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SV40 Large Tumor Antigen and the Tumor Suppressor p53 Are Phosphorylated by a DNA-Activated Protein Kinase from Human Cells

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Many of the proteins that regulate cell growth and gene expression are phosphoproteins, and protein phosphorylation appears to be an important modulator of the functions that regulate and coordinate cell growth and differentiation. Recent examples of phosphorylation events that regulate cell growth come from studies of oncogene and anti-oncogene products and the cell cycle regulated gene, the cdc2 protein kinase (reviewed by Sager, 1989; Murray, 1989). The retinoblastoma susecptibility gene product (RB), the p53 tumor suppressor, and the SV40 large tumor antigen (TAg) all are phosphorylated by the cdc2 kinase. Phosphorylation of SV40 TAg by the cdc2 kinase activated its ability to promote SV40 DNA replication in vitro (McVey et al., 1989). To understand how these and other regulatory proteins are themselves regulated, it will be necessary to identify the other protein kinases that act on them and to identify the sites which are phosphorylated.

We recently found that SV40 TAg and mouse p53 produced in insect cells are efficiently phosphorylated by a newly discovered serine/threonine protein kinase that we have partially purified from human cells. The unique property of this protein kinase is that its activity is dramaticly enhanced by double-stranded DNA (dsDNA). Similar kinase activities appear to be present in a wide variety of multi-cellular animals (Walker et al., 1985; Carter et al., 1988).

The human dsDNA-activated kinase has been purified ~50,000-fold from

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extracts of HeLa cells. Activation of the dsDNA-kinase is specific for dsDNA but is largely independent of DNA sequence. Poly(dA-dT) and poly(dG-dC) activated the kinase as well as natural dsDNAs. Neither ssRNA nor dsRNA activate the kinase. SsDNA, phosphate, pyrophosphate, and heparin inhibit dsDNA-dependent phosphorylation. Apparent kinase binding constants (K_m) are 1 ug/ml for calf thymus DNA and 24 uM for ATP; GTP is utilized poorly by the kinase. The dsDNA-kinase chromatographs during gel filtration in 1 M KCl as a protein of 350-kDa. HeLa cell polypeptides of 52-kDa, 65-kDa, 81-kDa, 110-kDa, and 350-kDa are phosphorylated in a dsDNA-dependent manner and co-purify with dsDNA-kinase activity through early purification steps including dsDNA-cellulose chromatography. Only the 350-kDa polypeptide co-purifies with kinase activity at later steps, and the 350-kDa polypeptide is the most abundant polypeptide in our most highly purified preparations.

The first dsDNA-kinase substrate to be identified was the heat shock protein, hsp90 (Walker et al., 1985, Lees-Miller and Anderson, 1989b). Hsp90 is an abundant cytoplasmic protein that forms complexes with the steroid hormone receptors, several retrovirus tumor gene products (e.g $pp60^{src}$), dioxin receptors, and actin (Linquist and Craig, 1988). HeLa cells express two hsp90 genes that encode closely related proteins (Rebbe et al., 1989; Hickey et al., 1989) Both are phosphorylated by casein kinase II but only the larger (hsp90(alpha)) is phosphorylated by the dsDNA-kinase (Lees-Miller and Anderson, 1989a, 1989b). We recently showed that the dsDNA-kinase phosphorylates two threonine residues at the N-terminus of hsp90(alpha) in the sequence PEETQTQDQPM- (Lees-Miller and Anderson, 1989b).

SV40 TAg and p53 that were produced with baculovirus vectors and purified by immuno-affinity chromatography were found to be even better substrates for the dsDNA-kinase than was hsp90. The HeLa dsDNA-kinase phosphorylates both

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TAg and p53 exclusively on serine residues. For TAg, there are at least two dsDNA-kinase phosphorylation sites. One is located on an N-terminal 17-kDa trypsin-produced fragment that contains 5 serines phosphorylated in vivo and the cdc2 kinase phosphorylation site ($threonine_{124}$). A second dsDNA-kinase phosphorylation site is located distal to the TAg nuclear localization signal. The C-terminal region of TAg is phosphorylated at 3 serine residues including one that inhibits TAg binding to origin sequences (Fanning et al., 1989). Sequence analysis to determine the precise sites in TAg and p53 phosphorylated by the dsDNA-kinase are in progess. The effects of dsDNA-kinase phosphorylation on biochemical properties of TAg and p53 are being studied.

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References.

Carter, T. H., Kopman, C. R., and James, C. B. L. 1988. DNA-stimulated protein phosphorylation in HeLa whole cell and nuclear extracts. Biochem. Biophys. Res. Comm. 157, 535-540.

Hickey, E., Brandon, S. E., Smale, G., Lloyd, D., and Weber, L. A. 1989. Sequence and regulation of a gene encoding a human 89-kilodalton heat shock protein. Mol. Cell. Biol. 9, 2615-2626.

Lees-Miller, S. P., and Anderson, C. W. 1989a. Two human 90-kDa heat shock proteins are phosphorylated in vivo at conserved serines that are phosphorylated in vitro by casein kinase II. J. Biol. Chem. 264, 2431-2437 (1989).

Lees-Miller, S. P., and Anderson, C. W. 1989b. The human dsDNA-activated protein kinase phosphorylates the 90-kDa heat shock protein, hsp90alpha, at two N-terminal threonine residues. J. Biol. Chem. 264, 17275-17280.

Lindquist, S., and Craig, E. A. 1988. The heat-shock proteins. Ann. Rev. Genet. 22, 631-677.

McVey, D., Brizuela, L., Mohr, I., Marshak, D. R., Glutzman, Y., and Beach, D. 1989. Phosphorylation of large tumour antigen by cdc2 stimulates SV40 DNA replication. Nature 341, 503-507.

Murray, A. W. 1989. The cell cycle as a cdc2 cycle. Nature 342, 14-15.

Reebe, N. F., Hickman, W. S., Ley, T. J., Stafford, D. W., and Hickman, S. 1989. Nucleotide sequence and regulation of a human 90-kDa heat shock protein gene. J. Biol. Chem. 264, 15006-15011.

Sager, R. 1989. Tumor suppressor genes: The puzzle and the promise. Science 246, 1406-1412.

Schneider, J., and Fanning, E. 1988. Mutations in the phosphorylation sites of simian virus 40 (SV40) T antigen alter its orgin DNA-binding specificity for sites I or II and affect SV40 DNA replication activity. J, Virol. 62, 1598-1605.

Walker, A. I., Hunt, T., Jackson, R. J., and Anderson, C. W. 1985. Double-stranded DNA induces the phosphorylation of several proteins including the 90,000 M_r heat-shock protein in animal cell extracts. EMBO J. 4, 139-145.

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