Tailless keratins assemble into regular intermediate filaments *in vitro*

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Summary

To study the influence of the non α-helical tail domain of keratins in filament formation, we prepared a truncated keratin 8 mutant, K8/tailless. Using site-directed *in vitro* mutagenesis we introduced a stop codon in the position coding for amino acid number 417 of the K8/wild-type sequence, thereby deleting 86 amino acids of the non α-helical tail domain but leaving the consensus sequence at the end of the rod domain intact. Expression of the truncated keratin 8 in *Escherichia coli* allowed us to purifiy the protein by a two-step procedure.

The filament-forming capacity of the truncated K8 with wild-type K18 and K19 was analyzed using *in vitro* reconstitution. The *in vitro* assembly studies with K8/tailless and K18 wild-type indicate that the C-terminal tail domain of a type II keratin, including the homologous subdomain H2, is not required for filament formation. Moreover, reconstitution experiments with K8/tailless and K19, a naturally occuring tailless keratin I, show that the tail domains of type I as well as type II keratins are not an essential requirement for *in vitro* filament formation. Our results suggest that *in vitro* filament elongation does not depend on interactions between head and tail domains, although the tail domain might have a role in stabilization of intermediate filaments arising from certain keratin pairs.

Key words: intermediate filaments, keratins, deletion mutants, *in vitro* assembly.

Introduction

Intermediate filament (IF) proteins are members of a large multigene family. The keratins are the largest group of IF proteins, comprising the type I IF proteins represented by the smaller acidic keratins and the type II IF proteins represented by the larger neutral to basic keratins (Fuchs *et al.* 1981; Hanukoglu and Fuchs, 1983; Schiller *et al.* 1982; Tseng *et al.* 1982). In all epithelia at least two different keratins are coexpressed and form specific type I and type II keratin pairs (Moll *et al.* 1982; Tseng *et al.* 1982). Although *in vitro* any type I keratin forms filaments with any type II keratin (Hatzfeld and Franke, 1985), characteristic pairs are generally coexpressed *in vivo* in a tissue-specific manner. The type III group of IF proteins is represented by vimentin, desmin, GFAP and peripherin (for references, see Fliegner *et al.* 1990). In contrast to the obligatory heteropolymer character of keratin filaments, type III proteins can form homopolymeric filaments. The neurofilament proteins and α-internexin are classified as type IV IF proteins, whereas the nuclear lamins belong to the type V IF proteins (for review, see Steinert and Roop, 1988). Recently, a sixth class of IF proteins, represented by nestin, has been described (Lendahl *et al.* 1990). All IF proteins share the same subunit organization consisting of a non-α-helical N-terminal head, a central α-helical rod and a C-terminal tail (Geisler and Weber, 1982; Geisler *et al.* 1982; Steinert *et al.* 1983, 1985*a*; Hanukoglu and Fuchs, 1983; Weber and Geisler, 1984).

The *in vitro* assembly process of IF seems based on a hierarchical series of association steps involving different protein domains. First, two protein chains associate to form a coiled-coil molecule, in which both chains are parallel and arranged in register (Geisler and Weber, 1982; Woods and Gruen, 1981; Woods and Inglis, 1984; Parry *et al.* 1985; Quinlan *et al.* 1986). In the keratins this coiled coil is a heterodimer of a type I and a type II keratin (Hatzfeld and Weber, 1990). In the second step, two coiled coils form a tetramer representing the predominant stable intermediate, which can be isolated by dissociation of IF (Ahmadi and Speakman, 1978; Geisler and Weber, 1982; Quinlan *et al.* 1984; Parry *et al.* 1985). The geometric arrangement of the two coiled coils in the tetramer is not clearly established. Several laboratories prefer an antiparallel orientation with a stagger (Geisler *et al.* 1985; Parry *et al.* 1985; Fraser *et al.* 1985; Potschka, 1986; Stewart *et al.* 1989; Potschka *et al.* 1990) but a recent report favors a parallel arrangement, where antiparallelity and stagger are entered at the octamer stage (Hisanaga *et al.* 1990).

Formation of dimers as well as tetramers requires only the α-helical rod domains, since proteolytical removal of head and tail domains does not prevent the formation of these complexes (Kaufmann *et al.* 1985; Traub and Vorgias, 1983). Moreover, the heterotypic requirement of type I and type II keratins for coiled-coil formation is retained in the isolated α-helical domains and even in the individual coil 1 and coil 2 domains of type II keratins (Hatzfeld *et al.* 1987; Magin *et al.* 1987). The final assembly step, i.e. the formation of filaments, requires some contribution from the non-helical domains, since isolated rod domains are not capable of assembling into IF (Geisler *et al.* 1982; Sauk *et al.* 1984). Removal of the N-terminal head domain of desmin leads to a polymerization-incompetent derivative, indicating that the head region is essential for

the elongation reaction. Removal of about half of the C-terminal tail domain of desmin did not interfere with filament formation (Kaufmann *et al.* 1985). However, a particular recombinant derivative of GFAP lacking the entire non-helical tail domain failed to form IF (Quinlan *et al.* 1989). Taken together these data seem to suggest that the part of the tail domain proximal to the rod may also have an essential role in IF assembly.

In order to analyse the importance of the tail domain more precisely, we have constructed a mutant keratin 8 cDNA that has only the first six amino acids of the tail domain. We have expressed this mutant keratin 8 cDNA in *Escherichia coli* and examined the filament-forming capacity of the keratin in combination with the type I keratin 18 as well as the only naturally occuring tailless keratin 19. Here we show that the complete tail region can be deleted from keratins without impairing the process of filament formation *in vitro*.

Materials and methods

Site-directed mutagenesis and cloning procedures: K8/tailless

Restrictions enyzmes, bacteriophage T4 DNA ligase and bacteriophage M13 mpl8 RF (replicative form) DNA were obtained from NEN Biolabs (Beverly, MA). The mutagenesis kit was from Amersham (Amersham Buchler GmbH, Braunschweig, FRG) and a bacteriophage T7 polymerase-based sequencing kit from Pharmacia (Pharmacia LKB, Uppsala, Sweden). General cloning procedures were carried out according to Maniatis *et al.* (1982).

The cDNA clone pK XL 1/8 (Franz and Franke, 1986) with a *Bam*HI restriction site at the start of the coding sequence (Magin *et al.* 1987) was cloned into M13 mpl8. The 33-mer oligonucleotide TGT CTT GGT CTG AAA GCT TCA GTT CTG AAA GCC was used to introduce a TGA stop codon immediately followed by a *Hin*dIII restriction site at position 417 of the amino acid sequence of the *Xenopus* keratin 8. Site-directed mutagenesis was performed as described by Nakamaye and Eckstein (1986) and *E. coli* strain JM101 was used for transformation. Mutants were identified by sequence analysis and cloned into the expression plasmid pINDU (Bujard *et al.* 1987) using the *Bam*HI and *Hin*dIII restriction sites as described. *E. coli* strains expressing K8/wild-type and K18/wild-type have been described (Magin *et al.* 1987; Hatzfeld and Weber, 1990; for the original sequence of K18, see Alonso *et al.* 1987). All recombinant proteins K8/wild-type, K8/tailless, as well as K18/wild-type carry the modified N-terminal amino acid sequence MRGSP (one-letter code) instead of MS (keratin 8) and MRGS instead of MSF (keratin 18), respectively.

Keratin purification procedures

Bacterial cultures expressing K8/wild-type, K8/tailless or K18/wild-type were grown overnight in LB-medium containing 200 $\mu g\,ml^{-1}$ ampicillin. Bacteria were harvested and inclusion bodies were isolated as described (Nagai and Thogerson, 1987). High molecular weight DNA was destroyed by shearing in a Dounce homogenizer in detergent-containing buffer prior to centrifugation. Purified inclusion bodies were dissolved by addition of 9.5 M urea, 5 mM EDTA, 1% 2-mercaptoethanol, 10 mM Tris–HCl, pH 8.6, to give a final concentration of ~8.5 M urea and ~10 $mg\,ml^{-1}$ protein as determined by the method of Bradford (1976). The following protease inhibitors (Sigma Chemical Comp., Deisenhofen, FRG) were added: 0.5 μM E64 (L-3-*trans*-carboxyoxiran-2-carbonyl)-1-leu-agmatin), 100 $\mu g\,ml^{-1}$ ovomucoid and 2 mM PMSF (phenylmethylsulphonyl fluoride).

K8/wild-type and K8/tailless keratins were further purified by ion-exchange chromatography on Mono Q (Pharmacia Fine Chemicals) in 8.5 M urea, 5 mM EDTA, 10 mM Tris–HCl, pH 8.6, with a linear gradient from 0 to 200 mM guanidine–HCl (Hatzfeld and Weber, 1990). The solution of solubilized inclusion bodies was diluted 1:10 in chromatography buffer and directly applied to the column, which was connected to an FPLC apparatus. Peak fractions were analysed by gel electrophoresis, relevant fractions were pooled and stored at −20°C. K18/wild-type inclusion bodies were diluted 1:10 in 8.5 M urea, 10 mM Tris–HCl, pH 7.5, and applied to a single-stranded DNA affinity column (Gibco/BRL Life Technologies, Eggenstein, FRG) pre-equilibriated in the same buffer (Hatzfeld and Weber, 1990). Bound keratin was step eluted by 200 mM guanidine–HCl in equilibriation buffer. Human keratin 19 was purified from MCF-7 cytoskeletal fractions by ion-exchange chromatography on DEAE-cellulose as described (Achtstätter *et al.* 1986).

Gel electrophoresis and immunoblotting

One-dimensional SDS–PAGE electrophoresis was performed according to Laemmli (1970) using 10% acrylamide gels. Polypeptides were transferred to nitrocellulose according to the method of Kyhse-Anderson (1984). Immunoblots with the IFA-monoclonal antibody (Pruss *et al.* 1981) were developed using alkaline phosphatase-coupled anti-mouse Ig as described previously (Achtstätter *et al.* 1986; Hatzfeld and Weber, 1990).

In vitro *assembly and electron microscopy*

Equimolar amounts of type I keratins 18 or 19 and the K8/wild-type or K8/tailless keratins II were mixed in 8.5 M urea-containing buffer at a protein concentration of about 0.5 $mg\,ml^{-1}$. Samples (30–50 μl) were dialysed at room temperature against standard filament assembly buffer (50 mM Tris–HCl, pH 7.5, 5 mM EDTA) using microdialysis filters (Millipore GmbH, Eschborn, FRG) as described (Hatzfeld and Weber, 1990). In some experiments with K19 the pH of the filament buffer was 7.0 (K19 filament buffer) instead of 7.5. Filament formation was analysed after negative staining with 2% uranyl acetate as described (Hatzfeld and Weber, 1990).

Results

Construction of K8/tailless and its purification from E. coli

In order to analyse whether the tail domain of keratins participates in filament formation we have created a truncated form of the *Xenopus laevis* keratin 8, which consists of the N-terminal head domain and the helical rod domain. Using site-directed mutagenesis we have introduced a stop codon into the K8 cDNA followed by a *Hin*dIII restriction site (Fig. 1A). The stop codon corresponding to position 417 of the amino acid sequence provides a mutant keratin called K8/tailless, which lacks the C-terminal 86 amino acids of the wild-type protein. The *Hin*dIII site permitted the cloning of the mutant cDNA into the expression vector pINDU and transfection into *E. coli* as described (Magin *et al.* 1987; Hatzfeld and Weber, 1990). Immunoblot experiments (Fig. 2) of crude bacterial lysates with the monoclonal antibody IFA (Pruss *et al.* 1981) confirmed the expression of a truncated keratin with the expected molecular weight of about 47 000 and the presence of the consensus sequence at the end of the helical rod domain, which contributes to the epitope of the antibody (Geisler *et al.* 1983; Magin *et al.* 1987).

For filament reconstitution experiments K8/tailless was purified by a two-step procedure. K8/tailless is concentrated in the *E. coli* strain as inclusion bodies. These were purified using detergent extractions (Nagai and Thogersen, 1987). Fig. 3, lane 3, shows that the inclusion body preparation is highly enriched in K8/tailless. Inclusion body preparations were solubilized in 9.5 M urea and the keratin mutant was purified by ion-exchange chromatography on a Mono Q column in the presence of 8.5 M urea. The peak fraction eluting from the Mono Q

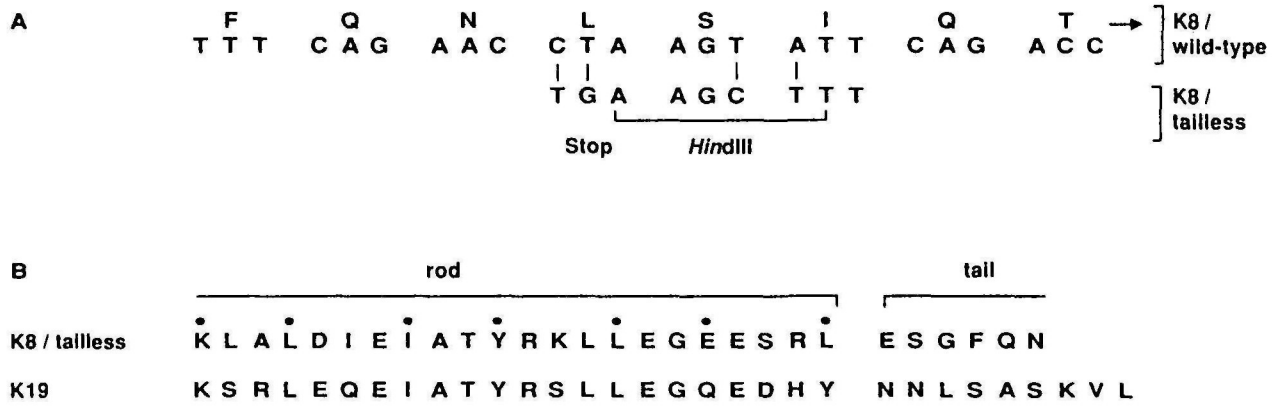

Fig. 1. Comparison of the nucleotide and amino acid sequence of K8/wild-type and K8/tailless in the region of mutagenesis. Bars indicate those nucleotides that have been changed by site-directed mutagenesis, creating a stop codon and a *Hin*dIII site as indicated. For the original sequence of K8 see Franz and Franke (1986). (B) Comparison of the amino acid sequence of K8/tailless and the naturally occuring tailless human keratin K19 (Stasiak *et al.* 1989), starting with the consensus sequence at the end of the rod domain. The dots mark the positions of hydrophobic amino acids in positions 'a' and 'd' of the coiled coil heptad repeat pattern. The mutant keratin K8/tailless is 3 amino acids shorter than K19 and stops at position 417 of the original sequence.

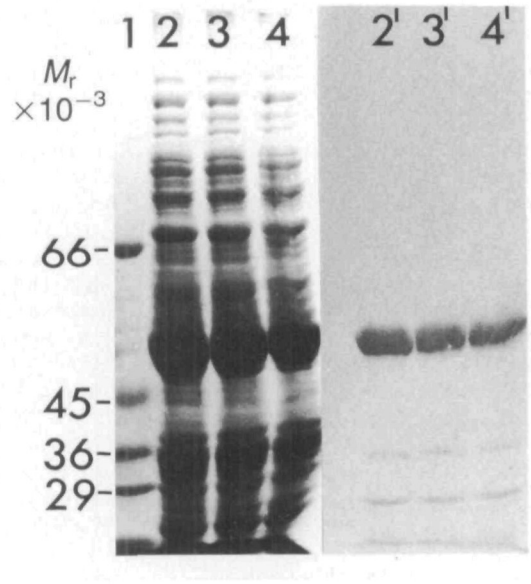

Fig. 2. SDS–PAGE and immunoblot analysis of 3 different clones of K8/tailless expressed in *E. coli*. Lanes 2–4 show total extracts from bacteria expressing K8/tailless stained with Coommassie Blue. Lanes 2′–4′ show the corresponding immunoblot reaction after incubation with the monoclonal antibody IFA (Pruss *et al.* 1981) and an alkaline phosphatase-coupled second antibody. Reference proteins in lane 1 are (M_r): BSA (66 000), ovalbumin (45 000), glyceraldehyde-3-phosphate dehydrogenase (36 000) and carbonic anhydrase (29 000).

column at a concentration of ~125 mM guanidine–HCl contained K8/tailless at a purity of at least 95% (Fig. 3, lane 4).

Although *in vitro* reconstitution experiments of keratins do not require pure protein preparations, and cycles of disassembly and assembly can be used as a purification tool (Steinert *et al.* 1980; Magin *et al.* 1983), the chromatography on Mono Q was recognized as an essential step in the study of K8/tailless. Inclusion body preparations contain at least one bacterial protease that is inactive in 8.5 M urea and therefore does not interfere with the purification steps performed in urea. However, upon dialysis against filament buffer without urea, the protease regains activity and converts keratins to their rod domains or even smaller fragments, which cannot assemble into filaments (data not shown). In the case of K8/tailless the protease is removed by the ion exchange purification step.

Filament assembly from K8/tailless and K18

As a filament partner for K8/tailless we first chose K18, which represents the typical *in vivo* partner of K8 (Moll *et al.* 1982). This protein was also expressed as a recombinant protein using a mouse K18 cDNA clone. It was purified from bacterial lysates as inclusion bodies followed by single-stranded DNA affinity chromatography as described (Hatzfeld and Weber, 1990). Wild-type K8 or K8/tailless were mixed with equimolar amounts of wild-type K18 and dialysed against assembly buffer. Fig. 4A and B document the typical appearance of reconstituted keratin filaments when normal K8 was used together with K18. After a 2 h dialysis, short filaments as well as yet unpolymerized material could be seen (Fig. 4A). Overnight dialysis provided practically complete filament as-

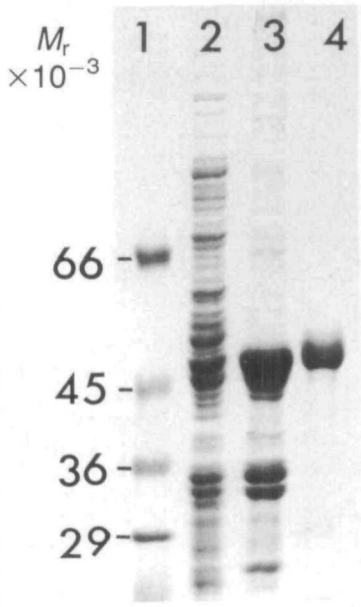

Fig. 3. SDS–PAGE demonstrating the purification of K8/tailless. Reference proteins in lane 1 are (M_r): BSA (66 000), ovalbumin (45 000), glyceraldehyde-3-phosphate dehydrogenase (36 000) and carbonic anhydrase (29 000). Lane 2, total extract from bacteria expressing K8/tailless. Lane 3, preparation of inclusion bodies from the same bacterial culture. Lane 4, peak fraction from Mono Q chromatography containing essentially pure K8/tailless (≥95%).

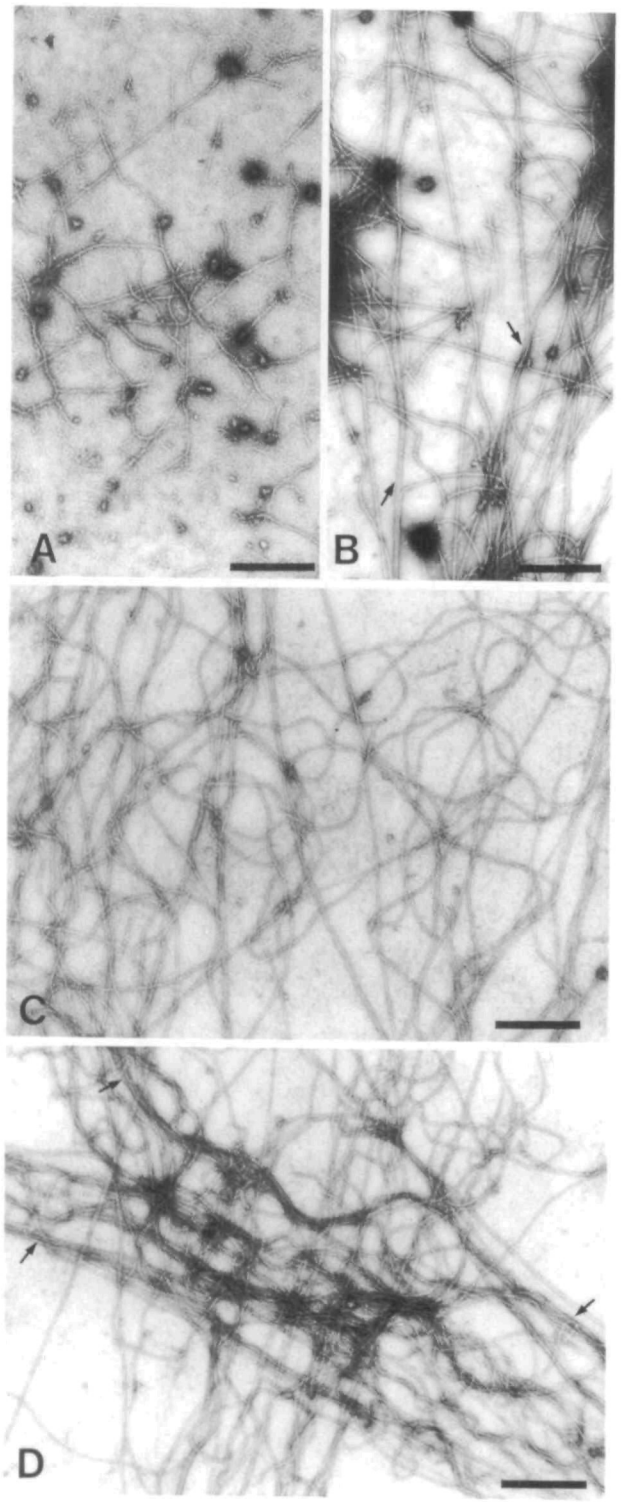

Fig. 4. Electron micrographs of IF assembled from K18 mixed with either K8/wild-type or K8/tailless. Purified keratins were mixed in equimolar amounts in 8.5 M urea and dialysed to standard filament buffer at pH 7.5. Samples were negatively stained with 2% uranyl acetate. (A) IF containing K8/wild-type and K18 stained after a 2 h dialysis. (B) Same sample as in A after overnight dialysis. (C and D) IF formed by K8/tailless and K18 after overnight dialysis; C shows long individual IF whereas D shows an area where IF tend to associate to small filament bundles. Some of these bundles are indicated by arrows. Bars: in A–D, 0.4 μm.

sembly (Fig. 4B). Most of the filaments were of considerable length and in some places on the grid several filaments were laterally associated into small bundles (arrows in Fig. 4B). Fig. 4C and D shows the corresponding filament preparations obtained by K8/tailless and K18/wild-type under identical assembly conditions. After overnight dialysis extended networks of filaments could be seen with negligible protofilamentous material present in the background. These filaments showed the same tendency to associate into small bundles (arrows in Fig. 4D) as the wild-type keratins. As observed with K8/wild-type, the mutant K8/tailless assembled only into filaments when mixed with the complementary type I keratin, K18. Thus, the tail domain of the type II keratin 8 is not essential for filament formation with its typical *in vivo* filament partner, the type I keratin 18.

Filament formation from two tailless keratins

To explore the possibility that *in vitro* filament elongation could require only the intact tail domain of one of the keratins in the heterodimer (Hatzfeld and Weber, 1990), we also analyzed the filament-forming capacity of the mixture of K8/tailless and keratin 19. This type I keratin is the only naturally occuring tailless keratin. It has a 13 amino acid extension of the rod domain but lacks the typical non-α-helical tail domain (Bader *et al.* 1986; Stasiak *et al.* 1989; see also Fig. 1B).

Fig. 5A shows the assembly products obtained from K8/tailless and human K19 after overnight dialysis against standard filament buffer, pH 7.5, which allowed the complete assembly from K8/tailless and K18 (Fig. 4). While K8/tailless and K19 were clearly able to form filaments, these structures were relatively short and were visualized against a strong background of protofilaments, suggesting that the *in vitro* reconstitution conditions were not optimal. To increase the relative yield of filaments, we changed the pH of the assembly buffer from 7.5 to 7.0, since optimal reconstitution conditions can vary for each specific pair of keratins (Hatzfeld and Franke, 1985; Eichner *et al.* 1986). After overnight dialysis against filament buffer at pH 7.0, K8/tailless and K19 formed long filaments with very little protofilamentous material seen in the background (Fig. 5B–D). Areas with extended networks of individual filaments (Fig. 5C) as well as some bundles of several laterally associated filaments (Fig. 5D) were typical for the assembly of the two tailless keratins.

Discussion

In this study we examined the importance of the C-terminal tail domains for keratin filament assembly *in vitro*. Using site-directed mutagenesis and expression of the mutant keratin 8 in *E. coli* we produced a truncated type II keratin lacking the tail domain. Purification of the mutant keratin 8 and *in vitro* assembly studies with keratins 18 and 19 indicate that there is no obligatory requirement for the tail domains of either type I or type II keratins in the *in vitro* filament formation.

Previous studies on the function of the non-helical terminal domains in IF assembly showed that the N-terminal head plays an essential role in IF assembly (Kaufmann *et al.* 1985; Traub and Vorgias, 1983; Sauk *et al.* 1984). The observation that proteolytic removal of half of the tail domain of desmin did not interfere with filament formation (Kaufmann *et al.* 1985) suggested that the entire tail domain is possibly not involved in filament

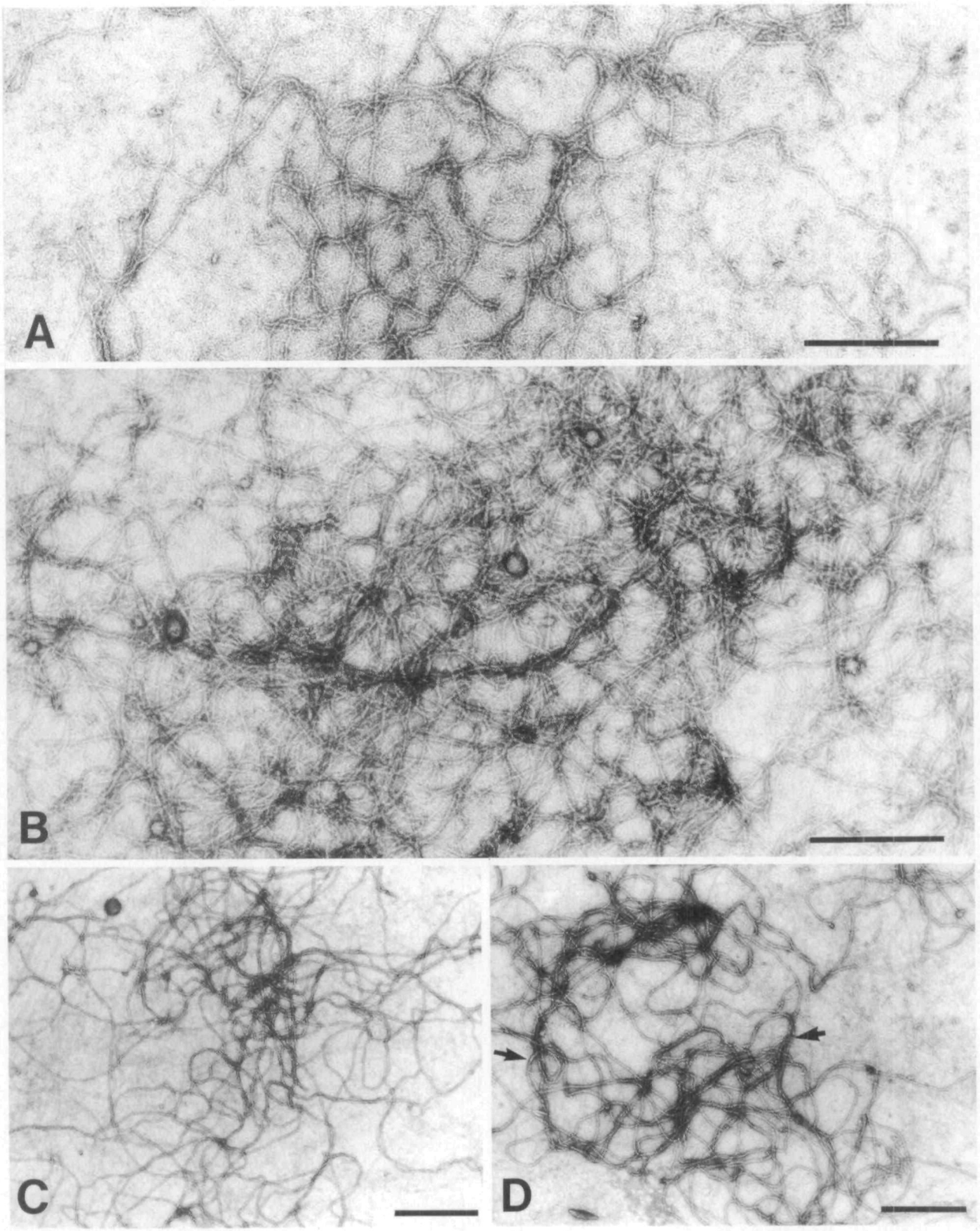

Fig. 5. Electron microscopy of IF assembled from K8/tailless and K19. (A) An equimolar mixture of K8/tailless and K19 was dialysed overnight against standard filament buffer at pH 7.5. The preparation shows rather short IF and a large amount of protofilamentous material indicating that polymerisation was not complete. (B–D) Same sample as in A dialysed to filament buffer at pH 7.0. Extended networks of typical long IF can be seen in B; C shows some individual filaments whereas in D some filament bundles can be detected, as indicated by arrows. Bars: A–D, 0.4 μm.

formation. In contrast, Quinlan *et al.* (1989) demonstrated that removal of the entire tail domain of a particular recombinant GFAP resulted in an assembly-incompetent molecule. Taken together the two *in vitro* reconstitution studies suggested that the part of the tail domain close to the rod region carries an important function in filament assembly.

The functional importance of the three-domain structure of IF proteins has also been previously explored with cDNA clones, which had been altered by site-directed mutagenesis. When mutant cDNAs encoding less than the entire rod domain of desmin and keratin 14 were introduced by transfection into fibroblasts and epithelial cells, respectively, the endogeneous system of vimentin and keratin filaments was perturbed or destroyed (Albers and Fuchs, 1987; van den Heuvel *et al.* 1987). In contrast, mutant cDNAs lacking the coding sequence of the tail domain left the endogeneous system of vimentin and keratin filaments intact and the tailless mutant proteins were readily incorporated. The latter results did not distinguish between two different structural models. Either the tail domains do not directly contribute to IF assembly or they are only needed in substoichiometric quantities and thus the endogeneous IF proteins of the transfected cells compensate for the lack of tails in the mutant proteins. A precedent for a substoichiometric contribution of the terminal domains is known from *in vitro* studies on desmin. While a headless desmin cannot form filaments on its own it is incorporated into filaments when mixed with intact desmin prior to renaturation from urea (Kaufmann *et al.* 1985). Keratin 19, a type I keratin, is a natural tailless IF protein, since it has only 13 residues past the rod domain (Bader *et al.* 1986; Stasiak *et al.* 1989). Nevertheless, it is effectively incorporated into filaments even in those epithelial cells where it accounts for the majority of keratin I molecules. It also forms *in vitro* filaments with all keratin II molecules tested (Hatzfeld and Franke, 1985). The existence of K19 and the transfection experiments with tailless keratin 14 mutants leave the possibility that the tail domain of type II keratins, in contrast to the tail domain of type I keratins, directly participates in assembly, since the tail domains of type I and type II keratins are quite distinct in sequence. Only type II keratins carry a unique 20-residue homology sequence (H2 domain) proximal to the rod domain (Steinert *et al.* 1985*b*; Steinert and Roop, 1988). By deleting the tail domain of K8 except for the first six residues we have now designed a tailless keratin II molecule. Its isolation from transformed *E. coli* has provided the possibility of assessing the importance of the tail domain directly by *in vitro* assembly experiments.

As K8/tailless and the intact recombinant K18 form morphologically normal IF under standard keratin assembly conditions, the entire tail domain of type II keratins is clearly not required for filament formation *in vitro*. Parallel experiments with K8/tailless and K19, a naturally tailless keratin I molecule, revealed suboptimal filament formation under the same assembly conditions. However, when the pH of the assembly buffer was lowered from 7.5 to 7 the mixture of the two tailless keratins showed normal filament formation. We therefore conclude that at least *in vitro* the tail domains of keratins are not an obligatory structural requirement for IF formation. Nevertheless, the differences in assembly at pH 7.5 and 7.0 make it possible that the presence of a single tail domain on a keratin heterodimer can provide additional stabilization of the filament formed by a specific keratin pair. Therefore, one could speculate that the epidermal keratins with their long (head and) tail domains form particularly stable filaments.

Our results on tailless keratins are not readily compatible with the recent report of Quinlan *et al.* (1989) that a recombinant GFAP-fusion protein lacking the tail domain did not form IF *in vitro*. However, in their discussion the authors raise the possibility that the substitution of the head domain by a CII sequence may already have introduced an inherent instability, which is further amplified once the tail domain is deleted. In addition, the GFAP derivate used by Quinlan *et al.* (1989) lacked the tail entirely, while K8/tailless still contains six additional residues. These few residues might stabilize the conformation at the end of the coiled coil rod domain containing the consensus sequence and thus allow IF assembly (see also Phillips *et al.* 1986, for the molecular ends of the tropomyosin coiled coil).

Given our experience with keratins we note the importance of exploring slight changes in assembly conditions. This observation is not without precedent. In studies of normal keratins, various assembly conditions have been used, depending on the keratin composition of the sample (Eichner *et al.* 1986; Hatzfeld and Franke, 1985). Finally, we note a possible technical problem arising with recombinant IF proteins in *E. coli*. Owing to their segregation into inclusion bodies, relatively pure preparations of IF protein can be readily obtained by dissolving these bodies in 8 M urea. In a study of five keratins or mutant keratins as well as vimentin we have observed that such preparations include at least one bacterial protease. While this enzyme is inactive in 8 M urea it can complicate assembly studies. Upon removal of the denaturant in the assembly reaction the enzyme is reactivated and can now degrade the IF proteins to the rod domain and even smaller fragments, which cannot form filaments. This problem is readily overcome by additional chromatography steps (see Materials and methods) and monitoring of the assembly products by gel electrophoresis.

In conclusion, our results indicate that *in vitro* keratin assembly and probably the assembly of other IF proteins as well does not involve an obligatory contribution by the tail domains. Thus the current evidence focuses on intact rod domains and an additional contribution from the head domain (for references, see Introduction). However, *in vivo* the situation might be more complex: if *in vivo* IF assembly is indeed a vectorial process, which involves a contribution from the nuclear compartment, the situation may be changed as the tail domains are thought to provide an interaction with lamin B that is not yet understood (Georgatos and Blobel, 1987). While we expect tailless keratin filaments also to be formed *in vivo* they may not necessarily traverse the cytoplasm in the way normal IF are seen to do. Moreover, cell type-specific interactions with cytoplasmic structures or proteins might depend on tail domains.

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