Cell Research (2012) 22:1707-1711.

www.nature.com/cr

LETTER TO THE EDITOR

Molecular basis of the acetyltransferase activity of MEC-17 towards α-tubulin

Cell Research (2012) **22**:1707-1711. doi:10.1038/cr.2012.154; published online 6 November 2012

Dear Editor,

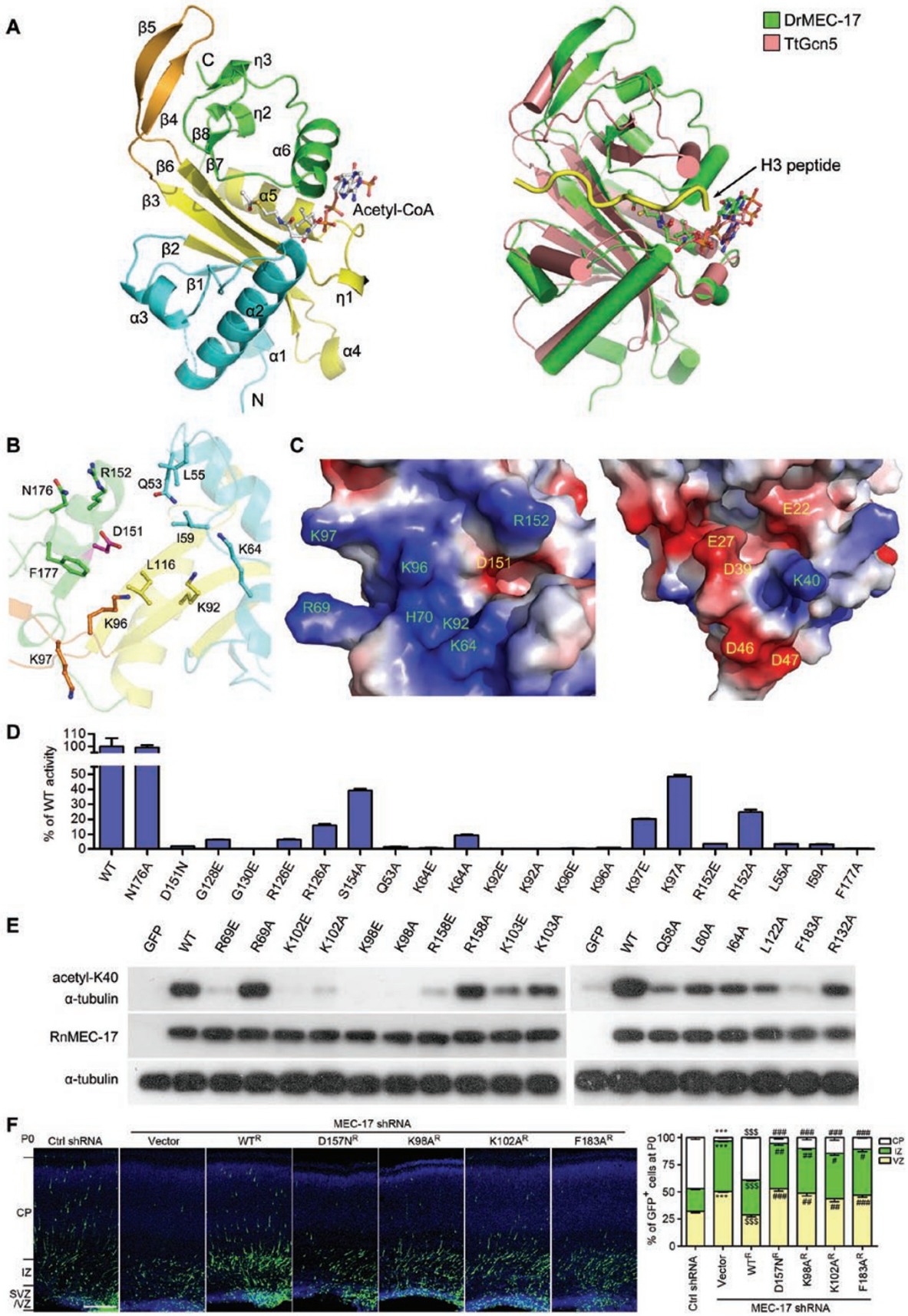
Adaptation of microtubules to the structural and functional complexities involves diverse post-translational modifications of tubulins, including acetylation, tyrosination, detyrosination, polyglycylation, polyglutamylation, palmitoylation, and phosphorylation. In particular, acetylation of α-tubulin affects the cell motility and plays an important role in neuronal migration and differentiation [1]. Specifically, acetylation of Lys40 of α-tubulin has prominent impacts on polarized protein trafficking and hence protein transport by promoting the binding of dynein/dynactin and kinesin-1 motor complexes [2, 3]. Recently the *Caenorhabditis elegans* protein mechanosensory abnormality 17 (MEC-17) and its homologues were shown to harbor intrinsic and specific acetyltransferase activity towards Lys40 of α-tubulin, which is critical in several biological processes such as cilium assembly of ciliated organisms, mechanosensation of *C. elegans*, and embryonic development of zebrafish [4, 5].

MEC-17 belongs to the Gcn5-related N-acetyltransferase (GNAT) superfamily. However, MEC-17 has very low sequence homology compared with other known GNAT superfamily acetyltransferases, and the molecular mechanism underlying its catalysis of α-tubulin acetylation is largely unknown. Here we report the structure of the catalytic domain of *Danio rerio* MEC-17 (DrMEC-17) in complex with the cofactor acetyl-coenzyme A (acetyl-CoA) at 1.7 Å resolution (Supplementary information, Table S1). As shown in Figure 1A, left panel, three antiparallel β-strands (β2, β3 and β6) and two helices (α4, α5) form the core region of the catalytic domain of DrMEC-17 that is structurally conserved in all lysine acetyltransferases (Supplementary information, Data S1).

Two pronounced clefts are formed between the N- and C-terminal segments of the protein, and the cofactor acetyl-CoA is bound in the smaller cleft surrounded by motif A (encompassing the β6-η1-α5 segment) and motif B (encompassing the β7-α6 segment) (Supplementary information, Figure S1). As in the Gcn5/acetyl-CoA [6] and PCAF/CoA complexes, the cofactor assumes a sharply bent conformation and the interactions between the cofactor and DrMEC-17 are largely conserved (Supplementary information, Figures S1, S2 and S3, and Data S1). Mutations of some involved residues, including Arg126, Gly128, Gly130 and Ser154, resulted in significant decreases in the enzyme activity (Figure 1D).

In the GNAT family, the position equivalent to Phe56 in AANAT (PDB code, 1CJW) is usually occupied by a hydrophobic residue in histone lysine acetyltransferases, which is proposed to be important for the bent conformation of acetyl-CoA [7]. In yeast Gcn5p, this position is occupied by Leu128, and the KQL mutant that contains a triple mutation of Lys126, Gln127 and Leu128 to Ala is completely defective [8]. Intriguingly, in DrMEC-17, a hydrophilic and highly conserved Gln residue (Gln53) takes this position (Supplementary information, Figure S2, left panel and Figures S4 and S5). The Q53A mutant exhibited only 1.5% activity compared with the wild-type enzyme (Figure 1D), demonstrating a crucial role of Gln53. On the other hand, the side chain of Gln53 points toward the substrate-binding cleft (Supplementary information, Figure S2, left panel). In the structure of the Gcn5/CoA/H3 peptide complex, several hydrophobic residues at this region that bind to CoA also interact with the substrate peptide [6]. Previously, the Chalfie group reported two missense mutations in allele of *C. elegans* MEC-17, u265, one of which (Leu46) corresponds to Gln53 of DrMEC-17 [9]. Intriguingly, although the acetyltransferase activity of MEC-17 is not required for touch sensation [10], the *mec-17* (u265) worms are not touch sensitive [9], indicating that Leu46 of *C. elegans* MEC-17 and probably the equivalent Gln53 of DrMEC-17 might contribute to the substrate binding.

In the Gcn5 structures, the cofactor is located in a small cleft and the substrate peptide is bound in a big cleft formed by the N- and C-terminal segments and the antiparallel β3-β4 sheet of the core region [6, 7, 11]. In the DrMEC-17/acetyl-CoA complex, the cofactor is bound with a similar mode (Supplementary information, Figure S2) and a presumed substrate-binding cleft is also detected at an equivalent position (Figure 1A, right


A
β5
C
η3
β4
η2
β8
α6
β6
β7
α5
Acetyl-CoA
β3
β2
β1
η1
α3
α2
α1
α4
N
DrMEC-17
TtGcn5
H3 peptide
B
R152
Q53
L55
N176
D151
I59
K64
F177
L116
K92
K96
K97
C
K97
R152
K96
D151
R69
H70
K92
K64
E22
E27
D39
K40
D46
D47
D
% of WT activity
110
100
50
40
30
20
10
0
WT
N176A
D151N
G128E
G130E
R126E
R126A
S154A
Q53A
K64E
K64A
K92E
K92A
K96E
K96A
K97E
K97A
R152E
R152A
L55A
I59A
F177A
E
GFP
WT
R69E
R69A
K102E
K102A
K98E
K98A
R158E
R158A
K103E
K103A
GFP
WT
Q58A
L60A
I64A
L122A
F183A
R132A
acetyl-K40
α-tubulin
RnMEC-17
α-tubulin
F
P0
Ctrl shRNA
MEC-17 shRNA
Vector
WT^R
D157N^R
K98A^R
K102A^R
F183A^R
CP
IZ
SVZ
/VZ
% of GFP+ cells at P0
100
75
50
25
0
CP
IZ
VZ
Ctrl shRNA
Vector
WT^R
D157N^R
K98A^R
K102A^R
F183A^R
MEC-17 shRNA

panel), although significant differences in the size and electrostatic properties are observed. The N-terminal segment of MEC-17 structurally resembles that of Gcn5 and in particular, the α2-α3 loop, equivalent to the α1-α2 loop of Gcn5, defines the lower side of the substrate-binding cleft. Inspection of the residues on the α2-α3 loop reveals two residues (Leu55 and Ile59) that are conserved in MEC-17 but not in the other GNAT family members (Supplementary information, Figures S4 and S5). In the DrMEC-17 structure, the two residues point their side chains towards the substrate-binding cleft (Figure 1B). Mutation of either Leu55 or Ile59 to Ala decreased the enzymatic activity of MEC-17 by about 95%, showing an essential role of the two hydrophobic residues (Figure 1D). The core region is conserved across the GNAT family, and at the bottom of the substrate-binding cleft, strictly conserved Leu116 on strand β6 (equivalent to strand β4 of Gcn5) might also contribute to the substrate binding. As the L116A mutant DrMEC-17 could not be readily purified, the impact of mutation of the equivalent Leu in *Rattus norvegicus* MEC-17 (RnMEC-17) was analyzed with cell biological assays (see later).

On the other hand, the C-terminal segment of MEC-17 displays substantial divergence compared with Gcn5 (Figure 1A, right panel), and there is no structural equivalent of the α5-β6 loop of Gcn5 that has been shown to be important for the histone binding [6, 11]. Gcn5 functions in large protein complexes and two regions, one flanking the histone-binding cleft and the other at the C-terminal surface, have been proposed to interact with other proteins to modulate the acetyltransferase activity of Gcn5. In contrast, MEC-17 acetylates highly purified microtubules or free tubulin *in vitro* ([4, 5] and this study) at a rate comparable to that in mammalian cells [4], indicating that MEC-17 can directly interact with and acetylate tubulin without facilitation of other proteins. MEC-17 preferentially acetylates tubulin in the polymerized state; however, the *K*m values for tubulin and microtubules were comparable [4]. Therefore, the structure of the αβ-tubulin heterodimer might provide useful hints for the interaction between α-tubulin and MEC-17.

Based on the available structures of α-tubulin, a 19-residue, Lys40-centered α-tubulin substrate peptide was synthesized (Supplementary information, Data S1). However, MEC-17 has no binding or activity for the synthesized peptide. It is noted that Lys40 of α-tubulin is primarily surrounded by several acidic residues, in particular Asp39, Asp46 and Asp47, that form a negatively charged surface patch (Figure 1C, right panel and Supplementary information, Figure S6). Intriguingly, a large part of the substrate-binding site of the MEC-17 catalytic domain is defined by positively charged residues, including Lys64, Arg69, His70, Lys92, Lys96, Lys97 and Arg152 (Figure 1C, left panel). In addition, this region and particularly most of these basic residues are strictly conserved in MEC-17 across different species (Supplementary information, Figure S7). The functional importance of the identified key residues was further examined. As a negative control, the mutation of Asn176 that is adjacent to the substrate-binding site has little effect on the acetyltransferase activity of MEC-17 (Figure 1D). In contrast, mutations of Lys64, Lys92, Lys96 and Arg152

Figure 1 Molecular basis of the acetyltransferase activity of MEC-17 towards α-tubulin. **(A)** Left panel, overall structure of the catalytic domain of DrMEC-17 in complex with acetyl-CoA. The N-terminal segment, core region, inserted β-hairpin and C-terminal segment are colored in cyan, yellow, orange and green, respectively. The disordered region is indicated with a dotted line. Right panel, structural comparison of the catalytic domain of DrMEC-17 (green) with that of Gcn5 (salmon, PDB code 1QSN). Superposition of the DrMEC-17 and Gcn5 catalytic domains was performed based on the core region. The H3 peptide bound with the Gcn5 catalytic domain is shown with a ribbon representation and colored in yellow. The cofactors are shown with ball-and-stick models and colored accordingly. **(B)** A detailed view of the conserved residues located at the substrate-binding site of DrMEC-17. The color coding is the same as **A**. **(C)** Electrostatic potential surface of the substrate-binding site of DrMEC-17 (left panel) and the Lys40-containing segment of α-tubulin (PDB code 1TUB). The surface charge distribution is displayed as blue for positive, red for negative, and white for neutral. **(D)** *In vitro* acetyltransferase assays of the wild-type DrMEC-17 and the mutants carrying mutations at the cofactor-binding site and the substrate-binding site. The acetyltransferase activities of DrMEC-17 are shown as the relative activity normalized to that of the wild-type protein. The experiments were performed in triplicates and the error bars indicate the standard deviation. **(E)** *In vivo* cell biological assays of the wild-type RnMEC-17 and the mutants. GFP-conjugated full-length RnMEC-17 proteins were overexpressed in COS7 cells. The acetylation level of α-tubulin Lys40 was assayed with western blotting using an anti-α-tubulin K40Ac monoclonal antibody (6-11B-1). The inputs of α-tubulin and the RnMEC-17 proteins are assayed with western blotting using anti-α-tubulin and anti-GFP antibodies. **(F)** Requirement of the acetyltransferase activity of MEC-17 for the migration of cortical projection neurons during development. Left panel, coronal sections of rat cortex at P0 electroporated at E16.5 with a GFP-expressing plasmid along with the indicated plasmids. Scale bars: 200 μm. Right panel, a histogram showing the distribution of the transfected cortical projection neurons in electroporated brain across different cortical regions at P0. *** $P < 0.001$ versus control shRNA. $^{\$\$\$}P < 0.001$ versus MEC-17 shRNA. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus MEC-17 shRNA with WT^{R}. All data represent the means ± SEM.

to Glu resulted in decrease of MEC-17 acetyltransferase activity by more than 90%, while mutation of Lys97 to Glu also had a significant impact on the MEC-17 acetyltransferase activity (Figure 1D). Consistently, the acetyltransferase activity of MEC-17 decreased to less than 25% as a result of mutations of Lys64, Lys92, Lys96 and Arg152 to Ala (Figure 1D). Intriguingly, mutations of Lys92 and Lys96 to Ala almost entirely abrogated the enzymatic activity of MEC-17 towards α-tubulin.

Glu122 of Gcn5, a residue immediately upstream of the β bulge on β4, has been proposed to serve as a general base to facilitate the proton abstraction of a structurally stabilized water molecule from the primary amine of the substrate [7]. In DrMEC-17, the equivalent position is occupied by Cys114, which is unlikely to function as a general base. Cys114 is also too far from the cofactor to form a covalently bound acetylated enzyme intermediate, excluding a possibility of utilization of a ping-pong mechanism by MEC-17. Therefore, some other residue at the catalytic site in DrMEC-17 should act as a general base. Asp151 on the β7-α6 loop is in the vicinity of the acetyl group of acetyl-CoA, and mutation of Asp151 of DrMEC-17, the equivalent Asp157 of *H. sapiens* MEC-17 or Asp144 of *C. elegans* MEC-17 to Asn, resulted in abolishment of the enzymatic activity [4, 10] (Figure 1D), indicating that this highly conserved Asp might be the general base. In addition, the side chain of Phe177, a strictly conserved residue on strand β8, flanks the left side of Asp151 to create a locally hydrophobic environment for Asp151 (Figure 1B), which might raise the p*K*a of Asp151 to facilitate the proton extraction of the substrate lysine. This notion is supported by the result that the F177A mutant almost completely lost the activity towards α-tubulin (Figure 1D).

Furthermore, we examined the effects of mutations of the equivalent residues in mammalian RnMEC-17 with cell biological experiments. Overexpression of the wild-type RnMEC-17 in COS7 cells substantially increased the acetylation level of α-tubulin Lys40 compared with the negative control (Figure 1E). In contrast, mutation of Gln58 (equivalent to Gln53 of DrMEC-17, Supplementary information, Table S2) to Ala did not have a significant impact, which combined with the *in vitro* biochemical data (Figure 1D) confirms an essential role of the residue. Several positively charged residues line up the substrate-binding cleft (Figure 1C, left panel) and were shown to be important for the acetyltransferase activity of DrMEC-17 (Figure 1D). Consistently, overexpression of the R132A mutant RnMEC-17 (Arg132 of RnMEC-17 is equivalent to Arg126 of DrMEC-17) led to moderate increase in the acetylation level (Figure 1E). Similarly, mutations of Arg69, Lys98, Lys102 and Arg158 of RnMEC-17 (which are equivalent to Lys64, Lys92, Lys96 and Arg152 of DrMEC-17, respectively) to Glu led to little changes compared with the negative control (Figure 1E). Mutations of Lys98 and Lys102 to Ala had a similar impact, while mutations of Arg69 and Arg158 to Ala had a minor effect (Figure 1E). These results are essentially in agreement with the results of the *in vitro* acetyltransferase assays (Figure 1D), indicating that these positively charged residues, particularly Lys98 and Lys102 of RnMEC-17, are important for the substrate binding. On the other hand, mutation of Lys103 of RnMEC-17 (equivalent to Lys97 of DrMEC-17) to either Glu or Ala retained some of the acetyltransferase activity of MEC-17 towards α-tubulin (Figure 1D and 1E), indicating a less important role of this residue for the substrate binding.

In addition, mutations of the conserved hydrophobic residues including Leu60 and Ile64 (which are equivalent to Leu55 and Ile59, respectively) on the α2-α3 loop of the lower side of the substrate-binding cleft and Leu122 (equivalent to Leu116 of DrMEC-17) on strand β6 of the substrate-binding pocket increased the acetylation level of α-tubulin Lys40 to a level significantly lower than that of the wild-type enzyme (Figure 1E), supporting their contribution to the substrate binding of MEC-17. For the catalysis, the indispensability of the Phe residue is again supported by the result that the F183A mutant of RnMEC-17 (corresponding to Phe177 of DrMEC-17) exhibited no detectable acetyltransferase activity towards α-tubulin (Figure 1E).

MEC-17 is highly expressed in the cerebral cortex during development and MEC-17 deficiency leads to impaired migration of cortical neurons [1]. To examine whether the acetyltransferase activity of MEC-17 is required for radial migration of cortical projection neurons during development, the rats at embryo day 16.5 (E16.5) were electroporated with GFP-expressing plasmid and indicated plasmids expressing MEC-17 shRNAs (Figure 1F). Four days later, the positions of cells in the cortical wall of the developing somatosensory cortex were examined. Consistent with our previous report [1], at postnatal day 0 (P0), 47% of the neurons expressing the control shRNA (Ctrl shRNA) migrated to the cortical plate (CP) and only 53% remained in the intermediate zone (IZ) and ventricular/subventricular zones (VZ/SVZ) (Figure 1F). In contrast, 97% of the MEC-17-deficient neurons remained in IZ and VZ/SVZ (Figure 1F). At P7, a substantially higher pecentage of the control cortical neurons reached their final destination, namely the layer II/III, than the MEC-17-deficient neurons (97% *vs*. 46%) (Supplementary information, Figure S8). Coexpression of the untargeted and shRNA-resistant (R) wild-type MEC-17 (WT^R), which was refractory to silencing by MEC-17

shRNA, almost completely rescued the migratory defect (Figure 1F and Supplementary information, Figure S8).

The strictly conserved Asp at the active site of MEC-17 has been proposed to act as a general base for deprotonation of the substrate lysine and the D157N mutant of RnMEC-17 has no detectable acetyltransferase activity towards α-tubulin [4]. In our study, we further show that a highly conserved Phe in the vicinity of Asp157 is critical (Figure 1B, 1D and 1E), probably through raising the p*K*a value of Asp157. Overexpression of $D157N^R$ or $F183A^R$ hardly restore the migratory defect induced by MEC-17 shRNA (Figure 1F and Supplementary information, Figure S8), indicating that the acetyltransferase activity of MEC-17 is required for its function in the migration of projection neurons during development. Similarly, overexpression of $K98A^R$ and $K102A^R$ that have mutations at the assumed binding site of α-tubulin could not largely rescue the migratory defect (Figure 1F and Supplementary information, Figure S8). These data indicate that the acetyltransferase activity of MEC-17 towards α-tubulin is essential for radial migration of cortical projection neurons.

Taken together, MEC-17 exhibits significant differences from Gcn5 in the substrate-binding cleft and the catalytic site, which correspond to the divergence in their substrates (Supplementary information, Data S1). Aberrations of acetylation of α-tubulin and protein transport have been reported in Huntington disease, Alzheimer's disease, and Charcot-Marie-Tooth disease. In addition, inhibition of histone deacetylase 6 compensates for the defect in the transport and release of brain-derived neurotrophic factor [2]. Due to the primary role of MEC-17 in the acetylation of α-tubulin, modulation of the MEC-17 activity and hence the acetylation level of α-tubulin might have an impact on the pathogenesis of diseases with altered intracellular transport such as Huntington disease. The molecular basis of the acetyltransferase activity of MEC-17 revealed here provides valuable information for further investigation on the function of MEC-17 and its potential involvement in neural disorders.

Accession code

The crystal structure of the catalytic domain of MEC-17 with bound acetyl-CoA has been deposited with the Protein Data Bank under accession code 4HKF.

Acknowledgments

We thank the staff members at the Shanghai Synchrotron Radiation Facility (SSRF), China for technical support in diffraction data collection. This work was supported by the Ministry of Science and Technology of China (2011CB966301), the National Natural Science Foundation of China (31221001), the Chinese Academy of Sciences (KSCX2-EW-Q-1-03).

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)