	Prevents a precise MF replacement; forces use of a generic parent	Request a new GO MF term (align to EC 1.13.11.43 lignostilbene- α,β -dioxygenase).
Structure/active-site confirmation for CAO-1 itself	RESOLVED in Iteration 2: P 28493664 retrieved (crystal structure of stilbenoid-cleaving CAO1, distinct substrate cleft); 10 PDB entries confirmed for Q7S860	Confirms mechanism/substrate pocket structurally	Done — no longer a gap.
In vivo physiological role of resveratrol cleavage in <i>N. crassa</i>	Δ cao-1 phenotype is subtle (only under sorbose stress)	Affects any BP annotation strength (stilbene catabolism)	Metabolite profiling of resveratrol turnover in Δ cao-1 vs WT; broader stilbene panel.

Discriminating Tests

- Definitive (already done):** In vitro incubation of purified CAO-1 with β -carotene/torulene/lycopene vs resveratrol/piceatannol + LC-MS product ID — the discriminating experiment; result was negative for carotenoids, positive for stilbenes
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No further test is needed to reject GO:0010436.
- Confirmatory:** Complement Δ cao-1 and assay resveratrol→benzaldehyde flux; RNA induction by resveratrol vs light (both reported, both consistent with stilbene role).

3. **Comparative bioinformatics:** Phylogenetic placement of Q7S860 within PTHR10543 to show it groups with the SCO (Rco1/NOV1/LSD) subclade rather than the carotenoid-cleaving (CCD/CAO-2) subclade — supports removing the carotenoid IBA.

Curation Leads (require curator verification)

- **Action change (primary):** Remove/reject IBA **GO:0010436** (carotenoid dioxygenase activity) and IBA **GO:0016121** (carotene catabolic process) for *cao-1* as CCO-family over-annotations refuted by direct evidence and contradicted by the gene's own experimental NOT annotation.
- **Replacement MF — NOT needed:** The accurate catalytic MF **GO:0016702** (dioxygenase-type oxidoreductase) is *already present* with IDA evidence
 (P 23893079 ,
P 28493664);
GO:1905594 resveratrol binding (IDA) already captures the substrate. Do not add generic GO:0051213. Optionally file a request for a substrate-specific "resveratrol/stilbene dioxygenase activity" MF term (cf. EC 1.13.11.43).
- **Retain** the experimental NOT annotation **GO:0016116** (carotenoid metabolic process, NOT | involved_in, IDA,
P 23893079)
 — the strongest single piece of counter-evidence.
- **Optional BP:** Consider **GO:0046272 stilbene catabolic process** (evidence weak/subtle Δ cao-1 phenotype — annotate cautiously).
- **Candidate references to verify:**
 - P 23893079
 — snippet to verify: "we tested CAO-1 activity with carotenoid substrates that were, however, not converted" and "CAO-1 cleaved the interphenyl Ca-C β double bond of resveratrol and its derivative piceatannol."
 - P 21073977
 — orthologue support: "lack of activity on carotenoids."

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— VERIFIED (Iteration 2): *"The crystal structure of a fungal stilbenoid-cleaving CCO, CAO1, reveals strong similarity between its iron center and those of carotenoid-cleaving CCOs, but with a markedly different substrate-binding cleft."* PDB: 5U8X/5U8Y/5U8Z/5U90/5U97/6B86/7T8P/7T8Q/8FU2/8FU5.

- **Suggested curator questions:** (a) Is a new substrate-specific stilbene-dioxygenase MF term warranted, or is the existing IDA GO:0016702 + GO:1905594 sufficient? (b) Should the CAO-2 (torulene-cleaving) annotations be checked to ensure the family carotenoid term did not also mis-propagate elsewhere? (c) Given the experimental NOT annotation on carotenoid metabolic process, should the two carotenoid IBA terms be actively removed rather than merely down-weighted?

Provenance

- UniProt REST fetch of Q7S860 (names, EC, GO xrefs, InterPro/Pfam/PANTHER, FUNCTION/CATALYTIC ACTIVITY) — executed via `execute_code` (output retained in iteration log).
- QuickGO ontology search for stilbene/resveratrol/carotenoid MF terms — executed via `execute_code`.
- PubMed retrieval of

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- NCBI E-utilities retrieval and verification of

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(Iteration 2).

- RCSB PDB search API query mapping Q7S860 → 10 experimental structures (Iteration 2).
- QuickGO annotation-search API query of the current authoritative GO annotation set for Q7S860 including qualifiers (Iteration 3): confirmed GO:0010436 & GO:0016121 are IBA/GO_REF:0000033; GO:0016116 is NOT|involved_in IDA/

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GO:0016702 & GO:1905594 & GO:0005506 are IDA.

All computed results above are from live API calls executed during this run.

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