Journal of Structural Biology 177 (2012) 14–23

Contents lists available at SciVerse ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Inroads into the structure and function of intermediate filament networks

Robert D. Goldman *, Megan M. Cleland, S.N. Prasanna Murthy, Saleemulla Mahammad, Edward R. Kuczmarski

Department of Cell and Molecular Biology, Northwestern University's Feinberg School of Medicine, 303 E. Chicago Avenue, Chicago, IL 60611, USA

ARTICLE INFO

Article history:
Available online 18 November 2011

Keywords:
Intermediate filaments
Vimentin
Keratin
Cell motility
Phosphorylation

ABSTRACT

Although intermediate filaments are one of three major cytoskeletal systems of vertebrate cells, they remain the least understood with respect to their structure and function. This is due in part to the fact that they are encoded by a large gene family which is developmentally regulated in a cell and tissue type specific fashion. This article is in honor of Ueli Aebi. It highlights the studies on IF that have been carried out by our laboratory for more than 40 years. Many of our advances in understanding IF are based on conversations with Ueli which have taken place during adventurous and sometimes dangerous hiking and biking trips throughout the world.

1. Introduction

This paper is written as a tribute to Ueli Aebi's remarkable career in the fields of structural and cell biology. In honor of his formal retirement from the University of Basel we have chosen to relate our own history of discovery in the area of intermediate filament structure and function, a field of research in which Ueli has made major contributions.

2. Historical overview

Intermediate filaments (IF) remain the least studied and understood of the three major cytoskeletal systems that are expressed in virtually all vertebrate and many invertebrate cell types. From a historical perspective, this is probably related to the fact that even though their constituent subunits typically form 10 nm filaments, the protein composition of these subunits is not conserved as they are encoded by ~75 different genes. Contributing to the complexity is the fact that the expression of these genes is developmentally regulated in a cell and tissue type specific fashion. As a result of the enormous diversity in their subunit composition, it took many years for cell biologists and biochemists to realize that all of the 10 nm IF seen in different types of cells were derived from the same large protein family. It is likely that the first descriptions of IF were made over a hundred years ago by the pioneering studies of cytologists and histologists who developed various methods to generate contrast in the bright field microscope (for review see (Wilson, 1928)). These methods employed various fixatives and stains which revealed extensive arrays of filamentous structures in nerve cells and in epithelial cells (Wilson, 1928). In the former, the fibrillar patterns are highly reminiscent of the organization and distribution of neurofilaments composed of the Type IV IF proteins (e.g., neurofilament triplet proteins) and in epithelial cells, the "fibrillae" or "tonofibrils", are now known to be bundles of closely packed Types I and II keratin IF (Fig. 1A).

Following the introduction of techniques developed for studying cell fine structure using electron microscopy, 10 nm IF were described in numerous cell types (Goldman and Follett, 1969) (see Fig. 2). However, the nomenclature used in the 1960s and 1970s to describe them caused significant confusion. During this period IF were called 10 nm and 100 Å filaments, decafilaments, 9 nm filaments, 110 nm filaments, 80–100 Å filaments, 'intermediate filaments' in muscle cells (the latter name is based on the cross sectional diameter of IF which lies between actin and myosin filaments), neurofilaments in neurons, tonofilaments for the keratin IF in epithelial cells, glial fibrillary acidic filaments in glial cells and sarcoplasmic filaments for skeletal muscle cell IF. In addition, the names of their purported protein subunits varied widely and included muscle skeletin or desmin; glial fibrillary acidic protein (GFAP); fibroblast 10 nm filament protein, fibroblast intermediate filament protein, vimentin or decamin; peripheral and brain neurofilament protein; keratin and tonofilament protein, etc. (for review see (Eriksson and Thornell, 1979)). Eventually, the name of intermediate filaments became widely accepted, based mainly on the fact that the diameter of IF was between that of actin/microfilaments and microtubules.

It was not until the late 1970s and early to mid-1980s that cell biologists began to realize that, although there were differences,

* Corresponding author. Fax: +1 312 503 0954.
E-mail address: r-goldman@northwestern.edu (R.D. Goldman).

1047-8477/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jsb.2011.11.017

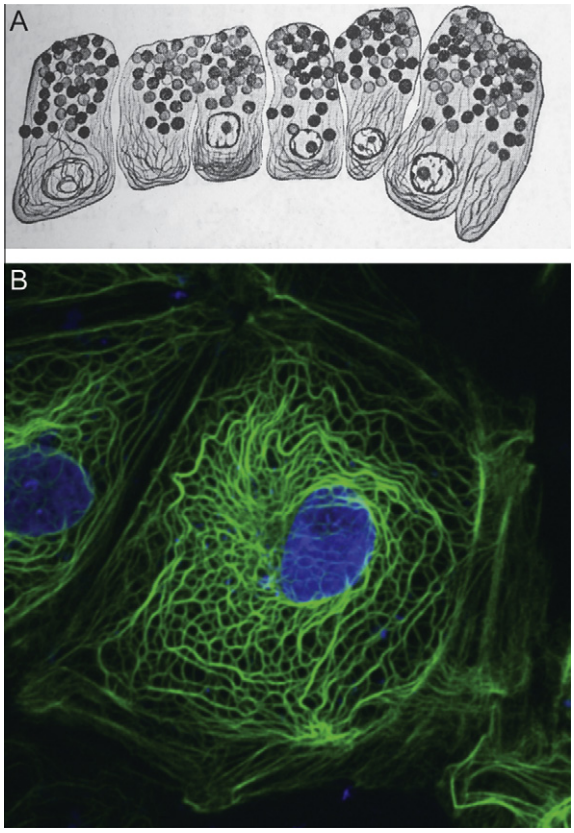

Fig.1. (A) Early observations of secretory epithelial cells in the mudpuppy pancreas. Note the "fibrillae" which most likely represent keratin IF bundles or tonofibrils. Work of Mathews in the laboratory of E.B. Wilson. From reference Wilson, 1928. (B) A PtK2 epithelial cell fixed and processed for immunostaining with anti-keratin. Note the similarities in the tonofibrils seen in the preparations spanning 80 years of research.

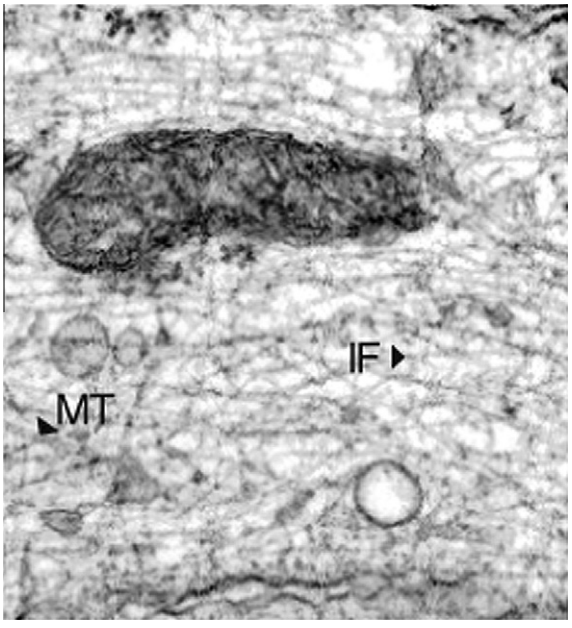

Fig.2. Electron micrograph of a human fibroblast showing the cytoplasm containing numerous 10 nm vimentin IF (IF) and a few microtubules (MT).

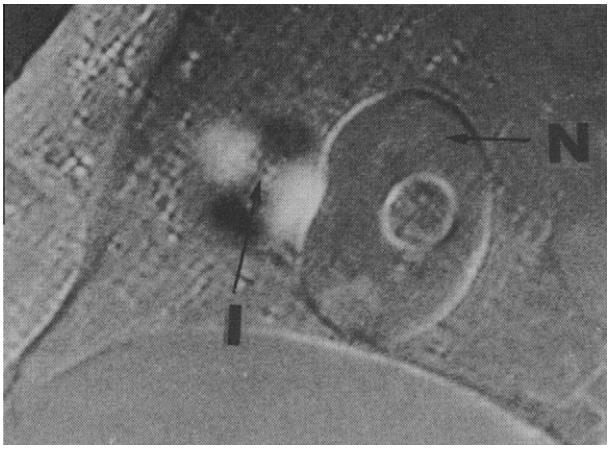

Fig.3. A living BHK-21 cell observed during the early stages of cell spreading by polarized light microscopy. The birefringent sphere comprised of dark and bright sectors surrounds an isotropic region (I). This structure is closely associated with the nucleus (N). From reference Goldman and Follett, 1970.

there were also very significant similarities in the proteins comprising the different IF systems. These similarities began emerging when comparative structural and biochemical methodologies, along with the introduction of cloning and sequencing of cDNAs, made it possible to determine the specific properties of IF derived from a wide range of cell types. Now we know that IF assemble from a coiled–coiled dimer. The region responsible for dimer formation is the highly conserved, α-helix rich, central rod domain which is a common feature of all cytoskeletal IF proteins. These dimers associate in a hierarchical fashion to form IF which differ from each other primarily in the size and the amino acid composition of their N- and C-terminal non-α-helical domains. These discoveries are based primarily on work from the laboratories of Peter Steinert, David Parry, Ueli Aebi and Harald Herrmann (Dowling et al., 1983; Herrmann et al., 1996, 1999; Parry et al., 1985, 1986, 2007; Steinert et al., 1976). During this same period, the development of antibodies directed against IF proteins and advancements in fluorescence microscopy revealed the presence of extensive arrays of complex cytoskeletal networks of IF, typically extending from the perinuclear region to the cell surface (Starger et al., 1978; Sun and Green, 1978) (Fig. 1B). We now know that these complex networks engage in many functions ranging from cell shape determination and mechanics to signal transduction and cell motility.

3. Early observations leading to the isolation and initial biochemical characterizations of IF

Our first IF isolation procedures were based upon observations of live cells using polarized light microscopy. We discovered that during the early stages of the spreading of fibroblasts such as BHK-21 cells, there was a prominent juxtanuclear "birefringent sphere" (or juxtanuclear cap) which became elongated into birefringent fibers as cells flattened and took on the shape of fibroblasts. The middle of this spherical region was isotropic while the surrounding anisotropic region exhibited positive birefringence with respect to its circumferential axis (Fig. 3). This latter finding indicated that filamentous components were oriented with their long axes wound around the center of the isotropic core. Electron microscopy revealed that the birefringent sphere consisted exclusively of closely associated masses of IF organized in the fashion predicted by the polarized light findings. No microtubules or microfilaments were detected within these juxtanuclear arrays of