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The Migrating Thermodynamic Quantum Hypothesis for Cytoplasmic Streaming, Sodium Pumping and Other Cell Biological Phenomena, Deduced from Biofunctional Considerations of the Ultrastructure of Brush Border Microvilli

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ABSTRACT

An attempt is made to reconcile experimental data dealing with, *inter alia*, cytoplasmic streaming in Characean algae, contraction in actomyosin systems, Na^+- and -K^+ - stimulated ATPase activity and the ultrastructure of brush border microvilli. It is postulated that myosin molecules transfer energy from ATP to an actin-containing filament and that a high energy conformation is subsequently propagated along the filament. At regularly spaced intervals corresponding to the length of an actin-tropomyosin subunit, the propagation of high energy involves rejection of a pressure pulse in the direction of cytoplasmic streaming. Proteins in solution capable of storing the thermodynamic energy represented by the pressure pulse will either migrate in the opposite direction or conserve the quantized cytoplasmic flow generated by the actin-containing filaments. At sites where actin filaments are attached to the plasma membrane the high energy is propagated in another direction leading to expulsion of sodium ions and neutralization of the vectorial pressure pulse.

INTRODUCTION

In 1956–57 (9, 12) it was discovered by darkfield microscopy that protoplasma droplets from *Characeae* contain fibrils, rapidly moving forward or in circle, longitudinally dissociating and associating. The multiply associated state was also observed to display undulating movement. Cytoplasmic particles that came close to these fibrils were captured and without Brownian motion translocated in the opposite direction and it was therefore concluded that the observed fibrils are responsible for cytoplasmic streaming in this and other species (9, 10, 12.) The moving fibrils generating cytoplasmic streaming have recently been shown by decoration (22) with myosin to contain F-actin oriented as if able to pull myosin filaments in the direction of the cytoplasmic flow (14).

During recent years, actin and actin-like proteins have been found in a number of nonmuscle cells (4, 15, 17, 19, 24, 28, 32), including bacteria (21), and their interaction with myosin from distant unrelated species suggests that actin has maintained its original molecular properties during the course of evolution pointing to a general function not restricted to cytoplasmic streaming or muscular contraction (15). In cases where a transmembraneous gradient of sodium is suggested to play a main role in a physiological function, such as propagation of action potentials in nerve cells and sodium-dependent transport of certain metabolites in intestinal epithelial cells (25), actin-containing filaments are found in close association with the plasma membrane (19, 24). Electron microscopic investigations of nerve cell axones (19) and brush border microvilli (24) indicate that the actin-containing filaments may be involved in excitation (19) and in functional cooperation with proteins in the plasma membrane of brush border microvilli. In the latter case this is because the attachment sites are regularly spaced at a distance that corresponds to the length of an actin-tropomyosin subunit (16, 22, 24). Involvement of actin-containing filaments in plasma membrane phenomena is also suggested by the findings that release from "contact inhibition" of cultured cells may be accompanied both by a decreased transmembraneous potential (8) and a diminished association of actin-containing fila-

ments to the plasma membrane (17). Furhter, a mutant strain of *E. coli* lacking the ability to accumulate potassium from a potassium-depleted medium is characterized by a mutant actin that fails to polymerize at that particular potassium concentration (21). When taken together, these and other findings suggest that sub-plasmamembraneous actin-containing filaments may be part of phenomena involved in generating a transmembraneous electrochemical gradient of sodium and potassium without reference to the mechanism of this involvement.

The polarity of the actin-containing filaments in brush border microvilli (see Fig. 1) has recently been determined by decoration (22) with myosin subfragment 1, by means of immunologic localization of α-actinin predominantly in the distal tips of the microvilli and by ultrastructural evidence for myosin filaments in the proximal terminal web (24). The diverging observations on microvillar contraction, which has been described as either shortening or undulating (cf: 29) movement, could be unified by assuming that actin-containing filaments behave in brush border microvilli as they do in protoplasma droplets from *Characeae (cf:* 9, 10, 12) carrying with them a cytoplasmic flow. The direction of this cytoplasmic flow can be deduced from the polarity of the actin-containing filaments in brush border microvilli by analogy with the corresponding filaments in *Characeae* (14). This deduction is complicated by the fact that the mechanism generating cytoplasmic streaming must be inhibited, or directed towards other functions, along the entire length of at least some actin-containing filaments, in order to ascertain restreaming into the cell.

Evidence has been obtained that transport of certain amino acids and sugars against a gradient into brush border microvilli is dependent on co-migration of sodium (25), a phenomenon that is a fundamental principle for the transport of many metabolites into different types of cells (3). In order to ascertain restreaming in brush border microvilli it will therefore be postulated in the present hypothesis that the mechanism generating cytoplasmic streaming is used in the plasma membrane for generating a transmembraneous gradient of sodium. The direction of the cytoplasmic flow that will result from this assumption, is illustrated in Fig. 1 B. Actin-containing filaments are attached to the plasma membrane in regularly spaced intervals at a distance that corresponds to the length of one actin-tropomyosin subunit (24) indicating functional cooperation between the filaments and membraneous proteins. Since no other binding sites have been observed it will be assumed in this hypothesis that both cytoplasmic streaming and sodium expulsion are governed from these regularly spaced intervals. An additional function of the observed attachement-sites (24) is probably that their presence counteract the propulsion of the actin core into the cell interior that would occur in their absence as a result of cytoplasmic streaming in the distal direction.

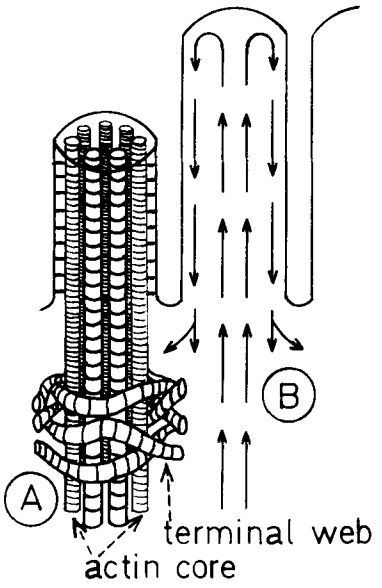

Fig. 1. Schematic illustration of the anatomic structure of a cut microvillus (A, *cf.*: 24) and the direction of cytoplasmic streaming deduced by analogy with Characean algae (B, *cf.*: 14).

When brush border microvilli are demembranated by treatment with Triton-X 100 (23) the subsequent addition of Ca^{++} and ATP results in propulsion of the actin core into the cell interior (23). This finding does not support that the sliding filament model for muscular contraction (7,

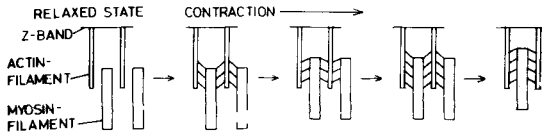

Fig. 2. Schematic illustration of the sliding filament model according to which contraction of skeletal muscle is mediated by active translocation of myosin filaments along the actin-containing filaments by a mechanism that implies ATPase activity for every translocation step past an actin dimer. (*cf.*: 7).

see Fig. 2) is applicable to contraction of brush border microvilli, since no myosin was reported to be located along the axis of propulsion (23) or is even found in the vicinity of the villous part of the actin core in contracted intact brush border microvilli preparations (29), necessitating the postulation of invisible myosin (29). Filaments reminiscent of striated muscle myosin are instead located perpendicular to the actin core in the underlying terminal web (24) initiating the shortening and undulating movement of microvilli at distance. They apparently do not migrate distally towards the α-actinin-containing tips of microvilli (corresponding to the Z-line of muscle (24)) as they should do according to the sliding filament model for muscular contraction. An alternative interpretation of myosin action is also required in view of failures of the sliding filament model to explain contraction in actomyosin preparations lacking myosin filaments (6) and in regenerated actomyosin containing conversely oriented actin filaments (6). Further, the excess of actin in comparison with myosin in many non-muscle cells (15) and the mechanochemical coupling in actomyosin systems catalyzed by globular myosin subfragments (27), evoke the suspicion that the sliding filament model is not generally applicable to contractile phemomena (15). This model has also been criticized on the basis of ultrastructural observations on contracted striated muscle (30).

It is very difficult to devise a mechanism by which the sliding filament model for muscular contraction can explain the cytoplasmic streaming in brush border microvilli that is deduced from their shortening or undulating (*cf.*: 29) movement by analogy with the movement of the corresponding fibrils in protoplasma droplets from *Characeae* (*cf.*: 9, 12). Since this model implies that myosin filaments sometimes return to a relaxed position it also implies that cytoplasmic streaming should be reversed at times contrary to what is known about this phenomenon in other systems (9, 10, 12, 14). If the sliding filament model is modified by assuming that myosin molecules of the myosin filaments upon reaching the α-actinin-containing distal tips of microvilli are detached and subsequently passively transported into the cell, other questions still remain unanswered. These questions relate to energetic considerations dealing with the considerable amounts of ATP that must be transported to and consumed in microvilli in order to mediate on one hand the myosin ATPase catalyzed translocation of myosin (7) and on the other hand the Na^+-and-K^+-stimulated ATPase-catalyzed (3, 20) generation of a transmembraneous electrochemical gradient of sodium and potassium. The sliding filament (7), the screwing filament (11) and the undulating filament (31) hypotheses are all unable to provide any physiological function to the observed contraction of brush border microvilli. The reason for this is that if any of these hypotheses is adapted, too complex and multiple interactions would be necessary in the binding of the filaments to the plasma membrane. In addition, any active mechanism for the assumed cytoplasmic straming in brush border microvilli would yield energy-requiring frictional flow.

One experimental fundament for the hypothesis that will be presented here is the original observation that cytoplasmic particles move closely along the protoplasmic filaments of Characean algae without Brownian motion (9, 12). Only a mechanism capable to non-randomize and vectorize (structurize) this random motion can thus be assumed to be responsible for cytoplasmic streaming. Any active mechanism such as the myosin ATPase catalyzed sliding of filaments (7), propelling of filaments (11), or undulation of filaments (31) must accordingly be rejected because it would on the contrary enhance Brownian motion in the vicinity of these filaments.

Thermal motion of solvent molecules can be absorbed by proteins in small amounts resulting in *e.g.* strained bond angles (*cf.*:5). It is postulated in the present hypothesis that increased utilization of similar or other possibilities of storing thermodynamic energy results in a defined conformation of a protein. By further postulating that this energy-storing conformation of a protein in one single step reverts to a low energy conformation a possibility is created to structurize the Brownian motion of the surrounding solvent molecules. Since the thermodynamic energy is rejected as a pressure pulse when used for generating cytoplamic streaming it must be assumed that a "condensed" form of a protein equivalents its energy storing, low entropy conformation. This idea is compatible with calculations indicating that all components of a protein do not oscillate simultaneously in all degrees of freedom of motion of the protein (26). A protein oscillating between two main conformations may be considered to have one main degree of freedom of motion, at least represented by the difference of entropy between the "condensed" and the "loose" form.

According to the present hypothesis, the one step release of thermodynamic energy from the low entropy conformation of a protein (or proteins) in the actin-containing filaments into the surrounding medium is brought about by the transfer to this conformation of the energy residing in a high energetic phosphate bond in an adjacent protein of the actin-containing filament. By this assumption a concept of propagating high energy equivalent to a phosphate bond in the actin-containing filament is introduced, which may explain why myosin apparently effects contraction of brush border microvilli at distance and why ATP enhances cytoplasmic streaming in several other cells (12). This idea is not contradicted by recent reports that in actin, high energy may be stored in two interchangeable forms, bound ATP and a labile conformation of protein (16). Furthermore the idea is compatible with considerations indicating that the nucleotide-binding region of actin is located in a β-pleated sheet (16), because this conformation of a polypeptide would be the most efficient in generating a pressure pulse. Since a low entropy conformation of a protein imposed by absorption of thermodynamic energy from the surrounding medium is labile and tends to reject this energy the postulation of such a conformation may be a requirement for the concept of a unidirectional propagation of high energy pulses which will be "pulled" by the thermodynamically labile conformation. It is further assumed in this hypothesis that a thermodynamically labile conformation of a protein that in addition binds phosphate in a high energetic bond tends to "push" the phosphate bond to adjacent proteins. It is inherent in the hypothesis that is outlined here that absorption of thermodynamic energy resulting in a defined low entropy conformation would confer on the propagating high energy pulse the possibility of an equivalent active physiological function at equivalent sites along the actin-containing filaments.

THE HYPOTHESIS

It is postulated that components of the actin-containing filaments formed *in vivo* can exist in two low energy conformations, L_2, L_1 and two

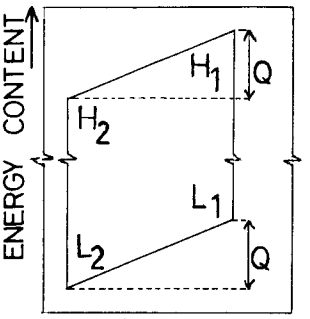

Fig. 3. Energy content of proteins that are of the same magnitude of size as the proteins constituting the actin-containing filaments. (L_1—L_2) is equivalent to (H_1—H_2) and defines the energy content of a protein that is exchangeable with the surrounding medium as one thermodynamic quantum (Q), that is, a single pressure pulse or a defined region of increased thermal motion of solvent molecules. The capital H denotes a high energy content derived from and exchangeable with a terminal phosphate bond of ATP. This high energy may reside in a protein in the form of a high energetic phosphate bond or an unstable conformation of a protein (*cf.*: 16). The capital L denotes that a protein is lacking this particular form of high energy content. The lines in this figure denote possible but not obligate energy transitions of a protein submitted to the postulates of this hypothesis.

high energy conformations, H_2, H_1 (see Fig. 3 and page 209 for explanation of the notations used). The energetic difference Q (=one thermodynamic quantum) between L_2, H_2 and L_1, H_1, respectively, is equal and exchangeable with the surrounding medium as described by the following reactions:

$$L_{2p} + Q_s \leftrightarrow L_{1p} \quad (1)$$

$$H_{2p} + Q_s \leftrightarrow H_{2p} \quad (2)$$

The thermodynamic quantum is generated by the actin-containing filaments as a mainly unidirectional cytoplasmic pressure pulse which is equivalent to a defined region of increased thermal motion of cytoplasmic solvent molecules. The energetic difference between L_2, L_1 and H_2, H_1, respectively is comparable to the energy content of the terminal phosphate bond of ATP. The main function of myosin is to transfer energy from ATP to the actin-containing filaments which as reflected by their polarity have the capability of propagating this energy in one direction. The unidirectional propagation of high energy is a requirement for the generation of thermodynamic quanta. Mainly two combinations of energy content in adjacent proteins of the actin-containing filament are unstable and responsible for the unidirectional propagation of high energy as described by the following reactions:

$$H_2\,(X)L_1\,(Y) \rightarrow L_2\,(X)H_2\,(Y) + Q_s \quad (3)$$

$$H_1\,(X)L_2\,(Y) \rightarrow L_1\,(X)H_2\,(Y) \quad (4)$$

These reactions have a physiological significance at the junction between two adjacent actin-tropomyosin subunits where they are involved in either the generation of cytoplasmic streaming or sodium carrier function. Cytoplasmic streaming is generated as illustrated in Fig. 4 and can be described by reaction (3) in the following form (see Fig 4 B-C):

$$H_2\,(DI)L_1\,(PII) \rightarrow L_2\,(DI)H_2\,(PII) + \vec{Q}_s \quad (5)$$

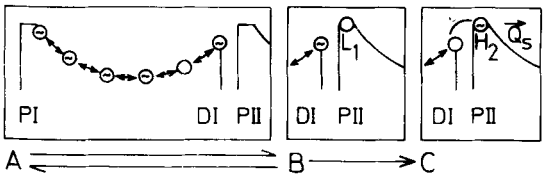

Fig. 4. A: schematic illustration of an actin-tropomyosin subunit. The equilibrium arrows indicate that the propagation of high energy in the intermediate part of the subunit not necessarily is unidirectional. B to C illustrate reaction (5). The notations used are explained on page 209.

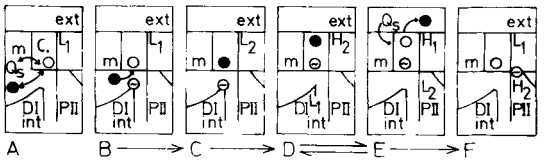

Fig. 5. Schematic illustration of sodium pumping from a sodium-depleted (int) medium across a plasma membrane (m) to a sodium-enriched medium without reference to the possible occurrence of proteins linking the carrier and the actin-containing filaments together. Filled circles represent sodium ions and empty circles represent carrier sites. The translocation of the carrier sites as illustrated in this figure only indicate differential accessability to either side of the membrane without any preference of molecular mechanism for this phenomenon. A illustrate reaction (6). B to C illustrate reaction (7). C to D illustrate reaction (8). D and E illustrate reaction (9). E to F illustrate reaction (10). The notations used are explained on page 209.

In order to ascertain that the propagating high energy pulse does not give rise to any pressure pulse when transferred from a sodium carrier molecule to PII it must be assumed that reaction (2) is involved in this transfer. It is further postulated that association and dissociation of sodium to the carrier sites are equilibrium reactions and that each energy content according to Fig. 3 of the carrier is preferentially represented by a sodium-associated or -dissociated carrier molecule exposing its carrier sites to either a sodium-depleted or sodium-enriched medium. By applying reactions (1) to (4), the consequences of these postulates for the sequential mode of action of a sodium carrier molecule will be described by the following reactions (see Fig. 5, and appendix for deduction).

$$Na^+_{int} + L_1\,(C) \rightleftarrows L_2\,(C, Na^+_{int}) + Q_s \quad (6)$$

$$L_1\,(C) + Na^+_{int} \rightarrow L_2\,(C, Na^+_{int}) + Q_s \quad (7)$$

$$H_1\,(DI)L_2\,(C, Na^+_{int}) \rightarrow L_1\,(DI)H_2\,(C, Na^+_{ext}) \quad (8)$$

$$H_2\,(C, Na^+_{ext}) + Q_s \rightleftarrows H_1\,(C) + Na^+_{ext} \quad (9)$$

$$H_1\,(C)L_2\,(PII) \rightarrow L_1\,(C)H_2\,(PII) \quad (10)$$

By the reactions (7) to (10) one cycle of the carrier is completed leading to expulsion of sodium ions and the propagation of one high energy pulse in the distal direction. In order to ascertain a sodium carrier function it must be assumed that the high energy pulse is preferentially transferred to a carrier which is associated with sodium as described in reaction (8). This property is inherent in the sequence of reactions that have been described in that the high energy pulse leaves the carrier in a sodium-dissociated L_1 conformation (reaction (10)) which according to reaction (7) (see appendix for interpretation) has a high affinity for sodium.

Since $L_{1(C)}$ is unstable, efficient pumping of potassium would be impossible if this ion were to associate with an L_1 conformation of the carrier in a potassium depleted external medium. By making the same postulates as were made for sodium, relating to different conformations of the carrier, the following transitions will operate in potassium pumping, in the sequence corresponding to the reactions (6) to (9): (see appendix for additional evidence).

$$L_1\,(C, K^+_{int}) \overset{(6b)}{\underset{Q_s, K^+_{int}}{\rightleftarrows}} L_2\,(C) \overset{(8b)}{\rightarrow} H_2\,(C) \overset{(9b)}{\underset{Q_s, K^+_{ext}}{\leftrightarrows}} H_1\,(C, K^+_{ext})$$

Since ouabain-binding is involved in reaction (9b) (see appendix) electrogenic action of the carrier is ascertained in the present hypothesis by competition of endogenous ouabain (1, 18, 35) with the potassium-binding sites when these are exposed to the external side of the plasma membrane. This view is further supported by the finding that high external potassium concentrations result in a diminished transmembraneous potential (32).

The "field" of migrating thermodynamic quanta generated by the actin-containing filaments will induce unusual properties of the surrounding medium as exemplified by the behaviour of proteins in solution that are capable of oscillating in harmony with the quantized flow. According to Boltzmanns law, $S=k \ln \Omega$ (*cf.*: 5), highly ordered states are less probable, and therefore accumulation of either high entropy forms or low entropy forms in adjacent coordinates (x-1), (x), (x+1) of the field is not favoured. Some consequences of this statement for proteins in solution, capable of storing the energy represented by Q are described by the following reactions where the sequence of notations indicate adjacent location in the direction of quantized cytoplasmic flow.

$$(Q_s + L_{2p}) - \vec{Q}_s \rightarrow L_{1p} - \vec{Q}_s \qquad (11)$$

$$(L_{1p} - \vec{Q}_s) \rightarrow L_{2p} \qquad (12)$$

$$\vec{Q}_s + (L_{1p} - Q_s) \rightarrow \vec{Q}_s + L_{2p} \qquad (13)$$

$$(\vec{Q}_s + L_{2p}) \rightarrow L_{1p} \qquad (14)$$

These reactions are all unidirectional because they occur in a field of migrating thermodynamic quanta. Fusion of the energy components in parenthesis in reaction (11) to (14) is either accompanied by migration of the protein in the backward direction or by extension of the quantized field.

DISCUSSION

According to this hypothesis ATP enhances cytoplasmic streaming (12) by increasing the number of high energy pulses per filament length while filamentous myosin probably influences cytoplasmic streaming by increasing synchrony in the quantized field surrounding the actin-containing filaments. A tendency of the protoplasmic fibrils of *Characeae* to join in circles (9, 12) is explained partly from considerations of fuel economy since only thermodynamic energy would be required to maintain the rotation of such circles provided the propagation of high energy pulses is unrestricted.

There are profound energetic differences between on one hand the present hypothesis and on the other hand the sliding filament hypothesis for myosin-actin interaction (7) and the Na^+ - and - K^+ -stimulated ATPase hypothesis for generation of a transmembraneous sodium gradient (*cf.*: 1, 3). These differences are illustrated by the following calculations which are based on an estimated number of 50 actin-tropomyosin subunits, (*cf.*: 24)

each containing 13 actin monomers (22) in their turn constituting spheres with a diameter of 55 Å (22), in one actin-containing filament along the entire length of one microvillus. According to the present hypothesis the hydrolysis of 2 ATP molecules optimally results in 50 cycles of the sodium carrier, and depending on the degree of quantization and on the magnitude of the oscillations of the proteins involved in generating a pressure pulse, results in a cytoplasmic translocation (flow) perhaps in the range of 10 Å to 50x10 Å. The latter estimation is based on the difference of width of the interchain cleft between tetrameric oxy- and deoxyhemoglobin (MW: 64 500) which is 7 Å (29) and which may be representative of the magnitude of oscillations of a protein of approximately that molecular weight (MW_{actin}: 42000). On the contrary, in order to bring about a comparable physiological effect according to the sliding filament and the Na^+-and-K^+-stimulated ATPase hypotheses, 10Å/55Å (0.2) to 50 x 10/55 (9.1) ATP molecules (see text to Fig. 3 and ref. 7) and 50 ATP molecules, respectively, are hydrolyzed (*cf.*: 7).

The present hypothesis does not *contradict* the idea that a Na^+-and-K^+-stimulated ATPase molecule is responsible for generation of an electrochemical gradient, but extends this idea in that it includes a mechanism for the reutilization of high energetic phosphate bonds. This considerably increases the efficiency for generation of an electrochemical gradient of sodium and potassium. It is notable that when the sequential mode of action of the carrier is deduced from the postulates of this hypothesis one "solution" (see appendix) is compatible with several experimental data. In addition this solution is also compatible with fundamental thermodynamic principles in that a low entropy form of the carrier is associated with potassium and dissociated from sodium on the potassium-depleted and sodium-enriched side of the membrane. This is a less probable state and should represent lower entropy according to Boltzmanns law $S = k \ln\Omega$. A high affinity for internal sodium is also accounted for in the present hypothesis since the low entropy form, L_1, is more unstable when adjacent to a high energetic phosphate bond in accordance with the same fundamental thermodynamic principles.

The finding that mitochondria may be located in horizontal planes at the level of the Z-band of striated muscle (2) is compatible with the postulate of the present hypothesis that high energetic phosphate bonds propagate towards regions enriched in α-actinin when combined with the generally held view that mitochondria are found where they are best needed. Since the propagating high energy would leave the filament at this site, low energetic phosphate metabolites would accumulate resulting in positive chemotaxis of mitochondria.

In skeletal muscle, the length of an actin-tropomyosin subunit determined by X-ray diffraction is approximately 365 Å (7) and the intercrosslink distance of equivalent spatial orientation between actin and myosin filaments is 429 Å (7). This difference of length is interpreted in the present hypothesis to indicate that myosin is arranged in the myofilaments so to, as far as possible, avoid contact with equivalent sites on the actin-containing filaments. This arrangement would guarantee, firstly minimal interference with the quantum flow generating mechanism located between adjacent actin-tropomyosin subunits, and secondly, transfer of ATP from myosin to the actin-containing filaments irrespective of their spatial orientation and the degree of shortening. This application of the present hypothesis is, in contrast with the sliding filament model for muscular contraction, compatible with the repetedly demonstrated fact that ATP weakens the interaction between myosin and actin (*cf.*: 22).

The contraction of regenerated actomyosin containing conversely oriented actin filaments (6) is explained by reactions (11) to (14) according to which proteins capable of storing the energy represented by Q are able to migrate opposite to the quantized flow generated by the actin-containing filaments. These reactions imply that conversely oriented actin-containing filaments slide past each other inside the regenerated actomyosin threads (*cf.*: 6) where the degree of quantization is high. The reassembly of myosin filaments simul-

taneously with contraction in regenerated actomyosin (6) and the tendency of myosin molecules to join in the A-band of striated muscle proximal (see notations, page 209) to the actin-containing filaments is explained by postulating that myosin molecules are capable of storing the energy represented by Q. By this assumption the occurrence of myosin-like filaments in the I-band proximal to the Z-band (30) and the apparent migration of material from the I-band to the A-band during contraction (30) would also be explained.

The present hypothesis gives an alternative explanation to the phenomenon that the ATP level remains unchanged after contraction in skeletal muscle (*cf.*: 18). It is further suggested that creatine and phosphocreatine interconversion constitute a mechanism for recycling of the high energy pulse when it leaves the distal ends of the actin-containing filaments. The main effect of ATP hydrolysis in muscular contraction would consequently be to supply energy in the form of thermal motion of cytoplasmic solvent molecules. Since no possibilities for restreaming close to the Z-band of the sarcomere of skeletal muscle can be discerned the propagation of high energy pulses along the actin-containing filaments in this system only results in the establishment of a quantized field without accompanying quantized cytoplasmic flow. Contraction of skeletal muscle will therefore be described by the following reaction:

$$H_{2(DI)}L_{1(PII)(x)} \rightarrow L_{2(DI)}H_{2(PII)(x-1)} \qquad (15)$$

The ubiquity of actin and actin-like proteins in animal and plant kingdoms (*cf.*:15) suggests that the evolution of many proteins has occurred under the influence of the quantum-generating mechanism described here. These conditions would counteract the establishment of protein mutants that do not oscillate in harmony with the quantum flow generated by actin-containing filaments, because the presence of such "distorsion" molecules would render impossible the optimal function of cellular enzymes, adapted to a quantized environment millions of years ago. Experimental support for this assumption is again found in electron-microscopic studies on brush border microvilli. In some of these studies the actin-core supposed to carry cytoplasmic flow in the distal direction apparently originates in the cell body (29). Since cytoplasmic enzymes are of the same size as the proteins constituting the actin core they would tend to obstruct the flow if they were to follow with it. However, the reaction (11) implies that proteins oscillating in harmony with the quantized flow are either stationary or translocated in the opposite direction. Entrance into microvilli of cytoplasmic enzymes adapted to a quantized flow would then be counteracted by both the central actin core and the terminal web, a network of actin-containing (29) quantum-generating filaments situated in the distal part of the cell but proximal to microvilli (see Fig. 1).

These considerations thus seem to be compatible with the view that actin-containing filaments in generating thermodynamic quanta constitute a pace-maker for cellular life and evolution and profoundly influence the behaviour of most cellular proteins.

SUMMARY STATEMENTS

A hypothesis for cell biological phenomena is presented according to which:

1. "Condensed", low entropy forms of a protein are capable of generating a pressure pulse when reverting in one single step to high entropy forms.

2. The actin-containing filaments generate vectorial pressure pulses each representing a defined thermodynamic energy content, thus explaining cytoplasmic streaming.

3. Proteins of actin-containing filaments generate vectorial pressure pulses in connection with the vectorial propagation of protein-bound high energy pulses along the filament. These phenomena are mutually dependent.

4. The evolution of many proteins has occurred under the influence of the quantum flow generated

by the actin-containing filaments, making them capable of oscillating in a quantized field, storing and rejecting the thermodynamic energy represented by the pressure pulse.

5. The protein(s) responsible for generation of a transmembraneous gradient of sodium and potassium is (are) in this sence adapted to a quantized environment.

6. A sequential mode of action of a sodium and potassium carrier molecule can be deduced from the postulates of the hypothesis. One of four solutions in this deduction is compatible with experimental data and fundamental thermodynamic principles.

8. A physiological significance is ascribed to the contraction of brush border microvilli. Concominantly with contraction, sodium ions are pumped out of the cell thus creating an electrochemical gradient which is necessary for sodium-dependent transport of certain metabolites across the microvillous plasma membrane. When reaching the intracellular space the transported metabolites are carried further into the cell by a submembraneous cytoplasmic flow in the microvilli.

9. The high energetic phosphate bond of ATP is reutilized several times for sodium pumping when propagated along an actin-containing filament bound to carriers in the plasma membrane, thus considerably increasing the efficiency of the sodium- and potassium-pumping mechanism.

10. The main effect of ATP hydrolysis in muscular contraction is to supply energy in the form of thermal motion of cytoplasmic solvent molecules.

NOTATIONS

L_1, L_2, H_1, and H_2: energy content of proteins as defined in Fig. 3.

The sequence of these denotions indicate the vectorial propagation of a high energy pulse.

Q: The thermodynamic quantum, that is, the energy differnce ($L_1 - L_2$) rejected as a pressure pulse from actin-containing filaments in connection with the unidirectional propagation of high energy residing in a labile phosphate bond of ATP.

-Q: The energy difference ($L_2 - L_1$)

Θ: A sign denoting energy comparable to and exchangeable with the energy residing in a terminal phosphate bond of ATP.

p: Index denoting the energy that resides in protein

s: Index denoting the energy that resides in solution

(x-1), (x) and (x+1): Indices occurring after p and s and denoting coordinates in a field om migrating thermodynamic quanta in the direction of quantized cytoplasmic streaming.

→: Migration of energy in the direction of quantized cytoplasmic streaming (= forward, distal)

←: Migration of energy in the direction opposite to quantized cytoplasmic streaming (=backward, proximal)

P: Proximal part (in the direction of quantized cytoplasmic streaming) of an actin-tropmyosin subunit. The part of an actin-containing filament located immediately distal to a binding site of the filament to the plasma membrane in brush border microvilli.

D: Distal part (in the direction of quantized cytoplasmic streaming) of an actin-tropomyosin subunit. The part of an actin-containing filament located immediately proximal to a binding site of the filament to the plasma membrane in brush border microvilli.

I: The intermediate part of an actin-tropomyosin subunit.

C: Sodium and potassium carrier molecule located in a biological membrane.

(X), (Y): Indices denoting location of energy or ions.

I, II: Sequence of actin-tropomyosin subunits in

the direction of quantized cytoplasmic streaming.

(C,Na^+_{int}), (C,Na^+_{ext}): indices denoting association of ions to the carrier sites when exposed to the sodium-depleted (int) or the sodium-enriched (ext) side of a membrane.

APPENDIX

The reactions (6) to (10) are deduced as follows.

Since $H_{1(C)}L_{2(PII)} \rightarrow L_{1(C)}H_{2(PII)}$ is the final reaction, the sequence of transitions of the carrier must be $L_2 \rightarrow H_2 \rightarrow H_1 \rightarrow L_1$. Since adsorption of sodium is an equilibrium reaction $H_{2(C)} \rightarrow H_{1(C)}$ is either accompanied by adsorption of Na^+_{int} or dislocation of Na^+_{ext}. In the former case $L_{1(C)}$ is associated with Na^+_{ext} and $L_{1(C)} \rightarrow L_{2(C)}$ or $H_2L_{1(C)} \rightarrow L_2H_{2(C)}$ is necessary for its dislocation. $L_{1(C,Na^+_{ext})}$ is thus on average thermodynamically unstable. On the contrary, when $H_{2(C)} \rightarrow H_{1(C)}$ is accompanied by dislocation of $Na^+_{ext(C)}$, $L_{1(C)}$ is an interior sodium-dissociated form. The thermodynamically spontaneous transition $H_2L_{1(C)} \rightarrow L_2H_{2(C)}$ is then impossible because $H_{2(C)}$ is associated with Na^+_{ext}. On average $L_{1(C)}$ will therefore be unstable increasing the affinity of the carrier for sodium when the carrier sites are exposed to the cell interior. This sequential mode of action of the sodium carrier is illustrated in reactions (6) to (10) because it is compatible with several experimental findings and interpretations made by researchers in this field as given by the following statements (1): 1. A single cation carrier has an absolute requirement for Na^+ to go to its "activated" form. 2. External ATP is not hydrolyzed. 3. The temperature dependent step is neither the phosphorylation nor the dephosphorylation step (reaction (8) and (10)). 4. Carriers have inwardly oriented cation sites of high affinity for Na^+.

When a corresponding deduction is made for potassium pumping, one "solution" is rejected because in that case the L_1 conformation is labile, making potassium accumulation from a potassium depleted medium improbable. This sequence, $L_{1(C)} \rightarrow L_{2(C,Na^+_{int}\,K^+_{ext})} \rightarrow H_{2(C,Na^+_{ext}\,K^+_{int})} \rightleftarrows H_{1(C)}$ is also improbable according to Boltzmanns law, $S = k \ln \Omega$, which implies that highly ordered states are not favoured. The other solution which is illustrated in the text as the sequence (6b) to (9b) is compatible with *inter alia* the following experimental findings and interpretations (1): 1. The cation carrier can return to its inactivated state either as a free carrier or in combination with K^+. 2. K^+ but not Na^+ decreases labeling with ^{32}P from $AT^{32}P$ at low temperatures (reaction (6), (6b), (8) and (8b)). In this case K^+ but not Na^+ stabilizes $L_{1(C)}$, and the terminal phosphate bond is looked upon as corresponding to $H_{1(DI)}$.).

Oubain competitively inhibits potassium-binding (18, 35) when the carrier sites for this ion are exposed to the external side of the plasma membrane (18) indicating that oubain-binding is involved in reaction (9b). This is compatible with experimental data indicating that oubain binds to a phosphorylated form of the enzyme (34).

ADDENDUM

Possible application of the present hypothesis to anabolic phosphorylation of ADP

Each actin-tropomyosin subunit in an actin-containing filament is considered to represent a functional unit attached to a sodium carrier molecule in the plasma membrane. When the concentration of Na^+_{int} is high and Na^+_{ext} is low, the $L_{2(c)}$ and $H_{1(C)}$ conformations are favoured. Under these conditions, a high ATP/ADP ratio will favour the generation of a transmembraneous gradient because of imposed unidirectional components for transitions of the carrier as illustrated by the sequence $L_{2(C)} \overset{ATP}{\rightarrow} H_{2(C)} \underset{Na^+}{\rightleftarrows} H_{1(C)} \rightarrow L_{1(C)} \overset{Na^+}{\rightarrow} L_{2(C)}$. On the contrary, if Na^+_{ext} is high and Na^+_{int} is low, the $H_{1(C)}$ and $L_{1(C)}$ conformations will be stabilized. Under these conditions, a low ATP/ADP ratio will favour the reverse action of the carrier as illustrated by another sequence of transitions, $L_{1(C)} \overset{ADP+P_I}{\rightarrow} H_{1(C)} \overset{Na^+}{\rightarrow} H_{2(C)} \rightarrow L_{2(C)} \underset{Na^+}{\rightleftarrows} L_{1(C)}$. The actin-tropomyosin subunit may thus be regarded as a coupling factor (*cf.*: 18), the main function of which could be to bind ADP and ortophosphate in an optimal spatial relationship

to the carrier. When the carrier associated with a coupling factor is operating in reverse due to a high transmembraneous gradient of the transported ions, ATP will be formed from ADP and ortophosphate. These functional considerations support the idea derived from structural similarities (16) that an actin-tropomyosin subunit is equivalent to the coupling factors of mitochondria (and chloroplasts). In both cases ATP is formed simultaneously with the reflux of ions participating in a transmembraneous gradient. These ions are evidently sodium (and potassium) in the plasma membrane and protons in the mitochondrial membrane (*cf.*: chemiosmotic hypothesis for oxidative phosphorylation, 18).

In addition to the already established photophosphorylation and the oxidative phosphorylation of ADP the present hypothesis is theoretically compatible with a third mechanism for generating ATP. This mechanism which may be denoted thermodynamic phosphorylation relates to the well-known tendency of actin filaments to depolymerize at high potassium concentrations (*cf.*: 6). Thermodynamic phosphorylation implies that potassium is accumulated and sodium pumped out of a cell by means of a high energy pulse propagating along an actin filament. When a critical potassium concentration is reached the actin filaments are enzymatically depolymerized to single actin-tropomyosin subunits, still attached to the plasma membrane. The sodium-potassium gradient is then used for generation of ATP by the reverse action of the carrier molecules with their associated actin-tropomyosin coupling factors. At the critical potassium concentration both polymerized and depolymerized actin-tropomyosin filaments may coexist and these mechanisms may be operating simultaneously. This type of phosphorylation may be phylogenetically older than photophosphorylation and oxidative phosphorylation.

The above considerations may be applicable to the phenomena that tumor cells can survive in a low oxygen environment practically devoid of fuel for conventional generation of ATP (36) and that fibroblasts exposed to a low oxygen tension begin to move about (*cf.*: 12, 36).

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