# FOR THE RECORD

# Novel domains in NADPH oxidase subunits, sorting nexins, and PtdIns 3-kinases: Binding partners of SH3 domains?

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Abstract: Two SH3 domain-containing cytosolic components of the NADPH oxidase,  $p47^{phox}$  and  $p40^{phox}$ , are shown by analyses of their sequences to contain single copies of a novel class of domain, the PX (*phox*) domain. Homologous domains are demonstrated to be present in the Cpk class of phosphatidylinositol 3-kinase, *S. cerevisiae* Bem1p, and *S. pombe* Scd2, and a large family of human sorting nexin 1 (SNX1) homologues. The majority of these domains contains a polyproline motif, typical of SH3 domain-binding proteins. Two further findings are reported. A third NADPH oxidase subunit,  $p67^{phox}$ , is shown to contain four tetratricopeptide repeats (TPRs) within its N-terminal Rac1<sup>GTP</sup>binding region, and a 28 residue motif in  $p40^{phox}$  is demonstrated to be present in protein kinase C isoforms  $\iota/\lambda$  and  $\zeta$ , and in three ZZ domain-containing proteins.

**Keywords:** homology; signal transduction; chronic granulomatous disease; tetratrico peptide repeats; phospholipase D

Patients with chronic granulomatous diseases (CGDs) are severely predisposed to infection by fungi and bacteria due to deficiencies of the component subunits of NADPH oxidase. Dysfunction of the oxidase reduces superoxide generation, thereby compromising a major non-specific host defense mechanism of phagocytes (reviewed in Segal, 1989). The most common cause of CGDs is an X-linked inheritance, resulting in the deficiency of the large  $\beta$  subunit of flavocytochrome b (gp91phox). In approximately 30-40% of cases, however, autosomally inherited deficiencies of other subunits with masses 47k and 67k (p47<sup>phox</sup>, and p67<sup>phox</sup>) lead to CGD (Clark et al., 1989; Leto et al., 1990). Stimulation of neutrophils or other phagocytes results in these two cytosolic proteins, along with the small GTPases Rac1 or Rac2, translocating to the plasma membrane; subsequently, they form the functional NADPH oxidase, in complex with the transmembrane and heterodimeric gp91<sup>phox</sup>-p22<sup>phox</sup> flavocytochrome b558. This is the minimum complex required to generate the microbicidal superoxide anion. An additional cytosolic factor, p40phox, is known also to associate with the NADPH oxidase complex (Wientjes et al., 1993), although its regulatory roles remain uncertain.

P40<sup>phox</sup>, p47<sup>phox</sup>, and p67<sup>phox</sup> each contain Src homology 3 (SH3) domains, which mediate multiple associations with proline-rich targets within the NADPH oxidase complex (Fuchs et al., 1995; de Mendez et al., 1996). These domains are also present in a diverse range of kinases, phosphatases, phospholipases, and cytoskeletal proteins, and bind left-handed polyproline type II helices (reviewed in Pawson, 1995). The presence of other domain types in p47<sup>phox</sup> and p67<sup>phox</sup>, however, has not previously been noted. Here I report the identification of a novel domain family that includes p40<sup>phox</sup>, p47<sup>phox</sup>, phosphatidylinositol (PtdIns) 3-kinases, homologues of a sorting nexin (SNX1), and several yeast proteins, including the SH3-containing protein, Bem1p. I also record the presence of tetratricopeptide repeats (TPRs) in p67<sup>phox</sup>, and note that a 28 residue (octicosapeptide) repeat (OPR), present in p40<sup>phox</sup> and other proteins (English et al., 1995), is also present in protein kinase C (PKC)  $\iota/\lambda$  and  $\zeta$  isoforms and three ZZ domain-containing proteins.

A continuing interest in C2 domain-containing proteins (Ponting & Kerr, 1996; Ponting & Parker, 1996) prompted Blastp (Altschul et al., 1994) comparisons with databases of a region of the Cpk class of PtdIns 3-kinases (MacDougall et al., 1995; Molz et al., 1996; Virbasius et al., 1996), which intervenes between their catalytic and C2 domains. These searches revealed moderate similarities within the N-terminal region of human p47phox (lowest probability of matching by chance, p = 0.02). Further evidence that Cpk-like PtdIns 3-kinases and p47<sup>phox</sup> contain an homologous domain was provided by SWise (Birney et al., 1996) database searches, which demonstrated p47<sup>phox</sup> to be the highest scoring sequence using a Cpkderived profile. A subsequent Blastp search with the p47<sup>phox</sup> N-terminal sequence demonstrated additional similarities with regions of p40<sup>phox</sup> and orthologous S. cerevisiae and S. pombe sequences, Bem1p and Scd2 (p-values < 0.02). These three molecules also scored highest in SWise database searches using Cpk- and/or p47<sup>phox</sup>-derived profiles. These results strongly suggest that these molecules contain an homologous domain, which I shall term the PX (phox) domain. This suggestion is compatible with previous observations of pairwise similarities between p47<sup>phox</sup> and Bem1p (Chenevert, 1994), and p47<sup>phox</sup> and p40<sup>phox</sup> (Wientjes et al., 1993).

Significantly, the top six highest scoring sequences in a SWise search using a subsequent profile (derived from Cpk, p40<sup>phox</sup>,

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p47<sup>phox</sup>, Bem1p, and Scd2p sequences) were human sorting nexin-1 (SNX1) and 5 *Saccharomyces cerevisiae* proteins, MDM1p, Mvp1p, D9461.13p, YDL113c, and P9513.1p. Using SWise, Blastp, and MACAW, each of these six was demonstrated to be a member of a 12-strong family of homologues (data not shown; Fig. 1a); previously, only SNX1 and Mvp1p had been proposed as homologues (Kurten et al., 1996). Multiple alignments (Schuler et al., 1991; Thompson et al., 1994) of SNX1-homologues showed that their regions of highest conservation coincided with their PX domain-

like sequences. Indeed, motif searches employing the MoST algorithm (Tatusov et al., 1994) identified PX domain-like motifs in SNX1 homologues and, conversely, SNX1-like motifs in previouslydefined PX domains (searches used  $\alpha$ 1 alignment blocks, *r*-values of 0.01 or 0.03, and parameter i = 80%); similar results were found using SWise (not shown). It is concluded that these SNX1homologues also contain single copies of the PX domain.

In a final SWise database search, using a newly derived profile, four of the top seven top-scoring sequences were phospholipase D

Predicted 2-Structure	бебебе инининининининининини	
Consensus		
CPK/Drome	MERFIMIILEVIRHGOPDPIHLERSIREFIERHORDOMHFPL	.VKLHSLPAGVHV
CPK/Cacel	ONKITWYKUEVEKNUAUS	(15)
NCF1_Human	PSOHTVYMFLVKWQDLSEKVVYRRFTLYEFHKTLKEMFPI(9)	RIIPHLPAPKWE
NCF1_Mouse	PSQHTVYMFLVKWQDLSEKVYYRKFTEIYEFHKMLKEMFPI(9)	. RVIPHLPAPRWE
Bem1_Yeast	EDEKTWFLVCCELSNGKTRQLKRYYQDFYDLQVQLLDAFPA(14	) RIMPYIPGPVPY
Sed2_Schpo	RDDQYWYLVRAVMSDGKHRNLCRYYEDFFNFQTKFLELFPN (8)	. RV <u>IPYMP</u> GPVDI
P40/Human	SHFVFVIEVKTKGGSK	. CTLPTLPAKVYV
D9401.13/Yeast	HAIGIVILIEN,,	TITEPTPEKASI
Snx1/Human	AYVATKYTTOTSLPLFRSKOFAVKRRFSDFLGLYEKLSEKHSO(2)	. FIVPPPPEKSLI
Yor059w/Yeast	IHVETTVISESSLLELKYAQVSRRYRDFRWLYRQLQNNHWG	. KVIPPPPEKQSV
Yk58_Yeast	KDSLWIIKISTQPDVEKTIARAFSDFYWLYHQLQNNHWG	. KTIPPPTRSNII
Mvp1_Yeast	KHANYLVKHLIALPSTSPSEERTVVRRYSDFLWLREILLKRYPF	.RM <u>IPELP</u> PKRIG
Yjd6_Yeast	GYVSYQISTKTNNTSFYD. (5)ESIIVVHRRYSDLLLLHDILLNRFPT	CI <u>IPPLP</u> DKKVF
Yor357c/Yeast	MFTDIEIICRTNLPSFH, KRVSKVRKRISDFEFFRKCLIKEISM (5)	.VM <u>VPHLP</u> GKILI
Y http://teast	TNDY VULVEUSTDN KET VERVENDER (2)	TEVEREPEVON
Mdm1 Yeast	EITYXIINIHHFNNGOVSSWDNARRYNIFFELNTYLKKNFRD	(23)
P9513.1/Yeast	KHHYFLIKIKKQDDDDQD(12)AGYFYVTRTYSDFKKLSHDLKSEFPG(2)	CPRLPHRNKK
PLD1/Human	SINLTTIELTHGEFKWQVKRKFKHTQEFHRELLKYKAF	IRIPIPTRRHI
PLD1/Cacel	NTLLTTIELEHGQFRWSVIRNYKDFTLLNNRLMAHRAR	(151)
Sp14/Yeast	ENSLFRIHLEYGIDEDRLKWSIIRSTKDIKSLHHKLKIVAFQ	
Ya2g_Schpo	IHSTFTIQVEYGTGPHAIRWLIYRQLRDFINLHSHFLFFEFQ	
R21206/riuman	RETTIELRVKINLEDEX COEKIUIBEVSKIKALSUBENERLSKV	TETPPYYEKNSU
D28076/Casel	SFIAYSITSSLINIO	IPIPPXPEKC
R56645/Human	DSLWRRYSEFELLRSYLLVYYPH	. IVVPPLPEKRAE
Z44623/Human	REFVWLRQRLQSNALL	. VOLPELPSKNLE
N48381/Human	KHVEYEVSSQRFKSSVYRRYNDFVVYQEMLLHKFPY	. RM <u>VPALP</u> PKRMI
R95144/Human	QVKRRFSDXLGLYEKLSEKHSQ(2)	. FI <u>VPPPP</u> EKSLI
H17597/Human	RSVNHRYKBFDWLYERLLVKFGS	AIPIPSLPDKC
LA4419/Human	TYALTAITVHRRNINSEEMWKTYRRISDFHDFHMRITEQFES(1)	. SSILKLPGKKTE
N82298/10Xg0	TTUNER VIEW NERVIEW NE	TENVEGPERTE
F11999/Human	GYTEYKYTAOFISKKDPEDVKEVVVWKRYSDFRKLHGDLAYTHRN (3)	. EEFPAFPRAOVE
Predicted 2-Structure	. (5). нийнийнийнийнийн ройнолсо В сьсь в 11	
Predicted 2-Structure Consensus CPK/Drome	. (5). ННИНИНИНИНИНИНИНИ <b>RL bL</b> . (5). KSVAEK MLPLIQOR TAKSLFDASEEIAH SELVYT <b>F</b> HPLLRD(	Q U52192 (1625-1725)
Predicted 2-Structure Consensus CPK/Droate CPK/Mouse	. (5). ННИНИНИНИНИНИНИНИ RL bL b b b L . (5). KSVAEKBLPLIQRFLKSLFDASEEIAH SELVYTFHPLLRD( . (5). KDVAAKEKIELNSYLQSLMNASTDVAE CDLVCTFHPLLRD)	Q U52192 (1625-1725) S U52193 (1410-1510)
Predicted 2-Structure Consensus CPK/Drome CPK/Mouse CPK/Caeel	. (S).HHHHHHHHHHHHHHHHHHHHHHH R L bL b b 1 . (S).KSVAEK BLPLIQRTLKSLFDASEEIAHSELVYTFHPLLRD( . (S).KSVAEK KIELNSYLQSLMNASTDVAECOLVCTFHPLLRD) RAVAQKBIIHVQKFLIYLFNQVDEICHCDLVTFFHSLLRD;	2 U52192 (1625-1725) 5 U52193 (1410-1510) 8 Z65660 (943-1041)
Predicted 2-Structure Consensus CPK/Drome CPK/Mouse CPK/Casel NCF1_Human	. (5).HHHHHHHHHHHHHHHHHHHHHHHHHHHHH R L bL b b FL . (5).KSVAEKRLPLIQRFLKSLFDASEEIAHSELVYTFFHPLLRD( . (5).KSVAAKRIELNSYLQSLMASTDVAECDLVYTFFHPLLRD( RUAQKEIIHVQKFLYLSLPDICHCDLVYTFFHSLRD) QRAAENRGTLFYCSTLMSLPTKISRCPHLIDFFKVRPDD)	2 U52192 (1625-1725) 5 U52193 (1410-1510) 8 Z69660 (943-1041) 6 U52665 (20-125) 6 U 456 (20-125)
Predicted 2-Structure Consensus CPK/Drome CPK/Mouse CPK/Cacel NCF1_Funnan NCF1_Mouse Benn1_Vest	. (5). HHHHHHHHHHHHHHHHHHH <b>R</b> L b L h . (5). KSVAEK RLPLIQRT LKSLFDASEEIAH SELVYT FHPLLRD( . (5). KDVAAK RKIELNSY LQSLMAST DVAE CDLVCT FHPLLRD( RAVAQK BII HVQKF LIYLFNQVDEICH CDLVYT FHSILRDI . (2). QRAAE NQGT LTEYFNGLMGLPVKISK CPHLLD FKVRPDDI . (2). QRAAE SQCT LTEYFNGLWALPVISK SFMU HSLFVUNNG( NST KKE MEDLNI LPUNISK SFMU HSLFVUNNG( 	2 U52192 (1625-1725) 5 U52193 (1410-1510) 7 Z69566 (2043-1041) 6 M25665 (20-125) 7 Z36056 (204-404) 7 Z36056 (204-404)
Predicted 2-Structure Consensus CPK/Droade CPK/Mouse CPK/Caeel NCF1_Human NCF1_Mouse Bern1_Yeast Scd2. Schpo	. (5). HHHHHHHHHHHHHHHHHHH hhHHHH R L L L h h f I . (5). KSVAEK HLPLIQRILKSLFDASEEIAH SELVYTIFHPLLRD( . (5). KDVAAK HKIELNSYLQSLMNASTDVAE COLVCTIFHPLLRD( 	2 U52192 (1625-1725) 2 U52193 (1410-1510) 2 Z59560 (943-1041) 2 M25665 (201-125) 2 L11455 (201-125) 7 Z36069 (294-404) 7 U12339 (209-413)
Predicted 2-Structure Consensus CPK/Drome CPK/Casel NCF1_Human NCF1_Mouse Bern1_Yeast Sod2_Schpo P40/Human	. (5).HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	<ul> <li>US2192 (1625-1725)</li> <li>US2193 (1410-1510)</li> <li>Z59660 (943-1041)</li> <li>M25665 (201-15)</li> <li>L11455 (20-125)</li> <li>Z56056 (294-404)</li> <li>V12539 (309-413)</li> <li>X77094 (37-140)</li> </ul>
Predicted 2-Structure Consensus CPK/Drome CPK/Casel NCF1_Human NCF1_Human NCF1_House Bern1_Yeast Sod2_Solpo P40/Human D9461.13/Yeast	. (5).HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	2 US2192 (1625-1725) 5 US2193 (1410-1510) 5 US2193 (1410-1510) 5 US2193 (1410-1510) 5 US2193 (1410-1510) 5 US2193 (150-140) 5 US2193 (150-413) 5 US2192 (1625-1725) 5 US2193 (1625-1725) 5 US219 (1
Predicted 2-Structure Consensus CPK/Mouse CPK/Cacel NCF1_Human NCF1_Human NCF1_Mouse Bern1_Yeast Scd2_Schpo P40/Human D9461.13/Yeast YDL113c/Yeast	. (5).HHHHHHHHHHHHHHHHHHHHH hhHHH <b>L LL L L L L L L L L L L L L L L L L L</b>	2 U52192 (1625-1725) 2 U52193 (1410-1510) 2 Z59560 (943-1041) 3 M25665 (20-125) 1 L11455 (20-125) 2 Z3606 (204-04) 4 U12339 (309-413) 5 X77094 (37-140) 4 U3209 (173-28) 4 Z74161 (173-301) 4 Z74161
Predicted 2-Structure Consensus CPK/Droade CPK/Cacel NCF1_Human NCF1_Human D9461.13/Yeast Scd2_Schp0 P40/Human D9461.13/Yeast Snx1/Human YerVfew/Yeast	. (5). HHHHHHHHHHHHHHHHHHH hhHHH hhHHhHHHHHHHHHH	<ul> <li>U52192 (1625-1725)</li> <li>U52193 (1410-1510)</li> <li>Z59660 (943-1041)</li> <li>M25665 (20-125)</li> <li>L11455 (20-125)</li> <li>Z36069 (294-404)</li> <li>U12598 (608-413)</li> <li>X77094 (37-140)</li> <li>U33007 (137-326)</li> <li>Z74161 (173-301)</li> <li>U33225 (161-272)</li> <li>Z7407</li> </ul>
Predicted 2-Structure Consensus CPK/Droate CPK/Casel NCF1_Human NCF1_Mouse Ben1_Yeast Sod2_Schpo P40/Human D9461.13/Yeast Stat1/Human Yor059w/Yeast YEast	. (5). HHHHHHHHHHHHHHHHHHHHH hhHHHH R L bL h h fL . (5). KSVAEKHIPLIQRFIKSIFDASEEIAH SELVTTFHPLLRO . (5). KSVAAKHIEINSYLQSLMNASTOVAE CDLVTTFHPLLRO RAVQXHIIHVQKFITIFPOTEICH CDLVTTFHFLLRO 	<ul> <li>US2192 (1625-1725)</li> <li>US2193 (1410-1510)</li> <li>Z59660 (943-1041)</li> <li>M25665 (20-125)</li> <li>L11455 (20-125)</li> <li>Z36069 (294-404)</li> <li>U12097 (137-236)</li> <li>U23007 (137-236)</li> <li>U33225 (161-272)</li> <li>Z28303 (131-247)</li> </ul>
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Predicted 2-Structure Consensus CPK/Drome CPK/Mouse CPK/Caeel NCF1_Funnan NCF1_Mouse Bern1_Yeast Scd2_Schpo P40/Human D9461.13/Yeast Srst/Human Yor059w/Yeast Yic58_Yeast Micp1_Yeast Yic58_Yeast Yic62_Yeast Yor37c/Yeast	. (5). HHHHHHHHHHHHHHHHHHH hhHH R L bL h h h FL . (5). KSVAEK RLPLIQRT LKSLFDASEEIAHSELVYTT FHPLLRD( . (5). KDVAAK RKIELNSYLQSLMAASTDVAECDLVCT FFHPLLRD( . (2). QRAAEN RQGT LEY GSLMASTDVAECDLVCT FFHPLRDD) . (2). QRAAEN RQGT LEY CSTLMSLPTKISKCPHLD FFKVRPDD) . (2). QRAAEN RQGT LEY NGLMGLPVKISKCPHLD FFKVRPDD) . (2). RNITKK RKED LNIY ADLVNLPDYISKSEMY HSLFVUNNG . (2). ELISSQ RAMD LOVY LKENCRLPARLE NELVKIT FLEDGOV . (3). QEIAEM FPALNYM KSLLSPVWVLMDEDVRIT FVQSPYDC . (13). SKIIST KKMLNSFLSNCLNIDEISNDIVT QKT INDEFNWI . (39). EKLIRH RIPALNYM KSLLSPVWLMDEDVRIT FYQSPYDC . (14). AEFLEK RAALSKYLQRIVNHTMLQDEDVRIT LEKEELP . (5). QLELKK RRIGLSKT INVMKPKLSNDDLVTT TVT KTDU . (5). QLELKK RRIGLSKT INVMKPKLSNDDLVTT TVT KTDU . (9). QRTQK RCHSLOMT KWQSVAGHPLLQSGSKVLVVT EAEKEV	2 U52192 (1625-1725) 2 U52193 (1410-1510) 3 Z59660 (943-1041) 4 M25665 (20-125) 4 L1455 (20-125) 5 Z5069 (204-404) 4 U1239 (309-413) 5 X77094 (37-140) 4 U33907 (137-236) 4 U33907 (137-236) 4 Z74161 (173-301) 8 U33925 (161-272) 5 Z74977 2 Z28303 (131-247) 7 Z48613 (146-247) 7 Z4823 (131-247) 7 Z4825 (56-162) 2 X7525 (56-162) 2 X7555 (56-162) 2 X7555 (56-162) 2 X7555 (56-162) 2 X7555 (56-162) 2 X75555 (56-162) 2 X75555 (56-162) 2 X7555555555555555555555555555555555555
Predicted 2-Structure Consensus CPK/Drome CPK/Casel NCF1_Human NCF1_Mouse Ben1_Yeast So2_Schpo P40/Human D9461.13/Yeast YDL113cYeast YDL113cYeast YDL113cYeast YDL13cYeast Mvp1_Yeast Mvp1_Yeast Yd58_Yeast Yd58_Yeast Yd58_Yeast Yd58_Yeast Yd58_Yeast Yd57.Yeast Yd57.Yeast	. (5). HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	<ul> <li>US2192 (1625-1725)</li> <li>US2193 (1410-1510)</li> <li>Z59560 (945-1041)</li> <li>M25665 (20-125)</li> <li>Z36069 (254-404)</li> <li>U1239 (306-413)</li> <li>X77094 (77-140)</li> <li>U33007 (137-236)</li> <li>Z74161 (173-301)</li> <li>U3322 (161-272)</li> <li>Z48613 (146-247)</li> <li>Z48613 (146-247)</li> <li>Z48613 (146-247)</li> <li>Z48613 (146-247)</li> <li>Z48613 (146-247)</li> <li>Z48622 (47-157)</li> <li>Z75265 (56-162)</li> <li>U1130 (22-15)</li> </ul>
Predicted 2-Structure Consensus CPK/Drone CPK/Mouse CPK/Caeel NCF1_Human NCF1_Human NCF1_Human D9461.13/Yeast Sod2_Schpo P40/Human D9461.13/Yeast YDL113c/Yeast SrstI/Human Yor069w/Yeast Yid5_Yeast Yid5_Yeast Yid5_Yeast Yid5_Yeast Yid5_Yeast Yag5_Yeast Yag7_Yeast Memory Yeast	. (5). HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	2 U52192 (1625-1725) 5 U52193 (1410-1510) 5 U52193 (1410-1510) 5 U52193 (1410-1510) 5 U5255 (20-125) 5 U5255 (20-125) 5 U5255 (20-443) 4 U5255 (20-443) 4 U5255 (161-72) 5 U5325 (161-7
Predicted 2-Simulare Consensus CPK/Mouse CPK/Mouse CPK/Cacel NCF1_Human NCF1_Human NCF1_Human Sod2_Schpo P40/Human D9461.13/Yeast Soz1/Human Yot069w/Yeast Yb58_Yeast Yb58_Yeast Yb68_Yeast Yb62_Yeast Yb62_Yeast Yb62_Yeast Yb62_Yeast Yb7_Yeast Yam7_Yeast Mam1_Yeast P511.1/Yeast P511.1/Yeast	. (5).HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	2 U52192 (1625-1725) 2 U52193 (1410-1510) 2 U52193 (1410-1510) 2 U52956 (043-1041) 3 U52656 (20-125) 1 L1455 (20-125) 2 U12539 (309-413) 3 X77094 (37-140) 4 U12539 (309-413) 3 X77094 (37-140) 4 U12539 (309-413) 4 U12539 (309-413) 4 U12539 (309-413) 4 U12539 (309-413) 4 U12539 (310-347) 5 Z48223 (47-157) 5 Z48223 (47-157) 5 Z48223 (47-157) 5 Z48223 (47-157) 5 Z48223 (47-157) 5 Z48223 (47-157) 5 Z4823 (48-247) 5 Z4823 (4
Predicted 2-Structure Consensus CPK/Droate CPK/Casel NCF1_Human NCF1_Mouse Bern1_Yeast Scd2_Schpo P40/Human D9461.13/Yeast Sta1/Human Yor059w/Yeast Yi26_Yeast Yi26_Yeast Yi26_Yeast Yu52_Yeast Yu52_Yeast Yu52_Yeast Yu52_Yeast Yu52_Yeast Yu53_Yeast P513.1/Yeast P513.1/Yeast P513.1/Yeast	. (5). HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	<ul> <li>US2192 (1625-1725)</li> <li>US2193 (1410-1510)</li> <li>Z59660 (943-1041)</li> <li>M25665 (20-125)</li> <li>L11455 (20-125)</li> <li>Z36069 (294-404)</li> <li>UJ239 (309-413)</li> <li>UJ3293 (309-413)</li> <li>UJ3207 (137-36)</li> <li>UJ3207 (137-36)</li> <li>Z28303 (131-247)</li> <li>Z48239 (471-57)</li> <li>Z75265 (36-162)</li> <li>UJ0399 (91-214)</li> <li>UJ1379 (22-125)</li> <li>X (56371 (116-221))</li> <li>UJ3033 (291-3077)</li> <li>UJ3245 (99-213)</li> </ul>
Predicted 2-Structure Consensus CPK/Drome CPK/Mouse CPK/Casei NCF1_Mouse Ben1_Yeast Sod2_Schpo P40/Human D9461.13/Yeast YDL113c/Yeast YDL113c/Yeast YDL13c/Yeast Yk58_Yeast Mvp1_Yeast Yid5.Yeast Yid5.Yeast Ya57_Yeast Ya57_Yeast Ya57_Yeast Ya57_Yeast Ya57_Yeast Ya513.1/Yeast PDD1/Human PLD1/Lasei	. (5).HHNHHHNHHNHHHNHHH R L bL h b fL . (5).KSVAEKELPLIQRFLKSLFDASEEIAH.SELVTTFHPLLRD( . (5).KSVAEKELPLIQRFLKSLFDASEEIAH.SELVTTFHPLLRD( . (5).KDVAAKERIELNSYLGSLMNASTDVAE.CDLVTTFHPLLRD( . (2).QRAAENEQGTLTEYCSTLMSLFTKISR.CPHLDJFKVRPDDI . (2).QRAAESEQGTLTEYCSTLMSLFTKISR.CPHLDJFKVRPDDI . (2).QRAAESEQGTLTEYCSTLMSLFTKISR.CPHLDJFKVRPDDI . (2).QRAAESEQGTLTEYCSTLMSLFTKISR.CPHLDJFKVRPDDI . (2).QRAAESEQGTLTEYCSTLMSLFTKISR.CPHLDJFKVRPDDI . (2).QRAAESEQGTLTEYCSTLMSLFTKISR.CPHLDJFKVRPDDI . (2).QEIAEMETPALNAYMKSLLSLPVVLM.OEDVRIFYQSPYDI . (3).QEIAEMETPALNAYMKSLSLSLPVVLM.OEDVRIFYQSPYDI . (4).QEIAEMETPALNAYMKSLSLSLPVVLM.OEDVRIFYQSPYDI . (5).QLFLKERAALSRYLQRIVNHTMLCDPVRFLEKEELPI . (4).ENFIENERROMSSMLKXICQDFVLQK.DKDFLIFLTSDFSS FKPEYIISLQIMAKIKRIFNDXVLRL.DSNFIDJSWSKLKXICQDFVLQK. . (5).QLFLKKRIGISRINKKIFNDXVLRL.DSVLVTFLVKNDDL . (5).QLFLKKRIGISRINNVMKHFKLSN.DLVTTJVKNDDL . (5).QLFLKKRIGLSTINLYNMKHFKLSN.DLVTTJVKSRWE . (12).KDWLAERGGLYTINHILNSSLVEM.TKDILIQFLQSKFV TLVCEKMRISLGEFFINELYNDFDSWRMDIKIAQUFLQSKFV TLVCEKMRISLGYLTNELLSISEICEDNIFRFLSGFLD TLVCEKMRISLQUFLKILKDAEVSQ.SSSIRFFISGELDT TLVCEKMRISLGVLATIKLSLSISECESSIRFFISGFID EQFLQRRKQLEDYTKILKWPMRN.YAATTEFLDISGLS VTNLHMKKELLSNYLQVVLINN.YAATTEFLDISGLS VTNLHMKKLENNLQVVLINNYAATTEFLDISGLS VTNLHMKKLENLSNYLQVVLINNYAATTEFLOSGLS VTNLHKKLLENNLQVVLINNYAATTEFLOSSLS VTNLHKKLLENNLQVVLINNYAATTEFLOVS	<ul> <li>US2192 (1625-1725)</li> <li>US2193 (1410-1510)</li> <li>US2193 (1410-1510)</li> <li>Z59560 (204-104)</li> <li>L11455 (20-125)</li> <li>Z36069 (294-404)</li> <li>UI3309 (137-236)</li> <li>Z7794 (377-140)</li> <li>UI33007 (137-236)</li> <li>Z74613 (146-247)</li> <li>Z48013 (146-247)</li> <li>Z48023 (416-247)</li> <li>Z48023 (416-247)</li> <li>Z48023 (416-247)</li> <li>Z48023 (416-247)</li> <li>Z4803 (146-247)</li> <li>Z4803 (146-247)</li> <li>Z4803 (146-247)</li> <li>Z15365 (56-162)</li> <li>U00059 (91-214)</li> <li>U1332 (291-507)</li> <li>U38245 (592-213)</li> <li>U35854 (141-370)</li> </ul>
Predicted 2-Structure Consensus CPK/Drone CPK/Mouse CPK/Caeel NCF1_Human NCF1_Human NCF1_Human D9461.13/Yeast Sod2_Schpo P40/Human D9461.13/Yeast YDL113c/Yeast Sox1/Human Yo059w/Yeast Yds5_Yeast Yds5_Yeast Yds5_Yeast Yds5_Yeast Yds5_Yeast Yds5_Yeast Yds5_Yeast P513.1/Yeast PLD1/Human PLD1/Human PLD1/Jeast	. (5).HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	2         U52192 (1625-1725)           2         U52193 (1410-1510)           2         U5295 (1410-1510)           2         U5295 (1410-1510)           2         U5295 (1410-1510)           1         M25655 (201-125)           1         L11455 (201-125)           2         Z3606 (294-404)           7         U1239 (100-413)           2         U33007 (137-236)           2         U33020 (131-247)           2         Z4803 (140-247)           2         Z48203 (416-247)           2         Z48203 (416-247)           2         Z4823 (41-157)           2         Z5255 (56-162)           4         U0039 (91-214)           7         U51033 (291-507)           1         U51033 (291-507)           1         U53254 (14-370)           2         U53554 (14-370)           1         U53254 (14-370)
Predicted 2-Simulare Consensus CPK/Mouse CPK/Mouse CPK/Cacel NCF1_Human NCF1_Human NCF1_Human Ps61_Yeast Sod2_Schpo P40/Human P961_13/Yeast YDL113/Yeast YdS2_Yeast Yds5_Yeast Yds5_Yeast Yds5_Yeast Yds5_Yeast Yad57C/Yeast Yam7_Yeast P9513_1/Yeast P1D1/Human P1D1/Cacel Sp14/Yeast Ye32_Schpo P2128_Kemp	. (5). HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	2 U52192 (1625-1725) 2 U52193 (1410-1510) 2 U52193 (1410-1510) 2 U52956 (043-1041) 3 M25665 (20-125) 1 L1455 (20-125) 2 J3606 (29-44-04) 4 U1239 (309-413) 5 X77094 (37-140) 4 U3207 (147-36) 4 Z74161 (173-301) 4 U33225 (161-272) 5 Z74977 2 Z8303 (131-247) 7 Z48623 (146-247) 5 Z49224 (47-157) 5 Z75255 (56-162) 4 U00059 (91-24) 4 U0059 (91-24) 4 U0059 (91-24) 5 U538254 (141-370) 8 U28256 (225-383) 1 Z58042 (215-477) 5 Z21305 4 U21307 1 U5103 (221-507) 5 U58545 (141-370) 8 U28256 (225-383) 1 Z50142 (131-447) 8 Z21306 (225-383) 1 Z50142 (131-447) 8 Z21306 (225-383) 1 Z50142 (131-447) 8 Z21306 (225-383) 2 Z5056 (225-383) 2
Predicted 2-Structure Consensus CPK/Drome CPK/Mouse CPK/Casel NCF1_Human NCF1_Mouse Bern1_Yeast Sot2_Schpo P40/Human D9461.13/Yeast YDL113cYeast YDL113cYeast YDL113cYeast YDL13cYeast Yd5_Yeast Yd5_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast PLD1/Casel Sp14/Yeast Yd2_Schpo R21308/Human D047264Deras	. (5). HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	<ul> <li>US2192 (1625-1725)</li> <li>US2193 (1410-1510)</li> <li>Z59560 (2943-1041)</li> <li>M25655 (20-125)</li> <li>L11455 (20-125)</li> <li>Z36069 (294-404)</li> <li>U1239 (105-413)</li> <li>X77094 (77-140)</li> <li>U33007 (137-236)</li> <li>Z74161 (173-301)</li> <li>U3322 (161-272)</li> <li>Z48613 (146-247)</li> <li>U35225 (61-22)</li> <li>U5033 (29-157)</li> <li>U55854 (141-370)</li> <li>Z25265 (22-383)</li> <li>Z50424 (121-247)</li> <li>R21108</li> <li>D24276</li> </ul>
Predicted 2-Structure Consensus CPK/Drome CPK/Mouse CPK/Caeel NCF1_Mouse Ben1_Yeast Sod2_Solpo P40/Human D9461.13/Yeast YDL113cyYeast YDL113cyYeast YDL113cyYeast YG8_Yeast Yu58_Yeast Yu65_Yeast Yi65_Yeast Ya57c/Yeast Ya57cyYeast Ya57cyYeast Ya57cyYeast Ya513.1/Yeast P2D1/Human PLD1/Caeel Sp14/Yeast Ya2g_Solpo R21308/Human D24276/Crysa D28076/Caeel	. (5).HHNHHHNHHNHHHNHHH hhHhhe R L bL h b J I . (5).KSVAEK BLPLIQRTIKSLFDASEEIAH.SELVTTFHPLLRD( . (5).KDVAAK RKIELNSYLGSLMNASTDVAE.CDLVTTFHPLLRD( . (2).KDVAAK RKIELNSYLGSLMNASTDVAE.CDLVTTFHPLLRD( . (2).QRAAESRQGTITEYCSTIMSLPTKISR.CPHLDJFKVRPDD) . (2).QRAAESRQGTITEYCSTIMSLPTKISR.CPHLDJFKVRPDD) . (2).QRAAESRQGTITEYCSTIMSLPTKISR.CPHLDJFKVRPDD) . (2).WSTTKKKREDINIYVADLVNLDVISR.SEMVHSLTVVLNNG . (2).ELISSQRAMDLDVYLKENCRPARLE.NELVKITFLPLGD) . (3).QEIAEMRIPALNAYMKSILSLPVWLM.OEDVRITFYGSPYD . (3).GEIAEMRIPALNAYMKSILSLPVWLM.OEDVRITFYGSPYD . (4).ENTENRRPGNESMIKHICQDPVLQK.DKDFKIFNDFFNN . (4).ENTENRRPGNESMIKHICQDPVLQK.DKDFLIFISDFSS FKPEYIISLQIMAKIKRIFNCKLR.DSNFIDFISWDDLI SNFIDTISMRFGMESMIKHICGDPVLQK.SKVFKTLVSRDWE 	<ul> <li>US2192 (1625-1725)</li> <li>US2193 (1410-1510)</li> <li>US2193 (1410-1510)</li> <li>Z59660 (243-1041)</li> <li>M25665 (20-125)</li> <li>Z36069 (294-404)</li> <li>U12339 (309-413)</li> <li>X77094 (37-436)</li> <li>Z74807 (137-236)</li> <li>Z74977</li> <li>Z38303 (131-247)</li> <li>Z748071 (146-247)</li> <li>Z48403 (146-247)</li> <li>Z48226 (47-157)</li> <li>Z75265 (56-162)</li> <li>U00059 (91-214)</li> <li>U151033 (291-507)</li> <li>U352845 (146-213)</li> <li>U35284 (141-370)</li> <li>Z2556 (225-383)</li> <li>Z5546 (141-370)</li> <li>Z25042 (14-247)</li> <li>Z50142 (14-247)</li> <li>Z50142 (14-247)</li> <li>Z50142 (14-247)</li> <li>Z50142 (14-247)</li> <li>Z2138</li> <li>Z2476</li> <li>D28076</li> </ul>
Predicted 2-Structure Consensus CPK/Drone CPK/Mouse CPK/Caeel NCF1_Human NCF1_Human NCF1_Human Po401-Yeast Sod2_Schpo P40/Human P040F1.33/Yeast YDL113c/Yeast Soz1/Human Yo0059w/Yeast Yb45_Yeast Yo45_Yeast Yo45_Yeast Ya45_Yeast Ya45_Yeast Ya45_Yeast Yb45_Yeast P513.1/Yeast P513.1/Yeast PLD1/Human PLD1/Human PLD1/Jeast Sp14/Yeast Ya28_Schpo R21308/Human D24276/Oryss D28076/Caeel R56045/Human	. (5).HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	2 U52192 (1625-1725) 5 U52193 (1410-1510) 5 U52193 (1410-1510) 5 U52193 (1410-1510) 5 U5265 (20-125) 5 L11455 (20-125) 5 L11455 (20-125) 5 Z3606 (294-404) 7 U1239 (130-413) 5 X77094 (37-140) 4 U3302 (137-236) 4 Z74161 (173-301) 5 U3302 (137-236) 4 Z74161 (173-301) 5 Z48451 (146-247) 5 Z48451 (146-247) 5 Z4825 (46-247) 5 Z4825 (46-247) 5 Z4825 (46-247) 5 Z4825 (46-247) 5 U53854 (16-221) 5 U53854 (16-221) 5 U53854 (14-370) 8
Predicted 2-Structure Consensus CPK/Droate CPK/Casel NCF1_Human NCF1_Mouse Bern1_Yeast Scd2_Schpo P40/Human D9461.13/Yeast YDL113cyYeast YDL113cyYeast YDL113cyYeast Yd62_Yeast Yd62_Yeast Yd62_Yeast Yd62_Yeast Ym52_Yeast Ym52_Yeast Ym52_Yeast Ym513.1/Yeast PDD1/Casel Sp14/Yeast Ya62_Schpo R21308/Human D2427c/Orysa	. (5).HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	<ul> <li>US2192 (1625-1725)</li> <li>US2193 (1410-1510)</li> <li>Z59660 (943-1041)</li> <li>M25665 (20-125)</li> <li>IL1455 (20-125)</li> <li>S7094 (73-140)</li> <li>UT393 (20-125)</li> <li>Z7405 (17-361)</li> <li>UT3925 (161-272)</li> <li>Z48707 (13-301)</li> <li>US3225 (161-272)</li> <li>Z48239 (47-157)</li> <li>Z75265 (26-162)</li> <li>UU3025 (46-147)</li> <li>UT332 (21-27)</li> <li>IS3254 (41-370)</li> <li>US3254 (41-370)</li> <li>US3254 (41-370)</li> <li>US3254 (41-370)</li> <li>US3254 (41-370)</li> <li>US354 (41-370)</li> <li>US354 (41-370)</li> <li>Z2856 (25-383)</li> <li>Z30142 (131-247)</li> <li>R21108</li> <li>D24276</li> <li>D24076</li> <li>Z44623</li> </ul>
Predicted 2-Structure Consensus CPK/Drome CPK/Mouse CPK/Casel NCF1_Huntan NCF1_Mouse Ben1_Yeast Sot2_Schpo P40(Human D9461.13/Yeast YDL113c/Yeast YDL113c/Yeast YDL113c/Yeast YDL13c/Yeast Yd58_Yeast Mvp1_Yeast Yd58_Yeast Yd58_Yeast Yd57_Yeast Yd57_Yeast Yd57_Yeast Yd57_Yeast Yd513.1/Yeast P513.1/Yeast P1D1/Lasel Sp14/Yeast Ya28_Schpo R21308/Human D24276/Cryas D28076/Casel R56645/Human Z44623/Human	. (5).HHNHHHNHHNHHHNHHH R L bL h h fL . (5).KSVAEK BLPLIQRTLKSLFDASEEIAH.SELVTTFHPLLRD( . (5).KSVAEK BLPLIQRTLKSLFDASEEIAH.SELVTTFHPLLRD( . (5).KDVAAK KRIELNSYLGSLMASTDVAE.CDLVTTFHPLLRD( . (2).KDVAAK KRIELNSYLGSLMASTDVAE.CDLVTTFHPLLRD( . (2).QRAAESBQGTLTEYCSTLMSLFTKISR.CPHLLDFFKVRPDDI . (2).QRAAESBQGTLTEYCSTLMSLFTKISR.CPHLLDFFKVRPDDI . (2).NSTTKK KRDLNIYVADLVNLDVISR.SEMVHSLFVVLNNG . (2).QEIAEMBTPALNAYMKSLLSLPVWLM.OEDVRITFYGSPDO . (3).QEIAEMBTPALNAYMKSLSLSPVWLM.OEDVRITFYGSPDO . (3).QEIAEMBTPALNAYMKSLSLSPVWLM.OEDVRITFYGSPDO . (4).ENFIENBRFQMSSNLKNICQDFVLQK.OKDFLIFTSDFSS FKPEYIISLQMAMIKNICDDVILNLENSEITK.SITTDLDPNNMM 	<ul> <li>US2192 (1625-1725)</li> <li>US2193 (1410-1510)</li> <li>Z59560 (945-1041)</li> <li>M25655 (20-125)</li> <li>L11455 (20-125)</li> <li>Z36069 (254-404)</li> <li>U1239 (105-413)</li> <li>X77094 (77-140)</li> <li>U33007 (137-236)</li> <li>Z74161 (173-301)</li> <li>U35225 (161-272)</li> <li>Z48613 (146-247)</li> <li>Z48613 (146-247)</li> <li>Z48613 (146-247)</li> <li>Z48613 (146-247)</li> <li>Z48613 (146-247)</li> <li>Z48228 (47-157)</li> <li>Z75265 (56-162)</li> <li>M0059 (91-214)</li> <li>D11379 (22-125)</li> <li>X65671 (116-221)</li> <li>U153854 (141-370)</li> <li>Z5256 (22-383)</li> <li>U55854 (141-370)</li> <li>Z5256 (22-383)</li> <li>D24276</li> <li>D24076</li> <li>R26645</li> <li>Z44623</li> <li>N48381</li> <li>P05144</li> </ul>
Predicted 2-Structure Consensus CPK/Drome CPK/Mouse CPK/Caeel NCF1_Human NCF1_Mouse Ben1_Yeast Sod2_Schpo P40/Human J9461.13/Yeast YDL113cyYeast YDL113cyYeast YDL113cyYeast YDL113cyYeast YDL113cyYeast YG8_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast Yd6_Yeast P513.1/Yeast P513.1/Yeast P1D//Human PLD1/Caeil Sp14/Yeast Ya2g_Schpo R21308/Human D2427c/Caeil S56645/Human Z44623/Human R4531/Human R45514/Human	. (5).HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	2 US2192 (1625-1725) 5 US2193 (1410-1510) 5 US2193 (1410-1510) 5 US2193 (1410-1510) 5 US2595 (121-15) 5 US255 (121-15) 5 US255 (121-15) 5 US255 (121-15) 5 US255 (121-15) 5 US255 (146-172) 5 US2
Predicted 2-Simulare Consensus CPK/Mouse CPK/Mouse CPK/Caeel NCF1_Human NCF1_Human NCF1_Human Yest Sod2_Schpo P40/Human Yo005w/Yeast YDL113c/Yeast YDL113c/Yeast Suz1/Human Yo005w/Yeast Yd52_Yeast Yd52_Yeast Yd52_Yeast Yd52_Yeast Yd52_Yeast Yd52_Yeast Yd52_Yeast Yd52_Yeast Yd52_Yeast Yd52_Yeast Yd52_Yeast Yd52_Yeast PLD1/Human PLD1/Caeel Sp14/Yeast Y628_Schpo R21308/Human D24276/Oryss D28076/Caeel R56645/Human R95144/Human H17597/Human H2544/Human	. (5).HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	2         U52192 (1625-1725)           2         U52193 (1410-1510)           2         U5295 (1410-1510)           2         U5295 (1410-1510)           1         M25665 (20-125)           1         L11455 (20-125)           2         L5305 (294-404)           7         U1239 (100-413)           1         X77094 (37-140)           1         U13239 (100-413)           2         X7094 (37-140)           1         U3205 (161-272)           2         Z4803 (146-247)           2         Z48230 (131-247)           2         Z48230 (146-247)           2         Z4823 (291-507)           2         U53254 (146-247)           2         U53254 (146-247)           1         U5133 (291-507)           1         U53854 (146-247)           1         U5133 (291-507)           1         U53854 (141-370)           2         U53854 (141-370)           1         Z5256 (225-383)           2         Z0142 (131-247)           2         Z1304           2         Z266 (225-383)           2         Z0142 (141-370)           2         Z28256 (22
Predicted 2-Structure Consensus CPK/Drome CPK/Mouse CPK/Casel NCF1_Human NCF1_Mouse Bern1_Yeast Sot2_Schpo P40/Human D9461.13/Yeast YDL113c/Yeast YDL113c/Yeast YDL113c/Yeast Yd62_Yeast Ylx62_Yeast Ylx62_Yeast Ylx62_Yeast Ylx62_Yeast Ylx62_Yeast Ylx62_Yeast Ylx62_Yeast Ylx62_Yeast Ylx72_Yeast Mdm1_Yeast Ylx72_Yeast Mdm1_Yeast PLD1/Casel Sp14Yeast D24276(Yryss D24076(Yeast D24276(Yryss D24076(Yryss D24076(Yryss D24076(Yryss) D240776(Yryss) D2407776(Yryss) D24077776(Yryss) D240777776(Yryss) D2407777776(Yryss) D240777777777777777777777777777777777777	. (5). HHNHHHNHHNHHNHHHNH hhnh h R L bL h (5). KSVAEK BLPLIQRFIKSIFDASEEIAH. SELVTTFHPLLRO . (5). KSVAEK BLPLIQRFIKSIFDASEEIAH. SELVTTFHPLLRO . (5). KDVAAK KRIELNSYLQSLMASTOVAE CLVCTFHPLLRO . (2). QRAAC BLQCTITEYCSTIMSIFTKIER CPHLLOFFKVRPDD . (2). QRAAC BLQCTITEYCSTIMSIFTKIER CPHLLOFFKVRPDD . (2). QRAAC BLQCTITEYCSTIMSIFTKIER CPHLLOFFKVRPDD . (2). QRAAC BLQCTITEYCSTIMSICSIC PARLEE NELVKIFTLELDGD . (2). NSITKK NKEDINIYVAL VNLPDYISR. SEMVHSLFVVLNNG . (2). GLAEMETPALNAYMKSILSIPVVLN. OEDVRIFTYGSPYD . (3). QEIALMETPALNAYMKSILSIPVVLN OEDVRIFTYGSPYD . (3). QEIALMETPALNAYMKSILSIPVVLN OEDVRIFTYGSPYD . (4). AEFLEK KRAALSRYLQRIVNHTMLC DPUNETLEKEELP . (4). ENFIEN RRFQMSSMIKNICQDPVLQK DKDFLJFINDEFNN 	<ul> <li>US2192 (1625-1725)</li> <li>US2193 (1410-1510)</li> <li>Z59660 (943-1041)</li> <li>M25665 (20-125)</li> <li>Z36069 (294-404)</li> <li>U1239 (105-413)</li> <li>X77094 (77-140)</li> <li>U33007 (137-236)</li> <li>Z74161 (173-301)</li> <li>U35225 (161-272)</li> <li>Z48613 (146-247)</li> <li>Z5255 (256-162)</li> <li>A U00059 (91-214)</li> <li>U1139 (21-25)</li> <li>X56371 (116-21)</li> <li>U1333 (29-160)</li> <li>Z5255 (225-383)</li> <li>Z5042 (131-247)</li> <li>R21308</li> <li>D24276</li> <li>D24076</li> <li>R55144</li> <li>H17597</li> <li>L44419</li> <li>N82288</li> </ul>
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Fig. 1. See caption on facing page.

#### The PX domain family

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Fig. 1. (Continued from facing page.) Multiple alignments of (a) PX domain, (b) TPR, and (c) OPR sequences. Secondary structure predictions, calculated using PHD (Rost & Sander, 1994), are shown above the alignments [H/h denotes an  $\alpha$ -helix and E/e a  $\beta$ -strand, predicted with expected accuracies of  $\geq$ 82% (upper case) or otherwise  $\geq$  72% (lower case)]. EMBL database accession codes and residue limits are shown following the alignments, except in (b) [human p67<sup>phox</sup>: M32011 (residues 6–154); Synechocystis sp. ORF: D10474 (61–230); and, S. cerevisiae Ssn6p: M23440 (224–397)]. (a) Alignment of PX domain sequences constructed using ClustalW (Thompson et al., 1994). Residues conserved in ≥60% of sequences are shown in outline; hydrophobic residues (A, C, F, L, M, V, and Y), present in  $\geq$ 90% of sequences are in bold. Dots indicate insertions/deletions, whereas dashes indicate incomplete sequences. Intervening sequences are represented by numbers in parentheses. In closely similar paralogues (e.g., p47<sup>phox</sup> and Cpk PtdIns 3-kinases) further sequence similarity is evident within regions N-terminal to the sequences shown here, implying the presence of (an) additional secondary structure(s); construction of accurate alignments in this region, however, was not possible. The C-terminal limit shown is coincident with the C-termini of Yor357c/Yeast and Yhq5\_Yeast. The last 12 sequences represent translations of expressed sequence tags (ESTs); of these, some contain contributions from overlapping ESTs. (b) Four TPR motifs in  $p67^{phox}$ . An initial Blastp (Altschul et al., 1994) search using the human  $p67^{phox}$  sequence ("NCF2\_HUMAN," residues 1–223) produced probabilities p(n) of matching by chance over n alignment blocks of  $p(2) = 6.5 \times 10^{-4}$  for Synechocystis sp. ORF248 and  $p(2) = 3.1 \times 10^{-3}$  for S. cerevisiae Ssn6p; other TPR-containing sequences (e.g., S. cerevisiae OM70, human Cdc27, and E. nidulans BirnA) were also represented as top scoring sequences. Four-way alignments of two  $p67^{phox}$  TPRs and either two Synechocystis sp. ORF248 or two S. cerevisiae Ssn6p TPRs produced typical p-values of  $10^{-3}$ - $10^{-5}$  (Schuler et al., 1991); aligning the four p67<sup>phox</sup> TPRs produced a p-value of  $3.7 \times 10^{-5}$  (Schuler et al., 1991). Positions where amino acids form the hole (positions 4, 7, 8, and 11) and the knob (positions 20, 24, and 27) of TPRs are shown in outline; the three most frequent residues at these positions in TPRs (Sikorski et al., 1990) are also given. P67phox TPR1 appears to contain only 31 residues, and a truncated TPR appears to follow TPR3 (underlined). The amino acid Gly<sub>78</sub> substituted in an autosomal recessive CGD (de Boer et al., 1994) is in lower case (p67<sup>phox</sup> TPR3 position 8). (c) Alignment of p40<sup>phox</sup>-like OPR sequences (conserved acidic residues shown in outline and hydrophobic residues conserved in all but a single sequence shown in bold). These sequences scored highly in a profile database search (Birney et al., 1996) with the addition of Ref(2)p, which appears to be an orthologue of p62B, a Lck SH2 domain-binding ligand. Four-way alignments of diverse OPRs yielded typical p-values of  $10^{-7}$ - $10^{-14}$  (Schuler et al., 1991), demonstrating that these sequence similarities are significant.

(PLD) orthologues from *H. sapiens*, *S. pombe*, *S. cerevisiae*, and *C. elegans* (cf. Ponting & Kerr, 1996). These similarities could not be demonstrated to be significant using MoST, SWise, or Blastp searches. However, three-way MACAW alignments of PLD1 orthologues with two PX domain-containing proteins revealed two alignment blocks with low probabilities of aligning by chance (e.g., for human PLD1, p47<sup>phox</sup>, and SNX1,  $p = 3 \times 10^{-3}$  and  $1 \times 10^{-2}$ ). Assignments of PX domains in N-terminal regions of PLD1, therefore, are made tentatively, and further data are required to clarify this issue.

PX domains may possess SH3 domain-binding functions since it is noted that the majority of PX domains contain a polyproline motif X-P-p-X-P (where X is an aliphatic residue, particularly Ile or Leu, or else Met) within the polypeptide linking  $\alpha$ -helices 1 and 2 (Fig. 1a). This motif is identical to the left-handed polyproline type II helices that bind many SH3 domains (reviewed in Pawson, 1995) and may also bind WW domains (Chan et al., 1996). Other potential ligands of PX domains that do not contain SH3 domains include Cdc24p (Petersen et al., 1994) and Ste20p (Leeuw et al., 1995), since these are known to bind Bem1p via regions that overlap with its PX domain (Fig. 2). The PX domains of SNX1 and Mvp1p have been suggested to bind vesicular trafficking machinery (Kurten et al., 1996), including perhaps dynamin homologues (Ekena & Stevens, 1995). In contrast to this potential role in vacuolar protein sorting, the PX domain in Vam7p may, instead, function by regulating S. cerevisiae vacuolar assembly (Wada & Anraku, 1992).

The presence of PX domains in SH3-containing proteins Bem1p, Scd2, and phox proteins provides an additional common feature in eukaryotic signalling pathways involving Rho-related GTPases. In *S. cerevisiae*, Bem1p binds to the putative scaffolding protein Ste5p and to the Rho-type GTPase Cdc42p, thereby linking GTPase activity with Ste20p, the kinase initiator of the mitogen-activated protein kinase (MAPK) cascade (Peterson et al., 1994; Leeuw et al., 1995; Lyons et al., 1996). A similar pathway has been shown in *S. pombe* (Chang et al., 1994). In humans, the scaffold is provided by the sum of NADPH oxidase subunits that bind both a GTPase, Rac1, and Pak, a human homologue of Ste20p (Diekmann et al., 1994; Manser et al., 1994; Prigmore et al., 1995). PX domain-containing PtdIns 3-kinases and PLD isoforms may further regulate the mammalian pathway via the activation of protein kinases that phosphorylate p47<sup>phox</sup> (Ding et al., 1995; McPhail et al., 1995).

Examination of other regions of phox proteins using Blastp (Altschul et al., 1994) revealed two further observations. The N-terminal region of p67<sup>phox</sup> was found to contain four imperfect copies of the TPR motif (Fig. 1b). These are 34 amino acid motifs, predicted to form amphipathic  $\alpha$ -helices, that are thought to self-associate via a 'knob and hole' mechanism whereby a bulky hydrophobic residue (position 24; Fig. 1b), flanked by two small residues (positions 20 and 27), fits into a depression formed by residues at positions 4, 7, 8, and 11 (Sikorski et al., 1990). This is likely to account for the absence of the p67<sup>phox</sup> protein in an autosomal recessive CGD patient who is homozygous for a mutation causing the amino acid change  $Gly_{78} \rightarrow Glu$  (de Boer et al., 1994). This residue maps to position 8 of TPR3, which is critical for formation of the non-polar 'hole.' Disruption of TPR self-association as a consequence of this mutation is likely to destabilize p67<sup>phox</sup> folding and/or structure in a manner previously predicted for similar TPR position 8 Gly  $\rightarrow$  Asp substitutions in temperature-sensitive nuc2 and CDC23 mutants (Hirano et al., 1990; Sikorski et al., 1993). The p67<sup>phox</sup> TPRs (residues 6-154) are wholly contained within the Rac1 GTP-binding region (residues 1-199; Diekmann et al., 1994), suggesting that destabilization of TPRs results in abrogation of Rac1-mediated signalling.



**Fig. 2.** The domain architecture of representative PX domain-, TPR-, and OPR-containing proteins. Abbreviations: DH, dbl-homologous domain; PH, (split) pleckstrin homology domain; PIK, a domain common to PI3Ks. (a) Phox subunits, Bem1p and Cdc24p. Protein-protein interactions, determined using domain deletion experiments (Diekmann et al., 1994; Petersen et al., 1994; deLeo et al., 1995; Fuchs et al., 1995; Bender et al., 1996; de Mendez et al., 1996; Matsui et al., 1996), are indicated by brackets; P denotes proline-rich regions; S denotes serine residues in p47<sup>phox</sup> that are phosphorylation targets of kinases. A putative calponin-homology (CH) domain (Castresana & Saraste, 1995) in Cdc24p has not been proposed previously. (b) Human SNX1, yeast Vam7p, and *Drosophila* Cpk. The majority of SNX1-homologues contain coiled coil regions, as predicted using COILS (Lupas et al., 1991). The C-terminal SNX1 coiled coil has been shown to bind the epidermal growth factor (EGF) receptor (Kurten et al., 1996).

P40phox and the Bem1p-binding protein, Cdc24p, each possess single OPR motifs, as do Scd2 and the MAP kinase kinase, MEK5 (English et al., 1995). Further searches (Altschul et al., 1994; Birney et al., 1996) using these as query sequences yielded similar motifs in atypical PKC  $\iota/\lambda$  and  $\zeta$  isoforms (Ono et al., 1989), a TRK-fusion gene product (Greco et al., 1995) and three ZZ domaincontaining proteins, CA125, ref(2)P and p62, a p56lck-binding protein (Dezelee et al., 1989; Campbell et al., 1994; Park et al., 1995; Ponting et al., 1996). The Bem1p-binding region of Cdc24p (residues 780-854; Petersen et al., 1994) contains its OPR (residues 814-841), suggesting that this function may be mediated by the OPR motif. Similarly, data (Fuchs et al., 1995) indicate that p40<sup>phox</sup> may bind p67<sup>phox</sup> via its OPR sequence (Fig. 2). OPRs, which do not fit the consensus sequence of EF-hands, possess four conserved acidic residues (positions 7, 9, 11, and 20; Fig. 1c), suggesting that these repeats bind divalent cations, such as Ca2+. This is supported by observations that the interaction between Cdc24p to Bem1p is inhibited by Ca2+ (Zheng et al., 1995).

In conclusion, a novel domain was found to occur in two component subunits of NADPH oxidase (p47<sup>phox</sup> and p40<sup>phox</sup>) and in a variety of other eukaryotic molecules; in addition, four TPRs were identified in the Rac1<sup>GTP</sup>-binding region of p67<sup>phox</sup>. The latter observation is likely to account for the molecular defect in a well-characterised case of CGD (de Boer et al., 1994). This contribution to the definition of domains in NADPH oxidase subunits is expected to facilitate characterization of their structures and functions, and the understanding of the molecular basis of autosomally inherited CGDs.

### Note added in proof

Since completion of this work several PX domain-containing sequences have been deposited in databases (a human ORF, and *C. elegans* C05d9.1, F25h2.2, and F17h10.3). Using a revised alignment, profile methods now indicate the presence of PX domains in a second bud emergence protein, Bem3p, and in vacuolar protein sorting-associated protein VPS17p. These sequences have been added to Figure 1A.

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