Crystal structures of the human adiponectin receptors

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Adiponectin stimulation of its receptors, AdipoR1 and AdipoR2, increases the activities of 5′ AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor (PPAR), respectively, thereby contributing to healthy longevity as key anti-diabetic molecules. AdipoR1 and AdipoR2 were predicted to contain seven transmembrane helices with the opposite topology to G-protein-coupled receptors. Here we report the crystal structures of human AdipoR1 and AdipoR2 at 2.9 and 2.4 Å resolution, respectively, which represent a novel class of receptor structure. The seven-transmembrane helices, conformationally distinct from those of G-protein-coupled receptors, enclose a large cavity where three conserved histidine residues coordinate a zinc ion. The zinc-binding structure may have a role in the adiponectin-stimulated AMPK phosphorylation and UCP2 upregulation. Adiponectin may broadly interact with the extracellular face, rather than the carboxy-terminal tail, of the receptors. The present information will facilitate the understanding of novel structure–function relationships and the development and optimization of AdipoR agonists for the treatment of obesity-related diseases, such as type 2 diabetes.

Adiponectin (encoded by ADIPOQ in humans)1–4 is an anti-diabetic adipokine. Plasma adiponectin levels are reduced in obesity and type 2 diabetes5, while the replenishment of adiponectin reportedly ameliorates insulin resistance and dyslipidaemia in mice6–8. These beneficial effects of adiponectin are likely to be exerted, at least in part, by the activation of AMPK9–11 and PPAR-α,β,γ.12,13

We previously reported the expression cloning of the complement-arity DNAs encoding adiponectin receptors 1 and 2 (ADIPOR1 and ADIPOR2)14. AdipoR1 and AdipoR2 are predicted to contain a seven-transmembrane (7TM) domain14, with an internal amino terminus and an external C terminus, which is the opposite configuration to G-protein-coupled receptors (GPCRs). Therefore, AdipoR1 and AdipoR2 are thought to be structurally and functionally distinct from GPCRs15. AdipoR1 and AdipoR2 serve as the major receptors for adiponectin in vivo, with AdipoR1 activating the AMPK pathways and AdipoR2 the PPAR-γ pathways such as increased expression of uncoupling protein 2 (UCP2)15. Thereby, they regulate glucose and lipid metabolism, inflammation and oxidative stress in vivo. Recently, the small-molecule AdipoR agonist AdipoRon was shown to ameliorate diabetes and increase exercise endurance, and at the same time prolong the shortened lifespan in obesity16. It should also be noted that adiponectin receptors are conserved in evolution from mammals to plants and yeasts (http://www.ncbi.nlm.nih.gov/guide/proteins/), strongly suggesting that they have essential biological roles.

It is extremely difficult to crystallize GPCRs, owing to their conformational flexibility. By achieving technical breakthroughs, the crystal structures of the human β2 adrenoceptor (β2AR) were reported19–21. First, the conformational complexity of β2AR was controlled with high-affinity ligands (nanomolar dissociation constants), agonists and inverse agonists, to fix β2AR in the active and inactive forms, respectively20,21. Second, the crystallization was performed with antibody fragments and/or a protein fusion, in the lipidelic mesophase. These technical advancements enabled the structure determination of many other GPCRs, and an understanding of their ligand specificities24. Furthermore, the first crystal structure of the active-state complex of an agonist-occupied β2AR with a nucleotide-free Gs heterotrimer was reported25. Thus, the β2AR structures greatly promoted the fields of GPCR research and drug development26,27.

In contrast to the GPCRs, no information is available about the conformational states of AdipoR1 and AdipoR2 with respect to transmembrane signalling. Although the AdipoR agonist AdipoRon was successfully developed28, further refinement of the AdipoR agonists to achieve nanomolar dissociation constants is still underway. The structural information about AdipoR1 and/or AdipoR2, if available, would be very important for understanding the AdipoR signalling mechanisms, and for developing and optimizing AdipoR agonists.

We optimized the properties of human AdipoR1 and AdipoR2 by deleting their N-terminal tails, and then used the Fv fragment of an anti-AdipoR monoclonal antibody and the lipidic mesophase for crystallization29. In this study, we successfully determined the crystal structures of human AdipoR1 and AdipoR2 at 2.9 and 2.4 Å resolution, respectively.
The structure of AdipoR1 (residues 89–375) (Fig. 1a) contains the 7TM architecture, the zinc-binding site, the proline-induced kink20–21, of the transmembrane helices, such as the proline-induced kink20–21, of the transmembrane helices of GPCRs in classes A, B and C (refs 20, 21, 32, 33) are not observed in the AdipoR1/AdipoR2 structures, the transmembrane helices are not kinked, while helix V is slightly curved owing to three Gly residues (Fig. 2). Consequently, we concluded that the AdipoR1 and AdipoR2 structures are novel.

The zinc-binding sites of AdipoR1 and AdipoR2
Remarkably, we found a zinc ion bound within the 7TM domain in the AdipoR1 and AdipoR2 structures (Fig. 3a), by X-ray absorption spectroscopy (data not shown) and the anomalous difference Fourier map (Fig. 3b). The zinc-binding site is located in the intracellular layer of the membrane. The zinc ion is coordinated by three His residues, His 191 in helix II and His 337 and His 341 in helix VII of AdipoR1, and His 202 in helix II and His 348 and His 352 in helix VII of AdipoR2, at zinc–nitrogen distances of 2.1–2.6 Å (Fig. 3c, d). The zinc ion is thus located approximately 4 Å deep from the inner surface of the plasma membrane (Fig. 3a). Furthermore, a water molecule is observed between the zinc ion and the side-chain carboxyl group of Asp 219 in helix III of AdipoR2. Thus, the zinc ion has a tetrahedral coordination (Fig. 3d). The zinc ion binds helices II, III and VII together (Fig. 3c, d), and probably stabilizes the structure of the subdomain consisting of helices I, II, III and VII (Extended Data Fig. 2). The three His and Asp (3 × His + Asp) residues of AdipoR1 and AdipoR2 are strictly conserved in the homologues from mammals to plants and bacteria (Extended Data Fig. 4a, b).

Figure 1 | Overall structures of AdipoR1 and AdipoR2. a, The 2.9 Å resolution structure of AdipoR1. b, The 2.4 Å resolution structure of AdipoR2. The structures were determined for their complexes with an Fv fragment, but the Fv fragments are omitted here for clarity. The structures are viewed from the extracellular side (left) and parallel to the membrane (right). The NTR, helix 0, transmembrane helices I–VII and the CTR of AdipoR1 (a) and AdipoR2 (b) are indicated.

Figure 2 | Sequence alignment of human AdipoR1 and AdipoR2. Amino acid residues that are not conserved between these receptors are shown in green (AdipoR1) and cyan (AdipoR2). The deleted residues in the constructs and the disordered residues in the crystal structures are shown in grey and yellow, respectively. The helices in the crystal structures are surrounded by blue squares. The identical and similar residues between the two proteins are indicated with red asterisks and black colons, respectively. The characteristic Gly residues in helix V and in the CTR are indicated with red and blue number signs, respectively.
We mutated the zinc-coordinated 3× His + Asp residues of AdipoR1 (residues 89–375) (Fig. 3e). As compared with the parent AdipoR1 molecule (89–375), the adiponectin-stimulated AMPK phosphorylation was reduced by the triple mutant His191Ala/His337Ala/His341Ala (3Ala) and more seriously by the quadruple mutant His191Ala/Asp208Ala/His337Ala/His341Ala (4Ala), while none of the single His191Ala, Asp208Ala, His337Ala and His341Ala mutations affected it (Fig. 3e and Extended Data Fig. 4c). Therefore, the results suggested that zinc binding is not directly required for the adiponectin-stimulated AMPK phosphorylation, but exerts a putative structure-stabilizing effect.

By contrast, the adiponectin-stimulated UCP2 upregulation by AdipoR2 was markedly reduced by each of the single mutations Asp219Ala and His348Ala, and nearly completely eliminated by the triple mutation His202Ala/His348Ala/His352Ala (3Ala) and the quadruple mutation His202Ala/Asp219Ala/His348Ala/His352Ala (4Ala) of AdipoR2 (residues 1–386 and 100–386), as compared with the wild-type AdipoR2 (Fig. 3f and Extended Data Fig. 4d, e). Correspondingly, the single mutations His202Ala and His352Ala of AdipoR2 (residues 100–386) did not decrease the amount of bound zinc ion, whereas the single mutations Asp219Ala and His348Ala decreased it moderately, and the multiple mutations 3Ala and 4Ala reduced it markedly (data not shown). These results suggested that the zinc ion is directly involved in the adiponectin-stimulated UCP2 upregulation in the case of AdipoR2, in addition to structural stabilization.

An attractive hypothesis is that AdipoR2 has zinc-ion-dependent hydrolytic activity, and uses the water molecule fixed between the zinc ion and the side-chain carboxyl group of Asp219 of AdipoR2 for the nucleophilic attack on the carbonyl carbon atom of substrates. Free fatty acid might be produced from lipid hydrolysis by the adiponectin-stimulated AdipoR2, and PPAR-α activation by the produced free fatty acid would increase the expression of the target genes, such as UCP2.

The zinc-binding structures in the transmembrane domains of AdipoR1 and AdipoR2 are novel. The only previously reported membrane protein structure with a zinc ion within the transmembrane domain is that of a site-2 protease family intramembrane metalloprotease34. The protease consists of six transmembrane segments, and the catalytic zinc ion is coordinated by two His residues and one Asp residue, and is approximately 14 Å deep from the inner surface of the plasma membrane. Therefore, the site-2 protease and AdipoR structures are not homologous. By contrast, some globular zinc enzyme structures share architectural similarity, in terms of the coordination of three His residues and a water molecule35,36 (Extended Data Fig. 5). Although the transmembrane alkaline ceramidases share negligible sequence homology...
with AdipoR, three His residues and one Asp residue are conserved in these proteins. However, their crystal structures have not been solved. Therefore, we presently cannot completely exclude the possibility that the AdipoRs have ceramidase activity.

The large internal cavities of AdipoR1 and AdipoR2

In both the AdipoR1 and AdipoR2 structures, the seven transmembrane helices surround a large internal cavity, including the zinc-binding site (Fig. 4a, b). This large internal cavity is formed between the four- and three-helix subdomains (helices VII–I–II–III and IV–V–VI, respectively) of the 7TM domains of AdipoR1/2 (Extended Data Fig. 2). The cavities extend from the cytoplasmic surface to the middle of the outer lipid layer of the membrane (Fig. 4a, b), and contain unidentified extra electron densities, which are weaker than those of the protein (Fig. 4c, d). In the cavity of AdipoR2, the extra electron densities are observed along with helices III, V and VI (Fig. 4d). By contrast, in the cavity of AdipoR1, even weaker electron densities are observed on the cytoplasmic side of the cavity (Fig. 4c). These weak electron densities might be relevant to the substrates/products of the hypothesized hydrolytic activities of AdipoR1/AdipoR2.

The cavity has small openings between helices V and VI within the outer lipid layer and between helices IV and VI on the cytoplasmic side (Fig. 4a, b). Intriguingly, a much larger opening at helices III–VII would be uncovered on the cytoplasmic side, if the NTR was displaced from its present position (Extended Data Fig. 6). These openings might serve as the entrance/exit for the substrate/product of the hypothesized hydrolytic activity. Notably, the shorter constructs (residues 102–375 and 120–375) of AdipoR1 are also as active as the full-length AdipoR1 with respect to adiponectin-stimulated AMPK phosphorylation (Extended Data Fig. 1a), indicating that the NTR, which covers the large internal cavity, is not required for this activity.

The amino acid sequences of the ICL2 regions are significantly different between AdipoR1 and AdipoR2 (Fig. 2). In particular, AdipoR1 has a cluster of positively charged residues, Arg 257, Lys 262 and His 263, in the ICL2 region (Extended Data Fig. 7), unlike AdipoR2. Consequently, this structural difference in the cytoplasmic face may reflect the distinct signalling pathways downstream of these adiponectin receptors.

The extracellular faces of AdipoR1 and AdipoR2

The ECL1–3 and the CTR are exposed on the extracellular faces of AdipoR1 and AdipoR2. The three extracellular loops exhibit high conservation between AdipoR1 and AdipoR2 (Fig. 5a–d). Helices VII and helix VI to the very C terminus in the present structures. On the other hand, in this study, the CTR deletion after residue 366 or 370 of AdipoR1 did not affect the adiponectin-stimulated AMPK phosphorylation via AdipoR1 (Fig. 5e), indicating that the flexible CTR is not necessarily required for AMPK phosphorylation by adiponectin. By contrast, the longer deletion of the C-terminal thirteen residues up to Tyr 363 and Gly 364, the last two residues of helix VII, reduced the adiponectin-stimulated AMPK phosphorylation via AdipoR1 (residues 1–362; Fig. 5e and Extended Data Fig. 9a), indicating that the protruding C-terminal turn of helix VII may be involved in adiponectin signalling. Furthermore, the extracellular loop residues conserved between AdipoR1 and AdipoR2 were mutated to Gly/Ser (Fig. 5f, g): the three-loop mutation (ECL1/2/3) combined with the C-terminal 13-residue deletion (1–362) remarkably decreased adiponectin-stimulated AMPK phosphorylation via AdipoR1 (Fig. 5g and Extended Data Fig. 9b). The other mutants with fewer Gly/Ser mutations (ECL1, ECL2, ECL3 and ECL1/3) or with no C-terminal deletion showed correspondingly smaller decreased (Fig. 5f, g and Extended Data Fig. 9a, b). These data raised the possibility that AdipoR1 may recognize adiponectin by the extensive use of its extracellular face, including the three extracellular loops and the C-terminal turns of helix VII.

Conclusions

The structural and functional characteristics of AdipoR1 and AdipoR2 revealed by this study are completely different from those of GPCRs, and therefore the AdipoRs represent an entirely new class of receptor. The present crystal structures are expected to provide a strong basis for the development and optimization of adiponectin receptor agonists, such as Adioporon37, as well as for understanding the roles and mechanisms of the AdipoR1/AdipoR2 homologues from animals and plants in putative signalling, such as in defence systems and lipid metabolism (Extended Data Fig. 4a, b).
et al. 23. Rosenbaum, D. M.
et al. 2. Hu, E., Liang, P. & Spiegelman, B. M. AdipoQ is a novel adipose-specific gene
7. Berg, A. H., Combs, T. P., Du, X., Brownlee, M. & Scherer, P. E. The adipocyte-
11. Kahn, B. B., Alquier, T., Carling, D. & Hardie, D. G. AMP-activated protein kinase:
1. Scherer, P. E., Williams, S., Fogliano, M., Baldini, G. & Lodish, H. F. A novel serum
6. Yamauchi, T.
et al. 19. Rasmussen, S. G.
et al. 18. Lyons, T. J.
et al. 26. Shimamura, T.
et al. 20. Cherezov, V.
et al. 27. de Graaf, C. et al. Crystal structure-based virtual screening for fragment-like ligands of the human histamine H2 receptor. J. Med. Chem. 54, 8195–8206 (2011).
et al. 28. Tanabe, H. et al. Expression, purification, crystallization, and preliminary X-ray crystallographic studies of the human adipopectin receptors, AdipoR1 and AdipoR2. J. Struct. Funct. Genomics 16, 11–23 (2015).
et al. 29. Holm, L. & Rosenstrom, P. Dalai server: conservation mapping in 3D. Nucleic Acids Res. 38, W545–W549 (2010).
et al. 30. Palczewski, K. et al. Crystal structure of rhodopsin: a G protein-coupled receptor.
text editor 2002, 267–274 (1996).
et al. 37. Mao, X. et al. APPL1 binds to adiponectin receptors and mediates adiponectin signaling and function. Nature Cell Biol. 8, 516–523 (2006).
36. S. Y., T. K., K. Ishii for expression plasmid preparation, M. Nishimoto, Y. Tomabechi and
35. Wu, H. et al. Cloning of adiponectin receptors that mediate antidiabetic effects. Nature Med. 11, 346–354 (1997).
et al. 36. S. Y. and M. Y., a Grant-in-Aid for Scientific Research (B) (26293216) (T.K.), a Grant-in-Aid for Specially
34. Wu, H. et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nature Med. 8, 1288–1295 (2002).
et al. 35. Wu, H. et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nature Med. 8, 1288–1295 (2002).
et al. 20. Cherezov, V. et al. Structure of a nanobody-stabilized active state of the
29. Holm, L. & Rosenstrom, P. Dalai server: conservation mapping in 3D. Nucleic Acids Res. 38, W545–W549 (2010).
et al. 26. Shimamura, T. et al. Structure of the human histamine H2 receptor complex with doxepin. Nature 475, 65–70 (2011).
et al. 25. Rasmussen, S. G. et al. Crystal structure of the β2 adrenergic receptor-G protein coupled receptor. Science 318, 1258–1265 (2007).
et al. 24. Venkatakrishnan, A. J. et al. Molecular signatures of G-protein-coupled receptors. Nature 494, 185–194 (2013).
et al. 23. Rosenbaum, D. M. et al. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nature Med. 13, 332–339 (2007).
et al. 22. Okada-Iwabu, M. et al. Small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. Nature 503, 493–499 (2013).
et al. 21. Rasmussen, S. G. et al. Crystal structure of the human β2 adrenergic G-protein-coupled receptor. Science 318, 1258–1265 (2007).
et al. 20. Cherezov, V. et al. High-resolution crystal structure of an engineered human β2-adrenergic G protein-coupled receptor. Science 318, 1258–1265 (2007).
et al. 19. Rasmussen, S. G. et al. Activation of the human β2-adrenergic receptor by a nanobody. Nature 494, 353–357 (2007).
et al. 18. Lyons, T. J. et al. Metalloregulation of yeast membrane steroid receptor homologs. Proc. Natl Acad. Sci. USA 101, 5506–5511 (2004).
et al. 17. Okada-Iwabu, M. et al. A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. Nature 503, 493–499 (2013).
et al. 16. Yamauchi, T. et al. Cloning of adiponectin receptors that mediate anti-diabetic metabolic effects. Nature 423, 762–769 (2003).
et al. 15. Wess, J. G-protein-coupled receptors: molecular mechanisms involved in receptor activation and selectivity of G-protein recognition. FASEB J. 11, 346–354 (1997).
et al. 14. Yamauchi, T. et al. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nature Med. 13, 332–339 (2007).
et al. 13. Kaino, K. et al. Adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423, 762–769 (2003).
et al. 12. Scherer, P. E., Williams, S., Fogliano, M., Baldini, G. & Lodish, H. F. A novel serum
et al. 11. Kahn, B. B., Alquier, T., Carling, D. & Hardie, D. G. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab. 1, 1–25 (2005).
et al. 10. Kersten, S., Desvergne, B. & Wahli, W. Roles of PPARs in health and disease. Nature 405, 421–424 (2000).
et al. 9. Yamauchi, T. et al. Globular adiponectin protected ob/ob mice from diabetes and Apo-ε-deficient mice from atherosclerosis. J. Biol. Chem. 278, 2461–2468 (2003).
et al. 8. Yamauchi, T. et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nature Med. 8, 1288–1295 (2002).
et al. 7. Yamauchi, T. et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nature Med. 8, 1288–1295 (2002).
et al. 6. Yamauchi, T. et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nature Med. 8, 1288–1295 (2002).
et al. 5. Hotta, K. et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nature Med. 8, 1288–1295 (2002).
et al. 4. Scherer, P. E., Williams, S., Fogliano, M., Baldini, G. & Lodish, H. F. A novel serum
et al. 3. Maeda, K.
et al. 2. Hu, E., Liang, P. & Spiegelman, B. M. AdipoQ is a novel adipose-specific gene
et al. 1. Scherer, P. E., Williams, S., Fogliano, M., Baldini, G. & Lodish, H. F. A novel serum
**METHODS**

Preparation of the AdipoR1–Fv and AdipoR2–Fv crystals. The human AdipoR1 and AdipoR2 proteins and the Fv fragment of an anti-AdipoR1 monoclonal antibody were prepared as described. In brief, human AdipoR1 and AdipoR2 (residues 89–375 and 100–386, respectively) were expressed in High Five insect cells. The proteins were purified by Flag antibody affinity chromatography followed by anion exchange chromatography, metal ion affinity chromatography after cleaving the N-terminal Flag tag by His-tagged tobacco etch virus (TEV) protease, and size-exclusion chromatography. The Fv fragment was cloned from hybridoma cells. The Fv fragment was synthesized by the Escherichia coli cell-free protein synthesis method, and purified by Ni-affinity chromatography followed by size-exclusion chromatography. The purified AdipoR1 and AdipoR2 proteins were mixed with the Fv fragment, and the AdipoR1–Fv and AdipoR2–Fv complexes were purified by size-exclusion chromatography, and crystallized by the lipidic mesophase method.

**X-ray data collection.** Data collection was performed on beamline BL32XU at SPring-8, using an MX225HE CCD detector. X-ray diffraction data were collected at 100 K by the helical scan method, with a beam size of 1 × 10 μm (horizontal × vertical) using 1° oscillation. The AdipoR1 and AdipoR2 crystals diffracted up to 2.8 Å and 2.2 Å resolution, respectively. Data collection from the AdipoR1 crystals was limited to 10–30 images per crystal, owing to radiation damage in the microcrystals, and data from five crystals were merged to complete the data set. For AdipoR2, diffraction data were collected from a single crystal. The data from the AdipoR1 crystals and the AdipoR2 crystal were indexed, scaled and merged with the HKL2000 program, and the XDS package, respectively. The data collection statistics are shown in Extended Data Table 1. The AdipoR1 crystals belonged to the space group P2_12_1_2, with unit cell parameters a = 92.3, b = 194.1, c = 74.3 Å, and the AdipoR2 crystal belonged to the space group P2_1_2_1_2, with unit cell parameters a = 74.6, b = 108.6, c = 101.0 Å.

**Structure solution and refinement.** The initial phases for the AdipoR2–Fv complex were obtained by molecular replacement, using Fv (the V_1 and V_2 fragments from PDB accessions 1E6 and 1FDL, respectively) in Phaser as a search model. The resulting phases were improved by density modification using the program RESOLVE, and thereby the electron density map around the helix bundle region of AdipoR2 became clearly visible. The initial model (all of Fv and about 80% of AdipoR2) was automatically built using the program AutoBuild, and the rest of the model (the loops connecting the transmembrane helices) was built manually using COOT. Refinement was performed with phenix.refine, and the refined coordinates were rebuilt with COOT. The structure of the AdipoR2–Fv complex was refined with final Rwork/Rfree values of 0.25/0.29. The structure of the AdipoR1–Fv complex was determined by molecular replacement, using that of the AdipoR2–Fv complex as a search model, and was refined with the secondary structure restraints in phasenx.refine, as a reﬁnement of the AdipoR1–Fv complex was performed similarly to that of the AdipoR2–Fv complex. The structure of the AdipoR1–Fv complex was refined with final Rwork/Rfree values of 0.24/0.30. Ramachandran statistics were analysed with MolProbity. In the AdipoR1–Fv complex structure, 95.8% of residues were in favoured regions and 4.2% of residues were in allowed regions. In the AdipoR2–Fv complex structure, 95.6% of residues were in favoured regions and 4.4% of residues were in allowed regions. Each of the final models of the AdipoR1–Fv and AdipoR2–Fv complexes includes 281 residues of the receptor, 119 residues of V_1, and 107 residues of V_2. The data collection and refinement statistics are summarized in Extended Data Table 1. Structural illustrations were generated using PyMOL.

**Cell culture.** HEK293T cells (ATCC) were cultured in DMEM supplemented with 10% (v/v) FBS. Cells were transfected using Lipofectamine 2000 (Invitrogen), according to the manufacturer’s instructions. The cDNAs encoding the ADIPOR mutants were introduced into the pORI vector, for the expression of proteins tagged with a Flag epitope.

**Generation of recombinant adiponectin.** Recombinant mouse full-length adiponectin was generated as previously described. The expression of His-tagged adiponectin was induced by the addition of isopropyl (1-1-thiogalactopyranoside to the growth medium. Bacterial extracts were prepared using standard methods, and the fusion proteins were purified by elution through a nickel-1 agarose column. Western blot analysis and measurement of AMPK activities. Phosphorylation and protein levels of AMPK were determined as described. Western blot analyses were performed with anti-phosphorylated-AMPK (Cell Signaling Technology 2535) and anti-α-AMPK (Cell Signaling Technology 2532) antibodies. Protein levels of AdipoR were analysed by western blotting, using an anti-Flag antibody (Sigma-Aldrich F1804).

Real-time PCR. Real-time PCR was performed according to the method described previously. Total RNA was prepared from cells with Trizol (Invitrogen), according to the manufacturer’s instructions. We used the real-time PCR method to quantify the mRNAs, with slight modifications.

**Statistics.** Results are expressed as mean ± s.e.m. Differences between two groups were assessed using unpaired two-tailed t-tests. Data involving more than two groups were assessed by analysis of variance (ANOVA) followed by post-hoc comparisons. No statistical methods were used to predetermine sample size.

38. Hato, M., Hosaka, T., Tanabe, H., Kitsunai, T. & Yokoyama, S. A new manual dispensing system for in meso membrane protein crystallization with using a stepping motor-based dispenser. J. Struct. Funct. Genomics 15, 165–171 (2014).
39. Hirata, K. et al. Achievement of protein micro-crystallography at SPring-8 beamline BL32XU. J. Phys. Conf. Ser. 425, 012002 (2013).
40. Murakami, I. et al. Tumor volume and lymphovascular space invasion as a prognostic factor in early invasive adenocarcinoma of the cervix. J. Gynecol. Oncol. 23, 152–158 (2012).
41. Ueno, G., Kanda, H., Kumasaka, T. & Yamamoto, M. Beamline Scheduling Software: administration software for automatic operation of the RIKEN structural genomics beamlines at SPring-8. J. Synchrotron Radiat. 12, 380–384 (2005).
42. Ohnivinski, Z. & Minor, W. Processing of X-ray diffraction data collected in oscillation mode. Methods Enzymol. 276, 307–326 (1997).
43. Kabosch, W. Xds. Acta Crystallogr. D 66, 125–132 (2010).
44. McCoy, A. J. et al. Phaser crystallographic software. J. Appl. Crystallogr. 40, 658–674 (2007).
45. Terwilliger, T. C. Maximum-likelihood density modification. Acta Crystallogr. D 56, 965–972 (2000).
46. Terwilliger, T. C. Automated side-chain model building and sequence assignment by template matching. Acta Crystallogr. D 59, 49–49 (2003).
47. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of COOT. Acta Crystallogr. D 66, 486–501 (2010).
48. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D 66, 213–221 (2010).
49. Chen, V. B. et al. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr. D 66, 12–21 (2010).
50. Delano, W. L. The PyMOL molecular graphics system. http://www.pymol.org (Delano Scientific, 2002).
51. Iwabu, M. et al. Adiponectin and AdipoR1 regulate PGC-1α and mitochondria by AMPK activation and AMPK/SIRT1. Nature 464, 1313–1319 (2010).
52. Minokoshi, Y. et al. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 415, 339–343 (2002).
53. Tsao, T. S., Murray, H. E., Hug, C., Lee, D. H. & Lodish, H. F. Oligomization state-dependent activation of NF-κB signaling pathway by adipoctrome complement-related protein of 30 kDa (Acpr30). J. Biol. Chem. 277, 23393–23392 (2002).
54. Woods, A., Sait, I., Scott, J., Hardie, D. G. & Carling, D. The α1 and α2 isoforms of the AMP-activated protein kinase have similar activities in rat liver but exhibit differences in substrate specificity in vitro. FEBS Lett. 397, 347–351 (1996).
55. Hayashi, T. et al. Metabolic stress and altered glucose transport. Activation of AMP-activated protein kinase as a unifying coupling mechanism. Diabetes 49, 527–531 (2000).
56. Hanson, M. A. et al. Crystal structure of a lipid G protein-coupled receptor. Science 335, 851–855 (2012).
57. Lebon, G. et al. Agonist-bound adenosine A2A receptor structures reveal common features of GPCR activation. Nature 474, 521–525 (2011).
58. Vogeley, L. et al. Anabaena sensory rhodopsin: a photochromic color sensor at 2.0 Å. Science 306, 1390–1393 (2004).
Extended Data Figure 1 | Analysis of N-terminal deletion mutants of AdipoR1 and AdipoR2 and lattice packing of the AdipoR1–Fv and AdipoR2–Fv crystals. a, Phosphorylation and amounts of AMPK in HEK293 cells transfected with full-length AdipoR1 (residues 1–375) or N-terminally truncated mutants (residues 47–375, 77–375, 89–375, 102–375 and 120–375), treated for 5 min with adiponectin (15 μg ml⁻¹). b, UCP2 mRNA levels in HEK293 cells transfected with full-length AdipoR2 (residues 1–386) or N-terminally truncated mutants (residues 88–386 and 100–386), treated for 18 h with adiponectin (3 μg ml⁻¹). All values are mean ± s.e.m. n = 3–4, three independent experiments. **P < 0.01 compared to control cells or as indicated (see Methods for statistical tests used). NS, not significant. c–h, Lattice packing of the AdipoR1–Fv crystals (c–e) and the AdipoR2–Fv crystals (f–h). AdipoR1, AdipoR2 and Fv are coloured green, cyan and grey, respectively. The AdipoR1–Fv and AdipoR2–Fv complexes crystallized with an anti-parallel arrangement of the receptor molecules.
Extended Data Figure 2 | Comparison of the AdipoR1–Fv and AdipoR2–Fv structures. a–c, Superimposition of the AdipoR1–Fv and AdipoR2–Fv complexes: side view (a), extracellular view (b), intracellular view (c). Fv was omitted from the intracellular view for clarity. d, Superimposition of the subdomains consisting of helices I, II, III and VII between AdipoR1 and AdipoR2. The Cα r.m.s.d. value is 0.34 Å. e, Helix 0 (pink) interacts hydrophobically with the cytoplasmic ends of helices I–III, and the ICL1 (purple), as represented by those of AdipoR1. In addition, the zinc ion firmly connects helices VII, II and III (Fig. 3). Therefore, helices VII, I, II and III (d) constitute a rigid subdomain in the 7TM-domain structures. By contrast, helices IV, V and VI are superimposed between AdipoR1 and AdipoR2 with a Cα r.m.s.d. value of 0.73 Å, and are likely to constitute the other subdomain, with some conformational differences in helix V between AdipoR1 and AdipoR2.
Extended Data Figure 3 | Comparison of the AdipoR2 structure with other 7TM proteins. The structures of 7TM proteins in the N-terminus-out topology were inverted and superimposed on the AdipoR2 structure in the C-terminus-out topology. Superimpositions of AdipoR2 (cyan) with the β2AR (PDB code 2RH1) (r.m.s.d. 3.9 Å) (a), the glucagon receptor (PDB code 4L6R) (r.m.s.d. 3.8 Å) (b), the metabotropic glutamate receptor 1 (PDB code 4OR2) (r.m.s.d. 2.9 Å) (c), the sphingosine 1-phosphate receptor 1 (PDB code 3V2Y) (r.m.s.d. 3.0 Å) (d), the A2A adenosine receptor (PDB code 2YDV) (r.m.s.d. 3.4 Å) (e), and sensory rhodopsin (PDB code 1XIO) (r.m.s.d. 3.1 Å) (f) in orange. The AdipoR2 and other 7TM protein structures are viewed parallel to the membrane (top) and from the extracellular and intracellular sides, respectively (bottom).
Extended Data Figure 4 | The zinc-binding sites of AdipoR1 and AdipoR2. a, b, The zinc-binding sites of AdipoR1 (a) and AdipoR2 (b) are conserved from mammals to plants. The conserved residues, the 3× His + Asp residues and a Ser residue, are shown in red. The side chains of Ser 187 (AdipoR1) and Ser 198 (AdipoR2) in helix II are located 3.7 and 3.8 Å, respectively, away from the zinc ion (data not shown and Fig. 3b). c, d, The amounts of AdipoR1 (c) and AdipoR2 (d) in HEK293 cells transfected with AdipoR1 (residues 89–375), AdipoR2 (residues 100–386) or a variety of mutants of AdipoR1 and AdipoR2 were analysed by western blotting, using an anti-Flag antibody. The label 89–375 indicates no mutation, and the other labels, such as His191Ala and 4Ala, indicate the single and multiple mutations (see text). The label 100–386 indicates no mutation, and the other labels, such as His202Ala and 4Ala, indicate the single and multiple mutations (see text). e, UCP2 mRNA levels in HEK293 cells transfected with full-length AdipoR2 (residues 1–386) or a zinc-binding site mutant. All values are presented as mean ± s.e.m. n = 3–4, three independent experiments, *P < 0.05, **P < 0.01 compared to control cells or as indicated (see Methods).
Extended Data Figure 5 | The zinc-binding sites of soluble proteins.

a, b. The zinc-binding sites of Astacus astacus L. astacin (PDB code 1AST) (a) and human carbonic anhydrase II (PDB code 1CA2) (b). The zinc ion (magenta) is coordinated by three His residues and a water molecule (pink sphere).
Extended Data Figure 6 | The cytoplasmic side of AdipoR1. a, b, Structures of AdipoR1 residues 89–375 (a) and 120–375 (b) viewed parallel to the membrane. c, d, The cavity of AdipoR1 (residues 89–375 (c) and 120–375 (d)) viewed from the cytoplasmic side. Residues 120–375, including helix 0, the 7TM domain, and the CTR, are coloured green. The NTR (residues 89–119) is coloured orange.
Extended Data Figure 7 | The cytoplasmic faces of AdipoR2 and AdipoR1.

a, b, Intracellular views of AdipoR1 (a) and AdipoR2 (b). The openings of the cavities are circled in red. The N-terminal regions of the AdipoRs are represented as surface models (orange). c, d, The ICL2 s of AdipoR1 (c) and AdipoR2 (d).
Extended Data Figure 8 | Crystal packing of the CTRs of AdipoR1 and AdipoR2. a, b. Crystal packing of the CTR of AdipoR1 with Fv (a) and the CTR of AdipoR2 with Fv (b). The CTR of AdipoR1 is tucked between the two Fv fragments, whereas the C-terminal tail of AdipoR2 contacts the framework region 1 of V_{H} (orange). The CTRs are coloured purple.
Extended Data Figure 9 | Expression of the AdipoR1 mutant proteins.

(a, b) The amounts of AdipoR1 in HEK293 cells transfected with full-length AdipoR1 (residues 1–375) or a variety of mutants of AdipoR1 were analysed by western blotting, using an anti-Flag antibody. Full-length AdipoR1 (residues 1–375) and the C-terminally truncated mutant (residues 1–370, 1–366 and 1–362) were used in (a). AdipoR1 residues 1–375, MYFMAPL (residues 161–167) changed to SGSSGGS (ECL1); residues 1–375, YCS (residues 229–231) changed to GGG (ECL2); residues 1–375, FVKATTV (residues 291–297) changed to SSSGGGS (ECL3); residues 1–375, ECL1 and ECL3 (ECL1/3); and residues 1–375, ECL1, ECL2 and ECL3 (ECL1/3/2) were used in (b).
### Extended Data Table 1 | Data collection and refinement statistics (Molecular replacement)

|                         | AdipoR1–Fv complex | AdipoR2–Fv complex |
|-------------------------|--------------------|--------------------|
| **Data collection**     |                    |                    |
| No. of crystals         | 5                  | 1                  |
| X-ray source            | BL32XU, SPring-8   | BL32XU, SPring-8   |
| Wavelength (Å)          | 1                  | 1                  |
| Space group             | C222₁              | P2₁2₁2             |
| Cell dimensions         |                    |                    |
| $a$, $b$, $c$ (Å)       | 92.3, 194.1, 74.3  | 74.6, 108.6, 101.0 |
| $\alpha$, $\beta$, $\gamma$ (°) | 90.0, 90.0, 90.0  | 90.0, 90.0, 90.0   |
| Resolution (Å)          | 20.0–2.9 (3.0–2.9) | 19.5–2.4 (2.5–2.4) |
| $R_{merge}$             | 0.192 (0.930)      | 0.115 (1.297)      |
| $I/\sigma I$            | 6.73 (2.03)        | 8.55 (1.19)        |
| Completeness (%)        | 99.7 (100.0)       | 98.3 (99.4)        |
| Redundancy              | 7.4 (7.5)          | 4.5 (4.5)          |
| **Refinement**          |                    |                    |
| Resolution (Å)          | 19.9–2.9           | 19.5–2.4           |
| No. reflections         | 15098              | 32141              |
| $R_{weak}$/ $R_{free}$  | 23.9 / 30.0        | 24.8 / 29.0        |
| CC₁/₂                   | 0.976 (0.517)      | 0.996 (0.506)      |
| CC*                     | 0.994 (0.826)      | 0.999 (0.820)      |
| No. atoms               |                    |                    |
| AdipoR                  | 2294               | 2286               |
| Fv                      | 1747               | 1747               |
| Zn                      | 1                  | 1                  |
| Water                   | -                  | 52                 |
| B-factors               |                    |                    |
| Protein                 | 62.1               | 66.4               |
| Zn                      | 49.5               | 56.3               |
| Water                   | -                  | 61.5               |
| R.m.s deviations       |                    |                    |
| Bond lengths (Å)        | 0.004              | 0.003              |
| Bond angles (°)         | 0.8                | 0.76               |

*Highest resolution shell is shown in parenthesis.