Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 8567–8571, September 1992
Cell Biology

Primary structure of tektin A1: Comparison with intermediate-filament proteins and a model for its association with tubulin

(basal body/centriole/cilia/flagella/microtubule)

J. M. NORRANDER*, L. A. AMOS†, AND R. W. LINCK*

*Department of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455; and †Laboratory of Molecular Biology, Medical Research Center, Hills Road, Cambridge, CB2 2QH, United Kingdom

Communicated by M. F. Perutz, May 19, 1992 (received for review January 22, 1992)

ABSTRACT **Tektins are proteins that form filamentous polymers in the walls of ciliary and flagellar microtubules and that have biochemical and immunological properties similar to those of intermediate-filament proteins. We report here the sequence of a cDNA for tektin A1, one of the main tektins from *Strongylocentrotus purpuratus* sea urchin embryos. By hybridization analysis, tektin A mRNA appears maximally at ciliogenesis. The predicted structure of tektin A1 (M_r 52,955) is a series of α-helical rod segments separated by nonhelical linkers. The two halves of the rod appear homologous and are probably related by gene duplication. Comparison of tektin A1 with intermediate-filament proteins, including nuclear lamins, reveals a low amino acid homology but similar molecular motif, i.e., pattern of helical and nonhelical domains. This study indicates that tektins are unique proteins but may be evolutionarily related to intermediate-filament proteins, and suggests a structural basis for the interaction of tektins and tubulin in microtubules.**

Tektins A (≈55 kDa), B (≈51 kDa), and C (≈47 kDa) were originally identified as proteins comprising 2- to 3-nm-diameter filaments that remained insoluble after extraction of sea urchin sperm flagellar microtubules (MTs) with chaotropic solvents (1–3). Immunofluorescence microscopy demonstrated the presence of tektins in doublet MTs, and possibly in central pair MTs (4), and tektin-like components in centrioles, centrosomes, and mitotic spindles (4–6). Immuno electron microscopy suggested that tektins formed extended polymers in the walls of ciliary and flagellar MTs (2). Tektins probably interact directly with tubulin in the MT wall, since *N*-lauroylsarcosine detergent extraction of cilia and flagella yielded stable ribbons of three protofilaments composed of tubulin and tektins in a molar ratio of ≈2:1 (1–3, 7). We have determined the primary structure of tektin A, in order to better understand its structure and function in MTs.‡

Tektins appear to be similar to intermediate-filament proteins (IFPs) in terms of their relative insolubility, molecular weights, and fibrous, α-helical structure (1). Peptide mapping, amino acid analysis, and immunological studies showed that the three main tektins were related to each other but not to tubulin (2, 3) and substantiated the similarity between tektins and IFPs (3, 8, 9). We have begun to examine this relationship more closely by characterizing a cDNA for tektin A from sea urchin embryos, obtained by screening a λgt11 library from *Strongylocentrotus purpuratus* blastulae with antibodies against flagellar tektin A. Immunofluorescence microscopy (10) and immunoblotting analysis (11) have shown that blastula cilia contain tektins that appear identical to sperm flagellar tektins. Furthermore, a ciliary MT protein of ≈55 kDa is synthesized *de novo* at the onset of ciliogenesis in blastulae (12), and this has been identified as tektin A (13).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

S. purpuratus gametes were collected, handled, and cultured at 16°C, as described (14). At desired times, aliquots of eggs/embryos were isolated and frozen in liquid N_2. Total RNA and poly(A)$^+$ mRNA were isolated (15). A λgt11 cDNA expression library, constructed from blastulae (gift of T. L. Thomas, Texas A & M, College Station, TX), was screened with polyclonal antibodies (16) against *S. purpuratus* sperm flagellar tektin A (11). The largest clone isolated, tekA10-2, contains a 2055-base-pair insert. Rescreening of the library failed to uncover any larger tektin A clones. A second library was constructed from blastula mRNA in λZapII (Stratagene) and screened with radiolabeled tekA10-2 (15). Several clones were isolated, including tekA5-8. Inserts were transferred to the plasmids pUC118/119 (17), sequential deletion clones were constructed (18), and dideoxy sequencing was performed (19). Sperm flagellar tektins were purified by *N*-lauroylsarcosine/urea extraction and reversed-phase HPLC (3). Tektin A, whose purity was checked by SDS/PAGE–immunoblotting, was treated with *N*-chlorosuccinimide (NCS) (Aldrich), which cleaves primarily on the carboxyl side of tryptophan (20) but may also act on histidine and tyrosine. Protein was dissolved in 50–75% acetic acid and treated with NCS for 1 hr at 37°C (8 μmol of NCS per ml of tektin per A_{280} unit). The reaction was terminated with excess *N*-acetylmethionine. After evaporation to dryness, samples were dissolved in SDS sample buffer. Peptides were separated by SDS/PAGE and transferred to Immobilon-P (Millipore) (21), which was stained with Serva Blue R and dried. Excised bands were sequenced by automated Edman degradation on an Applied Biosystems 470 peptide sequencer by E. D. Eccleston, Microchemical Facility, University of Minnesota. Standard data bases were searched with the program FASTA (22). For detailed sequence comparison, the DIAGON program (23) was used; homology scores (using the Dayhoff score matrix, based on observed substitution frequencies for residues in related proteins) were calculated to compare pairs of residues, plus flanking sequences spanning 49 total residues. The program calculates a "double matching probability", i.e., that of obtaining a particular score, given two infinitely long sequences of the same composition as those being compared. Scores with a probability of occurring by chance alone of $<10^{-4}$ were plotted. Secondary structure was predicted (24) with the program ANALYSEP (25).

RESULTS

Two cDNA clones were fully sequenced. By several criteria (below), clone tekA5-8 includes the complete coding se-

Abbreviations: IFP, intermediate-filament protein; MT, microtubule; NCS, *N*-chlorosuccinimide.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M97188).

quence for tektin A, and tekA10-2 includes ≈70% of the coding sequence. Within their coding regions they show >97% nucleotide and >99% amino acid identity; however, differences in the noncoding regions suggest that tektin A belongs to a multigene family. The results for tekA5-8, which we have termed tektin A1, are shown in Fig. 1. The predicted molecular weight of 52,955, acidic pI, amino acid composition, and α-helical content all compare favorably with previous experimental measurements for the tektin A protein from sea urchins (1, 3, 11).

Direct evidence that tekA5-8 encodes tektin A was obtained by partial amino acid sequencing of the flagellar form of tektin A. Since the amino termini of SDS/PAGE-purified tektins are blocked, fragments of tektin A were obtained by cleavage with NCS. By SDS/PAGE, discrete peptide bands corresponded in molecular weight to predicted fragments that could be generated by complete and incomplete cleavage of tektin A at tryptophans (data not shown), corroborating the positions of the four tryptophans. Moreover, 12.5- and 26-kDa fragments yielded the following N-terminal sequences respectively (−, undetermined residues; alternative residues in parentheses):

AEFS(V)N(H)DNVVRAE-E-L(D)ADQQD(L)--LID-I(A)LTD,
A(ED)EFS(V)-DNVVRAE---LA-Q(V)Q(D)

The two sequences, probably identical because the 26-kDa peptide is partially cleaved at His-374 or -377, confirm 20–30 residues, starting from Ala-273. A secondary sequence obtained from the 12.5-kDa band (a unique, comigrating fragment), KF-LN(H)REIEDMI(D), matches ≈12 residues of a predicted fragment generated by NCS cleavage at Trp-128 and Trp-230. Clones tekA5-8 and tekA10-2 hybridize with a 2750-base embryonic mRNA, which reaches peak levels during ciliogenesis (Fig. 2), when tektin A is maximally synthesized (12, 13). These observations indicate that both clones encode tektin A, rather than a cytoplasmic IFP or a nuclear lamin. We also noted a low level of mRNA hybridizing to tektin clones at earlier points after fertilization (lanes 3–5). It is possible that this low-abundance mRNA is for components in centrioles, centrosomes, or mitotic spindles, given the evidence for tektins in these organelles (4–6).

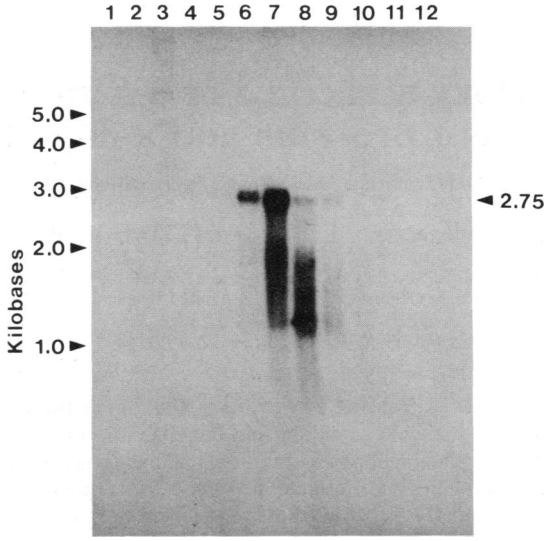

FIG. 2. Appearance of tektin mRNA during *S. purpuratus* embryogenesis. Eggs were fertilized and cultured. At selected times, poly(A)$^+$ mRNA was isolated, resolved, and probed with ^{32}P-labeled cDNA clone tekA10-2. Lanes: 1, unfertilized eggs; 2, 4-cell stage; 3, 32-cell stage; 4, 7.5 hr; 5, 9 hr; 6, 10.5 hr, beginning of ciliogenesis; 7, 12 hr, 50% rotating blastulae; 8, 13.75 hr, hatched and swimming; 9, 17.5 hr; 10, 25.5 hr, late blastula; 11, 44.5 hr, early gastrula; 12, 52.5 hr, gastrula. A major band of 2750 bases is labeled maximally in lane 7; weaker bands are present earlier. Tektin A mRNA may have been degraded in samples obtained after ciliogenesis.

Taken together, the predicted features of clone tekA5-8, its mRNA expression during ciliogenesis, and its coincidence with partial amino acid sequence in the native protein establish that the clone codes for tektin A.

The deduced amino acid sequence of tektin A was analyzed for secondary structure and internal repeats. Seventy percent of the central segment (residues 87–432, referred to here as the rod) is strongly predicted to be α-helical (Fig. 1) (24, 25), and much of this is likely to form coiled coil (26, 27). This prediction is consistent with the ≈70% α-helix estimated from the circular dichroism of tektin filaments (1) and with

```
ATG GAT GCT GGT GCA ACT CTC CTT TCC AGG AGC TAT GCT CCA ACG ATC CCT GTC TAT CCA ACA CAG ACC ACA GTA GGA ACC AAG ACA GAC
met asp ala gly ala thr leu leu ser arg ser tyr ala pro thr ile pro val tyr pro thr gln thr thr val gly thr lys thr asp  30

CAG GCC CTG TCT CAA GAT CTT GCC AAG ATG AGC GGC CTA GGT GAA ACC GGA GTC TAC GGT GTT CCC ACT GGG GCA CCG GCC GCA CAG GGT
gln ala leu ser gln asp leu ala lys met ser gly leu gly glu thr gly val tyr gly val pro thr gly ala pro ala ala gln gly  60

TTC CGC TCC GGC AAG CAC ACC ACT CAG GAA TGG CAC GAA TCC AAC TAC AAC AAA TAT TTC CAG AGT TTC ACA GAC CGC GAT AAC GCT GAG
phe arg ser gly lys his thr thr gln glu trp his glu ser asn tyr asn lys tyr phe gln ser phe thr asp arg asp asn ala glu  90

AGG CTA TGC CAC GAG TCC AAG CAG CTC TCA AAC GAG ACC CAC GCT CTG ACC ATG CGC ACA CAG GCC GAC GTC ACG AAG AAG CTC GGG GAC
arg leu cys his glu ser lys gln leu ser asn glu thr his ala leu thr met arg thr gln ala asp val thr lys lys leu gly asp 120

AGG ATG AAT GAC ATC AAC TTC TGG AAG TTT GAG CTC AAC CGT GAG ATT GAG GAG ATG ATC GAG GAG ACA GAC CTG CTA TGT GCA CAG AAG
arg met asn asp ile asn phe trp lys phe glu leu asn arg glu ile glu glu met ile glu glu thr asp leu leu cys ala gln lys 150

AAG CGG TTA GAG AAT GCG CTG GAT GCA ACA GAA GTG CCA CTG AAG ATC GCT AGG GAT AAT CTG ACC TGC AGG TCA AGG AGG CAA GAT ATT
lys arg leu glu asn ala leu asp ala thr glu val pro leu lys ile ala arg asp asn leu thr cys arg ser arg arg gln asp ile 180

GAT CTT GTT GGA GAC AGG GTA GAG ATG GCT CTA AAT AAG GAG GTA GAT ATC ATC ACA AAA GTT CAA GAT CTA CTC AAG AGA ACA TTA GAA
asp leu val gly asp arg val glu met ala leu asn lys glu val asp ile ile thr lys val gln asp leu leu lys arg thr leu glu 210

CAA TCT GAT AGA CAA ATC AAG CTC AAC CGT GGT TCA AAG CAC AAA TTG ACC ATG GAC TGG TCG GAT AAA CTC TCC GCC TTC AAG ATT GAT
gln ser asp arg gln ile lys leu asn arg gly ser lys his lys leu thr met asp trp ser asp lys leu ser ala phe lys ile asp 240

GAG AAA TGT ACT GGA CTT AAC AAC AAT TCA ACT GAG ATC CAG TAT AAG GAA GGA TCG GCC AAA TTT GAG GCA GTT CAA ACT AAT CCA CAA
glu lys cys thr gly leu asn asn asn ser thr glu ile gln tyr lys glu gly ser ala lys phe glu ala val gln thr asn pro gln 270

TCC TGG GCA GAG TTT TCG CAT GAC AAT GTC GTC CGT GCT GAG CAT GAG CGT CTT GCC TCC CAG CAG CTC CGC AAC CTG ATA GAC CAG ATC
ser trp ala glu phe ser his asp asn val val arg ala glu his glu arg leu ala ser gln gln leu arg asn leu ile asp gln ile 300

CTC ACA GAC ACC TCC AAC GAC ATG AGG GAA CAG TGC AAC ACC GTC AAC ACA GAG TTT GCT CGC CGC ATC GAA GAG ATG AAT GAC GCC AAG
leu thr asp thr ser asn asp met arg glu gln cys asn thr val asn thr glu phe ala arg arg ile glu glu met asn asp ala lys 330

ACG AAG ATG GAA AAC CAC TTG CTG AAG ACC GTT GAA GAT ATC GCT GGC ATG GAG AAG AAC ATC AAG GAT CTG ACC CAG GCC GTC AAG GAC
thr lys met glu asn his leu leu lys thr val glu asp ile ala gly met glu lys asn ile lys asp leu thr gln ala val lys asp 360

AAA GAG GCC CCA ATG AAG GTG GCC CAG ACC AGG TTG GAC CAC AGA ACA CAC AGG CCC AAT GTG GAA CTC TGC AGG GAC CCA GCT CAG TAC
lys glu ala pro met lys val ala gln thr arg leu asp his arg thr his arg pro asn val glu leu cys arg asp pro ala gln tyr 390

AGG ATG GTC CAA GAG GTC GGT GAG ATC CAG GAC TCC ATC GAC AAG CTC CAG CAG AAG CTG GCC GAG TCC AAG GCC TCG CTC AAG GAC CTG
arg met val gln glu val gly glu ile gln asp ser ile asp lys leu gln gln lys leu ala glu ser lys ala ser leu lys asp leu 420

ATG GAC ACC CGC ATG GCT CTG GAG AAG GAG ATT GCC CTT AAG AAG AAC AGC ATC TTC GTT GAC CGC GAC AAG TGC CTC AAG TTC CGC ACC
met asp thr arg met ala leu glu lys glu ile ala leu lys lys asn ser ile phe val asp arg asp lys cys leu lys phe arg thr 450

CGT TAC CCA TCC ACC TCC AAG CTG GTC GGC TAT CAG TAA
arg tyr pro ser thr ser lys leu val gly tyr gln
```

FIG. 1. Nucleotide and deduced amino acid sequence of tektin A1 from cDNA tekA5-8. Regions predicted to be α-helix (24, 25) are underlined; of these, residues 127–167, 190–216, 229–239, 319–373, and 388–432 show clear heptapeptide repeats (where first and fourth residues within a heptad are nonpolar), indicative of coiled-coil structure. The 5′ and 3′ noncoding sequences (not shown) can be obtained from GenBank.

their strong α-type x-ray patterns (28). A search of the sequence for internal repeats (23) indicates a significant homology between the two halves of the rod (Fig. 3).

The tektin A1 sequence was compared with other proteins. No high degree of homology was found to sequences in data banks (EMBL and GenBank as of January 1992); at most, tektin A shares 13–15% sequence identity with IFPs, myosins, and tropomyosins (i.e., coiled-coil proteins), but for reasons given in the *Discussion*, a more detailed comparison of tektin and IFP rod sequences was pursued. For convenience, we use IFP terminology (29, 30) to refer to parts of tektin A. The only consistent homology revealed in automated plots of tektin rod sequence versus IFP rods (equivalent to Fig. 3 but not shown) occurs near their carboxyl termini. By using this feature to fix its relative position, segment 2 of tektin can be consistently aligned manually with individual or consensus IFP sequences, so as to maximize amino acid identities and heptad repeats. The best match of segment 1 was much less obvious, but we found a single alignment (Figs. 4*a* and 5) that produces a good match between helix and link structures and fits the two halves of each IFP rod sequence with each other as well as with each half of tektin A.

DISCUSSION

There are several points of comparison between tektin A1 and IFPs. First, there are clear differences. (*i*) Tektin lacks the IFP consensus sequence LNDRL(or F)AXYI at the start of rod domain 1A. (*ii*) Tektin has only part of the IFP consensus sequence LD(or E)XEIAXYRKLLEGEEXR(or K) at the end of rod 2B (29–32), lacking the LLEGEEXR motif. (*iii*) Overall, there is a low degree of sequence homology. However, a low sequence identity does not preclude structural and functional homologies; e.g., there is <30% sequence identity between the α-helical segments of different IFPs or between myosin isotypes (29, 33). Significantly, tektins and IFPs share several interesting features not common to other coiled-coil proteins. (*i*) The degree of homology found between the sequence LMDTRMALEKEIALKK, near the carboxyl terminus of the rod domain of tektin A1, and the carboxyl-terminal sequences of most individual IFP rods (see Fig. 5) should occur by chance with a probability of $\approx 10^{-5}$. (*ii*) Tektin A1 has a structural pattern similar to that of IFPs, based on a long central rod, which is largely α-helical and divided into two roughly equivalent segments; major portions of this rod contain heptapeptide repeats necessary to form coiled coils. The lengths and positions of predicted helices and nonhelical linkers are similar to those in IFPs; furthermore, the locations of two additional linker regions in tektin correspond to structural anomalies within the IFP rod (Figs. 4 and 5). (*iii*) Both tektins and IFPs consist of polypeptides of variable mass (46–68 kDa) and assemble into filaments of marked insolubility.

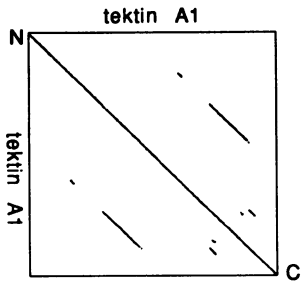

FIG. 3. DIAGON plot (23) comparing the tektin A1 amino acid sequence with itself. Each dot indicates a score, with a probability of $<10^{-4}$ of occurring by chance alone, for homology between different stretches of 49 residues. Off-diagonal rows of dots reveal a long-range homology between the two halves of the rod.

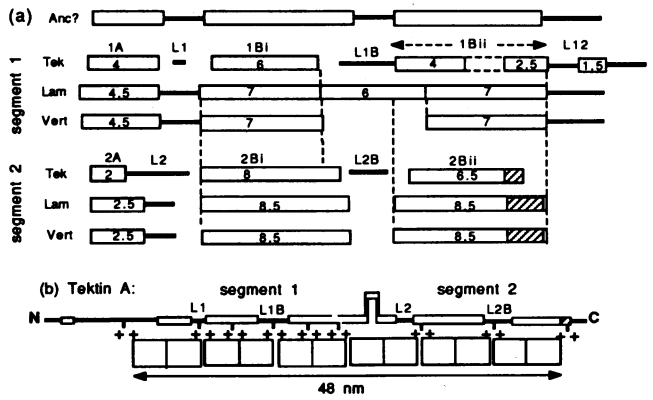

FIG. 4. (*a*) Summary of relationships among separate halves of tektin A1 (Tek) and IFP rods, with segment 1 (tektin residues 83–244) aligned over segment 2 (residues 277–432). Lam, lamins plus invertebrate IFPs; Vert, vertebrate IFPs. Boxes represent sequence strongly predicted as helical and show the number of heptad repeats included; solid lines represent nonhelical linkers. Lengths and positions of helices and linkers are similar for all three protein types. Hatched regions show where tektin matches half the IFP consensus sequence in segment 2B. Additional linkers in tektin A lie at sites of IFP structural anomalies: linker L1B occurs where lamins and invertebrate IFPs contain 42 additional amino acid residues compared with vertebrate IFPs; linker L2B occurs where IFPs contain a "stutter" in heptad phasing (29, 30). Anc?, predicted structure of an ancestral protein that may have duplicated to produce tektins and IFP. (*b*) Model for the structure of tektin A1 and its association with tubulin dimers (double boxes) of a MT protofilament. ++, Two or three consecutive positively charged residues in the tektin sequence. By analogy with tau and MAP2, two MT-associated proteins, such features may represent tubulin-binding sites. The spacing of basic groups corresponds roughly to that of tubulin dimers, with some intermediate sites at monomer spacings.

(*iv*) Finally, SDS/PAGE–immunoblotting and immunofluorescence microscopy show similarities between tektins and IFPs (5, 8, 9). (*a*) Affinity-purified polyclonal antibodies against four different tektins (from two sea urchin species) crossreact with IFP and nuclear envelopes (5, 8), where the crossreactions of anti-tektins with IFPs require only a 5-fold increase in the antibody concentration over that needed for comparable staining of tektins. (*b*) One monoclonal antibody against tektin C crossreacts with desmin and vimentin, and another crossreacts with nuclear lamins A and C (8). (*c*) A monoclonal anti-tektin B stains intermediate-filament patterns in cultured mammalian cells (W. Steffen and R.W.L., unpublished data). While crossreaction of a monoclonal antibody can occur between unrelated proteins, the specific crossreactions of four different polyclonal and three monoclonal anti-tektins against four different IFPs are probably significant.

Thus, although tektin A is too distinct to be classified in the IFP superfamily (29), tektins and IFPs could share a common ancestry (Fig. 4*a*). Overall structural similarity previously led to speculation that IFP segments 1 and 2 arose by gene duplication, although there was insufficient sequence identity to support this hypothesis (34). However, segments 1 and 2 of tektin A can be coaligned fairly unambiguously to give 35% homology (22% identity and 13% conservative substitutions) plus an overall alignment between α-helical and linker domains (Figs. 3 and 4). The probability of this degree of *long-range* homology occurring by chance is $\approx 10^{-5}$. When IFP rod sequences are compared with tektin A, it is possible (Figs. 4 and 5) to produce an alignment that also matches the two halves of the IFP rod with each other. The overall agreement is qualitatively best if we insert a break at the point

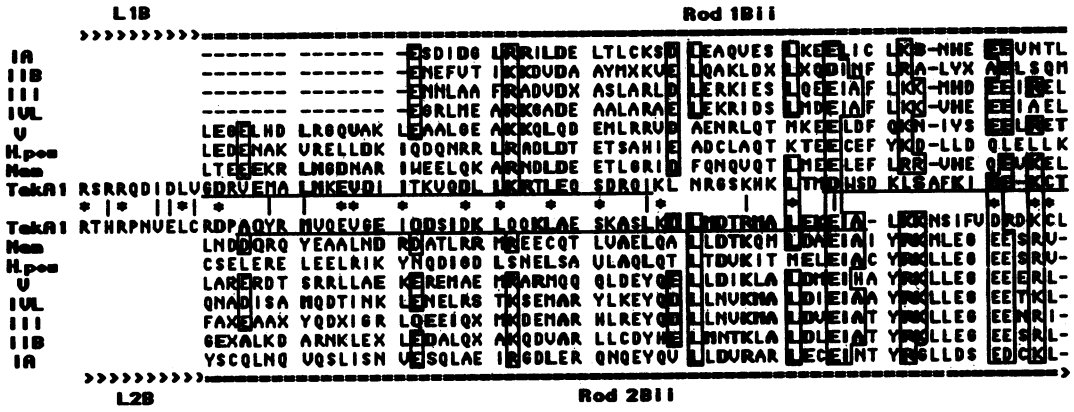

FIG. 5. Protein sequences (single-letter code) for regions 1Bii and 2Bii of some rod domains, aligned as in Fig. 4*a*. (Comparison of the complete rod sequences of tektin and IFPs will be published elsewhere.) The homology between segments 1 and 2 of tektin is illustrated for residues 174–244 vs. 375–446 (TekA1, center lines): asterisks indicate identical residues; vertical bars, similar residues (of like charge or hydrophobicity). Regions predicted to be α-helical are underlined, as in Fig. 1. The seven residues in a block are predicted to occupy positions *defgabc* of a coiled-coil structure (26, 27); most residues in positions *a* and *d* in helical regions 1B and 2B are hydrophobic (69% and 82%) or neutral (19% and 11%). Equivalent sequences from various IFPs are aligned above or below TekA1. Identical or similar residues, between tektin and any IFP, are in bold type; boxes indicate consistent homologies between segments 1 and 2. Consensus vertebrate sequences are simplified from ref. 30 (X, unspecified residues; -, inserted spaces): classes IA and IIB, two of the four keratin types; III includes desmin and vimentin; IVL, the smallest neurofilament IFP; V, nuclear lamins. Invertebrate sequences: Nem from a nematode, *Ascaris lumbricoides* (32), and Pom from a snail, *Helix pomatia* (31).

of the "stutter" in IFP segment 2B, as well as a break in the middle of segment 1B, where lamins and invertebrate IFPs have extra sequence compared with vertebrate IFPs (31, 32). Moreover, consistent differences between segments 1 and 2 of tektin A and IFPs (such as differences in size between regions 1A and 2A) suggest that IFP and tektins diverged after the postulated duplication event. The strong homology between lamins and invertebrate IFPs indicates that they are closer to the putative ancestral protein than are the vertebrate IFPs (31, 32). However, the greater similarity between the two halves of the tektin rod suggests that it may have diverged even less from the ancestral protein.

Aside from the possible tektin–IFP relationship, we are interested in the interactions of tektins with tubulin and other components of ciliary and flagellar MTs. Tektin A has only weak similarities with known MT-associated proteins (MAPs) (comparisons not shown). Putative tubulin binding sites in MAP2, tau, and a trypanosome MAP all include a cluster of positively charged residues apparently located on a loop between two helical segments (35–37). Two predicted loops in tektin A (L1B and L2B, Figs. 4 and 5) both share this feature and may represent novel tubulin-binding sites.

The arrangement of tektins in MTs has not been resolved, but evidence suggests that they exist as extended polymers in the walls of ciliary/flagellar doublet MTs, interacting directly with tubulin. Immunomicroscopy has shown that tektins are evenly distributed along flagellar MTs and that anti-tektin-stained filaments project axially from the ends of MTs (2, 4). X-ray diffraction of purified tektin filaments gives α-type patterns (28), indicating that α-helices are oriented axially in the isolated filament. The lengths of individual tektin molecules have not been measured directly, but by analogy with lamins we predict molecules ≈50 nm long. Tentative results, obtained by immuno electron microscopy with a monoclonal anti-tektin, suggest that similar molecules are arranged in filaments with a periodicity of roughly 48 nm (38). Based on the predicted structural motif (Fig. 4*b*), linear tektin subunits spanning several tubulin dimers might function to stabilize certain tubulin protofilaments, in agreement with the observed association of tektins with stable classes of MTs and MT protofilaments (1–5).

According to the model (Fig. 4*b*), α-helical regions of tektins might run alongside tubulin subunits. Many coiled-coil proteins, including myosin and IFPs, have patterns of charged residues with an average 9- to 10-residue repeat that, in association with the hydrophobic heptad repeat, produces an approximate overall periodicity of 28 residues (26, 29, 30). This widespread feature may be important in bundling coiled-coil molecules into filaments. The longitudinal spacing of tubulin monomers in the MT lattice (4.05–4.10 nm) (39) is very close to the length of 28 residues in a coiled-coil conformation (26, 38), and tubulin may interact directly with the α-helical regions of certain fibrous proteins, as proposed for giardin, a coiled-coil protein associated with MTs in *Giardia* (40). Indeed, tubulin may have originally adapted its longitudinal dimension to fit a preexisting fibrous cytoskeleton of tektin-like polypeptides. It is somewhat surprising, therefore, that Fourier analysis of charge distributions in tektin A1 (data not shown) shows it to be less periodic than members of the IFP family. Neither the spacing of positively charged residues nor that of negatively charged residues in the rod segments of tektin indicates an overall 28-residue or 56-residue periodicity; only if one looks at groups of two or three consecutive positive charges does the rough pattern in Fig. 4*b* appear. However, tektins may have evolved a more complex charge distribution, important for associations with other axonemal components, such as dynein arms, radial spokes, and nexin filaments (7, 41).

We are grateful to R. E. Stephens for helpful comments on this paper. This work was supported by Public Health Service Grant GM35648 and Minnesota Medical Foundation Grant SMF-527-86 to R.W.L. and by National Research Service Award 1F32-GM11669 to J.M.N.

1. Linck, R. W. & Langevin, G. L. (1982) *J. Cell Sci.* **58**, 1–22.
2. Linck, R. W., Amos, L. A. & Amos, W. B. (1985) *J. Cell Biol.* **100**, 126–135.
3. Linck, R. W. & Stephens, R. E. (1987) *J. Cell Biol.* **104**, 1069–1075.
4. Steffen, W. & Linck, R. W. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2643–2647.
5. Steffen, W. & Linck, R. W. (1989) in *Cell Movement*, eds. McIntosh, J. R. & Warner, F. D. (Liss, New York), Vol. 2, pp. 67–81.
6. Steffen, W. & Linck, R. W. (1992) *J. Cell Sci.* **101**, 809–822.
7. Stephens, R. E., Oleszko-Szuts, S. & Linck, R. W. (1989) *J. Cell Sci.* **92**, 391–402.

8. Chang, X.-j. & Piperno, G. (1987) *J. Cell Biol.* **104,** 1563–1568.
9. Steffen, W. & Linck, R. W. (1989) *Cell Motil. Cytoskel.* **14,** 359–371.
10. Amos, W. B., Amos, L. A. & Linck, R. W. (1985) *Cell Motil.* **5,** 239–249.
11. Linck, R. W., Goggin, M. J., Norrander, J. M. & Steffen, W. (1987) *J. Cell Sci.* **88,** 453–466.
12. Stephens, R. E. (1977) *Dev. Biol.* **61,** 311–329.
13. Stephens, R. E. (1989) *J. Cell Sci.* **92,** 403–413.
14. Schroeder, T. E., ed. (1986) in *Methods in Cell Biology* (Academic, New York), Vol. 27, p. 471.
15. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 326–328.
16. Young, R. A. & Davis, R. W. (1983) *Science* **222,** 778–782.
17. Vieira, J. & Messing, J. (1987) *Methods Enzymol.* **153,** 3–11.
18. Dale, R. M. K., McClure, B. A. & Houchins, J. P. (1985) *Plasmid* **13,** 31–41.
19. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5563–5567.
20. Schechter, Y., Patchornik, A. & Burstein, Y. (1976) *Biochemistry* **23,** 5071–5075.
21. Matsudaira, P. (1987) *J. Biol. Chem.* **262,** 10035–10038.
22. Pearson, W. R. & Lipman, D. J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2444–2448.
23. Staden, R. (1982) *Nucleic Acids Res.* **10,** 2951–2961.
24. Robson, B. & Suzuki, E. (1976) *J. Mol. Biol.* **107,** 327–356.
25. Staden, R. (1988) *Comput. Appl. Biosci.* **4,** 53–60.
26. McLachlan, A. D. & Stewart, M. S. (1982) *J. Mol. Biol.* **162,** 693–695.
27. Cohen, C. & Parry, D. A. D. (1986) *Trends Biochem. Sci.* **11,** 245–248.
28. Beese, L. (1984) Ph.D. thesis (Brandeis Univ., Waltham, MA).
29. Steinert, P. M. & Roop, D. R. (1988) *Annu. Rev. Biochem.* **57,** 593–625.
30. Conway, J. F. & Parry, D. A. D. (1988) *Int. J. Biol. Macromolec.* **10,** 79–98.
31. Weber, K., Plessmann, U., Dodemont, H. & Kossmagk-Stephan, K. (1988) *EMBO J.* **7,** 2995–3001.
32. Weber, K., Plessmann, U. & Ulrich, W. (1989) *EMBO J.* **8,** 3221–3227.
33. Vale, R. D. & Goldstein, L. S. B. (1990) *Cell* **60,** 883–885.
34. Quax-Jeuken, Y. E. F. M., Quax, W. J. & Bloemendal, H. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3548–3552.
35. Schneider, A., Hemphill, A., Wyler, T. & Seebeck, T. (1988) *Science* **241,** 459–462.
36. Lewis, S. A., Ivanov, I. E., Lee, G.-H. & Cowan, N. J. (1989) *Nature (London)* **342,** 498–505.
37. Goedert, M., Crowther, R. A. & Garner, C. C. (1991) *Trends Neurochem. Sci.* **14,** 193–199.
38. Amos, L. A., Amos, W. B. & Linck, R. W. (1986) *J. Cell Sci.* (Suppl.) **5,** 55–68.
39. Wais-Steider, C., White, N. S., Gilbert, D. S. & Eagles, P. A. M. (1987) *J. Mol. Biol.* **197,** 205–218.
40. Holberton, D., Baker, D. A. & Marshall, J. (1988) *J. Mol. Biol.* **204,** 789–795.
41. Amos, L. A., Linck, R. W. & Klug, A. (1976) in *Cell Motility*, eds. Goldman, R., Pollard, T. & Rosenbaum, J. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 847–867.