

## LETTERS

# An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis

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The sterol regulatory element binding protein (SREBP) family of transcription activators are critical regulators of cholesterol and fatty acid homeostasis<sup>1,2</sup>. We previously demonstrated that human SREBPs bind the CREB-binding protein (CBP)/p300 acetyltransferase KIX domain and recruit activator-recruited co-factor (ARC)/Mediator co-activator complexes through unknown mechanisms<sup>3–5</sup>. Here we show that SREBPs use the evolutionarily conserved ARC105 (also called MED15) subunit to activate target genes. Structural analysis of the SREBP-binding domain in ARC105 by NMR revealed a three-helix bundle with marked similarity to the CBP/p300 KIX domain. In contrast to SREBPs, the CREB and c-Myb activators do not bind the ARC105 KIX domain, although they interact with the CBP KIX domain, revealing a surprising specificity among structurally related activator-binding domains. The *Caenorhabditis elegans* SREBP homologue SBP-1 promotes fatty acid homeostasis by regulating the expression of lipogenic enzymes<sup>6,7</sup>. We found that, like SBP-1, the *C. elegans* ARC105 homologue MDT-15 is required for fatty acid homeostasis, and show that both SBP-1 and MDT-15 control transcription of genes governing desaturation of stearic acid to oleic acid. Notably, dietary addition of oleic acid significantly rescued various defects of nematodes targeted with RNA interference against *sbp-1* and *mdt-15*, including impaired intestinal fat storage, infertility, decreased size and slow locomotion, suggesting that regulation of oleic acid levels represents a physiologically critical function of SBP-1 and MDT-15. Taken together, our findings demonstrate that ARC105 is a key effector of SREBP-dependent gene regulation and control of lipid homeostasis in metazoans.

Cholesterol and fatty acids have important functional roles in metazoans, such as modulating membrane fluidity, serving as signaling molecules and providing energy storage in the form of triacylglycerides. Abnormal cholesterol and fat levels have been linked to prevalent diseases, including atherosclerosis, obesity, type 2 diabetes and hypertension (all associated with metabolic syndrome), underscoring the importance of understanding fully how cholesterol and lipid homeostasis are regulated and maintained<sup>8–10</sup>.

The mammalian SREBP family of basic helix–loop–helix zipper (bHLH-Zip) transcription factors promote adipocyte differentiation and are critical regulators of cholesterol and fatty acid homeostasis

by controlling the expression of cholesterogenic and lipogenic genes<sup>1,2</sup>. Human SREBPs can activate target genes by recruiting the chromatin-targeting CBP/p300 acetyltransferases and the polymerase-II-interacting ARC/Mediator co-activators<sup>3–5,11</sup>. A short sequence within the amino-terminal activation domain of SREBP-1a bound both CBP/p300 and the ARC/Mediator co-activator (Fig. 1a, e). We previously identified the KIX domain in CBP/p300 as a functionally important target of the SREBP activation domain<sup>3</sup> (Fig. 1b). Bioinformatics analysis revealed that the ARC105 subunit of the ARC/Mediator co-activator contains a region with significant sequence similarity to the CBP/p300 KIX domain<sup>12</sup>, raising the possibility that structurally related motifs present in both CBP/p300 and the ARC/Mediator co-activator might mediate interaction with SREBP-1a (Fig. 1b; see also Supplementary Fig. S1). Indeed, ARC105 bound to the SREBP-1a activation domain, but not to a number of other activators<sup>13,14</sup> (Fig. 1c, e and data not shown). The SREBP-1a activation domain did not bind significantly to other ARC/Mediator subunits tested (Fig. 1c and data not shown). The activation domains of the SREBP-1c and -2 isoforms also interacted with the ARC/Mediator co-activator and ARC105, albeit more weakly than SREBP-1a (Fig. 1e). SREBP-1a associated with ARC105 *in vivo*, as revealed by co-immunoprecipitation studies (Fig. 1d). Together, these results show that ARC105 is a direct target of the SREBP family of activators.

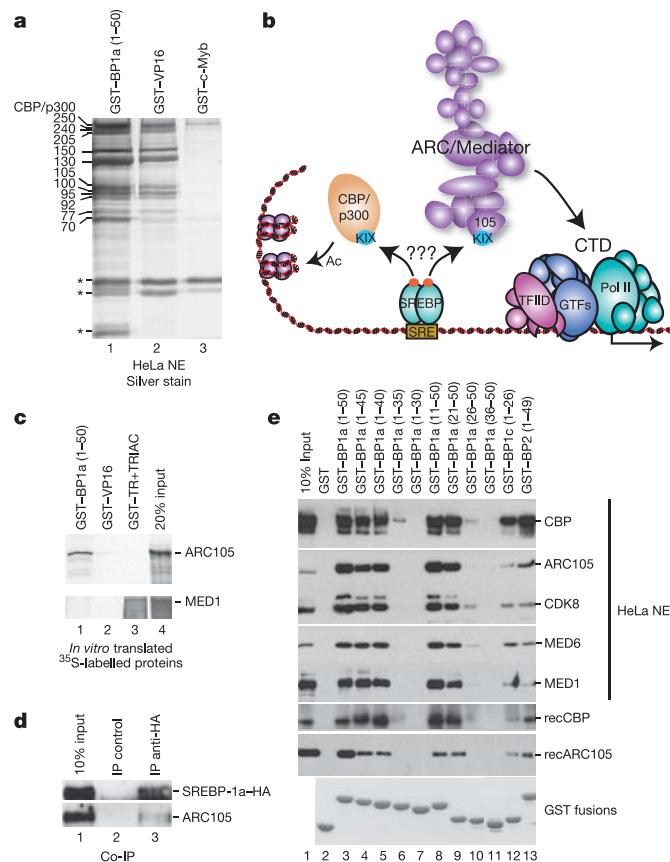
The N-terminal region of ARC105 containing the predicted KIX domain strongly interacted with recombinant SREBP-1a (Fig. 2c), supporting the hypothesis that SREBPs associate with distinct co-activators that harbour related KIX domains. Fluorescence polarization analysis also showed that the SREBP-1a activation domain interacts with high affinity (dissociation constant ( $K_d$ ) of ~120 nM) with the putative ARC105 KIX domain (Supplementary Fig. S2). Structural studies with NMR clearly demonstrated that the ARC105 SREBP-binding domain folds into a three-helix bundle with striking similarity to the CBP KIX domain (Fig. 2a, b). Deletion analysis and point mutagenesis provided evidence that the third  $\alpha$ -helix of the ARC105 KIX domain contains the primary SREBP-1a-binding site (Fig. 2c, e, f and data not shown).

The CBP KIX domain mediates interactions with a number of activators, including the cAMP/protein kinase A (PKA)-regulated CREB and the c-Myb transactivators<sup>15,16</sup>. However, although the

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activation domains of CREB and c-Myb bound strongly to the CBP KIX domain, they did not exhibit significant binding to the ARC/Mediator co-activator, nor to the ARC105 subunit or its KIX domain (Figs 1a and 2d and data not shown). Thus, the ARC105 KIX domain interacts with only a subset of CBP/p300 KIX-binding activators. Tyr 658 and Lys 662 in the CBP KIX domain have been implicated in the interaction with PKA-phosphorylated CREB and c-Myb activation domains<sup>17–20</sup>; however, these amino acids are not conserved in the ARC105 KIX domain, potentially explaining why CREB and the c-Myb activation domain did not bind the ARC105 KIX domain (Fig. 2e, f). To address this possibility, Ile64 and Asp68 in the ARC105 KIX domain were changed into the corresponding CBP amino acids (I64Y/D68K). The doubly mutated ARC105 KIX domain interacted with both CREB and the activation domain of c-Myb fused to the Gal4 DNA-binding domain (Gal4-MybAD), albeit more weakly than with the CBP KIX domain (Fig. 2d, e).



**Figure 1 | The activation domains of SREBPs interact with the ARC105 subunit of the ARC/Mediator co-activator.** **a**, The ARC/Mediator co-activator from HeLa cell nuclear extract (NE) bound to GST fusions of the activation domains of human SREBP-1a (BP1a) and VP16, but not to human c-Myb. All three activation domains interacted with the CBP/p300 co-activators. Molecular weights (kDa) of ARC/Mediator subunits are indicated to the left of the panel. Non-specific proteins are indicated with asterisks. **b**, Cartoon illustrating the question of whether the small SREBP activation domain recruits both CBP/p300 and the ARC/Mediator via KIX domains. **c**, The SREBP-1a activation domain bound to the ARC105 subunit, whereas the ligand-bound thyroid hormone receptor (TR) specifically interacted with MED1 (ref. 30). **d**, Haemagglutinin-tagged SREBP-1a (SREBP-1a-HA) expressed in U2OS cells co-immunoprecipitated with endogenous ARC105. **e**, CBP and the intact ARC/Mediator co-activator from nuclear extract, as well as recombinant CBP and ARC105, interact with the same sequence within the SREBP-1a activation domain. The activation domains of SREBP-1c and SREBP-2 also interacted with CBP and ARC105.

These results suggest that several amino acids located primarily in the third  $\alpha$ -helix of the CBP and ARC105 KIX domains provide activator-binding specificity. Notably, chemical shift analysis revealed that several amino acid resonances in the third helix were perturbed upon binding unlabelled SREBP, indicating that this area of the ARC105 KIX domain is the key SREBP docking site (Fig. 2e, f). The SREBP-1a binding site on the ARC105 KIX domain appears to be distinct from the surfaces on CBP KIX that are bound by CREB (pKID), c-Myb and in particular, MLL (Fig. 2f)<sup>17–22</sup>. Together with the lack of conservation of Tyr 658 and Lys 662 in the ARC105 KIX domain, these differences may account for why CREB (pKID) and c-Myb are unable to bind effectively to the ARC105 KIX domain (Fig. 2f).

Consistent with the findings suggesting that the third  $\alpha$ -helix of the ARC105 KIX domain constitutes the primary SREBP-binding site, addition of a peptide containing the third  $\alpha$ -helix strongly inhibited interaction of the SREBP-1a activation domain with both the ARC/Mediator co-activator and CBP/p300 (Fig. 3a). Moreover, the ARC105 peptide acted as a potent inhibitor of ARC/Mediator-dependent transactivation by SREBP-1a and Sp1 in a chromatin-based *in vitro* transcription reaction, while having no effect on transactivation by Sp1 alone (Fig. 3b; see also Supplementary Fig. S3). Together, these results show that ARC105 harbours a functionally important SREBP-binding domain that is structurally highly similar to the activator-targeted KIX domain found in the CBP/p300 acetyltransferases.

RNA interference (RNAi) was used to investigate the functional role of ARC105 in SREBP-dependent gene activation in human cells. Depletion of ARC105 by short interfering RNA (siRNA) strongly decreased cholesterol-regulated transcription of SREBP target genes, including low-density lipoprotein receptor (*LDLR*), fatty acid synthase (*FASN*), HMG-CoA synthase (*HMG-CoAS*) and HMG-CoA reductase (*HMG-CoAR*)<sup>1–2</sup> (Fig. 3c), as well as activation of a *FASN* promoter reporter by co-transfected SREBP-1a (Fig. 3d). In contrast, RNAi of *ARC105* did not affect gene activation by several other ARC/Mediator-targeting activators, including BMP-regulated SMAD1 (ref. 13), the viral activator VP16 (ref. 14) and the tumour suppressor p53 (data not shown), indicating that depletion of ARC105 does not globally impinge on the structure or function of the ARC/Mediator co-activator. Consistent with the lack of binding of c-Myb to ARC105, knockdown of ARC105 had no effect on transactivation by the activation domain of c-Myb (Fig. 3d). Next, we demonstrated by chromatin immunoprecipitation (ChIP) that epitope-tagged SREBP-1a and ARC105 co-expressed in U2OS cells co-occupy the *LDLR* and *FASN* promoters (Fig. 3e). Moreover, both CBP and the ARC/Mediator co-activator were found to be recruited to the endogenous *LDLR* and *HMG-CoAR* promoters in an SREBP-1a-dependent manner (Supplementary Fig. S4). These studies show that ARC105 co-localizes with SREBP on target genes and is specifically required for cholesterol-regulated and SREBP-1a-dependent gene activation in human cells.

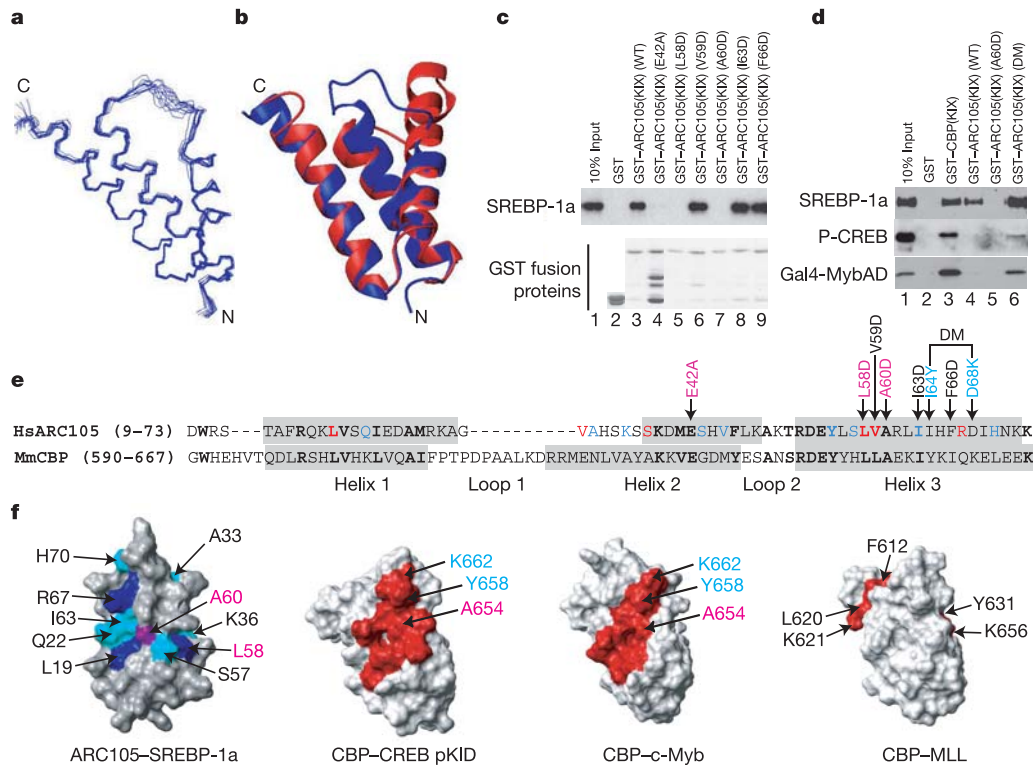
The ARC/Mediator co-activator is conserved in the nematode *C. elegans*<sup>23</sup>, and exploiting the genetic tractability of *C. elegans*, we have investigated the *in vivo* role of the ARC105 homologue MDT-15 in gene activation by the SREBP homologue SBP-1 and in the control of lipid homeostasis<sup>6,7,24</sup>. The activation domain of SBP-1 stimulated transcription in human cells and interacted with the conserved KIX domains of both MDT-15 and human ARC105, suggesting that the regulatory circuitry observed in human cells may be conserved in *C. elegans* (Fig. 4a; see also Supplementary Fig. S5). RNAi of either *mdt-15* or *sbp-1* produced a transparent or 'clear' phenotype, resembling starved *C. elegans* deprived of nutrients<sup>7,25</sup> (Fig. 4f). This phenotype has been suggested to possibly correlate with decreased fat storage in the intestinal compartment<sup>7</sup>. By contrast, RNAi depletion of many other ARC/Mediator subunits did not yield a clear intestinal phenotype, indicating that *mdt-15* may serve a specific role in maintaining lipid homeostasis and promoting fat storage (Fig. 4f; see also Supplementary Table 2).

Gas chromatography/mass spectrometry and thin liquid chromatography were used to gain a more detailed understanding of the alterations in lipid homeostasis upon removal of *mdt-15* and *sbp-1*. RNAi of either *mdt-15* or *sbp-1* resulted in decreased levels of triacylglycerides, the main form of fat stored in the intestinal compartment (triacylglycerides as percentage of total lipids: *mdt-15(RNAi)* 32%, *sbp-1(RNAi)* 23%, *mdt-4(RNAi)* 43%, *tbg-1(RNAi)* 47%, empty vector 44%). Moreover, RNAi of *mdt-15* or *sbp-1* brought about similar qualitative changes in the ratios of many different fatty acids, whereas control RNAi had little effect (Fig. 4b; see also Supplementary Fig. S6a).

The saturated fatty acid stearic acid (C18:0) was markedly increased in response to both *sbp-1* and *mdt-15* RNAi, whereas the monounsaturated fatty acid oleic acid was strongly decreased (Fig. 4b). In mammals, SREBPs directly activate transcription of a family of stearoyl-CoA desaturase genes that act in part to desaturate stearic acid to generate oleic acid<sup>26</sup>. As RNAi of *sbp-1* and *mdt-15* produced a marked increase in the stearic acid to oleic acid ratio (from ~2:1 for vector control to ~7:1 for *sbp-1(RNAi)* and ~16:1 for *mdt-15(RNAi)*), we investigated whether regulation of the *fat-6* and *fat-7* genes, homologues of the mammalian stearoyl-CoA desaturases<sup>27</sup>, was affected (Supplementary Fig. S6b). Indeed, expression of both *fat-6* and *fat-7* was markedly decreased upon RNAi of *mdt-15* or *sbp-1*, whereas depletion of *C. elegans mdt-4* (control) had little effect, indicating a pivotal and specific role of MDT-15 in the expression of SBP-1-regulated genes governing fatty acid desaturation (Fig. 4c). Moreover, intestinal expression of a *fat-7::GFP* reporter

construct was strongly and specifically reduced upon RNAi of either *sbp-1* or *mdt-15* (Fig. 4d; see also Supplementary Fig. S7).

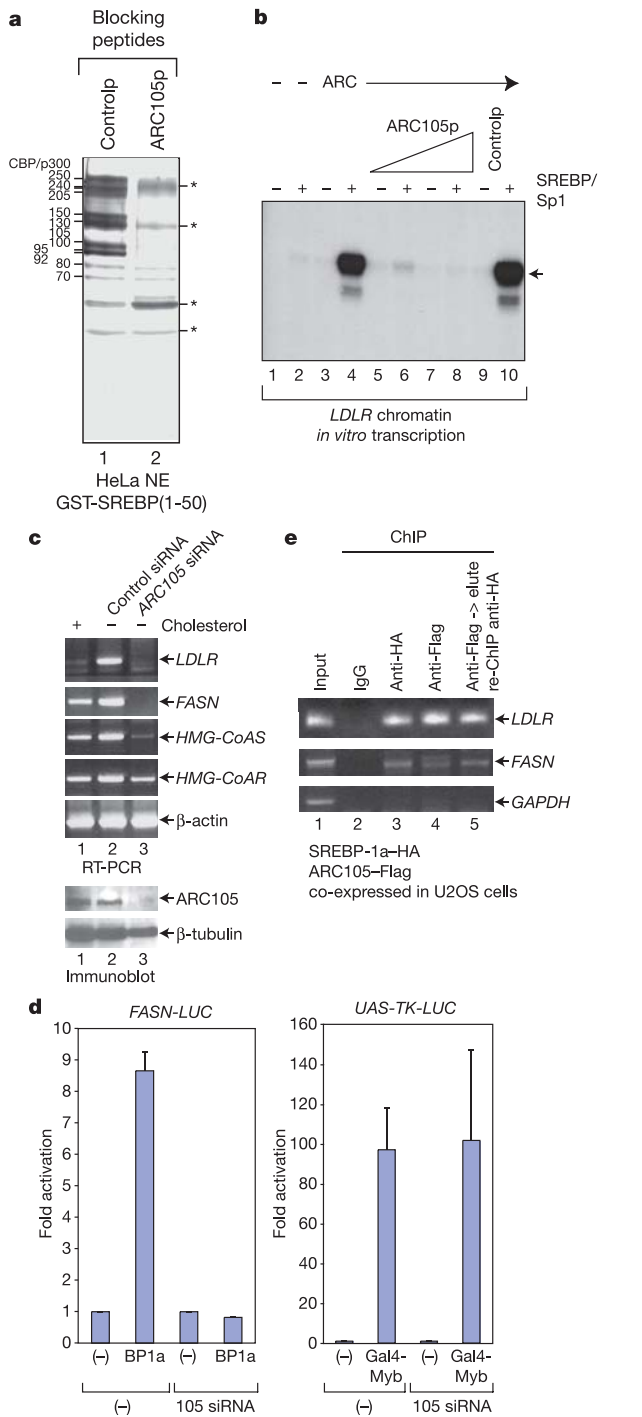
RNAi of *mdt-15* and *sbp-1* resulted in similar overt phenotypes, including clear intestines, growth defects, infertility, aberrant locomotion and decreased lifespan<sup>25</sup> (Fig. 4e, f; see also Supplementary Fig. S9, and data not shown). Oleic acid serves as a precursor for several physiologically important pathways, including synthesis of triacylglycerides destined for fat storage, membrane phospholipids, and polyunsaturated fatty acids (PUFAs)<sup>28</sup> (Supplementary Fig. S6b). Thus, the decreased levels of oleic acid due to impaired expression of the *fat-6* and *fat-7* stearoyl-CoA desaturases might contribute to some of the phenotypes in *mdt-15(RNAi)* and *sbp-1(RNAi)* nematodes. Notably, RNAi of both *fat-6* and *fat-7* (*fat-6/fat-7(RNAi)* nematodes) caused phenotypes that were similar to those resulting from RNAi of *mdt-15* and *sbp-1*, including clear intestines, infertility, decreased motility, smaller size and decreased lifespan<sup>25</sup> (Fig. 4f and data not shown). Moreover, dietary supplementation with oleic acid improved the fertility, darkened the intestines, increased locomotion and partially rescued the size defect of *mdt-15* and *sbp-1* RNAi-treated nematodes, and rescued all phenotypes of *fat-6/fat-7(RNAi)* animals (Fig. 4e, f; see also Supplementary Fig. S9). By contrast, stearic acid, which is elevated in response to RNAi of *mdt-15* and *sbp-1*, had little effect. These results collectively suggest that oleic acid deprivation due to decreased *fat-6* and *fat-7* desaturase expression is a primary cause of the clear intestines, small size, decreased fecundity and slow locomotion of *mdt-15(RNAi)* and *sbp-1(RNAi)* nematodes.



**Figure 2 | NMR solution structure of the ARC105 KIX domain.**

**a**, Representation of the 15 lowest energy structures of the ARC105 SREBP-binding domain reveals a three-helix bundle. **b**, The human ARC105 structure (blue) exhibits marked similarity to the mouse CBP KIX domain (red). **c**, Point mutagenesis revealed that E42, L58 and A60 are important for binding of recombinant Flag-tagged SREBP-1a to the ARC105 KIX domain. **d**, PKA-phosphorylated CREB (P-CREB) and Gal4-MybAD bound to immobilized CBP KIX, but not to the ARC105 KIX domain. Flag-SREBP-1a interacted with both KIX domains. Mutating two amino acids in the ARC105 KIX domain to the CBP KIX equivalents (I64Y/D68K, double mutant or DM) allowed partial binding of both P-CREB and Gal4-MybAD.

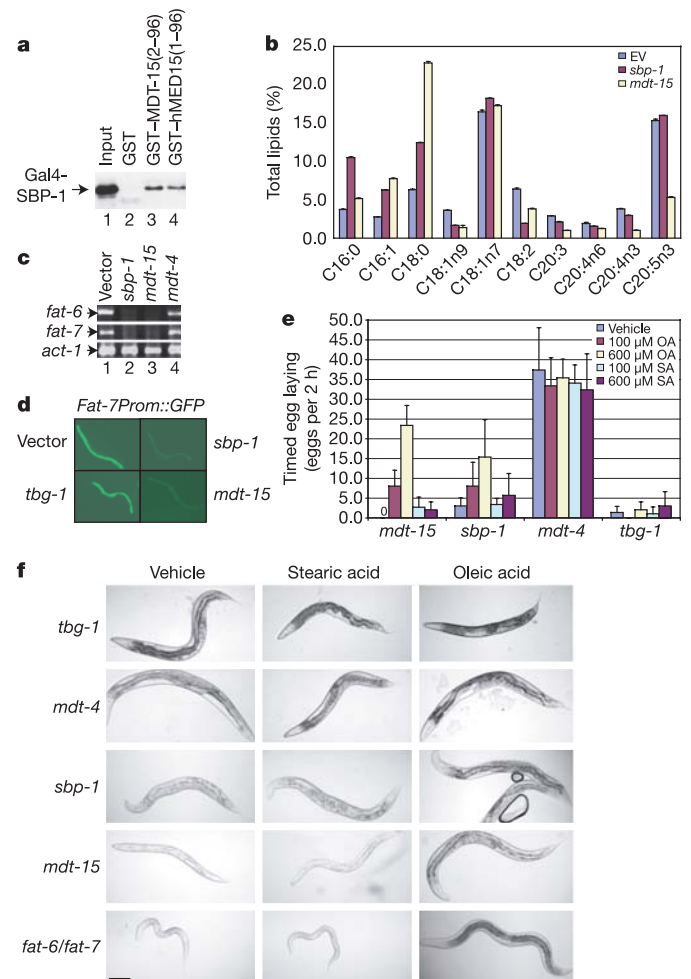
**e**, Alignment of the human ARC105 and mouse CBP KIX domains reveals conservation (bold) in the helical domains (grey). Point mutations in the ARC105 KIX domain tested for binding to SREBP-1a and other activators are indicated by arrows. Amino acids coloured red and blue were strongly and moderately affected, respectively, in chemical shift studies upon binding of the SREBP-1a activation domain. **f**, The SREBP-1a binding surface of the ARC105 KIX domain (dark blue/cyan) is distinct from the CBP KIX binding surfaces identified for the activation domains of CREB (pKID), c-Myb and MLL (red). Dark blue amino acids correspond to chemical shift >1.0 and cyan amino acids indicate chemical shift of 0.75–1.0.



**Figure 3 | ARC105 is required for SREBP-dependent transactivation and associates with SREBP target genes in human cells.** **a**, An ARC105 KIX peptide (ARC105p) inhibits binding of both CBP/p300 and the ARC/Mediator co-activator to the GST-SREBP-1a activation domain, whereas a control peptide (Controlp) has no effect. Molecular weights and asterisks are as in Fig. 1a. **b**, ARC105p inhibited ARC/Mediator-dependent activation by SREBP-1a and Sp1 in an *in vitro* transcription reaction with *LDLR* promoter-derived chromatin template. **c**, ARC105 knockdown by siRNA in HeLa cells results in decreased cholesterol-regulated expression of SREBP target genes, including *LDLR*, *FASN*, *HMG-CoAS* and *HMG-CoAR*. **d**, *ARC105* RNAi inhibited SREBP-1a-dependent transactivation of a *FASN* promoter reporter, without affecting Gal4-MybAD transactivation. **e**, Chromatin immunoprecipitation (ChIP) of SREBP-1a-HA and ARC105-Flag on SREBP target genes in U2OS cells. GAPDH was used as control. Re-ChIP with anti-HA after anti-Flag immunoprecipitation and Flag peptide elution showed that SREBP-1a and ARC105 co-occupy SREBP target genes. Error bars represent s.e.m.

Together with the role of ARC105 in activin/TGF- $\beta$ -induced gene activation by the SMAD2/3 activators<sup>13</sup>, the findings presented here suggest that ARC105 is a critical transducer of gene activation signals that control early metazoan development as well as cholesterol and fatty acid homeostasis. Tissue-specific changes in the expression of SREBPs, as well as SREBP polymorphisms, have been linked to the development of obesity, atherosclerosis, type 2 diabetes, fatty liver and lipodystrophy in humans and in mice<sup>2</sup>. As ARC105 is a downstream effector of SREBP gene regulation, altered expression levels or mutations/polymorphisms in ARC105 may also contribute to these diseases.

*Note added in proof:* A recent paper reported a requirement for *mdt-15* in *C. elegans* lipid homeostasis<sup>29</sup>.



**Figure 4 | The *C. elegans* ARC105 homologue MDT-15 is a critical regulator of *C. elegans* SREBP (SBP-1) target genes and is required for normal lipid homeostasis.** **a**, The SBP-1 activation domain interacted with the KIX domains of *C. elegans* MDT-15 and human ARC105. **b**, Gas chromatography/mass spectrometry analysis revealed altered fatty acid profiles in *sbp-1*(RNAi) and *mdt-15*(RNAi) nematodes, as compared with empty vector control. **c**, Transcription of the *fat-6* and *fat-7* stearoyl-CoA desaturases was strongly decreased upon RNAi of *sbp-1* and *mdt-15*. **d**, Intestinal expression of the *fat-7* promoter fused to GFP (*fat-7Prom::GFP*) decreased after RNAi of *sbp-1* and *mdt-15*. **e**, Dietary addition of oleic acid (OA), but not stearic acid (SA), partially rescued sterility due to *mdt-15* and *sbp-1* RNAi, while having no effect on controls (*tbg-1*(RNAi) and *mdt-4*(RNAi)). **f**, RNAi of *sbp-1*, *mdt-15* and *fat-6* and *fat-7* yielded pale intestines and smaller animals. Dietary addition of 600  $\mu$ M oleic acid, but not stearic acid, darkened the intestines and increased the size of *mdt-15*(RNAi), *sbp-1*(RNAi) and *fat-6/fat-7*(RNAi) nematodes. The scale bar in the lower left panel represents 0.1 mm. Error bars represent s.e.m.

## METHODS

Details of plasmids, primers for RT-PCR and ChIP, siRNA, antibodies, expression/purification of proteins in Sf9 insect cells, GST pull downs, immunoprecipitation, tissue culture, transfection, luciferase assay, RT-PCR, ChIP, NMR spectroscopy/structure determination, fluorescence polarization, chromatin-based *in vitro* transcription, RNAi in *C. elegans* and fatty acid/lipid analysis by gas chromatography/mass spectrometry and thin liquid chromatography are given in Supplementary Information.

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**Supplementary Information** is linked to the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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**Author Information** Atomic coordinates of the amino-terminal region of ARC105 containing the KIX domain has been deposited in the Protein Data Bank with the accession number 2GUT. Reprints and permissions information is available at [npg.nature.com/reprintsandpermissions](http://npg.nature.com/reprintsandpermissions). The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to A.M.N. ([naar@helix.mgh.harvard.edu](mailto:naar@helix.mgh.harvard.edu)).