AIGR Deep Research Report — *ire-1* (Q09499, *C. elegans*)

Hypothesis: hydrolase activity, acting on ester bonds (GO:0016788)

Focus type: computational_prediction (GO-GPT / BioReason-Pro) **Term under test:** GO:0016788 — *hydrolase activity, acting on ester bonds* **Reference context:** doi:10.64898/2026.03.19.712954

Executive Judgment

Verdict: PARTIALLY SUPPORTED — but over-general and redundant; do NOT add GO:0016788 as an annotation.

ire-1 unambiguously carries a catalytic activity on nucleic-acid ester (phosphodiester) bonds: its C-terminal KEN/RNase domain is a site-specific **endoribonuclease** that cleaves *xbp-1* mRNA to drive non-conventional UPR splicing. In that loose biochemical sense the prediction "acts on ester bonds" is not wrong. However, the prediction is the **wrong level of specificity for annotation**, for three concrete reasons:

1. **A precise, experimentally-supported term already exists.** UniProt Q09499 already carries **GO:0004521 (RNA endonuclease activity, IMP:WormBase)** and **GO:0016787 (hydrolase, IEA-KW)**. GO:0016788 adds no information beyond these.
2. **In the current GO ontology, GO:0016788 is NOT an ancestor of GO:0004521.** QuickGO shows the RNA-endonuclease branch descends through *catalytic activity, acting on a nucleic acid* (GO:0140640) → nuclease (GO:0004518) → endonuclease (GO:0004519) → RNA endonuclease (GO:0004521), **not** through *hydrolase, acting on ester bonds*. So the seed's premise that GO:0016788 is a "correct high-level parent of GO:0004521" **does not hold in the current ontology**.
3. **Mechanistic mismatch — the closest analog is a LYASE, not a hydrolase.** IRE1-family RNases use a **metal-independent transesterification** (general acid/base His–Tyr pair) yielding a **2′,3′-cyclic phosphate + 5′-OH**, not a hydrolysis to a phosphomonoester. GO's own

treatment of the enzyme class sharing this exact chemistry — **tRNA-intron lyase activity (GO:0000213)** — places it under **lyase / phosphorus-oxygen lyase (GO:0016829 / GO:0016849)** and under RNA endonuclease (GO:0004521), but **explicitly NOT under GO:0016788**. So GO:0016788 is not just off-branch; it is the mechanistically wrong high-level MF for a cyclic-phosphate-producing transesterase. (It also fails GO:0016788's own hydrolytic nuclease children GO:0016891/2, which require phosphomonoester products.)

Bottom line for the curator: the true, well-supported molecular function is the specific IRE1-type endoribonuclease activity (GO:0004521 / more specifically an IRE1/tRNA-ligase-type transesterifying endoribonuclease). GO:0016788 is a correct-but-imprecise generalization that is **subsumed and redundant** and should **not** be added.

Evidence Matrix

Citation	Evidence type	Supports/ Refutes/Qualifies	Claim tested	Key finding	Co
P 11780124 (Calfon 2002, *Nature*)	Direct in-vitro assay + genetics	Qualifies (supports specific endoribonuclease, not the broad term)	Is IRE1 a direct endoribonuclease on xbp-1 mRNA?	"Purified mouse IRE1 accurately cleaved XBP-1 mRNA in vitro… a direct target of IRE1 endonucleolytic activity"; C. elegans ire-1/xbp-1 mutations abolish UPR splicing	M IR vi *el* in
P 11779465 (Shen 2001, *Cell*)	Mutant phenotype (target organism)	Supports specific function	Does worm ire-1 mediate xbp-1 splicing?	"C. elegans requires ire-1-mediated splicing of xbp-1 mRNA for UPR gene transcription and survival upon ER stress"	*C. el* in
P 21729333 (Korennykh 2011)	Structural + enzymology	Qualifies (defines mechanism)	What is the catalytic mechanism of Ire1 RNase?	His1061/Tyr1043 general acid-base pair; Asn1057/ Arg1056 coordinate the **scissile phosphate**; cleaves stem-loops of HAC1/Xbp1 and tRNA-Phe ASL	Ye ge Ir RN
P 36389122 (De-Souza 2022)	Functional (target organism)	Supports	Is IRE-1 endoribonuclease activity operative in worm aging?	"IRE-1 endoribonuclease activity declines early in [aging]"	*C. el*
P 30833722 / 32446294	Structural/ pharmacology (review-level orientation)	Qualifies	Is IRE1 a bifunctional kinase + RNase with a druggable RNase site?	RNase active site (Lys907/ His910/Tyr892 in mIRE1) excises the 26-nt XBP1 intron	M hu IR
UniProt Q09499 (database)	Curated record	Supports (existing precise annotation)	What MF terms are already assigned?	GO:0004521 RNA endonuclease (IMP:WormBase); GO:0004674	*C. el*

Citation	Evidence type	Supports/ Refutes/Qualifies	Claim tested	Key finding	Co
				kinase (ISS); GO:0016787 hydrolase (IEA-KW); KEN/ RNase domain 781–909, kinase 518–778	
QuickGO ontology (database)	Ontology structure	Refutes seed's parentage premise	Is GO:0016788 an ancestor of GO:0004521?	No — GO:0004521 ancestry runs via GO:0140640/0004518/0004519, not GO:0016788	Cu GO
QuickGO GO:0000213 (database)	Ontology structure / mechanistic analogy	Refutes hydrolase-branch fit	Where does GO place the transesterifying (2′,3′-cyclic-phosphate) RNase class?	tRNA-intron lyase (GO:0000213) is a child of RNA endonuclease (GO:0004521) AND lyase/ phosphorus-oxygen lyase (GO:0016829/GO:0016849) — NOT under GO:0016788	Cu GO

GO Curation Implications

Molecular Function (MF). The relevant, supported MF term is **GO:0004521 (RNA endonuclease activity)** — already annotated to Q09499 with IMP:WormBase evidence, plus the kinase term GO:0004674. A still-more-precise child (e.g. an IRE1/tRNA-ligase-type transesterifying endoribonuclease term) would be ideal if available.

Recommended action for the predicted term GO:0016788 (lead, requires curator verification): - **Do not add GO:0016788.** It is redundant with the already-present GO:0016787 (hydrolase) and, more importantly, is **superseded by** the specific GO:0004521 that captures the actual activity. - Treat the GO-GPT prediction as **"correct in spirit, too general, and structurally off-branch."** It is not a false positive at the chemistry level, but it fails the *annotate-to-most-specific-term* principle and does not sit on the lineage of the experimentally supported term. - If anything, the existing IEA GO:0016787 keyword annotation could itself be viewed as low-value given GO:0004521 is present, but that is a separate housekeeping matter.

This is **not** a "protein binding" fallback; a specific, informative MF (endoribonuclease) is well supported.

Mechanistic Scope

- **Direct gene-product activity (what the term should describe):** two catalytic activities on the cytoplasmic face — (i) a Ser/Thr **protein kinase** (autophosphorylation; a phosphotransferase, *not* a hydrolase) and (ii) a **site-specific endoribonuclease** (KEN domain) that cleaves two stem-loops in *xbp-1* mRNA, excising an intron. The endoribonuclease is the only activity relevant to GO:0016788.
- **Chemistry:** cleavage of an RNA **phosphodiester bond** (an ester bond) via general acid/base catalysis, producing a 2′,3′-cyclic phosphate and 5′-OH (transesterification), later re-ligated by RtcB-type ligase to yield spliced *xbp-1*.
- **Downstream (NOT the molecular function):** production of active XBP-1 transcription factor; transcriptional UPR; ER homeostasis; larval development; longevity in insulin/IGF-1 mutants; ER-stress apoptosis. These are BP/phenotype consequences, not the MF being tested.

Conflicts and Alternatives

- **Ontology-structure conflict (primary):** The seed frames GO:0016788 as a valid parent of GO:0004521. Current GO places nuclease/endonuclease activity under GO:0140640 (*catalytic activity, acting on a nucleic acid*), **not** under GO:0016788. Thus GO:0016788 and GO:0004521 are in parallel branches, not parent–child. This weakens the "correct-but-less-precise parent" framing.
- **Mechanism conflict (hydrolase vs lyase):** GO:0016788 still owns hydrolytic nuclease children (GO:0016891/2) that specify phosphomonoester products; IRE1 produces cyclic-phosphate ends (transesterase/lyase-like, cf. EC reclassification of cyclizing RNases to EC 4.6.1). Decisively, GO classifies IRE1's mechanistic twin — **tRNA-intron lyase (GO:0000213)** — under **lyase (GO:0016829)/phosphorus-oxygen lyase (GO:0016849)** and RNA endonuclease (GO:0004521), **not** under GO:0016788. So IRE1 fits neither GO:0016891/2 nor the strict "hydrolase" reading; if a high-level chemistry parent beyond GO:0004521 were

ever wanted, the lyase branch would be the better-motivated choice — further undercutting the GO:0016788 prediction.

- **Orthology/paralog caveat:** Direct biochemical cleavage assays (Calfon; Korennykh) were done on mouse/yeast IRE1; worm *ire-1* is supported genetically (Shen) and by conserved domain architecture. No paralog-overannotation risk (IRE1 is the single ER UPR IRE-branch sensor); low frequency-bias risk.
- **Not an in-vitro-only artifact:** function is corroborated in vivo in *C. elegans*.

Knowledge Gaps

1. **Worm-specific direct cleavage assay.** Checked: worm evidence is genetic (IMP) + orthology; direct in-vitro cleavage was shown for mouse IRE1. Matters because MF assignment ideally rests on direct activity. Resolve with a purified *C. elegans* IRE-1 RNase cleavage assay on *xbp-1* stem-loops.
2. **Exact GO term granularity.** Checked: a QuickGO search found **no dedicated "IRE1 endoribonuclease" MF term**; GO:0004521 (RNA endonuclease activity) is the most specific applicable term and is already annotated (IMP:WormBase). A mechanism-precise child (analogous to the tRNA-intron *lyase* term GO:0000213) does not exist for IRE1. Matters only if maximal precision is desired; could be a new-term request but is not required for correct curation.
3. **Whether curators intend GO:0016788 as a mechanism-agnostic descriptor.** If a curation policy treats phosphodiester cleavage as "acting on ester bonds," the term becomes defensible-but-still- redundant. Resolve via GO editorial guidance.

Discriminating Tests

- **Ontology audit:** confirm in the review's GO release whether GO:0016788 is an ancestor of GO:0004521 (it is not in current GO) — decisive for the "correct parent" claim.
- **Point-mutant RNase-dead worm** (KEN-domain catalytic His/Tyr equivalent) that abolishes *xbp-1* splicing while retaining kinase activity — isolates the endoribonuclease MF in vivo.
- **In-vitro cleavage** of a *C. elegans xbp-1* stem-loop by recombinant IRE-1 cytoplasmic module, with 2′,3′-cyclic-phosphate product characterization — confirms mechanism class.

Curation Leads (require curator verification)

- **Lead 1 — Reject/de-prioritize GO:0016788 as an added annotation.** Rationale: redundant with existing GO:0016787 + GO:0004521; not on the lineage of the specific term in current GO; mechanism mismatch with its hydrolytic nuclease children.
- **Lead 2 — Retain/strengthen GO:0004521 (RNA endonuclease activity)** as the core MF, upgrading evidence with orthologous direct-assay references (P 11780124) if the review model permits ISS-with-mouse support alongside worm IMP.
- **Candidate references to verify:**
- PMID **11780124** — snippet: *"Purified mouse IRE1 accurately cleaved XBP-1 mRNA in vitro... a direct target of IRE1 endonucleolytic activity."*
- PMID **11779465** — snippet: *"C. elegans requires ire-1-mediated splicing of xbp-1 mRNA for UPR gene transcription and survival upon ER stress."*
- PMID **21729333** — snippet: Ire1 RNase uses His1061/Tyr1043 general acid/base and coordinates the *scissile phosphate*.
- PMID **36389122** — snippet: *"IRE-1 endoribonuclease activity declines early in"* (worm).
- **Suggested curator question:** "Does our GO release place GO:0016788 as an ancestor of GO:0004521? If not, GO:0016788 should not be represented as the specific term's parent."
- **Suggested experiment:** RNase-dead separation-of-function *ire-1* allele + in-vitro *xbp-1* stem-loop cleavage to nail the worm-specific direct MF and mechanism class.

Provenance

Computed checks run (executed code + outputs retained in the job log): UniProt Q09499 REST fetch (domains + 16 GO annotations); QuickGO ancestry queries for GO:0004521, GO:0004518, GO:0004540, and GO:0000213; children of GO:0016788; QuickGO term search for IRE1/endoribonuclease/tRNA-splicing terms (no dedicated IRE1 MF term exists). Literature via PubMed (PMIDs above). Where worm-specific direct biochemistry was unavailable, it is stated as such and not fabricated.

Artifact files (computed provenance): - `go_decision_table.csv` — per-term GO curation decision table (predicted vs annotated terms, recommended actions, rationale). - `ontology_relationship_tests.csv` — computed QuickGO ancestry tests (GO:0016788 is not an ancestor of GO:0004521; the mechanistic analog GO:0000213 is a lyase, not under GO:0016788).
