Analysis of the roles of the head domains of type IV rat neuronal intermediate filament proteins in filament assembly using domain-swapped chimeric proteins

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SUMMARY

Type IV neuronal intermediate filament proteins consist of α-internexin, which can self-assemble into filaments and the neurofilament triplet proteins, which are obligate heteropolymers, at least in rodents. These IF proteins therefore provide good systems for elucidating the mechanism of intermediate filament assembly. To analyze the roles of the head domains of these proteins in contributing to their differential assembly properties, we generated chimeric proteins by swapping the head domains between rat α-internexin and either rat NF-L or NF-M and examined their assembly properties in transfected cells that lack their own cytoplasmic intermediate filament network. Lαα and Mαα, the chimeric proteins generated by replacing the head domain of α-internexin with those of NF-L and NF-M, respectively, were unable to self-assemble into filaments. In contrast, αLL, a chimeric NF-L protein generated by replacing the head domain of NF-L with that of α-internexin, was able to self-assemble into filaments, whereas MLL, a chimeric NF-L protein containing the NF-M head domain, was unable to do so. These results demonstrate that the α-internexin head domain is essential for α-internexin’s ability to self-assemble. While coassembly of Lαα with NF-M and coassembly of Mαα with NF-L resulted in formation of filaments, coassembly of Lαα with NF-L and coassembly of Mαα with NF-M yielded punctate patterns. These coassembly results show that heteropolymeric filament formation requires that one partner has the NF-L head domain and the other partner has the NF-M head domain. Thus, the head domains of rat NF-L and NF-M play important roles in determining the obligate heteropolymeric nature of filament formation. The data obtained from these self-assembly and coassembly studies provide some new insights into the mechanism of intermediate filament assembly.

Key words: α-Internexin, Neurofilament triplet protein, Intermediate filament, Assembly, Head domain, Domain swapping

INTRODUCTION

α-Internexin and the neurofilament triplet proteins (NFTPs) designated NF-L, NF-M, and NF-H for low, middle and high molecular mass subunits, respectively, are members of the type IV intermediate filament (IF) protein family, which comprise most of the 10 nm filaments present in neurons (for reviews see Fliegner and Liem, 1991; Parry and Steinert, 1995; Lee and Cleveland, 1996). α-Internexin is expressed earlier and more abundantly than the NFTPs in the developing nervous systems and examines their assembly properties in transfected cells that lack their own cytoplasmic intermediate filament network. As development continues into adulthood, the expression level of α-internexin decreases, while those of the NFTPs increase (Kaplan et al., 1990; Fliegner et al., 1994). α-Internexin and the NFTPs are colocalized in most neurons in the adult central nervous system (Chiu et al., 1989; Kaplan et al., 1990).

Like all other IF proteins, α-internexin and the NFTPs conform to a tripartite structure consisting of a highly conserved α-helical rod domain flanked by non-α-helical amino-terminal head and carboxyl-terminal tail domains (Steinert and Roop, 1988; Fliegner and Liem, 1991; Shaw, 1991). The α-helical rod domain is composed of heptad repeats of hydrophobic residues that allow the formation of a coiled-coil dimer. The head and tail domains are variable in both length and primary sequence among different IF proteins. On the basis of data obtained from chemical cross-linking studies of some of the IF proteins, a model for IF assembly has been proposed: (1) in the first step of IF assembly two parallel, unstaggered IF polypeptide chains form a dimer via their α-helical rod domains; these dimers can be either homodimers or heterodimers; (2) the dimers may associate laterally to form antiparallel, unstaggered tetramers or antiparallel, staggered tetramers; (3) the dimers may also associate longitudinally with a short head-to-tail overlap of the α-helical rod domains; (4) these lateral and longitudinal associations lead to the formation of protofibrils (octamers) and ultimately 10 nm IFs (Stewart et al., 1989; Geisler et al., 1992; Steinert et al., 1993a,b; for reviews see Heins and Aebi, 1994; Parry and Steinert, 1995).

In vitro assembly studies have shown that α-internexin and
NF-L can each polymerize into IFs, whereas NF-M and NF-H require NF-L for copolymerization into normal filaments (Geisler and Weber. 1981; Liem and Hutchison, 1982; Chiu et al., 1989; Kaplan et al., 1990). However, transient transfection experiments performed in SW13cl.2Vim +, a cell line lacking any cytoplasmic IF network, have demonstrated that none of the rodent NFTPs can self-assemble into IFs and instead, formation of a filament network requires coassembly of NF-L with NF-M or NF-H (Ching and Liem, 1993; Lee et al., 1993). In contrast, α-internexin is able to self-assemble and coassemble with each of the NFTPs into filament networks (Ching and Liem, 1993). The assembly properties and developmental expression patterns of these proteins suggest that α-internexin may act as a scaffold upon which the NFTPs coassemble into filament networks during neuronal development.

The roles of the head and tail domains of α-internexin and the NFTPs have been examined by transient transfection studies in SW13cl.2Vim - cells. An α-internexin mutant missing 54% of the head domain is unable to self-assemble into an IF network (Ching and Liem, 1998). This result is similar to those obtained with head-deletion mutants of vimentin, desmin, glial fibrillary acidic protein and peripherin (Raats et al., 1990; Herrman et al., 1992; Chen and Liem, 1994; Cui et al., 1995; Ho et al., 1995). An α-internexin mutant lacking the tail domain self-assembles into aberrant thick filaments (Ching and Liem, 1998). Both the head- and tail-deletion mutants of α-internexin can form normal filament networks with wild-type NF-L or NF-M, but not with NF-H. In contrast, deletion mutants of NF-L missing some portions of the head domain fail to form IFs with wild-type NF-M or NF-H (Lee et al., 1993; Ching and Liem, 1998). Similar results are obtained from coassemblies of the head-deletion mutants of NF-M and NF-H with wild-type NF-L and NF-M, respectively (Ching and Liem, 1993; Lee et al., 1997). Coassembly of a tailless NF-H mutant with wild-type NF-L, however, results in the formation of an aberrant filament network (Sun et al., 1997).

The results obtained from self-assembly and coassembly studies with deletion mutants of α-internexin and NFTPs suggest that the presence of an intact (or a substantial portion of) head domain within the IF tripartite structure is essential for homopolymerization and heteropolymerization into IFs. However, it remains unclear whether the differences between α-internexin and the NFTPs in their abilities to self-assemble into IFs can be attributed to the intrinsic characteristics of their head domains. It also remains unclear whether the head domains of the NFTPs play any role in determining the obligate heteropolymeric nature of filament formation by these proteins. To answer these questions, we generated chimeric proteins by swapping the head domains between rat α-internexin and either rat NF-L or NF-M, and examined the assembly properties of the resultant chimeric proteins in transfected SW13cl.2Vim - cells. We also prepared antibodies that recognize these chimeric proteins. Our results demonstrate that the head domain of α-internexin can confer the ability for self-assembly into IFs and that the head domains of NF-L and NF-M play a contributory role in dictating the obligate heteropolymerization of NFTPs in IF assembly. Our present report thus provides additional information on the mechanism of IF assembly.

MATERIALS AND METHODS

Construction of plasmids
To construct pRSV-α.LL, a pGEM-α.L plasmid, which contains the sequences encoding the α-internexin head domain and the NF-L coil 1 region was first prepared by ligating a 0.3 kb HindIII-PstI fragment from pRSV-α (Ching and Liem, 1993) and a 0.2 kb PstI fragment from pRSV-NFL2(+) (Chin and Liem, 1989; Ching and Liem, 1998) to a HindIII-PstI-digested pGEM vector (Promega, Madison, WI). A 0.3 kb HindIII-PstI fragment from pGEM-α.L, a 1.7 kb PstI-HindIII fragment from pRSV-NFL2(+) and a HindIII-digested, phosphatase-treated pRSV-HindIII vector (Forman et al., 1988) were ligated together to generate pRSV-α.LL. To construct pRSV-α.MM, a pGEM-α.M, which encodes the α-internexin head domain and the NF-M coil 1 region, was first prepared by ligating a 0.3 kb HindIII-PstI fragment from pRSV-α and a 0.3 kb PstI fragment from pRSV-NF-M1(+) (Chin and Liem, 1989) to a HindIII-PstI-digested pGEM vector. A 0.6 kb HindIII-Avi fragment from pGEM-α.M, a 2.4 kb AviII-BamHI fragment from pRSV-NF M1(+) and a HindIII-BamHI-digested pRSV-HindIII vector were ligated together to generate pRSV-α.MM. To construct pRSV-PLL, a pGEM-PLL plasmid, which encodes the NF-M head domain and the NF-L coil 1 region, was first prepared by ligating a 0.3 kb HindIII-PstI fragment from pRSV-NF M1(+) and a 0.2 kb PstI fragment from pRSV-NFL2(+) to a HindIII-PstI-digested pGEM3 vector. A 0.3 kb HindIII-PstI fragment from pGEM-ML, a 1.7 kb PstI-HindIII fragment from pRSV-NFL2(+) and a HindIII-digested, phosphatase-treated pRSV-HindIII vector were ligated together to generate pRSV-PLL. To construct pRSV-LLα, a pGEM-LLα plasmid, which encodes the NF-L head domain and the α-internexin coil 1 region, was prepared by ligating a 0.3 kb HindIII-PstI fragment from pRSV-NF M1(+) and a 0.4 kb PstI fragment from pRS V-α to a HindIII-PstI-digested pGEM3 vector. A 0.4 kb HindIII-Xhol fragment from pGEM-Lα, a 1.4 kb Xhol-BamHI fragment from pRSV-α and a HindIII-BamHI-digested pRSV-HindIII vector were ligated together to generate pRSV-LLα. To construct pRSV-Mαα, a pGEM-Mαα plasmid, which encodes the NF-M head domain and the α-internexin coil 1 region, was first prepared by ligating a 0.3 kb HindIII-PstI fragment from pRSV-NF M1(+) and a 0.4 kb PstI fragment from pRSV-α to a HindIII-PstI-digested pGEM3 vector. A 0.4 kb HindIII-Xhol fragment from pGEM-Mαα, a 1.4 kb Xhol-BamHI fragment from pRSV-α and a HindIII-BamHI-digested pRSV-HindIII vector were ligated together to generate pRSV-Mαα. DNA sequencing was performed on pGEM-α.L, pGEM-α.M, pGEM-ML, pGEM-L and pGEM-M to deduce the amino acid sequences at all fusion junctions. pRSV-NFHI(+) was previously described (Chin and Liem, 1990).

DNA transfection and immunofluorescence staining
Human adrenal carcinoma SW13cl.2Vim - cells (Sarria et al., 1990) were grown in DMEM/F12 medium (Gibco BRL, Gaithersburg, MD) containing 5% fetal bovine serum at 37°C and 5% CO2. Cells were transiently transfected with 20 µg of a tested DNA construct (or 10 µg each when two DNA constructs were used) and then examined by indirect immunofluorescence staining as previously described (Ching and Liem, 1993).

Cell extraction and immunoblot analysis
Total cellular proteins were prepared from transfected cells, electrophoresed in SDS-10% polyacrylamide gels and analyzed by western blot immunostaining as previously described (Ching and Liem, 1998).

Antibodies
Rabbit polyclonal antibody AbαNX that specifically recognizes the amino-terminus of α-internexin was prepared against a histidine-tagged peptide containing the amino-terminal 123 amino acid residues of rat α-internexin. This peptide was expressed from a 0.4 kb Ndel-
Results of chimeric intermediate filaments

Antibodies AbNFLn, which specifically recognize the amino termini of rat NF-L, were purchased (Sigma Chem. Co., St Louis, MO). The mouse monoclonal antibody mAb135 and the rabbit polyclonal antibody AbNFMm, both of which can recognize the rod domain of internexin, NF-L and NF-M, respectively, have previously been described (Kaplan et al., 1991; Ching and Liem, 1993). Monoclonal antibodies to NF-L, NF-M, NF-H and vimentin (clones NR4, NN18, N52 and V9, respectively) were purchased (Sigma Chem. Co., St Louis, MO). The rabbit polyclonal anti-vimentin antibody was a gift from Dr Eugenia Wang (Lady Davis Institute for Medical Research, Montreal).

Previous transfection experiments performed in SW13cl.2Vim- cells have shown that rat α-internexin is able to self-assemble into a filamentous network, whereas none of the rat or mouse NFTP is able to do so (Ching and Liem, 1993; Lee et al., 1993). To determine if this difference in IF self-assembly can be attributed to the head domains of these proteins, we swapped the head domains of rat NF-L and NF-M with that of rat α-internexin and used the DNA constructs that express the resultant chimeric proteins to transfect the SW13cl.2Vim cells. The amino acid sequences at the fusion junctions of these chimeric proteins are shown in Fig. 1. Western blot analysis showed that the chimeric proteins were correctly produced in the transfected cells (Fig. 2). Immunofluorescence costaining with two antibodies showed the chimeric nature of these proteins (Figs 3 and 4). Since vimentin-expressing revertant cells may arise, the antibodies showed the chimeric nature of these proteins. Transfection experiments showed that when expressed individually, Lα, a chimeric protein which consists of the head domain of NF-L and the rod and tail domains of α-internexin, yielded a punctate immunostaining pattern in vimentin network.

Fig. 2. Western blot analysis of the chimeric proteins expressed individually in transfected SW13cl.2Vim- cells. The blots containing total cellular proteins from cells transfected with (A) lane 1, pRSV-Lαα; lane 2, pRSV-Mαα; and (C) lane 1, pRSV-αLL; lane 2, pRSV-MLL. Bars indicate the positions of the prestained protein markers with molecular masses of 221, 133, 93, 67, 56 and 42 kDa.
transfected SW13cl.2Vim\textsuperscript{−} cells (Fig. 3A,B). This punctate immunostaining pattern suggests that homopolymerization of L\textit{aa} was arrested at a low-order oligomeric state. A similar result was obtained with M\textit{aa}, a chimeric protein that consists of the head domain of NF-M and the rod and tail domains of \(\alpha\)-internexin (Fig. 3C,D). These results indicate that replacement of the head domain of \(\alpha\)-internexin with those of NF-L and NF-M renders \(\alpha\)-internexin incapable of self-assembly into IFs. Thus, the head domain of \(\alpha\)-internexin is essential for \(\alpha\)-internexin’s homopolymerization into filaments and its role cannot be replaced by the head domains of rat NF-L and NF-M, which are unable to self-assemble into filaments in vivo (Ching and Liem, 1993; Lee et al., 1993).

We further tested whether chimeric rat NF-L or NF-M proteins containing the head domain of \(\alpha\)-internexin can self-assemble into filaments. Transient transfection showed that \(\alpha\)LL, a chimeric protein that consists of the head domain of \(\alpha\)-internexin and the rod and tail domains of rat NF-L, was able to self-assemble into a filamentous network in transfected SW13cl.2Vim\textsuperscript{−} cells (Fig. 4A,B). However, \(\alpha\)MM, a chimeric protein that consists of the head domain of \(\alpha\)-internexin and the rod and tail domains of NF-M, yielded a punctate immunostaining pattern (Fig. 4C,D). To confirm that the ability of \(\alpha\)LL to self-assemble into filaments is attributed to the presence of the \(\alpha\)-internexin head domain, chimeric rat NF-L with the NF-M head domain was examined for comparison. MLL, a chimeric protein that consists of the head domain of NF-M and the rod and tail domains of NF-L, failed to form filaments and instead showed a punctate pattern (Fig. 4E,F). Overall, these results are consistent with the previously observed self-assembly properties of rodent NF-L and NF-M (Ching and Liem, 1993; Lee et al., 1993). These data suggest that the head domain of \(\alpha\)-internexin can enable \(\alpha\)LL to homopolymerize into filaments.

Since the rod domain and to a lesser extent the tail domain of IF proteins also play important roles in homopolymerization (Raats et al., 1991, 1992; Kouklis et al., 1992; Eckelt et al., 1992; McCormick et al., 1993; Chen and Liem, 1994; Ho et al., 1998; Ching and Liem, 1998), the inability of \(\alpha\)MM to self-assemble into filaments suggests that the intrinsic characteristics of the NF-M portion of the chimeric protein may not favor filament formation in the self-assembly of \(\alpha\)MM. To investigate this possibility, we further characterized \(\alpha\)LL and \(\alpha\)MM by examining their coassemblies with wild-type NFTPs. Transient transfections in SW13cl.2Vim\textsuperscript{−} cells showed that \(\alpha\)LL formed a normal filament network with each of the NFTPs (Fig. 5). \(\alpha\)MM, on the other hand, formed a normal filament network with NF-L, but yielded a punctate immunostaining pattern with NF-M or NF-H (Fig. 6). These coassembly properties of \(\alpha\)LL and \(\alpha\)MM are similar to those of \(\alpha\)-internexin.

Fig. 3. Self-assembly properties of L\textit{aa} and M\textit{aa}. SW13cl.2Vim\textsuperscript{−} cells transiently transfected with (A,B) pRSV-L\textit{aa} or (C,D) pRSV-M\textit{aa} were immunostained with polyclonal antibodies to NF-L (A) and NF-M (C), and monoclonal antibody to \(\alpha\)-internexin (B,D). Bar, 20 \(\mu\)m.

Fig. 4. Self-assembly properties of \(\alpha\)LL, \(\alpha\)MM and MLL. SW13cl.2Vim\textsuperscript{−} cells transiently transfected with (A,B) pRSV-\(\alpha\)LL; (C,D) pRSV-\(\alpha\)MM; or (E,F) pRSV-MLL were immunostained with polyclonal antibody Ab\textsubscript{oNX} to \(\alpha\)-internexin (A,C), polyclonal antibody to NF-M (E), and monoclonal antibodies to NF-L (B,F) and NF-M (D). Bar, 20 \(\mu\)m.
and NF-M, respectively. The similarities between αMM and NF-M in their self-assembly and coassembly properties suggest that the rod and perhaps a portion of the tail domain of NF-M play a dominant role in conferring NF-M-like assembly characteristics on αMM. Although the present studies do not address the tail domain of NF-M, previous experiments have shown that tailless NF-M can coassemble with either NF-L or α-internexin into filaments (Ching and Liem, 1993).

The observation that αLL behaved like α-internexin raises the possibility that the head domains of Lαα and Mαα may cause them to behave like NF-L and NF-M, respectively. This prompted us to examine the coassemblies of Lαα and Mαα with the wild-type NFTPs. Transient transfection in SW13cl.2Vim− cells showed that Lαα formed a normal filament network with NF-M, but yielded a punctate immunostaining pattern with NF-L or α-internexin into filaments (Ching and Liem, 1993).

In contrast, coassembly of Mαα with NF-L yielded aberrant, thick filaments as well as punctate immunostaining (Fig. 8A,B). All transfected cells showed thick filaments, with some variation in the level of punctate immunostaining. The punctate immunostaining could be due to the difference in the relative levels of expression of the two proteins. Nevertheless, the results indicate that Mαα and NF-L were able to coassemble into filaments. A previous study from our laboratory has shown by electron microscopy that thick filaments similar to those shown in Fig. 8A and B are composed of intermediate filament proteins (Ching and Liem, 1993, 1998; Lee et al., 1993).

Fig. 5. Coassemblies of αLL with wild-type NFTPs. SW13cl.2Vim− cells transiently transfected with (A,B) pRSV-αLL and pRSVi-NFL2(+); (C,D) pRSV-αLL and pRSVi-NFM1(+); or (E,F) pRSV-αLL and pRSVi-NFH1(+) were immunostained with polyclonal antibody AbαNX to α-internexin (A,C,E) and monoclonal antibodies to NF-L (B), NF-M (D) and NF-H (F). Bar, 20 μm.

Fig. 6. Coassemblies of αMM with wild-type NFTPs. SW13cl.2Vim− cells transiently transfected with (A,B) pRSV-αMM and pRSVi-NFL2(+); (C,D) pRSV-αMM and pRSVi-NFM1(+); or (E,F) pRSV-αMM and pRSVi-NFH1(+) were immunostained with monoclonal antibodies to NF-M (A,D) and NF-H (F), polyclonal antibody to NF-L (B) and polyclonal antibody AbαNX to α-internexin (C,E). Bar, 20 μm.
domains of NF-L and NF-M contribute to the obligate heteropolymerization of \( \alpha \)-internexin with NF-M and M\( \alpha \) with NF-L into filaments. However, the contribution of the NF-L and NF-M head domains to filament assembly is dependent on the rest of the molecule, since coassembly of \( \alpha \)-internexin with M\( \alpha \) yielded a punctate immunostaining pattern (not shown). In contrast to the coassemblies with wild-type NFTPs that involve alignment of two heterotypic rod domains, coassembly of \( \alpha \)-internexin with M\( \alpha \) involves alignment of two identical rod domains. The presence of the rod and tail domains of \( \alpha \)-internexin in the chimeric proteins presumably gave rise to an alignment of the \( \alpha \)-internexin rod domains that does not allow favorable alignments of the head domains of NF-L and NF-M for filament formation. As a result these chimeric proteins fail to coassemble into a filamentous network.

**DISCUSSION**

Although the roles of each domain within the IF tripartite structure in IF assembly have been extensively studied for various types of IF proteins, the use of deletion mutants in a majority of these studies limits the kinds of information obtained from these functional analyses. In consideration of these limitations, we decided to generate chimeric proteins for assembly studies by domain swapping. \( \alpha \)-Internexin and the rodent NFTPs differ in their self-assembly properties and can coassemble to form filaments. They are therefore ideal candidates for the generation of chimeric proteins and provide a useful system for elucidating the mechanism of neuronal IF assembly. In the present study, we generated several chimeric proteins by swapping the head domains between \( \alpha \)-internexin and either rat NF-L or NF-M and analyzed the roles of the head domains of these neuronal IF proteins in IF assembly. We examined the assembly properties of the chimeric proteins in a cell line that does not have its own cytoplasmic IF network. The results obtained from the self-assembly and coassembly studies (see Table 1) give us new insights into the roles of the head domains of the type IV IF proteins in homopolymerization and heteropolymerization of IFs.
Table 1. Coassemblies of chimeric proteins with NFTPs

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F: normal filament network; P: punctate immunostaining; ATF: aberrant, thick filaments within a punctate pattern.

The observation of punctate immunostaining patterns from the self-assembly of Lαα and Mαα is consistent with our previous study that shows that the partial deletion of the head domain abolishes the ability of α-internexin to homopolymerize into IFs (Ching and Liem, 1998). The inability of Lαα and Mαα to self-assemble into filaments indicates that the intrinsic characteristic of the α-internexin head domain is essential for the homopolymerization of α-internexin into IFs, since the head domain of NF-L or NF-M cannot substitute for the α-internexin head domain. In addition, the α-internexin head domain enabled αLL to self-assemble into a filamentous network, whereas the NF-M head domain did not confer on MLL this same ability. Taken together, these results demonstrate that the α-internexin head domain allows IF proteins to self-assemble, provided that their rod domains are compatible with self-assembly. In contrast, the NF-L and NF-M head domains do not have this property. Thus, the difference between α-internexin and the rat NFTPs in their abilities to self-assemble into IFs can be attributed in part to the intrinsic characteristics of their head domains.

Previous assembly studies have shown that the rod and tail domains of an IF protein are important in IF self-assembly (Raats et al., 1991, 1992; Kouklis et al., 1992; Eckelt et al., 1992; McCormick et al., 1993; Chen and Liem, 1994; Ho et al., 1998; Ching and Liem, 1998). Thus, it appears that the intrinsic characteristics of these domains of NF-M may not favor filament formation in the self-assembly of αMM, despite the presence of the α-internexin head domain. The similarities between αMM and NF-M in their self-assembly and coassembly properties with the NFTPs suggest that the rod and perhaps part of the tail domains of NF-M play a dominant role in conferring NF-M-like assembly characteristics on αMM. Our previous studies have shown that NF-L is present only in the Triton X-100 insoluble fraction of singly transfected SW13cl.2Vim- cells, whereas NF-M is found in both the Triton X-100 insoluble and soluble fractions (Ching and Liem, 1993). The differential solubilities in Triton X-100 indicate that NF-M does not self-assemble into higher-order polymeric structures as readily as NF-L. In vitro assembly studies have shown that NF-L, but not NF-M, polymerizes readily into IFs (Geisler and Weber, 1981; Liem and Hutchison, 1982). Although these in vitro assembly properties do not reflect the fact that both rodent NF-L and NF-M are obligate heteropolymer in vivo (Ching and Liem, 1993; Lee et al., 1993), they agree with the differential solubilities of NF-L and NF-M in Triton X-100 (Ching and Liem, 1993). These studies also indicate that NF-L, but not NF-M, can homopolymerize into IFs under appropriate experimental conditions. Taken together, these results suggest that the rod and tail domains of NF-L are intrinsically favorable to filament formation in self-assembly. In contrast, the rod and tail domains of NF-M are intrinsically unfavorable for filament formation. Yeast two-hybrid studies and cross-linking studies have also shown that NF-M prefers to form a heterodimer with NF-L than a homodimer, whereas NF-L can form homodimers as well as heterodimers with NF-M (Leung and Liem, 1996; Carpenter and Ip, 1996; Athlan and Mushynski, 1997). Therefore, homopolymerization of a chimeric NF-M is unlikely to occur even in the presence of an α-internexin head domain. In contrast, homopolymerization of NF-L appears to be prevented by its own head domain and therefore replacement with the α-internexin head domain allows for productive filament assembly by the chimeric NF-L.

Coassemblies of either Lαα or Mαα with wild-type NFTPs yielded additional interesting results (summarized in Table 1). While coassembly of Lαα with NF-M and coassembly of Mαα with NF-L led to formation of filaments, coassembly of Lαα with NF-L and coassembly of Mαα with NF-M yielded punctate patterns. Since Lαα and Mαα differ only in their head domains, the assembly results demonstrate that the head domains of NF-L and NF-M are responsible for the obligate heteropolymerization of Lαα with NF-M and Mαα with NF-L into filaments. It is apparent that these coassemblies result in filament formation only when one partner has the NF-L head domain and the other partner has the NF-M head domain, but not when both partners have the same kind of head domain. These assembly properties of Lαα and Mαα appear to be similar to those of NF-L and NF-M, respectively. The formation of aberrant thick filaments from coassembly of Mαα with NF-L is presumably due to the chimeric nature of Mαα, resulting in relaxed control of lateral and longitudinal associations during IF assembly. Since the NF-L-like and NF-M-like assembly properties of Lαα and Mαα, respectively, are attributed to the intrinsic characteristics of the NF-L and NF-M head domains, the coassemblies of these chimeric proteins with the NFTPs also provide evidence that the head domains of NF-L and NF-M play contributory roles in dictating the obligate heteropolymeric nature of filament formation by the NFTPs.

Unlike coassembly of Lαα with NF-M, coassembly of Lαα with NF-H did not result in filament formation. Our previous assembly study has shown that in contrast to NF-L and NF-M, NF-H does not form filaments with any deletion mutants of α-internexin, indicating that a largely intact α-internexin polypeptide is required for proper molecular alignment with NF-H to form filaments (Ching and Liem, 1998). In view of this requirement for alignment between α-internexin and NF-H, the chimeric nature of Lαα may not allow a proper molecular alignment between Lαα and NF-H for filament formation, since the intrinsic characteristics of the NF-L head domain differ from those of the α-internexin head domain. This may account for the observation of a punctate immunostaining pattern from coassembly of NF-H with Lαα.

Although our present study demonstrates the important roles played by the head domains of the type IV IF proteins in self-assembly and coassembly of IFs, it does not exclude any roles that the rod and tail domains of these proteins have in the two assembly processes. Indeed, our data also show the significant impact that the rod and tail domains of rat NF-L and NF-M have on filament formation. In the case of human NF-L, self-assembly has been observed. The divergent characteristics of human NF-L from rodent NF-L has been found to be due to several amino acids in coil 1b and the L12 linker region within the rod domain of NF-L (Carter et al., 1998), further confirming the importance of the rod domain in directing assembly. It would be interesting to know if the human NF-L
head domain could also help override the inability of rodent NF-L to self-assemble into filaments, similar to the data reported here with the α-internexin head domain. Overall, our assembly results are consistent with the current IF assembly model, which proposes that the head domain of one dimer may interact with both conserved ends of the rod domain of another dimer (Stewart et al., 1989; Geisler et al., 1992; Steinert et al., 1993a,b; for reviews see Heins and Aebi, 1994; Parry and Steinert 1995). The information obtained from this study will also help us to further understand the IF assembly processes of the type III IF proteins, which can self-assemble into IFs, and the cytoplakin, which are obligate heteropolymers.

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