Ultrastructural and Biochemical Properties of the 120-kDa Form of Chick Kinectin*

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Kinectin, an integral membrane protein (160 kDa), was identified as a kinesin-binding protein. Analysis of the predicted amino acid sequence of kinectin cDNA indicated an α-helical coiled-coil structure from amino acid 320 to 1310. A 120-kDa kinectin has been observed consistently, and N-terminal sequencing showed that 232 amino acids were missing from the N terminus of full-length kinectin. 120-kDa kinectin was distributed in the supernatant and a low density fraction of vesicles, whereas both forms were in the high density fraction of vesicles. In the electron microscope, the 120-kDa form appeared as a linear molecule of 133 nm in length. In hydrodynamic studies, the cytosolic 120-kDa kinectin was a dimer. Monoclonal antibody molecules (anti-kinectin KR160.9) bound asymmetrically to kinectin often with two antibodies/kinectin, indicative of a parallel coiled-coil. Metabolic labeling with [3H]myristic acid showed that both the 120- and 160-kDa kinectin are myristoylated in chick embryo fibroblasts. The myristoylation of 120-kDa kinectin may provide a mechanism for linking it to a low density fraction of vesicles. Immuno-precipitation with a 160-kDa kinectin-specific antibody brought down the 120-kDa kinectin. Thus, we suggest that kinectin is an extended parallel coiled-coil dimer, often a heterodimer.

Microtubules play an important role in intracellular organelle transport in various membrane trafficking events by supporting the movement of motor proteins bound to the vesicles (for reviews see Refs. 1 and 2). The microtubule-dependent motor proteins such as kinesin and cytoplasmic dynein have been implicated in the movement of melanophores (3), lysosomes (4), and axonal vesicles (5). For specific targeting of motor molecules to vesicles, there must be a specific attachment site on the vesicle surface. For instance, given a cytosol containing both motor proteins, purified secretory granules select kinesin, whereas purified phagosomes prefer cytoplasmic dynein in vitro (6, 7). Such results throw light upon the potential importance of organelle-specific proteins, which may act as regulatory receptors or activators for microtubule-dependent motors. A recent development in the study of the targeting of motor-protein interactions with membranes was the identification of a kinesin-binding protein, kinectin, on endoplasmic reticulum and evidence of kinectin’s functional involvement with in vitro vesicle motility (8, 9). However, the distribution of kinectin between different intracellular organelles is unclear. Further, the 120-kDa kinectin and other isoforms of kinectin may be differentially distributed between different membrane compartments.

Kinectin was originally purified from chick embryo brain (CEB) vesicles as an integral membrane protein of 160 kDa that associated with the motor protein, kinesin. A kinectin cDNA clone containing a 4.7-kilobase insert was isolated from a CEB library by immunoscreening with a panel of monoclonal antibodies to kinectin (10, 11). A human homologue of kinectin has been identified with 61–71% identity in the different domains (12). In chicken and human cells, kinectin appeared as two protein species of 160 and 120 kDa as defined by the relative molecular mass on SDS-PAGE. The 160-kDa kinectin corresponds to the full-length protein, but the actual mass of both forms has not been characterized previously.

The 160-kDa kinectin was identified in the high density fraction of vesicles in chick embryo brains. In addition, a series of mAbs against kinectin recognize the 160- as well as the 120-kDa protein on these vesicles. Previously, the structure of kinectin has been predicted on the basis of cDNA sequence data without any ultrastructural or biochemical evidence. In the present study, we show that the 160- and the 120-kDa forms distribute differently in low and high density vesicle fractions in chick embryo fibroblasts. Furthermore, we have analyzed the N-terminal sequence and myristoylation of both forms. The 120-kDa kinectin was further analyzed. The molecular mass was determined by hydrodynamic parameters, and the structure was demonstrated by electron microscopy.

EXPERIMENTAL PROCEDURES

Distribution of 160- and 120-kDa Kinectin on CEF Vesicles—Chick embryo fibroblast (CEF) vesicles were prepared as described previously (13). The cells were harvested by pelleting at 700 × g for 15 min at 4 °C. The pellet was washed with homogenization buffer, PMEE (55 mM PIPES, pH 7.4, 5 mM MgSO4, 5 mM EGTA, 0.5 mM EDTA, and 1 mM dithiothreitol), and a protease inhibitor mixture (1 μg/ml pepstatin A, 10 μg/ml p-tosyl-l-arginine methyl ester, 10 μg/ml 1-tosylamide-2-phenylethyl chloromethyl ketone, 1 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, and 1 μg/ml phenylmethylsulfonyl fluoride) and resuspended in an approximately equal volume of cold homogenization buffer. The homogenate was prepared by using a ball bearing homogenizer (14). The nuclei and unlysed cells were pelleted at 1400 × g, and the resulting supernatant (S1) was analyzed on a linear Nycodenz gradient.

Linear Nycodenz Gradient—A linear Nycodenz (Accurate Chemical and Scientific Co., NY) gradient (10–60% in homogenization buffer) was prepared by using a gradient maker. The CEF cells were collected by trypsinization, and a CEF supernatant (S1) was prepared as described above. The CEF supernatant (S1) was layered onto the Nycodenz gradient and centrifuged at 28,000 rpm for 15 h at 4 °C in an SW 40 rotor (Beckman Instruments). Fractions (8 drops in each) were

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1 The abbreviations used are: CEB, chick embryo brain; mAb, monoclonal antibody; PAGE, polyacrylamide gel electrophoresis; CEF, chick embryo fibroblast; kb, kilobase pair(s); PIPES, 1,4-piperazinediethanesulfonic acid.

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collected from top to bottom by using an automatic fraction collector, and the kinecin distribution was analyzed by Western immunoblot with mAb KR160.9.

**Immunofluorescence Purification of 160- and 120-kDa Kinectin**—Because the chick embryo brain high speed pellet contains both the 160- and 120-kDa forms of kinecin, we prepared both a high speed pellet and a low speed supernatant fraction as described earlier with slight modification (10). Chick embryo brains (frozen), typically 40 g, were homogenized in an equal volume of homogenization buffer. The homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C in a SA 600 (Sorvall Instruments, Wilmington, DE). The resulting supernatant was centrifuged at 45,000 rpm for 90 min in a Ti-50.2 rotor (Beckman Instruments). The supernatant (S2) was collected gently for immunofluorescence purification of the 120-kDa protein from cytosol. Furthermore, the pellet (P2) was resuspended in 10 ml of homogenization buffer. The pellet suspension was treated with 1% Triton X-100 and 0.5 M NaCl, subsequently diluted to 20 ml with homogenization buffer, and centrifuged at 45,000 rpm for 90 min in a Ti-50.2 rotor. The resulting Triton X-100 supernatant was subjected to immunoprecipitation using an antibody purified from ascites, mAb KR160.9, as described earlier (9). The protein purified by using protein A beads with mAbs contains mAb contamination because of elution at low pH. An alternative preparation, in which mAb KR160.9 was conjugated to Sepharose CL-4B (Amersham Pharmacia Biotech, instruction manual), gave a purified kinecin without contaminating mAb. For rotary shadowing and analysis of hydrodynamic properties, the 120-kDa protein was purified from CEB cytosol by using mAb KR160.9 Sepharose CL-4B. After elution, the 120-kDa protein was concentrated on a Centricon-30, and buffer was exchanged into 20 mM Tris-HCl buffer, pH 8.0. The 120-kDa protein (~20 μg/ml) was exchanged with buffer containing a final concentration of 20% glycerol and 0.2 M ammonium bicarbonate for rotary shadowing.

**Hydrodynamic Parameters and Molecular Mass of Cytosolic 120-kDa Kinecin**—The 120-kDa form of kinecin was purified from chick embryo brain cytosol by immunoprecipitation with mAb KR160.9. The hydrodynamic properties of the 120-kDa protein were analyzed according to the procedure of Seigel and Monte (16). The Stokes radius (Rs) and sedimentation coefficient (s) were estimated by gel filtration on a Superose 6 column (Amersham Pharmacia Biotech, 1 × 31 cm) and by using a 15–40% glycerol gradient at 38,000 rpm for 16 h in a Beckman SW 55 rotor, respectively, as described earlier (17).

The molecular mass was estimated by using Equation 1,

\[ M_r = s_N^2 (6 \pi \eta \rho R_s)^{-1} \times \left(1 - \frac{1}{2} \rho \eta \frac{R_o}{R_s}ight) \times 4,204 \times s' R_s \]  

(1)  

where \( M_r \) represents molecular mass, \( N_o \) is Avogadro’s number, \( \eta \) is viscosity of the solvent (0.01 poise), \( \rho \) is density of solvent (1.0 g/cm³), and \( R_o \) is partial specific volume of the protein (assumed to be 0.73 cm³/g). For this numerical formulation, \( s' \) is in Svedberg units and \( R_s \) is in nanometers.

**Electron Microscopy**—For rotary shadowing, the samples purified from CEB cytosol by mAb KR160.9 were exchanged into 0.2 M ammonium bicarbonate, 20% glycerol, sprayed onto freshly cleaned mica, dried under vacuum, and rotary shadowed with platinum (18). The micrographs were taken on a Philips 300 microscope at ×50,000 and printed at ×150,000 magnification.

**Co-immunoprecipitation of the 160- and 120-kDa Kinecin from Vesicles of CEB (P2)**—To analyze the association of 120-kDa kinecin with 160-kDa kinecin, the Triton X-100 supernatant of vesicles (CEB P2) was immunoprecipitated as described above except that the solubilized vesicles were diluted with an equal volume of homogenization buffer and centrifuged at 60,000 rpm in a TLA 100.3 rotor (Beckman Instruments) for 30 min at 4 °C. The resulting supernatant was used for immunoprecipitation with mAbs KR160.10 or KR160.9 or mouse IgG as a control. The immunoprecipitated material was eluted and analyzed on Western blot as described in the legend of Fig. 7.

**Northern Blot Analysis**—Total RNA and mRNA were purified from chick embryo fibroblasts by using RNeasy total RNA kit and oligotex mRNA kit (QIAGEN Inc.). The mRNA was separated on a 0.7% agarose-formaldehyde gel using a standard protocol (21). The mRNA was transferred to a nylon membrane and probed with 32P-labeled kinecin cDNA. After hybridization, the nylon membrane was washed two times for 30 min in 2 × SSPE (1 × SSPE is 150 mM sodium chloride, 10 mM sodium phosphate monobasic, 0.8 mM EDTA, pH adjusted to 7.4 with sodium hydroxide and autoclaved), 0.1% SDS at room temperature and once with 0.2 × SSPE, 0.1% SDS for 30 min at 45 °C. Bands were visualized by autoradiography.

**RESULTS**

**Distribution of 160- and 120-kDa Kinectin in CEF Vesicles**—The presence of 160- and 120-kDa kinecin could represent different vesicle subpopulations. Therefore, we analyzed the distribution of both forms of vesicle-associated kinecin by separating the low speed supernatant of CEF on a linear Nycodenz gradient (10–60%). The distribution of kinecin (Fig. 1) showed that the 120-kDa protein was separated into two different subpopulations of vesicles. Only the 120-kDa kinecin was found in the low density Nycodenz peak fractions, whereas high density fractions contained both the 120- and 160-kDa kinecin. To rule out the possibility that the low density 120-kDa kinecin was the result of soluble contamination, pelleted vesicles (CEF P2) were also separated on a stepwise Nycodenz gradient. A low density vesicle fraction from P2 was found with only 120-kDa kinecin (data not shown). Thus, the 120-kDa kinecin was associated with a low density vesicle fraction.

**Analysis of 160- and 120-kDa Kinecin by N-terminal Sequencing**—Most of the monoclonal antibodies against kinecin react with both the 160- and 120-kDa protein of kinecin on immunoblots of chick and human cells (10, 11). Antibodies against the C-terminal domain of kinecin recognize both 160- and 120-kDa kinecin, but antibodies against the N-terminal domain react only with the 160-kDa protein (12). This suggests that the 120-kDa kinecin may lack a large portion of the N-terminal sequence. Therefore, the N-terminal amino acid sequence of both forms of kinecin in chick embryo brain was determined. The sequence of the 160-kDa kinecin shows that it contains the N-terminal amino acid sequence predicted by the cDNA clone of kinecin, whereas the 120-kDa form starts 233 amino acids from the N-terminus of the cDNA clone (Fig. 2). The sequence and the antibody binding data are consistent with the hypothesis that the 120-kDa form is missing the N-terminal 232 amino acids of the 160-kDa kinecin.
the 120-kDa form of kinectin in CEB cytosol is so low (27 ng/mg of cytosol) that we cannot detect it by Western blot. Consequently, the hydrodynamic properties of the 120-kDa form of kinectin were determined with material purified and concentrated by immunopurification. Gel filtration chromatography on Superose 6 was used to determine the Stokes radius by extrapolating from the elution profiles of known proteins. The Stokes radius \( R_s \) of 120-kDa kinectin was estimated to be 11.9 nm, as shown in Fig. 3. The sedimentation coefficient was estimated by gel-erythrocyte gradient velocity sedimentation (Fig. 4). Purified 120-kDa kinectin co-sedimented with the peak of bovine serum albumin (4.6 S), and the \( s \) value was estimated to be 4.6. The \( R_s \) estimated from gel filtration and the \( s \) value from velocity sedimentation were used to estimate the mass of native 120-kDa kinectin using the Svedberg equation (see “Experimental Procedures”). A mass of 230,127 Da was determined by this equation, which is very close to the mass for a dimer of a 129,905-Da peptide. Thus, the native form of cytosolic 120-kDa kinectin appears to be a dimer of kinectin molecules.

**Hydrodynamic Properties of Kinectin**—The concentration of the 120-kDa form of kinectin in CEB cytosol is so low (27 ng/mg of cytosol) that we cannot detect it by Western blot. Consequently, the hydrodynamic properties of the 120-kDa form of kinectin were determined with material purified and concentrated by immunopurification. Gel filtration chromatography on Superose 6 was used to determine the Stokes radius by extrapolating from the elution profiles of known proteins. The Stokes radius \( R_s \) of 120-kDa kinectin was estimated to be 11.9 nm, as shown in Fig. 3. The sedimentation coefficient was estimated by gel-erythrocyte gradient velocity sedimentation (Fig. 4). Purified 120-kDa kinectin co-sedimented with the peak of bovine serum albumin (4.6 S), and the \( s \) value was estimated to be 4.6. The \( R_s \) estimated from gel filtration and the \( s \) value from velocity sedimentation were used to estimate the mass of native 120-kDa kinectin using the Svedberg equation (see “Experimental Procedures”). A mass of 230,127 Da was determined by this equation, which is very close to the mass for a dimer of a 129,905-Da peptide. Thus, the native form of cytosolic 120-kDa kinectin appears to be a dimer of kinectin molecules.

**Electron Microscopy of 120-kDa Kinectin**—Because the 160-kDa kinectin has a hydrophobic membrane attachment peptide, it cannot easily be purified from vesicles for structural studies. Therefore, the 120-kDa kinectin (purified from CEB cytosol) was chosen for analysis by electron microscopy. Initial studies used the 120-kDa kinectin purified by immunopurification with mAb KR160.9 on protein A-agarose beads. We found that antibodies released from the protein A by the low pH elution contaminated this material (Fig. 6). We therefore developed a new immunopurification procedure using mAb KR160.9 covalently coupled to Sepharose CL-4B followed by concentration and buffer exchange on a Centricon-30 (Amicon Inc., Beverly, MA). As seen in Fig. 5, the final preparation was quite pure, showing a single 120-kDa band on Coomassie Blue-stained SDS-PAGE.

This preparation was visualized by rotary shadowing and revealed a simple and uniform structure. The molecules were thin strands with a length of 133.0 ± 6 nm (Mean ± S.D. and no. of molecules \( n = 21 \)). Some molecules showed a hinge region with a length of 24.0 ± 6 nm (Mean ± S.D. and no. of molecules \( n = 17 \)). The diameter is too thin to measure reliably but is similar to that of myosin and other double-stranded coiled-coils we have visualized. Some molecules had a very small globular domain at one end.

The preparations contaminated with mAb KR160.9 provided an important additional insight. There were many free IgG molecules, visible as triangular shapes, in the electron microscope specimen. Most of the kinectin strands had mAbs at-
attached at a single site, which was 76.0 ± 8.4 nm from one end and 56.8 ± 6.2 nm (mean ± S.D. and no. of molecules \( n = 21 \)) from the other end of the molecule as shown in Fig. 6. The complex frequently appeared as a “bow tie,” which we interpret as two triangular IgG molecules attached at the same region (one to each strand of the coiled-coil) on opposite sides of the molecule. Because the mAbs attached at the same point on the strand, we suggest that the coiled-coil is parallel rather than anti-parallel.

**Co-immunoprecipitation of the 160- and 120-kDa Kinectin in Chick Embryo Brains**—Although it was not possible to obtain similar micrographs of the 160-kDa kinectin, we predict that it is also a dimeric protein because of co-precipitation with the 120-kDa form. The mAb KR160.10 selectively recognizes the 160-kDa kinectin, whereas mAb KR160.9 recognizes both the 160- and 120-kDa kinectin. As shown in Fig. 7, the immunoprecipitation of Triton X-100-solubilized CEB (P2) by mAb KR160.10 brought down both the 120- and 160-kDa forms of kinectin. This suggests that 120-kDa kinectin might be associated with 160-kDa kinectin.

**Post-translational Modification of Kinectin with Fatty Acids in CEF Cells**—The 120-kDa kinectin lacks the N-terminal, hydrophobic, membrane-binding domain but exists in a membrane-bound form in the low density fraction of vesicles. One possible membrane attachment mechanism of the 120-kDa kinectin might be the post-translational modification of kinectin with fatty acids. When CEFs were labeled with \(^{3}H\)myristic acid for 2 and 9 h, both 160- and 120-kDa kinectin were myristoylated after 9 h (Fig. 8). Parallel incubations with \(^{3}H\)palmitic acid did not show any labeling of either form. Furthermore, the myristoylation was not sensitive to hydroxylamine treatment, which indicated that it was amide-linked myristoylation of kinectin. Although the exact myristoylation site of kinectin is not known, myristoylation of 120-kDa kinectin suggests that post-translational modification may serve for membrane attachment even without the membrane-binding.
domain.

mRNAs of Kinectin in CEF—A Northern blot of CEF mRNA probed with kinectin cDNA identified two messages (Fig. 9). One message, ~5.3 kb, is consistent with the mRNA observed in chick embryo brain for 160-kDa kinectin (11). The second message appears to be ~4.4 kb in size but is a minor band in CEF. The presence of the second message suggests that there are other possible isoforms of kinectin in CEFs.

DISCUSSION

Because kinectin may function in several different vesicular compartments to attach motors to vesicles, we have characterized the two major species that react with most of the anti-kinectin antibodies in chick embryo fibroblast and brain extracts. The 120- and 160-kDa proteins are both clearly kinectin. From the molecular characterization of the 120-kDa soluble protein, kinectin appears to be an extended a-helical coiled-coil dimer. Myristoylation may serve to more tightly link the protein to the vesicle membrane. From the separation of the two major forms on vesicles of different densities, there may be different targets for the proteins in intracellular vesicle transport events.

Initial characterization of the anti-kinectin antibodies identified one monoclonal antibody that only reacted with the 160-kDa kinectin (10, 11). The mAb BR160.10 reacts with the bacteria-expressed N-terminal portion of kinectin (11). Antibody data and the N-terminal sequence of the 120-kDa kinectin indicate that it is formed by the deletion of 232 amino acids from the N terminus of full-length kinectin. The fact that 120-kDa but not 160-kDa kinectin is found in the cytosolic fraction further supports the hypothesis that the N-terminal domain functions in membrane binding.

Because the 120-kDa form is a soluble kinectin, we could use it to characterize the basic structure of most of the kinectin molecule. The soluble 120-kDa kinectin is very dilute (~27 ng/mg CEB cytosolic protein), and it is not detected in cytosol by Western blot analysis (10). By comparison, there is at least 50 times more found as membrane bound in CEB (P2) vesicles, as measured by densitometric quantitation (data not shown). We were able to concentrate material by immunoaffinity purification (~7.0 μg from cytosol after processing 50 g of CEB).

Under these conditions, we did not find any of the 160-kDa form of kinectin in CEB cytosol (Fig. 5). The hydrodynamic properties of 120-kDa kinectin indicated a molecular mass of ~230 kDa, which is close to the mass of 260 kDa predicted for a dimer of kinectin amino acid 233–1364 peptide. The molecule is ~133.0 ± 12 nm in length, which is considerably longer than kinesin (~80 nm in length) or cytoplasmic dynein. Sequence analysis predicts a coiled-coil extending from amino acid 320 to 1310 in the 160-kDa kinectin. This would mean that the 120-kDa kinectin should have an ~110-amino acid N-terminal domain, 890 amino acids of coiled-coil, and a small C-terminal domain. The electron microscopy shows a structure quite consistent with this, a long thin strand with a globular domain at one end, presumably the N terminus. The average length of 133 nm is predicted for an 890-amino acid a-helix. Some molecules show a pronounced bend about 24.4 nm from one end, which is consistent with the break in the predicted coiled-coil structure about 200 amino acids from the C terminus.

The different vesicular compartments within the cell typically have differentiated components such as specific small G proteins, and it is to be expected that the different kinectin isoforms would be localized on different compartments. Here, we find that the two-kinectin forms of 160 and 120 kDa are distributed in two subpopulations of vesicles of different density (Fig. 1). A recent analysis of the distribution of kinectin in chicken pancreatic tissue suggested that the microsomal fraction contains both 160- and 120-kDa kinectin, whereas the zymogen granules contain exclusively 120-kDa kinectin. The predominance of the 120-kDa kinectin in the light membrane compartments is consistent with localization to that compartment by a post-translational modification of the soluble form of kinectin, whereas the 160-kDa form would be restricted to movement through membrane compartments. The fatty acid derivatization of both forms of kinectin provides a simple means for affixing a soluble species to the vesicle membrane surface. It has been suggested that kinectin is myristoylated or palmitoylated on the basis of sequence analysis (11). Our metabolic labeling of CEF cells with myristic acid (Fig. 8) suggests that the 160- and 120-kDa forms of myristic acid are myristoylated, and myristic acid seems to be amide-linked because there is no effect of hydroxylamine (pH 7.0) treatment on the labeled proteins. It is known that proteins modified by amide-linked myristic acid are diversely distributed to plasma membrane, endoplasmic reticulum, nucleus, and cytoplasm (20). Our studies suggest that 120-kDa kinectin is distributed to the membrane as well as the cytoplasm, and it is possible that myristoylation of 120-kDa kinectin could provide a means to link it to membranes.

The origin of the 120-kDa form is not clear at this point. Although the Northern blot of CEF mRNA shows another isoform of kinectin (Fig. 9), the other message was too low in quantity with respect to the full-length 5.3-kb message of k-
nectin for the 120-kDa form to be detected by reverse transcriptase polymerase chain reaction techniques. In vitro translation studies have shown that a 120-kDa product is often produced, presumably at an alternate start site for translation. Alternatively, proteolysis perhaps induced by apoptotic signals may have caused the cleavage of 160-kDa kinectin into a 120-kDa form. The sequence shows several predicted Asp-protease sites that could produce an approximately 120-kDa protein. However, the N-terminal sequence from amino acid 229 of 160-kDa kinectin is IPLVDNSDAG (the 120-kDa kinectin begins at DNSDAG) and is not consistent with the Asp-protease site. The presence of two closely spaced 120-kDa bands raises the possibility that a C-terminal proteolysis had occurred. However, there is evidence of alternatively spliced forms of kinectin from two human kinectin sequences in the data base, and the 160-kDa kinectin appears as multiple bands as well. Further, the addition of protease inhibitors does not change the pattern, whereas the pattern does change between different tissues. There is reason to believe that the multiple forms of kinectin are present within cells and perhaps serve different functions.

The immunoprecipitation of 120-kDa kinectin from chick embryo brain vesicles with mAb KR160.10, which does not recognize the 120-kDa kinectin, shows that the two forms may exist in a heteromeric complex with 160-kDa kinectin as shown in Fig. 7. The simplest hypothesis is that the single N terminus of the 160-kDa subunit would be sufficient to bind both 160- and 120-kDa kinectin to the membrane. However, 120-kDa kinectin homodimers would lack this strong N-terminal membrane attachment but could attach to low density membranes through the myristate or could be soluble in the cytosol.

The characterization of kinectin structure and distribution is consistent with the postulated roles for kinectin in microtubule-dependent vesicle trafficking. Recent findings of small G protein interaction sites in kinectin near the sites where we find motor binding suggest that multicomplexes be formed on the vesicle surfaces that include kinectin (22). α-Helical coiled-coil domains interact in many motor-protein complexes involved in force production, and it is possible here that an extended interaction occurs between the motors and kinectin. The extended length of the coiled-coil may be designed to provide a flexible tether between the motor molecule and the membrane vesicles.

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