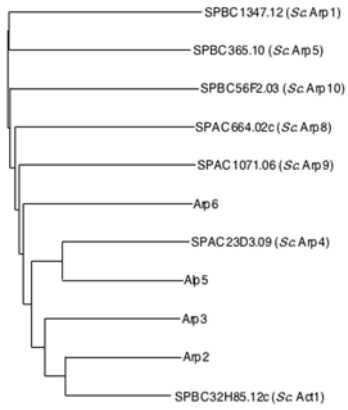
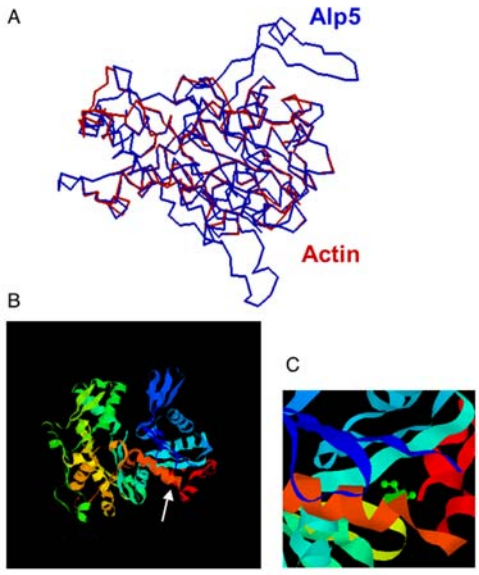
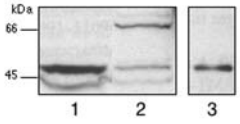


Supplementary Figure S1: Minoda *et al.*



Supplementary Figure S2: Minoda *et al.*



Supplementary Figure S3: Minoda *et al.*

Supplementary data: Minoda et al.

Supplementary Materials and methods

Cloning of the *alp5*$^+$ gene

An *S. pombe* genomic library in a *LEU2*-based multicopy vector pAL-KS (obtained from Taro Nakamura) was used for the isolation of the *alp5*$^+$ gene. 6 transformants of an *alp5-1134* strain, which were Ts$^+$ in a plasmid-dependent manner, were obtained from 10,000 Leu$^+$ transformants. Plasmid DNAs were recovered from each transformant and restriction mapping showed that all the plasmids contained an overlapping insert. Subcloning analysis indicated that one (SPBP23A10.08) of the ORFs, which is contained in the insert DNA, is responsible for complementation. Identification of SPBP23A10.08 as *alp5*$^+$ was confirmed by genetic crosses between C-terminally tagged strain (*alp5*$^+$-*kan*^{r}) and the original ts *alp5-1134* mutant. No recombinants (G418-resistant and Ts$^-$ or G418-sensitive and Ts$^+$) were obtained from 1,000 segregant colonies.

Determination of the mutation site in *alp5-1134*

Genomic DNA was cloned from an *alp5-1134* strain by PCR. To avoid potential mutations created during PCR, two independent reactions were performed in different tubes. Also the *alp5*$^+$ gene was amplified from a wild type strain. Sequencing of these amplified fragments showed that both DNA fragments derived from *alp5-1134* contain a point mutation at the same position, that is G1208A (A for initiator ATG is denoted as +1). This mutation would result in amino acid replacement at 402 from serine to asparagine (S402N).

Homology search and amino acid alignment of actin-related proteins

Clustalx and Macboxshade softwares were used to make amino acid alignment of sequences among homologous protein. Homology search against fission yeast genome database using Alp5 as a query shows that fission yeast contains, like budding yeast, 10 actin-related proteins in addition to the conventional actin (Act1). Phylogenetic tree is provided in Supplementary materials (Figure S1).

Production of anti-Alp5 antibody

Polyclonal rabbit anti-Alp5 antibody was prepared as follows. A whole ORF encoding *alp5*$^{+}$ was amplified by PCR and cloned into bacterial expression vector pET-14b (Novagen, Madison, WI). 6-His tagged Alp5 fusion protein was purified on Ni^{2+}-NTA beads (QIAGEN, Valencia, CA) as recommended by the manufacturer. Crude anti-Alp5 serum was affinity purified using Alp5 fusion protein immobilised on nitrocellulose filters.

Gel filtration chromatography

Soluble protein extracts were prepared in buffer A (20 mM Tris-HCl pH 7.5, 20% glycerol, 0.1 mM EDTA, 1 mM mercaptoethanol, 5 mM ATP plus a cocktail of inhibitors, Sigma). Gel filtration chromatography was performed on a Superose-6 column by FPLC (Pharmacia Biotech). The column was equilibrated with 2 column volumes of buffer A containing 100 mM NaCl. To determine molecular weight, a parallel column was run with standards consisting of dextran (2,000 kDa), thyroglobulin (669 kDa) and α-amylase (232 kDa). Fractions (50 µl each) were separated by SDS-PAGE on 10% gels and fractionated proteins were detected with individual antibodies.

Supplementary References

Kabsch, W., Mannherz, H.G., Suck, D., Pai, E.F., and Holmes, K.C. (1990). Atomic structure of the actin: DNase I complex. Nature *347*, 37-44.

Otterbein, L.R., Graceffa, P., and Dominguez, R. (2001). The crystal structure of uncomplexed actin in the ADP state. Science *293*, 708-711.

Legends for supplementary figures

Figure S1: Actin and Actin-related protein family in fission yeast

Phylogenetic tree was constructed among fission yeast actin-related proteins including conventional actin (Act1). Budding yeast proteins most similar to fission yeast proteins are shown in parentheses (e. g. *Sc.* Arp4). Homology search against the fission yeast genome database using budding yeast Arp4 as a query shows that Alp5 (SPB23A10.08) is the most homologous, and vice versa Arp4 is the most homologous protein to Alp5 in the budding yeast genome. However homology search using SPAC23D3.09 shows that this ORF is also most homologous to budding yeast Apr4. As fission yeast has no counterpart for budding yeast Arp7, SPAC23D3.09 could be a fission yeast homologue for Arp7. Indeed when homology search was performed using Arp7, SPAC23D3.09 is the first hit in the fission yeast genome.

Figure S2: Predicted Alp5 structure and mutation site in the *alp5-1134* mutation

(A) Structure of Alp5 (shown in blue) is superimposed to that of conventional actin (shown in red) (Kabsch *et al.*, 1990; Otterbein *et al.*, 2001). (B. C) 3-D structure of Alp5 is created based upon that conventional actin using 3-D Jigsaw ver. 2 software. Then

using RASMAC software, 3-D structure is visualized. Replaced amino acid (S402N) is shown with either white arrow (B) or green side chain (C).

Figure S3: Production of polyclonal anti-Alp5 antibody

Total cell extracts were prepared from wild type (lane 1, 20 μg), a diploid strain heterozygous for *alp5*$^{+}$ (*alp5*$^{+}$/*alp5*$^{+}$-*myc*) (lane 2, 20 μg) or *E. coli* containing plasmids expressing the 6His-Alp5 fusion protein (10 ng) were run and immunoblotting was performed with anti-Alp5 antibody. The positions of protein size markers are shown in the left corner.