TABLE S1
Purification of recombinant RcPPC4 from 16 g of *E. coli*

Step	**Activity**	**Protein**	**Specific Activity**	**Purification**	**Yield**
	units	*mg*	*units/mg*	*-fold*	*%*
Clarified Extract	434	3,999	0.11	1	100
Ni^{2+}-affinity FPLC	241	76	3.2	29	56
Superdex-200 FPLC	27	1.0	27	254	7

SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. **BTPC and PTPC sequence alignment.**

Several deduced PEPC sequences were aligned using the Clustal W software (http://www.ebi.ac.uk/Tools/clustalw2/index.html). Residues with known catalytic and regulatory importance (1-8) are highlighted. The corresponding NCBI protein accession numbers are as follows: Maize-C4, P04711; AtPPC3, AAC24594; RcPPC3, ABR29876; AtPPC4 (BTPC), CAD56727; RcPPC4 (BTPC), ABR29877; *E. coli,* NP_290593.

FIGURE S2. **Susceptibility of recombinant RcPPC4 to proteolysis by endogenous COS proteases or exogenous thrombin.**

A clarified extract from stage VII developing COS was preincubated for 2 h at 25 ^{o}C to allow for complete cleavage of native RcPPC4 p118 subunits by endogenous proteases (7). Recombinant RcPPC4 (30 μg) was then incubated at 25 ^{o}C in 200-μl of this extract (*COS extract*), or with 30 mU of thrombin protease (*Thromb*.) (Novagen) in 50-μl of thrombin reaction buffer (20 mM Tris-HCl, pH 8.4, 150 mM NaCl, 2.5 mM $CaCl_2$). Aliquots taken at the indicated times were boiled in SDS sample buffer and subjected to SDS-PAGE and immunoblotting with anti-(recombinant RcPPC4)-IgG (*A*) or anti-(RcPPC4 peptide)-IgG (*B*) (7). Each lane contains 700 ng of RcPPC4. It has been established that the 90-kDa immunoreactive polypeptide detected on the anti-(RcPPC4 peptide)-IgG immunoblots of clarified COS extracts arises from a non-specific cross-reaction of this IgG with a 90-kDa sucrose synthase subunit, rather than to a BTPC (7).

FIGURE S3. **pH-activity profiles of recombinant PEPC isoforms**.

Activities of recombinant AtPPC3 (●), RcPPC4 (▼), and chimeric Class-2 PEPC (○) were determined using standard assay conditions described under the "Experimental Procedures", except that the PEP concentration was 16 mM and the pH was varied using a mixture of 25 mM Mes and 25 mM Bis-Tris-propane as the buffer. All values in represent the means of three different experiments and reproducible to within ± 10% of the mean value.

REFERENCES

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Supplemental Fig. S1

X – catalytically critical residue
X – hydrophobic PEP binding pocket
X – glucose-6-P binding/interacting residue
X – conserved loops in plant-type PEPC, proximal to glucose-6-P binding site
X – Mg^{2+} binding site
S – phosphorylation site
X – aspartate/malate binding/interacting residue
X –intra-dimer salt bridge \
X – HCO_3 binding base
K – monoubiquitination site

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MaizeC4         MASTKAPGPGEKHHSIDAQLRQLVPGKVSEDDKLIEYDALLVDRFLNILQDLHGPSLREF 60
AtPPC3          ----MAGRNIEKMASIDAQLRQLVPAKVSEDDKLVEYDALLLDRFLDILQDLHGEDLRET 56
RcPPC3          ----MQPRNLEKLASIDAQLRLLVPAKVSEDDKLVEYDALLLDRFLDILQDLHGEDLKET 56
AtPPC4          ------------MTDTTDDIAEEISFQSFEDD-----CKLLGSLFHDVLQREVGNPFMEK 43
RcPPC4          ------------MTDTTDDIAEEISFQSFDDD-----CKLLGNLLNDVLQREVGSKFMEK 43
E. coli         ---------------------MNEQYSALRSN-----VSMLGKVLGETIKDALGEHILER 34
                                          .   .:       :* . : : ::   *  : *

MaizeC4         VQECYEVSADYE-----GKGDTTK--LGELGAKLTGLAPADAILVASSILHMLNLANLAE 113
AtPPC3          VQELYELSAEYE-----GKREPSK--LEELGSVLTSLDPGDSIVISKAFSHMLNLANLAE 109
RcPPC3          VQECYELSAEYE-----GKHDPRK--LDELGNLLTSLDPGDSIVIAKSFSHMLNLANLAE 109
AtPPC4          VERIRILAQSALNLRMAGIEDTANLLEKQLTSEISKMPLEEALTLARTFTHSLNLMGIAD 103
RcPPC4          LERNRILAQSACNMRLAGIEDTAELLEKQLALEISRMTLEEALTLARAFSHYLNLMGIAE 103
E. coli         VETIRKLSKSSR----AGNDANR----QELLTTLQNLSNDELLPVARAFSQFLNLANTAE 86
                ::    :: .       *          :*   :  :   : : ::  :: : *** . *:

MaizeC4         EVQIAHRRRNSKLKKGGFADEGSATTESDIEETLKRLVSEVGKSPEEVFEALKNQTVDLV 173
AtPPC3          EVQIAHRRRIKKLKKGDFVDESSATTESDIEETFKRLVSDLGKSPEEIFDALKNQTVDLV 169
RcPPC3          EVQIAYRRR-NKLKKGDFADENSATTESDIEETFKRLVIDLKKSPEEVFDALKNQTVDLV 168
AtPPC4          THHRMH-KVHNVTQL------------ARSCDDIFSQLLQSGISPDELYKTVCKQEVEIV 150
RcPPC4          THHRVR-KARSMTHL------------SKSCDDIFNQLLQSGISAEELYDTVCKQEVEIV 150
E. coli         QYHSISPKGEAASNP------------EVIARTLRKLKNQPELSEDTIKKAVESLSLELV 134
                  :    :     :                   :     :   * : : .:: .  :::*

MaizeC4         FTAHPTQSARRSLLQKNARIRNCLTQLNAKDITDDDKQELDEALQREIQAAFRTDEIRRA 233
AtPPC3          LTAHPTQSVRRSLLQKHGRIRDCLAQLYAKDITPDDKQELDESLQREIQAAFRTDEIRRT 229
RcPPC3          LTAHPTQSIRRSLLQKHARIRNCLAQLYAKDITPDDKQELDEALQREIQAAFRTDEIRRT 228
AtPPC4          LTAHPTQINRRTLQYKHIRIAHLLEYNTRSDLSVEDRETLIEDLVREITSLWQTDELRRQ 210
RcPPC4          LTAHPTQINRRTLQYKHIRIAHLLDYNDRPDLTHEDREMLIEDLVREITSIWQTDELRRH 210
E. coli         LTAHPTEITRRTLIHKMVEVNACLKQLDNKDIADYEHNQLMRRLRQLIAQSWHTDEIRKL 194
                :*****:  **:*  *  .:   *      *::  ::: * . * : *   ::***:*:

MaizeC4         QPTPQAEMRYGMSYIHETVWKGVPKFLRRVDTALKNIGINERLPYNVSLIRFSSWMGGDR 293
AtPPC3          PPTPQDEMRAGMSYFHETIWKGVPKFLRRVDTALKNIGIDERVPYNAPLIQFSSWMGGDR 289
RcPPC3          APTPQDEMRAGMSYFHETIWKGVPKFLRRVDTALKNIGINERVPYNAPLIQFSSWMGGDR 288
AtPPC4          KPTPVDEARAGLNIVEQSLWKAVPQYLRRVSNSLKKF-TGKPLPLTCTPMKFGSWMGGDR 269
RcPPC4          KPTPVDEARAGLNIVEQSLWKALPHYLRRVSTALKKH-TGKPLPLTCTPIRFGSWMGGDR 269
E. coli         RPSPVDEAKWGFAVVENSLWQGVPNYLRELNEQLEEN-LGYKLPVEFVPVRFTSWMGGDR 253
                 *:*  * : *:  ..:::*:.:*::**.:.  *::   .  :*     ::* *******
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MaizeC4         DGNPRVTPEVTRDVCLLARMMAANLYIDQIEELMFELSMWRCNDELRVRAEELHSSS--- 350
AtPPC3          DGNPRVTPEVTRDVCLLARMMAANLYYNQIENLMFELSMWRCTDEFRVRADELHRNS--- 346
RcPPC3          DGNPRVTPEVTRDVCLLARMMAANLYYSQIEDLMFELSMWRCSDELRVRADELHRSS--- 345
AtPPC4          DGNPNVTAKVTKEVSLLSRWMAIDLYIREVDSLRFELSTDRCSDRFSRLADKILEKD--- 326
RcPPC4          DGNPNVTAKVTRDVSLLSRWMAVDLYIREVDSLRFELSMVQCSDRLLKVANDILIEETSS 329
E. coli         DGNPNVTADITRHVLLLSRWKATDLFLKDIQVLVSELSMVEATPELLALVG--------- 304
                ****.**..:*:.* **:*  * :*:  ::: *  ***  ... .:   .

MaizeC4         ------------------------------------------------------------
AtPPC3          ------------------------------------------------------------
RcPPC3          ------------------------------------------------------------
AtPPC4          ------YDRGKSNFQKQQSSSCLPTQLPARAHLPACIDF--GESRHTKFEIATTDYMPPN 378
RcPPC4          EDHHESWNQPASRSQTKFPRKSLPTQLPPRADLPACTECNDGESQYPKLELPGTDYMPFN 389
E. coli         ------------------------------------------------------------

MaizeC4         ------------------------------------------------------------
AtPPC3          ------------------------------------------------------------
RcPPC3          ------------------------------------------------------------
AtPPC4          LQKQNEQDFSESDWEKIDNGS----------RSGLTSRGSFSSTSQLLLQRKLFEESQVG 428
RcPPC4          RQEALGSSYSESSSQDINHGLPKTTGNGSVANSSGSPRASFSS-AQLVAQRKLFAESKIG 448
E. coli         ------------------------------------------------------------

MaizeC4         GSKVTKYYIEFWKQIPPNEPYRVILGHVRDKLYNTRERARHLLASGVSEISAESSFTSIE 410
AtPPC3          RKDAAKHYIEFWKTIPPTEPYRVILGDVRDKLYHTRERSRQLLSNGISDIPEEATFTNVE 406
RcPPC3          KRDS-KHYIEFWKQVPPSEPYRVILGDLRDKLYQTRERSRQMLSHGNSDIPEEATFTNVE 404
AtPPC4          KTSFQKLLEPPPLKRAGSAPYRIVLGEVKEKLVKTRRLLELLIEGLPCEYDPKNSYETSD 488
RcPPC4          RSSFQKLLEPSLPQRPGIAPYRIVLGNVKDKLMRTRRRLELLLEDLPCEYDQWDYYETTD 508
E. coli         -------------EEGAAEPYRYLMKNLRSRLMATQAWLEARLKGEELPK-PEGLLTQNE 350
                                   *** :: .::.:*  *:   .  :                :

MaizeC4         EFLEPLELCYKSLCDCGDKAIADGSLLDLLRQVFTFGLSLVKLDIRQESERHTDVIDAIT 470
AtPPC3          QFLEPLELCYRSLCSCGDSPIADGSLLDFLRQVSTFGLSLVRLDIRQESERHTDVLDAIT 466
RcPPC3          QFLEPLELCYRSLCSCGDQPIADGSLLDFLRQVSTFGFSLVRLDIRQESDRHTDVMDTIT 464
AtPPC4          QLLEPLLLCYESLQSSGARVLADGRLADLIRRVSTFGMVLVKLDLRQEAARHSEALDAIT 548
RcPPC4          QLLDPLLLCYESLQSCGAGVLADGRLADLIRRVATFGMVLMKLDLRQESGRHADTLDAIT 568
E. coli         ELWEPLYACYQSLQACGMGIIANGDLLDTLRRVKCFGVPLVRIDIRQESTRHTEALGELT 410
                :: :**  **.**  .*   :*:* * * :*:*  **. *:::*:***: **::.:. :*

MaizeC4         THLGIG-SYREWPEDKRQEWLLSELRGKRPLLPPDLPQTDEIADVIGAFHVLAELPPDSF 529
AtPPC3          KHLDIGSSYRDWSEEGRQEWLLAELSGKRPLFGPDLPKTEEISDVLDTFKVISELPSDCF 526
RcPPC3          KHLEIG-SYREWSEERRQEWLLSELSGKRPLFGPDLQRTDEVADVLDTFHVIAELPADSF 523
AtPPC4          TYLDMG-TYSEWDEEKKLEFLTRELKGKRPLVPQCIKVGPDVKEVLDTFRVAAELGSESL 607
RcPPC4          KYLEMG-TYSEWDEEKKLEFLTRELKGKRPLVPPTIEVAPDVKEVLDAFRVAAELGSDSL 627
E. coli         RYLGIG-DYESWSEADKQAFLIRELNSKRPLLPRNWQPSAETREVLDTCQVIAEAPQGSI 469
                 :* :*  * .* *  :  :*  ** .****.        :  :*:.: :* :*    .:

MaizeC4         GPYIISMATAPSDVLAVELLQRECGVRQP-----------LPVVPLFERLADLQSAPASV 578
AtPPC3          GAYIISMATSPSDVLAVELLQRECHVKNP-----------LRVVPLFEKLADLEAAPAAV 575
RcPPC3          GAYIISMATAPSDVLAVELLQRECHVKQP-----------LRVVPLFEKLADLEAAPAAL 572
AtPPC4          GAYVISMASNASDVLAVELLQKDARLALTSEHGKPCPGGTLRVVPLFETVNDLRAAGPSI 667
RcPPC4          GAYVISMASNASDVLAVELLQKDARLAVSGELGRPCPGGTLRVVPLFETVKDLRGAGSVI 687
E. coli         AAYVISMAKTPSDVLAVHLLLKEAGIGFA-----------MPVAPLFETLDDLNNANDVM 518
                ..*:****. .******.** ::. :  .           : *.**** : **. *   :
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MaizeC4         ERLFSVDWYMDRIK----GKQQVMVGYSDSGKDAGRLSAAWQLYRAQEEMAQVAKRYGVK 634
AtPPC3          ARLFSIDWYKNRIN----GKQEVMIGYSDSGKDAGRLSAAWELYKAQEELVKVAKKYGVK 631
RcPPC3          ARLFSIDWYRNRIN----GKQEVMIGYSDSGKDAGRFSAAWQLYKAQEELIKVAKQFGVK 628
AtPPC4          RKLLSIDWYREHIQKNHNGHQEVMVGYSDSGKDAGRFTAAWELYKAQENVVAACNEFGIK 727
RcPPC4          RKLLSIDWYREHIIKNHNGHQEVMVGYSDSGKDAGRFTAAWELYKAQEDVVAACNDFGIK 747
E. coli         TQLLNIDWYRGLIQ----GKQMVMIGYSDSAKDAGVMAASWAQYQAQDALIKTCEKAGIE 574
                 :*:.:***   *     *:* **:*****.**** ::*:*  *:**: :  ..:  *::

                     Mobile Loop I:
MaizeC4         LTLFHGRGGTVGRGGGPTHLAILSQPPDTINGSIRVTVQGEVIEFCFGEEHLCFQTLQRF 694
AtPPC3          LTMFHGRGGTVGRGGGPTHLAILSQPPDTVNGSLRVTVQGEVIEQSFGEAHLCFRTLQRF 691
RcPPC3          LTMFHGRGGTVGRGGGPTHLAILSQPPDTIHGSLRVTVQGEVNEQSCGEEHLCFRTLQRF 688
AtPPC4          ITLFHGRGGSIGRGGGPTYLAIQSQPPGSVMGSLRSTEQGEMVQAKFGIPQTAVRQLEVY 787
RcPPC4          VTLFHGRGGSIGRGGGPTYLAIQSQPPGSVMGTLRSTEQGEMVQAKFGLPHTAIRQLEIY 807
E. coli         LTLFHGRGGSIGRGGAPAHAALLSQPPGSLKGGLRVTEQGEMIRFKYGLPEITVSSLSLY 634
                :*:******::****.*:: *: ****.:: * :* * ***: .   *  .  .  *. :

MaizeC4         TAATLEHGMHPPVSPKP-EWRKLMDEMAVVATEEYRSVVVKEARFVEYFRSATPETEYGR 753
AtPPC3          TAATLEHGMNPPISPKP-EWRALLDEMAVVATEEYRSVVFQEPRFVEYFRLATPELEYGR 750
RcPPC3          TAATLEHGMHPPVSPKP-EWRKLMDEMAVIATEEYRSIVFKEPRFVEYFRLATPELEYGR 747
AtPPC4          TTAVLLATLKPPQPPREEKWRNLMEEISGISCQHYRSTVYENPEFLSYFHEATPQAELGF 847
RcPPC4          TTAVLLATLRPPHPPREEQWRNVMEEISKISCQNYRSTVYENPEFLAYFHEATPQAELGF 867
E. coli         TGAILEANLLPPPEPKE-SWRRIMDELSVISCDVYRGYVRENKDFVPYFRSATPEQELGK 693
                * * *   : **  *:  .** :::*:: :: : **. * ::  *: **: ***: * *

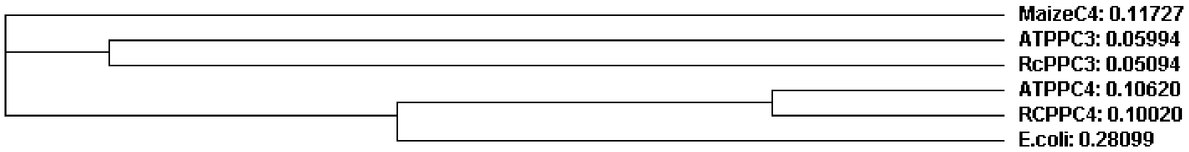
                   Mobile Loop II:        Characteristic Ser of C4 PEPC
MaizeC4         MNIGSRPAKRRPGGGITTLRAIPWIFSWTQTRFHLPVWLGVGAAFKFAIDKDVRNFQVLK 813
AtPPC3          MNIGSRPSKRKPSGGIESLRAIPWIFAWTQTRFHLPVWLGFGAAFRYAIKKDVRNLHMLQ 810
RcPPC3          MNIGSRPSKRKPSGGIESLRAIPWIFAWTQTRFHLPVWLGFGAAFKHVIQKDVRNLHMLQ 807
AtPPC4          LNIGSRPTRRKSSSGIGHLRAIPWVFAWTQTRFVLPAWLGVGAGLKGVSEKGHA--DDLK 905
RcPPC4          LNIGSRPTRRKSSTGIGHLRAIPWVFAWTQTRFVLPAWLGVGAGLKGACEKGFT--EDLK 925
E. coli         LPLGSRPAKRRPTGGVESLRAIPWIFAWTQNRLMLPAWLGAGTALQKVVEDGKQ--SELE 751
                : :****::*:.  *:  ******:*:***.*: **.*** *:.:: . ...      *:

MaizeC4         EMYNEWPFFRVTLDLLEMVFAKGDPGIAGLYDELLVAEELKPFGKQLRDKYVETQQLLLQ 873
AtPPC3          DMYKQWPFFRVTIDLIEMVFAKGDPGIAALYDKLLVSEDLWAFGEKLRANFDETKNLVLQ 870
RcPPC3          EMYNEWPFFRVTIDLVEMVFAKGDPGIAALYDKLLVSQDLWSFGERLRTNYEETKRLLLQ 867
AtPPC4          EMYKEWPFFQSTLELIEMVLAKADIPMTKHYDEQLVSEKRRGLGTELRKELMTTEKYVLV 965
RcPPC4          AMYKEWPFFQSTIDLIEMVLGKADIPIAKHYDEVLVSESRRELGAELRSELLTTEKYVLV 985
E. coli         AMCRDWPFFSTRLGMLEMVFAKADLWLAEYYDQRLVDKALWPLGKELRNLQEEDIKVVLA 811
                 * .:****   : ::***:.*.*  ::  **: ** :    :* .**       . :*

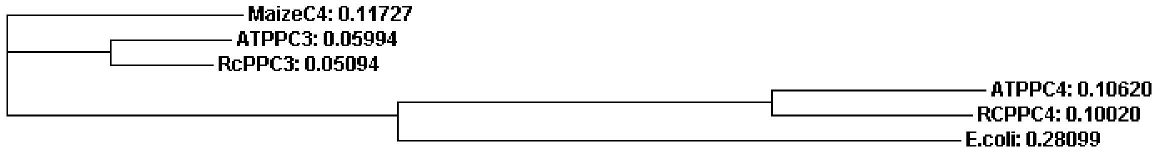
MaizeC4         IAGHKDILEGDPFLKQGLVLRNPYITTLNVFQAYTLKRIRDPNFKVTPQPPLSKEFADEN 933
AtPPC3          TAGHKDLLEGDPYLKQRLRLRDSYITTLNVCQAYTLKRIRDANYNVTLRPHISKEIMQSS 930
RcPPC3          IAGHKDLLEGDPYLKQRLRLRDSYITTLNVCQAYTLKRIRDPNYNVTLRPHISKEIMESS 927
AtPPC4          ISGHEKLLQDNKSLKKLIDSRLPYLNAMNMLQVEILKRLRRDED---------------- 1009
RcPPC4          VSGHEKLSQNNRSLRRLIESRLPYLNPMNMLQVEVLKRLRRDDD---------------- 1029
E. coli         IANDSHLMADLPWIAESIQLRNIYTDPLNVLQAELLHRSRQAEKEGQ------------- 858
                 :....:  .   : . :  *  *  .:*: *.  *:* *  :

MaizeC4         KPAG-LVKLNPASEYPPGLEDTLILTMKGIAAGMQNTG 970
AtPPC3          KSAQELVKLNPTSEYAPGLEDTLILTMKGIAAGLQNTG 968
RcPPC3          KPADELVKLNPKSDYAPGLEDTLILTMKGVAAGLQNTG 965
AtPPC4          ---------------NNKLRDALLITINGIAAGMRNTG 1032
RcPPC4          ---------------NNKLRDALLITINGIAAGMRNTG 1052
E. coli         -------------EPDPRVEQALMVTIAGIAAGMRNTG 883
                                  :.::*::*: *:***::***
```

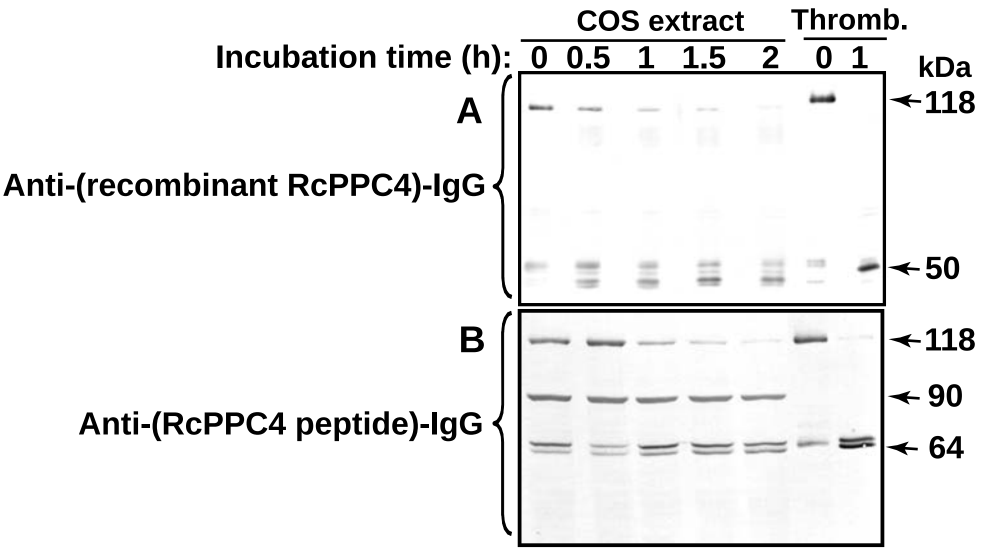
Cladogram



Phylogram



Supplemental Fig. S2



Supplemental Fig. S3