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Protein kinase CK2 interacts with adiponectin receptor 1 and participates in adiponectin signaling

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ABSTRACT

Adiponectin is an adipokine with anti-atherogenic, anti-diabetic and insulin sensitizing properties. Its effects on energy homeostasis, glucose and lipid metabolism are mediated by two ubiquitously expressed seven-transmembrane receptors, AdipoR1 and -R2. With the exception of APPL1 and RACK1, no intracellular binding partners of adiponectin receptors are reported and thus signaling pathways downstream of these receptors remain largely unknown. To incorporate adiponectins protective potential in drug development it is essential to understand adiponectin signaling cascades in detail. A yeast two-hybrid approach employing AdipoR1s cytoplasmatic N-terminus led to the identification of the regulatory subunit of protein kinase CK2. We confirmed the interaction in co-immunoprecipitation, ELISA experiments and co-localization analysis in mammalian cells. Furthermore we could localize the interaction site in an N-terminal basic region close to the transmembrane domain. In adiponectin stimulation experiments of C2C12 mouse myotubes and MCF7 cells incorporating CK2 inhibitor 2-dimethylamino-4,5,6,7-tetrabromo-1H-benz-imidazole (DMAT) we found a modulator role of CK2 in adiponectin signaling. Accordingly we identified the regulatory subunit of protein kinase CK2 as a novel intracellular partner of AdipoR1 and have strong evidence of CK2 as an effector molecule in adiponectin signaling. Since CK2 is involved in signaling cascades of other adipokines and hormones, e.g. leptin and insulin, our findings suggest a possible key function in crosstalk between adiponectin and insulin signaling pathways and could provide further insight into the anti-diabetic effects of adiponectin.

1. Introduction

Adiponectin belongs to the group of adipose tissue-derived hormones implementing the role of adipose tissue as an endocrine organ. A growing body of evidence indicates adiponectin as a key player in regulation of insulin sensitivity, inflammation and energy homeostasis [1–3]. Reduced serum concentrations correlate with obesity and type 2 diabetes in humans [4,5]. These findings underline the great attention received by adiponectin and make it a promising candidate for drug development and treatment of metabolic disorders.

Adiponectin is present in a full length form (fAd) and to a lesser extent as a globular fragment (gAd). The full length protein forms a wide range of multimers from low, medium to high molecular weight complexes. Adiponectin variants appear to be involved in different and tissue-specific signaling pathways [6]. Recently two receptors have been identified as adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) with AdipoR1 acting as a high affinity receptor for gAd with a reduced affinity for fAd, while AdipoR2 seems to possess medium affinity for both variants [7]. Both receptors comprise seven transmembrane helices but are structurally and functionally distinct from G-protein coupled receptors. We have already confirmed the inverse integration of AdipoRs in the plasma membrane with an intracellular N-terminus and an extracellular C-terminus (Fig. 1*A*) [8].

Obviously, AMP dependent kinase (AMPK) exerts a central role in mediating the stimulatory effect of adiponectin on fatty acid oxidation via phosphorylation and inhibition of the acetyl-CoA carboxylase (ACC). Furthermore adiponectin induces GLUT4 transport and subsequent glucose uptake via activation of AMPK. With the exception of the adaptor molecule APPL1 [9] and the receptor for activated C-kinase 1 (RACK1) [10] no interaction partners of the receptors have been identified. Thus, the intracellular signaling cascades accounting for the various effects of adiponectin remain poorly understood.

Here we report the protein kinase CK2 β subunit (CK2β) as a novel interaction partner of AdipoR1. CK2β is the regulatory subunit of the protein serine/threonine kinase CK2. In its most abundant form CK2 appears as a holoenzyme consisting of two regulatory (β) and two catalytic subunits (α and/or α′) [11]. There is growing evidence that both subunits may also occur separately and mediate effects individually [12–14]. CK2 is involved in a complex series of cellular processes such as proliferation, apoptosis and differentiation [15], exhibiting a magnitude of substrates and interaction partners [16].

Abbreviations: α-protein AB, anti-protein antibody; ACC, acetyl-CoA carboxylase; AdipoR, adiponectin receptor; AMPK, AMP dependent kinase; CK2β, protein kinase CK2 β subunit; DMAT, 2-dimethylamino-4,5,6,7-tetrabromo-1H-benz-imidazole; DSP, dithiobis[succinimidylpropionate]; fAd, full length adiponectin; gAd, globular adiponectin; IRS1, insulin receptor substrate.

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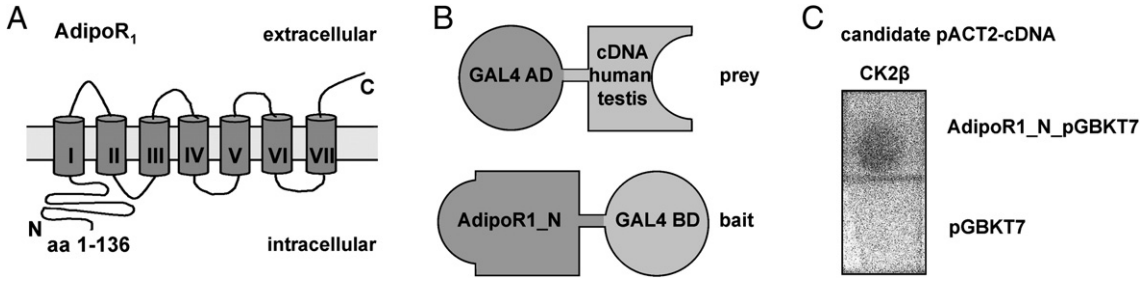

Fig. 1. Screening of interaction partners of AdipoR1-N in a yeast two-hybrid assay. (*A*) Schematic diagram illustrating the structure of AdipoR1 with its intracellular N-terminus, 7TM domains and the extracellular C-terminus. (*B*) Components of the yeast two-hybrid assay: bait (AdipoR1-N fused to GAL4-DNA binding domain) and prey (human testis cDNA library fused to GAL4 activation domain. N = N-terminus, C = C-terminus, GAL4 BD = GAL4-DNA binding domain, GAL4 AD = GAL4 activation domain. (*C*) Results from co-transformation of the Yeast strain AH 109 with the prey-candidate (CK2β) and either AdipoR1_N-pGBKT$_7$ (bait) or pGBKT$_7$ (control plasmid). Transformed colonies were spotted and grown on nutritionally selective plates (-Ade, -Leu, -His, -Trp) and examined by α-galactosidase activity. Blue colonies indicate reporter gene expression in AH109. When AH109 is co-transformed with the candidate plasmid pACT$_2$-cDNA and the insert-less pGBKT$_7$ absent colony growth proved for the correctness of the candidate.

Accordingly, in addition to the interaction, we could also identify a functional role of CK2 in adiponectin signaling, as adiponectin signaling was modified by the specific CK2 inhibitor DMAT. Thus, our findings represent a promising starting point to gain deeper insight into the complexity of adiponectin signaling.

2. Material and methods

2.1. Plasmids, adiponectin, antibodies and recombinant protein

Cloning of the full length cDNA encoding AdipoR1 from human MCF7 cells and subsequent subcloning of cDNAs encoding AdipoR1 and its intracellular N-terminal fragment (aa 1-136) AdipoR1_N into mammalian expression vector pEYFP-N1 from Clontech (Mountain View, CA) was performed as described previously [8]. CK2 was cloned into a Clontech expression vector with an N-terminal HA-tag. Full length and globular adiponectin were cloned into pET-15b bacterial expression vector and expressed as His-tag fusion proteins in *E.coli* BL21(DE3) pLysS_Rare. Fusion proteins were purified by Ni-NTA affinity chromatography and purity and identity were confirmed by SDS-PAGE, western blot and mass spectrometry. YFP-tagged AdipoR1 constructs were immunoprecipitated using an antibody (AB) against the tag (rabbit polyclonal α-GFP AB, Abcam). Protein specific antibody α-AdipoR1 was obtained from Acris (Hiddenhausen, Germany), α-CK2β AB was from Jena Bioscience (Jena, Germany) and α-HA-TRITC AB, α-β-tubulin AB, as well as control IgGs, were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). ACC and phospho-ACC specific antibodies as well as 5-aminoimidazole-4-carboxyamide ribonucleoside (AICAR) were from Cell Signaling Technologies (Danver, MA). Recombinant human CK2β was obtained from BiAffin (Kassel, Germany). InSolution™ casein kinase II inhibitor 2-dimethylamino-4,5,6,7-tetrabromo-1H-benz-imidazole (DMAT) was purchased from Calbiochem (San Diego, SC).

2.2. Bacterial and yeast strains

E.coli BL21(DE3)pLysS_Rare was used for bacterial expression of His$_6$-tagged proteins. The yeast *Saccharomyces cerevisiae* strain AH109 based two-hybrid reagents were purchased from Clontech. All yeast culture media, supplements and reagents were obtained from Invitrogen (Carlsbad, CA), Clontech or Sigma-Aldrich (St. Louis, MO).

2.3. Yeast two-hybrid cDNA library screen

AdipoR1 N-terminal cDNA (aa 1-136) was cloned into yeast expression vector pGBKT$_7$ (Clontech) containing a GAL4-DNA binding domain (GAL4 BD) (Fig. 1*B*). A pretransformed human testis cDNA library/pACT$_2$ in Y178 (Clontech) was used and the two-hybrid screening was carried out as described (Clontech Matchmaker Two-Hybrid System Protocols). Briefly, the bait plasmid AdipoR1_N_pGBKT$_7$ was transformed into the *Saccharomyces cerevisiae* strain AH109 and transformants were selected on artificial medium lacking tryptophane. The strain AH109 containing AdipoR1_N_pGBKT$_7$ was mated with the human testis cDNA library containing strain Y178. After 24 h incubation time of the mated strains in 2*YPDA/Kan media suspension the diploid strains were plated onto medium lacking histidine, leucine and tryptophane and incubated colonies side down, at 30 °C for 8 days. Subsequent colonies from those plates were replica-plated under more stringent conditions. In this approach plates lacking adenine, histidine, leucine and tryptophane and containing X-α-Gal were used. False positives were eliminated by using the reporter set ADE2, HIS3 and MEL1 encoding α-galactosidase with X-α-gal for indication. The pACT$_2$-cDNAs of identified positive clones were rescued and double checked by a co-transformation protocol. Yeast strain AH 109 was co-transformed with the prey candidates and either AdipoR1_N-pGBKT$_7$ (bait) or pGBKT$_7$ (control plasmid). The transformed colonies were spotted and grown on nutritionally selective plates (-Ade, -Leu, -His, -Trp) and controlled for α-galactosidase activity. Blue colonies indicate a reporter gene expression in AH109 due to the interaction of prey and candidate. When AH109 was co-transformed with the candidate plasmid pACT$_2$-cDNA and the insert-less pGBKT$_7$ absent colony growth verified the correctness of the candidate AdipoR1_N-terminal protein interaction (Fig. 1*C*). The library plasmids were recovered from selected clones and sequenced with an automated DNA sequencer (ABI Prism 3100 Genetic Analyzer, Applied Biosystems, Foster City, CA) using a dye terminating method.

2.4. Cell culture and transfection

C2C12 myoblasts were obtained from ATCC (Manassas, VA) and were grown in DMEM (PAA, Pasching, Austria) supplemented with 10% fetal calf serum (PAA) at 37 °C in a humidified 10% CO_2 atmosphere and differentiated as previously described with only minor modifications [9]. MCF7 cells were grown in DMEM Ham's F-12 medium (PAA) supplemented with 10% FCS. Cells were serum starved for 5 h before stimulation experiments. All transfections were performed using Lipofectamin2000 (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol.

2.5. Peptide synthesis

Peptides were synthesized by solid-phase synthesis on a Syro II robot (Syro, MultiSynTech, Bochum, Germany) using fluorenyl-methoxycarbonyl/tert-butyl (Fmoc/t-Bu) strategy on a Wang resin (Novabiochem) as previously described [17]. Peptides were analyzed by RP-HPLC (Vydac RP18-column, 4.6×250 mm; 5 µm/ 300 °A, Merck