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STD and TRNOESY NMR studies for the epitope mapping of the phosphorylation motif of the oncogenic protein β-catenin recognized by a selective monoclonal antibody

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Abstract The interaction of the P-β-Cat$^{19–44}$ peptide, a 26 amino acid peptide (K^{19}AAVSHWQQQSYLDpSGIHpSGATTTAP^{44}) that mimics the phosphorylated β-Catenin antigen, has been studied with its monoclonal antibody BC-22, by transferred nuclear Overhauser effect NMR spectroscopy (TRNOESY) and saturation transfer difference NMR (STD NMR) spectroscopy. This antibody is specific to diphosphorylated β-Catenin and does not react with the non-phosphorylated protein. Phosphorylation of β-Catenin at sites Ser33 and Ser37 on the DSGXXS motif is required for the interaction of β-Catenin with the ubiquitin ligase SCF$^{β\text{-TrCP}}$. β-TrCP is involved in the ubiquitination and proteasome targeting of the oncogenic protein β-Catenin, the accumulation of which has been implicated in various human cancers. The three-dimensional structure of the P-β-Cat$^{19–44}$ in the bound conformation was determined by TRNOESY NMR experiments; the peptide adopts a compact structure in the presence of mAb with formation of turns around Trp25 and Gln26, with a tight bend created by the DpS^{33}GIHpS^{37} motif; the peptide residues (D32-pS37) forming this bend are recognized by the antibody as demonstrated by STD NMR experiments. STD NMR studies provide evidence for the existence of a conformational epitope containing tandem repeats of phosphoserine motifs. The peptide's epitope is predominantly located in the large bend and in the N-terminal segment, implicating bidentate association. These findings are in excellent agreement with a recently published NMR structure required for the interaction of β-Catenin with the SCF$^{β\text{-TrCP}}$ protein.

Keywords: β-Catenin oncogenic protein; P-β-Catenin phosphorylated peptide; Epitope mapping; Antibody; P-β-Catenin/antibody complex; STD NMR; TRNOESY; Restrained molecular dynamics; Bound structure; Binding fragment

1. Introduction

β-Catenin (β-Cat) is an oncogenic protein that plays an important role in the Wnt signaling pathway [1,2] and is an important component of the cadherin cell-adhesion complex (Fig. 1). Wnt genes encode secreted signaling molecules that play important roles in development and tumorigenesis [3,4]. Deregulation of Wnt signaling is responsible for several human malignancies [5,6]. It is well known that serine-phosphorylation of β-Catenin by the Axin-glycogen synthase kinase (GSK)-3β complex targets β-Catenin for degradation by the ubiquitination–proteosome pathway [7–10], and mutations at critical phosphoserine residues stabilize β-Catenin and cause human cancers [11–13]. β-Catenin phosphorylation results in its degradation when phosphorylated β-Catenin is specifically recognized by β-transducin repeat-containing protein (β-TrCP), an F-box/WD40-repeat protein that also associates with Skp1, an essential component of the ubiquitination apparatus [14].

It has been demonstrated that β-Catenin binds to the F-box WD40 protein β-TrCP [15,16], the receptor component of the multi subunit Skp1-Cullin-FBox (SCF)$^{β\text{-TrCP}}$E3 ubiquitin ligase complex through its phosphorylated serine residues at positions 33 and 37 [17]. β-TrCP is also involved in the ubiquitination and proteasome targeting of: (i) the HIV-1 protein Vpu [17], which enhances the release of new virus particles from the plasma membrane of cells infected with HIV-1 [18] whereas it induces the degradation of the CD4 receptor in the endoplasmic reticulum; (ii) IκBα, the inhibitor of master transcription factor NF-κB [16,19,20]; and (iii) ATF4, a member of the family of transcription factors [21]. The antigenic peptides containing the **DpSGXXpS** motif constitute β-TrCP-associated epitopes. The SCF$^{β\text{-TrCP}}$ complex specifically recognizes a 22-residue β-Catenin polypeptide, a HIV-1 encoded virus protein U (Vpu) peptide fragment of 22 amino acids, and a 19-amino acid motif in IκBα in a phosphorylation-dependent manner

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Abbreviations: ARIA, ambiguous restraints for iterative assignment; mAb, monoclonal antibody; β-Cat, β-Catenin protein; P-β-Cat, phosphorylated β-Catenin; β-TrCP, β-transducin repeat containing protein; SCF, Skp1-Cullin-FBox; NOESY, nuclear Overhauser effect spectroscopy; Vpu, HIV-1 encoded virus protein U; rmsd, root-mean-square deviation; STD, saturation transfer difference; TRNOESY, transferred nuclear Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy

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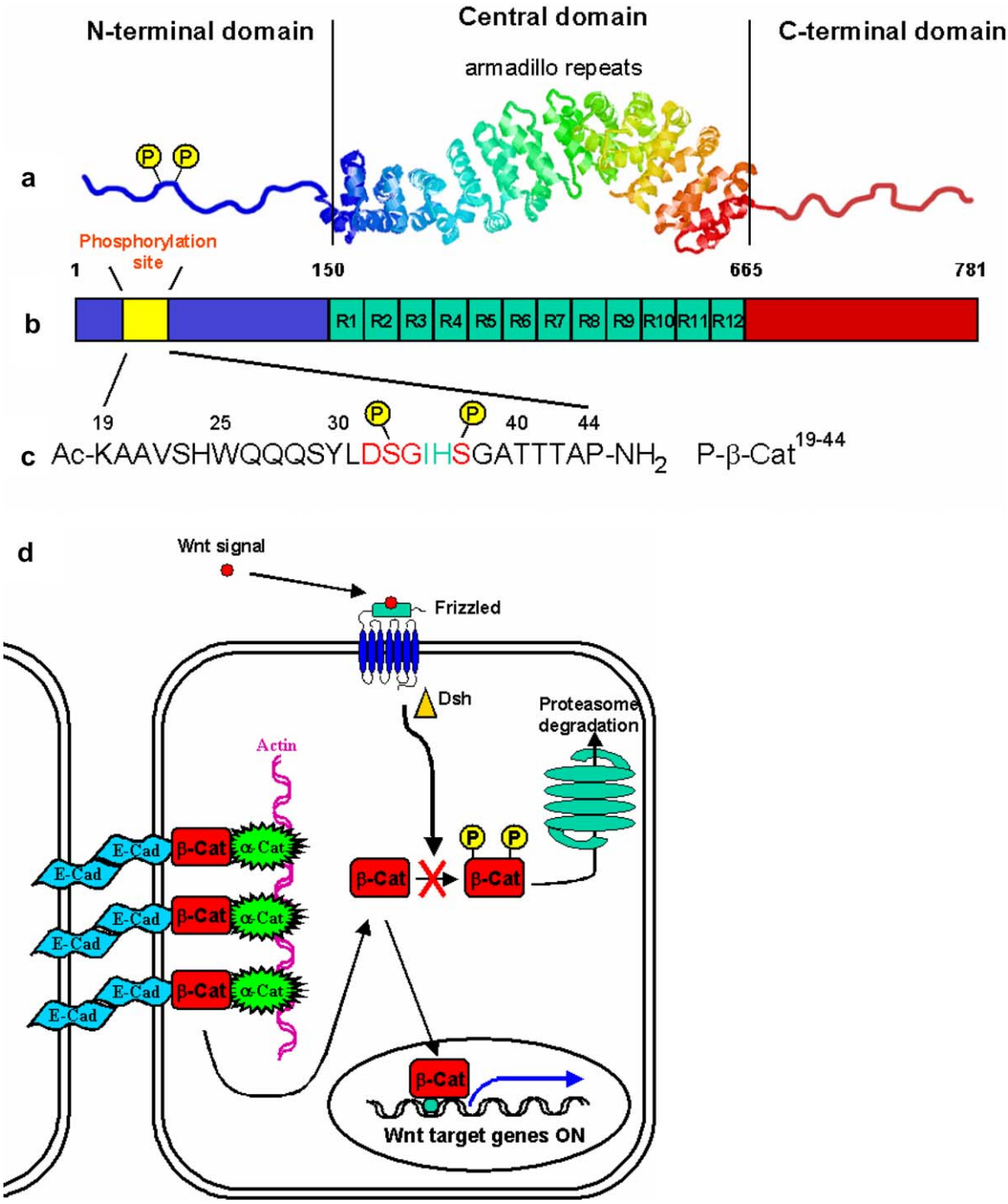

Fig. 1. (A) Schematic representation of the β-Catenin. (a) The three-dimensional structure of a protease-resistant fragment of β-Catenin containing the armadillo repeat region. The core region of β-Catenin is composed of 12 copies of a 42 amino acid sequence motif known as an armadillo repeat. The 12 repeats form a super helix of helices that features a long, positively charged groove of the proteolyse resistant fragment [52]. The structure of the N and C terminal domains remain unresolved. (b) Primary structure sequence of the full β-Catenin protein. The 12 armadillo repeats are shown in green. The phosphorylation site containing the consensus motif DpSGXXpS is shown in yellow. (c) The sequence of the phosphorylated β-Catenin fragment, P-β-Cat$^{19-44}$ which was investigated in the present work. (d) The α-cadherin/β-Catenin complex connects to the actin via α-Catenin and some actin-binding proteins, forming a rigid cytoskeleton. β-Catenin is involved in the Wingless/Wnt signaling pathway. When cells are exposed to Wnt signal, cell surface receptors are activated and block β-Catenin phosphorylation and its subsequent ubiquitination. β-Catenin is thus diverted from the proteasome, and it accumulates and enters the nucleus, where it finds a partner of the TCF/LEF family. Together, they activate new gene expression programs.

[16]. The signal for the recognition of all these cellular ligands by β-TrCP is the phosphorylation of the serine residues present in a conserved motif, D**pS**GXX**pS** for β-Catenin, Vpu, IκBα and D**pS**GXXX**pS** for ATF4. It was recently shown that Vpu is a competitive inhibitor of β-TrCP that impairs the degradation of SCF$^{\beta\text{-TrCP}}$ substrates as long as Vpu has an intact D**pS**GXX**pS** phosphorylation motif and can bind to β-TrCP [22].