

Tissue-specific expression and post-translational modifications of plant- and bacterial-type phosphoenolpyruvate carboxylase isozymes of the castor oil plant, *Ricinus communis* L.

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Supplemental Fig. S1. Sequence alignment of cDNA-deduced primary structures of castor PTPC (RcPPC1 and RcPPC3) and BTPC (RcPPC4) isozymes. Experimentally verified *in vivo* phosphorylation (Ser-11 and Ser-425 of RcPPC3 and RcPPC4, respectively) and monoubiquitination (Lys-628 of RcPPC3) sites are indicated with arrows and a bold/red font. RcPPC4's highly divergent ~10-kDa domain that was predicted to exist largely as an intrinsically disordered region (O'Leary et al., 2011) is enclosed in a red rectangle. Boxes I-III denote conserved subdomains essential for PEPC catalysis (Izui et al., 2004). The predicted M_r of each deduced polypeptide and their % sequence identity with RcPPC1 is also indicated. The corresponding NCBI protein accession numbers are as follows: RcPPC1 (PTPC), ABR29877.1; RcPPC3 (PTPC), ABR29876; RcPPC4 (BTPC) ABR29876.1. Semi-colons and asterisks indicate identical and conserved amino acids respectively. Sequences were aligned using the Clustal W software (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>).



RcPPC1 RSLCACGDRPIADGSLLDLFLRQVSTFGLSLVRDLRQESERHTDVLDAITKHLGIGFYRE 474
RcPPC3 RSLCSCGDQPIADGSLLDLFLRQVSTFGFSLVRDLRQESDRHTDVMdTITKHLIIGSYRE 474
RcPPC4 ESLQSCGAGVLADGRLADLIRRVATFGMVLMLKLDLRQESGRHADTLDAITKYLEMGTYSE 578
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RcPPC1 WSEEHRQEWLLTELGRKRPLFGPDLPKTDEIADVLDTFHVIAELPADNFGAYIISMATAP 534
RcPPC3 WSEERRQEWLLSELSGKRPLFGPDLQRTDEVADVLDTFHVIAELPADSFGAYIISMATAP 534
RcPPC4 WDEEKKLEFLTRELGKRPLVPPTIEVAPDVKEVLDAFRVAEELGSDSLGAYVISMASNA 638
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RcPPC1 SDVLAVELLQRECRVKQP-----LRVVPLFEKLADLEAAPAAVARLFSIDWYRN 583
RcPPC3 SDVLAVELLQRECHVKQP-----LRVVPLFEKLADLEAAPAALARLFSIDWYRN 583
RcPPC4 SDVLAVELLQKDARLAVSGELGRPCPGGTLRVVPLFETVKDLRGAGSVIRKLLSIDWYRE 698
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II ↓ III

RcPPC1 ----RINGKQEVMI GYSDSGKDAGRLSAAWQLYKAQEELVKVAKQYGVKLTMFHGRGGTV 639
RcPPC3 ----RINGKQEVMI GYSDSGKDAGRFSAAWQLYKAQEELIKVAKQFGV KLTMFHGRGGTV 639
RcPPC4 HIIKNHNGHQEVMV GYSDSGKDAGRFTAAWELYKAQEDVVAACNDFGIKVTLFHGRGGS 758
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RcPPC1 GRGGGPTHLAISLQPPDTIHGSLRVTVQGEVIEQSFGEELCFRTLQRFATAATLEHGMP 699
RcPPC3 GRGGGPTHLAISLQPPDTIHGSLRVTVQGEVNEQSCGEEHLCFRTLQRFATAATLEHGMP 699
RcPPC4 GRGGGPTYLAIQSPPGSMVMTLRSTEQGEMVQAKFGLPHTAIRQLEIYTTAVLLATLRP 818
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RcPPC1 PVSPKPE-WRTLLDEMAYIATKEYRSIVFQEPREFVEYFRLATPELEYGRMNIGSRPSKRK 758
RcPPC3 PVSPKPE-WRKLMDEMAYIATEEYRSIVFKEPREFVEYFRLATPELEYGRMNIGSRPSKRK 758
RcPPC4 PHPPREEQWRNVMEIEISKISQNYRSTVYENPEFLAYFHEATPQAEELGFLNIGSRPTRRK 878
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RcPPC1 PGGIESLRIPWIFAWTQTRFHLPVWLGFGPAFKHVIKDVRLHMLQEMYNQWPPFRV 818
RcPPC3 PGGIESLRIPWIFAWTQTRFHLPVWLGFGAAFKHVIQKDVRLHMLQEMYNEWPPFRV 818
RcPPC4 SSTGIGHLRIPWVFAWTQTRFVLPWLVGAGLKGACEKGF--EDLKAMYKEWPPFFQS 936
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RcPPC1 TIDLVMVFAKGDPGIAALYDKLLVSEELWPFGERLRVNYEETKHLLLQVAGHKDLLEGD 878
RcPPC3 TIDLVMVFAKGDPGIAALYDKLLVSDLWSFGERLRTNYEETKRLLLQIAGHKDLLEGD 878
RcPPC4 TIDLIEMLVGLKADIPIAKHDEVLVSESRRELGAELRSELLTTEKYVLVSGHEKLSQNN 996
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RcPPC1 PYLKQRLRLRDYITTLNVCQAYTLKRIRDPDYHVTLRPHLSKEHMESSKPAELVKLNP 938
RcPPC3 PYLKQRLRLRDSYITTLNVCQAYTLKRIRDPNYVTLRPHISKEIMESSKPADELVKLNP 938
RcPPC4 RSLRRLIESRLPYLNPMMMLQVEVLKRLRRDDNNKLR----- 1034
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		<u>Identity (%)</u>	<u>M_r (kDa)</u>
RcPPC1	RSEYAPGLEDTLILTMKGIAAGMQNTG 965	100	110.5
RcPPC3	KSDYAPGLEDTLILTMKGVAAGLQNTG 965	91	110.6
RcPPC4	-----DALLITINGIAAGMRNTG 1052	41	118.5

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References

Kai Y, Matsumura H, Izui K. 2003. Phosphoenolpyruvate carboxylase: Three-dimensional structure and molecular mechanisms. *Archives of Biochemistry and Biophysics* **414**, 170-179.

O'Leary B, Rao SK, Plaxton WC. 2011. Phosphorylation of a bacterial-type phosphoenolpyruvate carboxylase at serine-425 provides a further tier of enzyme control in developing castor oil seeds. *Biochemical Journal* **433**, 65-74.