rpoC (β′ subunit of RNA polymerase) in *Pseudomonas putida* KT2440 — Functional Annotation Report

Gene: rpoC | **Ordered locus:** PP_0448 | **UniProt:** Q88QP1 **Organism:** *Pseudomonas putida* (strain ATCC 47054 / DSM 6125 / NCIMB 11950 / KT2440), abbreviated PSEPK **Enzyme:** DNA-directed RNA polymerase subunit β′ (RNAP β′) | **EC 2.7.7.6**

Summary

rpoC encodes the β′ subunit, the largest (1,399 aa; ~154.8 kDa) and catalytic subunit of the multisubunit DNA-dependent RNA polymerase (RNAP) of *Pseudomonas putida* KT2440. Its primary function is template-directed synthesis of RNA (EC 2.7.7.6): using a DNA template and the four ribonucleoside triphosphates (NTPs) as substrates, it catalyzes the reaction RNA(n) + NTP → RNA(n+1) + diphosphate (Rhea:21248). β′ carries the catalytic center of the enzyme: it chelates the essential catalytic Mg^{2+} ion through an absolutely conserved aspartate-triad motif (**NADFDGD**), which in the Q88QP1 sequence lies at residues 458–464, placing the metal-coordinating aspartates at positions 460, 462, and 464. The subunit also binds two structural Zn^{2+} ions. Because *P. putida* has a single bacterial RNAP, rpoC is essential and non-redundant: every mRNA, rRNA, and tRNA in the cell is made by an enzyme containing this subunit.

Structurally, β′ is far more than a catalytic scaffold — it is the organizing and regulatory hub of the enzyme. It forms one pincer of the "crab-claw"-shaped RNAP and builds the mobile **clamp** that opens to load DNA and closes to confer the high stability of initiation complexes and the processivity of elongation complexes. The β′ **switch regions** couple clamp motion to promoter DNA opening; the β′ **secondary channel** is the conduit for incoming NTP substrates and the docking port for regulatory factors (GreA/GreB, DksA) and the stringent-response alarmone (p)ppGpp; and the β′ **clamp-helices domain** is the universal platform onto which σ factors dock during initiation and NusG/Spt5 dock during elongation. β′ is the last subunit recruited during core assembly, a step chaperoned by the ω subunit.

The enzyme functions in the **cytoplasm, associated with the nucleoid**, and concentrates into RNAP condensates at sites of high ribosomal-RNA synthesis. In *P. putida* KT2440 specifically, the single β′-containing core partners with **24 σ factors** (19 of them extracytoplasmic-function, ECF, σ factors) to generate a large repertoire of promoter-specific holoenzymes that drive this metabolically versatile soil bacterium's adaptation to solvents, iron limitation, oxidative stress, and stationary-phase transitions. β′ is thus both the catalytic engine and the master regulatory node of gene expression in this organism, and its clamp, switch, and secondary-channel elements are validated antibiotic targets.

Gene/Protein Identity Verification

Before reporting function, the target identity was rigorously confirmed. **The gene symbol "rpoC" matches the UniProt protein description exactly**, and all literature located is consistent with the correct gene, organism, and family:

Criterion	UniProt reference	Verification result
Gene symbol	rpoC	✓ Matches — rpoC universally denotes RNAP β′ across bacteria
Protein	DNA-directed RNA polymerase subunit β′ (EC 2.7.7.6)	✓ Confirmed by sequence, family, and catalytic motif
Organism	*P. putida* KT2440 (PSEPK)	✓ PP_0448 is the KT2440 ordered locus for rpoC
Family	RNA polymerase β′ chain family	✓ IPR000722, IPR012754 present
Catalytic motif	β′ aspartate triad	✓ NADFDGD found at residues 458–464 in Q88QP1
Operon context	—	✓ rpoB (Q88QP2, PP_0447) immediately upstream (rpoBC operon)

The gene symbol is **not** ambiguous in this case: rpoC is one of the most conserved and well-annotated genes in all of biology. Much of the deep mechanistic literature comes from model systems (*Escherichia coli*, *Thermus aquaticus*, *Bacillus subtilis*, *Salmonella*), but the extraordinary sequence and structural conservation of β′ — high enough that the *E. coli* core structure was interpreted directly from the *T. aquaticus* crystal model PMID: 11904365 — makes these findings directly transferable to the *P. putida* orthologue. Organism-specific findings are drawn from KT2440 studies where available.

Key Findings

F001 / F011 — β′ is the catalytic subunit carrying the Mg^{2+} active site, encoded in the rpoBC operon

Q88QP1 is a 1,399-amino-acid, ~154.8-kDa polypeptide catalyzing the core reaction of transcription (EC 2.7.7.6): **RNA(n) + a ribonucleoside triphosphate → RNA(n+1) + diphosphate** (Rhea:21248). The defining catalytic feature is the **absolutely conserved aspartate-triad motif NADFDGD**. Computational re-verification of the Q88QP1 sequence localized this motif to residues 458–464, positioning the three Mg^{2+}-chelating aspartates at **positions 460, 462, and 464**. These aspartates coordinate the catalytic Mg^{2+} ion that performs the two-metal-ion nucleotidyl-transfer reaction. The subunit additionally binds two structural Zn^{2+} ions. Annotation is governed by HAMAP-Rule MF_01322, and the protein belongs to the RNA polymerase β′ chain family (IPR000722, IPR012754).

The gene sits in the classic bacterial **rpoBC operon**: UniProt locus-tag adjacency confirms that rpoB (Q88QP2 = PP_0447, encoding the β subunit) lies immediately upstream of rpoC (Q88QP1 = PP_0448, β′). This gene arrangement — the two large catalytic subunits co-transcribed — is conserved across bacteria and ensures stoichiometric co-production of the β/β′ catalytic pair.

F002 — β′ forms the "crab-claw" active-center wall and houses the catalytic Mg^{2+}

The 3.3 Å crystal structure of *Thermus aquaticus* core RNAP (Zhang et al., *Cell* 1999) revealed a **"crab claw"-shaped molecule with a 27 Å-wide internal channel**; a catalytic Mg^{2+} ion sits on the back wall of the channel, chelated by an absolutely conserved motif — the β′ NADFDGD aspartate triad — that is conserved in **all bacterial and eukaryotic cellular RNA polymerases** PMID: 10499798. β′, the largest subunit, forms one pincer of the claw and the mobile clamp. The conservation is so high that the *E. coli* core enzyme structure could be interpreted directly from the *T. aquaticus* model PMID: 11904365. This establishes that the *P. putida* β′ likewise builds the active-center wall and holds the catalytic metal.

F005 — β′ builds the secondary channel: the NTP-substrate conduit and regulator docking site

The RNAP **secondary channel** — a β′-lined pore — is the route by which incoming NTP substrates reach the catalytic center and through which the 3′ RNA end threads during backtracking. It also accepts the transcript-cleavage factors **GreA/GreB** PMID: 15200953. In proteobacteria (which include *P. putida*), the stringent-response alarmones **(p)ppGpp bind two RNAP sites**: site 1 at the interface of the β′ and ω subunits, and site 2 at the interface of the β′ secondary channel and the transcription factor **DksA** PMID: 30200857; this two-site architecture is corroborated in *Azotobacter* PMID: 38574051. Gre factors entering the β′ secondary channel improve elongation fidelity and resolve transcriptional pauses in metabolic-gene transcription during oxidative stress PMID: 37014914. Thus β′ is not only catalytic but the physical gateway through which substrate entry and the most important stress-signaling regulators act.

F003 — The β′ mobile clamp confers initiation-complex stability and elongation processivity

Single-molecule FRET studies show that the β′-formed **clamp is open in free RNAP and early initiation intermediates, and closes upon formation of a catalytically competent initiation complex, remaining closed through initial transcription and elongation**. Clamp closure "accounts for the high stability of initiation complexes and the high stability and processivity of elongation complexes," while clamp opening allows DNA loading and unwinding in the active-center cleft PMID: 22859489. The clamp interconverts dynamically among three conformational states on 0.1–1 s timescales, and the alarmone **ppGpp selectively stabilizes a partly-closed-clamp state**, thereby modulating promoter opening PMID: 29878276. Clamp dynamics are therefore a central regulatory lever on the transcription cycle.

F009 — β′ switch regions couple clamp motion to promoter DNA opening and are validated antibiotic targets

The RNAP **switch regions** are the hinges connecting the mobile clamp to the enzyme body; they control the clamp conformational change required for open-promoter-complex formation and template-DNA loading. The natural-product antibiotic **myxopyronin** binds the switch regions to block these rearrangements PMID: 22965125. X-ray cocrystal structures of *E. coli* RNAP with **squaramide** inhibitors show displacement of switch 2, "predicted to interfere with the conformational change of the clamp domain and/or with binding of template DNA, a mechanism akin to that of natural product myxopyronin" PMID: 25798859. β′ additionally forms the secondary-channel target of the peptide antibiotic **microcin J25** PMID: 15200953. These findings establish β′ conformational machinery as a druggable surface.

F010 — The β′ clamp-helices domain is the universal docking hub for σ (initiation) and NusG/Spt5 (elongation) factors

Among the core RNAP elements contacting downstream duplex DNA that stabilize initiation and persist into elongation, "**the β′ clamp domain plays the most prominent role**" PMID: 27956639. The universally conserved **NusG/Spt5** elongation factors "bind to the conserved clamp helices domain of RNA polymerase, which also interacts with non-homologous initiation factors in all domains of life, and reach across the DNA channel to form processivity clamps that enable uninterrupted RNA chain synthesis" PMID: 25640595. Cryo-EM shows the NusG N-terminal domain binding the β′ clamp helices (together with the β protrusion/lobe), constraining the clamp and enhancing processivity PMID: 27899640; key β′ clamp-helix residues R270/R278/R281 form the NusG interface, itself a validated antimicrobial target PMID: 39196895. Rho-dependent termination requires opening of the β′ clamp and engages the β′ Zn-binding domain/clamp helices PMID: 33243850, PMID: 39730149. This mutually exclusive docking on a single β′ surface is what makes the clamp helices the master switchboard of the transcription cycle. A separate, earlier study mapping the σ^{70}-binding site to β′ residues 260–309 (a coiled-coil, equivalent to the clamp-helices region) reinforces this: point mutations there (R275Q, E295K, A302D) disrupt holoenzyme formation and are lethal PMID: 10764785.

F006 — β′ is the last subunit recruited during RNAP assembly; ω chaperones its folding

The bacterial core enzyme is $\alpha_2\beta\beta'\omega$. The smallest subunit, ω, "has a structural role in maintaining the conformation of the largest subunit, β′, and its recruitment in the enzyme assembly" PMID: 33238579. ω is universally conserved, with orthologues even in obligate intracellular bacteria PMID: 36719197. The canonical *E. coli* assembly order is $\alpha \rightarrow \alpha_2 \rightarrow \alpha_2\beta \rightarrow \alpha_2\beta\beta' \rightarrow$ holoenzyme, with β′ added after the β subunit; a noncanonical branch in *B. subtilis* allows the α-dimer to associate with either β or β′ before core formation PMID: 42001948. β′ is therefore the capstone subunit whose correct incorporation completes the catalytically competent core.

F007 — Localization: cytoplasm, on the nucleoid, in rRNA-synthesis condensates

As the core transcription enzyme acting directly on chromosomal DNA, RNAP (and thus β′) is a **cytoplasmic, nucleoid-associated enzyme**. Bacterial RNAP condensates colocalize with pre-rRNA synthesis, assemble in proportion to high rRNA synthesis, and dissolve upon nutritional stress; the ω subunit (which contributes to (p)ppGpp binding site 1 with β′) is critical for condensate maintenance during acid stress, and condensate persistence correlates with survival during acid-stress recovery PMID: 41959101. This positions β′-containing RNAP conceptually close to the eukaryotic nucleolus in its spatial organization.

F008 — In *P. putida* KT2440, the single β′-containing core partners with 24 σ factors for environmental adaptation

Genome analysis of KT2440 identified **24 σ factors, 19 of them ECF σ factors, with 13 FecI-like (iron-acquisition) σ factors** PMID: 12534467. A genome-wide microarray study of the KT2440 transcriptional machinery examined the RNAP core-subunit genes and the 24 σ factors, showing that expression of the RNAP core and the vegetative σ decreased in stationary phase while σ^S (RpoS) increased PMID: 16343331. Specific ECF σ factors reprogram the β′-containing holoenzyme to control physiology: **RpoT/ECF-Pp12** governs solvent (toluene) tolerance and efflux-pump expression PMID: 17071759, and **ECF-10** controls stress resistance, multidrug efflux (TtgABC), and biofilm formation PMID: 24907323. The single, essential β′ subunit is the common core onto which all these regulatory σ factors dock (via the clamp-helices platform of F010) to steer this versatile soil bacterium's adaptation.

F004 — Organism context: essential housekeeping subunit of an industrial chassis

PP_0448 = rpoC is the single-copy β′ subunit of the sole bacterial RNAP in *P. putida* KT2440, a "versatile soil bacterium" and emerging industrial cell factory that uses the Entner–Doudoroff pathway for glucose metabolism PMID: 39837196. As the catalytic subunit responsible for all mRNA/rRNA/tRNA synthesis, rpoC is essential; genome-scale omics and fitness studies treat the core transcription machinery as essential housekeeping genes PMID: 36853682, PMID: 32826213.

Mechanistic Model / Interpretation

The dual identity of β′: catalytic engine + regulatory switchboard

β′ can be understood as two integrated machines built into one polypeptide. First, it is the **catalytic engine**: its NADFDGD aspartate triad (residues 460/462/464 in Q88QP1) holds the Mg^{2+} that performs nucleotidyl transfer at the back wall of the crab-claw channel. Second, it is the **regulatory switchboard**: its clamp, switch regions, secondary channel, and clamp-helices are the moving parts and docking surfaces through which the entire transcription cycle is controlled.

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                  P. putida RNA polymerase core (a2 b b' w)
                  ============= "crab claw" =============

                          b' CLAMP  (mobile pincer)
                         /  \    <- opens to load DNA
     s / NusG dock ->  CLAMP HELICES   -> closes for processivity
                         \  /
      downstream DNA  ---+          SWITCH REGIONS (hinges)
      contacts (b')  ----|          <- myxopyronin, squaramides bind
                         |
           +-------------+------------+
           |   ACTIVE CENTER          |
           |   Mg2+ <= NADFDGD        |  <- catalysis
           |   (D460/D462/D464)       |
           +-------------+------------+
                         |
                  SECONDARY CHANNEL (b'-lined)
                  <- NTP substrate entry
                  <- GreA/GreB (cleavage/fidelity)
                  <- DksA + (p)ppGpp site 2 (stringent response)
                  <- microcin J25 (antibiotic)

    (p)ppGpp site 1  =  b'-w interface
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How the parts work together across the transcription cycle

Phase	β′ element in action	Consequence
Assembly	β′ recruited last; ω chaperones its fold	Completes catalytically competent core
Holoenzyme formation	Clamp-helices (~res 260–309) bind σ factor	Promoter specificity conferred (24 σ options in KT2440)
Promoter opening	Switch regions transmit clamp closure	Open complex forms; DNA loaded/ melted
Initiation → elongation	Clamp closes and stays closed	Stability + processivity
Substrate loading	NTPs enter via secondary channel	Nucleotide addition at Mg^{2+} center
Fidelity / pausing	GreA/GreB enter secondary channel	Proofreading, pause resolution
Stress signaling	(p)ppGpp at β′–ω (site 1) + DksA/β′ channel (site 2)	Stringent response reprograms transcription
Elongation control	NusG/Spt5 bind clamp-helices	Enhanced processivity, coupling
Termination	Rho opens β′ clamp; engages β′ Zn-domain	Enzyme inactivation, transcript release

Why this matters for *P. putida* biology

Because *P. putida* KT2440 possesses only one RNAP core but 24 σ factors, β′ is the physical convergence point of the organism's regulatory logic. Environmental cues — solvent stress (RpoT), iron limitation (13 FecI-like ECF σ factors), general stress/stationary phase (RpoS), oxidative stress (Gre/stringent response) — all ultimately act by loading a particular σ or regulator onto the same β′ clamp-helices/secondary-channel surfaces. This makes β′ simultaneously the most conserved housekeeping enzyme and the master integrator of adaptive gene expression in this industrially important chassis.

Evidence Base

PMID	Study (system)	Supports finding	Key contribution
10499798	*T. aquaticus* core RNAP crystal, 3.3 Å	F002	Crab-claw shape; catalytic Mg^{2+} chelated by conserved β′ motif
11904365	*E. coli* cryo-EM	F002	β′ conservation lets Taq model interpret *E. coli*; clamp flexibility
22859489	smFRET clamp (*E. coli*)	F003	Clamp open→closed transition = stability + processivity
29878276	smFRET clamp	F003	Three clamp states; ppGpp stabilizes partly-closed clamp
15200953	Microcin J25 mechanism	F005, F009	Secondary channel = NTP/Gre route; antibiotic target
30200857	Review (ppGpp/DksA)	F005	Two (p)ppGpp sites: β′–ω and β′ channel/DksA
38574051	*Azotobacter* stringent response	F005	Corroborates two-site (p)ppGpp architecture
37014914	*Salmonella* Gre factors	F005	Gre in β′ channel improves metabolic-gene fidelity
22965125	Myxopyronin / switch regions	F009	Switch regions control clamp/open-complex; drug target
25798859	Squaramide cocrystals (*E. coli*)	F009	Switch-2 displacement blocks clamp/DNA loading
27956639	Gate loop / clamp study	F010	β′ clamp "most prominent" downstream-DNA contact
25640595	NusG/Spt5 review	F010	Clamp-helices = universal σ + NusG docking hub
27899640	NusG–RNAP cryo-EM	F010	NGN binds β′ clamp helices, constrains clamp
39196895	NusG–CH inhibitors	F010	β′ R270/R278/R281 = NusG interface, drug target

PMID	Study (system)	Supports finding	Key contribution
33243850	Rho termination cryo-EM	F010	Clamp opening + β′ Zn-domain in termination
39730149	Rho genetic network	F010	β′ Zn-finger/clamp helices functionally in termination
10764785	β′260–309 σ^{70} mutants	F010	β′ coiled-coil is σ-binding site; mutations lethal
33238579	ω subunit review	F006	ω maintains β′ conformation, aids β′ recruitment
36719197	ω in intracellular bacteria	F006	ω universally conserved
42001948	*B. subtilis* assembly	F006	β′ added after β; noncanonical branch exists
41959101	RNAP condensates	F007	RNAP condensates at rRNA sites; ω-dependent under acid stress
12534467	KT2440 σ-factor genome analysis	F008	24 σ factors (19 ECF, 13 FecI-like)
16343331	KT2440 microarray	F008	RNAP core + σ expression across growth phases
17071759	RpoT regulon (*P. putida*)	F008	ECF σ RpoT controls solvent tolerance/efflux
24907323	ECF-10 knockout (KT2440)	F008	ECF σ controls efflux/biofilm/stress
39837196	Industrial chassis review	F004	KT2440 as versatile industrial platform

Supporting mechanistic detail on the nucleotide-addition cycle and active-site dynamics (trigger loop, bridge helix, F loop) further underpins the catalytic model: PMID: 19855007, PMID: 17679091, PMID: 19481445, and PMID: 23283976 (active-center "tuning" and the two-Mg^{2+} mechanism, with Gre-factor acceleration of intrinsic cleavage 3,000–4,000-fold).

Limitations and Knowledge Gaps

1. **Species of experimental evidence.** The deep structural and mechanistic data derive from model organisms (*T. aquaticus*, *E. coli*, *B. subtilis*, *Salmonella*, *Azotobacter*), not from *P. putida* β′ directly. The inference to Q88QP1 rests on the extreme conservation of β′ (the catalytic motif and clamp/switch/secondary-channel architecture are universal), which is very strong but remains inference for the KT2440 orthologue specifically.
2. **No *P. putida* β′ structure.** There is no experimentally determined structure of the *P. putida* KT2440 RNAP reported here; residue-level annotations (e.g., D460/462/464, clamp-helix residues) are mapped by homology/sequence to Q88QP1 rather than solved crystallographically for this protein.
3. **σ-factor pairing specifics.** While 24 σ factors are documented for KT2440, the precise β′ contact residues for each ECF σ and the quantitative promoter repertoires are not fully resolved for most of the 19 ECF σ factors.
4. **Essentiality is inferred, not directly demonstrated here.** rpoC essentiality is treated as established housekeeping biology and is consistent with genome-scale fitness data, but a clean conditional-knockout demonstration in KT2440 was not located in this investigation.
5. **Stringent-response nuances.** The (p)ppGpp/DksA two-site model is well established in proteobacteria generally; some downstream effects (e.g., polyphosphate, PHB synthesis) show that DksA and (p)ppGpp can act via distinct routes PMID: 30745375, PMID: 38574051, so the precise regulon in *P. putida* would need dedicated study.

Proposed Follow-up Experiments / Actions

1. **Cryo-EM structure of *P. putida* KT2440 RNAP** (core and representative ECF-σ holoenzymes) to confirm the β′ active-center geometry, clamp-helix docking residues, and any *Pseudomonas*-specific insertions.
2. **Site-directed mutagenesis of D460/D462/D464** (or conditional depletion) to experimentally validate the catalytic aspartate triad and quantify the effect on RNA synthesis in KT2440.
3. **ChIP-seq / genome-wide occupancy of β′-containing holoenzymes** paired with each of the 24 σ factors to map the promoter repertoires and directly link β′ to the adaptive regulons (solvent tolerance, iron acquisition, biofilm).

4. **Stringent-response profiling** (relA/spoT and dksA mutants) with transcriptomics to define the *P. putida* (p)ppGpp/DksA regulon acting through the β′ secondary channel and β′–ω interface.
5. **Antibiotic susceptibility mapping** targeting the β′ switch regions (myxopyronin/squaramide class), secondary channel (microcin J25), and clamp-helix–NusG interface, given the intrinsic multidrug-efflux phenotypes of *P. putida* — to assess whether transcription inhibitors bypass efflux-based resistance.
6. **In vivo imaging of RNAP condensates in KT2440** under nutrient shift and acid/solvent stress to test whether the ω/β′-dependent condensate behavior observed in other bacteria governs stress survival in this chassis.

Conclusion

rpoC (PP_0448; Q88QP1) encodes the **β′ subunit** — the largest, catalytic subunit of the single essential DNA-dependent RNA polymerase of *Pseudomonas putida* KT2440. It catalyzes template-directed RNA synthesis (EC 2.7.7.6) through a Mg^{2+}-dependent, two-metal-ion mechanism anchored by the absolutely conserved NADFDGD aspartate triad (D460/462/464). Beyond catalysis, β′ forms the crab-claw active-center wall, the mobile clamp and switch regions that load DNA and confer processivity, the secondary channel that admits NTP substrates and stringent-response regulators, and the clamp-helices platform that universally docks σ factors (initiation) and NusG/Spt5 (elongation). It functions in the cytoplasm on the nucleoid, concentrating in rRNA-synthesis condensates, and in KT2440 it is the common core for 24 σ factors that reprogram transcription for environmental adaptation. β′ is thus both the catalytic engine and the master regulatory hub of gene expression in this organism, and a validated antibiotic target.
