Structural features underlying the multisite phosphorylation of the a domain of the NF-AT4 transcription factor by protein kinase CK1

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The phosphorylation and dephosphorylation of the NF-AT family of transcription factors play a key role in the activation of T lymphocytes and in the control of the immune response. The mechanistic aspects of NF-AT4 phosphorylation by protein kinase CK1 have been studied in this work with the aid of a series of 27 peptides, reproducing with suitable modifications the regions of NF-AT4 that have been reported to be phosphorylated by this protein kinase. The largest parent peptide, representing the three regions A, Z, and L spanning amino acids 173-218, is readily phosphorylated by CK1 at seryl residues belonging to the A2 segment, none of which fulfill the canonical consensus sequence for CK1. An acidic cluster of amino acids in the linker region between domains A and Z is essential for high-efficiency phosphorylation of the A2 domain, as shown by the increase in Km caused by a deletion of the linker region or a substitution of the acidic residues with glycines. Individual substitutions