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The Third 20 Amino Acid Repeat Is the Tightest Binding Site of APC for β-Catenin

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Adenomatous polyposis coli (APC) plays a critical role in the Wnt signaling pathway by tightly regulating β-catenin turnover and localization. The central region of APC is responsible for APC-β-catenin interactions through its seven 20 amino acid (20aa) repeats and three 15 amino acid (15aa) repeats. Using isothermal titration calorimetry, we have determined the binding affinities of β-catenin with an APC 15aa repeat fragment and each of the seven 20aa repeats in both phosphorylated and unphosphorylated states. Despite sequence homology, different β-catenin binding repeats of APC have dramatically different binding affinities with β-catenin and thus may play different biological roles. The third 20aa repeat is by far the tightest binding site for β-catenin among all the repeats. The fact that most APC mutations associated with colon cancers have lost the third 20aa repeat underlines the importance of APC–β-catenin interaction in Wnt signaling and human diseases. For every 20aa repeat, phosphorylation dramatically increases its binding affinity for β-catenin, suggesting phosphorylation has a critical regulatory role in APC function. In addition, our CD and NMR studies demonstrate that the central region of APC is unstructured in the absence of β-catenin and Axin, and suggest that β-catenin may interact with each of the APC 15aa and 20aa repeats independently.

Keywords: adenomatous polyposis coli (APC); β-catenin; isothermal calorimetry; unstructured protein; phosphorylation

Introduction

The tumor suppressor adenomatous polyposis coli (APC) is considered to be a gatekeeper in colorectal tumourigenesis.[1,2] Truncational mutations of APC are found in Familial Adenomatous Polyposis (FAP) and more than 80% of sporadic colonic tumors.[3–7] In addition to its roles in cytoskeletal and cell adhesion regulation,[8,9] it is well established that APC plays an essential role in the Wnt-regulated degradation of β-catenin.[10–14] *APC* encodes a large 310 kDa protein with multiple domains. The central region of APC includes three 15 amino acid (15aa) repeats and seven 20 amino acid (20aa) repeats.[15,16] Mutations that truncate APC in the 20aa repeat region, such as that in SW480 colon cancer cells, lead to the accumulation of high levels of β-catenin.[17] When full-length APC or the central region of APC were introduced into SW480 cells, β-catenin levels were reduced.[12] Furthermore, human APC2, an APC analogue containing five 20aa repeats and no 15aa repeats, has also been shown to interact with β-catenin and can decrease β-catenin levels and signaling activity in SW480 cells.[18,19] All these results suggested a critical role for the APC 20aa repeats in β-catenin turnover.

Each of the APC 15aa repeats, which is not regulated by phosphorylation, binds to the structural groove formed by β-catenin armadillo repeats 5–10.[20] In comparison, every APC 20aa repeat region contains a highly conserved 20aa sequence with potential phosphorylation sites in a consensus motif SXXSSLSXLS (Figure 1(a)). Phosphorylation of APC by CK1ε and GSK-3β enhances its ability to bind and down-regulate β-catenin.[21,22] *In vitro* isothermal calorimetric (ITC) analysis showed that phosphorylation of the third APC 20aa repeat increases its β-catenin binding affinity by ~300-fold.[23,24] Recently the crystal structures of phosphorylated APC 20aa repeat 3 in complex with β-catenin revealed that one single APC 20aa repeat together with its flanking

Abbreviations used: APC, adenomatous polyposis coli; aa, amino acids; ITC, isothermal titration calorimetry; CD, circular dichroism; NOE, nuclear Overhauser effect.

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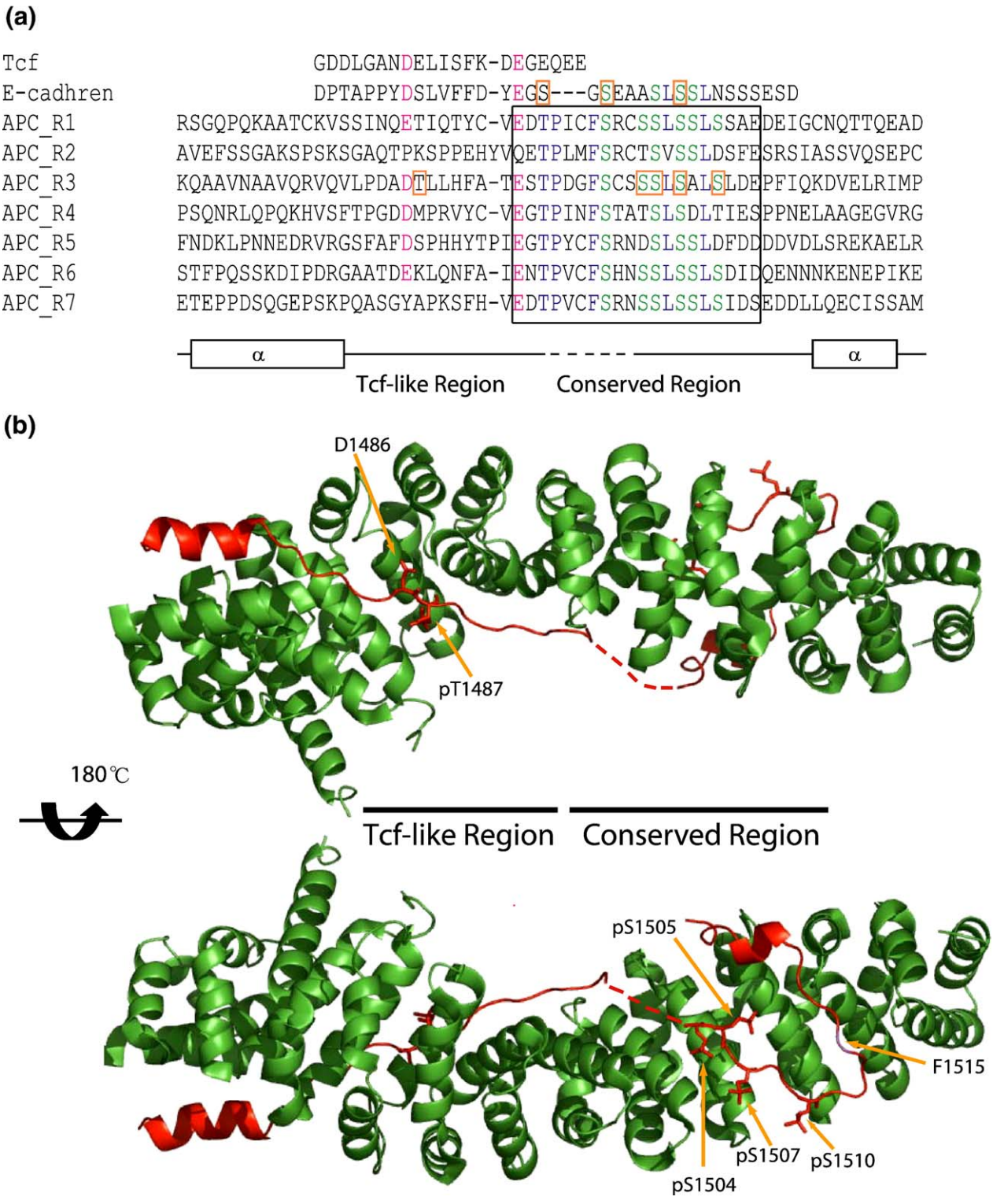

Figure 1. Structural basis of the interaction between β-catenin and APC 20aa repeats. (a) Sequence alignment of Tcf, E-cadherin and APC 20 amino acid repeats. The conserved 20aa repeat regions of APC are framed by a black rectangle. Conserved Ser residues are shown in green. The two acidic residues that may interact with the charged buttons of β-catenin are highlighted in red.[25] Residues phosphorylated in the β-catenin/phospho-APC 20aa repeat and the β-catenin/phospho-E-cadherin complex structures[23,24,26] are boxed in orange. The secondary structure was labeled as observed in the APC-R3 crystal structures. (b) Crystal structure of the β-catenin in complex with a phosphorylated APC-R3. Two views of the overall β-catenin/pAPC-R3 complex[23,24] are related by a 180 degree rotation. The armadillo repeat region of β-catenin and p-APC-R3 are shown in green and red, respectively. Asp1486, the APC residue that forms a critical salt-bridge with the first charged button of β-catenin, and five residues that are phosphorylated *in vitro*, are shown in red sticks. Residue F1515 was also labeled to indicate the position where we made a nonsense mutation to test the contribution of the C-terminal flanking region.