The number of proteins identified to be involved in the cytoskeleton is steadily increasing. For insight into their assembly in situ, muscle fibres offer special advantages related to their regular organization of myofibrils, composed of interdigitating thick and thin filaments. The thick filaments, mainly composed of myosin molecules, form the A-band, whereas the thin filaments composed of actin, tropomyosin and troponin project from either face of Z-discs in the I-band and interdigitate with the thick filaments in the A-band. The repeating unit, the sarcomere, extends from a Z-disc to a Z-disc.

The arrangement of the filamentous muscle cytoskeleton is illustrated schematically in Fig. 1. It shows the myofibrils and two non-contractile filamentous systems, the intermediate filament lattice, which is an exosarcomeric lattice surrounding the myofibrils, which links them to the sarcolemma, to the nucleus and the titin lattice, which is an endosarcomeric lattice present within the myofibrils (for review, see [1, 2]). In muscle cells and especially in working myocardial cells an extensive lattice of microtubules is also present (for review, see [3]).

The intermediate filament lattice
The intermediate filaments in striated muscle are in general composed of desmin, a protein with a molecular mass of 55 kDa belonging to the type 3 class of intermediate filaments [4]. The subunits form 10 nm filaments which extend as a three-dimensional lattice of transverse and longitudinal elements, encircling and interlinking the peripheral regions of Z-discs of individual myofibrils and connecting adjacent myofibrils [5–8]. The transverse lattice of intermediate filaments is continuous with intermediate filaments attaching to the sarcolemma and to the nuclear pores, where they may be continuous with intermediate filaments composed of lamins and which extend into the nuclear matrix (for review, see [9]). The attachment of the intermediate filaments to the sarcolemma may involve a number of proteins. Pardo et al. [10, 11], coined the term costamere, a periodic structure at the sarcolemma, in register with the myofibrillar Z-band and containing vinculin. At the same location spectrin, ankyrin, talin and γ-actin have been observed in various muscles (for review, see [2]). The sarcolemma of this area has also been reported to contain a special sialoglycoprotein of molecular mass 130 kDa [12]. Furthermore, interactions through transmembrane proteins of the integrin superfamily with the extracellular matrix seem to preferentially take place at this site (for review, see [13, 14]). So far it is not known how the intermediate filaments are linked to the Z-discs. Spectrin and ankyrin have been suggested to be anchoring proteins. In addition synemin and paranemin are such proteins in chicken skeletal muscle fibres and myocardial cells respectively (for review, see [2]).

Another transverse set of filaments can sometimes be observed to link to the myofibrils at the M-band level and possibly also the myofibrillar M-band to the sarcolemma (for review, see [2]). A pair of closely related proteins (200 kDa and 220 kDa, pI 5–5.1), referred to as skelemins [15], may be the organizing/anchorage proteins for linking the exosarcomeric lattice of filaments with myofibrils at the level of the M-band.

The intermediate filaments have also been suggested to form a longitudinally oriented lattice. Such a lattice is seen in chicken skeletal and cardiac myocytes [16–18], as well as in mammalian cardiac myocytes, especially at the intercalated discs (Fig. 2), where the intermediate filaments are attached to the desmosomes [19, 20]. The intermediate filaments have also been proposed to be associated with mitochondria and the sarcotubular system (for review, see [8]). However, as the intermediate filaments only comprise 0.35% and 2% of the total protein of mammalian skeletal and cardiac muscle, respectively [21, 22], and the intermediate filaments are dispersed and obscured by other structures, conclusive evidence for direct association with different organelles is still lacking.

Purkinje fibres as a model system for studies on structure and function of the cytoskeleton
The Purkinje fibres belong to the ventricular part of the conduction system which initiates and regulates the sequential contraction of the atria and the ventricles in the heart. In ungulate hearts the Purkinje fibres form large cables of tightly connected cells

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surrounded by connective tissue. Interestingly, the major portion of the cytoplasm of Purkinje fibres is composed of intermediate filaments which attach to desmosomes, myofibrils and the sarcolemma [19, 23–25]. Strong indirect evidence that the intermediate filament network in Purkinje fibres does function as a cytoskeleton is provided by the observation that isolated cables of Purkinje fibres retain their three-dimensional shape [19], even after extraction with detergent and low- and high-salt solutions, which extract membranes and myofibrillar proteins, respectively. These extracted cables also retain their intracellular organization with intermediate filaments linking the Z-discs of the myofibrils with either the sarcolemma facing the interstitium or with desmosomes at cell-cell junctions [19]. Using antibodies against desmoplakin, vinculin, skelemins, $\alpha$-actinin and $\alpha$- and $\beta$-spectrin, we have shown that all these proteins are putative candidates for being linkage protein for the Purkinje fibre intermediate filaments [19, 26]. The Purkinje fibre intermediate filament is of the desmin type but has special properties in that the desmin is more phosphorylated than desmin from ordinary atrial or ventricular myocardium [27]. Furthermore, monoclonal antibodies have been

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**Fig. 1**

Schematic model of the intermediate filament cytoskeleton in relation to the myofibrillar lattice of thick myosin filaments, thin actin filaments and titin filaments, the newly discovered third type of sarcomeric filaments

obtained which exclusively recognize Purkinje fibre desmin of aridactylia [28].

To obtain further insight into the expression and function of the intermediate filament core, and intermediate filament-associated proteins in Purkinje fibres, we have recently studied bovine embryonic and fetal hearts [29]. We have observed that diversity in cytoskeletal organization was already present at an early stage (4 weeks of gestation) of bovine cardiac development. Desmin was the main component of the intermediate filaments of the myocytes, although vimentin was co-expressed in areas which might develop into parts of the conduction system; however, Purkinje fibres were not yet discernible. At this stage of development, desmin and vimentin did not form a myofibrillar-related pattern as in the mature myocytes. The intermediate filament-associated proteins, desmoplakin, vinculin, skelensins and spectrin, on the other hand, were present in their mature locations at desmosomes, the sarcolemma and at myofibrils, indicating that anchoring sites for the intermediate filaments were available. Thus, although the intermediate filaments might not be involved in the myofibrillogenesis, as a number of intermediate filament-associated proteins were differentially distributed from early stages of cardiac development, we propose that the cytoskeleton is of major importance for the morphogenesis of the heart.

Another exciting feature of cardiac development and the cardiac conduction system is that antibodies against different neurofilament proteins [4], in addition to antibodies against desmin, react with all parts of the conduction tissue of rabbit hearts [29–32]. Furthermore, as antibodies against neural crest-related proteins are expressed in early stages of both rabbit and human hearts [30, 33, 34], the conducting tissue with its nerve-like properties might in fact be of neural crest origin [30].
The endosarcomeric titin lattice

As seen in Fig. 1, titin filaments run parallel to the long myofibrillar axis along the periphery of the myosin filaments from their bare zone, past the actin filaments and terminate at the Z-disc. Thus, the titin lattice provides continuity throughout the myofibrils and serves as a third filament system which might provide the intracellular passive series elastic property sought by muscle biophysicists (for review, see [1]).

Titin [35], which is synonymous to connectin [36], is a very large molecule of molecular mass 2.7×10^6 Da and is prominent only in striated muscle. It is 4 nm in diameter and about 1 μm long, indicating that one titin filament could consist of one molecule. Titin is expressed early in myofibrillogenesis [37, 38, 48] and is rapidly incorporated into the insoluble cytoskeleton, presumably via its connection to the Z-discs [39].

Exo- and endo-sarcomeric lattices in human skeletal and cardiac muscle cells

Human skeletal and cardiac muscle cells contain a transversely oriented lattice of desmin-containing intermediate filaments [25, 40, 41]. Vimentin and desmin are co-expressed both during early phases of skeletal muscle development and in activated satellite cells participating in regeneration of muscle fibres [40, 42]. Alterations in the intermediate filament cytoskeleton have been observed in a number of congenital myopathies, as well as in hereditary diseases [40, 41, 43–45]. From a functional point of view, it is noteworthy that in myofibrillar lesions and distortions with so-called nonius periods [46], an enhanced staining for desmin is observed in longitudinal strands within the myofibrils [41]. Desmin-stained strands of the same appearance have also been observed in biopsies from persons having delayed muscle soreness [47]. The ultrastructural correlate to this type of lesion is abnormalities in the myofibril of type Z streaming or myofibrillar disorganization [41]. So far it has not been clarified whether these lesions reflect a repair phase following myofibrillar damage, with the desmin strands acting as reinforcement for the myofibril while myofilaments are being formed, or providing a zone of myofibrillar growth for insertion of new sarcomeres into the myofibril. This type of lesion seems well suited for further studies aimed at understanding how the endosarcomeric titin lattice is affected and how intermediate filament-associated proteins are involved in myofibrillar organization; such studies are in progress in our laboratory.

Reorganization and turnover of actin filament architectures in cells

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Introduction

In higher eukaryotic cells, actin filaments are arranged into complex three-dimensional arrays by a number of overlapping subsets of actin-binding proteins. Many of the structural actin-binding proteins have two actin-binding sites and it appears that the spatial constraints imposed by these binding proteins on the relative orientation of cross-linked actin filaments, together with competition between different actin-binding proteins for similar binding sites on actin, largely determine the nature of the filament array formed. In higher animal cells, characteristic subset of actin-binding proteins. (i) In the cortical meshwork which signals received via receptors are converted into changes in architecture via a transduction system which is as yet poorly characterized; for example, during chemotactic, phagocytic or extra-cellular-matrix-derived signals. (ii) Within the leading lamellae of cells, bundles of actin filaments exist in which the filament polarity is parallel and that, by assembly at their distal (barbed) ends, contribute to the forward displacement of the leading edge of cells or to the shape change of platelets by forming microspikes or filopodia. (iii) The third level of architecture, stress fibres, is most obvious in cells cultured on plastic substrata. Stress fibres are bundles of up to several hundred actin filaments.

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