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The Sarcoplasmic Calcium Pump – A Most Efficient Ion Translocating System*

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Abstract. In contrast to the sodium-potassium transporting plasma membranes, the sarcoplasmic membranes (SR) are highly specialized structures into which only two major intrinsic proteins, a calcium transporting protein and a calcium binding protein are embedded. The calcium transporting protein is a highly asymmetric molecule. It binds two calcium ions with a very high affinity at its external, and two calcium ions with low affinity at the internal section of the molecule. ATP is bound with high affinity to an external binding site, inducing a conformational change. When the vesicular membranes are exposed to solutions containing Ca^{++}, Mg^{++} and ATP, ATP is hydrolyzed and simultaneously calcium ions are translocated from the external medium into the vesicular space. When calcium ions are translocated in the opposite direction, ATP is synthesized. The calcium-ATP ratio for ATP cleavage as well as for ATP synthesis is 2. Thus, the SR membranes can transform reversibly chemical into osmotical energy. Inward and outward movements of calcium ions are relatively slow processes connected with the appearance and disappearance of different phosphorylated intermediates. One phosphorylated intermediate is formed by phosphoryltransfer from ATP when calcium ions are present in the medium. In contrast, when calcium ions are absent from the external medium, two different intermediates can be formed by the incorporation of inorganic phosphate. Only when calcium ions present in the internal space of the vesicles are released, the incorporation of inorganic phosphate gives rise to an intermediate whose phosphoryl group can be transferred to ADP.

Key words: Sarcoplasmic calcium transport – Calcium transport protein – Energy requirement of calcium transport – Stoichiometry of calcium transport – Transport intermediates.

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Performance and Properties of Ion Transport Systems: Natrium-Kalium Transport Versus SR Calcium Transport

The osmotic and ionic balance in eucaryotic cells is essentially maintained by membrane bound ion transport systems. The most important representatives are the sodium-potassium and the calcium pump. The existence of an active transport mechanism for sodium ions was first demonstrated by Krogh [27], and evidence for the involvement of potassium ions was given by Harris [13]. The coupling between the energy yielding reactions of the cell and the vectorial movement of sodium and potassium ions across the cell membrane was controversal until 1960, when Caldwell et al. [4] and Dunham and Glynn [5] demonstrated that the sodium-potassium pump is fueled by energy rich phosphate compounds. The capacity of the sodium-potassium pump is especially high in membranes of kidney, salt glands and brain. Osmoregulation on the one hand, and the maintenance of excitability on the other hand, require permanently active and intense ion transport systems.

The existence of a special active transport mechanism for calcium ions was first evidenced by Gilbert and Fenn [11] for muscle tissue. Shortly later, membrane structures able to accumulate calcium ions using ATP as energy donator were discovered in sceletal muscle by Hasselbach and Makinose [18]. These membranes which were isolated as a fraction of microsomes were soon recognized as vesicular fragments of the sarcoplasmic reticulum (SR) [14]. SR membranes are characterized by a very high degree of specialization. They are intracellular structures by which muscle activity is regulated [7, 17]. During excitation calcium is liberated from special regions of the SR and to inactivate the contractile system the liberated calcium ions must be removed quickly and completely by the elaborated membrane network surrounding the myofibrils. During muscle activity, approximately 2 μmoles $Ca^{++} \cdot s^{-1}$ have to be reabsorbed by the membranes present in 1 g of muscle. More recently, a second calcium transport system was discovered [3, 36]. It is located in the plasma membrane and exchanges calcium ions for sodium ions. The energy for calcium extrusion is furnished by the osmotic energy of the sodium gradient maintained by the Na-K pump. Since the system cannot be studied in vitro its properties will not be discussed in the present context. Still another calcium transporting system will be excluded. It is the ATP dependent calcium pump in the membranes of the erythrocyte discovered by Schatzmann [38]. Its activity is very low and preparations of erythrocyte ghosts filled with TP and calcium have to be used for its analysis.

Table 1 illustrates some features of the working conditions of the sodium-potassium and the calcium transport system in vivo. The sodium-potassium pump maintains concentration ratios in the order of 30 across the plasma membrane at relatively high absolute concentrations for both ions. Even in the resting tissue the sodium binding sites at the internal and the potassium binding sites at the external surface of the membranes are nearly fully saturated. Consequently, the pump works with full speed. Obviously, in most cells the sodium-potassium pump has to work against a considerable leak in the membranes.

The concentration ratio maintained by the sarcoplasmic calcium pump and the sodium-calcium exchange system in the plasma membrane approaches 10,000. In the resting muscle the calcium activity is very low so that the pump works with less

Table 1. Performance and properties of Na-K and SR Ca transport

Steady state	Na-K pump				SR Ca pump	
	Na^+		K^+		Ca^{++}	
	internal	external	internal	external	internal	external (myoplasma)
Concentration (mM) [17]	5[a]	140[a]	130[a]	4[a]	1–10[b]	$5 \cdot 10^{-5}$[b]
Apparent affinities at the internal Km and the external Km membrane surface (mM) [35, 8, 23]	1	110	35	0.1	$3 \cdot 10^{-1}$	$3 \cdot 10^{-4}$
Number of binding sites per transport and unit [35, 8]	3	–	2	–	2	2 (?)
Molecular weight of transport unit [24, 28, 15]	a) 96,000 b) 50,000				100,000 130,000	
Lipid requirement [21, 41, 42]	phosphatidylcholin				75 fatty acid residues/10^5 g	
ATP binding sites moles/10^5 g [24, 34]	0.4				0.3–0.7 (?)	
Phosphorylation sites moles/10^5 g [24, 31, 34]	0.7				0.3–0.7 (?)	

[a] Internal and external erythrocyte compartments
[b] Ca-concentration in the internal space of the SR and the myoplasma, respectively

than 1% of its maximum activity. In the resting muscle the backflux of calcium into the myoplasma across the SR membranes as well as across the plasma membranes is very small.

Sodium-potassium and calcium transport differ not only with respect to their affinities for the respective ions but also for the affinity of the energy furnishing substrate ATP. Furthermore, in different cells the activity of the sodium-potassium pump related to the unit surface area differs considerably. For instance, the squid axon membrane displays an activity which is 1000-fold higher than that of the erythrocyte membrane. The maximum transport activity of the sodium potassium pump and of the calcium pump of the SR membranes are of the same magnitude when related to unit membrane area. Since the purified transport enzymes are characterized by very similar maximum turnover numbers, one must conclude that in the SR and in the squid axon membrane the density of the transport units must also be very similar.

Classical objects for studying ion transport are the squid axon and suspensions of erythrocytes which allow to measure ion fluxes across the membranes and to monitor changes in the extra- and intracellular ion concentrations (c.f. 12). New

aspects emerged when methods were introduced which allowed to manipulate reproducibly the internal composition of the squid axon as well as of the erythrocyte with respect to ions and energy furnishing substrates like ATP [43]. Both preparations, however, are not well suited for studying the chemical events connected with the transport processes. Firstly, it is quite combersome to supply the cytoplasmic surface of these cells with ATP or to change the ion concentration in their internal space, and secondly, the proteins which are specifically involved in ion translocation can be isolated from both preparations only in minute quantities. Therefore, those preparations do not allow to study simultaneously transport and transport chemistry. A new approach in transport chemistry was initiated by Skou [39] who demonstrated that in membrane fragments isolated from kidney and brain an ATP cleaving enzyme was present which needs for optimal activity the simulaneous presence of sodium and potassium ions at concentrations as they are required for active sodium-potassium transport. In the following, these membranes were used to study the sodium-potassium dependent reactions leading to ATP hydrolysis. However, the various chemical events cannot be correlated to ion transport since the fragmented membranes although of spherical shape never formed tightly sealed vesicles as it is required to study ion translocation. Recent attempts to incorporate membrane fragments or solubilize membrane constituents into tight liposomes could not overcome the described difficulties [21]. Most important for the development of a coherent scheme of the sodium-potassium transport proved to be the finding that the specific and potent inhibitor of the sodium-potassium transport in living cells, ouabain, suppresses the sodium-potassium ATPase of fragmented membranes with the same effectiveness.

Structural Features of SR Calcium Pump

In contrast to the fragmented plasma membranes the isolated fragments of the SR form tightly sealed vesicles of mostly correct sideness. Isolated SR membranes are the only preparation which allows to study in vitro ion transport and its chemistry simultaneously.

Approximately 3 mg of SR protein can be isolated from 1 g of rabbit skeletal muscle which corresponds to approximately 10,000 cm^2 of membrane area. SR membranes isolated from skeletal muscle are contaminated only by traces of cytoplasmic and mitochondrial enzymes. Even the activity of the ubiquitous adenylkinase is very low. The membrane consists of 65% protein and 35% lipid [1, 16]. The most prominent lipid compound is phosphatidylcholin characterized by high content of unsaturated fatty acids [9]. The protein matrix contains two major proteins, the calcium translocase (80%) [15, 28] and a calcium precipitating protein (calsequestrin) [22]. The relevance of other minor protein constituents is questionable. The calcium translocase has two high affinity binding sites and probably two low affinity calcium binding sites which face the external and the internal space of the vesicle, respectively [8]. The presence of high affinity sites for ATP and ADP has been deduced from binding and labeling studies. For the enzymatic function of the calcium translocase, the calcium dependent splitting of ATP and probably also for the ion translocating function the translocase molecule must be embedded in a fluid lipid

matrix. It is provided by approximately 70 unsaturated fatty acids by the same number of lysolecithin molecules [41] or 35 phosphatidylcholin molecules [42] which correspond to approximately 50% of the phospholipid content of the membrane. The translocase is a highly asymmetric molecule as evidenced by its high intrinsic birefringence in the membrane perpendicular to the plane of the membrane [40].

ATP Dependent Calcium Binding Versus Calcium Transport

When ATP, magnesium and calcium ions are added to suspensions of SR membranes, calcium is rapidly removed from the solution. An essential prerequisite for calcium removal is the presence of vesicular membrane structures. When open fragments are used calcium remains in the solution. Furthermore, when the permeability of the membranes for calcium is increased either by detergents or by calcium ionophores, calcium taken up by the vesicles is rapidly released. Consequently, it can be excluded that the ATP dependent uptake of calcium is brought about by an ATP dependent binding of calcium to the membranes [29]. Calcium storage leads to an increase of the total calcium concentration inside the vesicles to approximately 10 mM.

Energy Requirement of SR Calcium Transport

However, the concentration of soluble calcium as well as its activity inside the vesicles can only be guessed because the properties and the amounts of both the internal calcium binding sites of the membrane and the calcium binding protein are not well known. When, however, the medium is supplemented with anions which can form insoluble calcium salts and can permeate through the membranes, calcium and the respective anion are taken up together and calcium precipitates are formed in the internal space of the vesicles. Naturally, the concentrations of calcium and of the anions must be adjusted to such values that their ion product does not exceed the solubility product in the solution. Under these conditions the energy required for calcium translocation can be determined at every moment. That is because after the formation of calcium precipitates inside the vesicles the activity product in the internal space is identical with the solubility product of the precipitating anions and, furthermore, the decline of the ion product in the external space can be followed analytically. At steady state the ratio of the ion products $\frac{Ca_i \cdot A_i}{Ca_0 \cdot A_0} = \frac{L}{Ca_0 \cdot A_0}$ approaches 3,000–10,000 which corresponds to an energy requirement of 4,000–5,000 calories for the uptake of 1 mol of calcium salt.

The Stoichiometry of the SR Calcium Transport

As a result of calcium precipitation the internal calcium activity remains low and constant and, therefore, the rate of calcium efflux becomes very low. During the

initial phase of calcium uptake the influx exceeds calcium efflux considerably so that the latter can be neglected. As long as net uptake of calcium occurs ATP is split concomitantly very rapidly. For every 2 calcium ions transported into the vesicles 1 molecules of ATP is hydrolyzed. This stoichiometry holds not only for ATP but also for other substrates which are consumed by the pump as ITP, other NTPs, acetylphosphate or pNPP. In agreement with the calcium transport ratio of two the dependence of calcium transport and ATP splitting on the concentration of ionized calcium is characterized by a Hill coefficient of 2 [31].

Osmochemical Energy Interconversion

The high efficiency of the accumulation process indicates that the passive efflux of calcium across the sarcoplasmic membranes must be very slow. In fact, when calcium loaded vesicles are exposed to solutions containing EGTA the observed release rates remain far below 1% of the uptake rate. Both lipids and protein contribute to this low calcium permeability. The high resistence of the lipid matrix for calcium breaks down and calcium evades rapidly when the lipid matrix is disturbed by low concentrations of detergents or when calcium ionophores are incorporated into the membrane [37]. The role of the calcium translocase molecules as a gate for evading calcium clearly emerges when Barlogie et al. [2] showed that the slow rate of release is accelerated 10—50-fold on addition of phosphate and ADP to the EGTA containing release medium. The finding that ADP together with phosphate could induce a fast calcium release indicated that the sarcoplasmic calcium pump could possibly run in the reverse direction. In fact, the ADP-P_i induced release proved to be connected with a net synthesis of ATP. For two calcium ions released one molecule of ATP is synthesized [32]. Thus, in SR membranes an energy interconversion can most easily be demonstrated and since the external and the internal concentration of calcium as well as the concentration of the reaction constituents ATP, ADP and phosphate are known the energetic conditions of the process are well defined.

Transport Intermediates

In contrast to the hydrolysis and synthesis of ATP connected with proton translocation in mitochondrial, chloroplast and bacterial membranes the nucleotid dependent movement of sodium and potassium and of calcium ions gives rise to the appearance of phosphorylated intermediates in the membrane. However, due to the inability to correlate phosphorylation and transport in the sodium-potassium transporting membranes, membrane phosphorylation has been questioned repeatedly as being a true step in the reaction sequence [44]. Only the SR calcium transport system allows to correlate directly and quantitatively ion translocation and phosphoryl transfer. With respect to the various similarities between calcium and sodium-potassium transport direct evidence obtained for the participation of a phosphorylated intermediate in calcium transport indirectly support the concept that the observed phosphorylated compounds in the sodium-potassium transporting membranes are also true transport intermediates. The transport protein in the SR membranes can be phosphorylated by

ATP as well as by inorganic phosphate [30, 33]. In the forward running mode of the pump ATP is the phosphate donor while in the reverse mode the protein accepts inorganic phosphate.

Since in the SR membranes the translocase comprises 80% of the total membrane protein the yield of phosphoprotein is very high. 3—4 nmoles of phosphate are incorporated under steady state conditions per mg of protein which corresponds to approximately one phosphorylation side in every second transport molecule. When the pump runs forward, the phosphoryl transfer reaction starts with the binding of magnesium-ATP to high affinity binding sites (Km = 3 μM) [34]. The initial step in the reverse reaction is the binding of phosphate (Km = 0.3 mM) in the presence of magnesium ions. The phosphoryl transfer from ATP is specifically activated by magnesium und calcium ions and proceeds very rapidly [10, 20]. The phosphoryl residue is either hydrolytically split or it is transferred back to ADP giving thus rise to an ATP-ADP exchange reaction [19]. Both reactions proceed only when magnesium and calcium ions are present simultaneously. The level of phosphoprotein as well as the rate of calcium translocation, the rate of ATP hydrolysis and the rate of exchange reaction exhibit an identical dependence on the free calcium concentration in the medium [31]. The parallelism between ATP-ADP exchange reaction and calcium transport provided direct and first evidence that the phosphoprotein is a true intermediate and not an artefact formed during protein denaturation by acid quenching. The kinetic analysis of protein formation presented by Froehlich and Taylor [10] revealed that the stable intermediate is transformed into an acid label species prior to the liberation of inorganic phosphate. The assumption that in the forward reaction phosphorylation preceeds ion translocation agrees with the fact that identi-

Table 2. Decay of SR phosphoprotein and ATP formation of sonicated SR membranes[a]

	EP-level nmoles/mg	ATP formation after	
		2 s	2 min
Before addition of EGTA	0.6	—	—
2 s after addition of 2 mM EGTA	1.6	—	—
2 s after addition of ADP			
0.05 mM	1.3	0.1	0.3
5 mM	0.4	0.2	0.5
2 s after addition of Ca^{++}			
6 mM	0.3	—	—
2 s after addition of 6 mM Ca^{++}			
+ 0.05 mM ADP or	0.3	—	—
+ 5 mM ADP	0.4	0.1	0.2

[a] Hasselbach and Beil (unpublished results)

Sonicated SR vesicles were incubated in a solution containing 5 mM $MgCl_2$, 5 mM K-phosphate (^{32}P), pH 6.0, and 1 mM AP_5A P-P-di(adenosine-5′-)pentaphosphate. E-P formation was initiated by the addition of 5 mM EGTA = ethyleneglycol-bis (2 aminoethyl)-N-N′ tetraacetic acid. Subsequently the indicated additions were made. E-P was determined after acid quenching. ATP formed from ADP by $^{32}P_i$ incorporation was measured in the aqueous extract by scintillation counting after removing phosphomolybdate complex with benzene isobutanol

cal phosphorylated intermediates are formed irrespective of the ability of the preparation to store calcium. Since in the reverse mode of the pump P_i incorporation is preceeded by ion translocation, the ability of the preparation to store calcium must be preserved. However, even when open vesicular fragments are exposed to calcium free solutions at pH 7.0 a small quantity of inorganic phosphate is rapidly incorporated into the transport protein. Monovalent salts, ADP and ATP compete effectively with this reaction and the intermediate rapidly equilibrates with phosphate in the medium (25, Hasselbach and Beil, unpublished results). This latter property is rather difficult to reconcile with the existence of an acylphosphate bond. Most important, all attempts to transfer the phosphoryl group to ADP were unsuccessful. It has been reported that the sudden application of high concentrations of ADP together with high concentrations of calcium ions result in the formation of a small amount of ATP. When the experiments are performed with sonicated membrane fragments in the presence of powerful myokinase inhibitor AP_5A, only traces of ATP were slowly formed (Table 2). The phosphorylated intermediate disappears rapidly what, however, does not require the simultaneous presence of ADP and calcium. We, therefore, must conclude that the phosphoprotein formed when calcium is removed from open membrane fragments does not give rise to the formation of a useful phosphate bound. A phosphate intermediate is formed with complete different properties when phosphorylation is performed under conditions which correspond to the reverse

Table 3. Properties of the phosphorylated intermediates formed from P_i[a]

Properties	Phosphoprotein formed	
	Without calcium gradient (E-P)	With calcium gradient (E~P)
Acid stability	+	+
Calcium sensitivity (Km)	0.5 μM	0.3 μM
Substrate affinity (20° C) $H_2PO_4^-$ (Km)	10 mM	0.3 mM
Competing ions 50% inhibition		
Cl^-	0.3 M	no competion
ADP	0.05 M	–
ATP	0.05 M	0.1
Heat of formation	48000 KJ/mol	> 12000 KJ/mol
Rate of formation pseudo first order rate constant (10° C)	30 s^{-1}	0.3 s^{-1}
Sensitivity of Ca ionophores	none	+
ADP-phosphate donor	none	+ ADP Km 2 μM pH · 7.0

[a] Hasselbach, Beil and Rauch (unpublished results)

3 E-P was formed as described in the legend of Table 2 mostly from native SR vesicles. E~P was formed from SR vesicles loaded with Ca-phosphate in solutions containing 5 mM phosphate (^{32}P), 5 mM Mg, and 0.5 mM acetylphosphate for 20 min at 25° C E~P formation was started by the addition of 5 mM EGTA

mode of the calcium pump, i.e. when calcium is removed from vesicles loaded with calcium. The properties of two phosphorylated intermediates are compared in Table 3. The apparent affinity of the system for P_i increases considerably when a gradient exists. The competitive effect of monovalent ions has disappeared and the enthalpie change is strongly reduced. At 10° C the rate of formation can easily be measured. When the calcium gradient is destroyed by the addition of an ionophore, the gradient dependent EP disappears while the considerably smaller gradient independent fraction remains. The gradient dependent intermediate can momentarily be transferred to ADP giving rise to an ATP burst followed by a continuous ATP formation. Apart from the many distinct differences between the two phosphorylated intercompounds, one important feature is common to both intermediates. They are characterized by the same high calcium sensitivity. At pH 7.0 none of them is formed in the presence of calcium concentration in the system higher than 1 μM. Therefore, it seems suggestive to assume that the slow formation of calcium gradient dependent phosphorylated intermediate is preceeded by the rapid formation of the gradient independent species.

References

1. Balzer, H., Makinose, M., Hasselbach, W.: The inhibition of the sarcoplasmic calcium pump by Prenylamine, Reserpine, Chlorpromazine and Imipramine. Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. **260**, 444–455 (1968)
2. Barlogie, B., Hasselbach, W., Makinose, M.: Activation of calcium efflux by ADP and inorganic phosphate. FEBS Lett. **12**, 267–268 (1971)
3. Baker, P. F., Blaustein, M. P., Hodgkin, A. L., Steinhardt, P. A.: The influence of calcium on sodium efflux in squid axons. J. Physiol. (Lond.) **200**, 431–458 (1969)
4. Caldwell, P. C., Hodgkin, A. L., Keynes, R. D., Shaw, T. I.: The effects of injecting energy-rich phosphate compounds on active transport of ions in the giant axous of loligo. J. Physiol. (Lond.) **152**, 561–590 (1960)
5. Dunham, E. T., Glynn, I. M.: Adenosine triphosphatase activity and the active movements of alcali metal ions. J. Physiol. (Lond.) **156**, 274–293 (1961)
6. Ebashi, S., Lipmann, W.: Adenosine triphosphate-linked concentration of calcium ions in a particulate fraction of rabbit muscle. J. cell. Biol. **14**, 389–400 (1962)
7. Ebashi, S., Endo, M.: Calcium ion and muscle contraction. Progr. Biophys. **18**, 125–183 (1968)
8. Fiehn, W., Migala, A.: Calcium binding to sarcoplasmic membranes. Europ. J. Biochem. **20**, 245–248 (1971)
9. Fiehn, W., Hasselbach, W.: The effect of phospholipase A on the calcium transport and the role of unsaturated fatty acids in ATPase activity of sarcoplasmic vesicles. Europ. J. Biochem. **13**, 510–518 (1970)
10. Froehlich, J. P., Taylor, E. W.: Transient state kinetic studies of sarcoplasmic reticulum adenosine triphosphatase. J. biol. Chem. **250**, 2913–2013 (1975)
11. Gilbert, D. L., Fenn, W. O.: Calcium equilibrium in muscle. J. gen. Physiol. **40**, 393–408 (1957)
12. Glynn, J. M., Karlish, S. J. B.: The sodium pump. Ann. Rev. Physiol. **37**, 13–55 (1975)
13. Harris, J. E.: The influence of the metabolism of human erythrocytes on their potassium content. J. biol. Chem. **141**, 579–595 (1941)
14. Hasselbach, W.: Relaxation and the sarcotubular calcium pump. Proc. Soc. exp. Biol. (N.Y.) **23**, 309–312 (1964)
15. Hasselbach, W.: The sarcoplasmic calcium pump. In: Molecular bioenergetics and macromolecular biochemistry, Meyerhof Symp. 1970, pp. 149–171. Berlin-Heidelberg-New York: Springer 1972

16. Hasselbach, W.: Sarcoplasmic membrane ATPase. In: The enzymes X, pp. 431–467. New York: Academic Press 1974
17. Hasselbach, W.: Release and uptake of calcium by the sarcoplasmic reticulum. 26. Colloq. Mosbach, pp. 81–92. Berlin-Heidelberg-New York: Springer 1975
18. Hasselbach, W., Makinose, M.: Die Calciumpumpe der „Erschlaffungsgrana" des Muskels und ihre Abhängigkeit von der ATP-Spaltung. Biochem. Z. **333**, 518–528 (1961)
19. Hasselbach, W., Makinose, M.: ATP and active transport. Biochem. biophys. Res. Commun. **7**, 132–136 (1962)
20. Hasselbach, W., Suko, J.: Calcium and phosphate turnover in the sarcoplasmic membranes. Biochem. Soc. Spec. Publ. **4**, 159–173 (1974)
21. Hokin, L. E.: Reconstitution of the coupled transports of Na^+ and K^+ in vesicles containing the purified NaK ATPase from the rectal gland of squalus acanthias. FEBS Symp. on Biochemistry of Membrane Transport, Zürich (in press) (1976)
22. Ikemoto, N., Bhatnagar, G. M., Gergely, J.: Fractionation of solubilized sarcoplasmic reticulum. Biophys. Res. Comm. **44**, 1510–1517 (1971)
23. Ikemoto, N.: Transport and inhibitory Ca^{2+} binding sites on the ATPase enzyme isolated from the sarcoplasmic reticulum. J. Biochem. (Tokyo) **249**, 659–651 (1974)
24. Jørgensen, P. L.: Purification and characterisation of $(Na^+ + K^+)$-ATPase. III. Purification from the outer medulla of mammalian kidney after selective removal of membrane components by SDS. Biochim. biophys. Acta (Amst.) **356**, 36–52 (1974)
25. Kanazawa, T.: Phosphorylation of solubilized sarcoplasmic reticulum by orthophosphate and its thermodynamic characteristics. J. biol. Chem. **250**, 113–119 (1975)
26. Knowles, A. P., Racker, E.: Properties of a reconstituted calcium pump. J. biol. Chem. **250**, 1949–1951 (1975)
27. Krogh, A.: The active absorption of ions in some freshwater animals. Z. vergl. Physiol. **25**, 335–356 (1938)
28. Maclennan, D. H., Wong, P. T. S.: Isolation of the calcium sequestering protein from sarcoplasmic reticulum. Proc. nat. Acad. Sci. (Wash.) **68**, 1231–1235 (1971)
29. Makinose, M., Hasselbach, W.: Der Einfluß von Oxalat auf den Calcium-Transport isolierter Vesikel des sarkoplasmatischen Retikulum. Biochem. Z. **343**, 360–382 (1965)
30. Makinose, M.: Die Phosphorylierung der Membran des sarkoplasmatischen Retikulum unter den Bedingungen des aktiven Ca-Transportes. Proc. Int. Biophys. Congr. 2nd, Vienna, N. 276, Wiener Universitätsverlag 1966
31. Makinose, M.: The phosphorylation of the membranal protein of the sarcoplasmic vesicles during active calcium transport. Europ. J. Biochem. **10**, 74–83 (1969)
32. Makinose, M., Hasselbach, W.: ATP synthesis by the reverse of the sarcoplasmic calcium pump. FEBS Lett. **12**, 271 (1971)
33. Masuda, H., de Meis, L.: Phosphorylation of the sarcoplasmic reticulum membrane by orthophosphate. Inhibition by calcium ions. Biochemistry **12**, 4581–4585 (1973)
34. Meissner, G.: ATP and Ca^{2+} binding by the Ca^{2+} pump protein of sarcoplasmic reticulum. Biochim. biophys. Acta (Amst.) **298**, 906–926 (1973)
35. Post, R.: Titration of sodium against potassium by the action on (Na^+, K^+) ATPase. FEBS Symp. on Biochemistry of Membrane Transport, Zürich (in press) (1976)
36. Reuter, H.: Divalent cat ions as charge carrier in excitable membranes. Progr. Biophys. **26**, 1–43 (1972)
37. Scarpa, A., Baldassare, J., Inesi, G.: The effect of calcium ionophores on fragmented sarcoplasmic reticulum. J. gen. Physiol. **60**, 735–749 (1972)
38. Schatzmann, H. J.: Dependence on calcium concentration and stoichiometry of the calcium pump in human red cells. J. Physiol. (Lond.) **235**, 551–569 (1973)
39. Skou, J. C.: Relationship of a $(Mg^{2+} + Na^+)$-activity, K^+-stimulated enzyme or enzyme system to the active, linked transport of Na^+ and K^+ across the cell membrane. 1960
40. Stromer, M., Hasselbach, W.: Fusion of isolated sarcoplasmic reticulum membranes. Z. Naturforsch. **31c**, 703–707 (1976)
41. The, R., Hasselbach, W.: Unsaturated fatty acids as reactivators of the calcium-dependent ATPase of delipidated sarcoplasmic membranes. Europ. J. Biochem. **39**, 63–68 (1973)

42. Warren, G. B., Toon, P. A., Birdsall, N. J. M., Lee, A. G., Metcalfe, J. C.: Reconstitution of a calcium pump using defined membrane components. Biochemistry **13**, 5501–5507 (1974)
43. Whittam, R., Ager, M. E.: The connection between active cat ion transport metabolism in erythrocytes. Biochem. J. **97**, 214–227 (1965)
44. Whittam, R., Chipperfield, A. R.: The reaction mechanism of the sodium pump. Biochim. biophys. Acta (Amst.) **415**, 149–171 (1975)

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Discussion

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During this conference considerable attention has been given to the possible role of calcium as an intracellular transmitter in the photoreceptor process (see in particular the presentations by M. Chabre, J. J. Korenbrot, and J. E. Brown). In view of still existing uncertainties and lack of factual information, it is good to look at other biological systems.

It then becomes apparent that calcium plays an intracellular transmitter role in several systems. First of all there is the role of calcium in "stimulus-secretion coupling" (Douglas, 1968) in various secretory systems, like hormone stimulation of enzyme secretion by pancreas and salivary gland. Secondly, there is the role of calcium in muscle contraction (Ebashi and Endo, 1968). Thirdly, calcium appears to function in presynaptic nerve terminals in the release of transmitters upon depolarization (Blaustein et al., 1972).

In all these cases there is a basic similarity in the system. The resting cytoplasmic free Calcium concentration is very low, of the order of 10^{-6} M. There are intracellular stores: mitochondria or more specialized structures, like sarcoplasmic reticulum in muscle and presumably rod sacs in rod photoreceptors. The organelles acting as calcium stores possess an active calcium uptake system. In addition, there appears to be an active calcium extrusion system in the plasma membrane. The two systems maintain the low resting concentration of Ca^{2+} in the cytoplasm. When the cell is excited or stimulated, calcium ions are released from the store to the cytoplasm. The increased cytoplasmic calcium concentration triggers the particular process, whether muscle contraction, enzyme secretion or visual excitation. Afterwards the cytoplasmic calcium concentration is brought back to its low resting level by reuptake into the storage organelle and by extrusion across the plasma membrane, although the latter process appears to be rather slow.

The best studied calcium transport system is that of the sarcoplasmic reticulum. Therefore, it is good to have a presentation on this subject by Dr. Hasselbach, who has contributed greatly to our knowledge of this system. In order to relate this information to our understanding of what happens in the rod outer segments a comparison of similarities and differences between the two systems is in order.

Similarities between sarcoplasmic reticulum and rod sacs are:

1. both are closed structures,
2. both have inward Ca^{2+} transport,
3. both release Ca^{2+} to the cytoplasm upon stimulation.

Differences are:

1. the major and best understood membrane protein in sarcoplasmic reticulum is the Ca^{2+} pump; in rod sacs rhodopsin, which may function as the release system rather than the pump system,

2. in the sarcoplasmic reticulum all pump molecules work in parallel, while in rod sacs *one* rhodopsin molecule is sufficient for excitation (calcium release?), the others being there merely to confer the required high sensitivity to the system.

3. the rod sacs have a far narrower lumen (~ 20 Å) than the sarcoplasmic reticulum, which may give a much greater role to calcium binding in the storage process.

A comparison of a number of properties of the Ca^{2+} transport mechanisms in sarcoplasmic reticulum and in rod sacs (Bonting and Daemen, 1976; Schnetkamp, Daemen, and Bonting, to be published) is given in the table.

Table. Comparison of transport system

	Sarcoplasmic reticulum	Rod sac
Translocation	yes	yes
ATP requirement	yes	yes (for net uptake)
Inhibitors:		
oligomycin	no	no
ruthenium red	yes	no (little)
lanthanum ion	yes	no
ouabain	no	no
Ion effects:		
Na^+ external	no	inhibits uptake, speeds efflux
Ca^{2+} external	exchange (only with ATP)	exchange (also without ATP)
System	Ca-Mg ATPase	"Ca-Na exchange carrier"

References

Douglas, W. W.: Stimulus-secretion coupling, the concept and clues from chromaffin and other cells. Brit. J. Pharmacol. **34**, 451–474 (1968)

Ebashi, S., Endo, M.: Calcium ion and muscle contraction. Progr. Biophys. **18**, 123–183 (1968)

Blaustein, M. P., Johnson, E. M., Needleman, P.: Calcium-dependent norepinephrine release from presynaptic nerve endings in vitro. Proc. nat. Acad. Sci. (Wash.) **69**, 2237–2240 (1972)

Bonting, S. L., Daemen, F. J. M.: Calcium as a transmitter in the photoreceptor cells. In: Transmitters in the visual process (S. L. Bonting, ed.), pp. 59–88. Oxford: Pergamon Press 1976