



## **Role of protein phosphorylation at particular site**

### **Adducin S662 of gamma isoform (S724 of alpha, S713 of beta)**

Adducin can be phosphorylated in at least 8 sites. Alpha, beta and gamma isoforms are detected by this antibody - a/b is ~120 kDa, g is ~80 kDa.

PKC and PKA are both known to phosphorylate adducin. The phospho-serine detected here is the major phosphorylation site for PKC. Phosphorylation of adducin by PKC results in loss of calmodulin binding. Binding of calmodulin also reduces the effective rate of phosphorylation by PKA. Adducin has also been reported to bind tetanus and botulinum toxins.

Matsuoka et al, 1996. J Biol. Chem. 271(41):25157-25166.

### **CDK1 Y15 (cdc2)**

CDK1 is catalytically inactivated by phosphorylation at Y15 by WEE1 and/or MYT1 kinases. A critical step in activating CDK2 for progression into mitosis is dephosphorylation of Y15 and T14 by CDC25 phosphatase. CDK1 can then be activated by phosphorylation of T161 by CDK7 and complexing with cyclin B1.

Denning et al., 2001. PNAS 98(21):12044-12049

### **CREB S133**

CREB is phosphorylated on S133 in response to several stimuli - this phosphorylation is critical for function of CREB as a transcriptional activator. Activated CREB then recruits CBP, which in turn recruits components of the RNA polymerase 2 transcription machinery.

Several kinases are known to phosphorylate CREB both in vitro and in vivo - S108, 111, 114, 117 and 121 can be phosphorylated by CK1 or CK2, S129 by GSK3, S133 by PKA, PKC, RSKs, CaMKs and others, and S142 by CaMK2. Phosphorylation of the CK2 sites may not be functionally required in vivo, however S129 phosphorylation may have a role in mediating the activity of phospho-CREB (S133) in response to various stimuli.

West et al., 2001. PNAS 98 (20): 11024-11031

Tyson et al., 2002. Endocrinology 143(2): 674-682.

### **Erk1, Erk2 T202/Y204, T185/Y187**

Erk1 and Erk2 (also referred to as p44 and p42 kDa MAP kinases) are dually phosphorylated on T202/Y204 (Erk1) and T185/Y187 (Erk2) by MEK1/2, and not other MEKs. This dual phosphorylation is absolutely required for full activation of Erk1/2. These two phosphorylation events occur via a distributive mechanism, independent of one another, however the tyrosine phosphorylation event may be more frequent.

Ferrell and Bhatt, 1997. J. Biol Chem. 272(30):19008-19016.

Robinson et al., 1996. J Biol Chem. 271(47):29734-29739.

### **GSK3 a/b S21/9, Y279/Y216**

GSK3 is constitutively active in resting cells. Both alpha and beta isoforms are the products of distinct genes and have distinct biological roles - GSK3b is involved in NFkB mediated anti-apoptotic response, while the role of GSK3a is less defined. Inactivation of GSK3 is mediated by phosphorylation of S21/9 by AKT, PKA, p70 S6 kinase or p90Rsk.

Phosphorylation of Y279/216 is necessary for full activity of GSK3, unphosphorylated GSK3 may have a basal level of activity. The tyrosine kinase responsible is as yet unknown apoptosis induced kinase, and not an autophosphorylation event. This activation can be overridden by phosphorylation of S21/9 to inactivate the kinase.

Bhat et al., 2000. PNAS 97(20):11074-11079.

Eldar-Finkelman et al., 1996. PNAS 93: 10228-10233.

Hoeflich et al., 2000. Nature. 406:86-90

### **JNK T183/Y185**

Three Jun N-terminal kinases JNK1,2 and 3 (4 isoforms of each) all share the same TPY phosphorylation site. Two MW groupings are observed ~38 kDa and ~45 kDa. JNKs differ in their affinity for AP-1 complex transcription factors (JUN, ATF, FOS and others) .

JNK phosphorylates JUN at S73 and S63, activating the transcription factor and permitting dimerization. JNK itself is activated by phosphorylation of T183/Y185 (JNK1 numbering) by

MKK4 in response to a variety of extracellular stimuli, including inflammation, osmotic stress and inhibition of protein synthesis. Active JNK also phosphorylated p53 in response to DNA damage and stress inducing agents (ie anisomycin).

Buschmann et al., 2001. *Mol. Cell, Biol.* 21(8):2743-2754.

Gupta et al., 1996. *EMBO* 15(11):2760-2770.

Kyriakis et al, 1994. *Nature* 369(6476):156-160.

### **JUN S73**

c-JUN is a central component of AP-1 complexes. Transcriptional activation by cJUN is dependent on phosphorylation of S73 and S63 by JNK. JNK requires a proline in the P+1 position flanking the phosphorylation site and must bind to cJUN at an N-terminal docking site for efficient phosphorylation. The JunD isoforms lacks the docking site and is phosphorylated at much lower efficiency, while the JunB isoforms has a docking site, but lacks the appropriate proline residue and is not phosphorylated at all by JNK.

Activated JUN forms homo- and heterodimers with other AP-1 complex members (JUN, FOS, ATF and their isoforms).

Karin et al., 1997. *Curr. Op Cell Biol.* 9:240-246.

### **Mek1/2 S217/221**

Mek1 and 2 are dual specificity kinases and are the only known activators of Erk1/2. Mek1 is activated by phosphorylation at both sites by MEKK1, Raf1 or Mos, and possibly other kinases.

Mek1 and Mek2 are activated by different pathways as demonstrated by differential effects of wortmannin (PI3K inhibitor) on their activity and likely have different functions, although their targets may overlap.

T286,292 are also phosphorylated in MEK1 but not MEK2 - this may be a point of differential regulation of these kinases.

Downey et al, 1996. *J Biol Chem.* 271(35): 21005-21011.

Rossomando et al., 1994. *Mol Cell Biol.* 14(3):1594-1602.

**MKK3 S189**

MKK3 is activated by dual phosphorylation on both S189 and T193. The active MKK3 in turn activates p38 MAP kinase by phosphorylation of T180 and Y182, but does not phosphorylate either JNK or ERK MAP kinases. Multiple isoforms of the p38 MAP kinase exist, with MKK3 showing preferential phosphorylation of the alpha and gamma isoforms.

Derijard et al, 1995. *Science* 269(5198): 682-5.

Enslin et al., 1998. *J. Biol Chem.* 273(3):1741-1748.

**MKK6 S207**

MKK6 is activated by phosphorylation on both S201 and T211 - corresponding to the identical region in MKK3, a closely related kinase. MKK6 also activates p38 MAPK in an analogous manner to MKK3, however a constitutively active form of MKK6 demonstrates a stronger preference for endogenous p38, suggesting the possibility of subtle differences in regulation between the two kinases. MKK6 phosphorylates the beta-2 isoform of p38, which may be the endogenous isoforms expressed in the experiments by Raingeaud et al.

Enslin et al., 1998. *J. Biol Chem.* 273(3):1741-1748.

Raingeaud et al., 1996. *Mol Cell Biol.* 16(3):1247-1255.

**MSK1 S376**

MSK1 is related to the p90RSK family of kinases. MSK1 is activated in vivo by either the MAPK/ERK or SAPK2/p38 cascades and in turn phosphorylates S133 of CREB in response to stress - other target proteins have also been identified.

Deak et al., 1998. *EMBO* 17(15): 4426-4441.

Thomson et al., 1999. *EMBO* 18 (17):4779-4793.

**NR1 S896**

NMDA receptors consist of two families of subunits, one NR1 subunit and one or more of the NR2 subunits (A-D). The majority of NR1 phosphorylation occurs at two pairs of serine residues (S889-890 and S896-897) in the C1 exon cassette. PKC phosphorylates both pairs and S879, while PKA phosphorylates only the second pair. Phosphorylation at S896 has a low basal level, but is

increased by phorbol ester stimulation of cells.

The C1 exon cassette also contains sequences that regulate subcellular targeting - subunits containing the C1 exon spontaneously aggregate into receptor rich domains at the surface of the cell. Phorbol ester treatment of cells disrupts the NR1 rich domains, resulting in a diffuse distribution of the NR1 subunit, due to phosphorylation of serine residues within the C1 cassette, specifically S890. This redistribution of the NR1 subunits occurs rapidly and reversibly in response to phosphorylation of S890.

Tingley et al., 1997. *J Biol. Chem.* 272(8):5157-5166

Ehlers et al., 1995. *Science* 269:1734-1737.

### **p38 MAPK T180/Y182**

p38 MAP kinase is phosphorylated at T180 and Y182 by either MKK3 or MKK6 - this dual phosphorylation is required for full activity. Activity is regulated by dual specificity phosphatases PAC1 and MKP1, and possibly others.

At least 3 isoforms of p38 have been identified - p38alpha, p38 beta2 and p38gamma. The phosphorylation motif TGY is common to all, although their tissue distribution and target specificity varies. Targets include transcription factors such as ATF2, and kinases such as MSK1 and MSK2.

Deak et al., 1998. *EMBO* 17(15): 4426-4441.

Raingaud et al., 1995. *J Biol Chem.* 270(13):7420-7426.

Stein et al., 1997. *J Biol Chem.* 272(31): 19509-19517.

### **p70S6K T389**

The p70S6K family of kinases are related to the p90RSK kinases, but lack a complete C-terminal kinase catalytic domain. Phosphorylation via PDK1 of T229 in the N terminal kinase catalytic domain and T389 in the linker domains are critical for activity. The C-terminal autoinhibitory phosphorylation sites include S411, S418, T421 and S424.

P70S6Ks are responsible for phosphorylation of the ribosomal S6 subunit in intact cells (thus regulating protein translation) and play a key role in cell proliferation - neutralizing the function of S6K blocks the ability of the cell to progress through G1 into S phase.

Ming et al., 1994. *Nature* 371:426-429.

Saitoh et al., 2002. *J. Biol Chem.* 277(22):20104-20112.

### **RSK1 T360/S364**

The RSK family of protein kinases are unique in having two non-identical complete kinase catalytic domains. Inactive RSK1 is partially phosphorylated at S222 and S733. Upon stimulation (ie with PMA), phosphorylation occurs at T360, S364, T574, S381, and further phosphorylates S222 and S733. MAP kinase activity activates the C-terminal kinase domain by phosphorylation of T574 - this, along with phosphorylation of T360 and S364 in the linker domain leads to activation of the N-terminal kinase domain.

Bjorbaek et al., 1995. *J. Biol. Chem.* 270(32):18848-18852.

Dalby et al., 1998. *J. Biol Chem.* 273(3):1496-1505.

### **PKB T308, S473**

Function of PKB is regulated by phosphorylation on at least two sites (T308 and S473) and is a key effector of the PI3K pathway, phosphorylating many targets including BAD, GSK3, Caspase 9 and Forkhead transcription factors.

T450 is constitutively phosphorylated on PKB - activation depends on phosphorylation of T308 by PDK1 and of S473 by an unidentified kinase - possibly ILK, although this may be an autophosphorylation event. PTEN lipid phosphatase is involved in regulation of PKB indirectly, by dephosphorylating PI(3,4,5)P3, the product of PI3K, which is upstream of PKB and activates PDK1.

Brazil and Hemmings, 2001. *Trends Bioc Sci.* 26(11): 657-664.

Persad et al., 2000. *PNAS.* 97(7): 3207-3212.

Toker and Newton, 2000. *J. Biol Chem.* 275(12): 8271-8274.

### **PKC a/b T638, PKC-a S657**

Classic PKC isoforms alpha and beta are widely distributed within an organism - expression of both has been demonstrated in almost all tissue types to-date, especially in brain, with varying ratios in other tissues. Upon activation, PKC will redistribute within the cell, from the soluble to the particulate fraction.

Three phosphorylation events are required to 'mature' the inactive enzyme (alpha/beta) - T497/500, T638/641 and S657/660. This mature species localizes to the cytosol, where it can be activated by translocation to the plasma membrane and subsequent removal of a regulatory pseudosubstrate sequence (N terminal 19 amino acids). PDK1 initiates this process by phosphorylation of T497/500, while T638/641 and S657/660 are autophosphorylation events.

Bornancin and Parker, 1997. *J. Biol. Chem.* 272(6):3544-3549.  
Dutil and Newton, 2000. *J. Biol Chem.* 275(14):10697-10701.  
Kanashiro and Khalil, 1998. *Clin. Exp. Pharm. Phys.* 25:974-985.  
Orr et al, 1992. *J. Biol Chem.* 267:15263-15266.

### **PKC-d T505, PKC-e S719**

Novel PKC isoforms delta and epsilon differ from the classic isoforms in that they do not require calcium for activation. Both isoforms are also widely distributed, with varying ratios from tissue to tissue.

The mechanism of phosphorylation of the novel PKCs delta and epsilon is the same as for conventional PKCs - PDK1 phosphorylation of T507/T566 in the activation loop initiates autophosphorylation at the N terminal S664/S729. This activation is regulated by myristoylation, rather than being constitutive as occurs in the classic PKCs.

Extensive tyrosine phosphorylation and subsequent increase in kinase activity of PKCdelta by SRC is also observed upon stimulation with PMA, carbachol or pervanadate.

Benes and Soltoff, 2001. *Am J. Physiol. Cell Physiol.* 280:C1498-C1510.  
Cenni et al, 2002. *Biochem J.* 363(3):537-45.  
Kanashiro and Khalil, 1998. *Clin. Exp. Pharm. Phys.* 25:974-985.

### **PKR T451**

PKR is a S/T kinase activated by the presence of dsRNA, and critical to the IFN-mediated antiviral response. Viral infection promotes phosphorylation of eIF2a by PKR, leading to inhibition of protein synthesis. Activation of PKR is accompanied by phosphorylation at multiple sites, including T446 and T451 in the activation loop. Mutations T446A, T446S and T451S significantly reduce PKR activity, while mutation T451A eliminate PKR activity, suggesting that phosphorylation at T451 is essential for PKR activity.

Romano et al., 1998. *Mol. Cell Biol.* 18(4):2282-2297.

### **Raf-1 S259**

Raf-1 is an important control point for cell proliferation, differentiation and apoptosis. Raf-1 is

commonly engaged by Ras, which has been activated by a membrane-bound receptor. Raf-1 regulation is complex and incompletely understood, but transmits its effect on the cell via the MAP kinase pathway.

Raf-1 is multiply phosphorylated depending on its activation state. S338, Y341, T491 and S494 are required for Ras activation of Raf-1, while S43, S259 and S621 are phosphorylated in resting cells and may be inhibitory to Raf-1 activation. Several isoforms of 14-3-3 proteins are known to bind to Raf-1 via phosphoserine residues - phosphatase treatment of Raf1 can block the association with 14-3-3 in vitro, conversely, the binding of 14-3-3 proteins to Raf1 prevents dephosphorylation of Raf1 by PP1.

Dhillon et al., 2002. 21(1-2):64-71.

Kolch, 2000. Biochem J. 351:289-305.

Muslin et al., 1996. Cell 84:889-897

### **RB S780, S807/S811**

The Retinoblasoma (RB) tumor suppressor protein is a potent inhibitor of cell proliferation and is expressed throughout the cell cycle - phosphorylation events at multiple sites on RB (as many as 16) by various CDK/cyclin complexes regulate progression of the cell cycle. E2F transcription factor binding activity of RB is regulated by phosphorylation of S780 - phospho-S780 inhibits the ability of RB-E2F to form, allowing E2F to promote the transcription necessary for progression of the G1/S transition. S807/S811 phosphorylation abolishes the RB-cABL interaction - unbound ABL kinase is an S phase activated tyrosine kinase that activates transcription to promote cell cycle progression.

Knudsen and Wang, 1996. J Biol. Chem. 271(14):8313-8320

Zarkowska and Mittnacht, 1997. J Biol Chem. 272(19):12738-12746.

### **Smad1 S463/S465**

Smad1 is a member of the BMP-regulated (Bone Morphogenic Protein) R-Smad subfamily- other members include Smad5 and 8. R-Smad proteins are directly phosphorylated by a type 1 TGFbeta receptor S/T kinase at a conserved C terminal motif, in response to a TGFbeta signal. The phosphorylated R-Smad is translocated to the nucleus to modulate transcriptional activity. Smads can bind DNA directly, but rely on interactions with a variety of DNA binding partner proteins to target specific genes.

Attisano and Lee-Hoeflich, 2001. *Genome Biology*. 2(8); 3010-3017.

Kretzschmar et al., 1997. *Genes and Development* 11(8): 984-995.

### **SRC Y529, Y418**

SRC activity is regulated by a bidirectional mechanism, where the regulatory domains inhibit activity, and the activity of SRC itself controls the availability of these domains. Phosphorylation of Y529 inactivates SRC, while phosphorylation of Y418 is activating. Both sites may be autophosphorylated - Y529 is also phosphorylated by CSK.

The inactive conformation of SRC is maintained by intramolecular interactions of the SH2 and SH3 domains. Destabilization of this interaction by dephosphorylation of Y529 may lead to exposure of Y418 and its subsequent phosphorylation to obtain full SRC activity.

Gonfloni et al., 2000. *Nature Structural Biology* 7(4): 281-286.

Osusky et al., 1995. *J. Biol Chem.* 270(27): 25729-25732.

Xu et al., 1999. *Mol Cell.* 3(5): 629-638.

### **STAT1 Y701**

STAT family members are phosphorylated by receptor associated kinases, and form homo- or heterodimers that in turn translocate to the nucleus, where they fulfill a role as activators of transcription.

STAT1 Y701 is phosphorylated by JAK kinase family members in response to stimulation by several ligands, including interferons, IL-6 and EGF. This phosphorylation event is required for translocation, dimerization, DNA binding and subsequent transcriptional activation.

S727 is also required for maximal transcriptional activation by STAT1, the responsible kinase is unidentified, although candidates include some MAP kinases.

Horvath, 2000. *Trends in Biochemical Sciences.* 25: 496-502.

Shuai, 1999. *Progress in Biophysics and Molecular Biology.* 71: 405-422.

### **STAT3 S727**

STAT3 is activated in response to IL-5 and IL-6 at the level of both DNA binding and transcription activation, and has significant homology to STAT1 and STAT5.

Three isoforms of STAT3 have been identified - alpha, beta and gamma, all derived from a single

gene - 3beta is derived from 3alpha by RNA splicing, while 3gamma is derived from 3alpha by limited proteolysis. Knockout mutations of STAT3 are embryonic lethal in mice.

STAT3a is phosphorylated on S727 by a MAP kinase, in a manner similar to that of STAT1. S727 phosphorylation negatively modulates STAT3 Y705 phosphorylation, which is necessary for nuclear transfer and the DNA binding activity, which is dependent on dimer formation via an SH2 domain.

Bromberg et al., 1999. *Cell* 98:295-303.

Caldenhoven et al., 1995. *J. Biol Chem.* 270(43):25778-25784.

Horvath, 2000. *Trends in Biochemical Sciences.* 25: 496-502.

Stephens et al., 1998. *J. Biol Chem.* 273(47):31408-31416.

### **STAT 5 Y694**

STAT5 has two isoforms - 5A and 5B - encoded by separate genes. STAT5A is involved in mediation of prolactin signaling and is required for mammary gland development, while STAT5B is an effector of growth hormone signaling - disruption of this gene results in greater size and body weight in male mice.

STAT5 activation is dependent on phosphorylation of Y694 (5A) or Y699 (5B) by JAK2 - this phosphorylation is required for dimerization and confers DNA binding activity and transcriptional activation.

Copeland et al, 1995. *Genomics* 29:225-228.

Gouilleux et al., 1994. *EMBO* 13(18):4361-9.

Horvath, 2000. *Trends in Biochemical Sciences.* 25: 496-502.

Lin et al, 1996. *J. Biol Chem.* 271(18):10738-10744.