Final Report: SKN-1 GO:0006417 (Regulation of Translation) Over-Annotation Analysis

Executive Judgment

Verdict: Over-annotated. The GO:0006417 (regulation of translation) annotation on *C. elegans* SKN-1 (P34707) should be removed. Nine independent lines of evidence — spanning domain architecture, physical interactions, ortholog comparison, comprehensive literature analysis (53 papers), and GO term definition — converge on the conclusion that SKN-1 does not directly regulate translation. The annotation derives entirely from an automated electronic inference (IEA) via UniProtKB keyword KW-0810 ("Translation regulation"), with no experimental evidence code (IDA, IMP, IGI, etc.) supporting it. The most likely source of the erroneous keyword assignment is the well-documented biological relationship in which SKN-1 is activated *by* translation inhibition as a downstream transcriptional effector — a relationship that was apparently conflated with SKN-1 directly regulating translation. The most important caveat is that we cannot entirely exclude the possibility of an undiscovered, non-canonical, isoform-specific translational role; however, no current evidence supports such a function, and the protein lacks all recognized RNA-binding and translation-factor domains.

Summary

C. elegans SKN-1 is a well-characterized bZIP/CNC-family transcription factor orthologous to mammalian NRF1 (NFE2L1) and NRF2 (NFE2L2). It functions as a master regulator of the oxidative stress response, Phase II detoxification, proteasome homeostasis (via the SKN-1A isoform), and embryonic cell fate specification — all through transcriptional activation of target genes. The protein carries GO annotations for DNA-binding transcription factor activity (GO:0003700), positive regulation of transcription by RNA polymerase II (GO:0045944), and numerous biological process terms reflecting its transcriptional outputs, all supported by experimental evidence.

However, UniProt entry P34707 also carries an IEA annotation for GO:0006417 (regulation of translation), inferred from keyword KW-0810. This annotation has no experimental backing. Our investigation systematically evaluated whether SKN-1 could directly regulate translation through nine complementary analyses: (1) annotation provenance, (2) protein domain architecture, (3) physical interaction partners, (4) transcriptional target analysis, (5) ortholog comparison, (6) GO term definition analysis, (7) comprehensive literature review (53 papers), (8) isoform-specific function assessment, and (9) mechanistic pathway tracing. Every line of evidence supports the conclusion that this is an over-annotation arising from conflation of SKN-1's role as a transcriptional responder to translation perturbation with direct translation regulation.

The curation recommendation is to remove GO:0006417 from P34707 and to flag UniProtKB keyword KW-0810 for removal from this entry. No replacement GO term related to translation is warranted; the existing transcription-related annotations accurately capture SKN-1's molecular function.

Key Findings

Finding 1: GO:0006417 Annotation is IEA-Only with No Curator Support

The GO:0006417 (regulation of translation) annotation on SKN-1 (P34707) exists solely as an IEA (Inferred from Electronic Annotation) derived from UniProtKB keyword KW-0810 ("Translation regulation"). A comprehensive search of QuickGO annotations for P34707 reveals that none of the 78 manually curated or experimentally supported GO annotations include GO:0006417. No curation authority — not WormBase, UniProt manual curation, BHF-UCL, nor any other contributing group — has submitted this annotation based on experimental evidence. This IEA-only provenance is the weakest category of GO evidence and is explicitly understood to require verification before being treated as reliable. The absence of any corroborating evidence code (IDA, IMP, IGI, IPI, TAS, etc.) across the entire annotation history of this well-studied gene is itself strong evidence against the annotation's validity.

Finding 2: SKN-1 Lacks All RNA-Binding and Translation-Regulatory Domains

Comprehensive domain analysis of SKN-1 (P34707, 623 amino acids) across six major protein domain databases (InterPro, Pfam, Gene3D, SUPERFAMILY, PROSITE, and CDD) reveals that the protein contains exclusively DNA-binding and transcription factor domains:

Database	Domain	Accession	Function
Pfam	bZIP_Maf	PF03131	DNA-binding transcription factor
InterPro	Basic leucine zipper	IPR004827	Dimerization and DNA binding
InterPro	NFE2-like family	IPR047167	NRF/CNC transcription factor family
InterPro	Skn-1-like DNA-binding superfamily	IPR008917	DNA-binding domain
SUPERFAMILY	SSF47454	—	Eukaryotic TF DNA-binding domain
PROSITE	BZIP_BASIC	PS00036	Basic region of bZIP domain

Critically, **no RNA-binding domains** (RRM, KH, CCCH zinc finger, dsRBD, Pumilio, DEAD-box), **no translation factor domains** (eIF, eEF, or any ribosome-associated domain), and **no ribosome-interaction motifs** were detected by any database. Proteins that directly regulate translation invariably possess at least one RNA-binding or translation-machinery interaction domain. The complete absence of such domains in SKN-1 is strong negative evidence against direct translation regulation.

Finding 3: SKN-1 Functions Exclusively as a Transcription Factor Across All Isoforms

SKN-1 exists in three isoforms (SKN-1A, SKN-1B, SKN-1C), all of which function as transcription factors:

- **SKN-1C** is the canonical stress-responsive isoform that activates Phase II detoxification genes (gcs-1, gst-4, gst-7) in intestinal cells, functioning as the *C. elegans* ortholog of mammalian NRF2.
- **SKN-1A** is the longer isoform orthologous to mammalian NRF1/NFE2L1, which transcriptionally regulates proteasome subunit genes (e.g., rpt-3) in the conserved "bounce-back response" to proteasome dysfunction (PMID: 27528192). As stated in that paper: *"In Caenorhabditis elegans, this response requires SKN-1, a transcription factor related to mammalian Nrf1/2."*

- **SKN-1B** is expressed in ASI neurons and contributes to dietary restriction-mediated longevity, again through transcriptional regulation.

ChIP-seq data from the modENCODE project (PMID: 21177963) mapped SKN-1's genome-wide binding sites, confirming it binds DNA near transcription start sites as part of a study of 22 transcription factors. No isoform has been reported to bind RNA, interact with ribosomes, or directly modulate translation.

Finding 4: Translation Inhibition Activates SKN-1 Transcriptionally — The Likely Source of Keyword Confusion

The most probable explanation for the erroneous KW-0810 keyword assignment is the well-documented biological relationship in which perturbation of translation activates SKN-1 as a downstream transcriptional effector:

- **Wang et al. 2010** (PMID: 20700440) demonstrated that *"translation initiation factor RNAi robustly induces SKN-1 target gene transcription and confers skn-1-dependent oxidative stress resistance."* This paper explicitly shows SKN-1 is activated **by** translation inhibition and responds via transcription — not that SKN-1 regulates translation.
- **Rieckher et al. 2018** (PMID: 30282029) showed that *"The effects of mRNA decapping deficiency on stress resistance and longevity are orchestrated by a multimodal stress response involving the transcription factor SKN-1, which mediates lifespan extension upon reduced protein synthesis."* Again, SKN-1 is a transcriptional mediator of the cellular response to reduced protein synthesis.

The directionality is critical: translation perturbation → stress signaling → SKN-1 activation → transcriptional output. SKN-1 is downstream of translation regulation, not upstream. Automated keyword extraction likely captured the association between SKN-1 and "translation" without encoding the correct causal direction.

Additionally, during embryogenesis, SKN-1 protein itself is subject to translational regulation by MEX-3 and other CCCH zinc-finger proteins (PMID: 11830574). This is SKN-1 *being regulated* at the translational level, not SKN-1 *regulating* translation — another potential source of keyword confusion.

Finding 5: Mammalian Orthologs NRF1/NRF2 Lack GO:0006417 and KW-0810

Human NRF1/NFE2L1 (Q14494) and NRF2/NFE2L2 (Q16236) share the identical domain architecture with SKN-1 (bZIP, bZIP_Maf, NFE2-like, TF_DNA-bd_sf) and perform conserved transcriptional functions. Neither carries the KW-0810 keyword nor the GO:0006417

annotation. Given the strong functional conservation of the NRF/CNC transcription factor family, the absence of translation-regulatory annotation on the mammalian orthologs — which have been far more extensively studied — further supports the conclusion that the annotation on SKN-1 is erroneous rather than reflecting a *C. elegans*-specific function.

Finding 6: SKN-1 Transcriptional Target RIOK-1 Provides Only an Indirect Link to Ribosome Biogenesis

The closest connection between SKN-1 and translation machinery is its transcriptional regulation of *riok-1*, a RIO protein kinase involved in rRNA processing and 40S ribosomal subunit maturation (PMID: 25688864). As stated in that paper: *"the intestinal expression of riok-1 was dependent upon two putative binding sites for the oxidative and xenobiotic stress response transcription factor SKN-1."*

However, this represents a multi-step indirect pathway:

```
SKN-1 --[transcription]--> riok-1 mRNA --[translation]--> RIOK-1 protein
    --[rRNA processing]--> 40S ribosome maturation --[ribosome availability]--> translation capa
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By this logic, any transcription factor whose targets include ribosome biogenesis genes would qualify for GO:0006417, which would dramatically over-extend the term. The GO:0006417 definition requires **direct modulation** of translation frequency, rate, or extent — not transcriptional regulation of a gene whose protein product is two steps removed from translation.

Finding 7: Nucleolar Disruption Activates SKN-1 Independently of Translation

Leung et al. 2012 (PMID: 22240150) provided critical mechanistic evidence, demonstrating that disruption of nucleolar function (WDR-46, involved in 18S rRNA processing) activates SKN-1 and its target gene gst-4. Crucially, the paper states: *"disruptions to nucleolar function can activate SKN-1 by a mechanism that involves p53/cep-1 and is independent of protein translation."* This directly demonstrates that SKN-1 is a transcriptional responder activated by ribosome/translation perturbation through stress signaling pathways — not a protein that directly modulates translation.

Finding 8: No Physical Interactions with Translation Machinery

IntAct PSICQUIC database queries returned 10 interaction records for SKN-1 (P34707). These include protein-protein interactions (yeast two-hybrid) with NMAD-1 (RNA demethylase), and DNA-binding interactions (one-hybrid) with regulatory regions of target genes. UniProt records interactions with ELT-3 (GATA transcription factor), PGMA-5 (phosphoglycerate mutase), and MXL-3 (transcription factor). **No interactions were detected with translation initiation factors (eIFs), elongation factors (eEFs), ribosomal proteins, or any mRNA-binding proteins involved in translational control.** Proteins that directly regulate translation typically show robust physical interactions with translation machinery components.

Finding 9: GO:0006417 Definition Requires Direct Translation Modulation

GO:0006417 is defined as: *"Any process that modulates the frequency, rate or extent of the chemical reactions and pathways resulting in the formation of proteins by the translation of mRNA or circRNA."* This requires direct modulation of the translation process itself. SKN-1's only connections to translation are:

1. Being activated as a transcriptional effector when translation is inhibited (indirect; wrong direction)
2. Being itself subject to translational control during embryogenesis (SKN-1 is the target, not the regulator)
3. Transcriptionally regulating *riok-1*, a ribosome maturation factor (two-step indirect effect)

None of these constitute direct modulation of translation frequency, rate, or extent as required by the GO term definition.

Evidence Matrix

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
1	UniProt P34707; QuickGO	Database/ computational	Supports over-annotation	Is GO:0006417 experimentally supported?	IEA-only; no curator submission among 78 annotations	*C. elegans*, all tissues
2	InterPro, Pfam, PROSITE, SUPERFAMILY, Gene3D, CDD	Structural/ evolutionary	Supports over-annotation	Does SKN-1 have RNA-binding or translation domains?	Only DNA-binding/bZIP domains; no RNA-binding or translation factor domains	Sequence analysis
3	PMID: 27528192	Direct assay (mutant + molecular)	Supports over-annotation	Is SKN-1A a translation regulator?	SKN-1A is a transcription factor regulating proteasome genes	*C. elegans*, intestine
4	PMID: 20700440	Direct assay (RNAi screen)	Supports over-annotation	Does SKN-1 regulate translation?	Translation inhibition activates SKN-1 *transcriptional* response	*C. elegans*, whole organism
5	PMID: 30282029	Direct assay (genetic)	Supports over-annotation	Does SKN-1 regulate translation?	SKN-1 is transcriptional mediator of response to reduced protein synthesis	*C. elegans*, whole organism
6	PMID: 22240150	Direct assay (genetic)	Supports over-annotation	Is SKN-1 activation linked to translation?	SKN-1 activation by nucleolar disruption is *independent of translation*	*C. elegans*, intestine
7	PMID: 21177963	Direct assay (ChIP-seq)		Where does SKN-1 bind?	SKN-1 binds DNA near TSS;	

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
			Supports over-annotation		profiled as 1 of 22 TFs	*C. elegans*, L1-L2 larvae
8	PMID: 25688864	Direct assay (reporter/binding sites)	Qualifies	Does SKN-1 connect to ribosome biogenesis?	SKN-1 transcriptionally regulates *riok-1* (ribosome maturation factor)	*C. elegans*, intestine
9	PMID: 11830574	Direct assay (genetic)	Qualifies	Is SKN-1 involved in translational regulation?	SKN-1 protein is *translationally regulated* by MEX-3, not a translation regulator itself	*C. elegans*, early embryo
10	UniProt Q14494 (NRF1), Q16236 (NRF2)	Computational/ orthology	Supports over-annotation	Do orthologs have GO:0006417?	Neither NRF1 nor NRF2 carry KW-0810 or GO:0006417 despite identical domains	Human
11	IntAct PSICQUIC	Database/ interaction	Supports over-annotation	Does SKN-1 interact with translation factors?	No interactions with eIFs, eEFs, or ribosomal proteins	*C. elegans*
12	PMID: 32502151	Direct assay (genetic/ electrophysiology)	Supports over-annotation	Does SKN-1A regulate translation?	SKN-1A/NRF1 transcriptionally controls proteasome levels affecting ERAD	*C. elegans*, neurons
13	PMID: 38466732	Direct assay (genetic/immune)	Supports over-annotation	What does SKN-1A regulate?	SKN-1A regulates proteasomal gene expression;	*C. elegans*, epidermis/ intestine

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
					loss affects innate immunity	
14	PMID: 9015263	Mutant phenotype	Supports over-annotation	Do skn-1 mutants affect GLP-1 translation?	skn-1 mutants do not affect translational control of GLP-1 in embryo	*C. elegans*, early embryo
15	PMID: 25643626	Direct assay (genetic)	Qualifies	GCN-2 and SKN-1 in longevity	GCN-2 regulates translation; SKN-1 regulates transcription. Parallel pathways	*C. elegans*, lifespan
16	STRING network analysis	Computational (network)	Supports over-annotation	SKN-1 functional interaction partners	Top 20 partners are kinases, TFs, and target genes. No translation machinery	*C. elegans*, STRING
17	GO:0006417 definition (QuickGO)	Database/ computational	Supports over-annotation	Does SKN-1 activity fit the GO term?	Term requires direct modulation of translation; SKN-1 does not do this	GO ontology

GO Curation Implications

Primary Recommendation: Remove GO:0006417

Action: The IEA annotation GO:0006417 (regulation of translation) should be removed from P34707 (SKN-1). The supporting UniProtKB keyword KW-0810 (Translation regulation) should also be reviewed for removal from this entry.

Rationale: 1. The annotation has only IEA evidence from keyword mapping — no experimental evidence supports it. 2. SKN-1 lacks all expected molecular features of a translation regulator (no RNA-binding domains, no translation factor domains). 3. All established molecular functions are DNA-binding transcription factor activities. 4. No curation authority (WormBase, UniProt manual curators, etc.) has submitted this annotation. 5. The connection to translation is exclusively indirect — SKN-1 transcriptionally responds to translation inhibition. 6. Mammalian orthologs with identical domain architecture lack this annotation.

GO Decision Table

GO Term	Current Status	Evidence Codes	Recommendation	Rationale	Confidence
GO:0006417 (regulation of translation)	Annotated (IEA:UniProtKB-KW)	IEA only	**REMOVE**	No direct translation regulatory activity; IEA from erroneous KW-0810; no RNA-binding domains; no translation factor interactions; mammalian orthologs lack this annotation	Very High
GO:0003700 (DNA-binding TF activity)	Annotated (NAS)	NAS:UniProtKB	**RETAIN**	Well-established, foundational function	Very High
GO:0000981 (DNA-binding TF activity, RNAPII-specific)	Annotated (IBA)	IBA:GO_Central	**RETAIN**	Supported by phylogenetic inference and direct evidence	High
GO:0000978 (RNAPII cis-regulatory region DNA binding)	Annotated (IDA)	IDA:UniProtKB	**RETAIN**	Direct experimental evidence (ChIP-seq, EMSA)	Very High
GO:0045944 (positive regulation of	Annotated (IMP)	IMP:UniProtKB, IMP:WormBase	**RETAIN**	Multiple IMP evidence from	Very High

GO Term	Current Status	Evidence Codes	Recommendation	Rationale	Confidence
transcription by RNAPII)				independent groups	
GO:0006351 (DNA-templated transcription)	Annotated (IEA:UniProtKB-KW)	IEA only	RETAIN (but upgrade evidence)	Correct annotation but IEA; ample experimental support exists	High
KW-0810 (Translation regulation)	Present in UniProt	Keyword	**REMOVE from UniProt**	No basis in function description or literature; likely curation error	Very High

Possible New Annotation

If curators wish to capture SKN-1's role in the cellular response to translation inhibition (an indirect relationship), the most appropriate annotation would be **GO:0006950** (response to stress) with an annotation extension indicating the stimulus is translation inhibition, referencing PMID: 20700440. The term GO:0032055 (regulation of translation in response to stress) should **not** be used because SKN-1's response to translation stress is itself transcriptional, not translational.

Mechanistic Scope

Direct Molecular Activity

SKN-1 is a **sequence-specific DNA-binding transcription factor** of the CNC-bZIP family. Its immediate molecular activity is:

1. **DNA binding** via the bZIP/CNC domain to antioxidant response elements (AREs) and related cis-regulatory sequences
2. **Transcriptional activation** of target genes including Phase II detoxification enzymes (gcs-1, gst-4, gst-7), proteasome subunits (rpt-3, via SKN-1A), metabolic genes, and embryonic patterning genes (med-1, med-2)

Separation from Downstream Effects

The following are downstream consequences of SKN-1 transcriptional activity, NOT direct molecular functions:

Level	Example	Relationship to SKN-1
Direct MF	Binds ARE DNA sequences	SKN-1 performs this
Direct BP	Activates transcription of gcs-1	SKN-1 performs this
Downstream 1	GCS-1 synthesizes glutathione	Product of SKN-1 target gene
Downstream 2	Glutathione detoxifies ROS	Two steps removed
Downstream 1	RIOK-1 processes rRNA	Product of SKN-1 target gene
Downstream 2	Mature 40S subunits enable translation	Two steps removed
Phenotype	Lifespan extension, stress resistance	Multi-step consequence

The GO:0006417 annotation conflates SKN-1's indirect, transcription-mediated effect on ribosome biogenesis (via *riok-1*) and its role as a stress-responsive transcription factor activated by translation perturbation with direct regulation of translation.

Causal Pathway Diagram

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ACTUAL BIOLOGY (SKN-1 is DOWNSTREAM of translation perturbation):

Translation inhibition (e.g., eIF RNAi)
        │
        ▼
   Stress signaling (p38/PMK-1, mTOR)
        │
        ▼
   SKN-1 nuclear translocation & activation
        │
        ▼
   TRANSCRIPTIONAL activation of target genes
        │
        ├──► gcs-1, gst-4 (detoxification)
        ├──► rpt-3 (proteasome subunits, via SKN-1A)
        └──► riok-1 (ribosome maturation factor)

MISINTERPRETATION (what GO:0006417 implies):

   SKN-1 ──[DIRECTLY]──► Regulates translation
   (This does NOT occur)
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Conflicts and Alternatives

Potential Counterarguments Evaluated

1. Could SKN-1 have an undiscovered RNA-binding function? Some transcription factors have been found to moonlight as RNA-binding proteins. However, SKN-1 lacks any of the recognized RNA-binding domain folds (RRM, KH, CCCH zinc finger, dsRBD, Pumilio). The bZIP domain binds double-stranded DNA and has not been reported to bind RNA in any CNC/NRF family member. While we cannot entirely exclude a cryptic RNA-binding activity, the complete absence of domain evidence and the lack of any experimental report across decades of study makes this highly unlikely.

2. Could SKN-1A have an isoform-specific translation role? SKN-1A is the longest isoform and is orthologous to mammalian NRF1/NFE2L1. It has a unique N-terminal extension that mediates ER membrane association and DDI-1-dependent cleavage for proteasome gene

regulation (PMID: 27528192, PMID: 32502151, PMID: 38466732). This is a well-characterized transcriptional function. No evidence suggests any isoform has translational regulatory activity.

3. Could the RIOK-1 connection justify GO:0006417? SKN-1 transcriptionally regulates *riok-1* (PMID: 25688864), which is involved in ribosome maturation. However, this is a two-step indirect connection: SKN-1 → riok-1 transcription → RIOK-1 protein → ribosome maturation → translation capacity. GO annotation guidelines require direct participation in the process, not transcriptional regulation of an upstream factor. By the same logic, any transcription factor regulating ribosomal protein genes would carry GO:0006417, which would be clearly inappropriate.

4. Could the keyword have been assigned correctly based on now-superseded evidence? A thorough literature search of 53 papers, including the earliest SKN-1 studies from the 1990s through 2024, found no paper reporting direct translation regulation by SKN-1. The keyword assignment appears to be an automated inference error, not a reflection of historical evidence that was later overturned.

5. Paralog confusion or organism-specific differences? There are no close paralogs of SKN-1 in *C. elegans* that could cause confusion. The NRF/CNC family in worms consists of SKN-1 alone, unlike mammals which have NRF1, NRF2, NRF3, and other CNC members. No organism-specific translation-regulatory function has been reported.

6. SKN-1A ER association as a translational nexus? SKN-1A is ER-membrane-associated before cleavage (PMID: 27528192), and the ER is a site of co-translational protein insertion. However, SKN-1A's ER association is for regulated processing (glycosylation-dependent sensing of proteasome dysfunction), not for translation regulation. After cleavage by DDI-1, SKN-1A translocates to the nucleus where it functions as a transcription factor.

7. SKN-1 in embryonic translational regulation circuits? SKN-1 protein distribution in early embryos is controlled by translational regulation of skn-1 mRNA via MEX-3 and other RNA-binding proteins (PMID: 11830574; PMID: 9015263). However, SKN-1 is a **target** of translational regulation during embryogenesis, not a regulator of translation. Notably, Crittenden et al. 1997 (PMID: 9015263) found that skn-1 mutants do **not** affect translational control of GLP-1 in the early embryo, providing direct negative evidence.

Source of Keyword Confusion: Two Biological Contexts

Two biological contexts likely contributed to the erroneous KW-0810 assignment:

1. **Translation inhibition → SKN-1 activation pathway**: Wang et al. (2010, PMID: 20700440) and Rieckher et al. (2018, PMID: 30282029) demonstrated that inhibition of mRNA translation activates SKN-1-dependent transcription. This creates a functional link between SKN-1 and "regulation of translation" in the literature, but the directionality is reversed — SKN-1 is regulated BY translation status, it does not regulate translation.
2. **Embryonic translational regulation of SKN-1 itself**: SKN-1 protein distribution in early embryos is controlled by translational regulation of skn-1 mRNA (via MEX-3, PAR genes, etc.). SKN-1 is a TARGET of translational regulation during embryogenesis, not a regulator of translation.

Evidence Base: Key Literature

Papers Directly Testing the Relationship

- **Wang et al. 2010** (PMID: 20700440) — *"RNAi screening implicates a SKN-1-dependent transcriptional response in stress resistance and longevity deriving from translation inhibition."* This is the single most relevant paper. It demonstrates that inhibiting translation initiation factors activates SKN-1-dependent **transcription**, not that SKN-1 regulates translation. The title itself encodes the correct causal direction. Key quote: *"translation initiation factor RNAi robustly induces SKN-1 target gene transcription and confers skn-1-dependent oxidative stress resistance."*
- **Rieckher et al. 2018** (PMID: 30282029) — *"Maintenance of Proteostasis by P Body-Mediated Regulation of eIF4E Availability during Aging in Caenorhabditis elegans."* Shows SKN-1 mediates the transcriptional arm of the stress response to reduced protein synthesis. Key quote: *"The effects of mRNA decapping deficiency on stress resistance and longevity are orchestrated by a multimodal stress response involving the transcription factor SKN-1, which mediates lifespan extension upon reduced protein synthesis."*

- **Leung et al. 2012** (PMID: 22240150) — *"Depletion of a nucleolar protein activates xenobiotic detoxification genes in Caenorhabditis elegans via Nrf/SKN-1 and p53/CEP-1."* Provides critical evidence that SKN-1 activation by nucleolar disruption is *independent of protein translation*. Key quote: *"disruptions to nucleolar function can activate SKN-1 by a mechanism that involves p53/cep-1 and is independent of protein translation."*

Papers Establishing SKN-1 as a Transcription Factor

- **Lehrbach & Ruvkun 2016** (PMID: 27528192) — Establishes SKN-1A as a transcription factor in the proteasome bounce-back response, activated by aspartic protease DDI-1 cleavage.
- **Niu et al. 2011** (PMID: 21177963) — modENCODE ChIP-seq profiling of SKN-1 genome-wide binding sites confirms DNA-binding transcription factor function.
- **Doering et al. 2020** (PMID: 32502151) — Shows SKN-1A/NRF1-mediated transcriptional mechanism controls proteasome levels, which indirectly affects BK channel density via ERAD.
- **Grover et al. 2024** (PMID: 38466732) — SKN-1A regulates proteasomal gene expression; loss-of-function causes constitutive expression of innate immune programs.
- **Wu et al. 2021** (PMID: 34407394) — SKN-1 is a protective transcription factor in intestinal immunity, regulated by p38 MAPK phosphorylation.

Papers on SKN-1 Translational Regulation (SKN-1 as Target, Not Regulator)

- **Huang et al. 2002** (PMID: 11830574) — MEX-3 interacting proteins (MEX-6, SPN-4) link cell polarity to asymmetric gene expression; SKN-1 protein is translationally regulated during embryogenesis, but SKN-1 does not itself regulate translation.
- **Crittenden et al. 1997** (PMID: 9015263) — Mutants affecting anterior-posterior asymmetry disrupt GLP-1 translational control, but *skn-1 mutants do not affect the GLP-1 pattern* — negative evidence for SKN-1's role in translation regulation.
- **Mootz et al. 2004** (PMID: 15201219) — GLD-1 mediates translational control of PAL-1; relevant context showing translational regulation of embryonic determinants, but not by SKN-1.

Broader SKN-1/NRF Literature

Across all 53 papers reviewed — spanning topics from stress resistance and longevity, to detoxification, embryonic patterning, proteasome regulation, innate immunity, and metabolic regulation — **every paper describes SKN-1 exclusively as a transcription factor**. The papers from the nutraceutical/longevity field (constituting roughly half the literature) consistently describe SKN-1 as a transcriptional mediator of stress responses. Some papers use phrases like "up-regulating the translation of SKN-1 mRNA" (PMID: 37316035), referring to SKN-1 protein production (i.e., translation of SKN-1's own mRNA), not SKN-1 regulating translation of other mRNAs.

Limitations and Knowledge Gaps

Limitations of This Analysis

1. **Absence of evidence vs. evidence of absence:** While no evidence supports direct translation regulation by SKN-1, we cannot prove a negative with certainty. An undiscovered, non-canonical function remains formally possible, though all available evidence argues against it.
2. **Interaction database coverage:** Physical interaction databases (IntAct, BioGRID) have incomplete coverage. Undiscovered interactions with translation machinery cannot be fully excluded without comprehensive proteomics studies (AP-MS, BioID/TurboID).
3. **Literature search scope:** While 53 papers were reviewed, the complete *C. elegans* SKN-1 literature likely exceeds 200 papers. However, the most relevant mechanistic studies were captured, and the consistency of findings across all reviewed papers is strong.
4. **KW-0810 assignment rationale:** We could not determine exactly why or when UniProt assigned the KW-0810 keyword to P34707. Understanding the rationale would help prevent similar errors.

Knowledge Gaps Relevant to Curation

Gap	What Was Checked	Why It Matters	Resolution
Origin of KW-0810 assignment	UniProt entry, function description, all references	Understanding the historical basis could prevent recurrence	Contact UniProt curators
Whether SKN-1 transcriptional targets include core translation factor genes	Literature search; modENCODE targets; known regulon	If SKN-1 directly transcribes eIF/eEF genes, a qualified annotation might be warranted	Full cross-reference of ChIP-seq targets with translation machinery genes
No CLIP-seq/RIP-seq data for SKN-1	PubMed searches; domain analysis	Would definitively rule out RNA binding	eCLIP or iCLIP of SKN-1
Whether any SKN-1 isoform has non-transcriptional roles	Literature search for all isoforms	Isoform-specific functions could theoretically include translation regulation	Comprehensive molecular characterization of all isoforms
Polysome profiling in skn-1 mutants	Not performed	Would detect direct effects on translation	Ribo-seq comparing wild-type and skn-1(-)

Proposed Follow-up Experiments/Actions

Immediate Curation Actions

1. **Submit annotation correction** to UniProt/GOA to remove GO:0006417 from P34707
2. **Submit keyword correction** to UniProt to remove KW-0810 from P34707
3. **Consider adding a NOT annotation** for GO:0006417 with evidence code IEA-reviewed or TAS, citing PMID: 20700440 and PMID: 22240150, to explicitly record that this function was evaluated and rejected

Systematic Quality Control

1. **Audit other *C. elegans* transcription factors** for similar GO:0006417 over-annotations derived from KW-0810
2. **Review KW-0810 assignment criteria** — the keyword "Translation regulation" may be too broadly applied to proteins that participate in translation-related biological contexts without directly regulating translation

Discriminating Experiments (If Desired)

1. **eCLIP-seq of tagged SKN-1 isoforms** — Would definitively determine whether SKN-1 binds any mRNAs in vivo. Prediction: negative (no RNA-binding domains). A negative result closes the question.
2. **Polysome profiling in skn-1 mutants vs. wild-type** — If SKN-1 directly regulates translation, global or specific changes in polysome occupancy should be detectable. This would distinguish direct translation regulation from transcriptional effects.
3. **Ribosome footprinting (Ribo-seq) comparing skn-1 mutant and wild-type** — Would detect changes in translational efficiency at specific mRNAs, distinguishing transcriptional from translational effects.
4. **In vitro translation assay with purified SKN-1** — Adding recombinant SKN-1 to an in vitro translation system would test whether SKN-1 can directly modulate translation. This is the gold-standard for demonstrating direct translation regulation.
5. **Tethered function assay** — Fusing SKN-1 to a bacteriophage coat protein (e.g., MS2) and measuring its effect on translation of a reporter mRNA bearing coat protein binding sites would test for any intrinsic translational regulatory activity.

Curation Leads

Lead 1: Remove GO:0006417 (HIGH CONFIDENCE)

- **Action:** Remove GO:0006417 (regulation of translation) from P34707
- **Rationale:** IEA-only evidence from KW-0810; no experimental support; domain analysis contradicts; all known activities are transcriptional; mammalian orthologs lack annotation

- **Curator verification needed:** Confirm KW-0810 keyword should also be removed from UniProt entry

Lead 2: Remove KW-0810 from UniProt Entry (HIGH CONFIDENCE)

- **Action:** Contact UniProt to remove keyword KW-0810 (Translation regulation) from P34707
- **Rationale:** Function description mentions no translation-related activity; all references describe transcription factor function; keyword appears to be historical misassignment

Lead 3: Retain Existing Transcription-Related GO Terms (RETAIN)

- **Action:** Keep GO:0003700, GO:0000981, GO:0000978, GO:0045944 with current evidence codes
- **Rationale:** Well-supported by multiple experimental evidence types (IDA, IMP, NAS, IBA)

Lead 4: Candidate References for Verification

PMID	Exact Snippet	Relevance
20700440	*"translation initiation factor RNAi robustly induces SKN-1 target gene transcription and confers skn-1-dependent oxidative stress resistance"*	Demonstrates SKN-1 is activated BY translation inhibition as a transcriptional effector
30282029	*"The effects of mRNA decapping deficiency on stress resistance and longevity are orchestrated by a multimodal stress response involving the transcription factor SKN-1, which mediates lifespan extension upon reduced protein synthesis"*	Confirms transcriptional mediator role in response to reduced protein synthesis
22240150	*"disruptions to nucleolar function can activate SKN-1 by a mechanism that involves p53/cep-1 and is independent of protein translation"*	SKN-1 activation is independent of translation — it is a stress-responsive TF
27528192	*"In Caenorhabditis elegans, this response requires SKN-1, a transcription factor related to mammalian Nrf1/2"*	SKN-1A is a transcription factor in the proteasome bounce-back response
25688864	*"the intestinal expression of riok-1 was dependent upon two putative binding sites for the oxidative and xenobiotic stress response transcription factor SKN-1"*	Closest link to translation is indirect (transcriptional regulation of ribosome biogenesis factor)
21177963	*"we have used chromatin immunoprecipitation coupled with high-throughput DNA sequencing (ChIP-seq) to determine the genome-wide binding sites of 22 transcription factors"*	Genome-wide confirmation of DNA-binding TF function

Lead 5: Suggested Questions for Curator Review

1. Should WormBase flag P34707's KW-0810 keyword to UniProt for review?
2. Are there other *C. elegans* transcription factors carrying GO:0006417 via IEA that may represent similar over-annotations?
3. Should a NOT annotation (GO:0006417 with NOT qualifier) be added to explicitly record that this function was evaluated and rejected?

4. Does the indirect connection via *riok-1* transcriptional regulation warrant any annotation, or is the two-step distance too great?

Report generated from 3 iterations of systematic investigation, reviewing 53 papers and recording 9 confirmed findings. All evidence converges on the conclusion that GO:0006417 is an over-annotation on SKN-1 (P34707).
