STRUCTURE OF INTERMEDIATE FILAMENTS

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Intermediate filaments comprise a class of homologous but evolutionarily distinct cytoskeletal proteins. Extensive sequence determinations demonstrated the relatedness to wool keratines and provide a basic picture of the common domain organization (1). Because of the limited coherence length in x-ray fiber diffraction, intermediate filament structure has been subject to controversy for over 30 years. Characterizing subassemblies provides valuable boundary conditions for the elucidation of their quarternary topology.

Fig. 1 shows the principal building blocks of intermediate filaments and demonstrates the coexistence of two polymorphic forms of tetramers. The experimental study of the assembly pathway was complemented by theoretical analysis of the primary sequence (2) aimed at predicting nonrandom patterns of amino acids and attempting their physical interpretation in terms of function. Fig. 2 illustrates that the principal topology will be preserved for a range of geometries and this feature should be important to preserve filament integrity under torsional stress. It is conceivable that proteins with mechanoelastic functions may not be described by a conventional set of unique atomic coordinates. Fig. 3 shows the predicted low-resolution quarternary structure for the bonding type found in staggered tetramers consistent with experimental evidence. The nonstaggered tetramers presumably represent a 4 α-helix supersecondary structure, but details are not known. Fig. 4 summarizes the assembly of intermediate filaments, which initially proceeds mostly laterally. It reveals the existence of several distinct lateral bonding

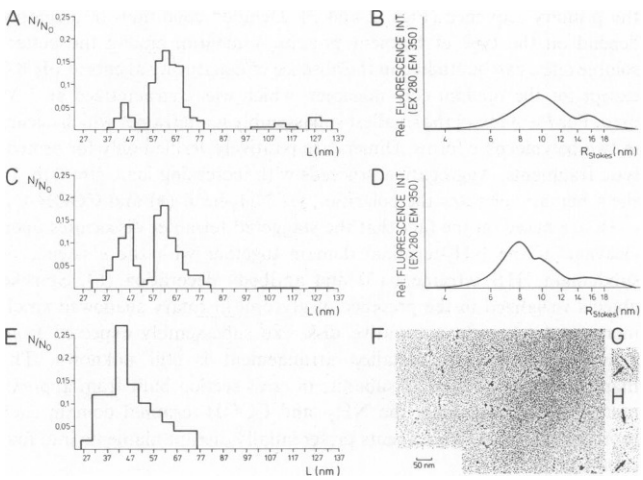

FIGURE 1 Vimentin was isolated from ATCC-C6 rat glioma tissue culture and purified by a novel HPLC scheme in the presence of 7 M urea.[1] The urea was removed by gel permeation chromatography (GPC) and the peak (*A*, *B*) and trailing edge (*C*, *D*) of the eluting oligomer analyzed by rotary shadowing electron microscopy (*A*, *C*) and calibrated GPC (*B*, *D*). The staggered tetramers are 63.9 ± 5.3 nm long with a thickened central overlap region (*G*) and molecular weight 200 ± 20 × 10^3 d and 70 nm hydrodynamic length derived from R_{Stokes} = 9.7 nm and 4.8S (3).[1] Proteolytic cleavage of NH_2- and COOH-terminal domains indicate that they are situated laterally in solution. Based on their Stokes radius the 44.8 ± 5.0 nm-long particles are a polymorphic form of nonstaggered tetramers that show terminal knobs in fortuitous cases (*H*). In 3.5 M urea the average length of the inhomogeneous population of oligomers is 47.0 ± 11.4 nm (*E*, *F*).

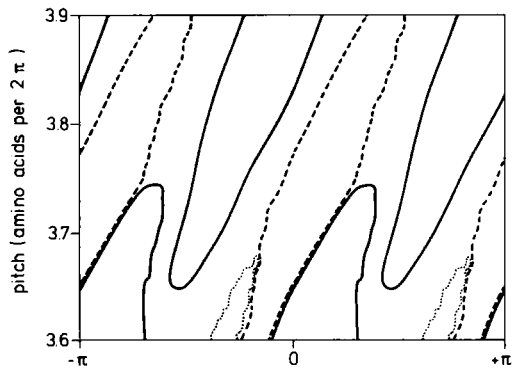

FIGURE 2 Angular distribution of amino acids on the surface of the dimer coiled coil averaged over vimentin subdomain H2(*C*) (amino acids 350–411). Shown are the contours of 10% significance at various pitches of the supercoil: devoid of charges (—), hydrophylic (---), charged but not hydrophylic (...). Hydrophobic and hydrophylic amino acids are segregated into distinct coaxial patches that are preserved for a range of pitches. This feature is strongest with subdomain H1B (data not shown). Based on current knowledge of protein folding, this hydrophobic girdle will provide the lateral interaction within the staggered tetramers and the subfilaments. It corresponds to a a distinct periodicity of 3.74 amino acids observed by auto correlation-Fourier transform analysis of the primary sequence (4).[2]

[1]Potschka, M., R. Timpl, F. Leichtfried, H. Winkler, and G. Wiche. 1985. The principal substructure of vimentin intermediate filaments. Manuscript submitted for publication.

[2]Potschka, M. 1985. Comparative analysis of hydrophobicity codes. Manuscript submitted for publication.

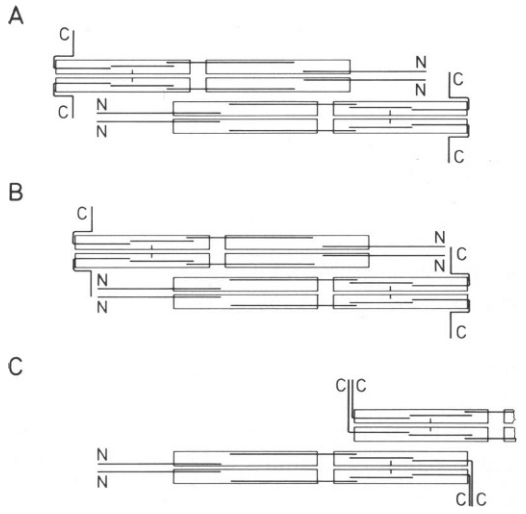

FIGURE 3 Schematic model of the predicted packing of subunits. Based on the angular distribution of amino acids (Fig. 2) and periodic distributions of side chain volume, only a limited number of three-dimensional models may be constructed. Among them only two alignments of subdomain H1 (*A*, *B*) and one along subdomain H2(*C*) are compatible with experimental evidence. This provides a structural resolution close to 1 nm for the core of the tetramer and octamer assemblies. A quasi-C_6 symmetry of positive or negative charges is presumably related to further lateral packing. Whether this contemporary knowledge of the forces responsible for polypeptide packing is correct remains subject to ongoing experimental verification.

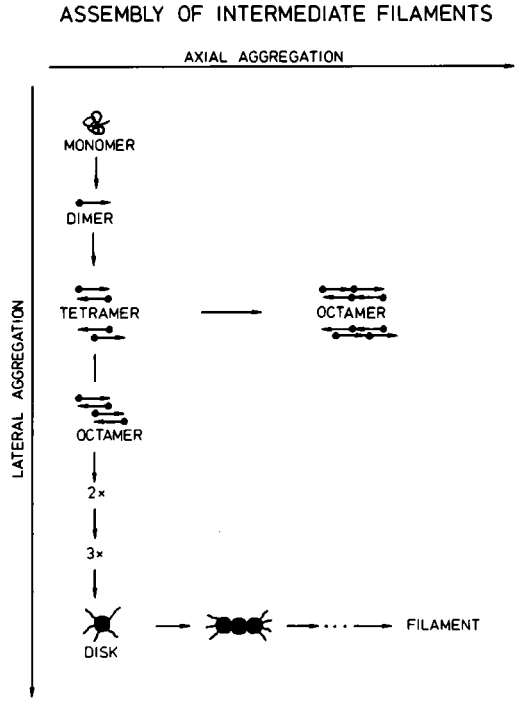

FIGURE 4 Tentative pathway of assembly based on hydrodynamic data and electron microscopy (Fig. 1) and supported by theoretical analysis of the primary sequence (Figs. 2 and 3). Detailed conditions of assembly depend on the type of filament protein. Vimentin, among the better-soluble ones, can be studied in the absence of denaturing agents at pH 8.0 except for the random coil monomer, which was characterized at 7 M urea. For $I \geq 3$ mmol the smallest subassembly is a tetramer which occurs in two polymorphic forms. Dimers are positively verified only for proteolytic fragments. Aggregation proceeds with increasing ionic strength. In the schematic pictures the polarities, viz NH_2-head (●) and COOH-tail (→), are based on the fact that the staggered tetramer dissociates upon cleavage of the NH_2-terminal domain together with the existence of subdomain H1B tetramers (5) and antibody decoration (6). Starlike objects visualized in the presence of glycerol in rotary shadowed specimens may represent a putative disk like subassembly expected from general principles. Its detailed arrangement is still unknown. The filaments contain 28 or 32 subunits in cross section built from approximately coaxial α-helices; the NH_2- and COOH-terminal domain each protrude laterally. Some agents preferentially disrupt filaments into four subfilaments.

hierarchies within the filament; those within dimers; those between dimers of subdomain H1 and H2; and those between dimers of equal subdomains. Subfilaments with eight subunits in crossection may be constructed with these features. To obtain filaments from these a further bonding level must be implicated. Thus a single subunit assembles with at least four nonequivalent lateral bonding patterns in hierarchical order.

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