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**STRUCTURAL STUDIES ON THE
NUCLEAR LAMINS
AND OTHER
INTERMEDIATE FILAMENT PROTEINS**

A thesis presented in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy in Biophysics at
Massey University.

James Frederick CONWAY

October, 1989

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ABSTRACT

A number of aspects of IF chain and molecular structure, as well as molecular aggregation, have been examined. These include the delineation of periodicities in the sequences of structural domains of IF proteins, the distribution of amino acid residues within the heptad substructure, the flexibility of the peptide backbone, the extent of homology among the IF proteins, the packing of chains in the dimeric molecule, and the axial packing of molecules in the IF. Particular focus has been placed on the newly sequenced type V IF proteins (the nuclear lamins and the *Helix pomatia* B protein) and on a type III IF protein (peripherin).

A parallel in-register arrangement of chains in the molecule is predicted for peripherin and the type V chains from a consideration of interchain ionic interactions. Also, periodicities in the linear distribution of charged residues in the rod domains of these proteins are shown to be comparable with periods in other IF chains. Ionic interactions between lamin molecules have been used to assess the likely modes of molecular aggregation in an *in vitro* assembly and a model is presented which also satisfies the constraints imposed by electron microscope data. In this model, antiparallel arrays of molecules are half-staggered and an extended conformation for the carboxy-terminal domains is predicted. Simple explanations are given for the transition between paracrystalline and lattice structures and for the disassembly of the lamin meshwork concomitant with hyperphosphorylation. The method of calculating intermolecular ionic interaction profiles is enhanced and a new, three-dimensional method is developed.

The inhomogeneous distribution of residues in the heptad substructure can be correlated to the coiled-coil structure and chain packing in the molecule. In particular, the ~75% occupancy rate of apolar residues in the internal a and d heptad positions is shown to be a general feature of α -fibrous proteins. Variability of residues in the outer b, c and f positions indicates that structural or functional specificity in the rod domain may be determined by these parts of the sequence. The predicted flexibilities of IF chains have been compared to the underlying structure for the chains.

Evidence from sequence homology studies suggests that several new subtypes are appropriate in the classification scheme. For the hard keratins the terms types Ia and IIa are proposed and for the soft keratins, types Ib and IIb; the need to separate the neurofilaments into the type IV class separate from the type III IF chains is confirmed; and division of the type V chains into cytoskeletal and karyoskeletal groups is indicated. A more detailed delineation is made of regions within the amino- and

carboxy-terminal domains than has been possible previously. Periodic features of the homology profiles for the rod domain are examined and found to be similar to those in the linear distribution of residues in the amino acid sequences. Comparison between amphibian and mammalian keratins, and also between hard and soft keratins reveals that type II chains are maintained at a higher level of fidelity than type I chains. Consensus rod domain sequences are derived for the various IF subtypes: absolutely conserved regions of primary structure identify types or subtypes.

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TABLE OF CONTENTS

1. An Overview of Intermediate Filament Structure	1
1.1..... Early Structural Work on Keratin IF	2
1.2..... Soft Keratin and Other IF.....	7
1.3..... Current Models of Intermediate Filament Structure	10
1.4..... Conservation of Amino Acid and Gene Sequences.....	15
1.5..... Lamin, Peripherin and the Helix A and B proteins	17
1.6..... Function of Intermediate Filaments.....	22
1.7..... Structural Form of the Thesis	25
2. Primary Structure.....	26
2.1..... Fourier Analysis	29
2.1.1..... Fourier Analysis - Method.....	30
2.1.2..... Fourier Analysis - Results	33
2.2..... Residue Distribution in the Heptad	37
2.3..... Flexibility.....	41
2.4..... Summary	47
3. Sequence Homology.....	50
3.1..... Homology Statistics.....	51
3.1.1..... Amino Acid Homology Score, h_a	52
3.1.2..... Residue Homology Score, h_r	52
3.1.3..... Segment Homology Score, h_s	54

3.2.....	Homology within the Rod Domain.....	54
3.2.1	Coiled-Coil Segments	56
3.2.2.....	Link Segments	57
3.2.3.....	Type V Chains	61
3.3.....	Subtyping on the basis of Homology	61
3.3.1	Hard and Soft Keratin IF Chains	61
3.3.2.....	Type IV IF Chains.....	62
3.4.....	Homology among the 'H' subdomains	63
3.5.....	Comparison of Amphibian and Mammalian Scores	66
3.6.....	Periodic Features in the Homology Score Distributions	69
3.7.....	Consensus Sequences for IF Chain Types	78
3.8.....	Summary	81
4.	Secondary and Tertiary Structure	84
4.1.....	1D Ionic Interactions Between Chains	85
4.2.....	1D Ionic Interactions Between Molecules	90
4.3.....	Modelling Lamin.....	96
4.4.....	3D Ionic Interactions Between Molecules	110
4.4.1.....	Generation of Coordinates for a Coiled-Coil Molecule.....	112
4.4.2.....	Determination of Interactions.....	113
4.4.3.....	Analysis of the Interaction Maps.....	117
4.5.....	Summary	120
5.	Summary	123

Appendices	131
Appendix A : Zero-Filling prior to Fourier Transformation	131
Appendix B : Fourier Transforms	135
Appendix C : Curve Smoothing	149
Appendix D : Intermolecular Ionic Interactions	150
Bibliography	161
Publications	189

LIST OF FIGURES

Figure 1-1.... Electron micrographs of IF <i>in vitro</i>	1
Figure 1-2.... Electron micrograph of wool microfibrils in cross-section	3
Figure 1-3.... Schematic representation of the IF protein chain.....	9
Figure 1-4.... Schematic representation of the heptad substructure	12
Figure 1-5.... Schematic comparison of IF and lamin chain structures	20
Figure 2-1.... Example of applying the baseline correction operation prior to the Fourier transform	32
Figure 2-2.... Comparison of the human and <i>Xenopus</i> lamin A protein sequences.....	35
Figure 2-3.... Flexibility profiles for a selection of IF chains.....	43
Figure 3-1.... Homology profiles for the IF chain types	55
Figure 3-2.... Comparison of the link segments L1, L12 and L2	59
Figure 3-3.... Fourier transforms of the homology profiles.....	70
Figure 3-4.... Consensus sequences of the rod domain	79
Figure 4-1.... Intermolecular ionic interaction curves for human lamin A	92
Figure 4-2.... Electron micrographs of lamin paracrystals	98
Figure 4-3.... Diagram of staggered arrays of lamin molecules in Model A..	100
Figure 4-4.... Diagram of staggered arrays of lamin molecules in Model B..	101
Figure 4-5.... The alignment of conserved sequences	106
Figure 4-6.... Comparison of Models A and B	108
Figure 4-7.... Schematic of a lattice collapsing into a paracrystal.....	109

Figure 4-8.... Electron micrograph of a tropomyosin lattice/paracrystal structure.....	110
Figure 4-9.... Schematic of the relative orientations of molecules	112
Figure 4-10 .. Schematic of a pair of segment 1B dimers.....	116
Figure 4-11 .. Intensity map of the 3D intermolecular ionic interactions	117
Figure A-1 ... Rectangle and sinc functions - Fourier pairs	133
Figure B-1 ... Fourier transforms for peripherin.....	136
Figure B-2 ... Fourier transforms for human lamins A and C.....	138
Figure B-3 ... Fourier transforms for <i>Xenopus</i> lamin A	141
Figure B-4 ... Fourier transforms for <i>Xenopus</i> lamin B	144
Figure B-5 ... Fourier transforms for <i>Helix pomatia</i> B	147
Figure D-1 ... Intermolecular ionic interaction curves for peripherin	151
Figure D-2 ... Intermolecular ionic interaction curves for human lamin A	154
Figure D-3 ... Intermolecular ionic interaction curves for <i>Xenopus</i> lamin A .	156
Figure D-4 ... Intermolecular ionic interaction curves for <i>Xenopus</i> lamin B .	158
Figure D-5 ... Intermolecular ionic interaction curves for <i>Helix pomatia</i> B ..	160

LIST OF TABLES

Table 1-1	Lengths of the rod domain segments for IF types I-IV	11
Table 2-1	IF amino acid sequences	27
Table 2-2	Comparison of periods present in peripherin and other type III IF proteins	33
Table 2-3	Peaks resulting from multiplying the Fourier transforms together	36
Table 2-4	Residue distribution in the heptad for IF and myosins	38
Table 2-5	Mean flexibility indices for chain segments from a selection of IF chains	44
Table 3-1	Look-up table for mixed homology scores	53
Table 3-2	Look-up tables for acidic homology, basic homology and large apolar homology scores	54
Table 3-3	Regions of high sequence homology ($h_r \geq 90\%$)	56
Table 3-4	Regions of low sequence homology ($h_r < 60\%$)	57
Table 3-5	Mean segment homology scores	58
Table 3-6	Mean segment homology scores for the rod domain segments in soft and hard keratins	62
Table 3-7	Lengths of the homologous subdomains H1 and H2	64
Table 3-8	Extents of the structural domains in IF chains	67
Table 3-9	Mean segment homology scores for the rod domain segments in amphibian and mammalian keratins	68
Table 3-10	A selection of the most significant periodicities in the homology profiles	75

Table 3-11.... Mean residue homology scores for each position in the heptad substructure.....	78
Table 3-12.... Percentage occurrence of highly conserved residues.....	81
Table 4-1 Interchain ionic interactions for peripherin and type V IF	86
Table 4-2 Interchain ionic interactions for a selection of IF	89
Table 4-3 Interchain ionic interactions per dual heptad.....	89
Table 4-4 Significant intermolecular ionic interactions between human lamin A molecules	95
Table 4-5 Significant ionic interactions between peripherin molecules.....	96
Table 4-6 Volume calculation for the C-terminal domains of human lamins A and C.....	104
Table 4-7 Ionic interactions used for Models A and B	105
Table 4-8 Values used to derive a set of five pitch lengths for the coiled-coil.....	111
Table 4-9 Starting set coordinates in an undistorted α -helix.....	113
Table 4-10.... Coordinates for a pair of segment 1B dimers	114
Table 4-11.... Selection of the highest 3D interaction scores (antiparallel) ...	118
Table 4-12.... Selection of the highest 3D interaction scores (parallel).....	119
Table B-1..... Peaks in the Fourier transforms for peripherin.....	135
Table B-2..... Peaks in the Fourier transforms for human lamins A and C...	137
Table B-3..... Peaks in the Fourier transforms for <i>Xenopus</i> lamin A	140
Table B-4..... Peaks in the Fourier transforms for <i>Xenopus</i> lamin B	143
Table B-5..... Peaks in the Fourier transforms for <i>Helix pomatia</i> B	146
Table D-1..... Peaks in the ionic interaction curves for peripherin.....	150

Table D-2..... Peaks in the ionic interaction curves for human lamin A.....	153
Table D-3..... Peaks in the ionic interaction curves for <i>Xenopus</i> lamin A....	155
Table D-4..... Peaks in the ionic interaction curves for <i>Xenopus</i> lamin B....	157
Table D-5..... Peaks in the ionic interaction curves for <i>Helix pomatia</i> B.....	159