

One-thousand-and-one substrates of protein kinase CK2?

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ABSTRACT CK2 (formerly termed “casein kinase 2”) is a ubiquitous, highly pleiotropic and constitutively active Ser/Thr protein kinase whose implication in neoplasia, cell survival, and virus infection is supported by an increasing number of arguments. Here an updated inventory of 307 CK2 protein substrates is presented. More than one-third of these are implicated in gene expression and protein synthesis as being either transcriptional factors (60) or effectors of DNA/RNA structure (50) or translational elements. Also numerous are signaling proteins and proteins of viral origin or essential to virus life cycle. In comparison, only a minority of CK2 targets (a dozen or so) are classical metabolic enzymes. An analysis of 308 sites phosphorylated by CK2 highlights the paramount relevance of negatively charged side chains that are (by far) predominant over any other residues at positions n+3 (the most crucial one), n+1, and n+2. Based on this signature, it is predictable that proteins phosphorylated by CK2 are much more numerous than those identified to date, and it is possible that CK2 alone contributes to the generation of the eukaryotic phosphoproteome more so than any other individual protein kinase. The possibility that CK2 phosphosites play some global role, e.g., by destabilizing α helices, counteracting caspase cleavage, and generating adhesive motifs, will be discussed.—Meggio, F., Pinna, L. A. One-thousand-and-one substrates of protein kinase CK2? *FASEB J.* 17, 349–368 (2003)

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BACKGROUND

AS MENTIONED in a recent historical review on the origins of protein phosphorylation (1), the first protein kinase activity was discovered in 1954 by Burnett and Kennedy (2) (for an amusing recollection, see E. P. Kennedy’s autobiography, ref 3). Such an activity was first detected in rat liver using casein as phosphorylatable substrate and later found ubiquitously in a variety of organisms and tissues. Curiously, it was not due to any of the classical protein kinases popular early in the protein phosphorylation era (namely, phosphokinase, PKA, PKG, PKC, etc.) but to two in some way unusual protein kinases, provisionally termed casein kinases or

phosvitin kinases, now mostly referred to as CK1 and CK2, whose physiological roles remained enigmatic for a long time (for an historical review, see ref 4) and still are incompletely understood. At variance with the majority of protein kinases, which are quiescent unless their activity is triggered in response to specific stimuli and effectors, CK2 is constitutively active and independent of either second messengers or phosphorylation events. Such a lack of “on/off” regulatory mechanism is especially striking in view of the quaternary structure of CK2, reminiscent of that of PKA, with two catalytic subunits (α and/or α') assembled with a dimeric “regulatory” β subunit that, however, neither switches on nor off catalytic activity monitored with specific peptide substrates. In contrast, as discussed elsewhere (5), the phosphorylation of a subset of protein substrates is deeply affected by the β subunit through mechanisms not yet understood that do not imply major changes in catalytic efficiencies or site specificity. Even more paradoxical is the fact that CK2, after having remained for more than two decades a “kinase in search of its substrates,” later turned out to be the probably most pleiotropic protein kinase existing in eukaryotic organisms. This, in conjunction with the observation that CK2 is essential to cell viability and appears to be implicated in global processes such as tRNA and rRNA synthesis (6), apoptosis (7), and cell survival (8) and transformation (9), accounts for the increasing popularity of CK2, especially among “outsiders.” As summarized in **Fig. 1**, the first physiological targets of CK2 were detected in the late 1970s to reach the number of 50 in 1990. This figure rose to ~100 in 1994 and to 160 in 1997. Although many structural and functional properties of CK2 are dealt with in other reviews and commentaries (e.g., refs 5, 8, 11, 13–16), this review article focuses on proteins that are phosphorylated by CK2. The updated repertoire of CK2 substrates presented here includes 307 proteins; this number is steadily increasing day after day, and from the analysis of potential phosphoacceptor sites it can be predicted that proteins phosphorylated by CK2 make up a substantial proportion of the “phosphoproteome”

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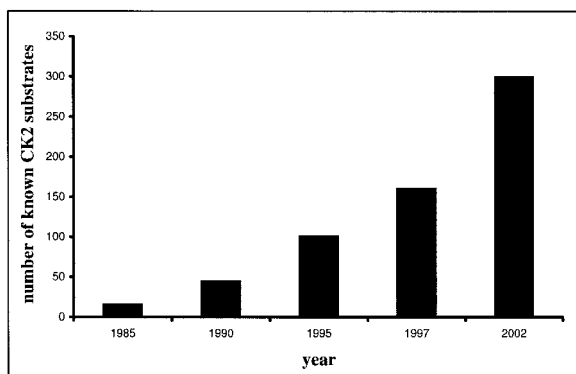


Figure 1. Time course of CK2 substrates detection. Data drawn from refs 10–13 and present paper.

and that CK2 dependent phosphorylation may play some general functions within the cell.

PROTEINS PHOSPHORYLATED BY CK2: AN UPDATED REPERTOIRE

To the best of our knowledge, there are at least 307 proteins whose phosphorylation by CK2 has been reported in the literature; but this is likely to be an underestimate since several proteins may have escaped our search. The proteins are listed alphabetically in **Table 1** together with a short description of their function, if known, and the pertinent reference(s). In the majority of cases, the actual implication of CK2 in the *in vivo* phosphorylation of these proteins was corroborated by the observation that the same residue(s) affected *in vitro* by CK2 are found phosphorylated in native proteins isolated from living cells. In many instances the physiological implication of CK2 is also supported by pharmacological and/or genetic criteria. For a detailed analysis of the individual situations, we suggest the reader consult the papers quoted in **Table 1** and references therein. In only 64 proteins (denoted in **Table 1** by italics) has phosphorylation by CK2 been demonstrated *in vitro*, whereas its *in vivo* occurrence is still a mere speculation. Sometimes, however, coincidental arguments are available based on analogy with physiological targets, favorable kinetic parameters, and conservation of optimal consensus sequence(s) that support the possible implication of CK2 in the phosphorylation of these proteins also *in vivo*.

More telling than their huge number perhaps is the nature of the proteins listed in **Table 1**. As summarized in **Table 2**, there are 60 transcriptional factors among them, and 50 proteins affecting the structure of DNA/RNA and/or implicated in RNA synthesis and translation, highlighting altogether the paramount importance CK2 must have in gene expression. Also striking is the number of signaling proteins, which, with the exclusion of transcriptional ones, are more than 80, including 11 calcium binding proteins, 10 protein

kinases, and 8 protein phosphatases. Finally, the presence of almost 40 viral proteins corroborates the view that by virtue of its constitutive activity, CK2 has been adopted by viruses as a phosphorylating agent of proteins essential to their life cycle and could therefore represent an enticing target for antiviral drugs. By comparison, metabolic enzymes (9 altogether) represent only a small tribe amid the population of CK2 substrates. At variance with PKA and other second messenger-dependent kinases, CK2 does not drastically affect the catalytic properties of these enzymes, consistent with its main implication in basal functions rather than in acute responses to transient stimuli. Pertinent to this may be the presence among CK2 targets of some proteins that play global functions within the cell, like calmodulin, RNA polymerases, topoisomerases I and II, the oncosuppressor protein p53, and a series of initiation and elongation factors. Consistent with this scenario, a recent systematic study of protein–protein interactions in yeast (181) has disclosed the participation of two or more CK2 subunits to seven protein complexes, four of which are implicated in transcription/DNA maintenance/chromatin structure, one in RNA metabolism, one in protein/RNA transport, and one in signaling.

THE STIGMATA OF CK2 PHOSPHOACCEPTOR SITES

In 175 of the protein substrates listed in **Table 1**, the sites affected by CK2 have been identified; since in many proteins the residues phosphorylated were more than one, we know the structure of 308 sites phosphorylated by CK2 (**Table 3**). This is probably the largest repertoire of sites phosphorylated by an individual kinase available to date. A cursory analysis reveals how reliable were predictions based on model peptide substrates derived from the first CK2 sites identified more than two decades ago, when only very few proteins phosphorylated by CK2 were known. Those pioneer studies showed that CK2 phosphorylation is specified by multiple acidic residues located mostly downstream from the phosphorylatable amino acid (serine being preferred over threonine), the one at position $n+3$ playing the most crucial function (205, 206). They also disclosed the negative role exerted by basic residues at any position close to Ser/Thr and of a prolyl residue at position $n+1$ (207), where it is instead absolutely needed to create the consensus sequence for different classes of proline-directed kinases. All the sites listed in **Table 3** conform to these rules, with only very few and partial exceptions. The average number of negatively charged side chains (either Glu or Asp or phosphorylated residues) surrounding the phosphorylated amino acid (which is serine in 265 sites, threonine in 42 cases and tyrosine in just one case) is 5.2. In only nine sites (<3%) is just one acidic determinant present (**Fig. 2A**), and in these cases it is invariably located at the crucial $n+3$ position, thus fulfilling the minimum consensus

TABLE 1. *Repertoire of proteins phosphorylated by CK2^a*

Number	Substrate	Description	Reference
1	<i>A-, B-Myb</i>	<i>Nuclear transcriptional activators</i>	17
2	<i>A6-related protein</i>	<i>Ephrin A family members tyrosine kinase receptor</i>	18
3	ABF-1	Multifunctional yeast nuclear transcription factor	13
4	ACC	AcetylCoA carboxylase	11
5	ADV E1a	Adenovirus type 5 E1A transactivator protein	12
6	<i>Annexin</i>	<i>Calcium-phospholipid binding, p58 protein</i>	19
7	ANTP	Homeotic Antennapedia transcription factor	20
8	APC	Tumor suppressor adenomatous polyposis coli protein	21
9	APE/Ref1	DNA repair apurinic protein endonuclease	22
10	APP- β	β -amyloid precursor neuronal receptor protein	23
11	<i>Apyrase</i>	<i>Nervous system, CaM binding, nuclear NTPase</i>	24
12	AQP4	Aquaporin 4 brain water channel	25
13	AR	Androgen receptor	11
14	Arrestin-3	G-protein-coupled receptors regulator	26
15	ATF/E4TF3	cAMP-dependent transcription factor 1	27
16	ATF-1	BZIP domain-containing transcription factor	28
17	B23	Nucleolar protein B23, NPM (nucleoplamin, nucleophosmin)	11
18	BDV P-protein	Borna disease virus RNA polymerase transcriptional cofactor	29
19	BID	FAS-mediated cytoplasmic apoptotic element	30
20	<i>bPrP</i>	<i>Bovine prion protein</i>	31
21	BPV-1 E1	E1 protein of bovine papilloma virus, ATP-dependent DNA helicase	32
22	BRCA1	Breast cancer nuclear protein type 1, transcriptional regulator	33
23	<i>BTF3a</i>	<i>RNA polymerase II transcription factor</i>	34
24	<i>C1r</i>	<i>Human complement proteinase</i>	13
25	C3	Human complement component	35
26	C9	Human complement component	36
27	<i>CaBP1, 2, 4</i>	<i>ER calcium binding proteins</i>	37
28	CaCBR	Calcium-channel blockers receptor	11
29	CACTUS	Cytoplasmic inhibitor of Rel-related transcription factor Dorsal	38
30	<i>Caldesmon</i>	<i>Calcium binding protein</i>	12
31	Calmodulin	Calcium binding protein	11
32	Calnexin	Calcium binding protein	39
33	<i>Cabreticulin (plant)</i>	<i>Calcium binding protein</i>	13
34	Calsequestrin	Calcium binding protein	12
35	<i>CarD</i>	<i>Bacterial transcriptional factor</i>	40
36	Catenin- β	<i>Wnt signaling component</i>	41
37	Caveolin	Membrane integral caveolae, G α -interacting component	13
38	CCA1	<i>Circadian clock-associated 1, Myb-related transcription factor</i>	42
39	<i>CD10/NEP24.11</i>	<i>CD10 neutral endopeptidase 24.11, neprilysin</i>	43
40	<i>CD163</i>	<i>Member of scavenger receptor cysteine-rich superfamily</i>	44
41	CD44	Yaluronate receptor	45
42	CD45	Receptor tyrosine phosphatase	13
43	CD5	T and B1 cell receptor-mediated immune response glycoprotein	46
44	Cdc28	Yeast homolog of eukaryotic cdc2	47
45	CDC34	Human ubiquitin-conjugating enzyme 2	48
46	CDC37	Yeast cell cycle regulatory protein	13
47	CDV-P	Canine distemper virus RNA polymerase transcriptional cofactor protein P	49
48	<i>CENP-B</i>	<i>Human centromere protein B</i>	13
49	CFOCF1-ATPase	Chloroplast ATPase β subunit	50
50	<i>CHAT</i>	<i>Choline acetyltransferase</i>	51
51	<i>CHPV-PP</i>	<i>Chandipura virus transcriptional cofactor phosphoprotein</i>	52
52	Chromogranin B	Acidic, acid-stable secretory granules component, secretogranin	53
53	c-Jun	AP-1 inducible transcription factor	12
54	CK2 β	Regulatory subunit of protein kinase CK2	54
55	Clathrin light chain b	Coated vesicles component	11
56	c-Myb	Nuclear transcriptional phosphoprotein	12
57	c-Myc	Nuclear transcriptional phosphoprotein	11
58	<i>Conglycinin-β</i>	<i>Soybean storage protein alpha subunit</i>	55
59	Connexin (Cx) 45.6	Avian lens GAP junctions component	56
60	<i>CPTP1</i>	<i>Chicken tyrosine phosphatase 1 homologue</i>	57
61	CREB	cAMP-responsive element binding protein, transcription factor	12
62	CREM	cAMP-responsive element modulator, transcription factor	12
63	Csx/Nkx2.5	Cardiac homeodomain-containing transcription factor	58
64	<i>CT</i>	<i>CTP: phosphocoline cytidyltransferase</i>	13
65	CTCF	Multi-Zn finger nuclear transcriptional repressor	59

TABLE 1. (continued)

Number	Substrate	Description	Reference
66	<i>CUT proteins</i>	<i>Drosophila and mammals DNA binding proteins</i>	60
67	<i>Cyclin H</i>	<i>CDK7-activating subunit</i>	61
68	DARPP-32	Dopamine and cAMP-regulated phosphoprotein	11
69	Dishevelled 1 and 2	Wnt signalling segmentation polarity proteins	41
70	<i>Dlk</i>	<i>DAP-like, leucine zipper-containing Ser/Thr protein kinase</i>	62
71	DNA ligase I	Mammalian isoform I of DNA ligase	12
72	DSIP	Delta sleep-inducing neuropeptide	12
73	<i>Dynamain</i>	<i>Phosphorylation-regulated, microtubule-associated GTPase</i>	13
74	<i>Dynein</i>	<i>Cytoplasmic mechanochemical ATPase</i>	63
75	Dystrophin	Cytoskeleton anchoring protein	12
76	E-47	Myogenic regulatory transcription factors (MRFs) interacting protein	13
77	EBNA-2	Epstein-Barr transcriptional regulator nuclear antigen 2	12
78	EBV ZEBRA	Epstein-Barr virus nuclear transactivator protein	12
79	EBV-SM	Epstein-Barr virus ribonucleotide reductase phosphoprotein	13
80	E-Cadherin	Calcium-dependent cell adhesion protein	64
81	EF-1 β	Rabbit elongation, GDP/GTP exchanging factor 1 beta	11
82	EF-1 δ	Rabbit elongation, GDP/GTP exchanging factor 1 delta	13
83	Egr-1	Early growth response transcriptional regulator	13
84	eIF2 α	Yeast eukaryotic translational initiation factor 2a	13
85	eIF2 β	Rabbit eukaryotic translational initiation factor-2 b	11
86	eIF3	Rabbit eukaryotic translational initiation factor-3	11
87	eIF4B	Rabbit eukaryotic translational initiation factor-4B	11
88	eIF4e/p20	Yeast eukaryotic translational initiation factor-4e	13
89	eIF5	Rabbit eukaryotic translational initiation factor-5	11
90	EKLF	Erythroid Kruppel-like transcription factor	65
91	EN	<i>D. melanogaster</i> segmentation polarity engrailed protein	13
92	ER (D. melanogaster)	Enhancer of rudimentary gene product	66
93	ER-E2	Human estrogen receptor	13
94	Factor Va	Prothrombinase essential cofactor	12
95	FAF-1	FAS-associated apoptotic factor 1	67
96	<i>Fibrinogen (α chain)</i>	<i>Blood coagulation protein</i>	11
97	Filaggrin	Keratin matrix developmental protein	11
98	FKBP25	FK506 binding receptor, nucleolin associated, peptidyl-prolyl cis-trans isomerase (PPIase), p25 protein	12
99	FKBP46	Insect FK506 binding PPIase, protein p46	68
100	FKBP52	FK506 binding, PPIase, protein p52	69
101	Fpr3	Yeast nucleolar FK506 binding, PPIase	70
102	Fragmin	Physarum polycephalum actin microfilament component	13
103	Furin	Paired basic residue cleaving endoprotease	13
104	GAIP	G α interacting protein, G-protein signaling regulator	71
105	GAR2	Yeast, nucleolin-like, nucleolar protein	72
106	GBF1	<i>A. thaliana</i> transcription factor	13
107	gbLOX	Soybean glycyrrhizin binding p96 and p94 lipoxygenase	73
108	GEF	Rabbit reticulocyte guanine nucleotide exchange factor (eIF2Be)	11
109	<i>GL-p100</i>	<i>Glycyrrhizin binding iron protein p100</i>	13
110	<i>Glycophorin</i>	<i>Erythrocyte cytoskeletal component</i>	11
111	GRO/TLE	Groucho/transducin-like enhancer transcriptional repressors	74
112	GRP94	Sarcoplasmic reticulum calcium binding protein	13
113	GS	Glycogen synthase	11
114	HASPP28	Heat and acid stable, PDGF-associated, P-protein of 28 kDa	13
115	HCI	Hemin-controlled translational inhibitor	75
116	HCMV gB	Human CMV major envelope glycoprotein B	76
117	HCMV pp65	Human CMV lower matrix P-protein	13
118	HCP	Histidine-rich calcium binding protein	13
119	HDAC1	Histone deacetylase 1, nuclear transcriptional regulator	77
120	HDV small AG	Hepatitis delta virus small antigen, nuclear phosphoprotein	13
121	<i>HIV-1 gp120</i>	<i>HIV-1 exterior membrane glycoprotein 120</i>	78
122	<i>HIV-1 gp41</i>	<i>HIV-1 transmembrane glycoprotein 41</i>	78
123	<i>HIV-1 p27, p17</i>	<i>HIV-1 capsid proteins</i>	78
124	HIV-1 Rev	HIV-1 nonstructural protein	13
125	HIV-1 RT p66	Reverse transcriptase p66 subunit	79
126	HIV-1Vpu	HIV-1 nonstructural protein	13
127	<i>HIV-2 Nef</i>	<i>Nonstructural protein of HIV-2</i>	12
128	<i>HMG-14</i>	<i>High mobility group nonhistone chromosomal protein 14</i>	11
129	<i>HMG-17</i>	<i>High mobility group nonhistone chromosomal protein 17</i>	11

TABLE1. (continued)

Number	Substrate	Description	Reference
130	HMG1A	<i>High mobility group protein 1A of Chironomus tentans</i>	80
131	HMGB1, 2/3	Maize chromosomal high mobility proteins of B family	81
132	HMG-I (Y)	High mobility group nonhistone chromosomal protein I (Y)	12
133	HMG-like protein P1	Nuclear phosphoprotein P1	11
134	<i>HnRNP A1</i>	<i>Major component of human eukaryotic hnRNP particles</i>	12
135	HnRNP A2	Heterogeneous nuclear ribonucleoprotein A2	82
136	HnRNP C	Heterogeneous nuclear ribonucleoprotein C	83
137	Hoxb-6	Murine homeodomain protein 6, transcription factor	84
138	Hoxb-7	Homeodomain protein b7, transcription factor	85
139	HPV-E7	Human papilloma virus transforming and transactivating oncoprotein E7	12
140	HS1	Haematopoietic lineage cell-specific protein 1	86
141	HSIX-1	Human class of homeodomain developmental proteins	87
142	<i>HSP90</i>	<i>Heat shock molecular chaperone protein 90 α and β</i>	11
143	HSV gE	Glycoprotein E of HSV	88
144	HSV RR1	Ribonucleotide reductase type 1 of HSV	89
145	<i>HSV α22</i>	<i>Herpes simplex virus type 1 transcriptional regulatory protein</i>	90
146	HSV-VP1/2, 13/14, 22	Transcription regulatory proteins of HSV	91
147	HSV-VP16	ICP25, transcriptional regulator of HSV	92
148	HY5	<i>A. thaliana</i> transcription factor	93
149	ICP27	IE63, transcriptional regulator of HSV	94
150	IFI16	Interferon-inducible myeloid differentiation transcriptional activator p16	95
151	IGF BP-3	Insulin-like growth factor binding protein 3	96
152	IGF-BP-1	Insulin-like growth factor binding protein 1	12
153	IGF-IIR	Insulin-like growth factor II receptor	11
154	I κ B α	NF- κ B cytoplasmic, transcriptional regulator, inhibitor α	97
155	I κ B β	NF- κ B cytoplasmic, transcriptional regulator, inhibitor β	98
156	Influenza virus PA	Viral RNA polymerase transcriptional cofactor	99
157	<i>INH-2</i>	<i>Inhibitor-2 of protein phosphatase 1</i>	11
158	IR	<i>Insulin receptor</i>	11
159	IRF-1	Interferon regulatory transcriptional factor 1	100
160	IRF-2	Interferon regulatory transcriptional factor 2	101
161	IRS-1	Insulin receptor substrate	12
162	Kell	Human erythrocyte membrane minor protein, metalloprotease	102
163	Kv3.1	Potassium channel	103
164	Kx	Human erythrocyte membrane transporter minor protein	102
165	L1	Neural cell adhesion molecule L1	104
166	<i>L5</i>	<i>RNA binding ribosomal protein</i>	105
167	La	Human autoimmune antibodies target La antigen	106
168	LACI	Lipoprotein-associated coagulation inhibitor serine protease	12
169	<i>Lactoferrin</i>	<i>Bovine and human iron binding protein</i>	107
170	Lamin-like proteins	Lamin-like proteins p71, p48, and p46 of pea lamina matrix	12
171	LDLR	LDL receptor	11
172	LHY	Late elongated hypocotyls, Myb-related plant transcription factor	108
173	LIS1	Lissencephaly microtubule associated P-protein	109
174	<i>m8</i>	<i>D. melanogaster basic helix-loop-helix type transcription factor</i>	110
175	MAP1B	Microtubule-associated protein 1B	11
176	Marburg virus NP	Nucleocapsid protein	111
177	Max	Heterodimeric partner of Myc oncoprotein, transcriptional regulator	12
178	MAZ	Myc-associated zinc finger transcription factor	112
179	<i>MDG4</i>	<i>Chromatin transposable gypsy element</i>	113
180	MDM-2	Murine double minute clone 2 oncoprotein-ubiquitin protein ligase E3	13
181	MDR1B gP	Multidrug resistance transporter 1B P glycoprotein, member of ABC transporter family	114
182	MEF2C	Myocyte enhancer transcription factor 2C	13
183	MPR300	Cation-dependent mannose-6-phosphate receptor of 300 kDa	115
184	MPR46	Cation-dependent mannose-6-phosphate receptor of 46 kDa	116
185	<i>MRLC</i>	<i>Sea urchin eggs myosin II regulatory light chain</i>	117
186	<i>mRNP</i>	<i>Reticulocyte messenger ribonucleoproteins</i>	118
187	<i>mSTII</i>	<i>Hsp70/Hsp90 organizing protein</i>	119
188	mUBF	Nucleolar RNA polymerase I transcription factor	12
189	MV-PP	Measles virus RNA polymerase transcriptional cofactor protein P	13
190	MVP	Major vault, lung resistance-related ribonucleoprotein	120
191	Myf-5	Muscle-specific transcription factor	121
192	MyoD-MRF4	Myogenic helix-loop-helix transcription factors	13

TABLE 1. (continued)

Number	Substrate	Description	Reference
193	Myosin HC	Skeletal and smooth muscle myosin heavy chain	12
194	Myosin LC	Gizzard myosin light chain	11
195	NAP-1	Nucleosome assembly protein 1 of <i>D. melanogaster</i>	122
196	NAP-1 and NAP-2	Nucleosome assembly human proteins 1 and 2	123
197	<i>NBP-60</i>	<i>Rat liver NLS binding protein</i>	13
198	NDPK A	Nucleoside diphosphate kinase A (nm23)	13
199	NDPK B	Nucleoside diphosphate kinase B	13
200	Neuroglycan C	Brain-specific transmembrane proteoglycan	124
201	Neuromodulin	B50 or GAP43, calmodulin binding protein	125
202	<i>NF-ATc</i>	<i>Activated T cell nuclear transcription factor</i>	126
203	NFκB/Rel p65	Nuclear κB transcription factor	127
204	NHCP p400	Nonhistone chromatin protein p400	128
205	NIPP-1	Nuclear inhibitor of PP-1	13
206	Nm HC myosin B	Nonmuscle (brain) myosin heavy chain B	12
207	NMDA channel	N-methyl-D-aspartate channel	129
208	N-Myc	Neuroblastoma nuclear transcription factor	12
209	NopA64	Allium cepa nucleolar phosphoprotein	130
210	Nopp140	Nucleolar shuttling P-protein	131
211	Nrfl	Nuclear respiratory transcription factor 1	132
212	NS5 protein	Nonstructural RNA polymerase transcriptional cofactor protein of dengue virus	133
213	NSP5	Nonstructural RNA binding phosphoprotein 5 of Rotavirus	134
214	Nucleolin	C23, p110, nucleolar chromatin-associated phosphoprotein	11
215	<i>Occludin</i>	<i>Membrane tight junction component</i>	135
216	ODC	Ornithine decarboxylase	11
217	Osteopontin	Glycosylated sialophosphoprotein	12
218	P0, P1, P2	Yeast ribosomal protein	12
219	p120	Human proliferation-associated nucleolar protein	12
220	p130	Nucleologenesis-associated nucleolar P-protein	136
221	<i>p21 (WAF1/CPI)</i>	<i>Cyclin-dependent kinase inhibitor</i>	137
222	<i>p210</i>	<i>Nuclear DNA binding protein</i>	12
223	p22	Epididymal fat cells acid soluble protein p22 (PHAS-1 homologue)	12
224	p32	ASF/SF2-associated transcriptional regulator, partner of ICP27 and of Rev	138
225	p34	Chloroplasts ribonucleoprotein	12
226	p34 cdc2	CDC2 kinase catalytic subunit p34	12
227	<i>p35, p15, p13</i>	<i>Bamboo shoots ribosomal P-proteins</i>	139
228	p47 (phox)	Component of leukocyte NADPH oxidase	140
229	p53 human	Tumor suppressor phosphoprotein p53, transcriptional regulator	12
230	p98 sea urchin	Sea urchin eggs DNA binding protein p98	12
231	Pacsin 1	FAP52-homolog brain cytoplasmic adapter, syndapin	141
232	PAM	Peptidylglycine a-amidating monooxygenase integral membrane protein	142
233	PC4	Human transcriptional regulator, positive coactivator 4	143
234	<i>PDH65</i>	<i>Pea DNA helicase</i>	144
235	PGD synthase	Prostaglandin D synthase	145
236	PHAS-I	Translational regulator, insulin and growth factor intracellular target	13
237	Phospholipase A1	Bovine testis, phosphatidic acid-preferring, soluble phospholipase	146
238	Phosphophorin	Dentin component	147
239	PIP kinase type IIa	Phosphatidylinositol phosphate kinase	148
240	PKA RII	Protein kinase A type II regulatory subunit	11
241	<i>PKC</i>	<i>Protein kinase C II β</i>	12
242	PLD1a	Phospholipase D1a	149
243	PP2Ca	Protein phosphatase type 2C	12
244	pp35, pp32	Proliferation-associated murine nuclear phosphoproteins	12
245	PR	Progesterone receptor	13
246	Proteasomes C8 and C9	Proteasome complex C8 and C9 components	13
247	Pro-Tα	Immune function mediator prothymosin α	12
248	PRS1/α7	Proteasome subunit 1	150
249	PRV pp62	Pseudorabies virus P-protein	11
250	PTEN	Tumor suppressor nonreceptor tyrosine phosphatase	151
251	PTP-S2	Chromatin-associated nuclear tyrosine phosphatase	152
252	PU-1	Ets-related transcription factor	12
253	PV VP1	Polyoma virus major capsid protein	13
254	RAB-17	Basic glycine-rich, abscisic acid-responsive, dehydration-induced gene product	12

TABLE 1. (continued)

Number	Substrate	Description	Reference
255	<i>RAD</i>	<i>CaM binding Ras-family GTPase</i>	153
256	RNA polymerase I	rRNA RNA polymerase	11
257	RNA polymerase II	mRNA RNA polymerase	11
258	RNA polymerase III	5S and tRNA RNA polymerase	13
259	<i>RPB6</i>	<i>Eukaryotic 14.4 kDa subunit of RNA polymerase</i>	154
260	RSV-PP	Respiratory syncytial virus, RNA polymerase transcriptional cofactor protein P	155
261	RVFV NSs	Rift Valley fever virus nonstructural protein	156
262	<i>SI00B</i>	<i>Glial-associated Ca²⁺ binding protein</i>	157
263	SAG	Ring-H2 zinc finger-containing antioxidant protein	158
264	SAPK1/JNK1	Stress-activated protein kinase-1/c-Jun amino n-terminal kinase-1	159
265	Sarcalumenin	Calcium binding protein	13
266	Simian CMV pAP	Assembly protein precursor peptidase	160
267	Sm MAK16	S. mansonii homologue of yeast cell cycle MAK16 protein	161
268	SMRT	Silencing mediator for retinoid/thyroid hormone receptor	162
269	Sp1	Zinc finger-containing transcription factor	163
270	<i>Spectrin</i>	<i>Erythrocyte cytoskeletal component</i>	11
271	<i>Spi-B/Spi-1(PU.1)</i>	<i>Hematopoietic transcription factors</i>	13
272	SRF	Serum response element (SRE) binding transcription factor	12
273	Srp1p	Yeast NLS receptor	164
274	SRP40	Yeast homolog of rat nucleolar Nopp140	13
275	SSR α	Signal sequence calcium binding receptor α for ER resident protein retention	39
276	<i>Stathmin</i>	<i>Multiphosphorylated microtubule-associated phosphoprotein</i>	13
277	STC1 and 2	Calcium and phosphate homeostasis regulatory proteins stannioalcin 1 and 2	165
278	SV40 large T Ag	SV40 large T antigen DNA binding protein	12
279	<i>Synaptotagmin</i>	<i>p65 synaptic vesicle protein</i>	12
280	<i>Syntaxin</i>	<i>Synaptotagmin-interacting, neurotransmitter transport SNARE protein</i>	166
281	Synuclein α	Parkinson's disease-associated brain amyloidogenic component	167
282	T-protein	Wheat germ T substrate	11
283	Tal-1	Basic helix-loop-helix transcription factor	13
284	Tau	Microtubule-associated protein	13
285	TBP	TATA box binding proteins (TFIID complex), transcription factor	168
286	<i>TCF-4</i>	<i>T cell transcription factor-4</i>	169
287	TFIIIA	X. laevis transcription factor IIIA	170
288	TFIIIB-TBP	Yeast TFIIIB complex TATA binding protein, transcription factor	171
289	TnT	Troponin T, calcium binding protein	11
290	Topoisomerase I	DNA structure affecting nuclear enzyme I	11
291	Topoisomerase II α	DNA structure affecting nuclear enzyme II	11
292	<i>TPS</i>	<i>Tryptophanyl-tRNA synthetase</i>	13
293	Treacle	Treacher Collins syndrome protein	172
294	TRHR	Tyrotropin-releasing hormone receptor	173
295	<i>Trypsin inhibitors</i>	<i>Soybean trypsin inhibitors</i>	174
296	Tr α 2	Thyroid hormone receptor variant	13
297	Tubulin β	Murine β -isoform microtubule main component	11
298	UBC3B	E2 ubiquitin-conjugating enzyme UBC3/CDC34 homolog	175
299	VAMP/synaptobrevin	Integral membrane protein of synaptic vesicles	13
300	VDR	1,25-Dihydroxy-vitamin D ₃ receptor	12
301	Vitronectin	Extracellular matrix adhesive glycoprotein	176
302	VMAT2	Vesicular monoamine transporter 2	177
303	VSV-P	Vesicular stomatitis virus, RNA polymerase transcriptional cofactor protein P	178
304	VZV-gpI	Varicella zoster virus glycoprotein I	12
305	XFG-5.1	X. laevis Kruppel-type zinc finger RNA binding protein	13
306	<i>XLC-OF 7.1</i>	<i>X. laevis ZFPs-FAX subfamily zinc finger protein</i>	179
307	ZFP47	D. melanogaster zinc finger protein	180

^a Italics denote protein substrates whose phosphorylation by CK2 is supported only by in vitro data.

S-x-x-E/D. The importance of this position is highlighted by the histograms of **Fig. 2B** showing that although acidic residues predominate at all positions between n-4 and n+7, they reach a peak approaching 90% at position n+3. The second most important position is n+1, where an acidic residue is found in 75% of the sites. Significantly, every time the acidic

determinant is lacking at n+1 it is found at n+3, and vice versa (**Fig. 2C, D**). Other important information that can be drawn from **Fig. 3B** is that basic residues are very rare at CK2 sites and tend to disappear, especially at positions n+1, n+2, and n+3, i.e., where the frequency of acidic residues is higher. The great majority of the 175 protein substrates whose phosphorylated

TABLE 2. Grouping of CK2 protein substrates according to function^a

Functional category	No. of proteins
Signaling proteins (with the exclusion of transcription factors) (e.g.: β -catenin, androgen receptor BID, calmodulin, caveolin, CD44, CD45, DARPP-32, disheveled, IRS1, MPR300, NLS receptor (Srp1p), PKA RII, I-2 of PP-1, PKC II β , SAPK1/JNK1, neuromodulin, NIPP-1, PTEN)	87
Transcription factors (e.g.: c-Myb, ABF-1, c-Myc, CREB, CREM, Hoxb-6 and 7, Ikb β , p53, MAX, mUBF, PU-1, SRF, Tal-1, TFIIIA,)	60
Proteins affecting DNA/RNA functions and protein synthesis (e.g.: B23, DNA ligase, DNA topoisomerase I, and II α , eIF2, nucleolin, RNA polymerase I, Nopp140, RNA polymerase III, hnRNP A2, TBP, ribosomal proteins P0, P1 and P2)	50
Viral proteins (e.g.: EBNA-2, HPV E7, HSV IE63, SV40 large T Ag, influenza virus PA, NSP5, EBV ZEBRA, HIV-2 Nef, HIV-1 Rev, HIV Vpu, HIV RT, HSV VP1, VZV-gpl)	38
Cytoskeleton and structural proteins (e.g.: spectrin, glycophorin, tubulin, connexin, vitronectin)	14
Metabolic enzymes (e.g.: acetylCoA carboxylase, glycogen synthase, ornithine decarboxylase, phospholipase D1a, PGD synthase)	9
Miscellaneous (e.g.: bPrP, CDC34, chromogranin, furin, myosin light chain, osteopontin, phosphophorin, α -synuclein, complement C3, immunophilin Fpr3)	49

^a See Table 1 for nomenclature and further details.

sites are listed in Table 3 belong to mammals (100 are human, 19 belong to rabbit, mouse, or bovine). In all these cases, the CK2 phosphorylation sites are conserved across the mammalian species considered.

A more detailed analysis of the data sequences of Table 3 is presented in Fig. 3 showing the frequency of individual amino acids at positions spanning between $n-4$ and $n+7$. It can be seen that glutamic and aspartic acids predominate over any other individual amino acid at all positions, although it is only at positions $n+3$, $n+1$, and, to a lesser extent, $n+2$ where acidic residues (including phosphorylated ones) appear to be critical, occurring with a frequency higher than that of *all the other residues collectively taken*. These data agree with the optimal sequence provided by an oriented library approach (208) (EDEESEDEE), although the relative frequency of Glu vs. Asp is slightly different. Note, however, that the library approach cannot take into account phosphorylated residues that are conversely found in several natural sites. This includes CK2 into that class of protein kinases whose targeting can be primed by another protein kinase (209), and accounts for the occurrence of multiphosphorylated sites among putative targets of CK2 (see also below, **Table 4**). A comparison between 500 top proteins selected as CK2 substrates by the Scansite prediction program (<http://scansite.mit.edu>) and the actual protein substrates listed in Table 1 highlights the potentials and limits of the oriented peptide library approach the Scansite program is based upon. Although the virtual optimal sequence of the library reflects quite faithfully the *overall* picture emerging from our analysis (Fig. 3), the majority of proteins listed in Table 1 (253 of 307) are *not* found in the 500 top list selected by Scansite. This is clearly due to the phenomenon of the "expanded

consensus sequence" (210), as the oriented peptide library approach tends to select the specificity determinants of a given kinase at positions where they are not really required. As already pointed out (210), this leads the Scansite program to select as first choice PKA substrates proteins with clusters of many consecutive arginines on the amino-terminal side of serine, whereas in most physiological substrates of PKA these are just two at positions $n-2$ and $n-3$. Likewise, the first choice substrates of CK2 appear to be those where the target residue is embedded between two entirely acidic sequences, a feature found only in relatively few CK2 targets, many of which are readily phosphorylated, although they include just 2 or 3 acidic residues at the most critical positions, notably $n+3$, $n+1$ and $n+2$ (see Fig. 2A and Table 3). The score of these latter sites is far away from the optimal score provided by the library and these are not considered by the Scansite analysis with due priority.

Mutational studies have shown that the tendency of CK2 to interact with negatively charged side chains at positions from $n-1$ to $n+4$ is determined by unique basic residues that in CK2 are present at the end of the activation loop, in the glycine rich loop, and at the beginning of helix-C, respectively (211). This would imply that typical peptide substrate will bind across the catalytic cleft, bridging between the lower and upper lobes of the kinase. It also accounts for the adverse effect of positively charged side chains that are consequently almost completely absent in CK2 sites. The architecture of the active site and the position of the basic residues involved in peptide substrate recognition are not significantly altered by association with the β subunit (212). This accounts for the observation that, although the β subunit may have profound effects on

TABLE 3. *Repertoire of phosphoacceptor sites phosphorylated by CK2*

No.	Protein	Sequence	Reference
1	ABF-1	EEDLS ₇₂₀ DENIQPE	13
2	ACC	SEDNS ₂₉ EDEISNLVKL	13
3	ADV E1a	GFPPS ₁₃₂ DDEDEEGEEFVLD	182
4	A-Myb	KRSRS ₇ EDEDDDLQY	17
5	ANTP	GNGVT ₃₅ DLDAQQMHHY GEPGS ₃₆₄ GGEDEITPPN	20
6	APC	LSIDS ₂₀₃₄ EDDLLQE	21
7	AQP4	QTKGS ₂₇₆ YMEVEDNRS	25
8	Arrestin-3	TNYAT ₃₈₂ DDDIVFEDFARL	26
9	ATF-1	VSSLS ₃₆ ESEESQDSSDSI	27
10	B23	EDAES ₁₂₅ EDEDEEDVK	11
11	BDV P-protein	REQLS ₇₀ NDELIKLV LAENS ₈₆ MIEAEVVRG	29
12	B-Myb	HYQDT ₁₈ DSDVPEQRD	17
13	bPrP	IHFSG ₁₅₄ DYEDRYRE	31
14	BPV-1 E1	DRYDS ₄₈ QDEDFVDNA EEEDS ₅₈₄ EEDGDSMRT	32
15	BRCA1	ISLFS ₁₅₇₂ DDPESDPSEDRA	33
16	Clr	SGYIS ₁₈₉ SLEYPSRYP	13
17	CaBP1	SALYS ₂₂ SSDDVIELTPSN EDDIDL ₄₂₇ S DVELDDLEKDE-COOH	37
18	CACTUS	TPPDS ₄₆₃ DYDSS ₄₆₈ DIEDLDDTKM	38
19	Caldesmon	AERLS ₂₆ YQRNDDDEEE NFQNS ₇₃ SVAEEETKRST ₈₃ DDEALLERLA	183
20	Calmodulin	KMKDT ₇₉ DS ₈₁ EEEIREAF	11
21	Calnexin	KKDDT ₇₄ DDEIAKYDGK EQQKS ₅₃₅ DAEEDGGTAS ₅₄₅ QEEDDRKP	184
22	Calsequestrin (hearth)	DGNNS ₃₇₈ DEES ₃₈₂ NDDS ₃₈₆ DDDDE-COOH	12
23	Calsequestrin (skeletal muscle)	GEINT ₃₆₃ EDDDDEDDDD	12
24	CD44	NGEAS ₃₂₃ KS ₃₂₅ QEMVHLVNK	45
25	CD45	SEHDS ₉₆₅ DES ₉₆₈ S ₉₆₉ DDDS ₉₇₃ DSEEPSK	13
26	CD5	PDNSS ₄₅₉ DS ₄₆₁ DYDLHGAQRL	46
27	Cdc28	IRLES ₄₆ EDEGVPSTAIRE	47
28	CDC34	DEDDS ₂₃₁ GT ₂₃₃ EES ₂₃₆ -COOH	48
29	CHPV-PP	EEEDS ₆₂ EEDDDNLPTE	52
30	Chromogranin B	YGEES ₃₈₅ EEERGLEPGK	53
31	c-Jun	EEPQT ₂₃₁ VPEMPGE TPPLS ₂₄₃ PIDMESQE	12
32	CK2 β	H₂N-MS₂S₃SEEVSWISWF	54
33	Clathrin light chain b	FFSSS ₁₁ ES ₁₃ GAREAAE	11
34	c-Myb	RHSIS ₁₁ S ₁₂ DEDDDEFEMC	17
35	c-Myc	PPTTS ₂₄₉ S ₂₅₀ DS ₂₅₂ EEEQEDEEE TSPRS ₃₄₇ S ₃₄₈ DTENVKRR	11
36	Conglycinin-β	GEKGS ₇₅ EEEDEDEEE QQRES ₁₁₇ EESDSELRR	55
37	Connexin (Cx) 45.6	EEVVS ₃₆₃ DEVEGSPAP	56
38	CREB	TIAES ₁₀₈ EDS ₁₁₁ QES ₁₁₄ VDS ₁₁₇ VTDSQKRRE	185
39	CREM	TIAET ₉₄ DDSADSE EEEKS ₁₄₀ EEEGTPPNIA	12
40	Csx/Nkx2.5	QRYLS ₁₆₃ APERDQLASVLKL	58
41	CT	AYDIS ₃₆₂ EDEED-COOH	186
42	CTCF	KEDSS ₆₀₉ DSENAEPDLDDNEDEEE	59
43	CUT proteins	LTQGS ₄₀₀ VSDLLARPKP	60
45	DARPP-32	MLRLS ₄₂ EHS ₄₅ SPEEEASP ENQAS ₁₀₂ EEDELGELRE	11
46	DNA ligase I	RVLGS ₆₆ EGEEDEALSP	187
47	DSIP	GGDAS ₇ GE-COOH	12
48	EBNA-2	TESPS ₄₆₉ S ₄₇₀ DEDYVEGP	12
49	EBV ZEBRA	QQPES ₁₆₇ LEECDDS ₁₇₃ ELEIKRY	12
50	E-Cadherin	DYEGS ₈₄₀ GSEAASLSSLN SLNSS ₈₅₃ ES ₈₅₅ DQDQDYD	64
51	EF-1β	DLFGS ₁₀₅ DEEESSE	11
52	EF-1δ	ILFGS ₁₆₂ DEEEDKEAA	188
53	eIF2α	LDNRS ₂₉₂ DS ₂₉₄ EDDEDES ₃₀₁ DDE-COOH	13
54	eIF2β	H₂N-MS₂GDEMIFDPTMS	11

TABLE 3. (Continued)

No.	Protein	Sequence	Reference
55	eIF5	AEEES ₃₈₇ S ₃₈₈ GGEEDDEDENIE NGSVS ₁₇₄ TSETPPPPPP	189
56	EKLF	PF PDT ₂₄ QDDFLKWRSEE	65
57	EN	PSAVS ₃₉₄ RDS ₃₉₇ GMES ₄₀₁ S ₄₀₂ DDTRSETGS	13
58	ER (<i>D. melanogaster</i>)	PETRT ₁₈ YCDYES ₂₄ VNECMEG	66
59	ER-E2	ERLAS ₁₆₇ TNDKGSMAAME	13
60	Factor Va	EDEES ₆₉₂ DADYDYQNRLAA	190
61	FAF-1	VHMVS ₂₈₉ DS ₂₉₁ DGDDFEDATE	67
62	Fibrinogen	GEFVS ₅₂₃ ETESRGSES DEAGS ₅₉₀ EADHEGTHS GEDLT ₁₄₃ EEEDGGIIRRI	11
63	FKBP52	DADIY ₁₈₄ DS ₁₈₆ EDYDLTPDEDE	69
64	Fpr3	AAGGS ₂₆₆ DLEADKKDQPAV	70
65	Fragmin	EECPS ₇₇₃ DS ₇₇₅ EEDEGRGERTAF	13
66	Furin	PPSMS ₂₄ SHDTASPAAPS	71
67	GAIP	S _n E _n motifs	72
68	GAR2	AEEES ₇₁₂ S ₇₁₃ EDD-COOH	191
69	GEF	SPHQ ₆₆₆ EDEEPRDGLPEED	11
70	GS	HLKDS ₉₀₀ DEEENV-COOH	76
71	HCMV gB	NH ₂ -MS ₂ RSESRKNRQWVA	13
72	HDV small AG	MAGRS ₅ GDS ₈ DEELIRTV	13
73	HIV-1 Rev	RAEDS ₅₂ GNS ₅₆ EGEISALVE	192
74	HIV-1 Vpu	DDVDS ₉₂ DDDDLIGVPV	12
75	HIV-2 Nef	ISNES ₁₀₂ S ₁₀₃ EEEQ-COOH	12
76	HMG1 (Y)	ESPAS ₈₉ DEAEEKEAK	11
77	HMG14	VDDADAEVNS ₉₆ S ₉₇ D-COOH	80
78	HMG1A	DEEGS ₁₄₉ EEDEDDDE	81
79	HMG1B	ASQLS ₂₁₄ AEKEEKPAAE	84
80	HoxB-6	PWMRS ₁₃₂ S ₁₃₃ GTDRKRGR	85
81	HoxB-7	AGPGT ₂₀₃ T ₂₀₄ GQDRAEAEAEAEAEAE	
82	HPV-E7	QLNDS ₃₁₃ S ₃₁₂ EEDEID	12
83	Hsp90α	DKEVS ₂₃₆ DDEAEEKEDK EDVGS ₂₆₃ DEEEKKDGD	11
84	Hsp90β	EKEIS ₂₂₆ DDEAEEKGE EDVGS ₂₅₅ DEEDDSGKDK	11
85	HSV RR-1	DASS ₁₉₄ DSDSDDSEDIDSE	89
86	HSV-VP16	NNYGS ₃₇₅ TIEGLLDLPDDDAPE	92
87	HY5	EGIES ₃₆ DEEIRRVE	93
88	ICP27	GLDLS ₁₆ DS ₁₈ DLDEDPPEPAE	193
89	IFI16	KRKKS ₁₃₂ TKEKAGP	95
90	IGF BP-3	PGNAS ₁₃₈ ES ₁₄₀ EEDRSAG	96
91	IGFBP-1	ESPES ₁₀₁ TEITTEELL LMAPS ₁₁₉ EEDHSILWDA AQETS ₁₆₉ GEEISKF	12
92	IκBα	MLPES ₂₈₃ EDEESYDT ₂₉₁ ESEFTEF FTEFT ₂₉₉ EDEL PYDD	97
93	IκBα	DRHDS ₃₂ GLDS ₃₆ MKDEEYEQM	194
94	IκBβ	GPCSS ₃₁₃ SS ₃₁₅ DSDGGDE	13
95	INH-2	DDAYS ₈₆ DTETTEAMTPD REQES ₁₂₀ S ₁₂₁ GEEDSDLSPEE	11
96	IRS-1	IAADS ₉₉ EAEQDSWYQAL SPALT ₅₀₂ GDEAAGAADLD	12
97	L1	RSLES ₁₁₈₁ DNEEKAFGSS	104
98	La	TKFAS ₃₆₆ DDEHDEH	106
99	LAC1	LNADS ₃₀ EEDEEHTIIT	12
100	LDLR	RQMVS ₈₃₃ LEDDVA-COOH	11
101	m8	SGYHS ₁₅₉ DCDSPPPTP	110
102	Max	H ₂ N-MS ₂ DDDIEVES ₁₁ DEEQPRFQ	12
103	MAZ	VGSLS ₄₆₀ GAEGVPVSS	112
104	MDM-2	GHELS ₂₆₇ DEDDEVYRV	115
105	MEF2C	FQYAS ₅₉ TDMDKVLLKYT	13
106	MPR300	DEQDS ₂₄₂₁ EDEVLTLP FHDDS ₂₄₉₂ DEDLLHV-COOH	12
107	MPR46	LGEES ₂₆₇ EERDDHLLPM	196
108	mSTII	VDLGS ₁₈₉ MDEEEEAATP	119

TABLE 3. (Continued)

No.	Protein	Sequence	Reference
109	MV-PP	GPGES ₈₆ DDDAETLGIPP ESENS ₁₅₁ DVDIGEPDTE GFRAS ₁₈₀ DVETAEGGEI	13
110	MYCN (N-Myc)	EDTLS ₂₆₁ DS ₂₆₃ DDEDDEEEDDEEEE SPRNS ₃₆₇ DSEDSERRRNH	12
111	Myf-5	FLQGS ₄₉ DEDEHVRPTG RYIES ₁₃₃ LQELLREQVE	121
112	Myosin HC (skeletal)	AcS ₁ S ₂ DADMAVFG E	12
113	Myosin HC	NADGS ₁₉₅₄ EEVDARDA	12
114	Myosin LC	GDRFT ₁₃₄ TEEVDEMYRE	11
115	NAP1	KEPES ₁₁₈ ST ₁₂₀ DNEADAE PEVPS ₂₈₄ DQEEIDDDSQQ	122
116	NDPK A	HGSDS ₁₂₂ VESAEKEIGL	13
117	Neuromodulin	ETAES ₁₉₁ S ₁₉₂ QAEKEEAVDEA DGSAT ₈₈ T ₈₉ DAAPATSP	125
118	NF-ATc	NSEAS ₁₈₇ SYESNYS	126
119	NFκB/Rel p65	NGLLS ₅₂₉ GDEDFSSIADMD	127
120	NIPP-1	RVTFS ₂₀₄ EDDEIINPED	197
121	nm HC Myosin B	LEGAS ₁₉₅₂ LELS ₁₉₅₆ DDDT ₁₉₆₀ ESKTS ₁₉₆₅ SDV ESKTS ₁₉₆₅ DVNETQPPQS ₁₉₇₅ E-COOH	198
122	NSP5	IEVDS ₁₅₃ DS ₁₅₅ EDYVLDD VLDD ₁₆₃ DS ₁₆₅ DDGKCKNCK	134
123	Nucleolin	AAPAS ₁₈₇ EDEDEEDDDDEDDDD EEDDS ₂₀₉ EEEEAMEITPA	11
124	Occludin	CGYTT ₃₇₅ G GES ₃₇₉ ADELEDDSWD	135
125	ODC	EQPGS ₃₀₃ DDEEDES	11
126	P0, P1, P2	KKEES ₁₀₂ EES ₁₀₅ DDDMGFGLD	12
127	p120	AGIWS ₁₈₁ EEET ₁₈₅ EDEEEKE	12
128	p34 cdc2	IRLES ₃₉ EEEGVPST	12
129	p35	LPQLSYLDGYDDE	12
130	p47 phox	WIPAS ₂₀₈ FLEPLDSPDET E YLQKS ₂₈₃ GQDVVSQQAQRQIK QSPGS ₃₄₈ PLEEERQTQRSK	140
131	p53	VGPDS ₃₈₉ D-COOH	12
132	PAM	DRVST ₉₄₆ E GS ₉₄₉ DQEKDEDDGTE	142
133	PHAS-1	SHLHS ₉₉ SPEDKRAGG GGEES ₁₁₁ QFEMDI-COOH	199
134	Phospholipase A1	NYDFS ₉₃ SAESGSSRLRYS ₁₀₅ EGESGGG PTSIS ₇₁₆ ENEGISTIPSP	146
135	PIP kinase type IIa	EEGES ₃₀₄ DSTHPIGTP	148
136	PKA RII	AVADS ₇₄ ES ₇₆ SEDEEDLD	11
137	PKC β	GPPTS ₁₁ EGEESTVRFA	12
138	PLD1α	LFHPS ₆₁₀ S ₆₁₁ ESEQQLTRSTD	149
139	PR	QQSL ₈₁ DVEGAYSRA	13
140	Proteasome C8, C9	YAKES ₂₄₃ LKEEDES ₂₅₀ DDDNM-COOH	13
141	Pro-Tα	AAVD ₇ SSEITTKDLKE	12
142	PTEN	TPDVS ₃₇₀ DN EPDHRY HYRYS ₃₈₀ DT ₃₈₂ T ₃₈₃ DS ₃₈₅ DPENEPFDE	151
143	PU-1	PLEVS ₁₄₈ DGEADGLE	12
144	PV VP1	PTPES ₆₆ LTEGGQYYG	13
145	Rab-17	SGSSSSSEEDGMG (4 sites)	12
146	RAD	SREVS ₂₁₄ VDEGRACA	153
147	RPB6	NH ₂ -MS ₂ DNEDNFDGDDFDDVEEDE	154
148	RSV-PP	EGNDS ₂₃₂ DNDLS ₂₃₇ LEDF-COOH	155
149	RVFV NSs	VEMES ₂₅₂ EEES ₂₅₆ DDDGfVE	156
150	S100B	ETLDS ₆₂ DGDGECDFQE	157
151	SAPK1/JNK1	STEQT ₄₀₄ LAS ₄₀₇ DTDSSLDA	159
152	Simian CMV pAP	ERDAS ₁₅₆ S ₁₅₇ DEEEDMSFP	160
153	SMRT	YETLS ₁₄₉₂ DSE-COOH	162
154	Sp1	GKRFT ₆₆₈ RSDELQRHKRHT	163
155	SRF	GALYS ₇₇ GS ₇₉ EGDS ₈₃ ES ₈₅ GEEELGAER	12
156	Srp1p	DGADS ₆₇ DEEDES VSA	164
157	STC 1 and 2	ASGSS ₂₈₈ EWEDQSEYSDIRR	165
158	SV40 large T Ag	NLFC ₁₀₆ EEMPS ₁₁₁ S ₁₁₂ DDEATAD	200
159	Synaptotagmin	ETGLT ₁₂₈ DGEEKEEP	201

TABLE 3. (Continued)

No.	Protein	Sequence	Reference
160	Syntaxin-1A	TLKDS ₁₄ DDDD VAVTV	202
161	Synuclein α	YEMPS ₁₂₉ EEGYQDY PEA	167
161	Tau	QEGDT ₃₉ DAGL KESPL	13
162	TCF4	TNQDS ₅₈ S ₅₉ S ₆₀ DSEA ERRP	169
163	TFIIIA	RYICS ₁₆ FAD CGAAYNK	170
164	Tn T	AcS ₁ DEEVE QVEE	11
165	Topoisomerase I	LHNDS ₁₀ QIEAD FRLNDS	203
166	Topoisomerase II α	KRKPS ₁₄₆₉ TSDD SDSNFE FDEKT ₁₃₄₂ DD EDFVPC	204
167	TRHR	DHFST ₃₆₃ ELDD IT ₃₇₁ VTDTYLS ATKVS ₃₈₃ FDD TCLA	173
168	Tr α 2	LSSSS ₄₇₄ S ₄₇₅ DED TEVCEDL	13
169	Tubulin β	DEEES ₄₄₄ ES QGPK-COOH	12
170	UBC3B	DDDDS ₂₃₃ GNEE S-COOH	175
170	VDR	NLDLS ₂₀₈ EED SDDPSVT	12
171	Vitronectin	GDVFT ₅₀ MP EDY ₅₇ VYDD GEEK	176
172	VMAT2	DDEES ₅₁₂ ES ₅₁₄ D -COOH	177
173	VSV-PP serotype Indiana	AADDS ₆₀ DT ₆₂ ES ₆₄ EPEI DNQGL	178
174	VSV-PP serotype New Jersey	EEESS ₅₉ DS ₆₁ DTD VNAEHL	12
175	VZV PP	DSEST ₅₉₆ DT ₅₉₈ EEEF GNAIF	12

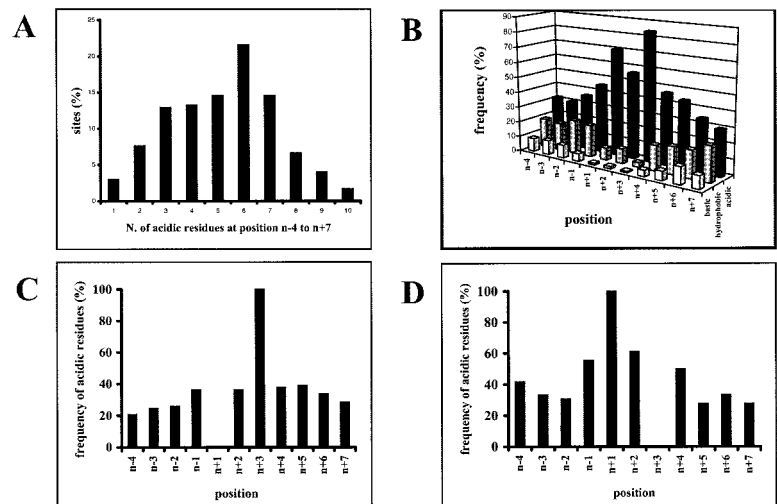
^a See Table 1 for nomenclature and description of the protein substrates. Phosphorylated residues are in boldface and denoted by their sequence number. Acidic residues are in boldface.

the targeting of some protein substrates, it does not significantly alter the site specificity determined using peptide substrates (e.g., ref 213).

Based on the common denominators outlined above (multiple negatively charged side chains downstream from Ser/Thr with special reference to the n+3 position, absence of positively charged residues nearby and of proline at n+1), it is possible to make reliable predictions about potential CK2 sites in proteins whose phosphorylation has not been yet reported and about the implication of CK2 in the generation of phosphoproteins whenever the responsible kinase is unknown. Interesting hints are provided by recent studies on the yeast proteome. In one (181), 24 proteins have been found to be associated with protein complexes containing one or both the catalytic subunits of CK2: with one exception, these proteins all include one or more

potential phosphorylation sites for CK2 in their sequence, although none had been previously reported to be a CK2 substrate. It is tempting to hypothesize that these proteins, given their interaction with CK2, will be also phosphorylated by this kinase. This would also mean that the list of known substrates displayed in Table 1, despite its crowdedness, represents just the tip of an iceberg. The same conclusion is supported by the outcome of a recent analysis of the phosphoproteome of *Saccharomyces cerevisiae* (214) leading to the identification of 216 phosphorylated sites, the majority of which include more than one phospho residue probably due to the methodological approach, expected to increase the yield of multiply phosphorylated peptides. As summarized in Table 4, 72 phospho residues (22.5%) display the specific hallmarks of CK2 phosphoacceptor motifs; the other 40, characterized by the

Figure 2. Frequency of negatively charged side chains at CK2 phosphoacceptor sites. *A*) Number of acidic and/or phosphorylated residues present at positions between n-4 and n+7 in all the sites listed in Table 3 is plotted against the number of sites expressed as percent of total. *B*) The frequency (%) of acidic and phosphorylated (solid bars), basic (empty bars), and hydrophobic residues (gray bars) found at the indicated positions relative to all the phospho residues listed in Table 3 is shown. *C, D*) Same analysis as in panel *B* has been applied only to sites lacking the acidic determinant at position n+1 (77 sites) and n+3 (37 sites), respectively. *C, D*) Only acidic residues are considered.



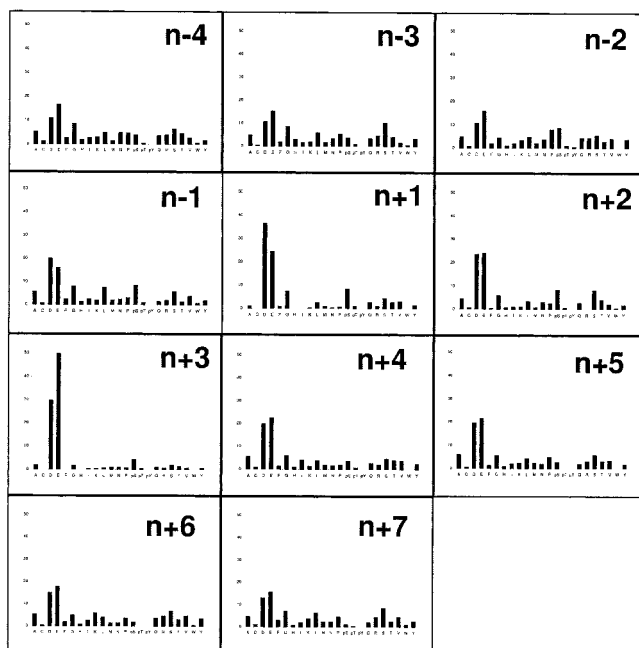


Figure 3. Frequency of individual amino acids within CK2 phosphoacceptor sites. The relative frequency (%) of amino acids found at positions between n-4 and n+7 in all the sites listed in Table 3 is shown. In each abscissa, individual amino acids are alphabetically listed after the one-letter code abbreviation.

motif pS/pT-x-x-pS/pT, can be considered either CK2 or CK1 potential sites depending on which the priming phospho residue is. Again, only in sporadic instances had these proteins been earlier reported to be phosphorylated by CK2, corroborating the view that the majority of CK2 substrates are still unknown and that CK2 together with few other protein kinases (notably proline directed kinases, GSK3 and CK1, see Table 4), primarily contributes to the generation of the eukaryotic phosphoproteome. It has to be assumed that the consensus sequences of protein kinases, with special reference to Ser/Thr specific ones, represent a necessary condition for phosphorylation; actual targeting of the individual protein substrates bearing the consensus will depend on additional features, notably recruitment to definite subcellular compartments and formation of supramolecular complexes. This also applies to CK2, with special reference to its nuclear translocation (215) and targeting to specific nuclear compartments, where many of its substrates are located (see Table 1) and where CK2 recruitment has been correlated with resistance to apoptosis (7).

DO CK2 PHOSPHOACCEPTOR SITES PLAY GENERAL FUNCTIONS?

The unique features of the sites phosphorylated by CK2 in conjunction with their variability within the overall high conservation of the acidic character suggest that they may serve different general functions in addition

to the obvious one of conferring susceptibility to phosphorylation by CK2. Of special interest is the observation of Zetina (216), who, reviewing recurrent motifs at α -helices in naturally unfolded proteins, pointed out their striking similarity to the consensus sequences specifically phosphorylated by CK2. Since transition between folded and unfolded is instrumental to the functionality of these proteins, this observation suggests that phosphorylation by CK2 could stabilize helix unfolding. Most of the motifs listed in ref 216 fulfill the CK2 consensus; in 6 cases the proteins are listed in Table 1 among the known substrates of CK2. More important, in the case of stathmin (217), calmodulin, and HIV Rev protein (Marin et al., unpublished results), the formal proof has been reached that phosphorylation of CK2 sites do act as helix breaker. What makes the connection between CK2 and protein unfolding an appealing field of investigation is the working hypothesis that CK2 might operate at a check point where folded proteins become unfolded and thereafter susceptible to aggregations that are highly toxic to cells (218). Pertinent to this could also be the presence among CK2 substrates of proteins implicated in neurodegenerative diseases, notably α -synuclein, β -amyloid precursor protein, the prion protein, and MAP-associated tau protein (see Table 1), whose pathological potential correlates with the ability to form insoluble aggregates.

Another intriguing possibility suggested by the frequency of aspartyl and glutamyl residues in its phosphoacceptor sites is that phosphorylation by CK2 could affect caspase cleavage that generally occurs at the carboxyl terminus of the acidic consensus E/D-x-D. At least five proteins (Max, Bid, connexin 45.6, HS1, and presenilin-2) have been reported to become refractory to caspase cleavage upon phosphorylation by CK2 (ref 219 and references therein). In the case of Bid, Max, and connexin, it has been shown that the residue

TABLE 4. Putative protein kinases implicated in the generation of the phosphorylated peptide sequences identified by Ficarro et al. (214) in yeast proteins^a

Putative kinase (consensus)	Number of phosphoresidues
CK2 (<u>S/T</u> -x-x-E/D/pS)	72 (22.5%)
CK2 (<u>S</u> -x-x-pS) or CK1 (pS-x-x- <u>S/T</u>)	40 (12.5%)
GSK3 (<u>S</u> -x-x-x-pS/pT)	25 (7.8%)
Pro-directed (<u>S/T</u> -P)	82 (25.7%)
Others	100 (31.3%)

^a 319 phosphorylated residues identified in 216 peptide sequences (214) have been analyzed for conforming to the consensus sequence of either CK2 (S/T-x-x-E/D/pS) or proline-directed kinases (S/T-P) or GSK3 (S/T-x-x-x-pS/pT). A number of doubly phosphorylated sites displaying the sequence pS-x-x-pS) can be assigned to either CK1 (consensus: pS-x-x-S) or CK2 (consensus S-x-x-pS) depending on whether the priming unknown kinase phosphorylates the amino- or carboxyl-terminal serine, respectively. Sites not conforming to any of the above motifs are included among "others." Underlining denotes the residue(s) targeted by the kinase; pS and pT denote phosphorylated serine and threonine acting as specificity determinants.

phosphorylated by CK2 is very close to the cleavage site, suggesting that the adverse effect of CK2 is site directed. The ability of CK2 to antagonize the action of caspases can account, at least partially, for its anti-apoptotic role (7).

Finally, a legitimate question would be whether CK2 contributes to the generation of protein-protein adhesion modules based on the recognition of phospho residues within given sequences. This kind of interaction is of paramount importance in signal transduction (220). Although the first detected adhesion domains (SH2 and PTB) recognize phosphotyrosine, several motifs whose recognition is based on Ser/Thr phosphorylation were identified later (221). Some of these are characterized by the pS/pT-P doublet, suggesting their generation by proline-directed kinases like CDKs or MAPKs and ruling out any implication of CK2. A scrutiny of the other Ser/Thr-phosphorylated consensus known to act as adhesive modules supports the view that although there is no necessary dependence of any of these motifs on CK2 mediated phosphorylation, in some cases they are quite compatible with sequences phosphorylated by CK2 for including no negative determinants while welcoming or tolerating an acidic residue at position $n+3$. A notable example is provided by the phosphorylated motifs recognized by the WD40 domains, whose consensus is DpSGxx(x)S. This motif is found close to the carboxyl-terminal end of the ubiquitin-conjugating enzyme-2 CDC34/UBC3, where it also fulfills the conditions for undergoing phosphorylation by CK2 (DSGteeS). It has recently been shown that its phosphorylation by CK2 either *in vitro* or *in vivo* is indeed a prerequisite for the anchoring of UBC3 to the F-box receptor protein β -TrCP (175). The same applies to a homologue of UBC3, UBC3B, whose phosphorylation by CK2 at a similar site also confers the ability to bind β -TrCP (175). Note that at least two more CK2 sites listed in Table 3, Ser-52 of HIV-1 Vpu protein and Ser-32 of I κ B α , are located in a context conforming to the consensus recognized by WD40 domains.

The sequence recognized by the FHA domains xxx-pTxxD/I/S/Y is also suited for CK2 mediated phosphorylation: it is actually found at 12 of the 42 Thr sites listed in Table 3, although we do not know whether any of these sites has the function of interacting with FHA domains. In principle, even the motifs recognized by the 14-3-3 proteins, supposedly generated by basophilic kinases because of a conserved arginine at either $n-3$ or $n-4$ position, could be phosphorylated by CK2: the arginyl residue is in fact far away enough to be tolerated by CK2 and the motif Rxx(x)pS is found in 25 CK2 sites listed in Table 3. In one case, homeodomain-containing transcription factor Csx, it is implemented by a proline at position $n+2$ that makes it a "perfect" 14-3-3 motif. In two other cases, DARPP-32 and PHAS-1, the motif is slightly altered (RxxxxpSxP and HxxxxpSxP, respectively; see Table 3) but still reminiscent of the canonical one.

CONCLUSIONS AND PERSPECTIVES

In the light of present knowledge, there is little doubt that CK2 is the most pleiotropic among the individual members of the protein kinase superfamily. Although "only" 307 proteins phosphorylated by CK2 are known (Table 1), it is likely they are the tip of an iceberg. In fact, this figure is steadily increasing day after day at a rate that does not tend to decline. Second, the analysis of phosphoproteins in *S. cerevisiae* (214) suggests that CK2 phospho sites are more frequent than those generated by any other individual protein kinase and may contribute to as much as a quarter of the whole eukaryotic phosphoproteome. Third, a search based on CK2 consensus sequence discloses the possibility that many proteins that interact *in vivo* with CK2 are also potential substrates, although their phosphorylation has not been reported so far. In the future, we shall see a further dramatic increase of the already long list of CK2 substrates.

Such an extreme pleiotropism may provide the rationale to explain why CK2 is also constitutively active, and these two properties when taken together support the view that CK2 cannot be compared with any of the classical protein kinases that play specific roles by being turned on and off at individual steps of signaling pathways. Expectedly evidence will be accumulating that CK2 is committed to a global constitutive role within the cell, where it takes care of a wide variety of basal cellular functions, notably housekeeping gene expression, RNA synthesis, protein synthesis, and degradation, and survival response (7). As discussed elsewhere (5) these physiological commitments of CK2 can become instrumental to dysregulated cell proliferation and virus infection under special circumstances. Whereas the majority of protein kinases operate in a hierarchical and "vertical" manner, along signaling cascades coming down from the membrane to the nucleus, CK2 intervenes laterally, like a free lance impinging on many signaling pathways at different levels. As first surmised 12 years ago (11) and recently corroborated by experimental data (6) in the case of CK2, the control of activity, if any, would take place in the opposite way from the other kinases, the paradigm "regulation = more activity" being subverted to "regulation = less activity." Using basket terminology, one would say that CK2 looks like a "playmaker" not a "pivot": hardly ever does it make scores; nevertheless, it is essential to the team game.

The systematic identification of all the proteins that are phosphorylated by CK2 (the "CK2 dependent phosphoproteome") will provide the pieces of the puzzle; next and not trivial task will be to put the pieces together and solve an enigma that has been lasting for nearly 50 years. FJ

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