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# Crystallization and preliminary crystallographic analysis of the motor domain of human kinetochore-associated protein CENP-E using an automated crystallization procedure

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Human centromere-associated protein E, a member of the kinesin superfamily, is a microtubule-dependent motor protein involved in cell division that has been localized transiently to the kinetochore. The protein is thought to be responsible for the correct attachment and positioning of chromosomes to the mitotic spindle during the metaphase. The 312 kDa protein comprises four different domains. In this study, the focus was on the N-terminal motor domain, which includes the ATP-binding site and a region for microtubule binding. Crystals of the CENP-E motor domain have been obtained by high-throughput crystallization screening using an automated TECAN crystallization robot. The crystals (737 × 132 × 79 µm) belong to the space group $P2_1$, with unit-cell parameters $a$ = 49.35, $b$ = 83.70, $c$ = 94.16 Å, $\beta$ = 103.05°. They diffract to 2.1 Å resolution using synchrotron radiation.

## 1. Introduction

The molecular motors of the kinesin superfamily are proteins that use the energy from ATP hydrolysis to perform a large number of cellular processes such as intracellular transport (*e.g.* moving along microtubules carrying a cargo) and cell division. *Homo sapiens* centromere-associated protein E (HsCENP-E) is a kinesin-related protein involved in mitosis (Yen *et al.*, 1992). CENP-E is located at the external fibrous corona of kinetochores (Cooke *et al.*, 1997; Yao *et al.*, 1997). It is involved in the correct positioning and attachment of chromosomes to spindle microtubules during the metaphase (Schaar *et al.*, 1997; Yao *et al.*, 2000). Homologous CENP-E from *Xenopus* extracts has been proposed to play a role in the mitotic checkpoint (a process that controls the critical step from the metaphase to the anaphase) *via* its binding to the checkpoint kinase BubR1 (Yao *et al.*, 2000; Mao *et al.*, 2003).

The human CENP-E full-length protein consists of 2663 amino acids, with a theoretical molecular weight of 312 kDa, and is predicted to function as a dimer. The protein comprises four distinct regions: the N-terminal motor domain of 330 residues containing the ATP-binding site and a region for microtubule binding, a very long discontinuous $\alpha$-helix that forms a coiled-coil structure in the functional dimeric form, the C-terminal region of the protein containing a kinetochore-binding domain (Chan *et al.*, 1998) and a poorly understood second microtubule-binding region (Liao & Yen, 1994).

Here, we describe the first steps in an investigation of the structural properties of this important kinetochore-associated molecular motor, namely the crystallization and preliminary X-ray analysis of the human CENP-E motor domain.

## 2. Experimental

### 2.1. Construction of plasmids for protein expression

The DNA construct coding for the CENP-E motor domain was synthesized by PCR. The PCR product and the expression vector pET28a (Novagen) were double-digested with *Nco*I and *Xho*I (both from New England Biolabs) and ligated using the Rapid Ligation Kit from Boehringer. Positive clones were identified by testing for the presence of an insert of the expected size after digesting the purified plasmids with the restriction enzymes mentioned above. The sequence was confirmed by DNA sequencing. The expression plasmid codes for the CENP-E motor domain and eight additional residues (LEHHHHHH) at the C-terminus of the protein.

### 2.2. Expression and purification of the CENP-E motor domain

Recombinant CENP-E was expressed and purified as described for monomeric human Eg5 (DeBonis *et al.*, 2003). For crystallization, the protein was freshly prepared and used rapidly.

### 2.3. Crystallization experiments

CENP-E in 20 m*M* PIPES 7.3, 200 m*M* NaCl, 1 m*M* $MgCl_2$ and 1 m*M* Na EGTA was supplemented with 2 m*M* ATP and 10 m*M* $MgCl_2$ and concentrated (Amicon Ultra-15, 30 kDa cutoff) to 11 mg ml$^{-1}$. To remove insoluble material, the solution was centrifuged at 30 000*g* for 15 min. The freshly purified protein was crystallized using sitting drops.

The crystallization assays were performed automatically using a Genesis TECAN robot RSP 100/8, equipped with eight independent needles adapted for pipetting small volumes of down to 0.5 µl and equipped with a liquid-detection system. The robot was piloted using *GEMINI* standard software version 4.0. The following steps were performed using the robot: 200 µl of each solution from commercially available Hampton Research crystallization kits (delivered in 15 ml Falcon tubes) were transferred into Greiner round-bottom 96-well crystallization plates with three wells for each reservoir, sealed with aluminium silverseal film (Greiner Bio-One) and stored at 277 K. Altogether, six crystallization plates with 576 different crystallization conditions were used. CENP-E at three different protein concentrations (2.7, 5.5 and 11 mg ml$^{-1}$) was added to the first three rows of a 96-well Greiner plate. The crystallization plates as well as the 96-well plate were placed on predefined positions on the robot worktable. The eight needles in parallel first took 1 µl protein from the protein 96-well plate and then 1 µl reservoir solution from the prefilled 96-well Greiner crystallization plate and placed the drops in the eight wells of the first row (1A to 1H) of this plate. The needles were washed extensively and the procedure repeated for all 12 rows and all three protein concentrations. The complete procedure (preparation of 288 crystallization drops for each plate) takes 21 min. Finally, the plates were sealed with transparent viewseal film (Greiner Bio-One) and stored at 292 K.

**Figure 1**
Crystal of the human CENP-E motor domain.

## 3. Results and discussion

### 3.1. Crystallization

Crystals appeared after 2 d as long rods (Fig. 1). The crystallization conditions were improved manually by varying the initial conditions. The best results were obtained using 1 µl CENP-E at 11 mg ml$^{-1}$ and 1 µl reservoir solution containing 23% PEG 3350, 0.2 *M* $NaNO_3$, 0.1 *M* PIPES 7.0 in hanging and sitting drops at 292 K.

### 3.2. Data collection

Data were collected at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) on beamline ID14-2 equipped with an ADSC Quantum 4 CCD detector. The wavelength was 0.933 Å. The crystals were transferred for a few seconds to a cryoprotectant solution (as used for crystallization supplemented with 20% glycerol or 25% erythritol) and directly flash-frozen in the cryostream. In all cases the diffraction was highly anisotropic, with resolution that extended beyond 2.1 Å in the *b** direction but that was considerably poorer in the *c** direction (Fig. 2). The best data set yielded data to 2.5 Å resolution with an overall completeness of 98%. The crystal belonged to space group $P2_1$, with unit-cell parameters $a$ = 49.35, $b$ = 83.70, $c$ = 94.16 Å, $\beta$ = 103.05°. Details of the data-collection statistics are given in Table 1.

### 3.3. Data analysis and discussion

Data were processed with *DENZO* (Otwinowski & Minor, 1997) and scaled with *SCALA* from the *CCP*4 package (Colla-

**Table 1**
X-ray data collection of human CENP-E.

Data were collected on the ID14-2 X-ray beamline at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. Values in parentheses are for the last resolution shell (2.64–2.50 Å).

| | |
|---|---|
| Unit-cell parameters | |
| $a$ (Å) | 49.35 |
| $b$ (Å) | 83.70 |
| $c$ (Å) | 94.16 |
| $\beta$ (°) | 103.05 |
| Space group | $P2_1$ |
| Molecules per AU | 2 |
| Maximum resolution (Å) | 2.5 |
| Total No. observed reflections | 282620 |
| No. unique reflections | 25698 |
| Completeness (%) | 98 (86) |
| Multiplicity | 4.7 |
| $R_{sym}$† | 0.064 (0.158) |
| $I/\sigma(I)$ | 6.4 (4.4) |

† $R_{sym} = \sum|I_j - \langle I\rangle|/\sum\langle I\rangle$, where $I_j$ is the intensity of reflection $j$ and $\langle I\rangle$ is the mean intensity.

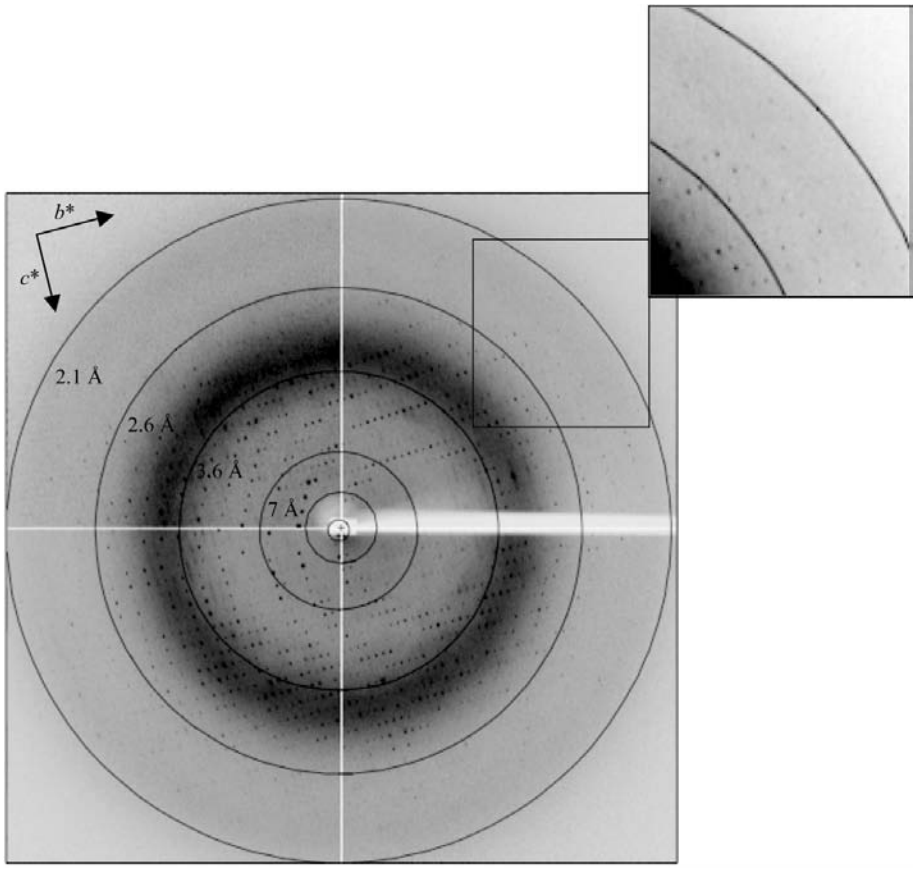


**Figure 2**
1° oscillation image of a CENP-E crystal. The crystal shows anisotropy, diffracting to 2.1 Å along the *b** direction and to 2.5 Å along the *c** direction.

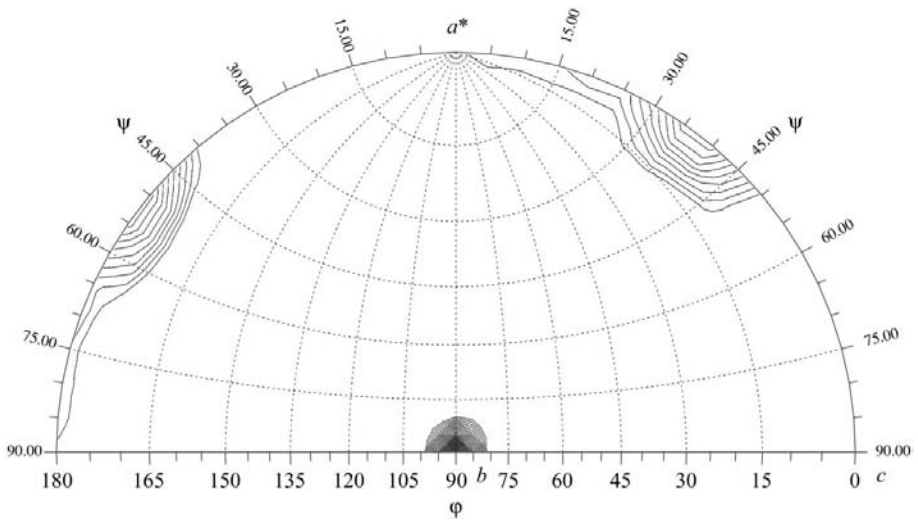


**Figure 3**
$\kappa = 180^\circ$ section of the self-rotation function calculated with *GLRF* (the resolution range used for calculation was 15–3.5 Å and the orthogonalization convention was *CXCAZ*). The peaks identifying the non-crystallographic twofold element relating monomer *A* and *B* were found at polar angles $\varphi = 0$, $\psi = 35$, $\kappa = 180^\circ$ and $\varphi = 180$, $\psi = 55$, $\kappa = 180^\circ$.

borative Computational Project, Number 4, 1994). The overall completeness of the data set to 2.5 Å resolution was 98%, with an $R_{\rm sym}$ of 6.4%. The mosaicity fitted by *SCALEPACK* was 0.7.

We found two molecules per asymmetric unit were related by a non-crystallographic twofold axis that is perpendicular to *b* and passes through $1/2c + a$. The self-rotation function calculated using *GLRF* (Tong & Rossmann, 1997) allowed the determination of the orientation; the angle of rotation of the non-crystallographic symmetry axis relating the two monomers gave polar angles $\varphi = 0$, $\psi = 35$, $\kappa = 180^\circ$ and $\varphi = 180$, $\psi = 55$, $\kappa = 180^\circ$ (Fig. 3).

The previously determined motor-domain structures of kinesin-superfamily members in *H. sapiens* are conventional kinesin (KHC; Kull *et al.*, 1996; Sindelar *et al.*, 2002), which is involved in intracellular transport, and Eg5 (Turner *et al.*, 2001), which is responsible for stabilization of the bipolar spindle in mitosis. CENP-E, Eg5 and KHC belong to distinct phylogenetic subgroups. The sequence identities between the motor domains of CENP-E and KHC and of CENP-E and Eg5 are 38.0 and 36.1%, respectively. Consequently, the CENP-E motor-domain structure was solved by molecular replacement using the structure of conventional human kinesin (PDB code 1mkj; Sindelar *et al.*, 2002) as a starting model. Further details of the three-dimensional structure of the CENP-E motor domain determined to 2.5 Å resolution will be reported elsewhere (Garcia-Saez *et al.*, 2004).

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## References

Chan, G. K. T., Schaar, B. T. & Yen, T. J. (1998). *J. Cell Biol.* **143**, 49–63.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Cooke, C. A., Schaar, B., Yen, T. J. & Earnshaw, W. C. (1997). *Chromosoma*, **106**, 446–455.
DeBonis, S., Simorre, J. P., Crevel, I., Lebeau, L., Skoufias, D. A., Blangy, A., Ebel, C., Gans, P., Cross, R., Hackney, D. D., Wade, R. H. & Kozielski, F. (2003). *Biochemistry*, **42**, 338–349.
Garcia-Saez, I., Yen, T., Wade, R. H. & Kozielski, F. (2004). In the press.
Kull, F. J., Sablin, E. P., Lau, R., Fletterick, R. J. & Vale, R. D. (1996). *Nature (London)*, **380**, 550–555.
Liao, H. & Yen, T. J. (1994). *Science*, **265**, 394–397.
Mao, Y., Abrieu, A. & Cleveland, D. W. (2003). *Cell*, **114**, 87–98.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Schaar, B. T., Chan, G. K. T., Maddox, P., Salmon, E. D. & Yen, T. J. (1997). *J. Cell Biol.* **139**, 1373–1382.
Sindelar, C. V., Budny, M. J., Rice, S., Naber, N., Fletterick, R. & Cooke, R. (2002). *Nature Struct. Biol.* **9**, 844–848.
Tong, L. & Rossmann, M. G. (1997). *Methods Enzymol.* **276**, 594–611.
Turner, J., Anderson, R., Guo, J., Beraud, C., Fletterick, R. & Sakowicz, R. (2001). *J. Biol. Chem.* **276**, 25496–25502.
Yao, X., Abrieu, A., Zheng, Y., Sullivan, K. F. & Cleveland, D. W. (2000). *Nature Cell Biol.* **2**, 484–491.
Yao, X., Anderson, K. L. & Cleveland, D. W. (1997). *J. Cell Biol.* **139**, 435–447.
Yen, T. J., Li, G., Schaar, B. T., Szilak, I. & Cleveland, D. W. (1992). *Nature (London)*, **359**, 536–539.