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Molecular Cloning of the Gene for p85 That Regulates the Initiation of Cytokinesis in *Tetrahymena*

Kohsuke Gonda, Kimiko Nishibori, Hiroyoshi Ohba,* Atsushi Watanabe, and Osamu Numata[1]
*Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan; and *Department of Biological Science and Technology, Science University of Tokyo, Noda, Chiba 278-0022, Japan*

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***Tetrahymena* p85 is localized to the presumptive division plane before division furrow formation; its molecular weight in SDS–polyacrylamide gel electrophoresis differs in wild-type and temperature-sensitive cell-division-arrest mutant *cdaA1* cells. At the restrictive temperature, p85 localization and division furrow formation are not observed in *cdaA1* cells. In this study, we purified p85 and cloned a wild-type p85 cDNA. The deduced amino acid sequence of p85 was composed mainly of two kinds of repeat sequences. One of these contained regions homologous to a calmodulin-binding site and a part of actin, and the other contained a region homologous to a part of a cdc2 kinase homologue. Moreover, we cloned a cDNA encoding the *cdaA1* p85. There was no difference in the predicted amino acid sequences of wild-type and *cdaA1* p85, suggesting that the difference in molecular weight between p85 in wild-type and mutant cells is caused by a disorder of posttranslational-modification mechanisms of p85 in the *cdaA1* cell.** © 1999 Academic Press

Cytokinesis, which is the final stage of the cell cycle, assures the equal distribution of segregated daughter chromosomes and cytoplasm into the two daughter cells. In the cytokinesis of animal cells, first, a division plane is determined at a cellular equator and then an actomyosin-based contractile ring appears at the division plane. The contractile ring constricts, generates a division furrow, and divides the cell (1, 2). In addition to actin and myosin, a number of actin-modulating proteins, for example α-actinin (3), cofilin (4), profilin (5), and 71-kDa F-actin-binding protein (6), are localized in the division furrow. These findings provide useful information on the structure and the dynamic organization of the contractile ring. However, the molecular mechanisms of the division plane determination and the contractile ring formation are not well understood.

To elucidate the mechanisms of cytokinesis, the employment of mutants affecting cytokinesis is a potentially powerful approach. In *Tetrahymena*, temperature-sensitive cell-division-arrest mutants (*cda* loci) have been isolated and partially characterized. One of the *cda* mutants, *cdaA1*, has been shown to have a defect in the formation of the division furrow (7, 8). In our previous study, we identified a single protein (Mr = 85 kDa, pI = 4.7, designated as p85) which differed slightly in mobility in two-dimensional SDS-polyacrylamide gel electrophoresis (2D SDS-PAGE) between wild type and *cdaA1* cell extracts (9). In the analysis of 2D SDS-PAGE, the molecular weight of *cdaA1* p85 was 89 kDa, whereas its isoelectric point was the same as that of wild type p85. At the permissive temperature, the p85 appeared at the presumptive division plane before the formation of the division furrow, and then was localized in the division furrow during cytokinesis in both wild type and *cdaA1* cells (9, 10). In addition, the contractile ring was formed along the localization of p85 to the presumptive division plane (11). On the other hand, in *cdaA1* cells at the restrictive temperature, localization of p85 and formation of a division furrow were not observed (9). These results suggest that p85 plays a key role in the mechanisms of division plane determination and contractile ring formation. Therefore, clarifying the primary structure and the function of p85 should help to solve the molecular mechanisms of *Tetrahymena* cytokinesis.

In this study, we purified p85 from *Tetrahymena* cell extracts and cloned a wild type p85 cDNA. The deduced amino acid sequence of wild type p85 was mainly composed of two kinds of 150-amino acid repeat sequences. N-terminal repeat sequences show homologies to a calmodulin (CaM)-binding site of Ca^{2+}/calmodulin dependent protein kinase Type II (CaM II kinase) and a part of actin, whereas C-terminal repeat sequences show homology to a part of a cdc2 kinase homologue. More-

[1] Address correspondence and reprint requests to Osamu Numata, Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan. Fax: +81-298-53-6648. E-mail: numata@sakura.cc.tsukuba.ac.jp.

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over, a *cdaA1* p85 cDNA was cloned and sequenced. The predicted amino acid sequence of *cdaA1* p85 was identical to that of wild type p85. Therefore, we suggest that the difference of molecular weight in SDS-PAGE between *cdaA1* and wild type p85 is caused by the disorder of posttranslational-modification mechanisms of p85 in *cdaA1* cells.

MATERIALS AND METHODS

Cell culture. The *Tetrahymena thermophila* wild type strain and *cdaA1* mutant strains used in this study were kindly provided by Dr. J. Frankel, University of Iowa. Cultivation of *Tetrahymena thermophila* was performed as described previously (12).

Antibodies. A guinea pig anti-p85 antiserum (p85GP1) was prepared and characterized in our previous study (9). A rabbit anti-p85 antiserum (p85R1) was prepared by the method of Ohba (9).

Immunoprecipitation. The wild type cells were lysed in 10 mM Hepes buffer (pH 7.2) containing 30 mM KCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), 3.6 μM pepstatin A, 540 μM *N*α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK), and 60 mM *n*-heptyl-β-D-thioglucoside, by sonication with a cell distributor (Ultrasonics Inst., Tomy Seiko, Japan), and then centrifuged at 15,000×g for 10 min. The soluble supernatant was mixed with p85R1-bound or preimmune serum-bound protein A-Sepharose (PIERCE, USA), and incubated for 2 h at 4°C. The mixture was centrifuged at 250×g for 1 min and then the precipitates were washed with 10 mM Hepes buffer (pH 7.2) containing 150 mM KCl, 1 mM PMSF, and 3.6 μM pepstatin A. After being washed, the p85 protein was eluted from immunocomplexes by incubating with 100 mM glycine-HCl (pH 2.5) and then neutralized with 2 M Tris-HCl (pH 9.0). The samples were separated by 8% SDS-PAGE and analyzed by silver staining and immunoblotting.

Purification of p85. The wild type cells were suspended in 20 mM Tris-HCl (pH 7.5), 100 mM KCl, 1 mM ATP, 0.5 mM DTT, 10% sucrose, 1% casein, 1 mM PMSF, and 0.5 M KI. The cells were sonicated using a cell distributor (Ultrasonics Inst., Tomy Seiko, Japan), and then ultracentrifuged at 250,000×g for 1 h. From the soluble supernatant, the fraction including p85 was purified through ammonium sulfate fractionation, a DEAE TYOPEARL PAK column (TOSOH, Japan), and a butyl TYOPEARL PAK column (TOSOH, Japan) based on monitoring of immunoblotting using the p85R1. The partially purified p85 fraction was separated by 2D SDS-PAGE and then the p85 spot was excised from the gel. The purified p85 was digested with V8 protease according to the methods of Cleaveland (13). The N-terminal amino acid sequence of the purified p85 and that of its fragment digested by V8 protease were determined by automated Edman degradation with an Applied Biosystem gas-phase sequencer (Foster City, CA, USA).

Construction of a cDNA library of cdaA1 cells. The poly(A)$^+$ RNA from *cdaA1* cells was prepared with Isogen (Nippon Gene, Japan) and OligotexTM-dT30 Super (TAKARA, Japan). The cDNA was synthesized from the poly(A)$^+$ RNA using SuperScriptTM Choice System for cDNA Synthesis (GIBCO BRL, USA). The resulting cDNA was cloned into the *Eco*RI site of lambda gt10 (Amersham Pharmacia Biotech, UK) and packaged *in vitro* by Gigapack II Gold Packaging Extract (STRATAGENE, USA). The recombinant phage was transduced into *E. coli* strain NM514 and maintained as liquid stock at 4°C. The cDNA library of wild type cells prepared in our previous study (14) was used.

Cloning of wild type and cdaA1 p85 cDNA. To prepare the probe for cloning the wild type p85 cDNA, a reverse transcription-polymerase chain reaction (RT-PCR) was carried out. With this method, the reverse transcription reaction was performed using the poly(A)$^+$ RNA and oligo(dT) primer (TAKARA, Japan). Two mixed primers, 5′-GCTACYGTYTTCGA-3′ and 5′-GTGACCRGTRGC-3′, were designed based on the 22-amino acid sequence of p85 (Fig. 2B-2), and then used to generate 62-bp nucleotides by PCR.

To clone the p85 cDNA of the wild type and that of *cdaA1*, recombinant phage plaques from cDNA libraries of the wild type and *cdaA1* cells were screened by plaque hybridization with the ^{32}P-labeled RT-PCR product probe and the ^{32}P-labeled wild type p85 cDNA probe, respectively, under the same conditions described previously (15). The *Eco*RI inserts of positive phages from wild type and *cdaA1* cDNA libraries were subcloned into the *Eco*RI site of pBluescript II (KS$^+$) plasmids (TOYOBO, Japan) and puc18 plasmids (TAKARA, Japan), respectively. Dual-directional deletion of these inserts was done using exonuclease III (TAKARA, Japan) and mung been nuclease (TAKARA, Japan), and the nucleotide sequence analyses were carried out according to the ABI 373A DNA sequencing system protocol.

Moreover, to clone the 5′ untranslated regions of wild type and *cdaA1* p85 cDNA, a rapid amplification of cDNA 5′-end (5′ RACE) was performed with 5′ RACE System version 2.0 (GIBCO BRL, USA). In this system, the reverse transcription reaction was carried out with poly(A)$^+$ RNA and the gene specific primer 5′-TTGTTATCCTTGAGG-3′ shown in Fig. 5. The PCR was done using the gene specific primer 5′-GATTTTGAGCGATGGAAGCTCC-3′ shown in Fig. 5 and the 5′ RACE Abridged Anchor Primer of this system. After 30 cycles using the wild type and the *cdaA1* cells, a single 150-bp band was observed. These PCR products of ten independent reactions were subcloned into pT7Blue T-Vector (Novagen, USA), and sequenced according to the ABI 377 DNA sequencing system protocol.

Southern and northern blot analyses. The genomic DNAs were prepared from the wild type cells by the method of Gorovsky (16). The poly(A)$^+$ RNA preparations of wild type and *cdaA1* cells were performed by the methods described above. Southern and Northern hybridization analyses were carried out under the conditions described previously (15).

Other procedures. 2D SDS-PAGE and SDS-PAGE were performed by the methods of Hirabayashi (17) and Laemmli (18), respectively. Immunoblot analysis was carried out according to the method of Towbin (19), using alkaline phosphatase-conjugated secondary antibodies.

RESULTS

Characterization of Anti-p85 Antisera

To clarify the primary structure and the function of p85, we attempted to purify it by monitoring immunoblotting with an anti-p85 antiserum. Since the amount of guinea pig anti-p85 antiserum (p85GP1) available was very low, we newly prepared rabbit anti-p85 antiserum (p85R1), and then investigated its properties. p85R1 specifically reacted to p85 in whole cell extracts, and also recognized the difference of molecular weight in SDS-PAGE between wild type and *cdaA1* p85 (Fig. 1A), just as p85GP1 did (9). Only p85 was isolated from the wild type cell extracts by immunoprecipitation assay using p85R1 (Fig. 1B, a) and immunoblotted with both antisera (Fig. 1B, b, c). These results indicate that p85GP1 reacted to the p85 immunoprecipitated with p85R1 (Fig. 1B, c). Both p85R1 and p85GP1 specifically recognize the p85 in *Tetrahymena* whole cell extracts.

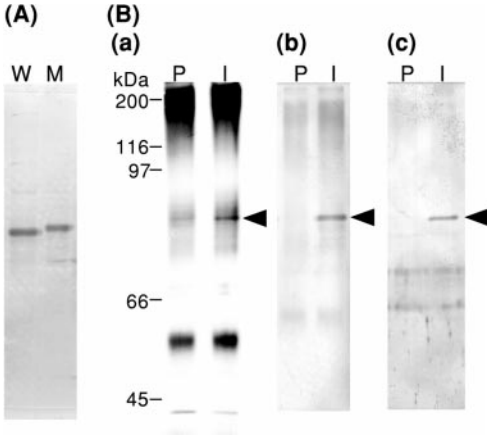

FIG. 1. Properties of anti-p85 antisera, p85GP1, and p85R1. (A) p85R1 recognizes the difference of molecular weight between wild type and *cdaA1* p85. The same numbers of wild type and *cdaA1* cells were lysed with guanidine HCl. The proteins were separated by 8% SDS-PAGE and analyzed by immunoblotting using p85R1. (W) and (M) lanes represent the wild type and the *cdaA1* cell extracts, respectively. (B) The specificity of p85R1 is identical to that of p85GP1. The wild type p85 was isolated from cell extracts by immunoprecipitation assay using a preimmune serum and p85R1, and then analyzed by (a) silver staining and immunoblotting with (b) p85R1 and (c) p85GP1. (P) and (I) lanes represent immunoprecipitates with the preimmune serum and p85R1, respectively. Arrowheads indicate p85. Other bands observed in both (P) and (I) lanes are derived from an immunoglobulin utilized for the immunoprecipitation assay (a, b) and caused by Alkaline phosphatase-conjugated goat anti-guinea pig IgG (CHEMICON, USA) (c).

Purification of p85

Since the specificity of p85R1 was confirmed, based on monitoring of immunoblotting using the p85R1, the fraction containing p85 was purified through ammonium sulfate fractionation, hydrophobic chromatography (Butyl TYOPEARL PAK column), and anion exchange chromatography (DEAE TYOPEARL PAK column) (Fig. 2A, a). The purified protein was partially degraded, and its molecular weight in SDS-PAGE was 83 kDa. The fraction after the DEAE TYOPEARL PAK column eluate was separated by 2D SDS-PAGE, and we then excised the p85 spot from the gel (Fig. 2A, b). The N-terminal amino acid sequence of the purified protein (Fig. 2B-1) and that of its fragment digested by V8 protease (Fig. 2B-2) were determined. Based on the latter sequence, two mixed primers were designed (Fig. 2B-2) and used to generate 62-bp nucleotides by RT-PCR. The predicted amino acid sequence of this PCR product was identical to the amino acid sequence shown in Fig. 2B-2. Using this PCR product as a probe, we screened the wild type *T. thermophila* cDNA library.

Cloning and Sequencing of Wild Type p85

As the result of screening, we isolated a cDNA clone that contained 2,595-nucleotides. Considering that *Tetrahymena* transcribes universal stop codons, TAA and TAG, as glutamine codons (14, 20), we determined one open reading frame of this cDNA which encoded an 803-amino acid polypeptide with a predicted relative molecular mass of 86,036 (Fig. 3A). The deduced amino acid sequence of wild type p85 contained interesting motifs. The p85 was mainly composed of two kinds of repeat sequences, which were designated as Repeats I, II, and III and Repeats A and B (Fig. 3A, B). Repeats I, II, and III consisted of 154, 154 and 149 amino acids, respectively. These repeat sequences showed more than 95% identity to one another. Repeats A and B consisted of 151 and 152 amino acids, respectively;

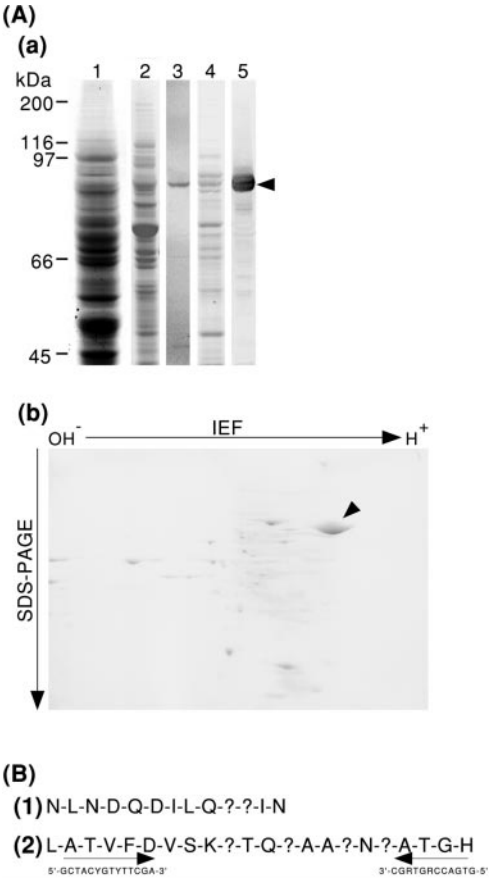

FIG. 2. Purification of wild type p85 and two amino acid sequences determined from p85. (A) The partially purified p85 fraction. (a) The wild type cell extract was fractionated, using ammonium sulfate fractionation, a butyl TYOPEARL PAK column, and a DEAE TYOPEARL PAK column. Lane 1, *Tetrahymena* cell extract. Lanes 2 and 3, 0.01–0.16 M $(KH_4)_2SO_4$-eluted fraction from the butyl TYOPEARL PAK column. Lanes 4 and 5, 0.23–0.3 M KCl-eluted fraction from the DEAE TYOPEARL PAK column. Lanes 1, 2, and 4 show Coomassie brilliant blue (CBB) staining. Lanes 3 and 5 show immunoblotting using p85R1. An arrowhead indicates p85. (b) The partially purified fraction after the DEAE TYOPEARL PAK column eluate was separated by 2D SDS-PAGE and then stained with CBB. An arrowhead indicates the p85 spot. The spot was excised from the gel and its amino acid sequence determined. (B) N-terminal amino acid sequences of purified p85 (1) and its fragment digested by V8 protease (2). Arrows show the sites of primers utilized for RT-PCR.

FIG. 3. Amino acid sequence of wild type p85 and comparison with other proteins. (A) A predicted amino acid sequence of wild type p85. The one letter amino acid code is used throughout. The 803-amino-acid protein contains two kinds of repeat sequences that are enclosed with solid and broken lines, respectively. A broken underline indicates the N-terminal amino acid sequence of purified p85, and underlines indicate that of its fragment digested by V8 protease. These data are available from GenBank/EMBL/DDBJ under Accession No. D84491. (B) A schematic drawing of the predicted primary structure of p85. The two kinds of repeat sequences are designated as Repeats I, II, and III and Repeats A and B. Regions homologous to parts of other proteins are underlined with numbers, 1: CaM II kinase in *S. cerevisiae;* 2: actin in *T. thermophila;* and 3: PCTAIRE-1 kinase in human. GenBank/EMBL/DDBJ accession numbers: CaM II kinase, X56961; actin, M13939; PCTAIRE-1, X66363. (C) Sequence alignments between the wild type p85 and parts of other proteins. 1, 2, and 3 are equivalent to those shown in (B). I, II, and III and A and B show the Repeats I, II, and III and the Repeats A and B, respectively. Asterisks indicate identity, and dots indicate similarity.

they showed more than 99% sequence identity to each other. However, there was no similarity between Repeats I-III and Repeats A and B.

A search of the most recent databases failed to detect any significant sequence homology over the full length of p85, but revealed sequence homologies to parts of several proteins (Fig. 3B, C). Repeats I, II and III contained sequences similar to a CaM-binding site of yeast CaM II kinase (21) and a part of *Tetrahymena* actin (22). In addition, Repeats A and B contained sequences similar to a part of human Ser/Thr protein kinase PCTAIRE-1, a cdc2 kinase homologue (23).

Southern and Northern Hybridization Analyses

Genomic Southern hybridization was performed to ascertain how many p85 gene copies exist in the macronucleus of *T. thermophila.* When the full-length cDNA of wild type p85 was used as a probe, four bands in *Hin*dIII-digested genomic DNA were detected (Fig. 4A). This result coincided with the patterns deduced from the restriction map of wild type p85 cDNA (Fig. 4C). These results show that *T. thermophila* possesses only a single copy of the p85 gene.

Northern hybridization using the full-length cDNA of wild type p85 as a probe detected a 2.6-kb transcript for p85 as a single band in wild type and *cdaA1* cells (Fig. 4B). Thus, p85 gene is actively transcribed as a single type transcript, in both types of cells.

Cloning and Sequencing of cdaA1 p85 cDNA

SDS-PAGE revealed that p85 from wild type and *cdaA1* cells differed in molecular weight (Fig. 1A). If the p85 gene of *cdaA1* cells is mutated, surely the mutation region can be detected, because *T. thermophila* possessed only a single copy of p85 gene and the gene was actively transcribed as a single type transcript (Fig. 4A–C). To examine the cause of the difference, we synthesized a cDNA library from *cdaA1* cells,

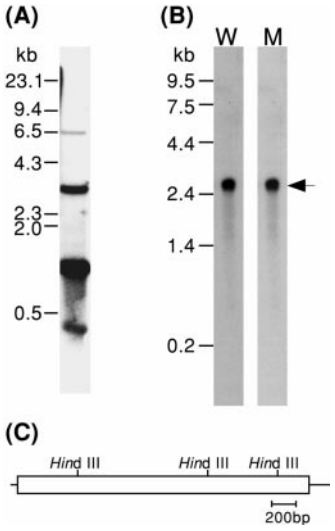

FIG. 4. Hybridization analyses using the wild type p85 cDNA as a probe. (A) Southern hybridization analysis of the p85 gene. Wild type genomic DNA (10 μg) was digested with *Hind*III, electrophoresed, and then blotted for the hybridization. The DNA size markers, determined from *Hind*III-digested lambda DNA, are shown at the left. (B) Northern hybridization analysis of the p85 transcript. Poly(A)$^+$ RNA (5 μg) was prepared from the wild type and the *cdaA1* cells, electrophoresed, and then blotted for the hybridization. (W) and (M) lanes represent wild type and *cdaA1* poly(A)$^+$ RNA, respectively. The approximately 2.6-kb bands indicated with an arrow represent the transcripts for wild type and *cdaA1* p85. The kilobase-markers are at the left, determined with RNA marker fragments (GIBCO BRL, USA). (C) Restriction maps of the wild type p85 cDNA. Protein coding regions are indicated by open boxes.

cloned the cDNA encoding the *cdaA1* p85, and sequenced it. The *cdaA1* p85 cDNA contained one open reading frame and its deduced amino acid sequence, *cdaA1* p85, was completely identical to that of wild type p85 (Fig. 5). On the other hand, there were some differences in the nucleotide sequence of the 3′ untranslated region (3′ UTR) between wild type and *cdaA1* p85 cDNA (Fig. 5).

5′ UTRs of wild type and *cdaA1* p85 cDNA isolated from the cDNA libraries were short. Therefore, it is possible that the translational initiation codon, AUG, newly appeared in the 5′ UTR of *cdaA1* p85 mRNA by point mutation, and that this induces the difference of molecular weight between wild type and *cdaA1* p85. To analyze the 5′ UTR of each cDNA, the 5′ UTR of p85 cDNAs for both cells were cloned by the 5′ RACE method and sequenced. Although a difference in nucleotide sequences between wild type and *cdaA1* cells existed (Fig. 5), the possibility that AUG newly appeared in the 5′ UTR of *cdaA1* p85 mRNA by point mutation was not demonstrated. These results show that there is no difference in the predicted amino acid sequences between wild type and *cdaA1* p85. Thus, we suppose that the difference in molecular weight between *cdaA1* and wild type p85 was caused by a disorder of posttranslational-modification mechanisms of p85 in *cdaA1* cells.

DISCUSSION

To clarify the primary structure and function of p85, which is concerned with the molecular mechanisms of *Tetrahymena* division plane determination and contractile ring formation, we partially purified p85 from *Tetrahymena* wild type cell extracts (Fig. 2A) and cloned wild type p85 cDNA (Fig. 3A). The deduced amino acid sequence was mainly composed of two types of repeat sequences, Repeats I, II, and III, and Repeats A and B (Fig. 3A, B). A search of the most recent databases found that the Repeats I, II, and III showed homology to the CaM-binding site of yeast CaM II kinase (Fig. 3B, C). In another recent study, we found that p85 directly interacted with *Tetrahymena* CaM in a Ca^{2+}-dependent manner and that both proteins colocalized in the division furrow during cytokinesis (see other submitted paper). In addition, the Ca^{2+}/CaM inhibitor N-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide HCl inhibited the direct interaction between p85 and Ca^{2+}/CaM and then also the localization of p85 to the presumptive division plane and formation of the contractile ring (see other submitted paper). Therefore, we think that the interaction between p85 and Ca^{2+}/CaM may regulate the division plane determination, the contractile ring formation and progression of the division furrow. The function of p85 speculated from its primary structure provides useful insights into the function of p85 in *Tetrahymena* cytokinesis. We further speculate the following two functions of p85 from its sequence motifs.

Repeats I, II, and III also showed homology to a part of *Tetrahymena* actin (Fig. 3B, C). The three homologous regions between p85 and actin corresponded to the surface region in the three-dimensional structure of the actin molecule (24). Although these homologous regions are not self-association sites of actin (24), p85 may bind directly to actin at these homologous regions. In addition, if these homologous regions are targeting sites of an actin-binding protein, it is also presumed that p85 may interact with actin indirectly through cross-linking to the actin-binding protein. In *Tetrahymena*, the contractile ring microfilaments composed of actin are formed along the localization of p85 to the presumptive division plane (11). From the analyses of primary structure and localization of p85, we speculate that p85 interacts directly or indirectly with actin in the presumptive division plane, and that these interactions serve as a polymerization center of actin in the formation of the contractile ring.

A computer search showed that Repeats A and B contained sequences similar to a cdc2 kinase homologue, PCTAIRE-1 kinase (Fig. 3B, C). The two homologous regions between p85 and PCTAIRE-1 kinase cor-

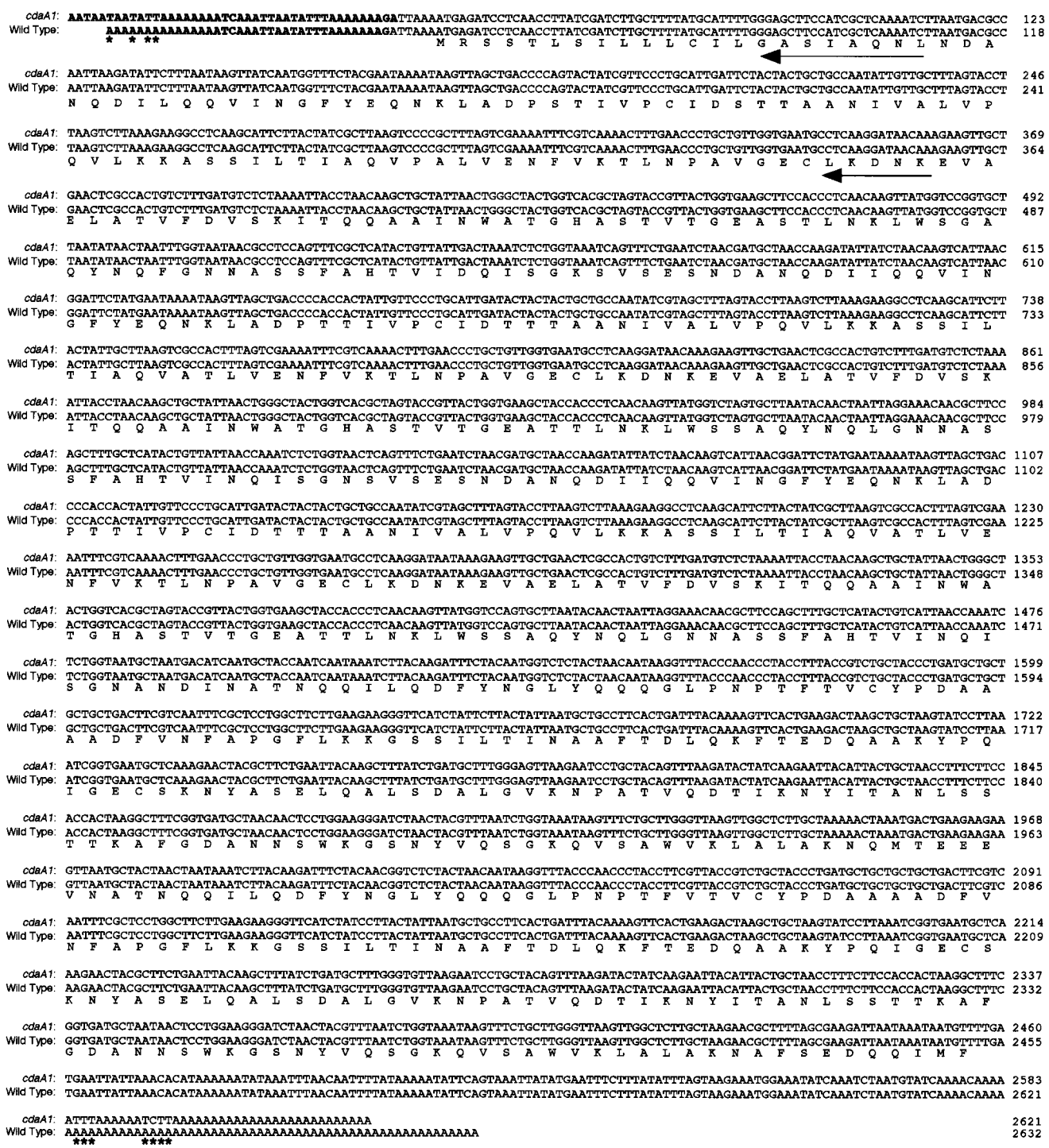

FIG. 5. Comparison of *cdaA1* and wild type p85 cDNA. The nucleotide sequences and deduced amino acid sequences of *cdaA1* and wild type p85 cDNA are shown in a single letter code. Arrows indicate the site of primers utilized for 5′ RACE methods. Boldface type shows the sequences found by 5′ RACE methods. Asterisks represent the nucleotide differences between *cdaA1* and wild type p85 cDNA.

responded to a part of the catalytic core region of this kinase (23, 25), suggesting that p85 may possess kinase activity. In unfertilized sea urchin eggs, Calyculin A, a protein phosphatase inhibitor, induced the formation of a contractile ring-like apparatus (26). This suggests that the phosphorylation process acts as a trigger of contractile ring formation. Therefore, we suppose that the p85 functions in contractile ring formation, as a kinase. Resolving how p85 and Ca^{2+}/CaM interact with actin and investigating whether or not p85 really

possesses kinase activity may facilitate understanding of the mechanisms of contractile ring formation.

cdaA1 p85 is larger than the wild type p85 (Fig. 1A), but has the same isoelectric point. To examine the cause of the difference in molecular weight, we cloned and sequenced the *cdaA1* p85 cDNA. Although there were differences in the nucleotide sequences of the 5′ and 3′ UTRs between the wild type and *cdaA1* p85 cDNAs, the deduced amino acid sequence of *cdaA1* p85 was identical to that of wild type p85 (Fig. 5). We expect that the differences in 5′ and 3′ UTRs do not affect the transcription and translation of the p85 gene, because the amounts of transcribed mRNA and translated protein of *cdaA1* p85 were equivalent to those of wild type p85 (Figs. 1A, 4B). These results suggest that the posttranslational-modification mechanisms of p85 are disordered in *cdaA1* cells and that this disorder may result in the difference of molecular weight between wild type and *cdaA1* p85. The disorder may inhibit the localization of *cdaA1* p85 to the presumptive division plane at the restrictive temperature, and because of this, *cdaA1* cells fail in the subsequent formation of a division furrow. Therefore, we speculate that the posttranslational modification of p85 is requisite for the division plane determination in *Tetrahymena*. It is well known that phosphorylation and glycosylation affect the mobility of proteins in SDS-PAGE. We treated the wild type p85 and the *cdaA1* p85 purified by immunoprecipitation with phosphatase and glycosidase. However, these treatments did not affect the molecular weight of wild type p85 and *cdaA1* p85 in SDS-PAGE (data not shown), suggesting some other modification is involved in the posttranslational modification of p85. Clarifying the posttranslational modification of p85 in detail and then elucidating the relation between this modification and the interaction of p85 and Ca^{2+}/CaM in the division plane determination is an interesting subject for future study.

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