PREFACE

The intermediate filament (IF) protein field has witnessed a remarkable growth since the initial description of filamentous arrays that were seen by electron microscopy in muscle cells. These initially described arrays were intermediate in size as compared to actin and myosin filaments or to microfilaments and microtubules (Ishikawa *et al.*, 1968). Hence the name that has ignited a field no one could have envisioned would evolve into its present and continuously growing status. Since those pioneering years of early characterization (Fig. 1), the complexity of this large and heterogeneous family of proteins and their interaction with other cytoskeletal elements continues to unfold and surprise present and past investigators (Helfrand *et al.*, 2003; Herrmann *et al.*, 2003; Hesse *et al.*, 2001; Lariviere and Julien, 2004; Lazarides, 1980; Leung *et al.*, 2002; Moll *et al.*, 1982; Oshima, 1992; Steinert and Parry, 1985). Among the three major categories of cytoskeletal proteins, intermediate filaments are fascinating in part because of their cell type-specific distribution (Table I) and their clearly defined function of protection from mechanical and environmental forms of stress (Fuchs and

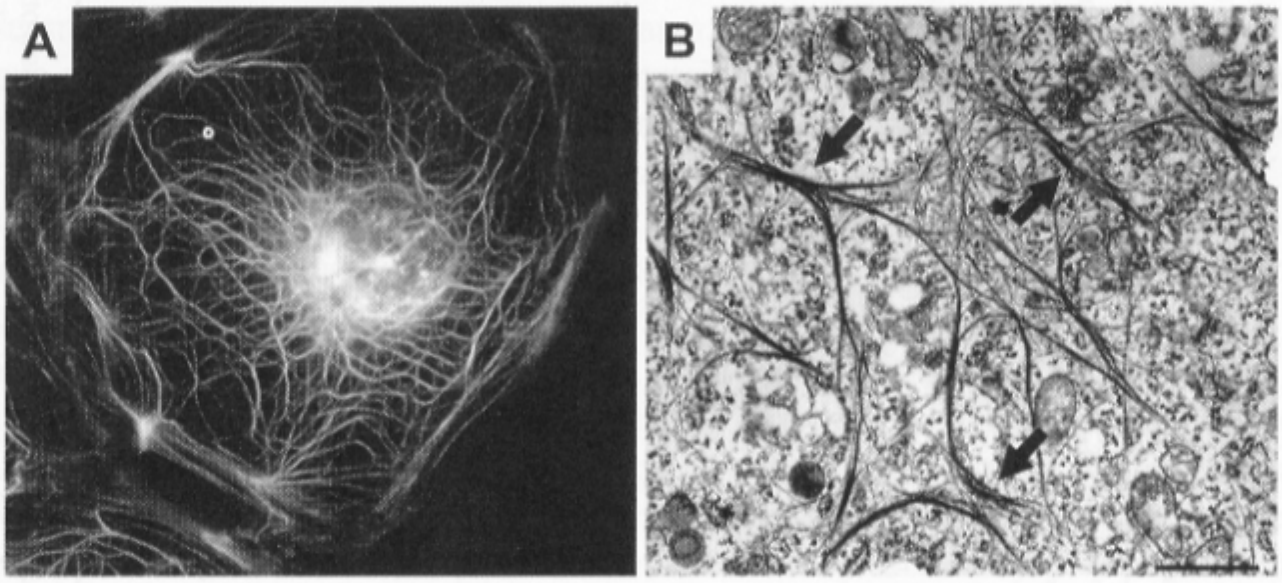

Fig. 1 Intermediate filaments-the early days. Two images reproduced from the pioneering work of Osborn, Franke and Weber (Osborn *et al.*, 1977), when intermediate filament proteins were just being recognized and tools developed for their study. Panel A: Intermediate filaments of methanol/acetone-fixed Pt K2 cells (derived from rat kangaroo kidney epithelium) as visualized by immunofluorescence staining using non-immune normal rabbit serum at a high concentration (1:6 dilution). The reactivity of the antibody was abolished after formaldehyde fixation (not shown). Panel B: Transmission electron microscopy of Pt K2 cells, with arrows highlighting the typically-noted bundles of intermediate filaments (in this case keratin and vimentin filaments). Bar = 1 μm. The images were generously provided by Dr. Mary Osborn.

Table I
Intermediate Filament Proteins

Name	Type[a]	Chromosome[b]	Size (kDa)[b]	No. genes[b]	Assembly group[c]	Cell/tissue distribution
Cytoplasmic						
Keratins	I	17	40–64	>25	A	K9-K20, K23 (Epithelia) Ha1-Ha8 (Hair, nail) Irs1-4 (Hair follicles)
Keratins	II	12	52–70	>25	A	K1-K8 (Epithelia) Hb1-Hb6 (Hair, nail) K6irs1-4 (Hair follicles)
Vimentin	III	10	55	1	B	Mesenchymal
Desmin	III	2	53	1	B	All muscle
GFAP	III	17	52	1	B	Astrocytes
Peripherin	III	12	54–62 [three splice variants]	1	B	Peripheral neurons
Neurofilaments	IV	8	61 (NF-L),	1	B	Central neurons
(L,M,H chains)		8	90 (NF-M),	1		
		22	110 (NF-H)	1		
α-Internexin	IV	10	61	1	B	Central neurons
Nestin	III, IV, VI	1	240	1	B	Neuroepithelial
Synemin	III, IV, VI	15	180 (α) & 150 (β) [two splice variants]	1	B	All muscle (β isoform mainly in striated muscle)
Syncoilin	III, IV	1	54	1	B	Muscle (mainly skeletal/cardiac)
Phakinin (CP49)	Orphan	3	46	1	D	Lens
Filensin (CP115)	Orphan	20	83	1	D	Lens
Nuclear						
Lamins A, C	V	1	62–78 [four splice variants]	1	C	Nuclear lamina
Lamins B1, B2	V	5, 19	62–78	1 each	C	Nuclear lamina

[a]IF sequence type is defined by gene substructure (position of introns) and nucleotide sequence homology for the region coding for the α-helical rod domain shared by all IF proteins. Some genes (nestin, synemin, syncoilin) can be ascribed to more than one sub-groupings depending on the criteria used.

[b]Chromosome assignment and protein size are given for human IF genes and proteins. The number of IF genes is nearly perfectly conserved in mammalian genomes that have been thoroughly investigated (human, mouse, rat).

[c]Assembly group refers to the ability of IF roteins to copolymerize to form 10–12 nm filaments. Keratins (group A) are strict heteropolymers involving type I and type II chains in a 1:1 molar ratio. Several proteins in group B can polymerize on their own to form IFs; however, they can copolymerize with one another *in vitro*. The nuclear lamins and lens-specific filensin and phakinin represent distinct groups.

Cleveland, 1998; Ku *et al.*, 1999) that is becoming understood in biophysical, structural and biochemical terms (Coulombe and Wong, 2004; Strelkov *et al.*, 2003). Intermediate filament proteins also take center stage when it comes to human disease, given the complex array of human diseases that they either cause or predispose to, which is a reflection of their nearly ubiquitous yet tissue specific expression (Omary *et al.*, 2004).

Our goal in assembling this volume was to provide a comprehensive treatise of methodology and systems analysis essentials for any investigator, actively working- or wishing to work- on IF genes and their gene products and associated proteins. Such a methodology resource is presently not available and the series editors felt, and we wholeheartedly agreed, that covering the topic of IF genes and proteins is timely. Our strategy in seeking expert investigators in the field was to cover state-of-the-art methodologies pertaining to 5 major aspects of IF proteins:

i. IF proteins from molecules to human disease: Here, the Herrmann and Aebi laboratories cover structural aspects of IF proteins in *Chapters 1 and 2*; Kole, Tseng and Wirtz cover, in *Chapter 3*, microrheology as a tool to study the mechanical properties of the cytoskeleton in live cells; in *Chapter 4* the Magin laboratory describe state-of-the-art methods to manipulate IF genes in the mouse genome, and comment on the large impact that such models have had on our understanding of IF-based disease and IF function; Marceau and colleagues cover the cleavage of IF proteins during apoptosis in *Chapter 5*; the McLean laboratory covers, in *Chapter 6*, methodologies and practical aspects pertaining to the genetic assessment of IF proteins in human diseases; Leers presents in *Chapter 7* how the tissue specificity and abundance of IF proteins, coupled with flow cytometry and other techniques, led to the use of IF proteins as tumor makers and a tool to assess tumor spread; in *Chapter 8* Zatloukal and colleagues discuss the ability of IF proteins to form cytoplasmic inclusions and how such inclusions can be studied; and *Chapter 9* by Lane and Pekny closes this section by describing various stress models that can be exploited to study IF function.

ii. IF proteins and gene regulation: Strategies that relate to the study of transcriptional control of IF gene expression are discussed by Sinha in *Chapter 10*. Fluorescence-based imaging modalities to study IF protein organization are covered by Flitney and Goldman in *Chapter 11* and by Windoffer and Leube in *Chaper 12*. Regulation of IF protein function and dynamics by posttranslational modifications, with an emphasis on the characterization of IF phosphorylation and the development of phospho-epitope-specific reagents, are discussed in *Chapter 13* by Kawajiri and Inagaki and in *Chapter 14* by Pant, Eriksson and colleagues.

iii. IF proteins in specific mammalian systems: This section of the volume focuses on hair and hair follicle epithelial keratins in *Chapter 15* by Langbein, Schweizer and colleagues; skin and simple epithelial keratins in *Chapter 16* and *Chapter 17* by the Coulombe and Omary laboratories, respectively; muscle IF

proteins in *Chapter 18* by Robson and colleagues; axonal transport of neuronal IF in *Chapter 19* by Millecamps and Julien; lamins in *Chapter 20* by Krohne; and the lens proteins vimentin, phakinin, and filensin in *Chapter 21* by the Quinlan laboratory.

iv. IF proteins in non-mammalian systems: This section recognizes the increasing importance of non-mammalian systems in providing insight into the properties and function of IF proteins, and discusses methods and assays that allow their study. As such, fish IF proteins are extensively discussed by Schaffeld and Markl in *Chapter 22*; Xenopus IF proteins are covered in *Chapter 23* by Gervasi and Szaro; and methodologies to study Caenorhabditis elegans IF proteins are discussed in *Chapter 24* by Fridkin, Karabinos and Gruenbaum.

v. IF-associated proteins: The last section of this volume covers the important topic of IF-associated proteins, an area that is likely to grow as functional and regulatory aspects of IF proteins unfold. Plectin is discussed in *Chapter 25* by the Wiche laboratory; Green, Borradori, Fontao, Weis and colleagues discuss methods to study desmoplakin-IF protein interactions in *Chapter 26*; in *Chapter 27* the Liem laboratory discusses cytolinker proteins and assays to study proteins that regulate IF-microfilament and IF-microtubule interactions; Listwan and Rothnagel discuss IF-binding proteins in the skin in *Chapter 28*; and Ostlund and Worman end this section with *Chapter 29* by covering lamin-associated proteins.

Not all IF proteins could be covered in this monograph due to space constraints, but every effort was made to cover broad categories and, when possible, emphasize a "systems"-oriented approach. We also opted to cover, in the first book of this kind in the field, the most practical and useful techniques to "old" and "new" hands alike. Most chapters include a "Pearls and Pitfalls" section that will hopefully meet its intended goal of providing helpful hints and advice that would otherwise not be routinely seen in the experimental section of manuscripts or in laboratory manuals. In addition, the authors included many useful and detailed tables with relevant reagents, vendors, and important resource websites.

Most if not all of us in this field believe that this is an exciting time for the study of IF genes and proteins. This is certainly reflected in the primary literature, in that the number of research articles dealing with intermediate filaments continues to increase steadily and to unfold their dynamic nature and nonstructural functions. Areas in which important revelations are likely to surface include and are not limited to IF structure, functions pertaining to signaling and other regulatory processes, interactions with other cytoskeletal elements and organelles, characterization of new IF genes and their products, and IF-related disease pathogenesis and treatment. This will be made possible in part through attracting new investigators to this field. We hope that this volume will serve its important purpose of helping new investigators as they establish themselves in the field, assist established investigators wishing to expand their studies on this topic, and disseminate

and possibly standardize the practice of many of the methods and assays that are commonly used when studying IF genes and proteins. In closing, we are most grateful to the series editors, all the contributing authors, and the editorial assistance provided by Elsevier to make this project possible.

M. Bishr Omary
Pierre A. Coulombe
Klaus Weber

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