Acetyl-CoA carboxylases (ACCs) catalyse the committed step in fatty-acid biosynthesis: the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA. They are important regulatory hubs for metabolic control and relevant drug targets for the treatment of the metabolic syndrome and cancer. Eukaryotic ACCs are single-chain multienzymes characterized by a large, non-catalytic central domain (CD), whose role in ACC regulation remains poorly characterized. Here we report the crystal structure of the yeast ACC CD, revealing a unique four-domain organization. A regulatory loop, which is phosphorylated at the key functional phosphorylation site of fungal ACC, wedges into a crevice between two domains of CD. Combining the yeast CD structure with intermediate and low-resolution data of larger fragments up to intact ACCs provides a comprehensive characterization of the dynamic fungal ACC architecture. In contrast to related carboxylases, large-scale conformational changes are required for substrate turnover, and are mediated by the CD under phosphorylation control.
Biotin-dependent acetyl-CoA carboxylases (ACCs) are essential enzymes that catalyse the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA. This reaction provides the committed activated substrate for the biosynthesis of fatty acids via fatty-acid synthase. By catalysing this rate-limiting step in fatty-acid biosynthesis, ACC plays a key role in anabolic metabolism. ACC inhibition and knock-out studies show the potential of targeting ACC for treatment of the metabolic syndrome. Furthermore, elevated ACC activity is observed in malignant tumours. A direct link between ACC and cancer is provided by cancer-associated mutations in the breast cancer susceptibility gene 1 (BRCA1), which relieve inhibitory interactions of BRCA1 with ACC. Thus, ACC is a relevant drug target for type 2 diabetes and cancer. ACC occurs in two closely related isoforms, ACC1 and 2, located in the cytosol and at the outer mitochondrial membrane, respectively. In addition to the canonical ACC components, ACC have been described already in the late 1960s for *Escherichia coli* (E. coli) ACC. Biotin carboxylase (BC) catalyses the ATP-dependent carboxylation of a biotin moiety, which is covalently linked to the biotin carboxyl carrier protein (BCCP). Carboxyltransferase (CT) transfers the activated carboxyl group from carboxybiotin to acetyl-CoA to yield malonyl-CoA. Prokaryotic ACCs are transient assemblies of individual BC, CT and BCCP subunits. Eukaryotic ACCs, instead, are multienzymes, which integrate all functional components into a single polypeptide chain of ~2,300 amino acids. Human ACC occurs in two closely related isoforms, ACC1 and 2, located in the cytosol and at the outer mitochondrial membrane, respectively. In addition to the canonical ACC components, eukaryotic ACCs contain two non-catalytic regions, the large central domain (CD) and the BC–CT interaction domain (BT). The CD comprises one-third of the protein and is a unique feature of eukaryotic ACCs without homologues in other proteins. The function of this domain remains poorly characterized, although phosphorylation of several serine residues in the CD regulates ACC activity. The BT domain has been visualized in bacterial carboxylases, where it mediates contacts between α- and β-subunits.

Structural studies on the functional architecture of intact ACCs have been hindered by their huge size and pronounced dynamics, as well as the transient assembly mode of bacterial ACCs. However, crystal structures of individual components or domains from prokaryotic and eukaryotic ACCs, respectively, have been solved. The structure determination of the 82-kDa CD. The crystal structure of the CD of *Saccharomyces cerevisiae* (Sac) ACC, intermediate- and low-resolution structures of human (Hsa) ACC CD and larger fragments of fungal ACC from *Chaetomium thermophilum* (Cth) reveal a comprehensive representation of the dynamic structure and regulation of fungal ACC.

**Results**

The organization of the yeast ACC CD. First, we focused on structure determination of the 82-kDa CD. The crystal structure of the CD of *Saccharomyces cerevisiae* (Sac) ACC was determined at 3.0 Å resolution by experimental phasing and refined to Rwork/Rfree = 0.20/0.24 (Table 1). The overall extent of the SacCD is 70 by 75 Å (Fig 1b and Supplementary Fig. 1a,b), and the attachment points of the N-terminal CT domain to the BCCP domain and the C-terminal CT domain are separated by 46 Å (the N- and C termini are indicated with spheres in Fig. 1b). SacCD comprises four distinct domains, an N-terminal α-helical domain (CDN), and a central four-helix bundle linker domain (CD1), followed by two α-β-fold C-terminal domains (CD1/CD2). CDN adopts a letter C shape, where one of the ends is a regular four-helix bundle (N23-6), the other end is a helical hairpin (N28,9) and the bridging region comprises six helices (N12,7,10-12). CD1 is composed of a small, irregular four-helix bundle (L1x1-4) and tightly interacts with the open face of CD1/CD2 via an interface of 1,300 Å 2 involving helices L2x3 and L2x4. CD1 does not interact with CDN apart from the covalent linkage and forms only a small contact to CD1/CD2 via a loop between 2x3-6 and the N-terminal end of 2x1, with an interface area of 400 Å 2. CDN shares a common fold; they are composed of six-stranded β-sheets flanked on one side by two long, bent helices inserted between strands β3/β4 and β4/β5. CD1/CD2 is extended at its C terminus by an additional β-strand and an irregular β-hairpin. On the basis of a root mean square deviation of main chain atom positions of 2.2 Å, CD1/CD2 are structurally more closely related to each other than to any other protein (Fig. 1c); they may have evolved by duplication. Close structural homologues could not be found for the CDN or the CD C domains.
Figure 1 | The phosphorylated central domain of yeast ACC. (a) Schematic overview of the domain organization of eukaryotic ACCs. Crystallized constructs are indicated. (b) Cartoon representation of the SceCD crystal structure. CD\textsubscript{N} is linked by a four-helix bundle (CD\textsubscript{L}) to two α-β-fold domains (CD\textsubscript{C1} and CD\textsubscript{C2}). The regulatory loop is shown as bold cartoon, and the phosphorylated Ser1157 is marked by a red triangle. The N- and C termini are indicated by spheres. (c) Superposition of CD\textsubscript{C1} and CD\textsubscript{C2} reveals highly conserved folds. (d) The regulatory loop with the phosphorylated Ser1157 is bound into a crevice between CD\textsubscript{C1} and CD\textsubscript{C2}, the conserved residues Arg1173 and Arg1260 coordinate the phosphoryl-group. (e) Structural overview of HsaBT-CD. The attachment points to the N-terminal BCCP domain and the C-terminal CT domain are indicated with spheres. All colourings are according to scheme a.
A regulatory loop mediates interdomain interactions. To define the functional state of insect-cell-expressed ACC variants, we employed mass spectrometry (MS) for phosphorylation site detection. In insect-cell-expressed full-length SceACC, the highly conserved Ser1157 is the only fully occupied phosphorylation site with functional relevance in S. cerevisiae. Additional phosphorylation was detected for Ser2101 and Tyr2179; however, these sites are neither conserved across fungal ACC nor natively phosphorylated for Ser2101 and Tyr2179; however, these sites are neither conserved across fungal ACC nor natively phosphorylated in yeast. MS analysis of dissolved crystals confirmed the phosphorylated state of Ser1157 also in SceCD crystals. The SceCD structure thus authentically represents the state of ACC, where the enzyme is inhibited by SNF1-dependent phosphorylation.

In the SceCD crystal structure, the phosphorylated Ser1157 resides in a regulatory 36-amino-acid loop between strands β2 and β3 of CD1 (Fig. 1b,d), which contains two additional less-conserved phosphorylation sites (Ser1148 and Ser1162) confirmed in yeast, but not occupied here. This regulatory loop wedges between the CD1 and CD2 domains and provides the largest contribution to the interdomain interface. The N-terminal region of the regulatory loop also directly contacts the C-terminal region of CD2 leading into CT. Phosphoserine 1157 is tightly bound by two highly conserved arginines (Arg1173 and Arg1260) of CD1 (Fig. 1d). Already the binding of phosphorylated Ser1157 apparently stabilizes the regulatory loop conformation; the accessory phosphorylation sites Ser1148 and Ser1162 in the same loop may further modulate the strength of interaction between the regulatory loop and the CD1 and CD2 domains. Phosphorylation of the regulatory loop thus determines interdomain interactions of CD1 and CD2, suggesting that it may exert its regulatory function by modifying the overall structure and dynamics of the CD.

The functional role of Ser1157 was confirmed by an activity assay based on the incorporation of radioactive carbonate into acid non-volatile material. Phosphorylated SceACC shows only residual activity ($k_{cat} = 0.4 \pm 0.2 \text{ s}^{-1}$, s.d. based on five replicate measurements), which increases 16-fold ($k_{cat} = 6.5 \pm 0.3 \text{ s}^{-1}$) after dephosphorylation by λ protein phosphatase. The values obtained for dephosphorylated SceACC are comparable to earlier measurements of non-phosphorylated yeast ACC expressed in E. coli.

### Table 1 | Crystallographic data collection and refinement statistics.

| Data collection | ScéCD | ScéCD Thimerosal | ScéCD Eu | HsaBT-CD | HsaBT-CD Cd2$^{\ddagger}$ | CthCD-CTclw1 | CthCD-CTclw2 | CthCD-CT | Cth\&BCCP |
|-----------------|-------|-----------------|----------|----------|------------------------|----------------|----------------|---------|---------|
| Space group     | P4$_1$2$_1$2    | P4$_1$2$_1$2    | P4$_1$2$_1$2 | 14:22    | 14:22                  | P2$_1$2$_1$2   | P2$_1$2$_1$2   | P2$_1$2$_1$2 | P6$_2$22 |
| Cell dimensions | a, b, c (Å)       | 110.86, 111.22, 108.65 | 110.86, 111.22, 108.65 | 131.12   | 131.49, 127.36, 210.61 | 110.86, 111.22, 108.65 | 110.86, 111.22, 108.65 | 131.12   | 131.49, 127.36, 210.61 |
| Resolution (Å)  | 3.0             | 90.90, 90.90, 90.90 | 90.90, 90.90, 90.90 | 90.90, 90.90, 90.90 | 90.90, 90.90, 90.90 | 90.90, 90.90, 90.90 | 90.90, 90.90, 90.90 | 90.90, 90.90, 90.90 | 90.90, 90.90, 90.90 |
| Completeness    | 99.9 (99.9)      | 99.6 (100)       | 99.9 (99.9) | 99.9 (99.9) | 99.9 (100)            | 99.9 (100)     | 99.9 (100)     | 99.9 (100) | 99.9 (100) |
| Redundancy      | 39.1 (39.8)      | 12.1 (14.3)      | 81.6 (65.2) | 13.7 (13.7) | 20.9 (19.1)           | 12.7 (13.5)    | 6.1 (6.5)      | 9.9 (10.4) | 18.5 (18.2) |
| Refinement      | Resolution (Å)   | 46.4–3.0         | —         | 84.5–3.7  | 49.2–3.6               | 49.1–4.5       | 49.9–7.2       | 50.0–8.4  |                     |
| Rmerge/Refree   | 0.20/0.24        | —                 | —         | 0.35/0.38 | 0.20/0.24             | 0.24/0.24      | 0.23/0.25      | 0.30/0.32 |                     |
| Number of atoms | Protein 5,465    | —                 | —         | 6,925     | 16,592                 | 16,405         | 22,543         | 22,445    |                     |
|                 | Waters 43        | —                 | —         | —         | —                     | —              | —              | —         |                     |
|                 | Ligand/ion 7     | —                 | —         | 5         | —                     | —              | —              | —         |                     |
|                 | R.m.s.d.         | Protein 130       | —         | 158       | 226                   | 275            | 272            | 250       |                     |
|                 |                  | Waters 84         | —         | —         | —                     | —              | —              | —         |                     |
|                 |                  | Ligand/ion 90     | —         | 189       | —                     | —              | —              | —         |                     |
|                 |                  | R.m.s.d.          | —         | —         | —                     | —              | —              | —         |                     |
| RMS (angles, °) | 0.97             | —                 | 0.83      | 1.07      | 1.11                  | 1.15           | 1.01           | —         |                     |
| RMS (bonds, Å) | 0.01             | —                 | 0.01      | 0.01      | 0.01                  | 0.01           | 0.01           | 0.01      |                     |

*a*Resolution cutoffs determined based on internal correlation significant at the 0.1% level as calculated by XDS.

*b*Highest-resolution shell is shown in parentheses.

*Modelled only as poly-alanine.

The variable CD is conserved between yeast and human. To compare the organization of fungal and human ACC CD, we determined the structure of a human ACC1 fragment that comprises the BT and CD domains (HsaBT-CD), but lacks the mobile BCCP in between (Fig. 1a). An experimentally phased map was obtained at 3.7 Å resolution for a cadmium-derivatized crystal and was interpreted by a poly-alanine model (Fig. 1e and Table 1). Each of the four CD domains in HsaBT-CD individually resembles the corresponding ScéCD domain; however, human and yeast CDs exhibit distinct overall structures. In agreement with their tight interaction in ScéCD, the relative spatial arrangement of CD1 and CD1 is preserved in HsaBT-CD, but the human CD1/CD1 didomain is tilted by 30° based on a superposition of human and yeast CD2 (Supplementary Fig. 1c). As a result, the N terminus of CD1 at helix L1, which connects to CDN, is shifted by 12 Å. Remarkably, CDN of HsaBT-CD adopts a completely different orientation compared with ScéCD.
With CD$_L$/CD$_C$ superposed, CD$_N$ in HsaBT-CD is rotated by 160° around a hinge at the connection of CD$_N$/CD$_L$ (Supplementary Fig. 1d). This rotation displaces the N terminus of CD$_N$ in HsaBT-CD by 51 Å compared with SceCD, resulting in a separation of the attachment points of the N-terminal linker to the BCCP domain and the C-terminal CT domain by 67 Å (the attachment points are indicated with spheres in Fig. 1e). The BT domain of HsaBT-CD consists of a helix that is surrounded at its N terminus by an antiparallel eight-stranded β-barrel. It resembles the BT of propionyl-CoA carboxylase$^{23}$; only the four C-terminal strands of the β-barrel are slightly tilted.

On the basis of MS analysis of insect-cell-expressed human full-length ACC, Ser80 shows the highest degree of phosphorylation (90%). Ser29 and Ser1263, implicated in insulin-dependent phosphorylation and BRCA1 binding, respectively, are phosphorylated at intermediate levels (40%). The highly conserved Ser1216 (corresponding to S. cerevisiae Ser1157), as well as Ser1201, both in the regulatory loop discussed above, are not phosphorylated. However, residual phosphorylation levels were detected for Ser1204 (7%) and Ser1218 (7%) in the same loop. MS analysis of the HsaBT-CD crystallization sample reveals partial proteolytic digestion of the regulatory loop. Accordingly, most of this loop is not represented in the HsaBT-CD crystal structure. The absence of the regulatory loop might be linked to the less-restrained interface of CD$_L$/CD$_C$ and CD$_N$2 and altered relative orientations of these domains. Besides the regulatory loop, also the phosphopeptide target region for BRCA1 interaction is not resolved presumably because of pronounced flexibility.

At the level of isolated yeast and human CD, the structural analysis indicates the presence of at least two hinges, one with large-scale flexibility at the CD$_N$/CD$_L$ connection, and one with tunable plasticity between CD$_L$/CD$_C$ and CD$_N$2, plausibly affected by phosphorylation in the regulatory loop region.

The integration of CD into the fungal ACC multienzyme. To further obtain insights into the functional architecture of fungal ACC, we characterised larger multidomain structures up to the intact enzymes. Using molecular replacement based on fungal ACC CD and CT models, we obtained structures of a variant comprising CthCT and CD$_C$1/CD$_N$2 in two crystal forms at resolutions of 3.6 and 4.5 Å (CthCD-CT$_{ter1/2}$), respectively, as well as of a CthCT linked to the entire CD at 7.2 Å resolution (CthCD-CT; Figs 1a and 2, Table 1). No crystals diffracting to sufficient resolution were obtained for larger BC-containing fragments, or for full-length Cth or SceACC. To improve crystallizability, we generated ΔBCCP variants of full-length ACC, which, based on SAXS analysis, preserve properties of intact ACC (Supplementary Table 1 and Supplementary Fig. 2a–c). For CthΔBCCP, crystals diffracting to 8.4 Å resolution were obtained. However, molecular replacement did not reveal a unique positioning of the BC domain. Owing to the limited resolution the discussion of structures of CthCD-CT and CthΔBCCP is restricted to the analysis of domain localization. Still, these structures contribute considerably to the visualization of an intrinsically dynamic fungal ACC.

In all these crystal structures, the CT domains build a canonical head-to-tail dimer$^{29}$, with active sites formed by contributions from both protomers (Fig. 2 and Supplementary Fig. 3a). The connection of CD and CT is provided by a 10-residue peptide stretch, which links the N terminus of CT to the irregular β-hairpin/β-strand extension of CD$_C$2 (Supplementary Fig. 3b). The connecting region is remarkably similar in isolated CD and CthCD-CT$_{ter}$ structures, indicating inherent conformational stability. CD/CT contacts are only formed in direct vicinity of the covalent linkage and involve the β-hairpin extension of CD$_C$2 as well as the loop between strands β2/β3 of the CT N-lobe, which contains a conserved RxxGxN motif. The neighbouring loop on the CT side (between CT β1/β2) is displaced by 2.5 Å compared to isolated CT structures (Supplementary Fig. 3c). On the basis of an interface area of ~600 Å$^2$ and its edge-to-edge connection characteristics, the interface between CT and CD might be classified as conformationally variable. Indeed, the comparison of the positioning of eight instances of the C-terminal part of CD relative to CT in crystal structures determined here, reveals flexible interdomain linking (Fig. 3a). The CD$_C$2/CT interface acts as a true hinge with observed rotation up to 16°, which results in a translocation of the distal end of CD$_C$2 by 8 Å.

The interface between CD$_C$2 and CD$_L$/CD$_N$1, which is mediated by the phosphorylated regulatory loop in the SceCD structure, is less variable than the CD–CT junction, and permits only limited rotation and tilting (Fig. 3b). Analysis of the impact of phosphorylation on the interface between CD$_C$2 and CD$_L$/CD$_N$1 in CthACC variant structures is precluded by the limited crystallographic resolution. However, MS analysis of CthCD-CT and CthΔBCCP constructs revealed between 60 and 70% phosphorylation of Ser1170 (corresponding to SceACC Ser1157).
The CD_N domain positioning relative to CD2/CD_C1 is highly variable with three main orientations observed in the structures of SceCD and the larger CthACC fragments: CD_N tilts, resulting in a displacement of its N terminus by 23 Å (Fig. 4a, observed in both protomers of CthCD-CT and one protomer of CthABCCP, denoted as CthCD-CT1/2 and CthABCCP1, respectively). In addition, CD_N can rotate around hinges in the connection between CD2/CD1 by 70° (Fig. 4b, observed in the second protomer of CthABCCP, denoted as CthABCCP2) and 160° (Fig. 4c, observed in SceCD) leading to displacement of the anchor site for the BCCP linker by up to 33 and 40 Å, respectively.

Conformational variability in the CD thus contributes considerably to variations in the spacing between the BC and CT domains, and may extend to distance variations beyond the mobility range of the flexibly tethered BCCP. On the basis of the occurrence of related conformational changes between fungal and human ACC fragments, the observed set of conformations may well represent general states present in all eukaryotic ACCs.

Large-scale conformational variability of fungal ACC. To obtain a comprehensive view of fungal ACC dynamics in solution, we employed SAXS and EM. SAXS analysis of CthACC agrees with a dimeric state and an elongated shape with a maximum extent of 350 Å (Supplementary Table 1). The smooth appearance of scattering curves and derived distance distributions might indicate substantial interdomain flexibility (Supplementary Fig. 2a–c). Direct observation of individual full-length CthACC particles, according to MS results predominantly in a phosphorylated low-activity state, in negative stain EM reveals a large set of conformations from rod-like extended to U-shaped particles. Class averages, obtained by maximum-likelihood-based two-dimensional (2D) classification, are focused on the dimeric CT domain and the full BC–BCCP–CD domain of only one protomer, due to the non-coordinated motions of the lateral BC/CD regions relative to the CT dimer. They identify the connections between CDN/CD1 and between CD2/CT as major contributors to conformational heterogeneity (Supplementary Fig. 4a,b). The flexibility in the CD2/CT hinge appears substantially larger than the variations observed in the set of crystal structures. The BC domain is not completely disordered, but laterally attached to BT/CDN in a generally conserved position, albeit with increased flexibility. Surprisingly, in both the linear and U-shaped conformations, the approximate distances between the BC and CT active sites would remain larger than 110 Å. These observed distances are considerably larger than in static structures of any other related biotin-dependent carboxylase. Furthermore, based on an average length of the BCCP–CD linker in fungal ACC of 26 amino acids, mobility of the BCCP alone would not be sufficient to bridge the active sites of BC and CT. Consequently, increased flexibility or additional modes of conformational changes may be required for productive catalysis. The most relevant candidate site for mediating such additional flexibility and permitting an extended set of conformations is the CD_C1/CD_C2 interface, which is rigidified by the Ser1157–phosphorylated regulatory loop, as depicted in the SceCD crystal structure.

Discussion
Altogether, the architecture of fungal ACC is based on the central dimeric CT domain (Fig. 4d). The CD consists of four distinct subdomains and acts as a tether from the CT to the mobile BCCP and an oriented BC domain. The CD has no direct role in substrate recognition or catalysis but contributes to the regulation of all eukaryotic ACCs. In higher eukaryotic ACCs, regulation via phosphorylation is achieved by combining the effects of phosphorylation at Ser80, Ser1201 and Ser1263. In fungal ACC, however, Ser1157 in the regulatory loop of the CD is the only phosphorylation site that has been demonstrated to be both phosphorylated in vivo and involved in the regulation of ACC activity. In its phosphorylated state, the regulatory loop containing Ser1157 wedges between CD1/CD2 and presumably limits the conformational freedom at this interdomain interface. However, flexibility at this hinge may be required for full ACC activity, as the distances between the BCCP anchor points and the active sites of BC and CT observed here are such large that mobility of the BCCP alone is not sufficient for substrate transfer. The current data thus suggest that regulation of fungal ACC is mediated by controlling the dynamics of the unique CD, rather than directly affecting catalytic turnover at the active sites of BC and CT. A comparison between fungal and human ACC will help to further discriminate mechanistic differences that contribute to the extended control and polymerization of human ACC.

Most recently, a crystal structure of near full-length non-phosphorylated ACC from S. cerevisiae (lacking only...
21 N-terminal amino acids, here denoted as flACC) was published by Wei and Tong. In flACC, the ACC dimer obeys twofold symmetry and assembles in a triangular architecture with dimeric BC domains (Supplementary Fig. 5a). In their study, mutational data indicate a requirement for BC dimerization for catalytic activity. The transition from the elongated open shape, observed in our experiments, towards a compact triangular shape is based on an intricate interplay of several hinge-bending motions in the CD (Fig. 4d). Comparison of flACC with our CthABCCP structure reveals the CD/C2/CT hinge as a major contributor to conformational flexibility (Supplementary Fig. 5b,c). In flACC, CD/C2 rotates ∼120° with respect to the CT domain. A second hinge can be identified between CD/C1/CD/C2. On the basis of a superposition of CD/C2, CD/C1 of the phosphorylated SceCD is rotated by 30° relative to CD/C1 of the non-phosphorylated flACC (Supplementary Fig. 5d), similar to what we have observed for the non-phosphorylated HsaBT-CD (Supplementary Fig. 1d). When inspecting all individual protomer and fragment structures in their study, Wei and Tong also identify the CD/CN/CD/C1 connection as a highly flexible hinge, in agreement with our observations.

The only bona fide regulatory phosphorylation site of fungal ACC in the regulatory loop is directly participating in CD/C1/CD/C2 domain interactions and thus stabilizes the hinge conformation. In flACC, the regulatory loop is mostly disordered, illustrating the increased flexibility due to the absence of the phosphoryl group. Only in three out of eight observed protomers a short peptide stretch (including Ser1157) was modelled. In those instances the Ser1157 residue is located at a distance of 14–20 Å away from the location of the phosphorylated serine observed here, based on superposition of either CD/C1 or CD/C2. Applying the conformation of the CD/C1/CD/C2 hinge observed in SceCD on flACC leads to CDN sterically clashing with CD/C2 and BT/CDN clashing with CT (Supplementary Fig. 6a,b). Thus, in accordance with the results presented here, phosphorylation of Ser1157 in SceACC most likely limits flexibility in the CD/C1/CD/C2 hinge such that activation through BC dimerization is not possible (Fig. 4d), which however does not exclude intermolecular dimerization. In addition, EM micrographs of phosphorylated and dephosphorylated SceACC display for both samples mainly elongated and U-shaped conformations and reveal no apparent differences in particle shape distributions (Supplementary Fig. 7). This implicates that the triangular shape with dimeric BC domains has a low population also in the active form, even though a biasing influence of grid preparation cannot be excluded completely.

Large-scale conformational variability has also been observed in most other carrier protein-based multienzymes, including polyketide and fatty-acid synthases (with the exception of fungal-type fatty-acid synthases), non-ribosomal peptide synthetases and the pyruvate dehydrogenase complexes, although based on completely different architectures. Together, this structural information suggests that variable carrier protein tethering is not sufficient for efficient substrate transfer and catalysis in any of...
and Sce (Fig. 4d). The phosphorylated regulatory loop binds to an allostERIC site at the interface of two non-catalytic domains and restricts conformational freedom at several hinges in the dynamic ACC. It disfavours the adoption of a rare, compact conformation, in which intramolecular dimerization of the BC domains results in catalytic turnover. The regulation of activity thus results from restricted large-scale conformational dynamics rather than a direct or indirect influence on active site structure. To our best knowledge, ACC is the first multienzyme for which such a phosphorylation-dependent mechanical control mechanism has been visualized. However, the example of ACC now demonstrates the possibility of regulating activity by controlled dynamics of non-enzymatic linker regions also in other families of carrier-dependent multienzymes. Understanding such structural and dynamical constraints imposed by scaffolding and linking in carrier protein-based multienzyme systems is a critical prerequisite for engineering of efficient biosynthetic assembly lines.

Methods

Protein expression and purification. All proteins were expressed in the Baculovirus Expression System. The MultiBac insect cell expression plasmid pACEBACI (Geneva Biotech) was modified to host a GATEWAY (Life-Technologies) cassette with an N-terminal 10xHis-tag, named pAB1GW-NH10 hereafter. Full-length HaAC (Genebank accession #Q13085), SceACC (#Q00955) and CthACC (#GI35935) were cloned into pAB1GW-NH10 using GATEWAY according to the manufacturer’s manual. Truncated variants were constructed by PCR amplification, digestion of the template DNA with DpnI, phosphorylation of the PCR product and religation of the linear fragment to a circular plasmid. The following constructs were used for this study: ACC (1–2,233), CthACC (1–2,297), CthABCCBP (1–2,297), CDABCCBP (1–1,144), CDABCCBP (1–1,203) and CD-CTCter1 (1,144–1,203). A full-length model was placed in the resulting maps. Since crystals derivatized with europium were slightly non-isomorphous with a c axis length of 127 Å, multicystrav averaging was averaged for density modification and provided directly interpretable maps. Iterative cycles of model building and refinement in Buster (version 2.10.2; Global Phasing Ltd) converged at Rmerge = 0.20/0.24. The model lacks the disordered N terminus (amino acids 768–789), an extended loop in the CDc1 domain (1,203–1,215), a short stretch (1,147–1,149) preceding the regulatory loop and the two very C-terminal residues (1,493–1,494). On the basis of temperature factor analysis, the start and end of the regulatory loop show higher disorder than the rest of the crystal. For all other phosphoproteins no disorder was detected and an initial model was placed in the asymmetric unit via the CDN and BT domains. Density on top of the 80%–90% of the model is visible on both crystals.

Protein crystallization. All crystallization experiments were conducted using sitting drop vapour diffusion. ScCoCD crystals were grown at 19 °C by mixing protein solution (0.1 M Bis-Tris pH 6.5, 0.05–0.2 M di-sodium malonate, 20–30% polyethylene glycol (PEG) 3350, 10 mM trimethylamine or 2% benzamidine) in a 1:1 or 2:1 ratio. Crystals appeared after several days and continued to grow for 200–200 days. Crystals were cryoprotected by short incubation in mother liquor supplemented with 22% ethylene glycol and flash-cooled in liquid nitrogen. For heavy metal derivatization the crystals were concentrated to 10 mg ml⁻¹. Two very C-terminal residues (1,493–1,494). On the basis of temperature factor analysis, the start and end of the regulatory loop show higher disorder than the rest of the crystal. For all other phosphoproteins no disorder was detected and an initial model was placed in the asymmetric unit via the CDN and BT domains. Density on top of the 80%–90% of the model is visible on both crystals.

Structure determination and analysis of phosphorylation. All X-ray diffraction data were collected at beamlines X06SA (PXI) or X06DA (PXIII) at the Swiss Light Source (SLS, Paul Scherrer Institute, Villigen, Switzerland) equipped with PILATUS detectors. The wavelength of data collection was 1.00 Å for native crystals, and 1.527 and 1.907 Å for crystals derivatized with europium and cadmium, respectively. Raw data were processed using XDS. Molecular replacement was carried out using Phaser 52 and resolve, multicrystal averaging 52 was carried out using phenix 50 and figures were prepared using PyMOL (Schrodinger LLC).

Discussion of initial BscACC crystals in space group Pa2,2 with unit cell dimensions of a = 110.3 Å and c = 131.7 Å was limited to 3.5 Å. The resolution was improved to 3 Å by addition of trimethylamine or benzamidine to the reservoir solution without significant changes in unit cell dimensions. Crystals derivatized with thimerosal and europium were used for initial SAD phase determination using the SHELXD package. Two merohedry and four euvromorphic sites were located, and an initial model was placed in the resulting maps. Since crystals derivatized with europium were slightly non-isomorphous with a c axis length of 127 Å, multicystrav averaging was averaged for density modification and provided directly interpretable maps. Iterative cycles of model building and refinement in Buster (version 2.10.2; Global Phasing Ltd) converged at Rmerge = 0.20/0.24. The model lacks the disordered N terminus (amino acids 768–789), an extended loop in the CDc1 domain (1,203–1,215), a short stretch (1,147–1,149) preceding the regulatory loop and the two very C-terminal residues (1,493–1,494). On the basis of temperature factor analysis, the start and end of the regulatory loop show higher disorder than the rest of the crystal. For all other phosphoproteins no disorder was detected and an initial model was placed in the asymmetric unit via the CDN and BT domains. Density on top of the 80%–90% of the model is visible on both crystals.
were determined by molecular replacement using a homology model based on ScCt (pdb 1od2) as search model in Phaser51,80,81. Multicrystal averaging was applied in density modification. The CT domain was rebuilt and a new homology model based on the ScCt structure was fitted into difference density for ChtCD-CTCter. Iterative cycles of rebuilding and refinement in Buster converged at Rwork/Rfree of 0.20/0.24. The refined Cdt model served as a starting model for obtaining ChtCD-CTCter coordinates. Coordinate refinement in Buster was additionally guided by reference model restraints and converged at Rwork/Rfree of 0.24/0.24. Residues 1,114–1,185, 1,213–1,252, 1,380–1,385, 1,465–1,468 and 2,188–2,195 were disordered in both crystal forms and are not included in the models. Helical regions C terminal to Glu2264 of both protomers of ChtCD-CTCter and C terminal to Arg2259 and Arg2261 of the two protomers of ChtCD-CTCter, respectively, could not be built unambiguously and were therefore interpreted by placing poly-alanine stretches. Conservation was mapped on the ChtCD-CTCter crystal structure using alcz30 based on a sequence alignment of 367 fungal ACC sequences calculated by Clustal Omega53.  

Although substantial difference density is observed, no defined positions of the BT loop were detected and 18,031 particles were re-aligned and classified into 36 classes using ML2F2D with a high-resolution cutoff of 30 Å. After 44 iterations the alignment converged and class averages were extracted.

**In vitro biotinylation and activity assay.** To ensure full functionality, ScACC was biotinylated in vitro using the E.coli biotin ligase BirA. The reaction mixture contained 10 μM ACC, 3.7 μM BirA, 50 mM Tris-HCl, pH 8, 5.5 mM MgCl2, 0.5 μM biotin, 80 mM NaCl, 1 mg/ml ATP and 10% glycerol, and the reaction was allowed to proceed for 7 h at 30 °C. The catalytic activity of phosphorylated and dephosphorylated ScACC was measured by following the incorporation of radioactive 14C into acid-stable non-volatile material50. Dephosphorylated ACC was prepared by overnight treatment with 500 units of phosphatase (NEB M0290B) of partially purified ACC before the final gel filtration step. The removal of the phosphoryl group from Ser1175 was confirmed by MS. The reaction mixture contained 0.5 μg recombinant ACC in 100 mM potassium phosphate, pH 8, 3 mM ATP, 5 mM MgCl2, 50 mM NaH14CO3 (specific activity 7.4 MBq mmol−1) and 1 mM acetyl-CoA in a total reaction volume of 100 μl. The reaction mixture was incubated for 15 min at 30 °C, stopped by addition of 200 μl 6 M HCl and subsequently evaporated to dryness at 85 °C. The non-volatile residue was redissolved in 100 μl of water, 1 ml Ultima Gold XR scintillation medium (Perkin Elmer) was added and the 14C radioactivity was measured in a Packard Tricarb 2000CA liquid scintillation analyser. Measurements were carried out in five replicates and catalytic activities were calculated using a standard curve derived from measurements of varying concentrations of NaH14CO3 in reaction buffer.

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Author contributions

M.H. cloned, expressed, purified and crystallized fungal ACC constructs, determined their structure and carried out SANS analysis. E.S. cloned, expressed and crystallized human ACC CD and determined its structure. EM analysis was carried out by E.S., M.H. and A.H. S.I. contributed to structural analysis and figure preparation. T.M. designed and carried out SAXS analysis. E.S. cloned, expressed and purified and crystallized fungal ACC constructs, determined their protein characterization and the Proteomics core facility for mass spectrometry and identification of post-translational modifications. M.H. acknowledges generous support by a Novartis Excellence Fellowship. A.H. is supported by a Fellowship for Excellence of the Biocenter Basel International PhD programme. This work was supported by Swiss National Science Foundation grants 13862/2, 159696 and 145023.

Additional information

Accession codes: Atomic coordinates and structure factors have been deposited in the Protein Data Bank with accession codes 5I6E (ScdCD), 5I87 (HuaBt-CD), 5I6F (HuaBtCD) and 5I6G (HuaBtCD-CT). Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

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