# Chemical dissection of the APC repeat 3 multistep phosphorylation by the concerted action of protein kinases CK1 and GSK3

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A crucial event in machinery controlled by Wnt signaling is the association of ?-catenin with the adenomatous polyposis coli (APC) protein, which is essential for the degradation of ?-catenin and requires the multiple phosphorylation of APC at six serines (1501, 1503, 1504, 1505, 1507, and 1510) within its repeat three (R3) region. Such a phosphorylation is believed to occur by the concerted action of two protein kinases, CK1 and GSK3, but its mechanistic aspects are a matter of conjecture. Here, by combining the usage of variably phosphorylated peptides reproducing the APC R3 region and Edman degradation assisted localization of residues phosphorylated by individual kinases, we show that the process is initiated by CK1, able to phosphorylate S1510 and S1505, both specified by non-canonical determinants. Phosphorylation of S1505 primes subsequent phosphorylation of S1501 by GSK3. In turn, phospho-S1501 triggers the hierarchical phosphorylation of S1504 and S1507 by CK1. Once phosphoryl