invited review

The cytoskeleton of digestive epithelia in health and disease

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Omary. The cytoskeleton of digestive epithelia in health and disease. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G1108–G1137, 1999.—The mammalian cell cytoskeleton consists of a diverse group of
fibrillar elements that play a pivotal role in mediating a number of
digestive and nondigestive cell functions, including secretion, absorption,
motility, mechanical integrity, and mitosis. The cytoskeleton of higher-
eukaryotic cells consists of three highly abundant major protein families:
microfilaments (MF), microtubules (MT), and intermediate filaments
(IF), as well as a growing number of associated proteins. Within digestive
epithelia, the prototype members of these three protein families are ac-
tins, tubulins, and keratins, respectively. This review highlights the impor-
tant structural, regulatory, functional, and unique features of the three
major cytoskeletal protein groups in digestive epithelia. The emerging
exciting biological aspects of these protein groups are their involvement
in cell signaling via direct or indirect interaction with a growing list of
associated proteins (MF, MT, IF), the identification of several disease-
causing mutations (IF, MF), the functional role that they play in
protection from environmental stresses (IF), and their functional integra-
tion via several linker proteins that bridge two or potentially all three of
these groups together. The use of agents that target specific cytoskeletal
elements as therapeutic modalities for digestive diseases offers potential
unique areas of intervention that remain to be fully explored.

THIS REVIEW FOCUSES ON THE three major cytoskeletal
protein families in epithelial cells of the digestive
system, including the liver, pancreas, esophagus, stom-
ach, intestine, and gallbladder. The term cytoskeleton
derives from "cyto" (kutos in Greek meaning "hollow
vessel") and "skeleton" (skelotos in Greek meaning
"dried up"). Cytoskeleton refers to the major fibrillar
elements that are found in cells if one removes the
soluble cytosol and intracellular organelles. The three
major protein families of the cytoskeleton fibrillar
systems are microfilaments (MF), microtubules (MT),
and intermediate filaments (IF) (Table 1), which in turn
interact with a growing list of associated proteins.
Although excellent reviews have addressed MF, MT, or
IF individually, we are not aware of any review that
addresses and contrasts side by side the three major
cytoskeletal protein groups within digestive epithelia.

Several cell types develop a polarized architecture
that is essential for their biological function. Typical
polarized cells are neurons and epithelial cells, which
partake in endocytosis, exocytosis, and vesicle trans-
port. In epithelial cells, the apical surface faces the
lumen or canalculus and is the site of secretion or
absorption. The basolateral surfaces refer to basal
areas, which contain hemidesmosomes and interact
with the extracellular matrix (ECM), and the lateral
sides, which interconnect neighboring cells via gap
junctions and desmosomes (Fig. 1). Secretory and mem-
brane proteins are transported in membrane-bound
vesicles from the endoplasmic reticulum (ER) to the
Golgi network and then are sorted in the trans-Golgi
network (TGN) to the plasma membrane or other
organelles. Basolateral sorting is mediated by signals
(such as specific amino acid sequences) that reside
within the protein to be transported (e.g., see Ref. 120).
There are also apical signals (such as N glycosylation
and glycosylphosphatidylinositol anchor) and late endo-
some sorting signals (120). Hepatocytes appear to be
unique among polarized cells in that most apical trans-
port of proteins occurs indirectly via initial transport to
the basolateral membrane followed by transcytosis,
except for the direct apical transport of sphingolipids
(277).

The three major activities of digestive epithelial cells,
namely, secretion, digestion, and absorption, require
the establishment and maintenance of cellular polarity
and intracellular transport, all functions intimately linked to the cytoskeleton. Other important roles for the cytoskeleton in digestive and nondigestive-type epithelia include its involvement in mitosis, protection from environmental stresses, cell and intracellular organelle anchorage, gene regulation, and motility during migration, differentiation, and wound repair. In addition, a new role for the cytoskeleton in signal transduction is emerging, as contrasted with the well-established reorganization of the cytoskeleton on signaling, since many signaling molecules associate with cytoskeletal proteins.

Although the three major cytoskeletal proteins do share some functions, which likely provide an important functional redundancy mechanism for cells, they also have several distinct properties (Table 1). For example, the diverse structure of IF proteins and their selective expression in higher eukaryotes imply one or more specialized evolutionary roles, which appear to include a protective role from a wide range of environmental stresses. In addition, some of the properties of IF proteins, compared with MF and MT, have made their study very difficult. For example, the absence of cytoplasmic IF proteins from yeast and Drosophila (which precluded functional genetic studies), their relative insolubility (which made biochemical separation for dynamic studies difficult), and the lack of selective stabilizers and destabilizers have contributed to the difficulty in appreciate their biological significance. Although many diseases result in modulation of all three cytoskeletal protein families, it is mainly mutations in IF proteins, and to a lesser extent actin, that are known to directly result in several human diseases. As our understanding of the cytoskeleton advances, it is hoped that targeted manipulation of cytoskeletal proteins will offer novel and effective therapeutic advantages. This review will highlight general physiological features of the three major cytoskeletal protein families and summarize their direct and indirect association with disease states of the digestive tract.

**MICROFILAMENTS**

**Actin and Regulation of Actin Dynamics**

Actin, actin-binding, actin-related proteins, and their regulation. The actin cytoskeleton is highly conserved in all eukaryotic cells, from protozoa to yeast to human, and is composed of actin as its major component and actin-binding proteins (220). The human actin gene family includes three classes, named α-, β-, and γ-actin. There are six different actin genes in mammals, two ubiquitous nonmuscle (β and γ), two striated muscle (α1 and α2 (cardiac)), and two smooth muscle (α2 or β of vascular smooth muscle and α3 or γ of enteric smooth muscle) isoforms (220). The amino acid sequences of these three gene product classes are almost identical (molecular mass of ~42 kDa), with 93–98% sequence identity between the α-, β-, and γ-isoforms. MF are formed by self-assembly of G actin and are decorated with actin-binding proteins. Polymeric actins (F actin) are further assembled into a filamentous network, with regulation by a large number of actin-binding proteins, which, in turn, are regulated by extracellular or intracellular signals.

More than 70 categorized types of actin-binding proteins have been identified (Table 2). They modulate the function of the actin cytoskeleton in terms of polymerization and dynamics, cross-linking and bundling, nucleation and branching, and the function of the actin cytoskeleton in terms of polymerization and dynamics, cross-linking and bundling, nucleation and branching, and the function of the actin cytoskeleton in terms of polymerization and dynamics, cross-linking and bundling, nucleation and branching, and cell-membrane interaction, cell-ECM interaction, cell-cell interaction, kinesis dynamics, contractility, scaffolding, and signaling (7). In addition to actin and its binding proteins, there are more than 34 actin-related protein (Arp) sequences identified from different species, with 35–55% sequence similarity to actins (220). In humans, Arp1 forms 37-nm-long filaments within a dynactin complex, which functions in vesicle transport. Arp2 and Arp3 complex with five other proteins to initiate the nucleation of actin polymerization.

The activities of actin-binding proteins are often modulated by phosphorylation, by signaling molecules such as Ca2+ and phosphoinositides, and by small GTPases including Rac, Rho, and Cdc42 (213). Rac is activated by the receptor tyrosine kinase pathway, including focal adhesion molecules such as integrins, and growth factors. Once activated, Rac controls the formation of membrane ruffles and lamellipodia (the outward sheet-like extension in the leading edge of a moving cell). In contrast, Rho controls the generation of adherens junction (A) and focal adhesions in polarized epithelial cells, whereas Cdc42 activation results in peripheral-actin microspike formation, including filopodia (finger-like protrusions consisting of actin bundles),

<table>
<thead>
<tr>
<th>Table 1. The cytoskeleton in epithelial cells</th>
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<tr>
<td><strong>Protein</strong></td>
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<tr>
<td>Actin</td>
</tr>
<tr>
<td>5–8</td>
</tr>
<tr>
<td>Conserved</td>
</tr>
<tr>
<td>Eukaryotes</td>
</tr>
<tr>
<td>+++</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>e.g., β-Thymosin</td>
</tr>
<tr>
<td>Many</td>
</tr>
<tr>
<td>Few known (cardiac)</td>
</tr>
<tr>
<td>Many</td>
</tr>
<tr>
<td>Phalloidin</td>
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<tr>
<td>Cytochalasins</td>
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and the reorientation of the Golgi apparatus in the direction of movement. Rac, Rho, and Cdc42 act on actin-binding proteins directly or affect them via phosphoinositides and protein phosphorylation, which in turn occur via phosphatidylinositol 3-kinase, phosphatidylinositol-4-phosphate 5-kinase, PKN (a fatty acid and Rho-activated serine/threonine kinase), protein kinase C (PKC)-related kinase 2, p21 GTPase-activated kinase, and Rho kinase. For example, Rho activates ROCK, a member of the Rho-kinase family. ROCK phosphorylates and activates LIM kinase, which phosphorylates cofilin, thereby inhibiting its actin-severing function (154). Other examples of actin-binding protein phosphorylation include adducin, ezrin/radixin/moesin (ERM), gelsolin, paxillin, plasmin, and cortactin (Refs. 68 and 115 and references therein). In addition, actin itself undergoes several posttranslational modifications, including phosphorylation, acetylation, glutamylation, ADP-ribosylation, and sulfation, but their functions are not well defined (220).

Actin cytoskeleton assembly and its organization. MF are 5- to 8-nm-wide helixes of uniformly oriented G actin. The two ends of the filament polymerize at different rates, such that the rate of the fast-growing “barbed end” is 20 times that of the opposing “pointed end.” In nonmuscle cells, ~50% of actin is filamentous and the remaining is monomeric. MF are highly dynamic, with a half time of minutes as determined by
Table 2. Classification of actin-binding proteins

<table>
<thead>
<tr>
<th>Actin-Binding Domain Structure</th>
<th>Example of Binding Proteins</th>
<th>Actin-Related Function</th>
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<tbody>
<tr>
<td>Gelsolin fold</td>
<td>Gelsolin</td>
<td>Cross-linking</td>
</tr>
<tr>
<td>Villin</td>
<td>Severing, capping</td>
<td></td>
</tr>
<tr>
<td>Profilin fold</td>
<td>Profilin</td>
<td>Severeing</td>
</tr>
<tr>
<td>VT</td>
<td>G actin binding</td>
<td></td>
</tr>
<tr>
<td>Villin headpiece</td>
<td>Villin</td>
<td>Cross-linking</td>
</tr>
<tr>
<td>CH domain</td>
<td>β-Spectrin</td>
<td>Contracting</td>
</tr>
<tr>
<td>Hisactophilin</td>
<td>Actin cross-linking</td>
<td></td>
</tr>
<tr>
<td>DNase I</td>
<td>Actin binding</td>
<td></td>
</tr>
<tr>
<td>Structure not known</td>
<td>Actin-Related</td>
<td></td>
</tr>
<tr>
<td>Adducin</td>
<td>Actin-binding</td>
<td></td>
</tr>
<tr>
<td>Arp2/3 complex</td>
<td>Nucleating</td>
<td></td>
</tr>
<tr>
<td>BPAG1-e, -n</td>
<td>MF-IF cross-linker</td>
<td></td>
</tr>
<tr>
<td>α-Catenin</td>
<td>Adhesion</td>
<td></td>
</tr>
<tr>
<td>Capping protein</td>
<td>Barbed end capping</td>
<td></td>
</tr>
<tr>
<td>Coronin</td>
<td>Actin and MF-MT cross-linker</td>
<td></td>
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<tr>
<td>Ema/VASP family</td>
<td>Actin assembly</td>
<td></td>
</tr>
<tr>
<td>Espin, small espin</td>
<td>Actin bundling</td>
<td></td>
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<tr>
<td>Ezrin/radixin/moesin</td>
<td>Actin cross-linking</td>
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<tr>
<td>Plectin</td>
<td>MF-IF and MT-IF cross-linker</td>
<td></td>
</tr>
<tr>
<td>Vinculin</td>
<td>Adhesion</td>
<td></td>
</tr>
<tr>
<td>WASP/Scar family</td>
<td>Actin assembling</td>
<td></td>
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Table 2 was adopted from Refs. 7, 196, and 250. Villin contains 2 different actin-binding structures. Some actin-related proteins are also actin-binding proteins, which function in filament nucleation (the rate-limiting step), anchoring, capping, severing, cross-linking, and bundling (Fig. 2). MF are nucleated at multiple sites beneath the plasma membrane, in association with the Arp2-Arp3 complex (Fig. 2) and in proximity to focal adhesions, AJ, or tight junctions (TJ) (Fig. 1).

MF are further bundled together via various actin cross-linking proteins, such as α-actinin and filamin, to form complex three-dimensional structures in the cell interior. These cross-linking proteins form extensions of high-angle branches or bind to MF sides between parallel filaments to generate actin bundles. On the basis of MF polarity, the actin cytoskeleton can be classified into three groups: antiparallel arrays, as in stress fibers and contractile rings (i.e., contractile bundles of actin filaments and myosin II); parallel arrays, which provide mechanical support for protrusive structures such as microspikes and microvilli (MV); and isotropic arrays, which form the actin terminal web (also called cortical actin filaments) beneath the plasma membrane. These three actin networks are compartmentalized and functionally distinct and are organized and regulated by different and overlapping actin-binding proteins.

Myosin-based force transduction. MF provide tracks for ATP-driven myosin motor proteins, which translocate along actin to generate forces necessary for contraction, vesicle trafficking, organelle localization, and signal transduction. There are at least 14 distinct classes of myosins (164), of which myosin I and V subfamilies (MyoI or MyoVa) play a role in vesicle/particle transport. As measured by an optical tweezer transducer, the chick intestinal apical MyoI moves along MF 11.5 nm in two steps during one ATP hydrolysis cycle (251). MyoVa interacts directly with the MT-based kinesin isofrom KcJu, which suggests that cellular transport may be coordinated through the direct interaction of different motor molecules on different polymer tracks. Current evidence suggests that MF and MT systems cooperate such that the long-range transport of cellular components in animal cells is based on MT networks, whereas the actin network appears to be critical for short-range transport (95). In support of this, MT do not extend to the cell periphery, whereas MF tend to be organized preferentially in cortical regions of the cell. Also, cytoplasmic vesicles from squid axoplasm translocate along both MF and MT, and a single vesicle is found to switch from MT to MF and vice versa. Thus one proposed model is that secretory vesicles budding off the Golgi complex travel to their destination at the cell periphery via long-range movements carried out by MT motor proteins along MT and then switch "track" to MF to traverse the actin-rich cortex with the help of myosins (10).

Function of the Actin Cytoskeleton in Digestive Epithelia

Cytoplasmic organization, cell shape, and polarity. The cytoplasm of a eukaryotic cell is spatially and temporally organized by MF, MT, IF, and their associated proteins, which form lattice-like mesh networks to restrict free diffusion of molecules larger than 500 kDa (149, 217). Distinct populations of actin filaments are associated with different cellular compartments, e.g., terminal web, stress fibers, filopodia, and lamellipodia. These specific actin compartments consist of different isoforms, which in turn can associate with unique actin-binding proteins in an actin isoform-specific fashion. For example, β- and γ-actins are differentially distributed in gastric parietal cells (269). The β-actin isoform is found along the entire gland lumen and associates preferentially with ezrin in gastric epithelial cells, whereas the γ-actin isoform is distributed preferentially near the basolateral membrane. In general, γ-actin is present in most, if not all, nonmuscle MF,
whereas β-actin is enriched in cytoplasmic and membrane structures undergoing or capable of undergoing rapid remodeling or polarized movement (81). MF provide a large charged surface area for potential localization of cytoplasmic components (103). This scaffolding function is supported by the fact that there is a 47,000-µm² surface area on MF in a 20-µm-diameter cell with a typical concentration of 10 mg/ml F actin, compared with only 700-µm² plasma membrane surface area. The MF-dependent localization of cellular components includes glycolytic enzymes, Src tyrosine kinase and PKC, organelles, and mRNA. Thus the actin cytoskeleton, in cooperation with MT and IF, provides a three-dimensional framework to structure the cytoplasm and to compartmentalize cellular events.

The actin cytoskeleton also plays a crucial role in cell shape maintenance by integrating the interior cytoskeleton with cell-cell and cell-ECM contacts through its binding proteins. For example, ERM family proteins link cortical MF to integrins of focal adhesions, whereas catenins bridge MF with AJ and zonula occludens (ZO)-1 binds MF to TJ. As such, cell shape is maintained by tension over the whole cell, which is generated, at least in part, by an integrated membrane-actin cytoskeleton. Another aspect of cell shape in digestive epithelial cells is their polarized cytostructure (Fig. 1). The sorting and targeting of apical and basolateral membrane proteins from TGN complex to the plasma membrane and the establishment of structural asymmetry and specialization within the plasma membrane (e.g., MV and different cell-cell and cell-matrix interactions) also involve the actin cytoskeleton (270).

The compartmentalized distribution of actin-binding proteins implies a functional significance in the morphogenesis and/or maintenance of these compartments. For example, the enterocyte “brush-border” apical domain is composed of two distinct arrays of well-organized MF that support the plasma membrane. One array is within the core of each MV and consists of MF that are bundled by actin-binding proteins, including
villin, fimbrin, and small espin. Another array, which is perpendicular to the long axis of MV, is the actin terminal web, wherein MF are cross-linked by myosin, fodrin, TW240, and TW260 (231, 257). Notably, separate, targeted disruption of mouse villin and moesin genes does not impair MV morphogenesis, which suggests that the function of the bundling proteins in MV is redundant (53). In support of this, antisense down-regulation of all three ERM proteins altered MV formation, whereas there was no significant effect of down-regulation of individual ERM proteins (232).

Cell-cell and cell-matrix interaction. Epithelial cells form highly specialized actin-anchoring membrane structures to facilitate cell-cell and cell-ECM contacts, which are likely essential for maintaining cell morphology and tissue integrity. Abnormalities in these contacts are associated with a variety of diseases, including diarrhea, carcinogenesis, and metastasis. All these contacts consist of transmembrane proteins that interact with neighboring cells or with ECM via their extracellular portions and with cytoplasmic adaptor molecules via their intracellular domains. Many of the cytoplasmic adaptor proteins interact with the actin cytoskeleton to strengthen the contacts. Examples of important epithelial actin-associated membrane structures include the following (see Fig. 1): 1) TJ, which cross-link cells to form a “fence” and are the gatekeepers that regulate the paracellular pathway and epithelial permeability. TJ (also called zonula occludens) consist of two families of four-transmembrane domain proteins, namely occludin (71) and claudin (177), and one single transmembrane domain protein, JAM (158), along with associated cytoplasmic proteins, including ZO-1, ZO-2, and ZO-3. The ZO proteins associate with each other via their PDZ domains (named for postsynaptic density protein 95, disks large, ZO-1), bind actin via the ZO-1 proline-rich tail or via α-catenin, and bind occludin via their guanylate kinase domains. The organization and function of TJ may be regulated by RhoA and Rac1 through regulation of MF in the apical pole of polarized enterocytes (112, 188). 2) AJ, which include the homotypically binding transmembrane protein E-cadherin that helps form a continuous adhesion belt around each interacting cell in the epithelial sheet, is another important epithelial actin-associated membrane structure. Other AJ cytoplasmic components include the actin-binding proteins vinculin, α-catenin, α-actinin, paxillin, talin, vasodilator-stimulated phosphoprotein, and adaptor proteins such as vinexin, β-catenin, and plakoglobin. α-Catenin plays a critical role in the transmembrane anchorage of cadherins, since deletion of α-catenin inactivates cadherin-mediated cell adhesion, resulting in a nonadhesive phenotype (99). Through these actin-binding proteins, a contractile bundle of actin-myosin filaments runs along the cytoplasmic surface of the junctional plasma membrane and links cell to cell via E-cadherin to generate an extensive transcellular network. The coordinated contraction of this actin network plays several roles in epithelial cells, including canalicular contraction during bile secretion. 3) Gap junctions, which connect neighboring cells by intercellular channels that consist of connexins, a family of four-transmembrane domain proteins with >14 different genes in mice, is a third important epithelial actin-associated membrane structure. Molecules less than ~1 kDa, including ions, metabolites, and messengers, pass freely through these channels. The role of gap junctions in digestive epithelia is important for coordinated tissue behavior, but their interaction with cytoskeletal elements is not clear. Although connexin mutations cause several human diseases, including nonsyndromic deafness and X-linked Charcot-Marie-Tooth disease, no digestive disease is known to be caused by connexin mutations (221). 4) Focal adhesions (i.e., focal contacts), which connect the actin cytoskeleton to the ECM through the integrins and their associated cytosolic actin-binding proteins, is a fourth important epithelial actin-associated membrane structure. The ectodomain of integrins binds to ECM, whereas their intracellular domain binds to many actin-binding proteins such as talin, vinculin, paxillin, and α-actinin, which in turn interact with actin (75).

Cell motility. Cell motility is a tightly integrated process between different components of the cytoskeleton (171). Actin-based cell motility plays important roles in epithelial cell functions, including secretary vesicle movement, cell movement during wound healing, and cell regeneration. Three types of actin-based epithelial cell motility can be categorized as those that 1) utilize already assembled MF as in cytosolic particle transport and during cytokinesis, 2) utilize new actin assembly as occurs during pathogenic intra- and intercellular bacterial movement, and 3) utilize preassembled and new MF assembly as occurs during cell locomotion. Examples of these categories are outlined as follows: 1) for cytokinesis, epithelial cells undergo a dramatic change in morphology during mitosis, which requires reorganization of all three cytoskeletal filaments and concludes with separation of daughter cells via cytokinesis. The cytoplasmic constriction at the equator during dividing cell separation is driven by a force generated by the preassembled contractile ring, which consists of membrane-associated actin and actin-binding proteins such as myosin II and cortezillins (256). This constriction draws the plasma membrane inward to form a “cleavage furrow,” which gradually deepens and finally breaks at each end to separate two daughter cells. Other cell-cell contacts are also affected during cytokinesis, such as AJ, which partially lose contact with the actin cytoskeleton. 2) For intracellular movement, Listeria monocytogenes and Shigella flexneri movement is driven by local catalysis of MF polymerization at one side of the bacteria that forms an actin comet tail to drive bacteria forward. Also, endocytic vesicle movement, after pinching off the plasma membrane into the cytosol, is advanced by a brief burst of “mini-comet tail” actin polymerization (165). 3) For locomotion, a moving cell is morphologically polarized and is often described as four cell regions from front to back: lamellipodium in the leading edge, lamella that is located immediately behind the lamellipodium and is...
thicker, cell body that contains the nucleus and other organelles, and the remaining cell rear (38). The lamellipodium protrudes outward by dynamic actin polymerization forces. The lamella appears to stay in place, whereas the cell body translocates by previously assembled actin-myosin filaments. The formation of an orthogonal MF network in lamellipodia is illustrated in Fig. 2. In some (see Ref. 253) but not all (Ref. 168) systems, MT enter newly formed lamellipodia and generate net plus-end growth that appears to be regulated by actin dynamics, whereas MT breakage and shortening near the cell body at the base of lamella depends on actin-myosin (253). This MT elongation in the lamellipodium locally activates Rac1 (255), and MT disassembling in cell body locally activates RhoA (202).

In turn, Rac1 promotes further MF assembly (i.e., dynamic component) at the leading edge (via Scar1 and Arp2- Arp3 complex pathway activation), which enhances MT growth in the lamellipodium, whereas RhoA drives actin-myosin contractility (via a preassembled component) and formation of focal adhesions in the cell body and near the cell edge, which provide the base for cytoplasmic movement (254).

Secrecion and absorption. The actin cytoskeleton is reorganized during epithelial cell secretion, as in exocytosis and transcytosis, to regulate vesicle transport in response to intra- and extracellular signals (163). The cell apex actin terminal web (Fig. 1) may provide a physical structure to anchor secretory vesicles in proximity to the plasma membrane. On stimulation, an actin-myosin network (including profilin, ADF/cofilin, and capping protein) reorganizes to release the physical barrier for granules and to allow movement to membrane docking sites (245). For example, stimulated acid secretion from parietal cells is inhibited by cytochalasins in a dose-dependent fashion (63), presumably by disrupting the cortex actin “tracks” for vesicle transport. Several vesicle transport-related myosins are found apically in enterocytes, hepatocytes, and pancreatic acinar cells. For example, MyoI binds to nascent, post-Golgi secretory vesicles that are transported to the enterocyte apex (222). Digestive epithelial cells can also transport nonpermeable molecules and particles from the apical to the basolateral membrane, or vice versa, via transcytosis (Fig. 1). It is assumed that MF plays a similar role in transcytosis, as it does in exocytosis and endocytosis, based in part on cytochalasin D inhibition of basolateral to apical transcytosis of sphingolipids in polarized Hep G2 cells (276).

In addition, luminal secretion of electrolytes to maintain intestinal content fluidity and mucosal defense is tightly regulated by ion channels and TJ-gated paracellular pathways, which also play a role in absorption (13).

Endocytosis and paracellular transport play major roles in epithelial cell absorption of nutrients (178). Coated-pit endocytosis in polarized epithelia occurs from the apical and basolateral membranes and is the primary intestinal absorption mechanism. Actin involvement in endocytosis is supported by inhibition of transferrin uptake in Madin-Darby canine kidney (MDCK) cells on depolymerization of actin filaments with cytochalasin D (79). The actin-binding protein epsin may help regulate endocytosis via its association with endocytic machinery components such as Eps15. Yeast genetic screening and biochemical analysis have implicated other actin-binding proteins in endocytosis, including the mammalian homologues Arp2/3, coflin, Wiskott-Aldrich syndrome protein (WASP), calmodulin, MyoI, and talin (261). Paracellular transport (i.e., molecule movement by diffusion between cells) also plays an important role in nutrient, water, and electrolyte absorption. The permeability properties of epithelia are gated by cell-cell interactions (e.g., TJ) in association with MF. Various absorption enhancers (e.g., EDTA, sodium caprate, and decanoylcarnitine) increase paracellular transport in human intestinal Caco-2 cells in association with MF. Various absorption enhancers (e.g., EDTA, sodium caprate, and decanoylcarnitine) increase paracellular transport in human intestinal Caco-2 cells in association with MF. Various absorption enhancers (e.g., EDTA, sodium caprate, and decanoylcarnitine) increase paracellular transport in human intestinal Caco-2 cells in association with MF. Various absorption enhancers (e.g., EDTA, sodium caprate, and decanoylcarnitine) increase paracellular transport in human intestinal Caco-2 cells in association with MF.
Actin Cytoskeleton in Digestive Diseases

Thus far, mutations in actin genes have not been implicated in digestive diseases. However, mutations in α-cardiac actin and its binding proteins (e.g., myosin) cause cardiac abnormalities (242). For example, two actin point mutations are found in patients with idiopathic dilated cardiomyopathy, in which the degenerative process of cardiac dilation may be due to the episodic defect in force transmission caused by the defective attachment of MF to the Z bands or to intercalated discs (191). Another actin point mutation in the putative myosin-binding site is found in patients with familial hypertrophic cardiomyopathy, in which compensatory hypertrophy may result from a defect in force generation caused by an alteration of myosin (172). In support of these pathophysiological mechanisms, α-cardiac actin knockout mice with transgenic replacement by γ-enteric smooth muscle actin show features of both idiopathic dilated and familial hypertrophic cardiomyopathy, including impaired systolic function and hypertrophy (142). This observation is explained by the five amino acid differences between α-cardiac actin and γ-enteric smooth muscle actin, which are located at the actin filament immobilized end and the putative myosin-binding site. As noted below, modulation of MF is associated with several digestive diseases.

Infection. A variety of microbes infect digestive epithelial cells or affect them indirectly via toxins, with dramatic reorganization of MF (61). For example, enteropathogenic E. coli (EPEC) attaches on the epithelial cell membrane and then secretes and translocates a membrane-bound protein, Tir (translocated intimin receptor), into the host cell membrane via a type III secretion system (72). The translocated extracellular domain of Tir binds to the EPEC surface protein intimin-α to anchor the bacteria to the epithelial cell membrane, whereas its cytosolic domain becomes tyrosine phosphorylated and recruits CHP (a Cdc42 homologous protein, Ref. 6). Then, CHP recruits N-WASP to initiate Arp2/3 complex-dependent actin polymerization. These EPEC-linked MF can extend up to 10 µm in length, are resistant to cytochalasin D, and form a pedestal-like structure to hold EPEC on the epithelial membrane and subsequently result in diarrhea. Similarly, Helicobacter pylori colonizes gastric surface cells and alters host cell actin organization to form a pedestal structure (37). Although the pathogen-induced changes in MF may play an important role in propagation of a given pathogen, it is unclear if these changes play a direct role in diarrheal secretion per se.

In contrast, Shigella flexneri enterocytes as part of a phagocytic vacuole followed by lysis of the phagosome membrane. The free bacteria in the cytoplasm multiply and move from cell to cell by the comet tail actin-based motility without encountering the host immune system. This actin polymerization is induced by the bacterial outer membrane protein IcsA, which recruits N-WASP to activate the Arp2/3 complex (184). Similarly, Listeria monocytogenes generates the propagative actin comet tail through its asymmetric expression of the bacterial surface protein ActA, which then triggers Arp2/3-dependent actin polymerization (259). Other diarrhea-inducing bacteria also appear to work via MF. For example, Salmonella typhimurium enters the host cell through the type III secretion of several bacterial proteins including SopE and SipA, which facilitate bacterial uptake by stabilizing MF, resulting in membrane ruffling (72). Many viruses also exploit the host cell MF during their life cycle (42). For example, vaccinia viruses move intra- and intercellularly by an actin comet tail behind the virus particle, in a fashion similar to Listeria and Shigella. Although no homologous sequences to IcsA or ActA are found in the viral genome, the vaccinia protein A36R and an unknown host phosphotyrosine protein are required for actin-based motility of vaccinia (206).

MF are also the target of pathogenic bacterial toxins (21). For example, Clostridium difficile toxins A and B, and Bacteroides fragilis toxin-2 damage human colonicocytes in association with disruption and condensation of MF, followed by TJ alterations, which may contribute to diarrheal pathogenesis (e.g., see Refs. 85 and 203). Similarly, the cytotoxic necrotizing factor type 1 from pathogenic E. coli induces a profound reorganization of MF into prominent stress fibers and membrane ruffles in human epithelial HEp-2 cells. This reorganization occurs in association with induction of a phagocytic activity, which may contribute to the mechanism by which E. coli invades epithelia (59). The understanding of pathogen-actin interactions provides a potential approach to interfere with infections by targeting such actin-dependent processes. Aside from providing a novel therapeutic approach, it is conceivable that antibiotics or antiviral drugs that block the interaction between a pathogen protein and MF may limit the potential for drug resistance development. One important caveat is that microbial infections of epithelial cells may, in some cases, selectively affect one component of the cytoskeleton (e.g., MF but not MT or IF). However, most reported studies tend to focus on one cytoskeletal group without assessing the consequences to the other two groups.

Cancer. The involvement of MF in cancer development and/or progression is likely to be indirect but nevertheless important. For example, loss of MF correlated with the transition of human noninvasive benign colonic tumors into invasive malignancies (64). The suppression of migration and proliferation of a human gastric cancer cell line by MF disruption suggests that there may be an association between changes in MF and gastric cancer metastasis in this cell line model (102). In addition, the actin-binding protein merlin functions as a tumor suppressor in schwannomas and meningiomas (82), but the role of merlin or other actin-binding proteins in epithelial cancers is unknown. Of note, wild-type p53 binds directly to F-actin with a dissociation constant of ~10 µM (166), but the role of this interaction in tumor biology is not known.

Inflammation. MF and its associated cell-cell interactions are involved in intestinal inflammation. For ex-
example, rearrangement of the epithelial cortical MF network in T84 cells is accompanied by polymorphonuclear leukocyte transepithelial migration in a physiological basolateral-to-apical direction or in the reverse direction. However, pretreatment of cells with the F actin-stabilizing agent phallacidin greatly enhanced migration in the reverse direction, which suggests that the epithelial actin rearrangement can selectively affect polymorphonuclear leukocyte migration (91). Also, E-cadherin expression and α-catenin expression during mucosal ulceration in inflammatory bowel disease decrease, which may promote cell migration during epithelial restitution of the gastrointestinal mucosa (116). E-cadherin/β-catenin complexes also rapidly dissociate in acute pancreatitis (145). In addition, TJ are disrupted in human intestinal HT-29 cells by tumor necrosis factor-β, with impairment of barrier function, which may play a pathogenic role in intestinal inflammation (214). The intestinal barrier function is also disrupted in rat experimental colitis in association with secretion of rat mast cell protease II, which may contribute to the pathogenesis of intestinal inflammation (225).

Apoptosis. Apoptotic cells undergo dramatic morphological changes to generate apoptotic bodies, the occurrence of which requires cytoskeletal reorganization. It is, however, unclear if cytoskeletal alterations during apoptosis are an epiphenomenon or if they play any direct role in the progression of apoptosis. Notably, actin and actin-binding proteins such as gelsolin and fodrin are caspase substrates in several tumor cell line apoptosis models (e.g., see Refs. 119 and 133). For example, caspase-3-dependent actin cleavage generates two fragments (15 and 31 kDa) in human tumor cell lines undergoing apoptosis, including HeLa (epithelioid), A431 (epidermoid), and U-937 (myeloid), presumably at the predicted Asp-244. Ecotopic expression of this apoptotic 15-kDa actin fragment in human embryonic kidney 293T cells induces morphological changes of actin localization resembling those of apoptotic cells independent of caspase activation (159). However, apoptosis-associated actin cleavage was not found in U937, HeLa, or human Burkitt lymphoma cell lines in an independent study (223). Hence, apoptosis-associated cleavage of actin and/or its binding proteins appears to depend on the system studied, but evidence to date supports an important role for actin/actin-binding protein reorganization and/or degradation during apoptosis.

Liver disease. In acute liver injury, there is expansion of the stellate cell population in conjunction with smooth muscle actin expression. During chronic liver injury, the stellate cell differentiates into a myofibroblast-like cell, which has a high fibrogenic capacity and is involved in ECM degradation (65). Analysis of smooth muscle actin from liver biopsies can identify the myofibroblastic transformation in injured liver, whereby the extent of smooth muscle actin-positive cells reflects progression to hepatic fibrosis and cirrhosis. In addition, autoantibodies against actin are found in association with autoimmune chronic liver disease and in chronic hepatitis C (27). Anti-actin antibodies are at least one of the components of the so-called anti-smooth muscle antibodies, and their presence may help differentiate hepatitis C-related vs. nonhepatitis C-related autoimmune liver disease (27). Presence of anti-actin antibodies may also correlate with a poorer prognosis and with HLA-DR3 expression (43). Other potentially useful serological markers include serum secretory gelsolin, which was decreased in patients with acute liver failure (228). However, the pathophysiological role of actin and its binding proteins in liver diseases is not known.

MICROTUBULES

Overview of MT

MT are composed of tubulin, which is found in all dividing eukaryotic cells and in most differentiated cell types (48). In hepatocytes, tubulin comprises ~1% of the soluble protein, with nearly 40% assembled into MT (Ref. 201 and references therein). MT are noncovalent polymers of tubulin and consist of heterodimers of α- and β-tubulin monomers (molecular mass = 57 and 54 kDa, respectively), which are ~50% identical at the amino acid level (48). A third tubulin, γ-tubulin, is expressed in animals, plants, fungi, and protist as part of the MT organizing center (MTOC), in pericentriolar areas, and in ciliate basal bodies (48, 150). A fourth tubulin, δ-tubulin, was described in Chlamydomonas, which functions in the maturation of basal bodies/centrioles (56). Most eukaryotic cells possess several isoforms of α-, β-, and γ-tubulins that arise as products of different genes or by posttranscriptional or posttranslational modifications and differ in their assembly, MT drug-binding, and dynamic properties (150). In vertebrates, six α- and seven β-tubulin gene products are known. Among the mammalian isoforms, several are expressed at high levels or specifically in brain and testis, whereas most isoforms are expressed at lower (although still abundant) levels, as found in the liver (150). The major posttranslational modifications of MT include detyrosination/tyrosination, acetylation/deacetylation, polyglutamylation, polyglycylation, and phosphorylation. The best-studied tubulin modification is detyrosination, which occurs on α-tubulin as a consequence of MT stabilization and is thought to mark MT for interaction with other proteins (80, 150).

Both α- and β-tubulin bind GTP, and, shortly after subunit assembly, the β-tubulin-bound GTP is hydrolyzed to release one phosphate followed by addition of an ßβ-dimer to the MT end (48). Tubulin dimers are added to the MT from head to tail, and the two MT ends are distinct in their polymerization rates in that one is faster growing (plus end) than the other (minus end). This polarity has very important physiological ramifications, including determining the directionality of cargo transport by motor proteins along the MT. The αβ-heterodimers assemble into linear protofilaments such that dimers are oriented with β-tubulin toward the plus end and α-tubulin toward the minus end of the MT (48). In vivo, MT consist predominantly of 13 protofila-
MT dynamics are governed primarily by a mechanism called “dynamic instability,” postulating that a single MT never reaches a steady-state length but persists in prolonged states of polymerization and depolymerization. In the dynamic instability model, MT stability is thought to depend on a β-tubulin-GTP-cap at the MT plus end where hydrolysis has not yet occurred (48). MT stability is also highly regulated by several binding proteins (Table 3). Each MT is oriented with the minus end at the nucleating site and the rapidly changing plus end free in the cytoplasm (48). Nucleation of most MT occurs from the MTOC (also called “centrosome” in animal cells), which is structurally diverse in different cell types but shares a common ability to nucleate MT (111). In nonpolarized cells, the MTOC is positioned at the cell center, to one side of the nucleus, whereas in polarized epithelial cells MT nucleation appears to occur from several noncentrosome-dependent sites near the apical membrane (83, 163). For example, hepatocyte MT minus ends originate from several organizing centers positioned at the apical pericanalicular region (187) and centrosomal structures are found just below the apical membrane in enterocytes and in the human intestinal cell line Caco-2 (83). Hence, due to the apical nucleation, most minus ends of MT in all studied polarized digestive epithelia face the apical area and the plus ends extend through the cell body to the basolateral surface (Fig. 1, also see General directionality issues that pertain to MT function below). This polarized distribution was demonstrated in Caco-2, WI-F1 (liver), and MDCK cells using a MT hook decoration assay and electron microscopy and is also likely present in enterocytes and pancreatic acinar cells (83, 163). Hepatocytes have, in addition to the apical to basal MT, some “curved” MT that intersect the “straight” MT, and it is hypothesized that the curved MT define TGN areas (83).

**Table 3. Major classes of MT-interacting proteins**

<table>
<thead>
<tr>
<th>Type of Protein</th>
<th>Example</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor Kinesin</td>
<td>Kin I, Kin N</td>
<td>Plus-directed vesicle/organellar transport</td>
</tr>
<tr>
<td></td>
<td>Kin C</td>
<td>Minus-directed vesicle/organellar transport</td>
</tr>
<tr>
<td>Dynein</td>
<td>DHC1–DHC3</td>
<td>Minus-directed vesicle/organellar transport</td>
</tr>
<tr>
<td>MAP</td>
<td>MAP1</td>
<td>MT stabilization, primarily neuronal</td>
</tr>
<tr>
<td></td>
<td>MAP2</td>
<td>MT stabilization, primarily neuronal</td>
</tr>
<tr>
<td></td>
<td>MAP4</td>
<td>MT stabilization, non-neuronal</td>
</tr>
<tr>
<td></td>
<td>tau</td>
<td>MT stabilization, neuronal</td>
</tr>
<tr>
<td>Destabilizer</td>
<td>Op18</td>
<td>MT destabilization by binding dimers</td>
</tr>
<tr>
<td></td>
<td>Katanin, EF-1α</td>
<td>MT destabilization by severing</td>
</tr>
<tr>
<td>Other</td>
<td>GMAP-210</td>
<td>Golgi positioning, binds minus ends</td>
</tr>
<tr>
<td></td>
<td>CLIP-170</td>
<td>Organellar positioning, binds plus ends</td>
</tr>
<tr>
<td></td>
<td>EB-1</td>
<td>Binds MT and APC</td>
</tr>
<tr>
<td>Signaling effector Protein kinase</td>
<td>MLK2</td>
<td>Binds KIF3 kinesins</td>
</tr>
<tr>
<td></td>
<td>PKA</td>
<td>Binds MAP2</td>
</tr>
<tr>
<td></td>
<td>ERK1/2</td>
<td>Binds MT for cytoplasmic retention</td>
</tr>
<tr>
<td>Small GTPase</td>
<td>Rac1</td>
<td>Binds and colocalizes with MT</td>
</tr>
<tr>
<td>Protein phosphatase</td>
<td>PP1</td>
<td>Binds tau</td>
</tr>
<tr>
<td></td>
<td>PP2A</td>
<td>Binds tau, prevents MT-tau binding</td>
</tr>
<tr>
<td>Transcription factor</td>
<td>c-myc</td>
<td>Binds MT, possible role in transport</td>
</tr>
<tr>
<td>Adaptors</td>
<td>cyclin B</td>
<td>Binds MAP4</td>
</tr>
</tbody>
</table>

MT Binding Proteins

MT stability is modulated by a growing list of effectors or binding proteins, which include motor proteins, the “classical” MT-associated proteins (MAP), and additional molecules that assist in scaffolding events important in cell signaling and mitosis (Refs. 28 and 80 and Table 3). As a group, these effectors help stabilize or destabilize MT and utilize MT as a scaffolding surface during signaling cascades (111).

**Table 3** was adopted from Refs. 28, 50, 80, 100, and 163. See, specifically, Ref. 50 for CLIP-170, Ref. 100 for GMAP-210, and Ref. 163 for DHC1–DHC3. In contrast with the other listed types of MT-binding proteins, the signaling effector category includes several groups of signaling proteins that interact with MT in a direct or indirect fashion. DHC, dynein heavy chain; MAP, MT-associated protein; MLK, mixed lineage kinase; PKA, protein kinase A; ERK, extracellular-regulated protein kinase; PP, protein phosphatase; APC, adenomatous polyposis coli.
kinesins, dynein supports the retrograde transport of membraneous organelles by powering transport toward the MT minus end (246). Dynein is a large enzyme complex and several human dynein heavy chain genes have been identified with apparently different distributions and functions (163). In digestive epithelia, dynein was isolated from liver (36) and pancreas (134), and a liver- and brain-specific dynein light chain, rp3, was reported (123). In contrast to kinesins, dyneins interact with distinct partners in order to be targeted and regulated instead of evolving differences in their heavy chain structure.

MAP and destabilizing effectors. MAP were initially identified by virtue of their copurification with MT and were shown to promote tubulin polymerization and to stabilize MT (54). For example, MAP overexpression inhibits vesicle transport and organelle movement and stabilizes and bundles MT. MAP2C and tau also act by virtue of their extension as a projection domain away from MT and as such can bundle MT and act as a scaffold for signaling elements or organelles. For example, several signaling molecules associate with MAP, such as protein kinase A, which binds to MAP2, and cyclin B, which binds to MAP4 (Ref. 80 and Table 3). Phosphorylation plays an important role in regulating MAP and usually weakens MAP-MT binding with subsequent MT destabilization on release of MAP. Some kinases may also be classified as MAP, as exemplified by the MT affinity-regulating kinases, which phosphorylate MAP and lead to increased MT turnover and perhaps facilitated transport along MT (54).

In neuronal tissue, tau is the best characterized MAP because of its association with Alzheimer’s disease. Some forms of tau are also expressed in mouse liver, and increased tau mRNA was noted in mouse hepatocytes after griseofulvin (GF) intoxication (121). MAP4 is well characterized and is present in all nonneuronal vertebrate cells (54), whereas a pancreatic MAP4-related 67-kDa protein was isolated but its endocrine and/or exocrine relationship and functional significance are not known (167). Another MAP, E-MAP-115, is predominantly found in an apicobasal distribution in differentiated epithelial cells, such as renal tubules, absorptive enterocytes, and testis (58).

MT function is also regulated by effectors that increase MT instability by severing and disassembling stable MT (28). One of the best-studied destabilizers, oncoprotein 18 (Op18 or stathmin), is expressed mainly in proliferating cells such as leukemias and some breast cancers (17). Op18 promotes MT depolymerization by binding and sequestering tubulin dimers, thereby favoring the equilibrium toward disassembly (94). Op18 is important during mitosis when its MT destabilizing activity is regulated by phosphorylation (28). Other MT destabilizers include katanin, an MT-severing protein, which is concentrated at centrosomes and is believed to mediate MT release from neuronal centrosomes for their subsequent axonal transport. Similarly, elongation factor-1α, which functions in peptide chain elongation during translation, severs MT but also bundles actin filaments (28).

Function of MT in Digestive Epithelia

MT are essential for many basic cellular functions, such as mitosis, vesicle transport, cell shape, cell polarity, and signaling. They also function in stable specialized structures such as processes of mature neurons and ciliary and flagellar axonemes (54). The general functions of MT in digestive epithelia appear to be similar to those for most other cell types (54, 80, 83). The vast majority of studies on MT-associated cellular processes are based on treating cells or animals with MT-stabilizing (e.g., taxol) and MT-destabilizing (e.g., colchicine, nocodazole) drugs.

General directionality issues that pertain to MT function. In polarized digestive epithelial cells, MT and their associated motor proteins are particularly important in facilitating and organizing vesicle transport during endocytosis and exocytosis (83, 163). Confusion may occur due to fundamental differences in MT organization between polarized and nonpolarized cells, which are based on the different localizations of the MTOC. For example, the vast amount of MT minus ends in most nonpolarized cells is found at the perinuclear MTOC, whereas the plus ends radiate out to all sections of the plasma membrane. This implies that exocytosis in nonpolarized cells is always MT-plus-end directed, whereas endocytosis is minus-end directed. In contrast, polarized epithelial cell MT are generally organized with their minus ends at the cell apex, indicating that apical exocytosis is expected to be MT minus-end directed and endocytosis from the apical regions (as in enterocytes) is expected to be plus-end directed. It should also not be overlooked that, although most MT are positioned with the minus ends at the apical region in polarized epithelial cells, there is a pool of MT that runs in the opposite direction (9). A further variation of this organization is found in neurons, which also are polarized cells, but in contrast to polarized epithelial cells neurons have their MTOC located in the cell body and the plus end in the axon, where secretion of synaptic vesicles occurs (8). These differences caution against generalizing MT-mediated transport results from different cell types. Attempts at unifying MT directionality with function are further complicated by the position of the Golgi and ER relative to MT in different cells. For example, hepatocyte Golgi are situated at the apical canalicular membrane at the MT minus ends, whereas pancreatic acinar cell and enterocyte Golgi are located supranuclearly at the MT plus ends (163). These differences may reflect the use of different motor proteins in different cells and/or the use of kinesins that can be plus or minus end directed.

Cell polarity and organelle positioning. Although MT do not directly interact with cell junctions as MF and IF do, they reorganize on cell junction formation with the aid of >50 MT-regulating proteins (80, 83). However, the functional significance of such reorganization is poorly understood. In contrast, MT and their motors are very important in organizing and positioning organelles, including Golgi, ER, endosomes, lysosomes, peroxisomes, and chromosomes, during mitosis (90, 111,
The importance of MT in Golgi positioning is supported by Golgi redistribution to peripheral cellular sites in the presence of MT-disrupting agents and the identification of a Golgi MT-associated protein (GMAP-210) that links cis-Golgi to MT minus ends in HeLa cells (100). Cytoplasmic dynein is also important for Golgi and vesicle positioning, in part because dynein-null murine blastocysts contain a highly vesiculated and redistributed Golgi complex throughout the cytoplasm with redistributed endosomes and lysosomes (84). Golgi membranes also associate with kinesin in several cell types, including rat hepatocytes (163). The reason for this association is unclear but could reflect a role for kinesin in plus-end MT-directed recycling of membranes back to the ER.

MT also associate with the ER in polarized epithelia and help localize the ER in proximity to the basolateral domain. This is supported by expansion of the ER toward the apical domain when MT are disrupted in rat pancreatic acinar cells when subjected to colchicine treatment (134). The ER appears to associate with MT plus ends via a dynein-based interaction (4) and with kinesin (244) and also interacts directly with MT through p63, an integral rough ER membrane protein (126). Other ER-MT interactions include the association with the MT-binding protein ch-TOG (31). These interactions and possibly other linkers contribute to the positioning of the ER. Peroxisomes may also associate with MT, since nocodazole alters peroxisomal morphology, subcellular distribution, and directional movement (265).

Absorption and endocytosis. Transport of endocytic vesicles from the basolateral plasma membrane of polarized epithelial cells is MT based and involves dynein (83). For example, hepatocyte dynein-driven receptor-mediated basolateral endocytosis of asialoglycoproteins (ASGP) follows the sequence of ASGP binding to their membrane receptor and then localizing in endosomal vesicles in association with MT. It appears that only ASGP, but not their receptors, bind to MT via dynein followed by transport toward pericentrosomal lysosomes (78, 83, 163). In other systems, MT also play a role in membrane and receptor recycling. For example, the apical pericentriolar endosomal compartment in Caco-2 or MDCK cells sorts membranes back to their site of origin after internalization (129) in a MT-dependent fashion using the small GTPases Rab25 and Rab11a (26). Rab proteins also associate with pancreatic zymogen granules (190), parietal secretory granules (24), and hepatocyte transcytotic vesicles (107). The roles of MT and their motors in apical endocytosis are still unclear, although it appears that polymerized MT are needed for apical endocytosis and for the convergence of both the apical and basolateral endocytosis pathways, which occurs at the level of late endosomes. In addition, the nonmotor protein, 170-kDa cytoplasmic linker protein (CLIP-170), may play a role, since it links endocytic vesicles to MT by binding MT plus ends in transfected cells (50). Other potential modes of regulating endocytosis includes MT phosphorylation, given that MT are substrates of Gprotein-coupled receptor kinase, which has a role in downregulating receptor signaling by promoting receptor endocytosis (80).

Secretion and exocytosis. Secretory proteins are transported from ER to Golgi and further packed into distinct vesicles that are destined for the apical or basolateral membrane compartments. Most data suggest that MT and MT motors facilitate the transport of apical proteins but are less involved in the transport of basolaterally destined proteins. MT involvement in these processes is based almost exclusively on studies using MT-disrupting drugs with resultant missorting of apical (e.g., gp80 glycoprotein) and basolateral (e.g., Ag525, albumin) proteins in hepatocytes or enterocytes (76, 83, 162). In contrast, Ig receptor and Na\(^{+}\)-K\(^{-}\)-ATPase transport to the basolateral membrane is not significantly affected by nocodazole in MDCK and intestinal cells, thereby supporting a minimal MT role in basolateral transport (22, 83). As for transcytosis, which is highly relevant in digestive epithelia, there is evidence for MT-dependent and -independent transcytotic transport (83, 276).

The major motor transporting vesicle from ER to Golgi and from Golgi to the apical plasma membrane in polarized epithelia is likely to be dynein, given that it is a minus end-directed motor (163). Dynein is approximately fifteen times more abundant in liver than in brain, which probably reflects the extent of MT-directed vesicle transport needed in hepatocytes (83). In cultured enterocytes, dynein, its motility activator dynactin, and myosin were found as part of TGN membranes but not Golgi stacks, which suggest that they are responsible for the minus end-directed post-Golgi movement of vesicles toward the apical surface (60). In support of this, dynein and the dynactin complex, but not kinesin, associate with zymogen granules at the apical membrane of pancreatic acinar cells, in a fashion that requires intact MT (134). This confirms previous studies that support MT involvement in pancreatic zymogen granule transport (114). The role of kinesin in pancreatic acinar cell secretion is unclear, since kinesin was located basally in one study (134) and associated with zymogen granules near the apical membrane in another (157). Zymogen granules also associate with Myol (197), which suggests a dual MT and MF role in apical secretion as shown in neurons (95). MT may also assist in hepatocyte bile acid transport from the sinusoidal surface to the apical canalicular membrane (83).

Posttranslational modifications of tubulin and potential tubulin binding proteins may also play roles in regulating secretion. For example, sorting in WIF-B hepatocytes suggests that only dynamic, unstable MT are involved in secretory protein transport to the plasma membrane and in transcytosis of membrane proteins to the apical surface, whereas stable MT are involved in membrane protein transport to the basolateral surface (198). In addition, syntaxins (e.g., syntaxin 1A), which have a polarized distribution in digestive epithelia and can function as plasma membrane vesicle receptors (69), have a tubulin binding motif and can bind tubulin in vitro (70). However, the role of MT, if
any, in vesicle docking at the plasma membrane is not known.

Mitosis and development. The classical and most dramatic reorganization of MT occurs during mitosis when MT help segregate the duplicated chromosomes to the two daughter cells. Although MT dynamics during mitosis have not been appreciably studied in digestive epithelia, their implications on such epithelia are clear during normal and abnormal cell division, as noted in regenerating hepatocytes (e.g., after partial hepatectomy) and enterocytes and during cancerous growth, respectively. The interphase radial array of MT changes during mitosis into bipolar MT arrays, which originate from the duplicated centrosomes and interact with the condensed chromosomes at the kinetochores (111). Most of the cell cycle-dependent regulation of MT assembly occurs in association with, and is likely due to, changes in phosphorylation of MT accessory proteins. For example, the MT destabilizer Op18 requires phosphorylation on four serines for mitotic spindle formation (28). Op18 expression also increases after partial hepatectomy (131) in association with hepatocyte MT depolymerization (19). In addition, cytoplasmic dynein is indispensable during mitosis due to its multiple roles in assembling and stabilizing the spindle pole, so that centrosomes, together with dynein and dynactin, play a cooperative role in chromosome alignment during mitosis (90, 117).

Little is known about the role of MT in development apart from their obvious role in cell division. Drosophila pb3-tubulin mutants manifest a lethal defect in midgut morphogenesis and development, likely due to inability of mutant larvae to absorb nutrients across the gut wall (49). There is evidence for differential posttranslational modifications of tubulin during development, as exemplified by tubulin acetylation during mouse preimplantation embryo differentiation (74, 93). In addition, MT have been implicated in zebra fish development, since MT disruption results in axis specification (106) and frog oocyte differentiation (74) defects.

Signal transduction. The enormous MT protein surface area and their organization in a polarized fashion from the apical membrane to the nucleus make them excellent candidates to regulate intracellular signaling events (80, 103). This is supported by the increasing number of characterized signaling proteins, including phosphatases, kinases, transcription factors, and adaptor molecules that interact with MT, MT motors, or MAP (Table 3). In most cases, the significance of MT-signaling protein interactions is not known, since the interacting domains have not been characterized to allow functional studies. Three types of MT signaling factor interactions can be envisioned. 1) The first is direct interaction with the MT. An example is the small GTPase Rac, which binds MT in vitro and colocalizes with MT in vivo. 2) The second is indirect interaction via a motor protein, as for the physical interaction of the mixed-lineage kinase (MLK2, an activator of the Jun kinase pathway) with the kinesin-like KIF3 family. 3) The third is indirect interaction via a nonmotor protein, such as protein kinase A binding with MAP2 and cyclin B binding with MAP4 (80). MT direct and indirect interactions with signaling molecules suggest that MT may act as a scaffold that brings together components of signaling pathways, thereby regulating their molecular availability (80). Although there are few MT-related signaling studies in digestive epithelia, there are some examples of changes in MT dynamics in association with alterations of cell protein phosphorylation, as noted in hepatocyte growth factor upregulation of kinesin, myosin, and tubulin gene expression in hepatocytes (240).

Digestive Disease Association of MT and Their Effector Proteins

Alzheimer’s disease is likely the most studied MT-associated human disease wherein the MAP tau becomes abnormally phosphorylated and forms neurofibrillary tangles. MT disease association is also well known from cancer treatment with drugs like taxol, which binds preferentially to MT and inhibits spindle pole formation and cell growth. There are no known diseases to our knowledge that are caused by MT mutations, although their identification would not be surprising given the accumulating keratin mutations and the recently described actin mutations. In addition, tau mutations that lead to its reduced ability to bind MT cause hereditary frontotemporal dementia and parkinsonism linked to chromosome 17 (also called FTDP-17, Ref. 92).

Liver diseases. Ethanol exposure affects several hepatic MT-regulated processes and is associated with decreased receptor-mediated endocytosis and biliary secretion and accumulation of proteins in the Golgi apparatus (83). Chronic alcohol consumption reduces MT in human hepatocytes (160), possibly due to interference with the ability of tubulin to polymerize when isolated in vitro from ethanol-treated rats (273). The effect of alcoholic liver disease on MT and their assembly competence may relate to the high affinity of acetaldehyde (an ethanol oxidation product) to \( \alpha \)-tubulin, particularly since acetaldehyde-conjugated \( \alpha \)-tubulin inhibits tubulin assembly into MT (83). Hence, the reduced vesicle movement in hepatocytes when subjected to alcohol exposure may be due to direct effects on MT or may be indirect such as via motor function impairment, as noted by the reduction of dynamin association with Golgi membranes (83). Impairment of MT functions has also been observed in intrahepatic cholestasis. For example, cholestatic concentrations of chenodeoxycholate conjugates inhibit the activity of MT motors, thereby suggesting a possible mechanism for vesicular transport impairment in cholestasis (83). In addition, antibodies to MT have been observed in sera from patients with alcoholic liver disease (143) and hepatitis delta (275) and hepatitis B (148) virus infections. The biological significance of tubulin autoantibodies and their clinical utility, if any, are unclear.

Pancreatitis. Caerulein-induced pancreatitis is an established experimental animal model that mimics some of the serological and histological events of hu-
man acute pancreatitis. Exposure of animals or isolated acinar cells to caerulein leads to MT disassembly (114, 243) and to increased amylase release (243), thus suggesting a role for MT in pancreatitis. However, both MF (Ref. 114 and D. M. Toivola, N.-O Ku, N. Ghori, S. A. Michie, and M. B. Omary, unpublished observations) and IF are altered in ways similar to MT in the pancreatic models. Hence, disruption of the cytoskeleton in general accompanies pancreatitis, and it is unknown if unique etiologies (e.g., alcohol) preferentially target one cytoskeletal group vs. another. Cytoskeleton-related treatment of acute pancreatitis has not been studied using MF- or IF-modulating drugs in humans, but taxol does protect rats from caerulein-induced pancreatitis (243). This raises the question of whether such drugs can be useful in treating human pancreatitis.

Cancer. Familial and sporadic colon cancers are associated with truncation of the COOH terminus of the human tumor suppressor adenomatous polyposis coli (APC). The COOH terminus of APC physically associates with the EB/RP family of MAP, such as EB1 and RP3, and also likely associates directly with MT, but the precise interacting domains have not been well defined (16). Normally, EB1 is localized with cytoplasmic and spindle MT and coprecipitates with dynein, dynamin, p150, and other members of the dynactin complex (16). Mutated APC cannot bind to EB1, which provides clues regarding loss of cell cycle control. APC also controls the Wnt signaling pathway by binding to β-catenin proteolysis via cascades that include GSK-3β (GSK-3β and axin/conductin (248). This binding leads to β-catenin polyadsysis via cascades that include GSK-3β phosphorylation of β-catenin, subsequent ubiquitination, and then proteasome-mediated degradation (248). Mutated APC in colonic polyps and cancer cannot bind β-catenin, which leads to its nuclear accumulation and then to binding to and activation of the transcription factor Tcf-4, thereby leading to constitutive transcription of target genes including myc and cyclin D (248). The association of MT with carcinogenesis, as occurs with APC, and with other MAP including tau, which is overexpressed in pancreatic exocrine tumors (compared with normal cells, Ref. 249), appears to be highly relevant but indirect.

Infection. Infection of epithelial cells with some viruses or bacteria disrupts MT and can also result in utilizing MT or MT motors as highways for intracellular transport. For example, type 1 human immunodeficiency virus causes MT disruption in human colonic HT-29 cells (46). In addition, dynne plays a role in facilitating adenovirus infection in HeLa and TC7 cells (229). MT may also be important for internalization of some bacteria, given that Campylobacter jejuni uptake was blocked in intestinal cells by MT depolymerization (189). Furthermore, polymerized MT are important in E. coli translocation across the intestinal epithelial barrier (41). The role of MT in microbial pathogenesis is likely cell type and pathogen specific, since nocardazole and colchicine had a limited effect on Listeria monocytogenes uptake in Caco-2 cells compared with other cell lines (141).

Potential use of MT-modifying drugs in digestive disease therapy. In general terms, MT-stabilizing (e.g., taxol) and -destabilizing (e.g., the vinca alkaloids vincristine and vinblastine and colchicine) agents have been used as therapy for several human digestive diseases, including cancer, cirrhosis, acute alcoholic hepatitis, primary biliary cirrhosis, and hepatitis B infection. As such, colchicine has well-proven benefits and is routinely used as therapy and/or prophylaxis in several human diseases, including familial Mediterranean fever and gout (15). Similarly, taxol appears to be relatively beneficial in several malignancies, including esophageal (63a) and gastric (122) adenocarcinomas, but on the basis of phase II studies may not be effective in hepatic (30), pancreatic (263), biliary tree (109), and colorectal (96) adenocarcinomas. The in vivo resistance to anti-MT agents, despite high biological activities in tumor cell lines (219), may be related to altered tubulin isotype expression and/or to alteration in the P glycoprotein multidrug resistance phenotype (e.g., Ref. 55). In nonmalignant liver disease, colchicine is not effective against severe acute alcoholic hepatitis (2) and has mixed results against chronic hepatitis B and cirrhosis (e.g., Ref. 62). However, colchicine may play a beneficial therapeutic role in primary biliary cirrhosis, particularly if used in combination therapy (15). Therefore, the use of MT-modulating agents remains an attractive possibility for certain digestive disease states but warrants further mechanistic, design, and optimum targetting studies.

INTERMEDIATE FILAMENTS
Overview of IF Proteins
IF are the third major cytoskeletal protein group and have several distinctive properties (Table 1). They are divided into five types based on genomic structure and amino acid sequence homology. This excludes the “hard” keratins found in hair and other appendages, the lens proteins phakinin and filensin, and the neuroepithelial and muscle protein nestin (67). Type I–IV IF proteins are cytoplasmic and are expressed in a tissue-specific manner, whereas type V IF consist of the nuclear lamins. The type I and II keratins (K) (also called “soft” keratins or cytokeratins) are found specifically in epithelial cells, and is routinely used as therapy and/or prophylaxis in several human diseases, including familial Mediterranean fever and gout (15). Similarly, taxol appears to be relatively beneficial in several malignancies, including esophageal (63a) and gastric (122) adenocarcinomas, but on the basis of phase II studies may not be effective in hepatic (30), pancreatic (263), biliary tree (109), and colorectal (96) adenocarcinomas. The in vivo resistance to anti-MT agents, despite high biological activities in tumor cell lines (219), may be related to altered tubulin isotype expression and/or to alteration in the P glycoprotein multidrug resistance phenotype (e.g., Ref. 55). In nonmalignant liver disease, colchicine is not effective against severe acute alcoholic hepatitis (2) and has mixed results against chronic hepatitis B and cirrhosis (e.g., Ref. 62). However, colchicine may play a beneficial therapeutic role in primary biliary cirrhosis, particularly if used in combination therapy (15). Therefore, the use of MT-modulating agents remains an attractive possibility for certain digestive disease states but warrants further mechanistic, design, and optimum targetting studies.

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Table 4. Keratins and their tissue distribution

<table>
<thead>
<tr>
<th>Keratin</th>
<th>Distribution in digestive epithelia</th>
<th>Distribution in other epithelia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I keratin (relatively acidic)</td>
<td>K9–K20</td>
<td>Hepatocytes</td>
</tr>
<tr>
<td>Type II keratin (neutral/basic)</td>
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</tr>
<tr>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Adinar pancreas</td>
</tr>
<tr>
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<td></td>
<td>Small intestine</td>
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<td></td>
<td></td>
<td>Colon</td>
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<td>Esophagus</td>
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<td></td>
<td></td>
<td>Stomach</td>
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<td></td>
<td></td>
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<td></td>
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*Minor keratins in indicated tissues. **K.19 is found in the apical but not cytoplasmic compartment of acinar cells (Toivola et al., unpublished observations).

Although there are more than 50 different IF proteins, they all share a common domain structure that consists of heterogeneous non-α-helical NH$_2$-terminal and COOH-terminal head and tail domains and a conserved α-helical central coiled-coil rod domain (Fig. 3). The rod domain consists of several segments (e.g., IA, IB, II) that are separated by so-called linkers (e.g., L1, L1–2). The segments consist of nonidentical heptad repeats, with the first and fourth amino acids of the heptads being nonpolar to generate the hydrophobicity in the presence of MT- and MF-altering agents, which induce other cytoskeletal and cellular changes that interfer with the assembly and indirectly interfere with MF and MT organization (77). Similarly, MT-selective agents also induce other cytoskeletal and cellular changes that need to be considered, depending on the duration of exposure, but rapid and significant effects on keratin organization in the presence of MT- and MF-altering agents.

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In contrast to MF and MT, which have been manipulated in cultured cells and intact animals using corresponding stabilizing and destabilizing compounds in hundreds of studies, the use of such compounds in studying IF proteins is limited. Acrylamide was utilized as a disrupter of IF organization as initially described in PtK1 cells, with disruption of vimentin and keratin filaments within 4 h while MT and IF filaments were left intact (57). However, it appears that a wide range of drugs, which in general act as metabolic inhibitors, can also cause preferential IF collapse, but caution was raised in their use, since they (including acrylamide) inhibit protein synthesis (127). Another specific method for disrupting IF is to use peptides that are derived from region IA of the rod domain, which interfere with IF assembly and indirectly interfere with MF and MT organization (77). Similarly, MT-selective agents also induce other cytoskeletal and cellular changes that need to be considered, depending on the duration of exposure, but rapid and significant effects on keratin organization in the presence of MT- and MF-altering agents.

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agents typically occur in the presence of both agents but not when either is used alone (128).

One important feature of MF and MT vs. IF assembly is the nonpolarized mode of the latter. For example, analysis of inducible chicken vimentin incorporation into preexisting mouse vimentin in 3T3 cells shows incorporation at numerous sites without any polarity (183). Similarly, microinjection of biotinylated keratins into cultured cells shows similar results of rapid incorporation throughout the cell (169). This indicates that IF turnover occurs throughout the filament network, whereas MF turnover and MT turnover occur generally at their ends. IF organization and assembly also appear to be regulated by interaction with MT via motor proteins. For example, microinjection of detyrosinated but not tyrosinated tubulin into NIH/3T3 cells results in IF collapse in a fashion that depends on the 14-kDa COOH-terminal portion of the detyrosinated tubulin and kinesin (136). It remains to be determined whether this is a general phenomenon for all IF proteins and whether motors display cell and/or protein selectivity.

Functional Studies and Regulation of Keratins in Digestive Epithelia

Although simple epithelial keratins were initially analyzed biochemically and by immunofluorescence staining more than 20 years ago (67, 192), understanding their function has not been very rewarding using cultured cell systems and, if anything, has led to their underappreciation as nondynamic structural proteins. However, the tissue-specific expression of keratins implies unique functions, which have begun to emerge. Use of transgenic animals made understanding the biology and disease association of IF proteins more tenable and has led to the appreciation that keratin and other IF protein functions are more evident in the context of a whole organ compared with a single cell. The relative solubility of digestive epithelial, compared with epidermal, keratins has also made using biochemical approaches that focused on their regulation via posttranslational modifications and associated proteins a useful adjunct to the transgenic animal approach. Several keratin functions in digestive epithelia have been either conclusively defined or suggested (Table 5).

Table 5. Functions and disease association of keratins in digestive epithelia

<table>
<thead>
<tr>
<th>Functions</th>
<th>Disease Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance of hepatocyte integrity (well established)</td>
<td>Human disease related</td>
</tr>
<tr>
<td>Protection from environmental stress in the liver (via keratin phosphorylation)</td>
<td>Mutation in a patient with cryptogenic liver disease (one patient described to date)</td>
</tr>
<tr>
<td>Regeneration of cytoplasmic protein availability (14-3-3 proteins and hsp70)</td>
<td>Mallory bodies (role in disease progression unknown)</td>
</tr>
<tr>
<td>Cell signaling (14-3-3 association, modulation of phosphorylation by mitogens)</td>
<td>Utility in cancer histological diagnosis as tissue-specific markers (very useful)</td>
</tr>
<tr>
<td>Cell-type specific (based on distribution, evidence indirect)</td>
<td>Keratin serum fragments (diagnostic/prognostic utility appears to be helpful)</td>
</tr>
<tr>
<td>Compartment specific (based on phosphorylation-dependent polarized distribution)</td>
<td>Anti-keratin antibodies (significance unclear)</td>
</tr>
<tr>
<td>Drug resistance (evidence indirect)</td>
<td>Animal model disease related</td>
</tr>
<tr>
<td>Tumor invasion and metastasis (evidence indirect)</td>
<td>Liver is a primary target in K8/18-modulated mice (based on several animal models)</td>
</tr>
<tr>
<td>“Cell surface” receptors (evidence indirect)</td>
<td>Mouse Mallory body models (well established, should help in characterization)</td>
</tr>
</tbody>
</table>
| K18 in the liver. Other liver-associated findings in aging K18-null mice included K8-containing aggregates of Mallory bodies (155), which were not seen in K8-null mice (see Mallory bodies). Mouse phenotypes that lack other digestive organ keratins vary from surprisingly benign to significant. For example, K19-null mice were reportedly normal (192), although their behavior under stress conditions that could affect biliary or pancreatic ductal epithelia (among potentially involved epithelia) has not been reported. In K4-null mice, esophageal, tongue, and corneal epithelia exhibited basal hyperplasia, hyperkeratosis, and abnormal nuclei. In addition, the esophagus had bacterial invasion, luminal hemorrhagic exudates, and neutrophil infiltration (182). This supports a functional role for K4 in the maintenance of epithelial cell integrity, as demonstrated for keratinocytes and hepatocytes of transgenic mice that overexpress mutant K14 or K18, respectively (192).

Important information regarding keratin function in digestive organs was also obtained by overexpression of mutant or ectopic keratins, which resulted in keratin filament disruption in specific organs, depending on the
expression level and the presence of other endogenous keratins. Although overexpression of human K8, K18, or K19 genes at modest levels had no detectable phenotype (192), liver-targeted ectopic overexpression of K14 in mice resulted in hepatocyte keratin filament disruption, chronic hepatitis, and decreased bile acid secretion. Similarly, transgenic mice that overexpressed K18 Arg-89→Cys (Table 6, K18 R89C mice) in a tissue-specific manner developed mild chronic hepatitis, cytoplasmic keratin filament disruption in hepatocytes and pancreatic acinar cells, and marked hepatocyte fragility (192). Of note, Arg-89 of K18 is a highly conserved residue among IF proteins and is a frequently mutated residue in the epidermal keratin diseases (66). The K18 R89C mice were also far more susceptible to drug-induced liver injury compared with mice that overexpressed wild-type human K18 (140, 192, 236). These results indicate that one function of keratins in hepatocytes is to maintain cellular integrity and resilience and that an intact keratin filament network is important for protection from drug-induced liver injury. However, the cell protective role that K8/18 filaments impart in the liver was not observed in the pancreas after pancreatitis was induced using caerulein or a choline-deficient ethionine-supplemented diet (Toivola et al., unpublished observations). Hepatocyte and acinar cell susceptibility differences to injury, despite having a similar cytoplasmic filament disruption, raise the possibilities of a keratin functional redundancy in the pancreas or that identical keratins may function differently in different cells. Notably, overexpression of human K8 in transgenic mice resulted in significant acinar cell atrophy, dysplasia, and progressive pancreatic exocrine dysfunction (25). However, it is unclear if these findings relate directly to K8 function or if they indirectly relate to variables such as high transgene copy number and/or differences in human vs. mouse K8 sequences.

Posttranslational modifications. Keratins are highly dynamic and reorganize in response to many stimuli. Potential mechanisms for regulating filament reorganization include keratin-associated proteins and posttranslational modifications such as phosphorylation, glycosylation, transglutamination, and proteolysis (193). Phosphorylation is the most studied and occurs within the head and/or tail domains (Fig. 3), which are the domains that impart most of the structural heterogeneity and presumed tissue-specific functions (193). Transglutamination occurs as a normal physiological modification in epidermal keratins but is a pathological modification in simple epithelial keratins (see Mallory bodies below). Characterization of keratin-associated proteins and posttranslational modifications, as two potential regulators of keratin function, has provided important leads in studying keratin function.

**Table 6. Transgenic animal models of simple epithelial keratins**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype in Transgenic Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overexpression</td>
<td></td>
</tr>
<tr>
<td>K8 wild-type</td>
<td>Normal or exocrine pancreatic disorders</td>
</tr>
<tr>
<td>K18 wild-type</td>
<td>Normal</td>
</tr>
<tr>
<td>K19 wild-type</td>
<td>Normal</td>
</tr>
<tr>
<td>K18 R89C</td>
<td>Chronic hepatitis, cell fragility, susceptibility to liver injury</td>
</tr>
<tr>
<td>K18 S52A</td>
<td>Susceptibility to stress-induced liver injury</td>
</tr>
<tr>
<td>K18 S33A</td>
<td>Reorganized filaments in liver and pancreas</td>
</tr>
<tr>
<td>K14 (ectopic)</td>
<td>Chronic hepatitis, decreased bile acid secretion</td>
</tr>
<tr>
<td>Knockout</td>
<td></td>
</tr>
<tr>
<td>K4 null</td>
<td>Basal hyperplasia, hyperkeratosis, and atypical nuclei in esophagus, tongue and cornea</td>
</tr>
<tr>
<td>K8 null (C57Bl/6)</td>
<td>Embryo lethality, massive liver hemorrhage</td>
</tr>
<tr>
<td></td>
<td>Colorectal hyperplasia, colitis, rectal prolapse, susceptibility to liver injury</td>
</tr>
<tr>
<td>K18 null</td>
<td>Mallory body accumulation in liver</td>
</tr>
<tr>
<td>K19 null</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Table 6 adopted from Refs. 182, 192 (and references therein), and unpublished observations.
are important chaperones that play a role in cell signaling via binding and modulation of the biological activity of several proteins, including PKC, Raf-1, and cdc25 phosphatase (1). In general 14-3-3 proteins bind to their partners in a phosphoserine-dependent fashion akin to the binding of phosphotyrosine to SH2 domains. The K18 phosphoserine motif that binds to 14-3-3 proteins is different from other 14-3-3 binding proteins, and it is speculated that one keratin/14-3-3 binding function is to displace other 14-3-3 binding partners in a cell cycle-dependent fashion to allow them to carry out their function (138). In addition, 14-3-3 proteins can act as keratin sequestering proteins and interfere with keratin filament assembly in vitro (193), which is the only known example of a potential keratin sequestering protein. Further support for the importance of K18 Ser-33 in keratin filament assembly in vivo is based on formation of short, disassembled filaments in hepatocytes and pancreatic acinar cells of transgenic mice that overexpress K18 Ser-33→Ala (K18 S33A, Table 6).

KERATIN DEGRADATION DURING APOPTOSIS. The linker L1–2 region of IF proteins contains caspase-specific consensus sequences (Fig. 3). Among IF proteins, nuclear lamins were initially identified as caspase substrates during apoptosis. Lamin cleavage occurs before packaging of the condensed chromatin into apoptotic bodies, and uncleavable mutant lamin A or B delays cell death and DNA fragmentation (Ref. 200 and references therein). In terms of cytoplasmic IF, K18 and K19 were initially reported as caspase substrates during apoptosis (29, 137). K14 can also undergo cleavage at the VEMD aspartate (cleavage site in bold) during apoptosis (Ku and Omary, unpublished observations), and mutations near or at the cleavage site of K14 (VEMD→MEMD, VERD, or VEMG) have been described in patients with epidermolysis bullosa simplex (Refs. 179 and references therein). Whereas VXXD→MXXD in K14, K18, and K19 does not affect the susceptibility to caspase-mediated fragmentation, VEMD→VERD in K14 or VEVD→VERD in K18 and 19 generates fragments with faster gel migration, which suggests the unmasking of an alternative cleavage site. In contrast, the VEMD→VEMG K14 mutation likely inhibits K14 fragmentation during apoptosis, since VXXD→VXXE in K14, K18, and K19 and VXXD→VXXA in K19 block caspase-mediated fragmentation in transfected cells (unpublished observations). The physiological relevance and disease association of inhibiting keratin degradation remain to be determined, but the data so far indicate that the location of the mutation within the caspase recognition motif is essential in dictating whether the keratin becomes degraded or not.

Associated proteins. At least six categories of keratin-associated proteins can be envisaged based on the types of association that have been described to date: 1) proteins that link keratin IF to other members of the cytoskeleton; this category includes proteins of the plakin family (see below) whose members are expressed in a variety of cell types, including neuronal and epithelial cells (208, 264); 2) proteins that are involved in large complexes, which may modulate cell anchorage and/or invasion. For example, K8 and K18 interact with the trophodermal protein bystin, which in turn binds to trophonin and tastin (230). Keratin-bystin binding enhances trophonin and/or tastin binding with bystin, which may play a role in adhesion of the trophoblast to the endometrial epithelium. The presence of analogous proteins in digestive epithelia is not known. 3) Proteins whose cytoplasmic availability may be regulated by interaction with the cytoplasmic heat shock protein 70-kDa family in an ATP-dependent fashion and K18 binding with the cytoplasmic heat shock protein 70-kDa family in an ATP-dependent fashion and K18 binding with the 14-3-3 family members (193); cells are thus provided with the ability to utilize abundant proteins to modulate each other in a regulated fashion. Other categories of keratin-associated proteins include 4) proteins that are involved in cell signaling, including 14-3-3 proteins, a PKC-related kinase, and annexin I (40, 193); 5) proteins that link the ectoplasmic to the cytoplasmic environments, such as the direct (or indirect) binding of K8/K18 with an 85-kDa cell surface protein, whose identity remains to be determined (35); and 6) keratin cross-linking proteins, such as filagrin (226), as found in the epidermis.

Other important features of epithelial cells are desmosomes and hemidesmosomes, and, in this context, the keratin-associated plakin family proteins play pivotal roles. The plakin family includes the hemidesmosomal plectin, BPAG1n/dystonin (neural protein), and BPAG1e (epidermal isoform) and the desmosomal proteins envoplakin, desmoplakin I, and desmoplakin II (208). Plectin and BPAG1e cross-link integrins (particularly α6β4) and keratins, and mutations of these linker proteins in human and animal models clearly demonstrate their significance. For example, plectin mutations cause autosomal recessive muscular dystrophy with epitheliosis (MD-EBS) due to the lack of IF-plasma membrane anchorage (66, 264). This underscores the important MF and IF binding and cross-linking features of BPAG and plectin and is supported by the presence of an actin-binding domain in their NH2 terminus and their direct binding and colocalization with keratin filaments (208). Plectin also cross-links IF and MT possibly via interaction with MAP (264), and BPAG1n3 cross-links IF to MT (268). With regard to desmosomal keratin binding proteins, desmoplakin-null mice form fragile, small embryos and have ~10x reduction in the number of AJ and a disorganized keratin network (73). It is unclear if simple epithelial cells also express other related linker proteins such as a unique BPAG.

Mechanical properties of IF proteins. The cytoskeleton provides a path for mechanical force transfer from the cell surface to the nucleus (104). Advancement in rheological methods (study of deformation and flow of biological polymers) is providing insights into the biophysical properties of cytoskeletal proteins and is unfolding the interplay of the three cytoskeletal groups, their associated proteins, and posttranslational modifi-
cations. MF mediate force transfer at low strain, but at high strain MF and MT rupture, whereas IF withstand a wide range of strain conditions and efficiently transfer the mechanical force. Even under conditions that lack intact MF and MT in bovine capillary endothelial cells treated with cytochalasin D and nocodazole, the IF network alone is able to resist large deformations and to transmit the mechanical force. This indicates that IF proteins are uniquely critical for the maintenance of cell integrity and response to mechanical stimuli (156) and supports the significant loss of mechanical integrity that is seen in hepatocytes and keratinocytes as a result of keratin mutations. The ability of IF proteins to resist high strain is likely related to their viscoelastic and structural properties. These properties can be classified as rodlike (MT), semiflexible (MF), and flexible (IF) (104). Another unique property of IF proteins is that they behave as viscoelastic solids that yield to shear strain rapidly as a manifestation of their flexibility, manifest strain hardening with repeated deformation, and recover quickly on cessation of an input strain. Viscoelastic differences do occur within IF proteins and possibly within keratins, which may correlate with their tissue-specific roles, although this remains to be tested. For example, sequential small deformations result in a progressively stiffer keratin biopolymer but less so for vimentin (151).

Cell-specific and compartment-specific roles. The tissue- and cell type-specific distributions of keratins suggest that they may play unique functional roles in such cell types. For example, K19 is expressed in basal cells of gastrointestinal epithelia, whereas K20 is expressed preferentially in the more differentiated suprabasal and tip cells (Ref. 173 and references therein), which correlates with proliferation differences in the two compartments. Although it is unclear what is the specific function of K19 or K20 in these different differentiation states, keratin replacement experiments in transgenic mice suggest that keratins are not completely redundant. For example, introduction of K18 into transgenic mice that lack K14, using the K14 promoter, only partially rescued the lethal and blistering phenotype that is typically noted in K14-null mice (98). This suggests that different keratins are likely to serve tissue-specific roles that reflect their unique expression. Another form of keratin compartmentalization is at the subcellular and cytoskeletal-associated vs. cytosolic levels, particularly when considering site-specific phosphorylated vs. unphosphorylated species (193). Similarly, K19 appears to be localized preferentially in the apical domain of several polarized cultured cells and downregulation of its expression using antisense nucleotides decreased the number of MV and slightly missorted the targeting of apically but not basolaterally distributed proteins (210). However, there were also effects on MT and MF in these cells, so it remains unclear if the observed changes were specific to K19 modulation.

The unique keratin expression also provides clues regarding the origin of metaplastic states such as Barrett’s esophagus (BE). For example, a small group of distinctive multilayered cells within the columnar metaplastic BE cells, which normally express K8/K18/K19, was found to express K8/K19 and K4/K13 (18). Coexpression of the normal squamous esophageal K4/K13 suggests that these cells may be precursor cells that populate BE and arise on denudation of the normal squamous epithelium and exposure to gastric acid injury. This provides evidence against the model that BE arises from an upward migration and growth of gastric epithelial cells after injury (18).

Drug resistance and tumor spread. Epithelium-derived tumors such as those arising from lung, breast, and colon have a drug resistance that blunts their sensitivity to chemotherapeutic agents in association with K8/K18 expression. For example, epithelium-derived carcinoma cell lines are 10–20,000 times more resistant to drugs such as doxorubicin and mitoxantrone compared with hematopoietic cell lines that do not express K8/K18 (39). Transfection of K8/K18 into keratin-negative cells imparts drug resistance to several agents that cause DNA damage via a variety of mechanisms but do not confer protection from ionizing and ultraviolet irradiation, which do not require interaction with the keratin network (5, 14). It is unlikely that drug resistance is due to direct drug sequestration via keratin-drug interactions, since only 4% of mitoxantrone associates with the keratin-enriched cytoskeleton (39). The presence of a normal filament network is not critical to drug resistance, since network-defective cells have similar resistance compared with cells with a normal filament network (39). The mechanism of drug resistance in epithelial cells is likely to be multifactorial, and the role of keratins in this may be indirect via regulation of multidrug resistance gene expression, modulation of cell signaling, and/or drug sequestration.

IF protein expression is maintained in tumor cells in most cases, although tumors can also coexpress mixed IF proteins and this coexpression may have functional implications (173). For example, most melanomas typically express vimentin, but K8/K18 coexpression in ocular melanoma is restricted to retino-invasive malignant melanoma (124). In vitro studies using membrane invasion culture systems demonstrate that K8/K18 overexpression in mouse L cells (which express vimentin) induces cell migration and invasion, and vimentin overexpression in human breast cancer cells by stable transfection results in increased mobility and invasiveness that can be significantly decreased by vimentin antisense oligomers (88). In vitro invasion measurements of human uveal melanoma cell lines show that uveal melanoma cells with K8/K18 and vimentin are 6-fold or 8- to 13-fold more invasive than the cells with only vimentin or normal uveal melanocytes, respectively (87). However, coexpression of keratins and vimentin is not sufficient to disseminate primary tumor cells to other organs (87). In addition, keratin expression in melanomas regardless of their metastatic potential may be a common finding (118). The functional significance of keratins or keratin/vimentin coexpression in tumor cell invasiveness remains poorly understood.
Potential role of keratins as cell surface receptors. Several reports have suggested the presence of cell surface keratins (only K8/K18 discussed here) that may act as receptors to a number of ligands. These include K8 on breast cancer cells as a putative plasminogen receptor (86) and K18 on hepatocytes as a thrombin-antithrombin complex receptor (260). In addition, several other reports have suggested the presence of cell surface K8/K18 or K10 (Ref. 205 and references therein) or modified K8/K18 (51). Although this is a potentially intriguing keratin function, the evidence for such a role is indirect and has not been demonstrated in vivo. For example, not completely excluded or assessed is the potential cross-reactivity of the analyzed anti-keratin antibodies to nonkeratin antigens, the nonspecific adsorption of “leaked” keratins (particularly because keratins are abundant and “sticky” proteins) to the cell surface, the dynamic nature for this putative interaction, and the method whereby the resident cytoplasmic protein reaches the cell surface. Mis-sorting and post-translational modifications are among potential mechanisms for keratin cell surface localization that have not been formally evaluated. Molecular mimicry is also a possibility, since antibodies to nonkeratin antigens can cross-react with keratins (258) and vice versa (unpublished observations) under what appears to be selective conditions such as differentiated states or exposure to cytokines. Although in vitro interaction domains have been defined for K8 binding to plasminogen (135), the in vivo significances of such interactions are not known. Hence, the putative cell surface receptor role for keratins and its biological relevance remain to be determined.

Disease Association of IF Proteins

Mutations in IF proteins account for several human diseases, most of which are autosomal dominant, although sporadic and recessive cases have been reported (66). Their dominant phenotype is based on the heteromeric and multistep assembly of the IF polymer, and manifestations of these diseases partly reflect the tissue-specific expression of IF proteins. Association of keratin mutations with epidermal diseases was suggested, in part, by the phenotype of transgenic mice that overexpressed truncated human K14, which showed a disease reminiscent of epidermolysis bullosa simplex (EBS). This led to identification of K14 and K5 mutations in EBS patients, a finding that was also determined independently by linkage analysis (66). Mutations in other epidermal keratins have since been identified as the cause of epidermolytic hyperkeratosis (K1/10), epidermolytic palmoplantar keratoderma (K9), ichthyosis bullosa of Siemens (K2e), and pachyonychia congenita (K6/16/17) (66). In addition, trichocyte keratin Hb6 mutations result in the beaded and fragile hair autosomal dominant hair defect monilethrix, desmin mutations result in cardioeskeletal myopathy (e.g., Ref. 180), and lamin mutations result in Emery-Dreifuss muscular dystrophy (20). To date, mutations in 14 of 20 keratins (excluding the trichocyte keratins) have been identified. Although neurofilament overexpression in transgenic mice results in a phenotype that mimics amyotrophic lateral sclerosis, it is unclear if neurofilament mutations can be causal in this neuromuscular disease (3, 113).

Digestive epithelial keratins K4/13 and K8/18. On the basis of the epidermal keratin diseases, the K4/13 preferential expression in the esophagus (174) suggests that K4 or K13 mutations may cause significant esophageal pathology. However, phenotypes of K4-null mice and patients with white sponge nevus (WSN) who harbor K4/13 mutations involve the oral mucosa with minimal esophageal involvement, although WSN patients can on rare occasions have esophageal manifestations (182). In K4-null mice, it appears that an undefined keratin, which could correspond to K6, becomes overexpressed and could compensate for the absence of K4 (182). In addition, other keratins that are expressed at low levels, such as K15 or K19, could also compensate functionally in WSN K13 mutations. Furthermore, esophageal epithelium may not experience the same type of mechanical "trauma" that oral and skin epithelia or cardiomyocytes (for desmin) endure repeatedly and hence may not be as susceptible to injury.

The K8/18 transgenic animal models (Table 6) suggested that the liver is the likely target of K8/18 mutations if they are to occur in humans. Hence, the most likely population for K8/18 mutations are patients with acute or chronic cryptogenic liver disease. Although inherited epidermal keratin diseases are relatively rare, there are no well-defined cases of autosomal dominant cryptogenic liver diseases. A search for K18 mutations in 28 patients with cryptogenic cirrhosis identified one patient with a K18 point mutation in the L1 region, which appeared to be sporadic and absent in >240 alleles. The same mutation in a K18 cDNA caused an in vitro filament assembly defect, which suggested that it was etiological or that it may have predisposed to the patient’s liver disease (192). The notion of a liver disease predisposition is attractive, since the K18 R89C transgenic mouse phenotype (which harbor the severe filament disrupter mutation) is relatively mild unless challenged by toxins (140, 192, 236). Genetic analysis of a large and broad cohort of liver disease patients, particularly cases with a strong family history and a phenotype that mimics the findings in the transgenic animal models (e.g., necrosis, hemorrhage, mild inflammation), should clarify the frequency and nature of K8/18 association with liver disease. In addition, an intestinal target, as in inflammatory bowel disease, may be associated with K8 mutations based on the intestinal phenotype of the K8-null mouse (Ref. 11 and Table 6). It is likely that simple epithelial keratin diseases are rare, but the predisposition model if correct has the potential to be significantly more common.

Utility as tissue and cellular differentiation markers. The wide range of antibodies that recognize specific keratins, coupled with tissue-selective keratin expression, make assessing keratin presence an important adjunct for histological diagnosis of tumor origin and subtyping carcinomas (173). We focus here on keratin
patterns in adenocarcinomas of digestive organs. Keratin expression, in general, distinguishes a lesion as a carcinoma and K8/18 expression indicates an adenocarcinoma, but the tissue source of different adenocarcinomas can be further refined. For example, pancreatic adenocarcinomas typically express K7/8/18/19 (suggesting a ductal origin), and have a significant focal increase in stratified epithelial keratins such as K4, K5, K13, and K17 compared with colorectal and gastric carcinomas (173). In addition, K7 is commonly found in pancreatic carcinomas compared with other gastrointestinal tumors. Although subtyping adenocarcinomas by their keratin expression has not reached the same clinical utility as the use of leukocyte-specific markers to subtype leukemias and lymphomas, the presence, absence, or altered expression of specific keratins can have useful diagnostic and prognostic vantages. For example, elevated K18 expression is associated with an improved prognosis in breast cancer patients (4.5% survival vs. 44.6% survival after 8 years of follow-up) (212), although this remains to be confirmed by other studies. In addition, K19 was detected in 7 of 34 patients with metastatic gastric cancer using nested PCR of peripheral blood, with a sensitivity of 1 cancer cell per 10^6 mononuclear cells, and may be associated with poor prognosis in patients who do not respond to chemotherapy (271). Micrometastatic esophagogastric carcinoma into resected rib segments (194) or gallbladder carcinoma into regional lymph nodes can also be detected using anti-K8/18 immunohistochemical staining and in some cases can be more sensitive (7 of 26 cases) in detecting metastatic foci not noted using routine hematoxylin and eosin staining (272). Examination of specific keratin pattern expression can also be helpful in determining the tumors that are metastatic to the liver (241). The broad utility of these methods in diagnosing cancer metastasis still requires further confirmation, but results to date are highly promising.

Keratin serum fragments and autoantibodies. Keratin serum fragments develop in patients with epithelial tumors and have a potential utility as tumor diagnostic markers. Serum components, referred to as “tissue polypeptide antigen” (TPA) were subsequently identified as K8-, K18-, and K19-related fragments. Additional keratin fragments include tissue polypeptide-specific antigen (TPS), a K18-soluble fragment (209), and anti-K19 fragments (called CYFRA 21-1) (Ref. 44 and references therein). In digestive organ-related tumors, several studies supported the utility of keratin fragment detection as prognostic or diagnostic markers. For example, TPS levels were more sensitive than carcinoembryonic antigen in predicting relapse in patients with colorectal cancer (e.g., Ref. 170). A ubiquitinated K8 fragment has also been detected in 25 of 31 surgically resected primary colorectal cancers but not in controls (185). In addition, CYFRA 21-1 may be a potentially useful marker for gastric adenocarcinomas (181) and for esophageal squamous cell carcinomas (267). In contrast, TPS and TPA levels do not appear to be helpful in pancreatic carcinomas (195) and do not appear to be as useful as α-fetoprotein in hepatomas (144), respectively. Their clinical use may increase as more studies test their potential utility, although their sensitivity and presence in nonmalignant conditions such as cirrhosis (176) and the appearance of some contradictory data (44) may be confounding. The site(s) of cleavage and mode of generation of serum K8/18/19 fragments and their relationship to already characterized apoptotic K18 and K19 fragments (29, 137) remain to be determined.

The presence of anti-keratin antibodies has been described in association with several diseases. The so-called “anti-keratin antibodies” (or anti-perinuclear factor) that strongly associate with rheumatoid arthritis recognize the epidermal protein filagrin (216). Anti-K8 antibodies are present in sera of patients with cryptogenic fibrosing alveolitis, but the significance of these antibodies is unknown (52). Anti-K8/18 antibodies also occur in association with autoimmune hepatitis and after interferon therapy. In particular, anti-soluble liver antigen (anti-SLA) antibodies that define a subpopulation of patients with autoimmune hepatitis appeared to recognize K8/18 (252). More recently, anti-SLA antibodies were shown to recognize glutathione S-transferase family members but not keratins (262), and the reason for the discrepancy is likely related to assay conditions (125). These antibodies appear to be distinct from anti-liver-kidney microsome antibodies and anti-liver-pancreas antigen (125, 247). Hence, the significance and diagnostic utility of keratin autoantibodies remain unclear.

Mallory bodies. Mallory bodies are hepatocyte cytoplasmic insoluble inclusions found in a variety of liver diseases (105). It is unclear whether Mallory bodies play a causative role in liver damage or whether they are simply a by-product of the damage process. Mallory bodies have a condensed tubular structure and contain ubiquitinated and aggregated K8/18 as major components with small amounts of RNA, phospholipid, and glycogen (105). The study of Mallory bodies has been facilitated by the availability of mouse models that can be induced to form Mallory bodies, such as after chronic intoxication with GF or 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) or after GF, DDC, or ethanol refeeding in GF or DDC drug-primed mice (47, 274, 278). In addition, Mallory bodies form spontaneously in hepatocytes of old K18 (155) but not K8-null mice (11), thereby indicating that K8 is sufficient for their formation. Accumulation of deposits that are reminiscent of Mallory bodies is also a feature of other IF proteins such as neurofilaments in Lewy bodies, which are found in several neurodegenerative diseases (113).

Although the mechanism(s) of Mallory bodies formation is poorly understood, their formation may be facilitated by multidrug resistance P-glycoproteins and transglutaminases (e.g., Ref. 199). After GF or DDC mouse feeding (>6 wk), many hepatocytes form Mallory bodies of aggregated K8/18 coupled with loss of P-glycoprotein expression, but some hepatocytes with normal K8/18 filaments still express P-glycoprotein. This suggests that Mallory body formation may be related to hepatocyte drug accumulation in association
CONCLUDING REMARKS

The goal of this review is to highlight unique aspects, common features, and functions of the three major cytoskeletal protein families, with emphasis on the digestive system (Tables 1 and 7). Most of the known functions involve MF and MT and in some cases all three cytoskeletal groups. In terms of known functions, IF proteins lag behind MF and MT but appear to possess the unique roles of allowing cells to cope with mechanical and environmental stresses. These more specialized IF functions are in line with the evolutionary development of higher and more complex organisms and correlate with IF expression. Although they are equally if not more abundant than MF and MT in some cell types, IF proteins also have other unique properties, including their mode of assembly, the presence of a small soluble pool, and a diverse family with members having a prominent cell-specific expression (Tables 1 and 4). The general preservation of the keratin normal tissue-specific expression during malignancy has made IF protein typing of tumor cells commonplace. Although as a group keratin mutations cause many epithelium-specific diseases, it is likely that MT mutations will be identified as a cause of one or more human diseases and more MF-associated mutations will be defined. In the case of simple epithelial keratins, it remains to be determined whether mutations in K7/8/18/19/20 will directly cause and/or predispose patients to digestive organ-related diseases.

One difference between IF and MF vs. MT is their susceptibility to caspase digestion during apoptosis as one potential mechanism that allows the cytoskeleton to reorganize. Interestingly, actins and keratins are well-described caspase substrates (e.g., Refs. 29, 119, 137, 159). However, we are unaware of any reports that describe tubulin degradation during apoptosis despite the presence of potential aspartate caspase cut sites. If tubulin is indeed not significantly degraded during apoptosis, then one potential explanation could be the high dynamic instability of tubulins, which renders their apoptotic cleavage unnecessary from a cellular rearrangement standpoint. Regardless, the dynamics of MF, IF, and MT are clearly different and are also mediated by unique associated proteins and modifications.

The not-so-distant past use of the term cytoskeleton as a "black box" is becoming far more refined. Hence, understanding the regulation of MF, MT, and IF at the level of associated proteins and posttranslational modifications is gaining momentum at a rapid pace. In addition, the intimate interaction of the three cytoskeletal groups is becoming magnified as mutations in linker proteins such as plectin are recognized (66, 264). Although many studies have focused on one particular cytoskeletal protein group in terms of a biological response to a stimulus, it is possible that the tested stimulus could also affect one or more of the two remaining cytoskeletal elements in a similar or different fashion. This is not to say that different stimuli cannot have a selective impact on a given cytoskeletal protein group, since there are many examples of such cases. For example, K18 dominant negative or K8-null mutations have a subtle or nondetectable impact on MF and MT (e.g., Ref. 236), although it is not known if the reverse would hold true. Indeed MF, MT, and IF behave as siblings in that they are related yet have their own identity.

Another biological and potentially clinically important aspect derived from understanding the three cytoskeletal protein groups is the ability to devise strategies to target drugs for specific disease states. Even a "general" anti-MT drug such as taxol appears to be very useful in the clinical arena. Using MT as an example, better understanding of the cytoskeletal-associated effects of known chemicals such as arsenic (146) and characterization of taxol-like compounds, which resist overexpression of the drug resistance P-glycoprotein (175), and other unique compounds such as lilmquinone, which disassembles unstable MT but leaves stable MT intact (198), should provide insights into MT-related biology and potential disease modulation. Finally, the study of the cytoskeleton in digestive epithelia is a broad and open area for investigation. Among the many relevant questions to address are which IF/MF/MT motor and nonmotor binding proteins are important in such epithelia and how their interactions are regulated. In addition, there is an accumulating list of signaling molecules that appear to bind

Table 7. Summary of MF, MT, and IF functions in digestive epithelia

<table>
<thead>
<tr>
<th>Function</th>
<th>MF</th>
<th>MT</th>
<th>IF</th>
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<tbody>
<tr>
<td>Cell proliferation</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Development and differentiation</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Cell polarity</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Vesicle transport</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Organelle positioning</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cell signaling</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Regulation of cytoplasmic protein availability</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cell shape and motility</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Protection from environmental stress</td>
<td>?</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Mechanical cell integrity</td>
<td>?</td>
<td>?</td>
<td>+</td>
</tr>
</tbody>
</table>

+, Significant evidence for listed function; -, no evidence for listed function; and ?, relationship to function unclear.
directly or indirectly with all three major cytoskeletal protein groups, which raises obvious questions regarding such interactions. For example, it appears that the cytoskeleton can function as a signaling regulator and/or a scaffold (80, 103, 193, 224). Although the IF field is still lagging in terms of the number of associated proteins that have been described, the MF and MT fields have an abundance of associated proteins that remain to be studied in more detail. As new target-specific modulators of cytoskeletal proteins, genetic models, advances in proteomics and genomics analysis, improved analytical and imaging techniques and culture methods, and progress in the human genome project become available, our understanding of these proteins will proportionately grow.

We are grateful to Drs. Pierre Coulombe (Johns Hopkins University), Gregg Gundersen (Columbia University), Paul Matsudaira (Massachusetts Institute of Technology), Robert Oshima (The Burnham Institute), and James Spudich, Timothy Stearns, and Hans Warrick (Stanford University) for comments and critical reading of the review and are grateful to Kris Morrow for preparing the figures and Sally Moorfield for help with text editing. Because the topic is broad, we apologize to authors whose work we inadvertently omitted or did not cite due to space limitations.

Our work is supported by Veterans Affairs Merit and Career Development Awards (M. B. Omary), National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-47918 and DK-52951 (to M. B. Omary), and a Postdoctoral Fellowship from The Academy of Finland (D. M. Toivola). N.-O. Ku, X. Zhou, and D. Toivola contributed equally to this review. Address for reprint requests and other correspondence: M. B. Omary, Palo Alto VA Medical Center, 3801 Miranda Avenue, 154, Palo Alto, CA 94304.

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