Recurring Views on the Structure and Function of the Cytoskeleton: A 300-Year Epic

Eugenio Frixione

Departamento de Biología Celular and Departamento de Fisiología, Biofísica y Neurociencias, Centro de Investigación y de Estudios Avanzados del IPN, México

Some unnoticed or seldom remembered precedents of current views on biological motion and its structural bases are briefly outlined, followed by a concise recapitulation of how the present theory has been constructed in the last few decades. It is shown that the evolution of the concept of fibers as main constituents of living matter led to hypothesizing microscopic structures closely resembling microtubules in the 18th century. At the beginning of this period, fibers sliding over each other and driven by interposed moving elements were envisioned as the cause of muscle contraction. In the following century, an account of the mechanism of myofibril contraction visualized longitudinal displacements of myosin-containing submicroscopic rodlets. The existence of fibrils in the protoplasm of non-muscle cells, a subject of long debate in the second half of the 19th century, was virtually discarded as irrelevant or fallacious 100 years ago. The issue resurfaced in the early 1930s as a theoretical notion—the cytosquelette—nearly two decades before intracellular filamentous structures were first observed with electron microscopy. The role originally assumed for such fibrils as signal conductors is nowadays being reappraised, although under new interpretations with a much wider significance including modulation of gene expression, morphogenesis, and even consciousness. Since all of the above ancestral conceptions were eventually abandoned, the corresponding current views are, to a certain extent, recurrent.


Key words: actin filaments; filament sliding; history; microtubules; motility; movement; neurofilaments

INTRODUCTION

Within the last 50 years cell biologists have hauled our comprehension of motility phenomena from a twilight laden with imaginative but unrelated or antagonistic conjectures, up to an experimentally supported and unified view in which most of the kinetic activities found in eukaryotic organisms are satisfactorily explained with a basic set of mechanical principles. Ratchet action of motor enzymes over linear polymers of highly conserved proteins is known to provide the impulse for such apparently different processes as cytoplasmic streaming, the beating of cilia or flagella, fast axoplasmic transport, chromosome migration, cytokinesis, and muscle contraction with its multiple expressions. Accordingly, the present century, on the eve of a new one, may feel justifiably proud of its key contributions to the long quest...
for understanding the fundamental nature of biological movement.

Ample credit has been acknowledged to the more distant forerunners who, amid the clatter of diverging theories, had the intellectual vision at least to point in the right direction [for historical reviews of related topics see Bastholm, 1950; Hughes, 1959; Fawcett, 1960; Mazia, 1961, 1975; Schmitt, 1968; Needham, 1971; Teich, 1973; Huxley, 1980; Allen, 1981; Franzini-Armstrong and Peachey, 1981; Gibbons, 1981; Haimo and Rosenbaum, 1981; Inoué, 1981; Pollard, 1981; Brinkley, 1982, 1997; Ochs, 1982; Brazier, 1984; Porter, 1984; Grigg, 1991; Satir, 1995; Huxley, 1996]. Perhaps the best known example of such good aiming is the persistent suspicion that ciliary activity should somehow be akin to that of muscle, an analogy that can be traced since the interpretation of the animalcula by van Leeuwenhoek down to our own day [van Leeuwenhoek, 1682; Sharpey, 1830, 1835; Müller, 1840; Engelmann, 1875, 1898; Kühne, 1888; Dellinger, 1909; Afzelius, 1959; Satir, 1995]. Yet, there are less obvious or rarely mentioned conceptual parallelisms across the centuries on the general subject of vital motion and its underlying structures, as well as a few authors whose achievements in this field are very close to oblivion if not actually forgotten.

This essay attempts to rescue and present in perspective some of these remarkable precedents, together with a cursory survey of the more recent developments that have forged our current outlook on the matter. An undertaking of this sort cannot aspire to anything more than sketching, and hence oversimplifying, a richly complex fabric full of communicating vessels. Despite the necessary bareness of the treatment, however, the reader will find in the included bibliography the clues for entering into the most significant periods in the history of the cytoskeleton. In the themes selected, certain ideas have tended to come back under a novel appearance and with usually scant or null memory of previous incarnations. Since they all have to do with the role of fibers in living matter, a brief account of the origin and initial evolution of this concept is given first.

FIBERS COMING OF AGE

The presence of fibrous parts in the animal body, plain to any butcher or cook, was already included in biological theory by the presocratic philosophers. Empedocles, in the 5th century BC, listed sinews or neura along with blood, flesh, and bone as one of the main bodily constituents [see Solmsen, 1950]. Two hundred years later, at the revolutionary medical school of Alexandria, Erasistratus taught that the neura themselves were in fact composite structures also containing veins and arteries, “like a rope formed by plaiting three strands of different kind” [cited by Galen; in Dobson, 1927]. However, the first extant discussion of living matter as being made up of minute fibers probably comes from Galen, at the peak of the Roman Empire. Regarding muscle as a blend of sensitive nerve and resistant ligament, he reasoned that the only way of adequately mixing the two is by dividing each into numerous fine branches that are then closely juxtaposed [Galen, 2nd century AD]. Of course—he continues—these delicate fibers need some protection to avoid erosion by their mutual rubbing, as well as defense against blows and harsh changes in temperature. This is the role of the flesh inserted between the fibers:

Accordingly, Nature, wise in all things, has placed this cushioning, which is itself not without its uses, all around the fibers as a protection from the cold and heat, and a covering very like felt [. . .] and everything taken together becomes the muscle.

This conception of flesh as mere protective packing, an echo of a description found in Plato’s Timaeus [Plato, 4th century BC], was rejected in the Renaissance by Galen’s revisionist and outspoken iconoclast Andreas Vesalius. In his celebrated treatise on anatomy, he accepted the double constitution of muscle out of fibers and flesh, but declared his conviction that the latter “is the chief agent” involved in contraction [Vesalius, 1543].

Further development of this issue, along with the realization that flesh and muscle fibers are one and the same thing, stemmed from microscopic studies in the 17th century. When Antoni van Leeuwenhoek first placed bits of cow muscles in front of his magnificent lenses, he found the substance composed of slender threads, each “made up of very small conjoined Globuls” [van Leeuwenhoek, 1674]. Eight years later he apologized for an error in judgement induced by the use of an “ordinary microscope”. A new look at muscle—presumably with a special microscope, which as usual he avoids specifying—revealed that, rather than as rows of globules, the “flesh-fibres” appear as regular series of “rings and wrinkles” [van Leeuwenhoek, 1682]. Moreover, having split and pressed flat one of these fibers, it became clear to him that they “in their turn consist of a great number of fibres, which for distinction I will call filaments.” Van Leeuwenhoek came to a logical conclusion from these observations:

I gave my mind to this subject and now say that, since we see that a large muscle in its turn consists of many thousands of minute muscles, each enclosed in membranes, and that each flesh-fibre again consists internally of filaments, [now I say] that each flesh-fibre is a muscle and that each filament (100 of which make a fibre) is in its turn a muscle of flesh. . . .

He did not see any reason for setting a limit to this fractal organization of muscles being composed of
smaller muscles and so on, simply because by then he had already startled himself and the world with the discovery of an apparently omnipresent microscopic jungle. It was pretty well established that there are many living creatures “thinner than one of the filaments of which a flesh-fibre is composed, and remember that such an animalcule must have a skin, veins, nerves and muscles, nay is as perfect as a large animal.”

While van Leeuwenhoek preferred to examine most specimens fresh, other microscopists were experimenting with procedures for preparing their samples so as to permit an easier observation. A pioneer in this effort was Marcello Malpighi, to whom a second line leading to the hegemony of fibers can ultimately be traced back. Having found a multitude of diminutive “glands” (i.e., neurons) in the cerebral cortex, he described nerve fibers associated with them and in passing alluded to his methods: “An intimate connection and continuation between these cortical glands and the nerve fibres is observed—wrote Malpighi—after boiling the brain. . . .” [Malpighi, 1666]. This approach was pursued by Malpighi’s outstanding pupil Giorgio Baglivi, who macerated and boiled fragments excised from diverse parts of the animal body in several liquids, in order to extract the stuffing of the animalcule must have a skin, veins, nerves and muscles, nay is as perfect as a large animal.

Based on his findings, Baglivi championed a radical paradigm shift in physiology. It was evident to everyone that living things consist of fluid and solid parts. Yet, for some reason, thus far the active role had always been assigned to the fluids. Since the Greeks, the body had been under the all-embracing influence of the four humors of the Hippocratic doctrine, and directly considered fluid-carrying pipes, yet so incredibly thin as to be close to the limit of resolution with the microscope. Consequentially, he concluded that fibers of varied sizes and assembled in different arrays should be the basic structural units of all organic materials [Baglivi, 1703].

Now, someone acquainted with cytoskeletal proteins will possibly notice a familiar design scheme in here. Not only scientists in the Enlightenment period imagined submicroscopic biological fibers as polymers, but they also conceived some of these structures as having a microtubule-like construction. If their elemental particles were allowed to represent tubulin monomers, and simple fibers stand for protofilaments, then vasa minima built of 13 simple fibers correspond to our current concept of microtubules. Mention of this curious coincidence is not to imply that microtubules were somehow anticipated in the 18th century, of course, because apart from obvious considerations there are important differences in scale and function. The vasa minima were considered fluid-carrying pipes, yet so incredibly thin as to be close to the limit of resolution with the microscope. Some of those minimal “vessels” (i.e., axons) had actually been discerned by van Leeuwenhoek as constituting the nerves [van Leeuwenhoek, 1717], so in all likelihood it was through them that the animal spirits or their liquefied equivalent, the spirituous fluid, were propelled by their pulsating hearts.

LONGITUDINAL SLIDING OF FIBERS

According to the traditional explanation inherited since Erasistratus, muscles shortened because they were caused to inflate by a sudden discharge of animal spirits flushed down through the nerves from the ventricles of the brain. Either the influx itself or an efervescent reaction of the spirits with another substance in the muscle would dilate the fleshy mass. This view, still sustained in the 17th century by René Descartes and many others, lost support following the demonstration by Jonathan Goddard and Jan Swammerdam that, instead of expanding, the muscles slightly reduce their volume when contracted [see Brazier, 1984]. Furthermore, the Dane Niels Stensen discovered while dissecting muscles that “not all the fibers are seen to move at the same time, but individual
fibers move separately at different times” [Stensen, 1667]. Therefore, the difficult question of muscle contraction had to be approached in a wholly new manner, leaving behind the deeply ingrained but now untenable theory of fluid infusion. This is where solidism stepped in.

Baglivi classified fibers in two main groups—membranaceous and motor—and endeavored to understand every physiological process in terms of predominantly solid interactions. Muscle contraction in particular was explained by him as a wavelike motion of the “muscular and motor fibers” over one another, just as Stensen had observed. Fiber gliding was driven and facilitated by the rush of blood red-corpuscles, which he found to be quite spherical after diluting the vital liquid with plain water in order to get a less crowded view for a better assessment of their actual shape. Assuming that the animal spirits leaving behind the deeply ingrained but now untenable traction had to be approached in a wholly new manner, the difficult question of muscle contraction 

Thus the concept of elongated structures sliding relative to each other through the assistance of moving interposed elements, established today as the mechanism of both sarcomere contraction and axoneme bending, has an old ancestor. Moreover, as can be read in the foregoing paragraph, Baglivi’s hypothesis also contains the notion of muscle contraction as a result of the fibers being pulled towards the middle, just like the actin filaments in each sarcomere. The refinement of this idea—i.e., whole muscle fibers gliding over each other—into that of internal filaments sliding for much shorter distances within the fibers themselves, can be reconstructed through a series of steps spanning the 18th and 19th centuries over several countries.

The long transition starts with the recognition of an approximation of the segments that constitute each myofibril, as deduced from van Leeuwenhoek’s description of the muscle fibers being full of “rings and wrinkles.” Boerhaave’s most distinguished pupil, the influential Albrecht von Haller, put forward a novel theory of muscle contraction in which the solid particles of the simple fibers, rather than being directly attached to each other, were connected by means of an irritable “glutinous mucus” able to pull them together [von Haller, 1753]. This proposal was vigorously objected to, most notably by Robert Whytt, on the grounds that the gluten or glue extracted from muscles “appears as inert and void of active powers as any other matter” [Whytt, 1761]. As a consequence, attention switched to the likely powers of the periodic “rings” or segments in which the fibrils of striated muscle appear divided.

This was the approach favored by the French researcher Henri Dutrochet, whose main goal was to discover a common basic mechanism for the movements displayed by both plants and animals, selecting the word motilité to designate such a general faculty of living things [Dutrochet, 1824, 1826]. Thrilled by his sagacious studies on the osmotic fluxes of water across biological membranes, Dutrochet imagined changes in the turgor of the “articulated muscle corpuscles” that according to him would constitute the myofibrils. Also William Bowman, in his classic treatise on the structure and function of striated muscles, concluded that contractility “resides in the individual segments” of which the fibers are composed [Bowman, 1840].

With the introduction of polarized light in microscopy, it was found that a striated muscle fiber so illuminated appears as a regular succession of birefringent and dark bands, the periodicity of which corresponded with that of the segments constituting the fibrils. Since the pattern of bands did not seem to change much when the muscle was stretched, but the birefringence was lost after the tissue was heated or treated with strong acids, Ernst von Brücke assumed that a labile ordered material was responsible for the regularity of the bright optical signal [von Brücke, 1858]. He speculated that each birefringent sector might contain submicroscopic elongated particles able to slide longitudinally, adjusting their relative positions as the muscle varied in length.

This hypothesis was modified when it was noticed that the birefringent bands maintain a nearly constant size irrespective of the fiber length, so they only approximate or get away from each other at the expense of the dark bands. Wilhelm Krause interpreted this fact by postulating periodic sets of submicroscopic rodlets of equal length and in register, each set being somehow pulled towards the contiguous ones along the fibril during muscle contraction [Krause, 1869]. Because he also observed that the birefringent bands disappeared after subjecting the muscle fibers to a certain extraction procedure, an educated guess could be made about the chemical identity of the moving material. The hypothetical sliding rodlets might be constituted of “myosin,” a major protein isolated from muscle and given its name a few years earlier by Wilhelm Kühne [1864].

Why these significant advances achieved in the middle third of the 19th century were dismissed or neglected for decades, so that the current sliding filament model was independently born again more than 80 years
later (see below), is a puzzle that has been penetratingly examined by Andrew Huxley [1980]. As it turned out, by the end of the century myosin and the myofibrils themselves were already out of favor in some scientific circles as the active factors involved in muscle contraction. Instead, excitement arose for a newly discovered orthogonal network of thin threads evenly distributed throughout the muscle fibers. This trellis, known today as the sarcoplasmic reticulum and its associated system of transverse tubules, became the leading contender for explaining contractility in a scheme where the myofibrils would serve a merely passive elastic function. Such opinion was held, among other outstanding specialists, by Kühne, the father of “myosin” [Kühne, 1888; see also Smith, 1961]. As we will next see, the presence of fine threads within other cells was also the matter of a protracted dispute.

FIBERS WITHIN NON-MUSCLE CELLS

Despite its confident long reign, the fiber theory went the way of wigs soon after the French Revolution. In the outcome of arduous debate, microscopists finally agreed that life was indeed a property related to an utterly different type of basic units: cells. And, as far as anyone could tell, these plump entities consisted of a viscous mass containing one or more smaller corpuscles, all of it surrounded by a controversial membranous envelope. It was then acknowledged that the ubiquitous fibers of previous histology were for the most part either artifacts caused by the crude techniques of specimen preparation, or fragments of a pervasive mesh in which the cells themselves were suspended. As for intracellular fibers or other elongated structures, they were a rare exception in the homogeneous transparency of the protoplasm. On the other hand, there were reasons for not attributing to them a role in contractility. Theodor Schwann himself, having found no trace of striae and very scarce fibrils in “involuntary” (smooth) muscles, suggested that “it may not be important that that [i.e., contractile] substance should consist of minute longitudinal fibres” [Schwann, 1839].

A presage of future storms came early, however, when a few years after Schwann’s book was first published Robert Remak announced his finding of a “primitive band” seemingly composed of hundreds of very fine fibers in fresh nerve cells of the crayfish [Remak, 1843, 1844]. He even provided a couple of illustrations of the same (Fig. 1). Perhaps because Remak warned that these tenuous structures were too fragile and tended to break down as free grains near the cut end of the axon. The fibrils tend to break down as free grains near the cut end of the axon. The straight vertical stripe is a crease mark in the folded plate. This drawing and another accompanying figure are the first known illustrations of the cytoskeleton. [Figs. 8 and 9 in Remak, 1844].

least known portion of the nerves [...] there is none, not even excepting Remak himself, its discoverer, who can boast that he has studied and learned its relations in every particular” [von Kölliker, 1852].

The subject was taken up later on by several authors including Max Schultze, who described bundles of an “exquisitely delicate fibrillar structure” crossing the nerve cell bodies of the torpedo fish from every offshoot to all others [Schultze, 1871]. This arrangement suggested to him the possibility that the constituent fibrils might act as conductive pathways for the nerve impulses. But then Thomas H. Huxley, in an exhaustive monograph about the crayfish as an example of a thorough zoological study, stated that the content of fresh axons in the ganglionic chain is “perfectly pellucid, and without the least indication of structure” [Huxley, 1880]. Thus, Huxley implicitly denied the presence of fibrils within the nerve cells of the same animal in which Remak originally claimed to have observed them. Even Carl Heitzmann, a so ardent believer in fibers as major constituents of tissues that he came near to revive the 18th century theory, recommended caution with regard to intracellular fibrils in nerve cells because he “could never discover any fibrillated structure in the axis-cylinders” [Heitzmann, 1883].
Accordingly, by the early 1880s the radical discrepancy about the existence of fibrils in nerve cells, not to mention its possible physiological significance, had lasted almost 40 years. It threatened to become a permanent schizophrenic condition worth a scrupulous Freudian analysis. “One author thinks of the nerve cell as granulated, the other as fibrillose; one thinks of the nerve fiber as a bunch of fibrils but another as a liquid column,” complained Sigmund Freud himself [Jones, 1953]. So he entered the fray while staying at Brücke’s laboratory as a research assistant, choosing also the crayfish nervous system for the study. His 1882 paper on the subject, one of the more unfairly overlooked contributions in the history of the cytoskeleton, left no room for ambiguity (Fig. 2):

The nerve cells in the brain and in the ventral ganglionic chain consist of two substances, one of which is arranged as a network in the fibrils of the nerve fibers, and the other is homogeneously continuous in between [Freud, 1882].

Schultze’s conception of the nerve fibrils as conductors, also entertained by Freud, was taken to its pinnacle by Stephan von Apáthy [1897] and Albrecht Bethe [1900], who introduced the term “neurofibrils.” In their view, these filaments would constitute continuous lines threaded through chains of nerve cells across the entire nervous system. This thesis seemed to offer a reasonable solution to the concomitant diatribe about the basic organization of the nervous system, which was alternatively visualized either as an uninterrupted reticulum or as a concatenation of individual cells. In the emerging third version of Apáthy and Bethe, the continuity required for fast impulse conduction would be guaranteed by the neurofibrils, while the tissue itself would consist of a community of cells just like any other in the body. Nevertheless, such a compromise certainly did not please Santiago Ramón y Cajal, the main force behind the neuron theory. He fully confirmed the presence of neurofibrils in nervous tissue from various sources, including the human brain, but insisted that the filaments never reach out of the individual neurons. Moreover, added Ramón y Cajal, the filaments could hardly have a specific function of conducting nerve impulses because

The existence of intraprotoplasmic fibrils is a general anatomical law of the cell. More or less modified in their disposition, intracellular threads have been found in skin epithelial cells, in the corpuscles of the lashes, in the egg cell [. . .] and nobody will think of inferring from this fact that the above mentioned threads constitute the obliged pathway for the light, heat, electric or mechanical waves [Ramón y Cajal, 1903].

This argument was itself a matter of acrimonious dispute at the time. In the wake of Brücke’s and Krause’s experiments with polarized-light microscopy in muscle, Theodor Engelmann, based upon his own observations of different contractile or motile specimens using the same technique, had reached the generalization that at least all cells endowed with the capacity for any sort of self-movement should contain elongated submicroscopic structures [Engelmann, 1875]. However, this kind of thinking was vehemently opposed by Lionel Beale, who put amoeboid cells as the best proof of the alleged fallacy. It took only an attentive watching of the movement of some free-living amoebae or white corpuscles of the blood—he retorted—to realize that the “perfectly transparent matter moves first,” before any particulate or otherwise structured material flowed into it [Beale, 1872 (italics as in the original)]. In order to avoid further confusion with the much abused term “protoplasm” for all cellular contents, structured or not, Beale named this motile homogeneous substance with the word “bioplasm.”

At around the same dates another startling new finding got in the discussion. Some microscopists had described the transient apparition of a large fusiform body within cells seen while in the process of division, and the spindle showed longitudinal striations that suggested a fibrillar composition. Walther Flemming, one of the leading experts on cell division, initially remained skeptical about such a structure but soon became an
indefatigable advocate of the importance of its “pale threads” for chromosome migration. In his 1880 milestone work on mitosis he wrote what is probably the first allusion to intracellular fibrils in relation to organelle transport:

When the two halves of the stainable substance move toward the poles they simply slide along the pale threads which therefore become visible at the area of the equator. [...] The achromatic figures deserve to be studied with as much attention as the chromatic ones, for if it is at all justified to assume the existence of either centers of direction or centers of attraction which control the shifts in position of the chromatic nuclear threads, and to speculate about the location of these centers, one must look for them in the domain of the achromatic figure [Flemming, 1880 (italics as in the original)].

Claims of protoplasmic fibrils other than those of the mitotic spindle and nerve cells, however, continued to face a multifront opposition. For example, the Belgian embryologist Édouard van Beneden, a leading proponent of a highly structured intracellular substance, gave the following description of the protoplasm of a living egg cell:

If one aparts with the imagination the hyaline spheres, the homogeneous droplets and the refringent corpules of the vitellus, it remains a reticulated framework, a system of layers, plates and beams anastomosed in a net; they are constituted by a finely dotted substance and together they form the protoplasmic body of the egg [...]. The dots are united with each other by extremely tenuous lines; it seems that the dot-shaped granules are strung on the filaments, or rather that the grains are but enlargements, sometimes equidistant, of beaded fibrils [van Beneden, 1883].

But Otto Bütschli held that such was just the appearance of protoplasm, not its real actual structure. In fact, he explained, the protoplasm is a microscopic foam or emulsion, and those interconnected lines would correspond to the extremely thin plates separating adjacent bubbles, because “the alveoli are so densely crowded that their real protoplasmic walls take on a honeycombed arrangement, which in optical section appears reticular” [Bütschli, 1892].

Conciliatory voices were heard by the end of the century. After thoroughly revising the whole subject, the young but authoritative American cytologist Edmund B. Wilson concluded that the main competing opinions on the structure of protoplasm were partly valid, although not very meaningful in the last analysis. Summarizing his own observations in living egg cells he stated that protoplasm “is an alveolar structure, as maintained by Bütschli,” but “the rays of the astral systems (astral rays and spindle-fibers) are actual fibrillae, and not merely the optical sections of lamellar plates” among alveoli [Wilson, 1899a]. Then, in a review of the topic published the same year, he declared:

I fully agree with the opinion of Kölliker, which has been adopted by an increasing number of later observers, that no universal or even general formula for protoplasmic structure can be given. The evidence indicates that alveolar, granular, fibrillar and reticular structures are all of secondary origin and importance, and that the ultimate background of protoplasmic activity is the sensibly homogeneous matrix or continuous substance in which those structures appear [Wilson, 1899b (italics as in the original)].

However, also exactly 100 years ago, the notion of fibrils or any other structured framework in the protoplasm was again seriously questioned. W. B. Hardy reported a critical study of protoplasm structure as seen in fixed cells, with which most descriptions of intracellular fibrils had been obtained. “It is notorious”—he remarked—“that the various fixing reagents are coagulants of the organic colloids, and that they produce precipitates which have a certain figure or structure” [Hardy, 1899]. In addition, microscopic fibril-looking striations such as those described radiating from the poles of the presumptive mitotic spindle in dividing cells, could easily be produced by merely stretching similar colloids. His general conclusion was devastating for many microscopists: “There is no evidence that the structure discoverable in the cell-substance [...] after fixation has any counterpart in the cell while living. A large part of it is an artifact. . . .”

Thus the maxim that fibrillar elements seen within fixed cells should be considered artifacts until proven true cellular components to everyone’s satisfaction—a requirement rarely demanded for vesicles or other corpuscular inclusions—got underway. Even the neurofibrils remained a belief of minorities. Early in this century there were suggestions that the fibrils might have a supportive role for the protoplasm [reviewed in Parker, 1929], but this conjecture was dismissed as unlikely because the structures seemed too flimsy for the purpose. “The neurone”—wrote G. H. Parker, one of the few defenders of the neurofibrils—“maintains its shape chiefly through its outer covering, and not in consequence of an inner skeleton” [Parker, 1929]. To his credit, though, Parker’s own farsighted hypothesis held that the neurofibrils possibly act as conveyors of “metabolic influences” from the neuronal cell body down to the distant terminals of its long processes.

As we all know, the concept of internal fibrous networks as standard gear of eukaryotic cells eventually came back. Yet, rather than through the common vindication by the use of a more sophisticated methodology, the return was heralded by theoretical considerations. And it was not just the microscopists, but also the biochemists, who kindled the new dawn.
THE CYTOSQUELETTE

The first hints came from observations of an astonishing physical resilience. The fast recovery of egg cells after being internally disorganized by centrifugation led Edwin Conklin to conclude the probable existence of a "spongiosomal framework," the elasticity of which would be able to properly redistribute again the displaced intracellular contents [Conklin, 1917]. Somewhat later, a structured nature of the apparently hyaline cellular substance was inferred also from a quite different biochemical perspective. As the complexity of the reactions taking place in the protoplasm was gradually realized, the view of the cell as an assortment of granules freely floating in a homogeneous juice became increasingly inadequate. The simple mass action of the reactants was esteemed insufficient to explain the coordinated sequence of steps involved in metabolism. This meticulous orchestration evidently required some type of support to maintain the appropriate topographical relations among enzymes and substrates. In 1930, Rudolph Peters postulated that such sustaining background might be constituted by

an organised network of protein molecules, forming a three dimensional mosaic extending throughout the cell. The enzymes would form part of this structure, their activity being largely controlled by the mosaic [Peters, 1930].

The following year the embryologist Paul Wintrebert, speculating on how the eggs of toads and other animals manage to keep their internal organization despite the vicissitudes undergone during the strenuous passage through the female reproductive tract, concurred with Conklin as regards the physical necessity for the presence of a "cytosquelette" [Wintrebert, 1931]. Then Joseph Needham, building upon these and many other premises, interpreted life in terms of a specific structural and chemical order kept to a large extent within cells by an admittedly difficult to demonstrate but undeniably existing "cytoskeleton" [Needham, 1936].

The word originally coined by Wintrebert lingered for about a quarter of a century, while electron microscopy made its debut in biological research. Once again, nervous tissue provided the first glimpse of the intimate architecture in non-muscle cells. The inspection of disrupted nerves with the new instrument showed long intra-axonal fibers, most likely tubular in shape, to which the name "neurotubules" was applied [De Robertis and Schmitt, 1948]. Soon after, though, these results along with the term neurotubules were recanted as observation of thin sections of similar samples showed filaments rather than tubules within the axons [Schmitt and Geren, 1950]. The word "neurotubules" resurfaced occasionally later on to denote a more defined class of tubules in nerve cells [e.g., Gonatas and Robbins, 1965], but these would eventually be called microtubules in all types of cells (see below). Further study of neuronal fine structure also introduced the term "neurofilaments" [Palay and Palade, 1955], some of which were finally identified as the neurofibrils of old [Gray and Guillery, 1961].

In 1957 we already find Keith Porter pondering whether some of the structures that electron microscopy had revealed in thin sections of many cells "could be regarded as 'cytoskeletal' in function" [Porter, 1957 (quote marks as in the original)]. Among the candidates, certain "canaliculi" particularly abundant in the protoplasm of cells fixed during mitosis were apparently involved in the achromatic spindle. By then any remaining doubts with regard to the reality of the spindle were dispelled for good, and mitotic apparatuses had even been isolated in quantity from dividing cells [Mazia and Dan, 1952], so the "canaliculi" should most probably correspond to the "pale threads" that had so intrigued Flemming.

More obviously cytoskeletal, in Porter's reckoning at the time, were the filaments seen extending within the full lengths of cilia and flagella. Such extraordinarily thin fibrils, originally hypothesized in the 19th century on the basis of studies with polarized-light microscopy [Engelmann, 1875], were later directly observed in fresh sperm flagella forced to burst open by simple pressure between two glass plates [Jensen, 1887; Ballowitz, 1890], and became the focus of intensive theorization in the following decades [Dellinger, 1909; Grave and Schmitt, 1925]. The first electron microscopical inspections of frayed flagella and cilia confirmed the presence of about 11 internal fibrils [Harvey and Anderson, 1943; Schmitt et al., 1943; Jakus and Hall, 1946], two of which were noticed to differ from the rest in general morphology and lability [Grigg and Hodge, 1949]. An accurate diagrammatic interpretation of the most probable internal architecture in intact cilia was offered [Manton and Clarke, 1952], but electron micrographs of the actual fibril arrangement appeared only when thin cross-sections of cilia in epithelial cells could be made [Fawcett and Porter, 1954]. Each cilium was then shown to contain a characteristic set of 11 fibrils, nine of them double like twin wires fused lengthwise and disposed around a pair of single ones.

The finding of tidy bundles of threads within the specialized appendages of certain cells, as well as the occurrence of abundant filaments in the highly idiosyncratic nerve processes, or those peculiar "canaliculi" seen in cells fixed while in the throes of dividing themselves, although undoubtedly interesting, surely did not compel everyone to include a cytoskeletal framework in the accepted notion of a typical cell in the normal condition. As I will attempt to summarize in the next few para-
TWO MAJOR SYSTEMS OF INTRACELLULAR FIBRILS

The mid-1950s was also the period when muscle research reached maturity, as the culmination of a rapid succession of brilliant discoveries that illuminated much of the subsequent development of ideas on the cytoskeleton. Following the rise and decline of “myosin” as a favorite to explain muscle contraction during the 19th century, this protein again took center stage in the early 1940s after its ability to hydrolyze ATP was demonstrated [Engelhardt and Ljubimowa, 1939]. For the first time a specific enzymatic activity had been unequivocally associated with a semi-purified and intracellularly localized molecule, and this relevant finding was almost immediately ratified by several laboratories [Needham, 1942; Bailey, 1942]. Still more interesting, long “myosin” threads reconstituted from muscle extracts proved to react mechanically, increasing their extensibility, when ATP was added to the medium [Engelhardt et al., 1941; see also Shen, 1947]. Work from Szent-Györgyi’s laboratory in Hungary then established that “myosin” was in fact composed of two proteins: myosin itself and a closely bound second component that they named “actin”1 [Straub, 1943]. Going even further in his chemical dissection of muscle, ten years later Szent-Györgyi reported that treatment of myosin with trypsin produces two distinct subunits or “meromyosins” [Szent-Györgyi, 1953].

As the stream of biochemical data on myosin and actin quickly expanded, the mutual relationship of the two proteins in muscle fibers began to be understood at the ultrastructural level. Early inspections of striated muscle fragments with the electron microscope had shown bundles of closely packed thin filaments, believed to be actin, each bundle seeming to run continuously through consecutive segments along the myofibrils, where periodical patches of an apparently amorphous material supposed to be myosin could be seen inserted [Hall et al., 1946; Rosza et al., 1950]. With the arrival of thin-sectioning technology, however, discrete sets of thick and thin filaments partly overlapping each other could be resolved in every segment or sarcomere [Hanson and Huxley, 1953; Hasselbach, 1953; Huxley, 1953; see also Hanson and Huxley, 1955]. This unexpected arrangement, plus observations of living muscle fibers with interference microscopy, prompted two research teams to simultaneously and independently conceive the fruitful idea that both types of filaments slide over each other, increasing their extent of overlap as the muscle contracts [Huxley and Niedergerke, 1954; Huxley and Hanson, 1954]. By then both the structural stability and the functional self-sufficiency of the filament system had been demonstrated, thanks to a helpful preparation procedure in which treatment with glycerol produced virtually demembranated muscle fibers that nevertheless contracted upon addition of ATP [Szent-Györgyi, 1951; see also Hanson and Huxley, 1955]. This method would later inspire countless experimental approaches in which permeabilized cells were used for directly stimulating cytoplasmic components, or to infiltrate a wide variety of chemical markers and other molecular probes.

An equally momentous corollary followed almost immediately. Since numerous short arms projecting sideways from the thick filaments seemed to connect with the thin filaments along the stretch of overlap, a cyclic action of these crossbridges was proposed to drive the sliding [Huxley, 1957]. That this sort of interaction could be possible as well in smooth muscle fibers, in spite of not having their filaments organized as a periodic series of sarcomeres, was soon intimated [Lowy and Hanson, 1962; Needham, 1962]. Eventually most of the ATPase activity in striated muscle myofibrils was located at the expanses where crossbridging between the thick and the thin filaments occurs [Tice and Smith, 1965]. And, as predicted also from the theory, the amount of tension generated by muscle was shown to depend on the extent of filament overlapping at the moment when the fiber is stimulated [Gordon et al., 1966].

The relative spatial disposition of the main myofilibrilar proteins was determined as the advance in biochemical and electron microscopy techniques allowed observations of isolated myofilaments at high magnifications. Actin filaments were found to consist of two intertwined chains of round particles, whereas myosin filaments appeared as spindle-shaped fibrous aggregates with bushy ends [Hanson and Lowy, 1963; Huxley, 1963]. These studies also showed that mixing free actin filaments with the heavier of the two meromyosins described by Szent-Györgyi, under appropriate conditions, resulted in an uniform “decoration” of the double chain of particles with distinctive “arrowheads” pointing all to the same end in each filament. Consequently, the periodical attachment of myosin molecules over actin filaments could be reconstructed as a three-dimensional model [Moore et al., 1970].

This unique molecular ornamentation became a decisive bridgehead sent out from muscle research to foster the nascent investigation on the cytoskeleton. The

1 Although unnoticed at the time, this finding was in fact a rediscovery, since in 1887 W. D. Halliburton had described a “myosin-ferment” that greatly accelerated the coagulation of myosin solutions obtained from muscle through variants of the procedure originally devised by Kühne. See also Finck [1968].
possibility of decorating actin filaments with heavy meromyosin provided an unequivocal means to test for the identity of thin filaments increasingly found in non-muscle cells, and suspected to contain actin ever since an actomyosin-like substance had been extracted from the slime-mold plasmodium [Loewy, 1952]. Thus, while this latter organism and certain amoebae were being used to carry out the first methodical biochemical analyses of actin and myosin in non-muscle motile systems [Hatano and Oosawa, 1966; Hatano and Tazawa, 1968; Adelman and Taylor, 1969; Weihing and Korn, 1969; Woolley, 1970], attempts to decorate thin filaments with heavy meromyosin succeeded in a wide variety of cells [Ishikawa et al., 1969].

Adding to the incipient repertoire of specific experimental tools, at around this time some fungal metabolites known as cytochalasins were found to inhibit motile processes in which actin filaments were thought to be involved. For example, the cleavage furrow zone in dividing cells, shown to exert measurable tension [Rappaport, 1967] and to be rich in thin filaments [Schroeder, 1968; Tilney and Marsland, 1969], proved sensitive to cytochalasin-B [Schroeder, 1969]. Shortly afterwards it was confirmed that such filaments can be decorated with heavy meromyosin [Perry et al., 1971; Schroeder, 1973]. Similar observations in diverse systems established that actin filaments are disrupted by the cytochalasins, which thereby interfere with a number of motile phenomena [Wessels et al., 1971].

The accumulation of morphological, biochemical, and pharmacological evidence led to the recognition of actin and myosin as responsible for contractile movement in many kinds of cells, besides muscle fibers [see Pollard and Weihing, 1974; Korn, 1978]. Furthermore, it was shown that an actin-based but myosin-independent thrusting motion, due to either a sudden formation or a straightening of actin filaments, can produce rapid and conspicuous changes in cell shape like the spectacular discharges of the acrosomal process by the sperm of some invertebrates [Tilney et al., 1973b; Tilney, 1975]. In the meantime, a parallel and ultimately convergent story had evolved in relation to the structure and activity of cilia and flagella. Only 2 years after the sliding filament theory of muscle contraction was published in its definitive form [Huxley, 1957], an analogous principle was suggested to explain flagellar movement. As each of the nine peripheral double fibrils in a flagellum appeared furnished with two rows of lateral projections extending toward one of the adjacent doublets (apart from radial spokes linking each doublet to the core where the central pair of single fibrils are located), these side arms were thought to perform a mechanical action similar to that proposed for the crossbridges seen between myofilaments [Afzelius, 1959].

This view gained substantial support when the autonomous beating of isolated flagella was found dependent on a local ATPase [Brokaw, 1961] and such enzymatic activity was soon localized at the side arms that protrude from the peripheral fibrils, in a specific protein called “dynein” [Gibbons, 1963; Gibbons and Rowe, 1965]. Concurrently, electron micrographs of cilia instantaneously fixed in different phases of movement provided convincing morphological evidence of restrained relative slipping of the peripheral fibrils [Satir, 1965, 1968]. Direct observation of full-length and ATP-dependent sliding of fibrils was finally achieved when structural restrictions were eliminated in demembranated and mildly trypsinized flagella [Summers and Gibbons, 1971]. The problem of explaining ciliary and flagellar motility then became one of figuring out just how this basic motion of the peripheral fibrils can translate into rhythmic bending or torsion of whole vibratile appendages [Warner and Satir, 1974], a difficult issue for which various models have since been elaborated [Sugrue et al., 1991; Cosson, 1996; Lindemann and Kanous, 1997].

Motor protein-powered active sliding of linear elements was thus proved to be the mechanism underlying a biological movement, though in flagella instead of muscle for which the idea had originally been surmised. At around the same time, research on other non-muscle systems also suggested that motion generation might have a common mechanical principle in diverse, apparently unrelated instances. Active longitudinal extension of Stentor, a ciliated protozoan, was tentatively explained as due to fiber sliding likely driven by dynein-like crossbridges [Huang and Pitelka, 1973]. Dynein or myosin were also pointed out as obvious candidates to investigate in relation to chromosome movement, which was observed to continue along mitotic spindles released from cells lysed while undergoing division [Cande et al., 1974].

Nevertheless, these various systems seemed to belong in either of two classes that had surprisingly little in common as regards the structures that slide. In contrast to the rod-like aggregates of myosin or the intertwined chains of actin molecules that constitute the myofilaments, for example, the fibrils contained within cilia and flagella seemed to be hollow cylinders with walls formed by beaded longitudinal filaments [André and Thiery, 1963; Pease, 1963]. Both the diameter—about four times that of an actin filament—and the overall aspect of these cylinders were remarkably similar to those of the “canalicular” that had continued to be found in an ever increasing number of different cells, and that, following an awkward period of nomenclatural indecisiveness, ended up being called “microtubules” [Slatterback, 1963].

Microtubules other than those in cilia and flagella seemed comparatively labile or unstable, but they be-
came a common feature of virtually every cell inspected with electron microscopy following the introduction of glutaraldehyde as a primary fixative [Sabatini et al., 1963]. Their topographical distribution in relation to other cytoplasmic organelles, some of which were known to travel within cells, suggested that microtubules could perhaps mediate intracellular motility [Porter and Tilney, 1965; Schmitt, 1968; Ochs, 1971]. This suspicion was upgraded to near certainty when colchicine, a compound long known to interfere with mitosis and to disorganize the fibers of the spindle in dividing cells [Inoué, 1952], became the standard probe for testing the function of cytoplasmic microtubules after its ability to also inhibit axonal transport in nerve was demonstrated [Dahlström, 1968; Karlsson and Sjöstrand, 1969; Kreutzberg, 1969].

Detailed knowledge about the nature of microtubules quickly came to a par with that of actin filaments. The wall of a microtubule was shown to consist of 13 longitudinal protofilaments [Ledbetter and Porter, 1964; see also Tilney et al., 1973a], each of them made up of particles aligned with an exact periodicity [Grimstone and Klug, 1966]. A major constitutive protein was isolated from various sources and named “tubulin” [Borisy and Taylor, 1967; Shelanski and Taylor, 1967; Wilson and Friedkin, 1967; Mohri, 1968; Weisenberg et al., 1968], but evidence that microtubules might in fact be composed of two closely resembling subunits soon emerged [Bryan and Wilson, 1971; Feit et al., 1971], and proof was offered that even individual protofilaments could be constituted of tubulin heterodimers [Meza et al., 1972]. Moreover, tubulin forming the sturdy doublets of microtubules in flagella was shown to be analogous to the protein that makes up the labile cytoplasmic microtubules, so the contrast in stability should probably be related to the different molecular assembly in each of the two alternative polymers [Wilson and Meza, 1973]. The precise arrangement of tubulin molecules in a tightly knit helical order around a microtubule wall could finally be reconstructed [Chasey, 1972; Amos and Klug, 1974; but see also Mandelkow et al., 1995]. This elegant structure, as mentioned above and with the allowances requested, evokes that of the *vasa minima* postulated by the theory of fibers in the 18th century.

Once microtubules and actin filaments were identified as widely distributed organelles with presumptive equivalent functions in many kinds of cells, including those of plants [Ledbetter and Porter, 1964; Palevitz et al., 1974; Williamson, 1974; Condeelis, 1974], the era of the cytoskeleton as a universally accepted notion formally began. The concept became at the same time more fascinating and complicated as twin cohorts of newly found proteins gradually recruited around actin and tubulin. A cytoplasmic dynein-like protein similar to that of flagella [Burns and Pollard, 1974; see also below], as well as other accessory proteins associated to microtubules [Weingarten et al., 1975; Murphy and Borisy, 1975], were discovered. Several types of myosin and a number of other actin-binding proteins were found in diverse non-muscle cells [Niederman and Pollard, 1975; Burridge and Bray, 1975; Elliot et al., 1976; see also Schliwa, 1981]. Therefore, not only were the cells endowed with intricate arrays of distinct linear polymers extending throughout their bodies, but each of these networks should in fact be considered a veritable mainstay for a vast installation of coordinated proteins.

This latter vision, arrived at mostly on the basis of biochemical data and a myriad electron micrographs that sampled just an infinitesimal plane or thin section of the specimens, was for a while a purely mental picture. The true panorama was unveiled to actual view when fluorescently labeled antibodies, raised against specific proteins, for the first time revealed the intracellular distribution of actin filaments [Lazarides and Weber, 1974] and microtubules [Fuller et al., 1975; Weber et al., 1975] in whole cells. Not only did the complete networks become readily visible in all their delicate complexity, but also their skeletal significance was clearly demonstrated, particularly in the case of microtubules. The changes of cell shape concomitant with division were accompanied by dissolution of the cytoplasmic microtubule network and formation of the mitotic spindle, whereas the subsequent disappearance of the spindle coincided with the organization of new microtubule networks in the cytoplasm of the daughter cells, thus suggesting the same tubulin subunit pool is used to build different microtubule constructions at separate moments in the cell cycle [Brinkley et al., 1975]. The immunofluorescence technique also allowed the localization of various actin-binding proteins [Lazarides and Weber, 1974] and microtubule-associated proteins [Connolly et al., 1978], all of which matched in distribution the splendid patterns of their respective supporting networks.

Being at last able to watch entire cytoskeletal organizations was encouraging, but the curtain had just been lifted to disclose the scenery. Neither all props nor the complete cast were yet on sight, and much remained to be learned about the deployment or operation of every part involved. Research in this realm—the molecular mechanics of the cytoskeleton—has taken up the last two and a half decades, and is still an ongoing effort.

**MOLECULAR DYNAMICS AND STILL MORE WEBS**

Studies on how the cytoskeletal polymers become assembled surged following the discovery of conditions conducive to the in vitro polymerization of both tubulin
from brain [Weisenberg, 1972; Borisy and Olmsted, 1972] and actin from non-muscle sources [Woolley, 1972; Spudich, 1974; Kane, 1975]. In each case the linear polymer was found to involve a labile association of the corresponding monomers, as suggested a few years earlier with regard to the microtubules of the mitotic spindle [Inoué and Sato, 1967]. But whereas actin filaments seemed to grow by simple annexation of monomers, evidence was obtained that subunits of fully solubilized tubulin come together through a sequence of steps in which ring-like forms, short protofilaments joined in parallel like flat sheets, and helical ribbons precede the cylindrical shapes typical of the microtubules [Borisy and Olmsted, 1972; Erickson, 1974; Kirschner et al., 1975].

The opposite ends of actin filaments and microtubules proved to have different propensities for the addition or removal of their respective monomers, so these polymerize or depolymerize preferentially in the same direction along the polymer axis, and can even travel in a continuous flux or treadmilling process down the structure at a steady state [Rosenbaum et al., 1975; Wegner, 1976; Margolis and Wilson, 1978; Bergen and Borisy, 1980; see also Kirschner, 1980; Cleveland, 1982]. This intrinsic polarity of the cytoskeletal polymers, which had been noticed for actin filaments ever since decoration with heavy meromyosin appeared as arrowheads pointing in the same direction over a given filament [Moore et al., 1970], could be demonstrated as well for microtubules when under certain conditions tubulin produced uniformly oriented hooks over their walls [Heidemann and McIntosh, 1980].

Then another important difference between the two types of cytoskeletal polymers was discovered. While actin filaments appeared to originate at, or at least to associate with, cell membranes [Pollard and Korn, 1973; Spudich, 1974], microtubules were shown to grow preferably from a few discrete and strategically located foci. Early electron microscopy work had found microtubules arranged as if they would emanate from small masses of a dense amorphous material, embedded in which short cylindrical arrays of nine triplets of microtubules, called centrioles or basal bodies, could be frequently seen [Szollosi, 1964; de-Thé, 1964; Gonatas and Robbins, 1965; see also Porter, 1966]. The fuzzy aggregates became known as “microtubule-organizing centers” or MTOCs [Pickett-Heaps, 1969; see also Brinkley, 1985], of which the more thoroughly studied example is perhaps the centrosome, a loose lump situated either close to the nucleus in animal cells or distributed into several domains in plant cells [Mazia, 1984; Moritz et al., 1995; Balczon, 1996; Vaughn and Harper, 1998]. Centrioles, characteristic of the centrosome in animal cells where they duplicate for eventually each set being present at one of the poles of the mitotic spindle, are currently thought to provide a structured focal support upon which the amorphous pericentriolar material is organized [Marshall and Rosenbaum, 1999].

In turn, this material amply justifies the term MTOC, since it was shown that such condensations not only seem to anchor the proximal ends of microtubules in certain cytoplasmic locations, but also act as sites for the initiation of microtubule assembly, which then proceeds by incorporating tubulin subunits preferentially at the distal ends [Inoué et al., 1974; Weisenberg and Rosenfeld, 1975; Brinkley et al., 1981; Deery and Brinkley, 1983; Mitchison and Kirschner, 1984; Soltys and Borisy, 1985; Rodionov et al., 1999]. Similarly, the basal bodies at the roots of cilia and flagella not only help to hold in place the proximal ends of the vibratile organelles, but also nucleate the assembly of tubulin upon themselves [Snell et al., 1974; Rosenbaum et al., 1975]. It is now being increasingly understood that this remarkable ability of promoting microtubule growth involves the participation of an intricate complex of proteins, of which centrin or caltractin [Salisbury, 1995; Schiebel and Bornens, 1995; Levy et al., 1996], pericentrin [Doxsey et al., 1994], centreactin [Clark and Meyer, 1992], and γ-tubulin [Zheng et al., 1991; Stearns et al., 1991; Joshi et al., 1992; Stearns and Kirschner, 1994; Zheng et al., 1995] have been well characterized.

The cytoskeleton became a still more complex system as it was realized that threads analogous to the neurofilaments [see Shelanski et al., 1976], each with a caliber intermediate between those of myosin filaments (or microtubules) and actin filaments, are also present in muscle fibers and other cells [Ishikawa et al., 1968; Cooke, 1976; Davison et al., 1977; Franke et al., 1978]. Some of these threads had been noticed long before by microscopists, who had referred to them by names such as “tonofilaments” or “gliafilaments” according to the cell types being studied [see Lazarides, 1980]. Now close inspection allowed the determination that they were of approximately the same width in a variety of tissues, and easily distinguishable from either microtubules, actin filaments, or myosin filaments. In addition to their mid-size diameter, these “intermediate filaments” exhibited peculiar properties that set them clearly apart from the other major cytoskeletal polymers. They were found to remain unaltered by colchicine, in contrast to the microtubules, and they also failed to become decorated like the actin filaments with heavy meromyosin [Ishikawa et al., 1968, 1969]. In fact, they proved so stable that quite drastic conditions, like extraction with 6–8 M urea, were needed to effectively solubilize them. Furthermore, contrary to actin filaments and microtubules, composed respectively of one and two types of subunits in every cell, the intermediate filaments turned out to be made of distinct...
proteins in different tissues [see Lazarides, 1982; Steinert and Roop, 1988; Albers and Fuchs, 1992], even though all such filaments share a common epitope that can be specifically recognized by a monoclonal antibody [Pruss et al., 1981]. As it had been with microtubules and actin filaments, research at the molecular level on this newly identified component of the cytoskeleton thrived as conditions for the in vitro assembly of various classes of intermediate filaments were defined [Steinert et al., 1976; Zackroff and Goldman, 1979; Renner et al., 1981; Zackroff et al., 1982]. Eventually a collection of proteins associated with these structures was discovered [see Foisner and Wiche, 1991]. In spite of the rapidly accumulating knowledge about these hardy filaments, however, convincing evidence of their functional significance remained pending to appear until quite recently.

Immunofluorescence microscopy had showed the intermediate filaments constituting dense intracellular meshworks of their own [Dahl and Bignami, 1973; Franke et al., 1978; Gordon et al., 1978], which from the outset were believed to serve an important role for the mechanical integration of the cytoplasm [Lazarides, 1980]. This early supposition was for a long time difficult to test, in part because no drug equivalent to colchicine or cytochalasin was available for experimentally destabilizing intermediate filaments [see Klymkowski et al., 1989], but it has now been supported by new data obtained with gene knockout approaches and other advanced techniques [see Galou et al., 1997; Capetanaki and Milner, 1998; Julien and Mushynski, 1998]. It was proven that cells lacking intermediate filaments are mechanically weaker and more fragile than normal. Even the inherently resistant muscle fibers are prone to rupturing when they are defective in the passive cytoskeletal network constituted by their intermediate filaments [Li et al., 1997]. Axons missing neurofilaments are narrower and take longer to regenerate upon damage [Eyer and Peterson, 1994; Zhu et al., 1997]. At the same time it is also being realized that, rather than static strings merely holding the cytoplasm together in place, the intermediate filaments are mobile elements that display wavy movements, extensions, and translocations at least in part dependent on microtubules [Marty et al., 1999]. These findings, along with an increasing understanding of their assembly mechanisms [Fuchs and Weber, 1994; Herrmann and Aebi, 1998; Hofmann, 1998], have revealed the intermediate filaments as unexpectedly dynamic structures.

Last added to the list of cytoskeletal components, and a particularly contentious matter, is a pervasive matrix of slender strands called “microtrabeculae,” which were found interlinking all other cytoplasmic structures when desiccated whole cells could be viewed in depth at high magnifications with stereo high-voltage electron microscopy [Buckley, 1975; Wolosewick and Porter, 1976]. These authors regarded the microtrabeculae as the ultimate configuration of the cytoplasmic ground substance, i.e., as a delicate web at once supporting and keeping the organelles properly distributed inside the cell. Alternatively, in those cells where the distribution of certain organelles is subject to physiological changes, like the massive migrations of pigment granules within chromatophores, the microtrabeculae would perform as an efficient collective carrier [Byers and Porter, 1977]. However, this lattice, which came closest to fit the original definition of the cytoskeleton as a three-dimensional assembly of proteins extended throughout the cell (see above), was soon the center of a debate concerning its real or artifactual nature [see Wolosewick and Porter, 1979]. The controversy revolved largely around the fine texture of intracellular surfaces and possible alterations introduced by specimen preparation. Cytoskeletons that were rapidly frozen and then freeze-dried, for instance, presented microtubules and filaments of definite calibers in complex arrangements, but no tapered and fused connections like the typical microtrabeculae [Heuser and Kirschner, 1980].

These observations suggested that microtrabeculae might form by a fixative-caused cross-linking of soluble proteins with each other and with adjacent structures, so that upon subsequent dehydration and drying such aggregates would shrink into a coarse coating continuous with short struts bridging among neighboring membranes or true cytoskeletal elements. Evidence was next offered that indeed much of the trabeculae may consist of a large number of polypeptides; still, on the basis of results obtained with differential extraction procedures, it was concluded that all of these molecules would likely be associated in a “metastable” structure-forming condition in the living cell [Schliwa et al., 1981]. Further strict tests aimed to either prove or disprove the existence of microtrabeculae as an independent cytoskeletal component, produced convincing data that such characteristic shapes probably do not represent the real appearance of the cytoplasmic ground substance in a standard cell [Kondo, 1984; Ris, 1985]. Nevertheless, the concept of a matrix filling the intracellular space in between the organelles gained support from the finding of a system of very fine (2–6 nm) linkers in various cells [Schliwa and van Blerkom, 1981; Hirokawa, 1982; Hirokawa and Tilney, 1982]. A finely fibrous, and yet mechanically resistant, material linking clusters of organelles to microtubules and intermediate filaments was also demonstrated in cells subjected to stepped disruption procedures [Frixione, 1983].

Learning how the cytoskeleton moves itself and other cell structures in terms of molecular interactions has understandably been the toughest challenge. Once
again, actin-myosin based movement provided the first lessons, as attempts to reconstitute simplified functional systems with these proteins extracted from either muscle or non-muscle sources began to be successful [Crooks and Cooke, 1977; Yano et al., 1978; Matsumura et al., 1980]. Furthermore, it was proven that such proteins taken from evolutionarily distant origins can constitute an operational hybrid system, useful as a quantitative assay, when fluorescent beads coated with heavy meromyosin from rabbit muscle migrated actively along stationary actin cables in opened cells of green algae [Sheetz and Spudich, 1983]. Conversely, free fluorescent actin filaments placed in contact with anchored myosin molecules could be observed to slide and bend across microscopic fields [Yanagida et al., 1984; Kron and Spudich, 1986].

As for the other major branch of cell dynamics, a decisive stride towards explaining microtubule-based motility was launched when video-enhanced contrast microscopy made it possible to look at single microtubules gliding over a glass surface, as well as serving like guiding tracks for bidirectional translocations of cytoplasmic particles in contact with them [Allen et al., 1985]. Experimental inquiry on the factors essential to support these motions led to the discovery of “kinesin,” the so far elusive force-generating protein responsible for anterograde axoplasmic transport in nerve [Brady, 1985; Vale et al., 1985a,b]. Kinesins were subsequently identified in other cells [e.g., Neighbors et al., 1988; Rodionov et al., 1991], and shown to play a role in mitosis and meiosis [see Yen, 1995]. In turn, the previously found cytoplasmic dynein (see above) was characterized as a microtubule-activated motor that translocates in a direction opposite to that of kinesin, and is therefore responsible for retrograde axoplasmic transport [Paschal et al., 1987; Paschal and Vallee, 1987; Schnapp and Reese, 1989; Schroer et al., 1989]. Like kinesin, cytoplasmic dynein is proving to be involved in a number of functions at different levels of cell physiology [Gonczy et al., 1999; Purohit et al., 1999; Karki and Holzbaur, 1999].

The 1990s were dominated by fundamental advances on the physics of the molecular motors and their interaction with cytoskeletal tracks, as a result of impressive methodological feats. The spatial relationships of myosin molecules with actin filaments, or the substructure of dynein arms, had been studied in superb high-magnification electron micrographs of quick-frozen and deep-etched specimens [Heuser and Cooke, 1983; Goodenough and Heuser, 1984]. However, only in the following decade were the detailed topography of the actin filament [Holmes et al., 1990], as well as that of the myosin head and the complex it forms with actin [Rayment et al., 1993a,b], modeled at the atomic level. The structural designs of kinesin and a related microtubule-dependent motor were found similar to that of myosin [Kull et al., 1996; Sablin et al., 1996], while functional analyses started to provide direct quantitative data on the mechanical properties of some of these molecules. By holding microscopic particles with so-called laser tweezers [Block et al., 1990; Vale, 1993; Finer et al., 1994; see also Block, 1995], and by nano-manipulation with glass micro-needles [Ishijima et al., 1994], the force and step length of individual molecular motors over their corresponding cytoskeletal tracks could be measured. According to all current indications these little machines, which move most of the living world, share a common set of structural mechanisms, i.e., an efficient combination of latch, swivel, and lever actions [Amos and Cross, 1997].

Newly developed technological approaches, like fluorescent speckle microscopy and fusion of green fluorescent protein to endogenous proteins [Waterman-Storer et al., 1999; Bloom et al., 1999; Marty et al., 1999], are bound to produce a fresh wave of quantitative data on the dynamics of specific cytoskeletal elements. On the other hand, the synergistic cooperation of these elements is also being disclosed [Gavin, 1997; Rodionov et al., 1998; Rogers and Gelfand, 1998], so at last the cytoskeleton is beginning to be understood as a highly integrated and coordinated machinery. A good measure of the rapid pace of meaningful research in this field is what has recently been accomplished on the study of mitosis, that most spectacular display of organized activity by the cytoskeleton, along such lines as the positioning of the mitotic spindle [Shaw et al., 1997], the arrangement of microtubules in the spindle [Purohit et al., 1999], centriole function [Marshall and Rosenbaum, 1999], or the role of kinetochores in attaching chromosomes to spindle microtubules [Rieder and Salmon, 1998; Maney et al., 2000].

LIFE (AND PERHAPS MORE) HANGS ON DELICATE THREADS

Today we may be witnessing the rise of a revised version of solidism that would probably meet Baglivi’s enthusiastic approval. The cytoskeleton, first seen as a neat arrangement of threads inside cells, then hypothesized as a definite distribution of proteins needed to maintain an ordered spatial organization within the cytoplasm, and subsequently understood as the machinery involved in nearly every cell movement, is in fact all of these things and much more. Recent data indicate that the cytoskeleton acts as an integrated system that generates, transmits, and senses mechanical events for the regulation of a variety of cell functions [see Ingber, 1997; Chicurel et al., 1998]. Tension developed by actin-myosin networks fastened to the plasma membrane appar-
ently exerts a constant pressure upon the elastic scaffold of microtubules and intermediate filaments, which in turn communicates the resultant stress to the nucleus and other internal structures. This influence, either at equilibrium or unbalanced by transient changes in response to stimuli and other circumstances, may modulate gene expression and, thus, the production of specific enzymes.

Therefore, the cell is able to react integrally to external or intrinsic conditions by a coordination largely dependent on forces directed and distributed through lines composed of cytoskeletal polymers. In tissues, the mechanical forces experienced by each individual cell are transmitted through trans-membrane proteins (integrins) and the extracellular matrix to those cells immediately attached to it, so the population can keep or modify its form and function as a harmonious ensemble. Mechanical interactions of this sort may play a critical role in guiding morphogenesis, even of such complex organs as the brain and other parts of the vertebrate nervous system [van Essen, 1997].

In summary, three full centuries after microscopic fibers were first postulated as the key to life processes, and 70 years after the necessity for the existence of a cytoskeleton as an ordering substratum of living matter was appreciated, the concept emerges once again with a new significance pointing in the same direction but with much wider and farther reaching implications. Organization, i.e., the formation and coordinated function of the organs, in living beings seems to depend largely on mechanical power and movement generated or transmitted by their constitutive cells. And most of such forces and motions take place primarily over tiny fibers that were virtually denied existence or importance 100 years ago.

Now history might be getting ready to start anew still another round of its cycles in this context. Signal transmission along intracellular fibrils, an issue over which controversy raged also a century ago, is again a fascinating front of investigation. Moreover, the highly regular molecular structure of the microtubules, which are found assembled as uniform bundles throughout all nerve cells and their branchings, has been propounded as uniquely propitious for the gradual superposition of quantum coherent states that could then undergo an “objective reduction” expressed as consciousness [Hameroff, 1994; Penrose, 1994]. Just like with the theory of conducting neurofibrils, this hypothesis has been seriously challenged [Grush and Churchland, 1995] and the critique refuted with matching alacrity [Penrose and Hameroff, 1995; Hameroff and Penrose, 1996].

Even if mind turns out not to reside in the neuronal microtubules, research on the cytoskeleton has clearly much in store to deal with for the forthcoming millennium. Given the tenacious recurrence of themes in the field, an occasional glance at previous developments might be instructive and perhaps even illuminating.

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