Subunit and Domain Requirements for Adenylate-Mediated Protection of Snf1 Activation Loop from Dephosphorylation*

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Supplementary Figure Legends

FIGURE S1. **HPLC analysis of nucleotides.** Adenylate nucleotides were analyzed for purity on an Altima HP-C18 HPLC column (5µm, 250 mm x 4.6 mm) developed with 50 mM sodium phosphate pH 6.0. ATP, ADP and AMP were clearly resolved when analyzed individually (A-C) or as a mixture of all three (D).

FIGURE S2. **Affinity purified preparations of PP2C and Snf1-TAP do not hydrolyze ADP**. In vitro dephosphorylation reactions containing 1 mM ADP, 20 mM Hepes (pH 7.0), 0.5 mM EDTA, 0.5 mM dithiothreitol and 5 mM magnesium acetate were incubated for 10 minutes at 37°C followed by phenol extraction and HPLC analysis. Reactions contained either no added protein (A), PP2C (B) or Snf1-TAP (C).

FIGURE S3. **Alignment of Snf1 with human AMPK α1.** The alignment was generated with the BLAST program for alignment of two sequences and shows Snf1 resides 42-585 and human α1 residues 14-481. Regions shaded are those deleted from the constructs Snf1-d381-488 and human α1-d377-411.

FIGURE S4. **Alignment of β Subunits.** The C-termini of β subunits from Saccharomyces cerevisiae (Gal83, Sip1 and Sip2) are aligned with the C-termini of β subunits from a variety of species from diverse lineages. The two histidine residues that contact the kinase activation loop in the phosphatase resistant conformation are conserved throughout all species and are marked with asterisks. Abbreviations used are as follows: Hs, Homo sapiens; Xl, Xenopus laevis; Dr, Danio rerio; Dm, Drosophila melanogaster; Ce, Caenorhabditis elegans; Mb, Monosiga brevicollis; Sp, Schizosaccharomyces pombe; At, Arabidopsis thaliana.

FIGURE S1

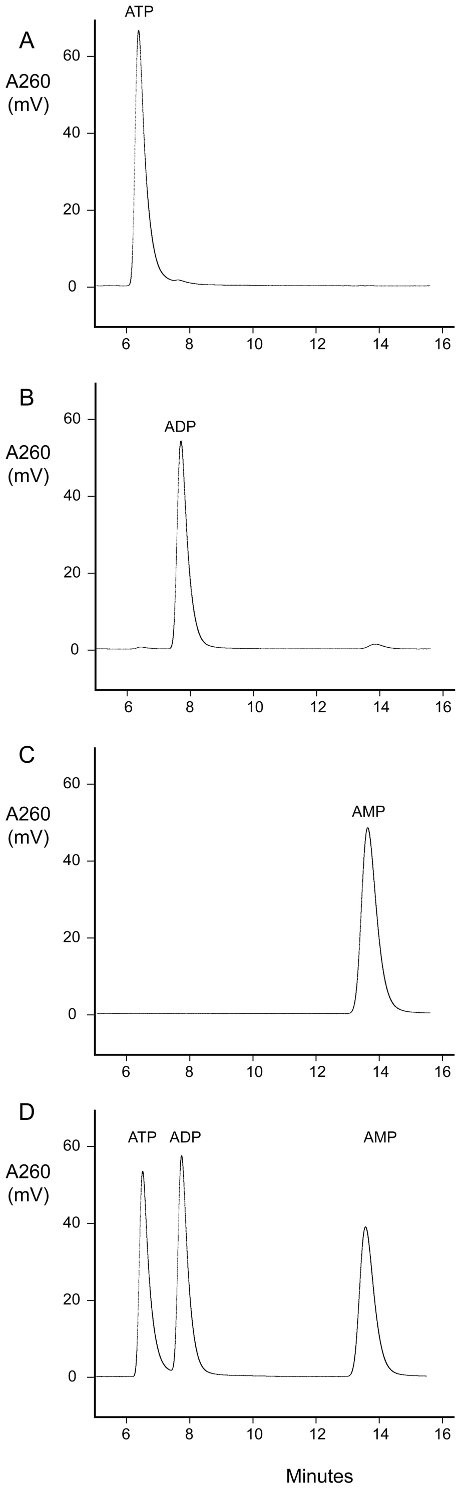
A
ATP
A260
(mV)
60
40
20
0
6
8
10
12
14
16
B
ADP
A260
(mV)
60
40
20
0
6
8
10
12
14
16
C
AMP
A260
(mV)
60
40
20
0
6
8
10
12
14
16
D
ATP
ADP
AMP
A260
(mV)
60
40
20
0
6
8
10
12
14
16
Minutes

FIGURE S2

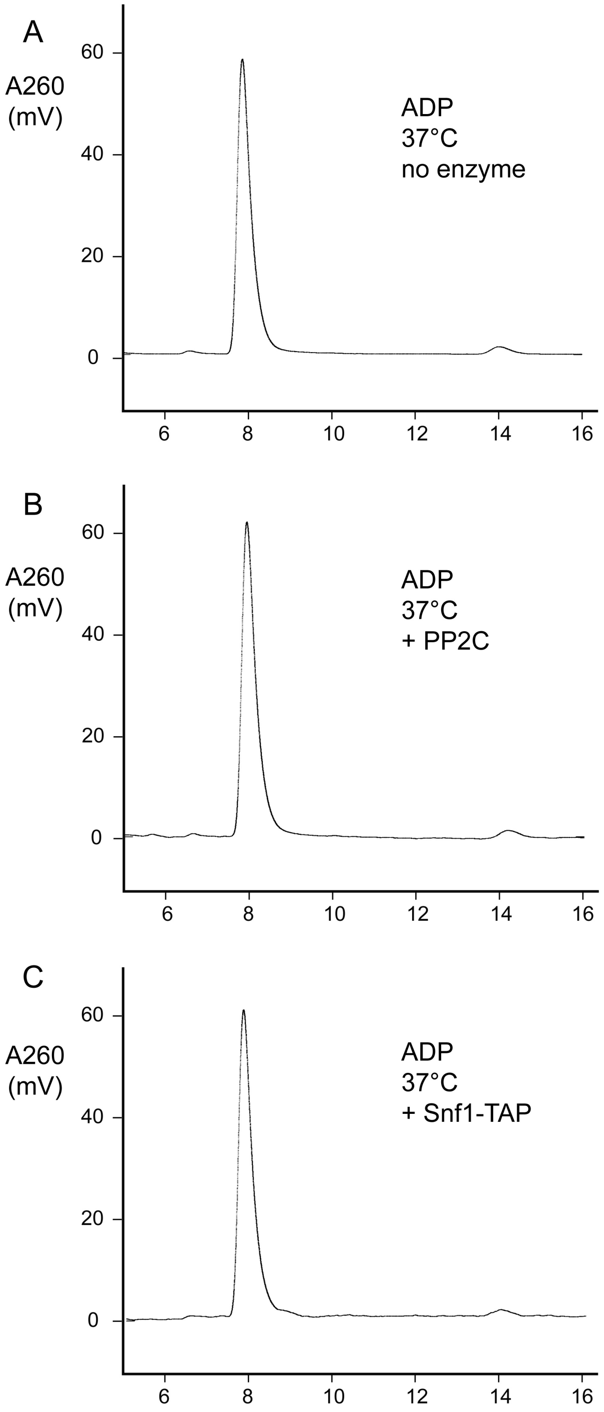
A
A260 (mV)
60
40
20
0
6
8
10
12
14
16
ADP
37°C
no enzyme
B
A260 (mV)
60
40
20
0
6
8
10
12
14
16
ADP
37°C
+ PP2C
C
A260 (mV)
60
40
20
0
6
8
10
12
14
16
ADP
37°C
+ Snf1-TAP

FIGURE S3

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Snf1   42   PKSSLA-----DG-AHIGNYQIVKTLGEGSFGKVKLAYHTTTGQKVALKIINKKVLAKSD  95
            P+ S+A     DG   IG+Y +  TLG G+FGKVK+  H  TG KVA+KI+N++ +   D
Hs a1  14   PRGSMAEKQKHDGRVKIGHYILGDTLGVGTFGKVKVGKHELTGHKVAVKILNRQKIRSLD  73


Snf1   96   MQGRIEREISYLRLLRHPHIIKLYDVIKSKDEIIMVIEY-AGNELFDYIVQRDKMSEQEA  154
            + G+I REI  L+L RHPHIIKLY VI +  +I MV+EY +G ELFDYI +  ++ E+E+
Hs a1  74   VVGKIRREIQNLKLFRHPHIIKLYQVISTPSDIFMVMEYVSGGELFDYICKNGRLDEKES  133


Snf1   155  RRFFQQIISAVEYCHRHKIVHRDLKPENLLLDEHLNVKIADFGLSNIMTDGNFLKTSCGS  214
            RR FQQI+S V+YCHRH +VHRDLKPEN+LLD H+N KIADFGLSN+M+DG FL+TSCGS
Hs a1  134  RRLFQQILSGVDYCHRHMVVHRDLKPENVLLDAHMNAKIADFGLSNMMSDGEFLRTSCGS  193


Snf1   215  PNYAAPEVISGKLYAGPEVDVWSCGVILYVMLCRRLPFDDESIPVLFKNISNGVYTLPKF  274
            PNYAAPEVISG+LYAGPEVD+WS GVILY +LC  LPFDD+ +P LFK I +G++  P++
Hs a1  194  PNYAAPEVISGRLYAGPEVDIWSSGVILYALLCGTLPFDDDHVPTLFKKICDGIFYTPQY  253


Snf1   275  LSPGAAGLIKRMLIVNPLNRISIHEIMQDDWFKVDLPEYLLPPDLKPHPEEENENNDSKK  334
            L+P    L+K ML V+P+ R +I +I + +WFK DLP+YL P D
Hs a1  254  LNPSVISLLKHMLQVDPMKRATIKDIREHEWFKQDLPKYLFPED----------------  297


Snf1   335  DGSSPDNDEIDDNLVNILSSTMGYEKDEIYESLESSEDTPAFNEIRDAYMLIKENKSLIK  394
               S  +  IDD  +  +       ++E+   L +       + +  AY LI +N+ ++
Hs a1  298  --PSYSSTMIDDEALKEVCEKFECSEEEVLSCLYNRNHQ---DPLAVAYHLIIDNRRIMN  352


Snf1   395  DMKANKSVSDELDTFLSQSPP-TFQQQSKSHQKSQVDHETAKQHARRMASAITQQRTYHQ  453
            + K         D +L+ SPP +F             DH   + H  R+
Hs a1  353  EAK---------DFYLATSPPDSFLD----------DHHLTRPHPERV------------  381


Snf1   454  SPFMDQYKEEDSTVSILPTSLPQIHRANMLAQGSPAASKISPLVTKKSKTRWHFGIRSRS  513
             PF+         V+  P +       + L + +P  SK   +     K +WH GIRS+S
Hs a1  382  -PFL---------VAETPRA------RHTLDELNPQKSKHQGV----RKAKWHLGIRSQS  421


Snf1   514  YPLDVMGEIYIALKNLGAEWAKPSEEDLWTIKLRWKYDIGNKTNTNEKIPDLMKMVIQLF  573
             P D+M E+  A+K L  EW   +    + +++R K         N       KM +QL+
Hs a1  422  RPNDIMAEVCRAIKQLDYEWKVVNP---YYLRVRRK---------NPVTSTFSKMSLQLY  469


Snf1   574  QIETNNYLVDFK  585
            Q+++  YL+DF+
Hs a1  470  QVDSRTYLLDFR  481
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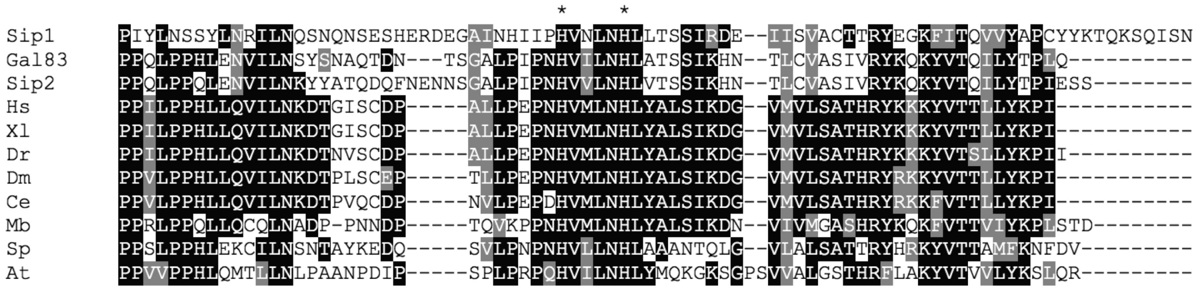
FIGURE S4



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Sip1      PIYLNSSYLNRILNQSNQNSESHERDEGAINHIIPHVNLNHLLTSSIRDE--IISVACTTRYEGKFITQVVYAPCYYKTQKSQISN
Gal83     PPQLPPHLENVILNSYSNAQTDN---TSGALPIPNHVILNHLATSSIKHN--TLCVASIVRYKQKYVTQILYTPLQ----------
Sip2      PPQLPPQLENVILNKYYATQDQFNENNSGALPIPNHVVLNHLVTSSIKHN--TLCVASIVRYKQKYVTQILYTPIESS--------
Hs        PPILPPHLLQVILNKDTGISCDP-----ALLPEPNHVMLNHLYALSIKDG--VMVLSATHRYKKKYVTTLLYKPI-----------
Xl        PPILPPHLLQVILNKDTGISCDP-----ALLPEPNHVMLNHLYALSIKDG--VMVLSATHRYKKKYVTTLLYKPI-----------
Dr        PPILPPHLLQVILNKDTNVSCDP-----ALLPEPNHVMLNHLYALSIKDG--VMVLSATHRYKKKYVTSLLYKPII----------
Dm        PPVLPPHLLQVILNKDTPLSCEP-----TLLPEPNHVMLNHLYALSIKDG--VMVLSATHRYRKKYVTTLLYKPI-----------
Ce        PPVLPPHLLQVILNKDTPVQCDP-----NVLPEPDHVMLNHLYALSIKDG--VMVLSATHRYRKKFVTTLLYKPI-----------
Mb        PPRLPPQLLQCQLNADP-PNNDP-----TQVKPPNHVMLNHLYALSIKDN--VIVMGASHRYKQKFVTTVIYKPLSTD--------
Sp        PPSLPPHLEKCILNSNTAYKEDQ-----SVLPNPNHVLLNHLAAANTQLG--VLALSATTRYHRKYVTTAMFKNFDV---------
At        PPVVPPHLQMTLLNLPAANPDIP-----SPLPRPQHVILNHLYMQKGKSGPSVVALGSTHRFLAKYVTVVLYKSLQR---------
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