AIGR Deep Research Report — SSB1 (P11484), *Saccharomyces cerevisiae*

Focus type: core_function **Seed hypothesis:** *"The four amino-acid differences between Ssb1 and Ssb2 confer a demonstrated paralog-specific substrate preference or cotranslational folding mechanism."*

Executive Judgment

Verdict: REFUTED as stated (over-annotation of a redundant paralog pair).

The hypothesis has two clauses. The **factual premise** — that Ssb1 and Ssb2 differ by four amino acids — is **correct** (verified directly: 613 aa each, 99.35 % identical, exactly 4 substitutions). The **functional claim** — that these differences *confer a demonstrated paralog-specific substrate preference or cotranslational folding mechanism* — is **not supported by any primary literature**. Across foundational and contemporary studies, Ssb1 and Ssb2 are treated as a single functional entity ("Ssb", "Ssb1/2p"), are deleted together, and no study assigns a distinct substrate spectrum or folding mechanism to one paralog versus the other. Three of the four differences do lie in the substrate-binding domain (SBD), which makes a paralog-specific effect *structurally conceivable*, but positional plausibility is not demonstration. The word **"demonstrated" is the failure point**: there is no such demonstration.

Most important caveat: absence of evidence for divergence is not the same as proof of perfect functional identity; a subtle, condition-specific difference has not been rigorously excluded. But for GO curation purposes, any paralog-specific MF/BP term would be unsupported and should not be asserted.

Evidence Matrix

Citation	Evidence type	Supports/ Refutes/ Qualifies	Claim tested	Key finding	Context	Confidence & limitations
This report (UniProt P11484 vs P40150, computed)	Structural/ evolutionary (sequence)	Qualifies (confirms premise)	Ssb1/Ssb2 differ by 4 aa	613 aa each, 99.35 % identical; E49Q (NBD), M413I, C435V, A436S (all SBD)	*S. cerevisiae* proteins	High for the count; residues not annotated as catalytic/ substrate-contacting sites
Nelson et al. 1992 (P 1394434)	Mutant phenotype / biochemistry	Refutes functional clause	Distinct roles for Ssb1 vs Ssb2	Characterizes "Ssb1/2p" jointly; *ssb1 ssb2* double mutant needed for phenotype (ribosome association, slow growth, drug hypersensitivity)	Yeast, translating ribosomes	High; classic study, treats pair as one
Willmund et al. 2013 (P 23332755)	Direct assay (global substrate mapping)	Refutes functional clause	Paralog-specific substrate preference	Defines cotranslational substrate specificity of "the yeast Hsp70 SSB" as one entity; SSB deletion → aggregation	Yeast, ribosome-nascent chains	High; the most direct substrate-specificity study — done at the SSB (not paralog) level
Chiabudini et al. 2012 (P 23007158)	Mutant phenotype	Refutes functional clause	Paralog-specific mechanism	RAC/"Ssb" (Ssb1 and Ssb2) jointly required for translational repression	Yeast	Medium-high; both deleted together

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Chiabudini et al. 2014 (P 25154418)	Mutant phenotype	Refutes functional clause	Paralog-specific mechanism	"Ssb (Ssb1 and Ssb2)" treated jointly in premature-termination assay	Yeast	Medium-high
Chen et al. 2022 (P 35701497); Kišonaitė et al. 2023 (P 37081320)	Structural (cryo-EM)	Qualifies	Mechanism of Ssb on ribosome	RAC–Ssb cotranslational folding mechanism resolved for "Ssb1/2" generically; no paralog distinction	Yeast / *C. thermophilum*	High for mechanism; not paralog-resolved

No competing paper asserting a demonstrated Ssb1-vs-Ssb2 functional difference was found.

GO Decision Table (grounded in current SSB1 annotations, QuickGO/P11484, verified Iteration 2)

SSB1 currently carries **39 GO annotations, none of which are paralog-specific**. Representative terms and the recommended action relative to the seed hypothesis:

GO ID	Aspect	Term	Evidence (ref)	Relation to hypothesis	Recommended action
GO:0044183	MF	protein folding chaperone	IBA	Shared core function	Retain (core)
GO:0016887	MF	ATP hydrolysis activity	IDA (P 9860955)	Shared core function	Retain (core)
GO:0005524	MF	ATP binding	IEA	Shared core function	Retain
GO:0031072	MF	heat shock protein binding	IBA	Shared (RAC co-chaperone interaction)	Retain
GO:0051083	BP	'de novo' cotranslational protein folding	IDA (P 9670014)	Shared core process	Retain (core)
GO:0002181	BP	cytoplasmic translation	IMP (P 1394434)	Shared; from *ssb1 ssb2* double mutant	Retain
GO:0005829	CC	cytosol	IBA	Shared localization	Retain (core)
GO:0005516	MF	calmodulin binding	IDA (P 17146552)	Not paralog-specific; likely non-core	Curator review (non-core?)
GO:0005515	MF	protein binding	IPI (P 11805837)	Uninformative	Do not treat as core
— (proposed)	MF/BP	*Ssb1-specific substrate preference / distinct cotranslational mechanism*	**none**	**The seed hypothesis**	**Do NOT add — unsupported**

Key point: the `IMP` process terms derive from **double-mutant (*ssb1Δ ssb2Δ*)** phenotypes, i.e., they document the *shared* Ssb function, not a paralog-resolved one. No existing term encodes a paralog-specific activity, and none should be added.

GO Curation Implications (leads — require curator verification)

- **Do NOT create or retain any paralog-specific MF/BP/CC term** implying Ssb1 has a substrate preference or cotranslational mechanism distinct from Ssb2. Such a term would be an over-annotation.
- **Appropriate, supported terms for SSB1** (shared with SSB2, evidence-backed):
- MF: *unfolded protein binding* (GO:0051082); *ATP binding* / *ATP hydrolysis activity* (Hsp70 NBD).
- BP: *protein folding* / *'de novo' cotranslational protein folding* (GO:0051083 / GO:0140719); *regulation of translation* / translational fidelity context.
- CC: *cytosol* (GO:0005829); *cytosolic ribosome* / *ribosome-associated* (polysome association, GO:0022626-adjacent).
- **Recommended action:** retain the general ribosome-associated Hsp70 chaperone annotations; **generalize/avoid** any qualifier that ascribes a *unique* substrate class to Ssb1. If a paralog-specific annotation currently exists, flag it for **removal or down-grading to NAS/ non-core**.
- Avoid "protein binding" (GO:0005515) as a final call; *unfolded protein binding* is the more informative supported MF.

Substitution Severity Analysis (computed, Iteration 3 — provenance: `ssb1_ssb2_substitution_analysis.csv`)

Physicochemical severity (Grantham distance) of the four Ssb1→Ssb2 substitutions:

Pos	SSB1	SSB2	Domain	Grantham	Severity
49	E	Q	NBD (ATPase)	29	conservative
413	M	I	SBDβ	10	conservative
435	C	V	SBDβ	**192**	**radical**
436	A	S	SBDβ	99	moderate

Mean Grantham = 82.5. **Interpretation:** three of four differences are conservative/moderate — consistent with near-neutral divergence between redundant WGD paralogs. The single striking substitution is **C435V**, which removes the only paralog-distinguishing cysteine (a redox-active thiol) in Ssb1, located in the substrate-binding β-subdomain. This makes C435 the **best candidate residue** for any hypothetical functional difference (e.g., redox-sensitive substrate handling), yet it remains **entirely untested** — no experiment links it to a substrate preference. This nuance neither rescues the "demonstrated" claim nor is dismissible; it is a lead, not evidence.

Mechanistic Scope

- **Immediate molecular function (both paralogs):** an ATP-dependent Hsp70 chaperone that, in complex with the RAC co-chaperone (Zuo1/Ssz1), binds hydrophobic segments of nascent polypeptides at the ribosomal exit tunnel to promote cotranslational folding and prevent misfolding/aggregation. This is a *shared* activity.
- **The tested increment** — a *paralog-specific* substrate preference or a *distinct* folding mechanism for Ssb1 — is the piece with **no direct gene-product evidence**.
- **Downstream/pleiotropic effects** (not core function, and not paralog-specific): translational repression of poly-lysine/nonstop transcripts, premature termination, prion ([PSI+]) modulation, TORC1-linked translational control. These are collective RAC/Ssb-system phenotypes.

Conflicts and Alternatives

- **Paralog confusion / carry-over is the central risk.** SSB1 and SSB2 arose from the whole-genome duplication and remain ~99 % identical; databases and papers routinely collapse

them to "Ssb". A curation asserting Ssb1-specific function would most likely be **database carry-over / inference, not demonstrated fact**.

- **Alternative interpretation (best-supported):** the 4 differences are **evolutionarily tolerated near-neutral substitutions** between redundant WGD paralogs, not adaptive functional divergence. The joint-deletion requirement for phenotypes (single deletions are near-silent) is classic redundancy.
- **Residue-level nuance:** C435V removes a cysteine unique to Ssb1 (potential redox/thiol difference) and M413I/A436S sit in the SBDβ — these *could* seed a testable hypothesis, but currently support only *speculation*, not a "demonstrated" claim.

Knowledge Gaps

1. **Has any assay directly compared Ssb1-only vs Ssb2-only cells/proteins?** Checked PubMed broadly; none found. Matters because it is the exact evidence the hypothesis claims exists. Resolve with paralog-swap strains + selective ribosome profiling (SeRP).
2. **Do the 4 residues alter substrate contacts?** Checked UniProt features — none are annotated substrate/nucleotide binding sites; three are merely within the SBD region. Resolve with structural modeling of the peptide-binding cleft and in vitro peptide-array affinity comparison.
3. **Expression/regulatory divergence?** Not resolved here; even if promoters differ, that would be regulatory, not the "substrate preference / folding mechanism" claimed. Resolve with paralog-specific expression datasets.

Discriminating Tests

- **Selective ribosome profiling (SeRP)** on FLAG-Ssb1-only vs FLAG-Ssb2-only strains (each in *ssb1Δ ssb2Δ* background) → directly tests differential nascent-chain substrate spectra.
- **Reciprocal complementation:** does *SSB2* fully rescue *ssb1Δ* phenotypes and vice versa across stress panels? Full rescue = redundancy (refutes); a specific non-complemented phenotype = paralog-specific (would support).
- **In vitro peptide-binding / ATPase kinetics** of purified Ssb1 vs Ssb2 (and site-swap mutants at 49/413/435/436) → isolates the causal contribution of the 4 residues.

- **Cys435 redox probe:** test thiol-dependent behavior unique to Ssb1.

Curation Leads (verify before applying)

- **Lead 1 — Reject paralog-specific functional annotation.** No primary evidence supports a distinct Ssb1 substrate preference or mechanism; treat as over-annotation.
- **Lead 2 — Candidate references to cite for shared function:**

  P 23332755
  (SSB cotranslational substrate specificity, as one entity),

  P 1394434
  (Ssb1/2p ribosome association),

  / P 35701497
  37081320 (RAC–Ssb cotranslational folding mechanism).
- Snippet to verify (P 23332755):
  "we use a sensitive and global approach to define the cotranslational substrate specificity of the yeast Hsp70 SSB."
- Snippet to verify (P 1394434):
  "The SSB hsp70s (Ssb1/2p) are associated with translating ribosomes."
- **Lead 3 — Suggested curator question:** Is any existing Ssb1-specific term backed by an experiment using an Ssb1-only reagent/strain, or is it inferred from the shared "Ssb" literature? If the latter, generalize the term to reflect shared function.
- **Lead 4 — Suggested experiment (if lab-backed curation desired):** paralog-swap SeRP (see Discriminating Tests).

Confidence

High confidence that the hypothesis is not *demonstrated* (verdict: refuted-as-stated / over-annotation). Moderate residual uncertainty that a subtle, untested paralog difference exists — relevant only if future experiments provide it; it should not drive current curation.
