Meetings

Endoplasmic Reticulum: United States–Japan Cooperative Science Program

A symposium on the structure, function, and biogenesis of the endoplasmic reticulum was held in Nara, Japan, 5–7 September 1966, under the U.S.–Japan Cooperative Science Program. Fifteen Japanese scientists, primarily cell biologists and histologists, and 11 Americans representing the same disciplines attended. A South Korean, a Chinese (Tiawan), and an Argentinean scientist participated as observers. About ten Japanese graduate students were also in attendance and helped effectively with the logistics of the symposium.

At the beginning of the proceedings, after a short historical introduction by K. Porter, Y. Watanabe reviewed present knowledge of the rough-surfaced endoplasmic reticulum (ER). He dealt primarily with species-dependent features and function-induced changes in this system. His main conclusion is that fragmentation (vesiculation) of rough ER cisternae occurs in a variety of conditions in which synthesis of secretory proteins is either increased or decreased. D. Sabatini and Y. Tashiro discussed structural and functional aspects of ribosome attachment to ER membranes. Sabatini presented recent evidence obtained by him and C. Redman on vectorial transport of proteins and peptides from attached ribosomes, across the ER membrane, to the cisternal space. These papers naturally led to a discussion of the function of the rough ER in the synthesis, segregation, and accumulation of secretory proteins. E. Yamada showed a remarkable example of "secretory" protein (peroxidase) demonstrated histochemically in all cell compartments involved in the "secretory" process (that is, rough ER cisternae, Golgi vesicles, Golgi cisternae, and secretion granules) in eosinophil promyelocytes. R. Kessel presented a series of striking examples of massive segregation and accumulation of vitellus proteins in rough ER cisternae followed by transport to smooth ER cisternae which occurs during oogenesis in various crustacea. Comparable cases were discussed by T. Yamamoto (Niigata), D. Fawcett, and W. Stoeckenius.

G. Palade stressed the necessity of looking for other functions of the system, more general than protein secretion, to explain the ubiquity of the rough ER in animal and plant cells. In view of the previous examples, "protein segregation for immediate or delayed use—either in the external medium or in an intracellular compartment separated from the cytoplasmic matrix"—would be a better formulation of the function of rough ER than the usual "segregation of proteins for export." Such a concept would include the production and segregation of hydrolases required for phagocytosis in heterotrophic cells and for controlled autolysis probably involved in regulatory processes in all cells. Another possible explanation could be the involvement of the rough ER in membrane biosynthesis, a fact now proven in the case of smooth ER membranes but possibly applicable to other cellular membranes (Golgi? plasmalemma?). The rough ER would make possible the asymmetric (polarized) assembly of the constitutive proteins of a membrane in distinct inner and outer leaflets ("inner" referring to the leaflet in direct contact with the cytoplasmic matrix). With this in mind, the proteins of the outer leaflet could be considered the "evolutionary precursors" of secretory proteins.

In a comprehensive review of smooth ER, E. Yamada set as identification criterion continuity with the rough ER. He added a few new cell types (sustentacular cells of olfactory epithelia and Clara cells of bronchial epithelia) to the already known examples of cells with a voluminous smooth ER (hepatocytes, steroid-secreting cells, pigmented retinal epithelia). These additions bring forth, of course, new questions as to the functions of the smooth ER. Yamada also showed good examples of direct rough ER continuity with piled Golgi cisternae in nerve cells (Purkinje cells). Numerous other examples of unusual forms of smooth ER or highly developed smooth ER were presented and discussed by: P. Hashimoto, in certain nerve endings; T. Nagano, in Sertoli cells and Tyson gland cells of mammals; H. Lin, in rat pinealocytes; and T. Yamamoto (Niigata), in various cell types of lower vertebrates. In the last example, the coexistence of elaborate infoldings of the cell membrane was not excluded.

Structural variations among cellular membranes were reviewed by T. Yamamoto (Sendai). He pointed out that in certain cell types (a photoreceptor cell in a primitive molluscan eye, in this case) membrane fusion (which results in the formation of "five-layered membrane structures") frequently affects outfoldings of the cell membrane in a primitive rabdomere as well as the intracellular membranes of the smooth ER, rough ER, and nuclear envelope. In the same cell, an apparently new type of cellular membrane, extremely thin and possibly single-layered, is found outlining a system of smooth-surfaced vesicles.

W. Stoeckenius discussed structural variations among various cellular membranes in terms of two basic premises: all these membranes are layered structures of the Davson-Danielli type (unit membranes) and differences in their thickness could be explained by differences in the chemical composition of their lipid phase, more specifically by differences in the proportion and types of phospholipids, and in the length and degree of unsaturation of their fatty acids. Stoeckenius also presented recent results on the effect of nearly completed lipid extraction (by acetone-water) upon the structure of various cell membranes. Some of them (ER membranes) collapse; some seem to be completely solubilized (outer mitochondrial membrane); while others retain their unit membrane structure (inner mitochondrial membrane), thus suggesting that in the latter case the protein leaflets of the membrane are cross-linked by protein or residual lipid.

K. Porter reviewed the function of the smooth ER (glucose mobilization in hepatocytes, steroid synthesis in various endocrine cells), and concentrated on a still controversial point: that is, the role of the system in lipid absorption by the intestinal epithelium. He reported experiments in which inert tracers (ferritin or colloidal silver) were introduced in the intestinal lumen concomitantly with a lipid emulsion,

and showed that the tracers and the lipid follow separate routes in the cell. The tracers appear in coated pits and vesicles and finally in lysosomes in the apical region of the cell, whereas lipid droplets are seen only in the smooth ER below the terminal web and subsequently spread to the rough ER. In agreement with most of the available biochemical evidence, the results were taken to indicate absorption of lipid in molecular form (fatty acids, monoglycerides), and to discount the often discussed hypothesis of absorption in particulate form.

A similar conclusion was drawn by R. Barrnett from cytochemical tests carried out during lipid absorption. Incubation of intestinal loops absorbing lipid in the presence of Pb^{2+} leads to heavy staining of small particles on and within microvilli as well as within the apical cytoplasmic matrix of the cell. These particles are interpreted as micelles made up of fatty acids on monoglycerides and bile acids, or as "lipid microcrystalline aggregates." Divalent lead staining is explained by the negative surface charge of the particles. Esterase activity is demonstrable in the smooth and rough ER and is connected with the resynthesis of triglycerides.

On the same general topic, that is, the function of the smooth ER, D. Fawcett presented interesting results recently obtained by Jones and Armstrong on rat livers perfused in vitro. When unsaturated fatty acids are added to the perfusate, small dense droplets appear in the cisternal space of both rough and smooth ER and are subsequently discharged in the space of Disse. Such particles were recovered from the perfusate, characterized by analytical centrifugation as very light lipoproteins, and shown by electron microscopy to have the same general morphology as the intracisternal particles. The role of the Golgi complex in the process is still uncertain. These results seem to rule out previous interpretations (uptake of chylomicrons, secretion of serum albumin) of similar appearances seen in the hepatocytes of intact animals. So far, it has not been decided whether the role of the smooth ER is limited to lipoprotein transport or encompasses also the synthesis of the lipid moiety of these macromolecules.

The cytochemistry of intracellular membranes was reviewed and discussed by R. Barrnett who, in addition to the work on intestinal lipid absorption already mentioned, concentrated on two other aspects of lipid metabolism: removal of lipid from adipose cells and synthesis of lipid in the muscle of starved-refed animals. During lipid mobilization from adipose cells, incubation in Pb^{2+} reveals a system of fine channels presumably related to smooth ER, and esterase tests stain a "membrane" or interface around the main lipid desposit. Lipid synthesis was explored by using thioacetate as a substrate. The results seemed to implicate again the smooth ER (sarcoplasmic reticulum) in the process, although the reactions involved remain to be ascertained and identified. K. Ogawo presented cytochemical preparations indicating that glucose-6-phosphatase activity is present in all ER membranes, but absent from the membranes of all Golgi elements in both normal rats and rats treated with phenobarbital or 3′-MeDAB. V. Mizuhira showed results obtained by labeling cell membranes with ^{3}H-cholesterol and ^{3}H-progesterone.

Relations between ER and the Golgi complex were discussed by K. Kurosumi who dealt primarily with the appearance of intracisternal granules in the rough ER in many cases in which production of secretory proteins apparently continues or increases, while the discharge of secretion is interfered with. This is the case with pituitary thyrotrophs after thyroidectomy, MSH-producing cells in the hypophysis of white adapted toads, and pancreatic exocrine cells during hibernation in bats. Y. Watanabe showed that intracisternal granules can be produced in exocrine pancreatic cells of the rat by combined treatment with pilocarpin and puromycin. It was agreed that such granules may indicate the presence of a block in intracellular transport affecting the "lock" that regulates movement from rough ER to the Golgi complex. G. Palade presented recent results, obtained with Jamieson, by autoradiography and cell fractionation of pancreatic slices. These results indicate that the small, smooth-surfaced vesicles at the periphery of the Golgi complex are involved in the transport of secretory proteins from the transitional elements of the rough ER to the condensing vacuoles of the Golgi complex. The vesicles apparently carry out an intermittent type of transport in bulk; the "lock" discussed in relation to intracisternal granules may be located at their level. Morphological findings suggestive of similar transport operations in the vitellogenesis of echinoderm oocytes and in the secretory process of silk spinning glands in insects were presented by R. Kessel.

In the discussion that followed it was agreed that the Golgi terminus of this type of transport could be a condensing vacuole, as in the exocrine cell of the guinea pig pancreas, or one or more piled Golgi cisternae as in pituitary thyrotrophs, mammotrophs, and many other cell types.

On behalf of A. Novikoff, who was unable to attend, R. Barrnett presented cytochemical evidence suggesting that certain experimental conditions result in a damming of the flow of acid phosphatase from the rough ER through the Golgi elements to the lysosomes. Under such conditions, the complex of connections (designated GERL by Novikoff) between these cell compartments becomes acid phosphatase positive.

Finally J. Revel discussed autoradiographic results obtained on chondrocytes by labeling with ^{3}H-glucose, ^{3}H-galactose, and $^{35}SO_4^{2-}$. He indicated that the polysaccharide moiety of muco- and glycoproteins is synthesized in the Golgi complex. Similar results have recently been reported from other laboratories, especially from that of C. Leblond. Such findings may implicate the Golgi complex not only in the production of muco- and glycoproteins for secretion, but also in the production and assembly of specific polysaccharides into a membrane eventually destined to become plasmalemma.

H. Swift's review of the nuclear envelope and annulate lamellae dealt primarily with: (i) nuclear "pore complexes" and evidence of movement of RNA-containing particles through them; (ii) behavior of the nuclear envelope during mitosis in various cell types, especially in protists; (iii) local differentiations (fusion, outpocketings followed by partial sloughing off, exposure to external medium following extensive sloughing off of the cytoplasm) which occur in certain protozoa and during spermiogenesis in certain invertebrate species; and (iv) general morphology and relations of annulate lamellae. In the ensuing discussion, T. Nagano and H. Lin showed new examples of annulate lamellae in normal Sertoli cells and pinealocytes of mammals, that is, in cell types in which it has generally been assumed that such lamellae are missing. Yasuzumi demonstrated a "specific" staining of nuclear pore complexes due, in his interpretation (questioned by others), to an adenosine triphosphatase reaction.

A comprehensive review of the sarcoplasmic reticulum (SR) by L. Peachey covered many species from many phyla. He stressed the progressive increase in the structural complexity of the system and in the regularity of SR-sarcomere relationships, and showed that these changes can be correlated with a progressive increase in the speed of the contraction-relaxation cycle. For reviewing fine structural details, Peachey chose the amphibian fast muscle (frog sartorius). In addition to the now familiar features of this system (T tubes, terminal cisternae, connecting tubules, and central collar, which were interpreted in terms of excitation-contraction coupling and subsequent relaxation), he stressed the presence of a granular content in the terminal cisternae and of a constricted passage between these cisternae and the longitudinal tubules. On the basis of these and other findings (see Ebashi), he postulated that during relaxation Ca^{2+} is pumped into the longitudinal tubules and subsequently accumulates in the terminal cisternae, presumably by complexing with their granular content.

S. Ebashi reviewed the development of current concepts concerning the function of SR from the initial finding of a muscle Mg^{2+}-activated adenosine triphosphatase by Kielley and Meyerhof, through the relaxing factor, to in vitro experiments with SR fractions isolated from muscle. The main conclusion of this line of work—that is, the ATP-dependent ability of SR fractions to accumulate Ca^{2+} in vitro—could lead to an understanding of the physiological role of the system in vivo provided that: (i) the accumulation of Ca^{2+} be extensive enough to reduce concentration in the cell sap below a critical value for relaxation; (ii) the movement of Ca^{2+} be fast enough to account for the known rapidity of relaxation; and (iii) the release of accumulated Ca^{2+} be induced by a change in electric field related to the arrival of a depolarization wave in the adjacent T tubules. Evidence obtained in Ebashi's and Weber's laboratories indicate that requirements (i) and (ii) are fulfilled; requirement (iii) is not yet satisfied by unquestionable evidence. Ultraviolet, microbeam irradiation carried out by Endo leads to contraction only when applied to the Z-band region in amphibian muscle. Since ultraviolet irradiation is known to affect SR permeability in vitro, the finding suggests that Ca^{2+} accumulation takes place in the terminal cisternae and that the SR is

functionally compartmented. Ebashi mentioned that cell membrane excitability is dependent on Ca^{2+} concentration in the cell sap; he postulated that Ca^{2+} accumulation in SR may have a double function: control of the relaxation-contraction cycle, as well as of cell membrane excitability. In this respect, he showed recent results indicating the presence of an ATP-dependent Ca^{2+} accumulation by brain microsomes.

K. Hama presented two new examples of sarcoplasmic reticulum in muscle fibers of the heart. His work may challenge current interpretations of the role of the T system in excitation-contraction coupling. The first example was found in the neurogenic muscle fibers of the heart in the mantis shrimp, and is characterized by two T systems for every sarcomere: one system is part of a triad at the Z-band level, while the other occurs in a dyad facing the middle of the A band. The second example is the myogenic muscle fiber of an avian myocardium in which no T system can be identified, although the equivalent of a terminal cisterna is recognized at the Z-band level.

D. Fawcett considered the biogenesis of ER membranes induced by drugs. His experiments, carried out in collaboration with Jones, confirm and extend to another species (the hamster) results obtained by Remer and Merker and by Orrenius and Ericsson on rats and rabbits. The main finding is the massive increase in smooth ER that follows the administering of drugs. The event has already been correlated with an increase in the activity of the enzymes of the detoxifying system of the liver; Fawcett reported that cholesterol synthesis is also increased under such conditions. The broad implications of the main finding (that is, the drug-induced proliferation of smooth ER) were discussed in terms of cellular pathology as well as in terms of a morphologically justified, basic distinction between smooth and rough ER.

G. Palade reported on work, carried on in collaboration with Dallner and Siekevitz, on a critical phase in the differentiation process of the rat hepatocyte. The phase occurs within a few days before and after birth and is characterized by: (i) the appearance (usually immediately after birth) of enzyme activities typical of the ER (microsomal) membranes of fully differentiated hepatocytes; (ii) asynchronous increase in activity even for enzymes which are

part of a common multienzyme system; and (iii) rapid proliferation of ER, primarily smooth ER membranes. Experiments with actinomycin D and puromycin indicated that these events reflect the synthesis of new proteins. Experiments which changed the lipid composition of the membranes (by dietary changes, or by the addition of various lipids to defatted membranes) suggested that these lipids do not play a critical role in the differentiation process. Kinetic experiments showed that the activity of the microsomal constitutive enzymes appears first in the rough and then in the smooth ER. Pulse-labeling experiments revealed that the same applies for total, newly synthesized membrane proteins. The results were considered in terms of a series of models which assume that new membrane is produced in the cell either in a one-step operation or in a multistep operation. In the latter case, a "primary" membrane is presumably laid down in a first step and various enzymes are added to it in subsequent steps. Each of these models could apply to a homogeneous membrane within which all constitutive proteins are randomly distributed, or, alternatively, to a mosaic membrane in which each tessera represents a multienzyme system or an aggregate of identical enzymes.

More recent results, which may narrow the choice between these various models, were reported by T. Omura. He showed that in fully differentiated hepatocytes the turnover of microsomal membrane lipids, as followed by regression of labeling with ^{14}C-glycerol, is much faster (half life ~ 40 hours) than the half life of the total membrane proteins (half life ~ 100 hours). Two constitutive enzymes which at present can be isolated and purified from microsomal membranes also proved to have different half lives. Hence, ER membranes appear to be continuously synthesized, even in the fully differentiated hepatocytes of the adult animal. The general turnover rate is relatively high and appears to be different for some of the membrane components. In this situation, it is possible to check on adult animals, under more favorable experimental conditions, some of the results previously obtained in newborns. For instance, kinetic studies on labeled membrane proteins can be carried out at the level of individual, satisfactorily purified enzymes (cytochrome b_5 and NADPH-cytochrome *c* reductase). The results show that these enzymes are produced in the rough ER, presumably by attached ribosomes, and subsequently transferred to the smooth ER. They also suggest a superimposed and relatively rapid process of membrane exchanges between the rough and the smooth ER, which leads in time to an equilibrium of the label.

So far, the findings exclude the existence of a homogeneous membrane in which all components turn over in synchrony, and favor a multi-step assembly model, probably of the mosaic version.

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Forthcoming Events

April

11–13. **Nursing Service and Hospital Administration**, American Hospital Assoc., Chicago, Ill. (E. J. Lanigan, AHA, 840 N. Lake Shore Dr., Chicago 60611)

12–14. **Optical Soc.** of Amer., Columbus, Ohio. (Miss M. Warga, OSA, 1155 16th St., NW, Washington, D.C. 20036)

12–14. **Shock Tube** Symp., 6th intern., Freiburg, West Germany. (R. G. Fowler, Dept. of Physics, Univ. of Oklahoma, Norman 73069)

13–14. **Teaching of Mathematics to Physicists**, Inst. of Physics and Physical Soc. and Inst. of Mathematics and Its Applications, conf., Exeter, England. (Meetings Officer, Inst. of Physics and Physical Soc., 47 Belgrave Sq., London, S.W.1, England)

13–15. American Assoc. for **Cancer Research**, 48th annual mtg., Chicago, Ill. (Secretary-Treasurer, The Association, 7701 Burholme Ave., Philadelphia, Pa.)

13–16. **British Medical Assoc.**, annual clinical conf., Londonderry, Northern Ireland. (Secretariat, Tavistock Sq., London, W.C.1, England)

14–15. **Echoencephalography**, intern. symp., Univ. of Erlangen-Nurnberg, West Germany. (W. Schiefer, 8520 Erlangen, Krankenhausstrasse 12, West Germany)

14–21. **French Physical Soc.**, 61st exhibition, Paris. (The Society, 33 rue Croulebarbe, Paris 13°)

15–16. American Soc. for **Artificial Internal Organs**, annual mtg., Atlanta, Ga. (P. M. Galletti, Dept. of Physiology, Emory Univ., Atlanta)

15–16. **Histochemical** Soc., 18th annual mtg., Chicago, Ill. (G. M. Lehrer, Div. of Neurochemistry, Mount Sinai School of Medicine, 11 E. 100 St., New York 10029)

15–16. **Nucleic Acids** Symp., Santa Monica, Calif. (M. S. Dunn, 9325 Venice Blvd., Culver City, Calif.)

17–19. **Elementary Particles**, Inst. of Physics and Physical Soc., conf., London,