Andrew P. Somlyo 1930-2004

Andrew P. Somlyo, M.D., Chairman and Charles Slaughter Professor of Molecular Physiology and Biological Physics and Professor of Internal Medicine (Cardiology) at the University of Virginia Health System, died suddenly on January 14, 2004. Dr. Somlyo served as Chairman of the University of Virginia Department of Molecular Physiology and Biological Physics from 1988 to the present, and built it into one of the preeminent physiology departments in the country, consistently ranking in the top five.

Born in Budapest and schooled by the Jesuits, he escaped Hungary during the Soviet occupation in 1948. Dr. Somlyo received his Doctor of Medicine degree and Master of Science in pathology from the University of Illinois in 1956, followed by internship and residency at Philadelphia General Hospital and Bellevue Hospital. He was chief resident at Mount Sinai in New York City, and served as Assistant Physician in Medicine at Columbia-Presbyterian Medical Center, where he received his fellowship in cardiology. He also received a Master's degree in biomedical engineering in 1963 from the Drexel Institute of Technology in Philadelphia. He then joined the faculty at the University of Pennsylvania, where he ascended the academic ladder and became Professor of Pathology in 1971 and also of Professor of Physiology in 1973. At the University of Pennsylvania, he founded the renowned Pennsylvania Muscle Institute, and led it as Director for nearly fifteen years. It became a prototypic interdisciplinary research group. At the same time, Dr. Somlyo was Senior Research Pathologist at the Presbyterian University of Pennsylvania Medical Center in Philadelphia, and was licensed to practice medicine in New York, Pennsylvania, and later Virginia

In 1988, he moved to the University of Virginia, where he became Chairman of the Department Physiology. He renamed the Department of Molecular Physiology and Biological Physics to reflect new areas of focus in research on structural biology and education. Dr. Somlyo's research interests covered many areas in the sciences from medicine to physics. Only a few of his accomplishments will be summarized here.

Spanning over more than five decades of research, Dr. Somlyo authored more than 300 articles in prestigious journals, as well as many reviews and book chapters. Their reviews provides a rich synthesis of information and remarkable insights, which continue to serve as valuable reference tomes and a road map for students and researchers of smooth muscle.

Through the course of his illustrious career, Dr. Somlyo trained over 58 postdoctoral fellows and students. Dr. Somlyo was well known for his insightful and often provocative discussions at scientific meetings. He always strove for excellence, and was as critical of his own work as he was of the work of others. He stimulated much collaboration and was a valued mentor to fellows, students as well as colleagues. Andrew Somlyo will be remembered for his scientific accomplishments, his scientific rigor, his unusually broad knowledge, pioneering spirit and infectious enthusiasm for research.

The name *Somlyo* emerged in the physiology literature in the late 1960's, a time when measurements of ionic fluxes and electrophysiological techniques were the major approaches taken for the study of the contractile responses of smooth muscles. Little was known about the ultrastructure of smooth muscle, largely due to shortfalls in tissue fixation techniques. Nor was there quantitative information on the distribution and translocation of calcium during contraction. The direction of Dr. Somlyo's research on smooth muscle followed two major pathways: studies on (1) activation and regulatory mechanisms of contraction of smooth muscle, and (2) the ultrastructure of smooth muscle and sites of calcium storage and release.

Dr. Somlyo's research, along with his wife and research partner, Dr. Avril Somlyo, defined and resolved the seminal questions in smooth muscle physiology, biochemistry and structural biology during the last 25 years. The work spanned ion fluxes and electrophysiological techniques, structural biology including electron microscopy, atomic force microscopy, light microscopy, advanced X-ray probe ultra-microanalysis and crystallography, muscle mechanics, and caged molecules. Advanced techniques were developed and applied to smooth, skeletal and cardiac muscles. They discovered some of the fundamental aspects of smooth muscle that we now take for granted, including that smooth muscles contain thick filaments, cross-bridges, and sliding filaments. The source of calcium release is the sarcoplasmic reticulum which can be triggered by either membrane potential changes or by receptor-effector coupling without depolarization (known as pharmacomechanical coupling, a term he and Avril coined). The mechano-chemical mechanism of energy economy in long force-bearing contractions was deduced. G-protein coupled signal transduction pathways, involving Rho, Rho kinase and myosin phosphatases were extensively characterized and the first crystal structure of Rho was solved. These contributions by the Somlyo lab placed smooth muscle on the same rigorous level of understanding with the other muscle types. They have enormous biomedical and healthcare impact in the cardiovascular, pulmonary and reproductive systems.

Dr. Somlyo's research has won many accolades, including the Louis and Artur Lucian Award for Research in Circulatory Diseases (1995), the CIBA Award for Hypertension Research from the American Heart Association (1991), the Distinguished Scientist Award for Biological Sciences from the Microscopy Society of America (1994), and the Presidential Award for Outstanding Scientific Contributions to the Theory and Practice of Microbeam Analysis (1996). He was awarded an honorary doctorate from the Université Catholique de Louvain, Belgium (1997), was a Fellow of the Biophysical Society and the American Association for the Advancement of Science, and Honorary Member of the Hungarian Physiological Society.

Dr. Somyo had editorial board positions at *Journal of Vascular Research* (1995-98), *American Journal of Physiology* (1979-83), *Journal of Applied Physiology* (1973-76), *Journal of Histochemistry and Cytochemistry* (1978-81), *Cell Motility* (1979-85), *Journal of Cardiovascular Pharmacology* (1977-86), *Cell Calcium* (1979-92), *Journal of Muscle Research and Cell Motility* (1980-2004; Editor, 1987-2004), *Circulation Research* (1978-86), *Journal of Structural Biology* (1979-93), *Magnesium: Experimental and Clinical Research* (1982-2004), *Journal of Microscopy Research andTechnique* (1984-93), *Journal of Microscopy Society of America* (1994-2004), *FASEB Journal* (1996-2004), and *Microscopy and Microanalysis* (2002-2004).

Excitation-contraction coupling and regulation of signal transduction

Although most of us in the Microscopy Society undoubtedly know Dr. Somlyo primarily for his pioneering work in biological microanalysis, this was only part of his work toward the goal of a comprehensive understanding of smooth muscle structure and function. An understanding of the mechanism and regulation of smooth muscle contraction and relaxation is essential for developing therapies for vascular diseases, asthma, and other diseases of smooth muscle.

In their early publications, the Somlyos showed that smooth muscles could be classified as *tonic or phasic* in terms of their contractile activity. It was generally accepted that phasic smooth muscles were activated by action potentials. But the Somlyos were the first to demonstrate, contrary to the prevailing view, that *graded depolarization*, not action potentials, activated tonic contractions. They introduced the idea that there were two mechanisms for the activation of smooth muscle: *electromechanical coupling*, involving a change in membrane potential and *pharmacomechanical coupling*, which was not associated with any obligatory change in membrane potential. We now know that the latter mechanism, first described by them in 1968, is based on signal transduction pathways. Investigation of the regulation of smooth muscle contraction through these pathways pervaded their research for many years, providing new information and insights along the way, including details of crossbridge kinetics in phasic and tonic muscles and signal transduction mechanisms. Using flash photolysis of the caged signaling molecule, InsP3, they demonstrated this important role in Ca2+ release in smooth muscle (in collaboration with Yale Goldman, David Trentham and Meredith Bond), and with caged nucleotides they showed that slow, tonic smooth muscles, unlike fast, phasic smooth muscle, have a high affinity for ADP and relatively low affinity for ATP, leading to a prolongation of the strongly bound state in the crossbridge cycle and marked slowing of relaxation of force by MgADP. From their research on pharmacomechanical coupling and regulation of contraction of smooth muscle, the concept of calcium-sensitization emerged. The Somlyos found that an underlying mechanism was the inhibition of myosin light chain phosphatase through a G-protein coupled process, and, to the present, their efforts were directed towards uncovering the messengers, pathways and mechanisms of this inhibition. Of particular interest was the small G-protein RhoA and Rho-kinase, which can phosphorylate and inhibit myosin phosphatase of smooth muscle and non-muscle cells, modulating not only contractile activity but also cell migration. This pathway is of fundamental importance in angiogenesis and in tumor cell metastasis. The Somlyos published a superb, comprehensive review of calcium sensitization mechanisms in October 2003 (Physiological Reviews). This work is represented here by figures from that review, and an earlier one in 1994.

Somlyo, A.V. and Somlyo, A.P. (1968) Electromechanical and pharmacomechanical coupling in vascular smooth muscle. *J. Pharmacol. Exp. Therap.* 159:129-145.
Somlyo, A.P. and Somlyo, A.V. (1994) Signal transduction and regulation in smooth muscle. *Nature* 372:231-236.
Somlyo, A.P. and Somlyo, A.V. (2003) Ca2+-sensitivity of smooth and non-muscle Myosin II: Modulated by G Proteins, Kinases and Myosin Phosphatase. *Physiol. Rev.* 83:1325-1358.

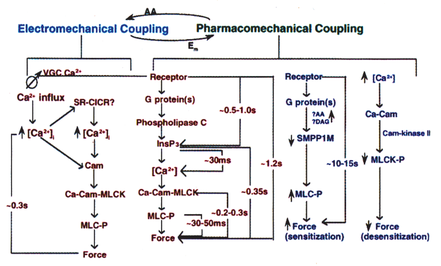


FIG. 1 The electromechanical and pharmacomechanical pathways of excitation–contraction coupling. The pathways on the left-hand side (electromechanical and phosphatidylinositol-cascade-mediated), shown in red, operate by increasing cytoplasmic free Ca^{2+}; this effect is reversed by removal of Ca^{2+}. The delays shown for the various steps of phosphatidylinositol-cascade-mediated Ca^{2+}-release are approximate, and were obtained at room temperature. The pathways (Ca^{2+}-sensitization and -desensitization) shown in blue on the right-hand side can operate at constant Ca^{2+}, and are the result of inhibition or activation of the enzymes phosphorylating (myosin light-chain kinase; MLCK) and dephosphorylating (smooth-muscle myosin light-chain phosphatase, SMPP1M) serine 19 on MLC_{20}. CICR, calcium-induced calcium release; MLC-P, phosphorylated myosin; MLCK-P, MLCK phosphorylated on site A; Ca-Cam, calcium-calmodulin; VGC Ca^{2+}, voltage-gated Ca^{2+} channel; SR, sarcoplasmic reticulum; $InsP_3$, inositol 1,4,5,-trisphosphate. Inhibition of voltage-gated calcium current by arachidonic acid (AA) and inhibition of $InsP_3$ production by hyperpolarization (E_m, membrane potential) represent bidirectional cross-talk between electromechanical and pharmacomechanical coupling. Short heavy arrows indicate increases or decreases in concentration or activity. DAG, diacylglycerol.

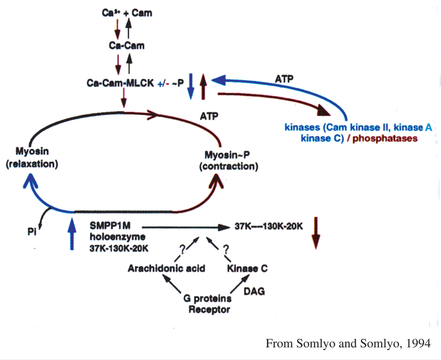


FIG. 2 Established and potential pathways modulating the Ca^{2+}-sensitivity of MLC_{20} phosphorylation and hence contraction. Ca^{2+}-sensitizing pathways are shown in red, desensitizing pathways in blue. Heavy arrows indicate increased or decreased enzyme (light-chain kinase or phosphatase) activity. Phosphorylation of MLC kinase (MLCK to MLCK-P) at site A by several protein kinases reduces its affinity for calcium-calmodulin (Ca-Cam) and, consequently, its activity at a given level of Ca^{2+}, whereas dephosphorylation of a phosphorylated MLC kinase would have the opposite effect. In contrast, inhibition of MLC phosphatase (SMPP1M) via a G-protein-coupled mechanism increases MLC_{20} phosphorylation and, consequently, force, at a given $[Ca^{2+}]_i$. The messengers mediating the effect of the plasma membrane-bound receptor–G-protein complex have not been definitely identified, but two of those implicated, arachidonic acid and protein kinase C, are shown. Mechanisms that would enhance dephosphorylation of myosin LC_{20} and relaxation through activation of MLC phosphatase have been proposed, but have yet to be experimentally demonstrated. Inhibition of SMPP1M is shown to result from reduced association between the 37K catalytic subunit and the targeting regulatory component (130K + 20K) of the enzyme[74,75]. Abbreviations as in Fig. 1.

From Somlyo and Somlyo, 1994

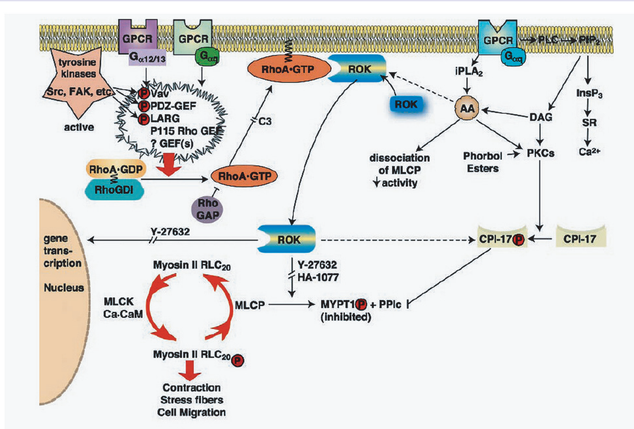
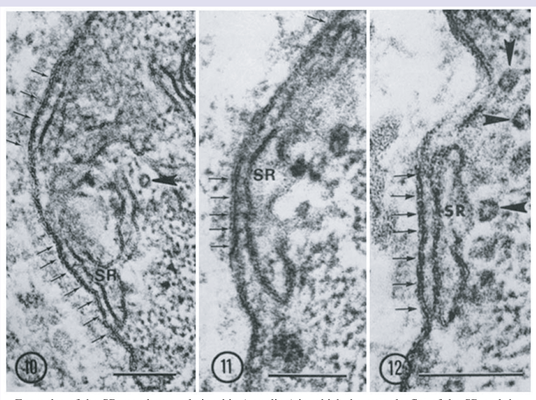


FIG. 4. Signaling pathways for Ca^{2+} sensitization in smooth muscle. Different smooth muscles respond to a large number of different agonists including catecholamines, muscarinic agonists, thromboxane, histamine, serotonin, as well as the sphingolipids: sphingosine 1-phosphate and sphingosylphosphorylcholine. Activation of their receptors initiates signaling through the illustrated cascades that inhibit myosin phosphatase (MLCP), increase RLC_{20} phosphorylation and contraction, stress fiber formation, and/or cell migration. The Rho/ROK pathway can also lead to activation of smooth muscle differentiation marker gene expression. GPCR, G protein-coupled receptors; GEF, guanine nucleotide exchange factor(s); RhoGDI, GDP dissociation inhibitor; RhoGAP, GTPase activating protein; ROK, Rho-kinase, ROKα/ROCK II, ROKβ/ROCK I; C3, clostridial C3 exoenzyme ADP ribosylates and inactivates RhoA; $iPLA_2$, Ca^{2+}-independent phospholipase A_2; CPI-17, PKC-potentiated inhibitor protein of 17 kDa; MYPT1 and PP1c, myosin phosphatase regulator and catalytic subunits; MLCK, myosin light-chain kinase; DAG, diacylglycerol; PKC, protein kinase C; AA, archidonic acid; SR, sarcoplasmic reticulum; $InsP_3$, inositol 1,4,5-trisphosphate; PLC, phospholipase C; PIP_2, phosphatidylinositol 4,5-bisphosphate.

From Somlyo and Somlyo 2003

Ultrastructural studies

From the Somlyos ultrastructural studies we learned much about the organization of contractile filaments in smooth muscles, including that smooth muscle myosin was organized into filaments, which were significantly longer than those of striated muscles; that in resting muscles, in which the regulatory light chains of myosin were unphosphorylated, myosin was organized into filaments (dispelling the notion that filaments were formed only on activation); and that actin filaments inserted into intracellular dense bodies as well as those associated with the plasma membrane. The Somlyos also demonstrated that inositol trisphosphate caused calcium release from the SR, a fundamental mechanism in Pharmacomechanical coupling. They also showed that smooth muscle contains sarcoplasmic reticulum, which serves as a Ca storage site although Ca influx was at the time considered as a sole source of Ca. More recently, together with Zhifeng Shao, they pioneered the use of cryo-atomic force microscopy for studies of contractile proteins providing the first images of the substructure of smooth muscle actin filaments and of myosin molecules in the context of conformational changes associated with the phosphorylation state of the regulatory light chains.



Examples of the SR-membrane relationship (coupling) in which the outer leaflet of the SR and the cell membranes are separated by an approximately 10 nm gap. Electron-opaque material present between the two apposing membranes has a "periodicity" of approximately 20-25 nm (small arrows). Some electron-opaque material is also present in the SR lumen. Microtubules are adjacent to the SR (arrowheads). Fig. 10, X 165,000; Fig. 11, X 207,000, Fig. 12, X 276,000.

From Devine et al., 1972

Devine, C.E., Somlyo, A.V. and Somlyo, A.P. (1972) Sarcoplasmic reticulum and excitation-contraction coupling in mammalian smooth muscle. *J. Cell Biol.* 52:690-718.
Ashton, F.T., Somlyo, A.V., and Somlyo, A.P. (1975) The contractile apparatus of vascular smooth muscle: Intermediate high voltage stereo electron microscopy. *J. Mol. Biol.* 98:17-29.
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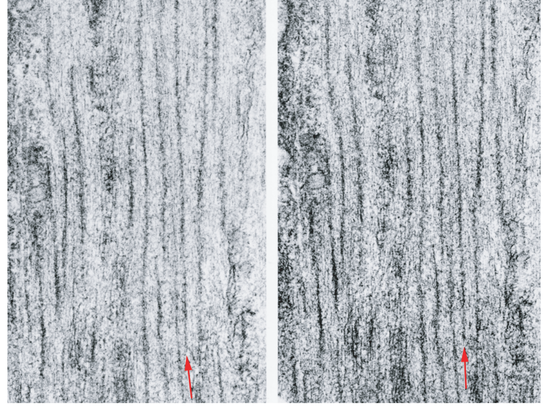
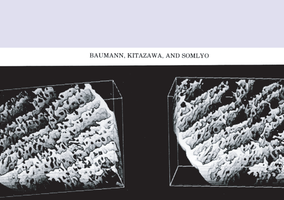
Shi, D., Somlyo, A.V., Somlyo, A.P. and Shao, Z (2001) Visualizing filamentous actin on lipid bilayers by atomic force microscopy in solution. *J. Miscrosc.,* 201:377-382.
Sheng, S., Gao, Y., Khromov, A.S., Somlyo, A.V., Somlyo, A.P., Shao, Z. (2003) Cryo atomic force microscopy of unphosphorylated and thiophosphorylated single smooth muscle myosin molecules. *J. Biol. Chem.*, 278:39892-39896.



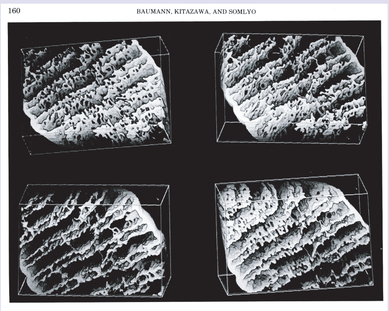
Stereo electron micrograph of 160 nm thick longitudinal section of vascular smooth muscle fiber. The ends of a complete myosin filament, 2.3 µm long, are indicated by the arrows. To the left, a "chain" of dense bodies with associated 10 nm filaments runs through the entire length of the Plate. 8% tannic acid in the fixative; lead citrate stained section. Tilt +/-10 degreees. Magnification 50,000X.

From Ashton et al., 1975



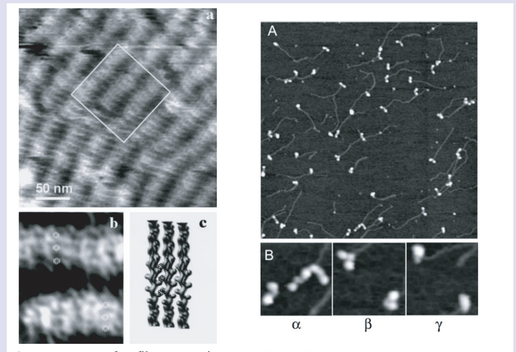
Transverse view of thick filament (myosin) and thin filaments (actin) connected by crossbridges.

From Ashton et al., 1975.



Several views of a depth gradient-shaded 3-D reconstruction of optical sections, 0.36-µm step each, obtained from a muscle. Note the connections between T-tubules within the same and between adjacent sarcomeres, and their "crossover" at a vernier dislocation. Box size is 34.0 µm x 22.5 µm x 10.1 µm, corresponding to collected data of 768 pixels x 512 pixels x 28 slices.

From Baumann et al., 1990



Arrangement of filaments in paracrystals. (a) Large-scale view. (b) zoomed at area marked by square frame, showing arrangements of individual filaments. (c) Model with 1 nm axial shift between neighboring filaments, in which the single F-actin is from electron microscopy reconstruction. The closest contacts between the filaments are marked by *.

From Shi et al., 2001

(A) Cryo-AFM image of freshly purified (dead heads removed) nonphosphorylated myosin on slightly negatively charged mica. (B) Examples of three different categories of myosin molecule conformations: α and β are essentially open conformations in which the two heads are separated; in the γ conformation, the two heads could not be distinguished in the iamge, suggesting that the two heads of these molecules cold be in close apposition. Image size = 1 µm.

From Sheng et al.,2003

Microanalysis

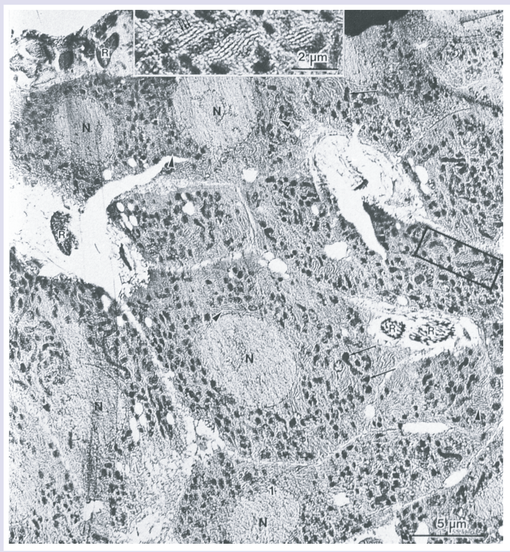
The Somlyos pioneered the application of a number of novel methods for biological research including electron probe X-ray microanalysis and electron energy-loss analysis. Together with Henry Shuman at the University of Pennsylvania, they developed new rapid freezing methods for the preservation of ion distributions in tissues and methods for processing x-ray spectra for the elemental detection of cellular calcium and other elements at high spatial resolution. It was through the use of such technology and electron microscopy that the Somlyos demonstrated the localization and quantified the amount of calcium stored in the sarcoplasmic reticulum (SR) of phasic and tonic smooth muscles. They also showed that SR of skeletal muscle was not in ionic communication with the extracellular space. Further, the elemental distribution indicated that there was no potential difference across the SR membrane; this finding negated the prevailing idea that depolarization of the SR triggered calcium release.

The Somlyos started using x-ray microanalysis in 1974, and added EELS in 1981. To answer important physiological questions, it is necessary to detect millimolar levels of diffusible ions, particularly calcium and phosphorus. This is difficult even today, but the Somlyos showed how it is possible (Schuman et al., 1976) and were major contributors to the techniques that are required. Specimen preparation is paramount, since useful results can only be obtained from tissue that faithfully reflects the *in vivo* condition. The carefully-controlled frozen-section methods they developed ensured this (Somlyo et al. 1977).

For EDX work, careful consideration of background subtraction, and peak fitting with reference spectra, led to methods capable of measuring concentrations below 10 mmol/Kg with 99% confidence level at nanometer-scale spatial resolution. To achieve the best possible precision with calcium, they found that direct fitting of unfiltered spectra was preferable to the standard use of the top hat filter to remove the continuum background (Ho et al., 2001). The filter was found to contribute errors by tending to create false peaks under weak signal conditions. Even a modest gain in precision is important because small changes in calcium concentration are physiologically significant.

Realizing early that EELS offers much higher sensitivity than EDX for detection of light elements, the Somlyos worked with Henry Schuman to build their own spectrometer. They recorded the first energy-filtered images from a post-column spectrometer in 1982. Important work was done in determining the optimal mean-free path (0.3) for detecting phosphorus (Wang et al. 1992), and accounting for errors due to thickness within the analyzed area by taking the average of the log-ratio method and the result from fitting the carbon edge/plasmon scattering (Ho et al., 1996). Development of the difference ratio method for extracting a weak edge (Feng, et al, 1999) freed EELS analysis from the limit to sensitivity that was imposed by detector gain variation.

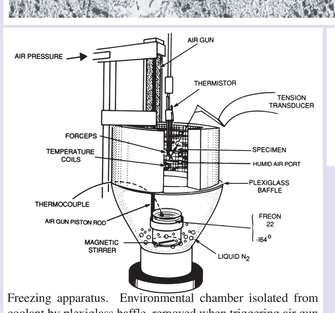
All of the experience and innovation in microanalysis accumulated over the years has been incorporated in an outstanding system for quantitative STEM-based EDX and EELS elemental mapping, described just this year (Feng et al., 2004).



Freeze-dried cryosection of rat liver. Nuclei (N) and numerous mitochondria (arrows) are indicated, as well as stacks of rough ER (arrowheads). The boxed region is enlarged (inset) to show the stacks of rough ER; some regions show periodic bumps (small arrows) along the membrane which are thought to be rebosomes. The EDX measurements of the Ca content of ER were obtained from such stacked tubules. Red blood cels (R) are also evident in the liver sinusoids.

For analysis, see table below.

From Somlyo et al., 1985.

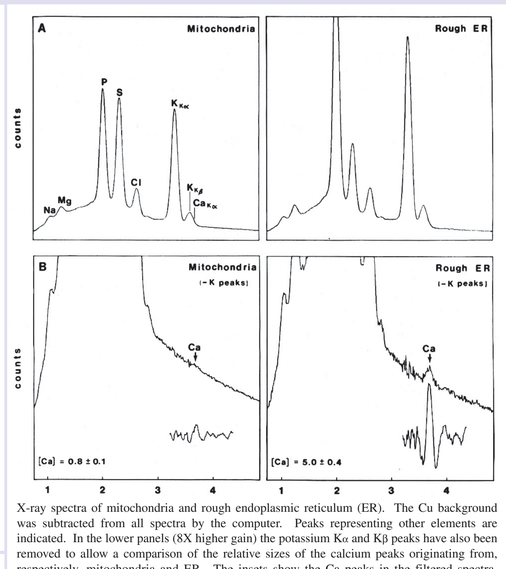


Freezing apparatus. Environmental chamber isolated from coolant by plexiglass baffle, removed when triggering air gun to raise Freon beaker at a speed of 80 cm/sec into chamber. Chamber maintained at 37 degrees with high humidity.

From Somlyo et al., 1989

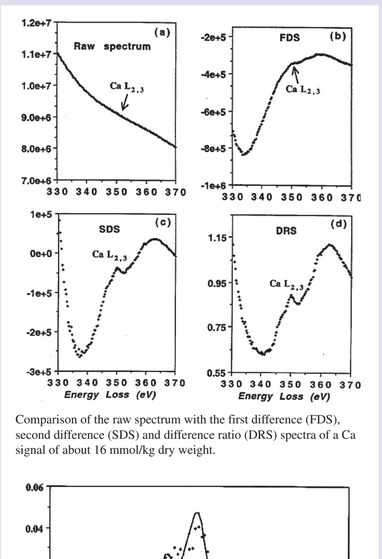
Subcellular distribution (mmol per kg dry weight)

(#Spectra)	Na	Mg	P	S	Cl	K	Ca
Mito(97)	54+/-3	43+/-1	360+/-7	316+/-4	75+/-3	314+/-5	0.8+/-0.1
ER(24)	80+/-8	76+/-2	920+/-21	224+/-8	94+/-5	579+/-17	5.0+/-0.4
Nuc(21)	77+/-6	53+/-2	557+/-16	245+/-8	117+/-6	625+/-22	0.8+/-0.4
Cyt(29)	75+/-6	53+/-2	653+/-20	241+/-9	103+/-5	465+/-15	4.0+/-0.4
Tot(20)	53+/-4	46+/-2	564+/-12	230+/-9	106+/-4	479+/-20	3.4+/-0.4

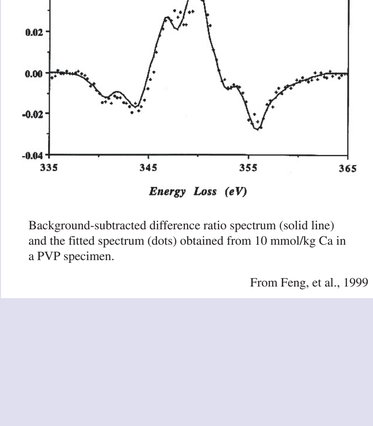


X-ray spectra of mitochondria and rough endoplasmic reticulum (ER). The Cu background was subtracted from all spectra by the computer. Peaks representing other elements are indicated. In the lower panels (8X higher gain) the potassium Kα and Kβ peaks have also been removed to allow a comparison of the relative sizes of the calcium peaks originating from, respectively, mitochondria and ER. The insets show the Ca peaks in the filtered spectra.

From Somlyo et al., 1985



Comparison of the raw spectrum with the first difference (FDS), second difference (SDS) and difference ratio (DRS) spectra of a Ca signal of about 16 mmol/kg dry weight.



Background-subtracted difference ratio spectrum (solid line) and the fitted spectrum (dots) obtained from 10 mmol/kg Ca in a PVP specimen.

From Feng, et al., 1999

Shuman, H., Somlyo, A.V. and Somlyo, A.P. (1976) Quantitative electron probe microanalysis of biological thin sections: Methods and validity. *Ultramicroscopy* 1:317-339.
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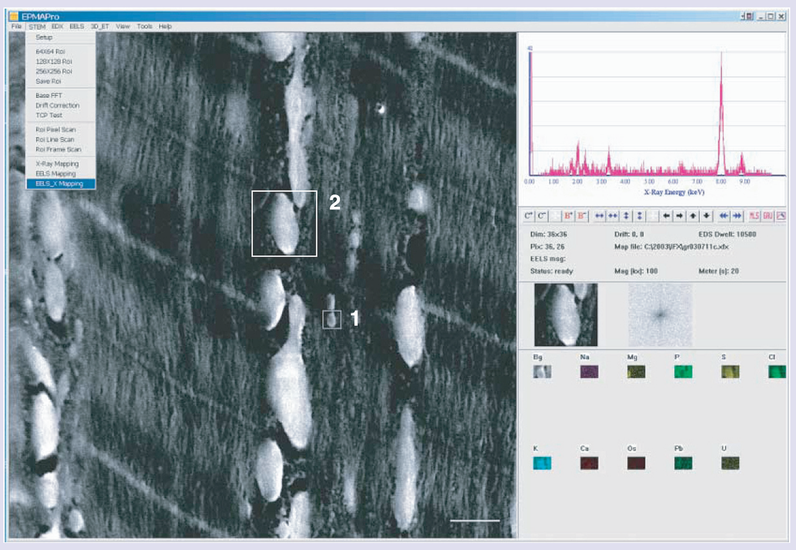
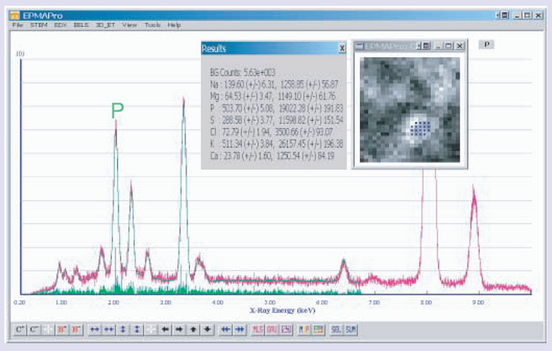
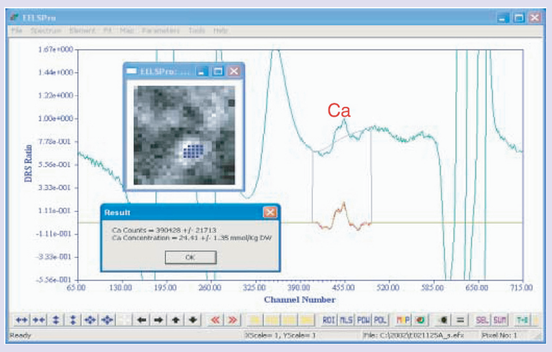
Wang, Y.Y., Ho, R., Shao, Z. and Somlyo A.P. (1992) Optimization of quantitative electron energy loss spectroscopy in the low loss region: Phosphorus L-edge. *Ultramicroscopy.* 41:11-31.
Ho, R., Shao, Z. and Somlyo, A.P. (1996) The effects of non-uniform specimen thickness on thickness determination and elemental quantitation with electron energy loss spectroscopy (EELS) *J. Microscopy Soc. Am.*2:87-97.
Feng, J.L., Ho, R., Shao, Z. and Somlyo, A.P. (1999) Parallel electron energy-loss spectroscopy free from gain variation. *Ultramicroscopy* 76:221-231.
Ho, R., Jia, Z., Somlyo, A.P., and Shao, Z. (2001) Improved precision of quantitating calcium in biological electron probe analysis. *J. Microsc.* 204:61-68.
Feng, J. Somlyo, A.V., and Somlyo, A.P. (2004) A system for acquiring simultaneous electron energy-loss and X-ray spectrum-images. *J. Microsc.* 215:92-99.



A screenshot of the main program EPMA_Pro in mapping mode. The left panel is an STEM image (1024x1024) of a cryosection (for sample preparation, see Somlyo et al., 1989) of myocardium. Boxed area 1 in the image is the mapping area, and area 2 over a mitochondrion is the reference area for drift-correction. The right panel contains four sections. The top section shown the collected X-ray spectrum at theprevious pixel position. The second section displays parameters: mapping area dimension, EDS dwell time (ms), current mapping pixel, specimen drift during the last acquisition, and message from EELS_Acq, etc. It also includes several buttons for image/spectrum manipulation. Section 3 shows the original reference image, its FFT, and the updated reference image (not shown here) for monitoring the sample drift and radiation damage. The bottom section displays the real-time compositional maps.





Off-line analysis of EDS (left panel) and EELS data from an element of corbular sarcoplasmic reticulum (cSR) in a cryosection of guinea-pig atrial muscle. The same mass-map is displayed for visual selection of pixels of interest. The pixels selected are marked by blue spots. EDS (left panel): the summed specgrum of the selected pixels is shown in pink, the multiple-least square fitted spectrum in green, and the (subtracted) residual spectrum in dark green. EELS (right panel): the summed spectrum (difference ration spectrum) is in green, the background-subtracted spectrum in red (Ca signal) and the fitted Ca spectrum in yellow.

Sources

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