CROSS-BRIDGE MECHANISM IN MUSCLE CONTRACTION

Edited by
Haruo Sugi and Gerald H. Pollack

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Explanation of Plates

Plate 1

(First row)	S. Okinaga/ H.E. Huxley, H. Sugi/ G.H. Pollack
(Second row)	S. Winegrad, G.H. Pollack, G.J. Steiger/ S. Ebashi, R. Natori, M. Endo
(Third row)	S. Winegrad/ D.C.S. White/ J. Dawson, E.M. Bartels, K. Yamada, Y. Umazume/ D.L. Brutsaert
(Fourth row)	T. Iwazumi, K.A.P. Edman/ E.M. Bartels, A. Drake, H.E.D.J. ter Kers, H.E. Huxley, M.I.M. Noble/ A. Oplatka

Plate 2

(First row)	T. Blangé/ J. Dawson, D.R. Wilkie, H. Shimizu, H. Mashima, R.T. Tregear, T. Sakai/ M.J. Kushmerick
(Second row)	M.M. Dewey/ D.R. Wilkie/ H. Shimizu/ R.C. Woledge
(Third row)	T.L. Hill/ During the session/ J.C. Rüegg
(Fourth row)	H. Sugi/ Dinner party at Happo-en/ M.I.M. Noble

Plate 1

Plate 2

Preface

Although more than twenty years have passed since the monumental discovery of a relative sliding motion between two sets of interdigitating filaments during length changes in striated muscle by H. E. Huxley, A. F. Huxley, and their co-workers, a question which still remains to be answered is, what makes the filaments slide? Among a number of possible causes for the relative sliding between the thick and thin filaments, the attention of most investigators has been focused on the cyclic interaction of the projections on the thick filaments—*i.e.* the cross-bridges—with the sites on the thin filaments to produce force and motion. In spite of considerable progress in the studies of muscle contraction, including mechanics, energetics, ultrastructural changes and enzyme kinetics of actomyosin ATPase, the cross-bridge mechanism is still a matter for speculation and controversy.

The purpose of this symposium is to thoroughly discuss various problems related to the current sliding filament/cross-bridge hypotheses. Since the validity of any contraction model should be tested on intact muscles or on other preparations in which three-dimensional filament-lattice structure is preserved, most papers presented are related to experiments on intact, skinned or glycerinated striated muscles. The results are discussed mainly in connection with the contraction models put forward by A. F. Huxley and A. F. Huxley and Simmons, which are central in the field of muscle physiology. In this connection, it is a great pity that we could not have Professor A. F. Huxley at this meeting, but it is our great pleasure to have Dr. H. E. Huxley, one of the founders of the current sliding filament hypotheses, with us.

This symposium consists of eight sessions. The following is an outline of the organization of the sessions.

Session I. Sarcomere dynamics. Studies on striated muscle at the level of sarcomeres are very important, since a sarcomere constitutes the functional unit in muscle contraction. Contrary to the general view that the length of the filaments does not change during contraction, Dewey *et al.* presents evidence that the thick filaments in *Limulus* striated muscle can shorten during contraction, suggesting a mechanism to produce force and motion other than the cross-bridges. By means of laser light diffraction technique, Pollack *et al.* demonstrate the stepwise shortening of sarcomeres in vertebrate cardiac and skeletal muscles; this suggests a synchronization of cross-bridge activity against the general concept of cross-bridges as independent force-generators.

Natori *et al.* show that overstretched skinned frog muscle fibers provide suitable material for studying the properties of the thick and thin filaments separately. Using the same preparations, Fujime *et al.* measure the flexibility of the thin filaments by analyzing optical changes when an electric field is applied.

Session II. Instantaneous elasticity and stiffness. The instantaneous elasticity or the stiffness of active muscle, as examined by tension changes in response to quick length changes (or *vice versa*), has generally been taken as a measure of cross-bridge elasticity. Pollack *et al.* describe a method of measuring the instantaneous elasticity in complex cardiac muscle tissues by use of laser light diffraction. Sugi demonstrates a uniform distribution of a highly compliant series elasticity along the length of single crayfish muscle fibers, indicating that it may originate mainly from structures other than the cross-bridges. He also shows a localized buckling at the released end of a frog muscle fiber during a quick release, suggesting that the tension changes in response to excessively quick length changes may not give correct information about the cross-bridges. Gott discusses the problems of measuring the instantaneous elasticity with quick length changes applied at one end, based on his multiple segment analysis of muscle responses. Rüegg *et al.* examine the stiffness of skinned or glycerinated cardiac, skeletal and insect fibrillar muscles in a variety of experimental conditions, including the replacement of ATP by AMP-PNP, to obtain information about cross-bridge properties. Another way of approaching cross-bridge properties is the so-called "sinusoidal analysis" technique, in which the tension changes in response to small sinusoidal length oscillations of varying frequency are studied. Kawai and Sagawa *et al.* apply this method to skinned skeletal muscle fibers and to intact cardiac muscles, respectively, and the visco-elastic elements inferred are discussed in connection with the cross-bridge properties.

Session III. Isotonic and isometric transients. The transient length changes after a quick change in load (isotonic transients) or the transient tension changes after a quick change in length (isometric transients) have been taken to reflect the kinetic properties of cross-bridge turnover. Though the isometric transients in insect fibrillar muscle is known to include a marked delayed rise in tension (stretch activation), its character is obscured by the presence of resting tension. In this connection, White *et al.* show that the mechanical properties of relaxed fibrillar muscle act functionally in parallel with the cross-bridges. Blangé and Stienen study the effect of metabolic inhibitors on the isometric transients in vertebrate skeletal muscle, showing that the quick tension recovery disappears after metabolic inhibition. Tsuchiya and Sugi's experiments are concerned with the isotonic transients in frog muscle fibers with special reference to marked oscillatory length changes following quick increases in load. Brutsaert *et al.* also study the isotonic transients in cardiac muscle in detail, including the time course of isotonic lengthening. Steiger's paper deals with the determination of rate constants on the time course of isometric transients and on the results of sinusoidal analysis in cardiac muscle.

Session IV. Length-tension relation. The well-known linear descending limb of the length-tension diagram in striated muscles has been taken to support the current sliding filament models in which the cross-bridges act as independent force-generators. ter Keurs *et al.* show, however, that the descending limb of the length-tension curve in single frog fibers is not linear at all, and that their results are not due to the dispersion in sarcomere length, thus challenging the concept of independent force-generators. Edman *et al.* demonstrate the enhancement of mechanical performance by stretch in single frog fibers; since the number of cross-bridges decreases with decreasing overlap between the filaments, this phenomenon is not readily explained by the current sliding filament models. Using rapid cooling contractures, Sakai also shows a non-linear descending limb of the length-tension relation in frog toe muscle. Maruyama reports the presence of a network of connectin, an elastic protein descovered by him, around the myofibrils in muscle, serving as a parallel elastic component. Winegrad and McClellan's paper deals with the control of contractile force by a cyclic AMP dependent mechanism in cardiac muscle.

Session V. Force-velocity relation. The independence of the shortening velocity at zero load (V_{max}) on the amount of overlap between the filaments has also been taken to support the concept of independent force-generators, though the measurement of V_{max} is technically difficult. Edman describes a new method of measuring the shortening velocity at zero load in single frog muscle fibers, and finds that it is length-independent over a wide range of sarcomere lengths but increases steeply at sarcomere lengths above 2.7 μm where resting tension introduces a compressive force. Endo studies the effect of viscosity of the surrounding medium on the mechanical performance of skinned frog muscle fibers in order to give information about the effect of viscosity on cross-bridge kinetics. Mashima studies the force-velocity relation in tetanized cardiac muscle under a variety of conditions, and determines the dynamic constants according to the Hill equation.

Session VI. X-ray diffraction studies. X-ray diffraction studies on resting, active and rigor muscles are extremely useful in obtaining information about the ultrastructural changes during contraction. The time resolution of X-ray diffraction studies has now been markedly improved by use of very intense X-ray sources such as synchrotron radiation. H. E. Huxley describes attempts to obtain direct evidence about the actual behavior of the cross-bridges by means of time-resolved X-ray diffraction of contracting frog skeletal muscle, including the recent finding that the 143 Å periodicity of the thick filaments increases during contraction. Another approach to the cross-bridge mechanism being made by Tregear *et al.* is to study the X-ray diffraction patterns from insect fibrillar muscle under various chemical conditions, with special reference to the effect of unhydrolyzable ATP analogs. Amemiya *et al.* show, with the time-resolved X-ray diffraction technique, that the intensity ratio of two equatorial reflections ($I_{1,0}/I_{1,1}$), which is generally taken as a measure of attached cross-bridges, does not change by stretching active frog muscle, and that the intensity ratio is smaller during an isotonic twitch under a small load than during an isometric twitch. The papers of Namba *et al.* and Maéda are related to the detailed structural analysis of the thin filaments in crab muscles in rigor to give information about the mode of attachment of the cross-bridges to the thin filaments. Their results indicate that crab muscle provides suitable material for studying the ultrastructure of the thin filaments.

Session VII. Energetics. Since the classic work of A.V. Hill, muscle energetics has constituted an important field of muscle physiology, providing information about how ATP splitting is coupled with cross-bridge performance. Woledge and Curtin review the problems on muscle energetics, and report their recent work on the effect of stretch on the rate of energy output in active frog skeletal muscle. Kodama and Yamada construct a model to explain the rate of energy output and the rate of ATP splitting in shortening muscle, based on enzyme kinetics of actomyosin ATPase. Hoh demonstrates the presence of myosin isoenzymes in cardiac and skeletal muscles, and shows that their profile in fast-twitch and slow-twitch muscles can be reversed by nerve cross-union. Kushmerick studies the relation between the high-energy phosphate utilization during contraction and the subsequent metabolic recovery reactions. Wilkie *et al.* describe their pioneering work in simultaneously recording the ^{31}P nuclear magnetic resonance spectra and the mechanical activity in intact skeletal muscles, and measure the time course of change in metabolite levels during contraction to give information about cross-bridge cycling.

Session VIII. Theories of contraction. For the future development of studies of cross-bridge mechanisms, it is very useful to construct new theories of muscle contraction which, since they can be tested experimentally, stimulate the interest of investigators. Hill describes a simplified theory which accounts for the results of Huxley and Simmons in terms of transitions between attached states of the cross-bridges. Shimizu illustrates his three-state contraction model, emphasizing the dynamic cooperativity of the cross-bridges—contrary to the general view that the cross-bridges act independently. Oplatka *et al.* propose a hydrodynamic theory for muscle contraction and for other primitive motile systems, which is fundamentally different from the current sliding filament hypotheses. Iwazumi's new field theory of muscle contraction is also fundamentally different from the general concept of cross-bridges, resting on the assumption that the cross-bridges are dipole field generators so that electrostatic forces are generated between the cross-bridges and the tips of dielectric thin filaments, and covers many phenomena which are not readily explained by the current sliding filament models, including the longitudinal and lateral stabilities of the filament-lattice structure.

As described above, this symposium contains many papers indicating various shortcomings of the current sliding filament/cross-bridge hypotheses. It underscores the necessity for careful reexamination of previous experiments which are generally regarded as supporting the current hypotheses. We believe that the papers and the controversial discussions following them will be useful for people interested in the cross-bridge mechanism, and hope that this volume will be an impetus for future progress in the study of muscle contraction.

H. Sugi
G. H. Pollack

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Participants

Symposium on the Current Problems of Sliding Filament Model and Muscle Mechanics held at the Hotel New Japan, Tokyo, 13-15 September, 1978

AMEMIYA, Y.
Engineering Research Institute, Faculty of Engineering, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan,

ARATA, T.
Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

BARTELS, E. M.
The Open University, Oxford Research Unit, Foxcombe Hall, Berkeley Road, Boars Hill, Oxford, England

BLANGÉ, T.
Fysiologisch Laboratorium, Jan Swammerdam Instituut, Universiteit van Amsterdam, Eerste Constantijn Huygensstraat 20, Amsterdam, The Netherlands

BRUTSAERT, D. L.
Department of Physiology, University of Antwerp, 2020 Antwerp, Belgium

DAWSON, J.
Department of Physiology, University College London, Gower Street, London WC1E 6BT, England

DEWEY, M. M.
Department of Anatomical Sciences, School of Basic Health Sciences, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York 11794, U.S.A.

DRABIKOWSKI, W.
Department of Biochemistry of Nervous System and Muscle, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, Warszawa 22 Poland

DRAKE, A.
The Midhurst Medical Research Institute, Midhurst, West Sussex GU29 OBL, England

EBASHI, S.
Department of Pharmacology, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

EDMAN, K. A. P.
Department of Pharmacology, University of Lund, Sölvegatan 10, S-223 62 Lund, Sweden

ENDO, M.
Department of Pharmacology, Faculty of Medicine, Tohoku University, Seiryo-cho, Sendai-shi 980, Japan

FUJIME, S.
Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan

GERGELY, J.
Boston Biomedical Research Institute, Boston, Massachusetts, U.S.A.

GILBERT, S. H.
Physilogie Générale des Muscles, Université Catholique de Louvain, Bruxelles, Belgium

GOTT, A. H.
Department of Physiology, Pharmacology and Biophysics, School of Medicine, Loma Linda Unversity, Loma Linda, California 92354, U.S.A.

HASHIZUME, H.
Engineering Research Institute, Faculty of Engineering, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

HILL, T. L.
National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014 U. S. A.

HOH, J. F. Y.
Department of Physiology, The University of Sydney, Sydney, N.S.W. 2006, Australia
HUXLEY, H. E.
MRC Laboratory of Molecular Biology, University Medical School, Hills Road, Cambridge CB2 2QH, England
ISHIDE, N.
Department of Internal Medicine, School of Medicine, Tohoku University, Seiryo-cho, Sendai-shi 980, Japan
IWAZUMI, T.
Anesthesia Research Center, Department of Anesthesiology, School of Medicine, University of Washington, Seattle, Washington 98195, U.S.A.
KAMIYAMA, A.
Department of Physiology, School of Medicine, Teikyo University, Itabashi-ku, Tokyo 173, Japan
KAWAI, M.
Laboratory of Neurophysiology, Department of Neurology, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York 10032, U.S.A.
KODAMA, T.
Department of Pharmacology, School of Medicine, Juntendo University, Bunkyo-ku, Tokyo 113, Japan
KOMETANI, K.
Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
KRAMER, A. E. J. L.
Department of Urology, Academisch Ziekenhuis-Leiden, Rijnsburgerweg 10, Leiden, The Netherlands
KUSHMERICK, M. J.
Department of Physiology, Harvard Medical School, Boston, Massachusetts 02115, U.S.A.
MAÉDA, Y.
Department of Pharmacology, Faculty of Medicine, Tohoku University, Sendai-shi 980, Japan
MARUYAMA, K.
Department of Biology, Faculty of Science, Chiba University, Chiba-shi 280, Japan
MASHIMA, H.
Department of Physiology, School of Medicine, Juntendo University, Bunkyo-ku, Tokyo 113, Japan
MATSUBARA, S.
Department of Neuropsychiatry, Kanazawa Medical University, Ishikawa-ken 920–02, Japan
MATSUMOTO, Y.
Service de Chimie Physique II, Campus Plaine U.L.B., Boulevard de Triomphe, 1050 Bruxelles, Belgium
MATSUMURA, M.
Department of Physiology, Kawasaki Medical College, Kurashiki-shi, Okayama 701, Japan
MITSUI, T.
Department of Biophysical Engineering, Osaka University, Toyonaka, Osaka 560, Japan
MOHAN, R.
Physiologisches Institut, Lehrstuhl für Klinische Physiologie, Universität Düsseldorf, 4 Düsseldorf, Moorenstrasse 5, West Germany
MORIMOTO, S.
Department of Physiology, Jikei University Medical School, Minato-ku, Tokyo 105, Japan
NAMBA, K.
Department of Biophysical Engineering, Osaka University, Toyonaka, Osaka 560, Japan
NATORI, Reiji
President, Jikei University Medical School, Minato-ku, Tokyo 105, Japan
NATORI, Reibun
Department of Physiology, Jikei University Medical School, Minato-ku, Tokyo 105, Japan
NISHIYAMA, K.
Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
NOBLE, M. I. M.
The Midhurst Medical Research Institute, Midhurst, West Sussex GU29 OBL, England

O'BRIEN, E. J.
Department of Biophysics, School of Biological Sciences, University of London King's College, 26–29 Drury Lane, London WC2B 5RL, England

OGAWA, Y.
Department of Pharmacology, School of Medicine, Juntendo University, Bunkyo-ku, Tokyo 113, Japan

OKINAGA, S.
President, Teikyo University, Itabashi-ku, Tokyo 173, Japan

OPLATKA, A.
Polymer Department, The Weizmann Institute of Science, Rehovot, Israel

OTSUKI, I.
Department of Pharmacology, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

POLLACK, G. H.
Anesthesia Research Center, Department of Anesthesiology, School of Medicine, University of Washington, Seattle, Washington 98195, U.S.A.

RALL, J. A.
Department of Physiology, The Ohio State University, 1645 Neil Avenue, Columbus, Ohio 43210, U.S.A.

RÜEGG, J. C.
II Physiologisches Institut, Universität Heidelberg, Im Neuenheimer Feld 326, 6900 Heidelberg 1, West Germany

SAEKI, Y.
Department of Physiology, School of Dentistry, Tsurumi University, Tsurumi-ku, Yokohama 230, Japan

SAGAWA, K.
Department of Medical Engineering, School of Medicine, The Johns Hopkins University, 720 Rutland Avenue, Baltimore, Maryland 21205, U.S.A.

SAKAI, T.
Department of Physiology, Jikei University Medical School, Minato-ku, Tokyo 105, Japan

SHIMIZU, H.
Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

STEIGER, G. J.
Department of Physiology, School of Medicine, The Center for the Health Sciences, University of California at Los Angeles, Los Angeles, California 90024, U.S.A.

STIENEN, G. J. M.
Fysiologish Laboratorium, Jan Swammerdam Instituut, Universiteit van Amsterdam, Eerste Constantijn Huygensstraat 20 Amsterdam, The Netherlands

SUGI, H.
Department of Physiology, School of Medicine, Teikyo University, Itabashi-ku, Tokyo 173, Japan

SUZUKI, S.
Department of Physiology, School of Medicine, Teikyo University, Itabashi-ku, Tokyo 173, Japan

TAMEYASU, T.
Department of Physiology, School of Medicine, Teikyo University, Itabashi-ku, Tokyo 173, Japan

TANAKA, H.
Department of Physiology, School of Medicine, Teikyo University, Itabashi-ku, Tokyo 173, Japan

ter KEURS, H. E. D. J.
Laboratory for Physiology, State University of Leiden, Wassenaarseweg 62, Leiden, The Netherlands

TSUCHIYA, T.
Department of Physiology, School of Medicine, Teikyo University, Itabashi-ku, Tokyo 173, Japan

TREGEAR, R. T.
Department of Zoology, ARC Unit of Insect Physiology, University of Oxford, South Parks Road, Oxford OX1 3PS, England

UMAZUME, Y.
Department of Physiology, Jikei University Medical School, Minato-ku, Tokyo 105, Japan

WAKABAYASHI, K.
Department of Biophysical Engineering, Osaka University, Toyonaka, Osaka 560, Japan

WAKABAYASHI, T.
Department of Physics, Faculty of Science, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
WHITE, D. C. S.
Department of Biology, University of York, Heslington, York YO1 5DD, England
WILKIE, D. R.
Department of Physiology, University College London, Gower Street, London WC1E 6BT, England
WINEGRAD, S.
Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, U.S.A.
WOLEDGE, R. C.
Department of Physology, University College London, Gower Street, London WC1E 6BT, England
YAMADA, K.
Department of Physiology, School of Medicine, Juntendo University, Bunkyo-ku, Tokyo 113, Japan
YAMADA, T.
Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
YAMAGUCHI, T.
Department of Biology, Division of Natural Sciences, College of Liberal Arts, International Christian University, Mitaka-shi, Tokyo 181, Japan
YAMAMOTO, T.
Department of Physiology, Faculty of Dentistry, Kyushu University, Fukuoka-shi 812, Japan
YANAGIDA, T.
Department of Biophysical Engineering, Osaka University, Toyonaka, Osaka 560, Japan
YOSHINO, S.
Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan