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Ji-Ying Song · Cornelis J.F. Van Noorden
Wilma M. Frederiks

Alterations of hepatocellular intermediate filaments during extrahepatic cholestasis in rat liver

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Abstract Intermediate filaments (IF) maintain the structural and functional integrity of cells. To investigate whether IF change as a consequence of increased mechanical pressure and what the significance of such alterations is for the integrity of hepatocytes, we investigated alterations of IF in rat liver following common bile duct ligation (CBDL). Immunofluorescence of cytokeratin 18 was performed on extracted cryostat sections which were also used for electron microscopy. Ultrathin sections of mildly extracted liver tissue were applied to reveal the relationship between IF and intercellular junctions and cytoplasmic organelles. Our results showed that hepatocellular IF underwent striking changes during CBDL. The so-called pericanalicular sheath disappeared and IF were rigidly rearranged at the cell periphery, appearing as honeycomb-like structures. Increased amounts of IF were found in close association with increased numbers of desmosomes at the lateral membranes of hepatocytes, and electron-dense desmosome-like bodies were even observed in the ectoplasm at bile canaliculi. Rearrangement of IF in the cytoplasm resulted in segregation of subcellular compartments. The increased density of the IF network and desmosomes are compensatory mechanisms of hepatocytes to resist increased mechanical load and disperse the tension. However, the intracellular rearrangement of IF leading to segregation of subcellular compartments may also have distinct effects on hepatocellular metabolic functions.

Key words Cholestasis · Cytokeratin · Cytoskeleton · Electron microscope · Intermediate filaments

J.-Y. Song · C.J.F. Van Noorden · W.M. Frederiks[1] (✉)
Laboratory of Cell Biology and Histology,
Academic Medical Center, University of Amsterdam,
The Netherlands

Mailing address:
[1] Academic Medical Center, University of Amsterdam,
Laboratory of Cell Biology and Histology, Meibergdreef 15,
1105 AZ Amsterdam, The Netherlands
Tel.: (31) 20-566 4971, Fax: (31) 20-697 4156

Introduction

Intermediate filaments (IF), together with microfilaments and microtubules, form the cytoskeleton (CSK) of eukaryotic cells. Understanding of the molecular components, the dynamics and the morphology of CSK has contributed a great deal to the current concepts of the principles of cell locomotion, cell division, transcellular transport, intercellular adhesion, and cellular morphogenesis [28, 36].

However, IF are still the least understood component of the CSK with respect to their physiological functions [14, 39]. During the last 15 years, the information available on IF protein composition [32], dynamic regulation [5, 27, 45] and structural organization [10, 21] has increased remarkably in volume. IF are thought to protect cells against mechanical stress and provide structural support [15]. The IF–desmosome complex allows transduction of shearing forces from one cell to another [40, 44]. Lazarides et al. [26] proposed that IF function as mechanical integrator of the cellular space regulating cell shape and distribution of organelles in the cytoplasm. It has also been suggested that IF are involved in cellular signalling [11], autophagy and transcellular transport [4].

IF in hepatocytes are composed of cytokeratins (CK) and form a meshwork extending from desmosomes at the lateral walls throughout the cytoplasm. They form a supportive network around bile canaliculi, the so-called pericanalicular sheath [21]. An intact pericanalicular sheath is considered to be essential for structural integrity of the bile canaliculus and for bile secretion [22, 23]. Pathological alterations of IF in liver have been investigated mainly in relation to their role in Mallory body formation in alcoholic liver disease [6, 13, 19, 30]. Recently, Ku et al. [25] introduced transgenic mice with mutant CK 18 which developed chronic hepatitis and fragile livers in association with CK8/CK18 filament disorders. French et al. [29] demonstrated focal disruption of the pericanalicular sheath during experimental cholestasis.

Cholestasis is usually defined as disturbance of normal bile secretion accompanied by accumulation of substances in the blood that are normally secreted in bile [7]. Malfunctioning of microfilaments and/or microtubules during cholestasis has been well documented and has been shown to play an important role in the failure of bile secretion and retention of bile fluid (for reviews see [34, 35]. In contrast, little attention has been paid to the structural organization of IF and its significance in obstructive cholestasis. Obstructive cholestasis increases biliary mechanical pressure, which may modulate hepatocellular IF. This may affect functions of IF as integrators of the cytoplasmic space and structural supporters in hepatocytes. In order to elucidate the role of IF in the pathogenesis of extrahepatic cholestasis induced by common bile duct ligation (CBDL), we investigated the subcellular organization of IF and its alterations in hepatocytes during CBDL. Morphological features of IF determined by light microscopy (LM) and electron microscopy (EM) were correlated with the increased number of desmosomes and changes in the spatial organization of cytoplasmic organelles.

Materials and methods

Twelve male Wistar rats, each weighing 200–250 g, were used. All animals had free access to water and food and were kept in a constant environment with respect to temperature, humidity and daylight cycle. In nine animals extrahepatic cholestasis was induced by CBDL as described elsewhere [3]. On the day of operation, the common bile duct was ligated twice under ether anaesthesia between the liver hilus and the head of the pancreas. The duct between the ligatures was then resected. Incisions of the abdominal muscle and skin were closed with a continuous suture. Antibiotics were not administered after operation and water and food were again freely accessible. Three sham-operated rats were kept under the same environmental conditions as rats with CBDL and were used as controls. After 2, 7 and 14 days of CBDL, rats were sacrificed under ether anaesthesia and livers were removed. Livers were cut into pieces which were either frozen in liquid nitrogen and used for cryostat sectioning for LM and EM purposes, or treated with saponin followed by conventional EM procedures.

In order to examine the architecture of IF, cryostat sections were intensively extracted using the method of Fey et al. [10] as modified by Katsuma et al. [21]. Cryostat sections (10 μm thick) were cut on a Bright motor-driven cryostat at a cabinet temperature of –25°C and mounted on grids attached to coverslips by a formvar film.

Cryostat sections were not allowed to dry before extraction. Sections were extracted for 6 min at 4°C. The extraction medium consisted of 100 mM NaCl, 3 mM $MgCl_2$, 300 mM sucrose, 0.5% Triton X-100 (Merck, Darmstadt, Germany), 1.2 mM phenylmethylsulfonyl fluoride (Sigma, St. Louis, Mo.), 0.1 mM iodoacetamide (Merck) and 10 mM Pipes buffer, pH 6.8. Sections were then rinsed in the same medium with 250 mM $(NH_4)_2SO_4$ as substitute for 100 mM NaCl for 6 min at 4°C, and were treated with this medium containing 100 μg/ml bovine pancreatic DNase-1 (Sigma) and 100 μg/ml bovine pancreatic RNase-A (Sigma) for 10 min at 22°C, and finally washed in a medium containing additional 250 mM $(NH_4)_2SO_4$ for 6 min at 22°C. After the extraction procedure, sections were fixed immediately with 2% glutaraldehyde in 100 mM cacodylate buffer, pH 7.4, for 30 min at 22°C followed by fixation with 1% osmium tetroxide in the same buffer for 10 min at 22°C. After fixation, samples were dehydrated through a graded series of ethanol, followed by CO_2 critical point drying and carbon coating. The specimens were observed with transmission electron microscopy (EM 10c; Zeiss, Oberkochen, Germany) at 80 kV.

For immunofluorescence cryostat sections 7 μm thick were cut on a Bright motor-driven cryostat at a cabinet temperature of –25°C and mounted on Superfrost glass slides. Unfixed cryostat sections were extracted with the same procedure as described above. After extraction, sections were rinsed three times in phosphate-buffered saline (PBS) and incubated with monoclonal mouse anti-human CK18 (RGE 53, IgG_1, undiluted; a gift from Prof. Dr. F.C.S. Ramaekers, Dept. of Molecular Cell Biology, University of Limburg, Maastricht, The Netherlands) for 60 min at 22°C followed by 30 min rinsing in PBS. Then sections were exposed to fluorescein isothiocyanate (FITC)-conjugated secondary antibody (Sigma,) 1:80 in PBS, for 60 min at 22°C. Finally, sections were rinsed in PBS and mounted in anti-fading mounting medium (Vectashield; Vector Lab., Burlingame, Calif.) and studied with a fluorescence microscope (Zeiss). Control staining was performed by incubating sections only with the FITC-conjugated secondary antibody and not with the primary antibody.

In order to investigate IF in relation to cytoplasmic organelles and intercellular junctions, a mild extraction method was applied according to Araki et al. [1]. Briefly, fresh liver tissue was cut into small pieces up to 1 mm^3, which were immediately immersed in a solution of 0.03% saponin (Sigma), 1 mM phenylmethylsulfonyl fluoride and 5 μM leupeptin (Sigma) in PHEM buffer (60 mM Pipes, 25 mM Hepes, 10 mM EGTA and 2 mM $MgCl_2$, pH 6.8) for 20 min at 22°C. After extraction, the tissue blocks were rinsed in PHEM buffer containing 8% sucrose for 30 min, and then fixed in a solution of 1% glutaraldehyde, 4% paraformaldehyde, 0.5% tannic acid and 8% sucrose in PHEM buffer, pH 6.8, for 2 h at 4°C and post-fixed with 1% osmium tetroxide in the same buffer for 1 h at 4°C. After block-staining with 1% uranyl acetate in distilled water for 30 min at 22°C, the specimens were dehydrated and embedded in LX-112 epoxy resin according to routine procedures. Ultrathin sections were stained with uranyl acetate and lead citrate and analysed with a Zeiss EM 10c transmission electron microscope at 80 kV.

Results

Morphological properties of IF were determined by means of immunofluorescence of CK18- and Triton X-100-extracted cryostat sections examined with EM. These approaches provided similar information on the structural organization of IF in rat livers. Immunofluorescence microscopy showed a filamentous meshwork of CK18 throughout the cytoplasm of hepatocytes of control liver with higher amounts at the cell periphery (Fig. 1A). Tube-like structures, the so-called pericanalicular sheaths, were observed at bile canaliculi. The fluorescence intensity at bile canaliculi was enhanced after 2 days of CBDL (Fig. 1B). CBDL for 7 induced largely increased amounts of CK18 in the cytoplasm of some hepatocytes and pericanalicular sheaths were hardly recognizable in these cells (Fig. 1C). Furthermore, the increased fluorescence intensity at the cell periphery gave rise to a honeycomb-like appearance of CK18 in hepatocytes following CBDL for 14 days (Fig. 1D).

The altered structural organization of IF was observed in more detail in extracted cryostat sections at the EM level. The network of IF was indeed present throughout the cytoplasm from the periphery of the cells to the surface of the nuclei in control hepatocytes (Fig. 2A). The organization of IF at the cell periphery was polarized: branching tube-like structures of IF, the so-called perica-

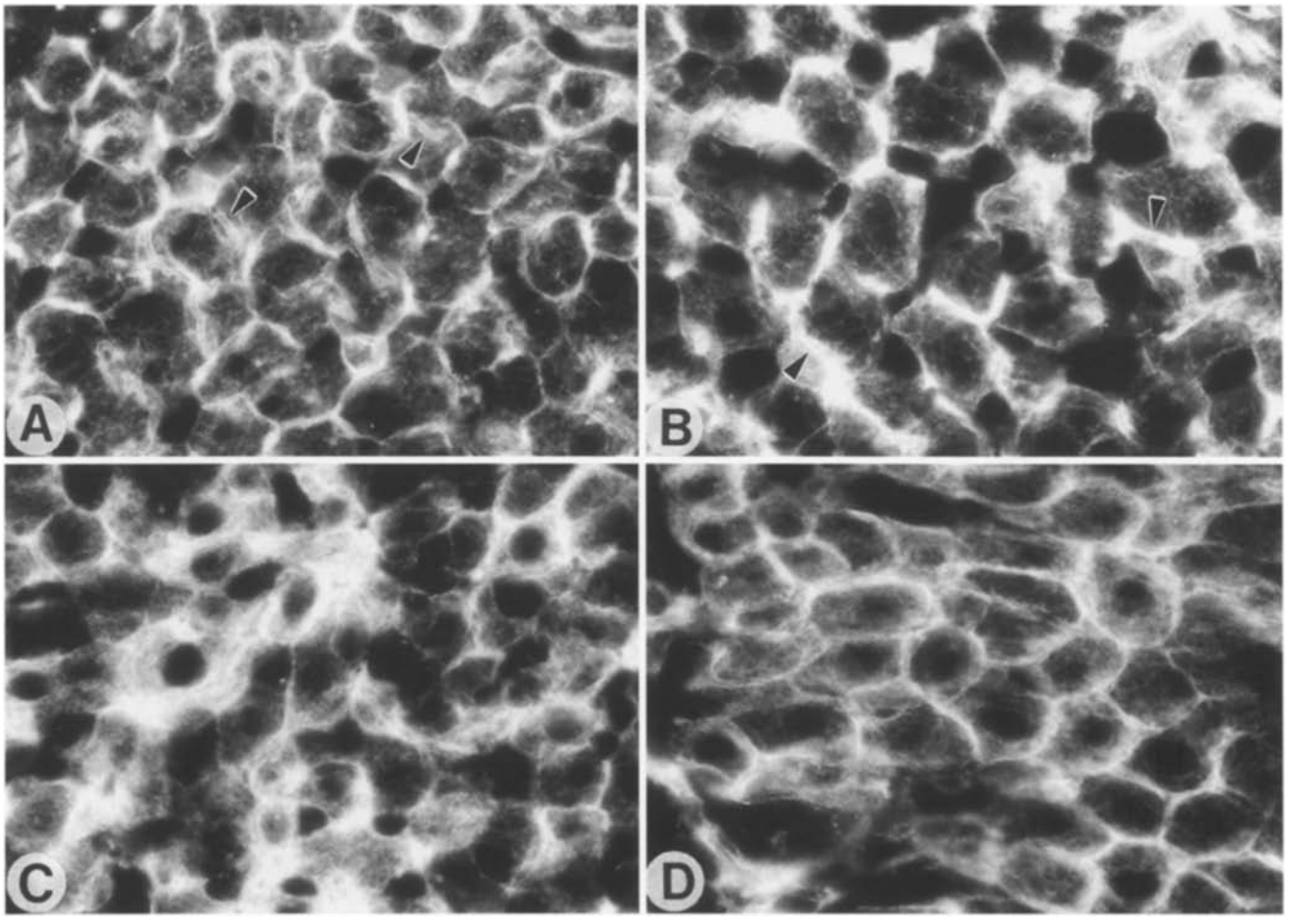

Fig. 1 LM micrographs of extracted cryostat sections showing immunofluorescence of CK18 **A** in control and **B** cholestatic liver after common bile duct ligation (CBDL) for **B** 2, **C** 7, and **D** 14 days. **A** CK18 is present in the cytoplasm of hepatocytes showing a filamentous network with high amounts at the cell periphery. Tube-like so-called pericanalicular sheaths are present at the bile canalicular region (*arrowheads*). **B** Increased amounts of CK18 are mainly detected at the bile canalicular and lateral membranes, with radial arrangements of filaments into the cytoplasm of hepatocytes. The pericanalicular sheath is thickened (*arrowheads*). **C** CK18 is expressed heterogeneously in liver lobules. Some hepatocytes show very high amounts of CK18 in the cytoplasm and then the pericanalicular sheath is hardly detectable. **D** CK18 is rearranged at the cell periphery as a honeycomb-like structure where the pericanalicular sheaths have disappeared. The amount of CK18 in the cytoplasm is also increased. Original magnifications: **A–B** ×50

nalicular sheath, were localized at the bile canalicular domain and corresponded to the tube-like structures of immunofluorescence of CK18 in Fig. 1A; IF sheets were present at the basolateral domain (Fig. 2A). This polarity of IF was gradually disturbed by CBDL. Pericanalicular sheaths became thickened (Fig. 2B), widened (Fig. 2C) and then undetectable (Fig. 2D). After 14 days of CBDL, IF sheets were arranged densely at the cell periphery in an unpolarized manner leading to a well-defined polygonal outline of IF in hepatocytes (Fig. 2D), which was in agreement with the honeycomb-like structures of immunofluorescence of CK18 as shown in Fig. 1D.

The mild extraction procedure using saponin as detergent showed the cytoplasm of hepatocytes to be electron lucent owing to loss of soluble cytoplasmic components after extraction (Fig. 3A). The intercellular junctions were readily observed. Cytoplasmic organelles, such as mitochondria, endoplasmic reticulum, Golgi complexes, lysosomes and peroxisomes, were well preserved. IF, which appeared as single filaments or thin bundles, were observed mainly at the lateral membrane of hepatocytes often connected with desmosomes, but were seldom found in the cytoplasm (Fig. 3A). Alterations of IF in livers after 2 days of CBDL were not obvious, but were clearly visible following 7 and 14 days of CBDL. Figure 3B shows that the amount of IF and the number of desmosomes at lateral plasma membranes of hepatocytes were considerably increased. Amorphous electron-dense material was frequently observed in the ectoplasm, either accumulated locally (Fig. 3C) or, together with the desmosomes at lateral membranes, arranged in a direction parallel to the axis of bile canaliculi (Fig. 3D). This electron-dense material was apparently associated with IF. Desmosome-like electron-dense material was also observed between microvilli in the lumen of bile canaliculi (Fig. 3, inset). Furthermore, the number of IF in the cytoplasm increased and appeared as thick bundles. In some areas of the cytoplasm, IF were arranged around cytoplasmic domains containing organelles such as mito-

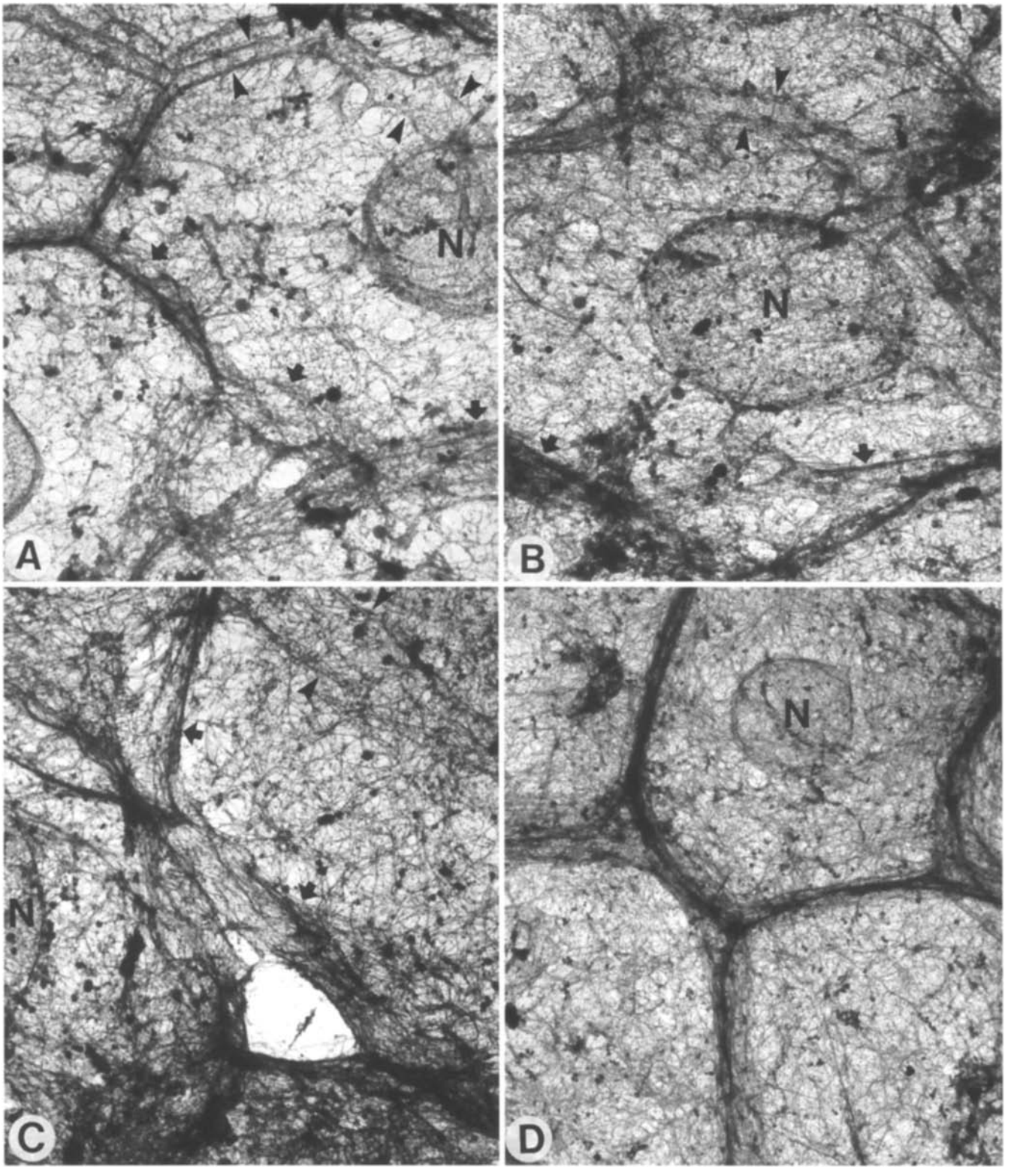

Fig. 2 EM micrographs of extracted unembedded cryostat sections of **A** control and **B** cholestatic liver after CBDL for **B** 2, **C** 7, **D** 14 (D) days. **A** Intermediate filaments (IF) are arranged as a meshwork throughout the cytoplasm from the cell periphery to the nucleus. At the cell periphery, IF are organized in a polarized manner, showing branching tube-like structures, the so-called pericanalicular sheath, at the bile canalicular region (*arrowheads*) and IF sheets at the basolateral domain (*arrows*). **B** The pericanalicular sheath is thickened and has become more electron dense (*arrowheads*); IF sheets at the basolateral membrane have also become more dense (*arrows*). **C** The pericanalicular sheath is enlarged (*arrowheads*) and IF sheets are widened extending into the cytoplasm (*arrows*). **D** The polarity of IF at the cell periphery has disappeared. IF at the cell periphery are densely arranged showing a honeycomb-like structure. IF in the cytoplasm are densely organized. *N* nucleus. Original magnifications: **A, B** ×4000, **C** ×5000, **D** ×2500

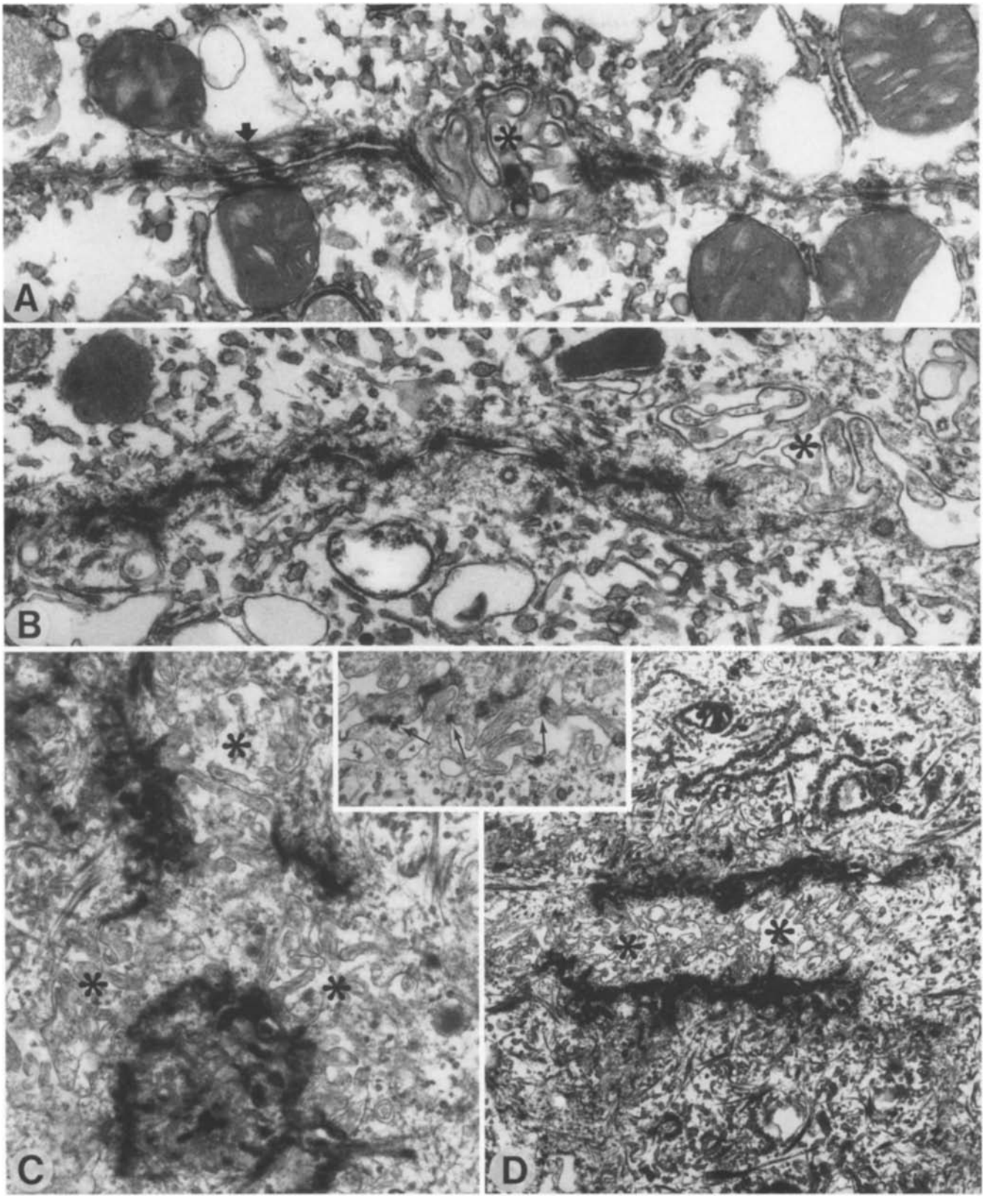

Fig. 3 EM micrographs of saponin-extracted and embedded ultrathin sections of **A** control and **B–D** cholestatic liver after CBDL for 14 days. **A** IF are observed mainly at the lateral membranes of hepatocytes in connection with desmosomes (*arrow*). **B** Increased numbers of desmosomes are present at the lateral membrane in association with increased amounts of IF. **C** Electron-dense material with insertion of IF accumulates locally in the ectoplasm in the vicinity of the bile canaliculi. **D** Electron-dense material in close relation to IF is present underneath the bile canaliculi, in part on the lateral membranes, and is arranged in a direction parallel to the axis of the bile canaliculi. *Inset* Desmosome-like electron-dense bodies (*arrows*) are observed between microvilli in the lumen of the bile canaliculi. *Asterisks*: bile canaliculi. Original magnifications: **A, B** ×20,000, **C** ×16,000, **D** ×10,000, *Inset* 31,500

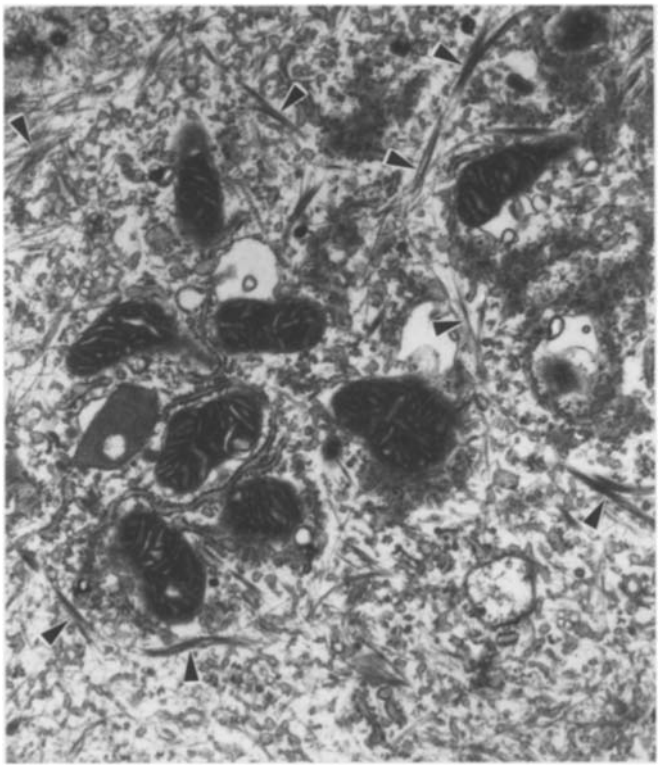

Fig. 4 EM micrograph of saponin-extracted and embedded ultrathin sections of cholestatic liver after CBDL for 14 days. Subcellular domains are segregated by increased amounts of IF appearing as thick bundles (*arrowheads*) in the cytoplasm of hepatocytes. Original magnification: ×12,500

chondria, endoplasmic reticulum or peroxisomes and seemed to encapsulate them from the rest of the cytoplasm (Fig. 4).

Discussion

The structural organization of IF in hepatocytes and its relation to intercellular junctions and cytoplasmic organelles were investigated by means of immunofluorescence of CK18 and EM using different extraction procedures. The results showed that CBDL for 14 days induced three significant changes in IF. There was rearrangement at the cell periphery and in the cytoplasm as a compensatory response of hepatocytes to the increased mechanical load, increased amounts of IF in close association with the increased number of desmosomes at the lateral membrane of hepatocytes, and reorganization resulting in segregation of cytoplasmic organelles.

IF consist of a filamentous meshwork that extends from the nucleus to the periphery of the cell [10]. In cells of the epidermis, this IF meshwork can resist mechanical tension efficiently. In hepatocytes, the IF network at the bile canalicular domain is organized into tube-like structures providing mechanical support and acting as a scaffold for the pericanalicular microfilaments [21]. This structure is considered to be important for the functional integrity of the bile canaliculus [21, 29]. Mechanical perturbation due to CBDL destroys the canalicular sheaths, which may lead to widening of the bile canaliculus. Our study has shown that the structure of pericanalicular sheaths is progressively impaired during CBDL. Rigid rearrangement of IF showing honeycomb-like structure appeared at the periphery of the cells. This rearrangement of IF is believed to enhance the mechanical strength of hepatocytes to withstand the increased biliary pressure. In the studies of Ingber et al. [18, 38, 43] using a tensegrity (tensional integrity) cell model, the force-induced rearrangement of CSK in living cells was demonstrated to be a response to mechanical perturbation. CSK stiffness (as measured by the ratio of stress to strain) increased proportionally with the external stress applied which required intact CSK elements such as IF [43]. The loss of IF caused by using anti-sense oligodeoxynucleotides reduced the mechanical rigidity of living tissues [41].

In our rat model of cholestasis, the mechanical load exerted upon hepatocytes increases after CBDL. As a response to this increased mechanical stimulus, IF were rearranged and condensed preferentially at the cell periphery, as shown in Fig. 2D. IF also appeared as thick bundles like tonofilaments in the cytoplasm. Tonofilaments are usually observed in keratinocytes and are important for strengthening and protecting tissues from stress and deformation. The present study has demonstrated that hepatocellular IF are capable of reorganizing to match higher biliary pressures due to CBDL. However, the mechanism by which regulations of dynamics of IF during CBDL take place is not clear. A series of in vitro studies of Goldman et al. (for a review see [8]) indicate that protein phosphorylation of IF is a major factor in the regulation of IF polymerization, subcellular organization and dynamics. For example, phosphorylation of keratin in liver resulted in a polar reorganization of IF at the basolateral domain of hepatocytes [27]. It has been shown that the kinase–phosphatase equilibrium of IF proteins is essential for the polymerization and depolymerization of IF [8, 9]. Therefore, it is assumed that high concentrations of bile acids and/or increased mechanical signalling due to CBDL may disturb the dynamic properties of IF proteins, leading to overpolymerization of IF, which either appear densely rearranged at the periphery of the cells or show tonofilament-like filaments in the cytoplasm of the cells. Bile acid was recently reported to be able to function as a cytokine causing overexpression of major histocompatibility complex class I in cholestatic hepatocytes [17]. Furthermore, the possibility of alterations in the molecular composition of IF proteins which may account for the thickened IF in CBDL liver cannot be ruled out.

Desmosomes are able to transfer mechanical stress applied from one cell to another and thus throughout the entire tissue [2]. They not only serve as a site of reinforcement of cell–cell adhesion but also function as an anchorage point for the IF scaffold in cells [16, 24, 33, 42]. The considerably increased numbers of desmosomes

at the lateral membranes of hepatocytes after CBDL are in agreement with previous observations [7]. Surprisingly, desmosome-like structures are also observed in the bile canalicular membranes between adjacent microvilli or in the ectoplasm where intimate cell–cell contacts were not present (Fig. 3). Moreover, the desmosome-like electron-dense bodies in ectoplasm together with the desmosomes at lateral membranes are arranged in a direction parallel to the axis of bile canaliculi. We believe that these phenomena are directly associated with the raised mechanical stress. Here, an increased number of desmosomes is required by hepatocytes to disperse tension from a local to a wider scale within the cell. In this case, desmosomes may assemble at places where cell–cell contact is absent. A study on transduction of mechanical force in living cells demonstrated that focal adhesion formation was correlated with local force transfer at the cell surface where cell–cell contact was not present [43]. Jones et al. [20] studied morphogenesis of desmosomes in relation to IF in cultured mouse epidermal cells and found that desmoplakin, a desmosome plaque protein, was preformed in the cytoplasm and appears as electron-dense material before desmosome assembly at the lateral membrane. High doses of Ca^{2+} in the culture medium initiated movement of IF accompanied by migration of desmoplakin from the cytoplasm to the cell surface where desmosomes assemble [20]. On the basis of this background information and our present findings, we hypothesize that desmosomes together with IF are capable of assembling at the surface of hepatocytes, where cell–cell contact may not be present but where mechanical tension is exerted as a result of CBDL. This hypothesis will be further investigated by in situ analysis of the presence of desmoplakin in desmosome-like electron-dense structures in cholestatic liver.

IF are not only associated with desmosomes but also interact with cellular organelles and function as integrators of the intracellular space. As demonstrated here and in other studies [12, 31, 37], IF form a filamentous network throughout the cytoplasm of cells from the nucleus to the cell surface, which may provide a direct pathway for mechanical and biochemical information transfer. Forgacs [11] took his percolation theory as the basis of his proposal that the CSK filamentous network plays a fundamental part in inter- and intracellular signalling. Our data indicate that the rearrangement of IF in the cytoplasm, resulting in segregation of subcellular domains, may interfere with normal metabolic pathways, leading to perturbation of hepatocellular function such as transcellular transport and bile secretion during CBDL.

In summary, CBDL results in significant changes in IF organization and its relationship with desmosomes and cytoplasmic organelles. On the one hand, this rearrangement of IF is required by hepatocytes to resist high mechanical stress caused by CBDL; on the other, the rearranged IF disturb normal structural organization of the cell, and this may lead to failure of hepatocellular metabolic functions.

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References

1. Araki N, Lee T, Matsubara H, Takashima Y (1993) An improved technique for observation of both membranous organelles and cytoskeleton in saponin-extracted cells. J Electron Microsc 42:51–54
2. Arnn J, Staehelin LA (1981) The structure and function of spot desmosomes. Dermatology 20:330–339
3. Aronson DC, De Haan J, James J Bosch KS, Ketel AG, Houtkooper JM, Heijmans HSA (1988) Quantitative aspects of the parenchyma-stroma relationship in experimentally induced cholestasis. Liver 8:116–126
4. Blankson H, Holen I, Seglen PO (1995) Disruption of the cytokeratin cytoskeleton and inhibition of hepatocytic autophagy by okadaic acid. Exp Cell Res 218:522–530
5. Chou CF, Omary MB (1993) Mitotic-arrest associated enhancement of *O*-linked glycosylation and phosphorylation of human keratins 8 and 18. J Biol Chem 268:4465–4472
6. Denk H, Franke WW, Eckerstorfer R, Schmid E, Kerjaschki D (1979) Formation and involution of Mallory bodies ("alcoholic hyalin") in murine and human liver revealed by immunofluorescence microscopy with antibodies to prekeratin. Proc Natl Acad Sci USA 76:4112–4116
7. Desmet VF (1987) Cholestasis: extrahepatic obstruction and secondary biliary cirrhosis. In: MacSween RNM, Anthony PP, Scheuer PJ (eds) Pathology of the liver. Churchill Livingstone, London, pp 364–413
8. Eriksson JE, Opal P, Goldman RD (1992) Intermediate filaments dynamics. Curr Opin Cell Biol 4:99–104
9. Eriksson JE, Brautigan DL, Vallee R, Olmsted J, Fujiki H, Goldman RD (1992) Cytoskeletal integrity in interphase cells requires protein phosphatase activity. Proc Natl Acad Sci USA 89:11093–11097
10. Fey EG, Wan KM, Penman S (1984) Epithelial cytoskeletal framework and nuclear matrix intermediate filament scaffold: three-dimentional organization and protein composition. J Cell Biol 98:1973–1984
11. Forgacs G (1995) On the possible role of cytoskeletal filamentous networks in intracellular signalling: an approach based on percolation. J Cell Sci 108:2131–2143
12. French SW, Kondo I, Irie T, Ihrig TJ, Benson N, Munn R (1982) Morphologic study of intermediate filaments in rat hepatocytes. Hepatology 1:29–38
13. French SW, Katsuma Y, Ray MB, Swierenga SHH (1987) Cytoskeletal pathology induced by ethanol. Ann NY Acad Sci 492:262–276
14. Geiger B (1987) Intermediate filaments. Looking for a function. Nature 329:392–393
15. Goldman RD, Goldman A, Green K, Jones J, Lieska N, Yang HY (1986) Intermediate filaments networks: organization and possible functions of a diverse group of cytoskeletal elements. J Cell Sci Suppl 5:69–97
16. Green KJ, Geiger B, Jones JCR, Talian JC, Goldman RD (1987) The relationship between intermediate filaments and microfilaments before and during the formation of desmosomes and adherens-type junctions in mouse epidermal keratinocytes. J Cell Biol 104:1389–1402
17. Hillaire S, Boucher E, Calmus Y, Gane P, Ballet F, Franco D, Moukthar M, Poupon R (1994) Effects of bile acids and cholestasis on major histocompatibility complex class I in human and rat hepatocytes. Gastroenterology 107:781–788
18. Ingber DE (1993) Cellular tensegrity: defining new rules of biological design that govern the cytoskeleton. J Cell Sci 104: 613–627

19. Irie T, Benson NC, French SW (1984) Electron microscopic study of the in vitro calcium-dependent degradation of mallory bodies and intermediate filaments in hepatocytes. Lab Invest 50:303–312
20. Jones JCR, Goldman RD (1985) Intermediate filaments and the initiation of desmosome assembly. J Cell Biol 101:506–517
21. Katsuma Y, Marceau N, Ohta M, French SW (1988) Cytokeratin intermediate filaments of rat hepatocytes: different cytoskeletal domains and their three-dimensional structure. Hepatology 8:559–568
22. Kawahara H, Marceau N, French SW (1989) Effect of agents which rearrange the cytoskeleton in vitro on the structure and function of hepatocytic canaliculi. Lab Invest 60:693–704
23. Kawahara H, Cadrin M, Perry G, Autilio-GambettiL, Swierenga SHH, Metuzals J, Marceau N (1990) Role of cytokeratin intermediate filaments in transhepatic transport and canalicular secretion. Hepatology 11:435–448
24. Kouklis PD, Hutton DE, Fuchs E (1994) Making a connection: direct binding between keratin intermediate filaments and desmosomal proteins. J Cell Biol 127:1049–1060
25. Ku NO, Michie S, Oshima RG, Omary MB (1995) Chronic hepatitis, hepatocyte fragility, and increased soluble phosphoglycokeratins in transgenic mice expressing a keratin 18 conserved arginine mutant. J Cell Biol 131:1303–1314
26. Lazarides E (1980) Intermediate filaments as mechanical integrator of cellular space. Nature 283:249–256
27. Liao J, Lowthert LA, Ku NO, Fernandez R, Omary MB (1995) Dynamics of human keratin 18 phosphorylation: polarized distribution of phosphorylated keratins in simple epithelial tissues. J Cell Biol 131:1291–1301
28. Lloyd C, Hyams J, Warn R (1986) The cytoskeleton: cell function and organization. J Cell Biol 5 Suppl (whole issue)
29. Ohta M, Marceau N, French SW (1988) Pathologic changes in the cytokeratin pericanalicular sheath in experimental cholestasis and alcoholic fatty liver. Lab Invest 59:61–74
30. Okanoue T, Ohta M, Ou O, Kachi K, Kagawa K, Yuki T, Okuno T, Takino T, French SW (1985) Relationship of mallory bodies to intermediate filaments in hepatocytes. Lab Invest 53:534–540
31. Okanoue T, Ohta M, Fushiki S, Ou O, Kachi K, Okuno T, Takino T, French SW (1985) Scanning electron microscopy of the liver cell cytoskeleton. Hepatology 5:1–6
32. Ouinlan R, Hutchison C, Lane B (1994) Intermediate filament proteins. Protein Profile 1:779–782
33. Ouyang P, Sugrue SP (1992) Identification of an epithelial protein related to the desmosome and intermediate filament network. J Cell Biol 118:1477–1488
34. Phillips MJ (1994) Biology and pathobiology of actin in the liver. In: Arias IM, Boyer JL, Fausto BN, Jakoby WB, Schachter DA, Shafritz DA (eds) The liver: biology and pathobiology. Raven, New York, pp 19–32
35. Phillips MJ, Poucell S, Oda M (1986) Biology of disease. Mechanisms of cholestasis. Lab Invest 54:593–608
36. Schliwa M (1986) The cytoskeleton. An introductory survey. In: Altert M, Beermann W, Goldstein L, Porter KR (eds) Cell biology monographs, vol 13. Springer, Vienna New York
37. Schliwa M, Van Blerkom J (1981) Structural interaction of cytoskeletal components. J Cell Biol 90:222–235
38. Sims JR, Karp S, Ingber DE (1992) Altering the cellular mechanical force balance results in integrated changes in cell, cytoskeletal and nuclear shape. J Cell Sci 103:1215–1222
39. Skalli O, Goldman RD (1991) Recent insights into the assembly, dynamics, and function of intermediate filament networks. Cell Motil Cytoskeleton 19:67–79
40. Staehelin LA (1974) Structure and function of intercellular junctions. Int Rev Cytol 39:191–283
41. Tropey N, Wylie CC, Heasman J (1992) Function of maternal cytokeratin in *Xenopus* development. Nature 357:413–415
42. Troyanovsky SM, Eshkind LG, Troyanovsky RB, Leube RE, Franke WW (1993) Contributions of cytoplasmic domains of desmosomal cadherins to desmosome assembly and intermediate filament anchorage. Cell 72:561–574
43. Wang N, Butler JP, Ingber DE (1993) Mechanotransduction across the cell surface and through the cytoskeleton. Science 260:1124–1127
44. White FH, Gohari K (1984) Desmosomes in hamster cheek-pouch epithelium: their quantitative characterization during epithelial differentiation. J Cell Sci 66:411–429
45. Yano T, Tokui T, Nishi Y, Nishizawa K, Shibata M, Kikuchi K, Tsuiki S, Yamauchi T, Inagaki M (1991) Phosphorylation of keratin intermediate filaments by protein kinase C, by calmodulin-dependent protein kinase and by cAMP-dependent protein kinase. Eur J Biochem 197:281–290