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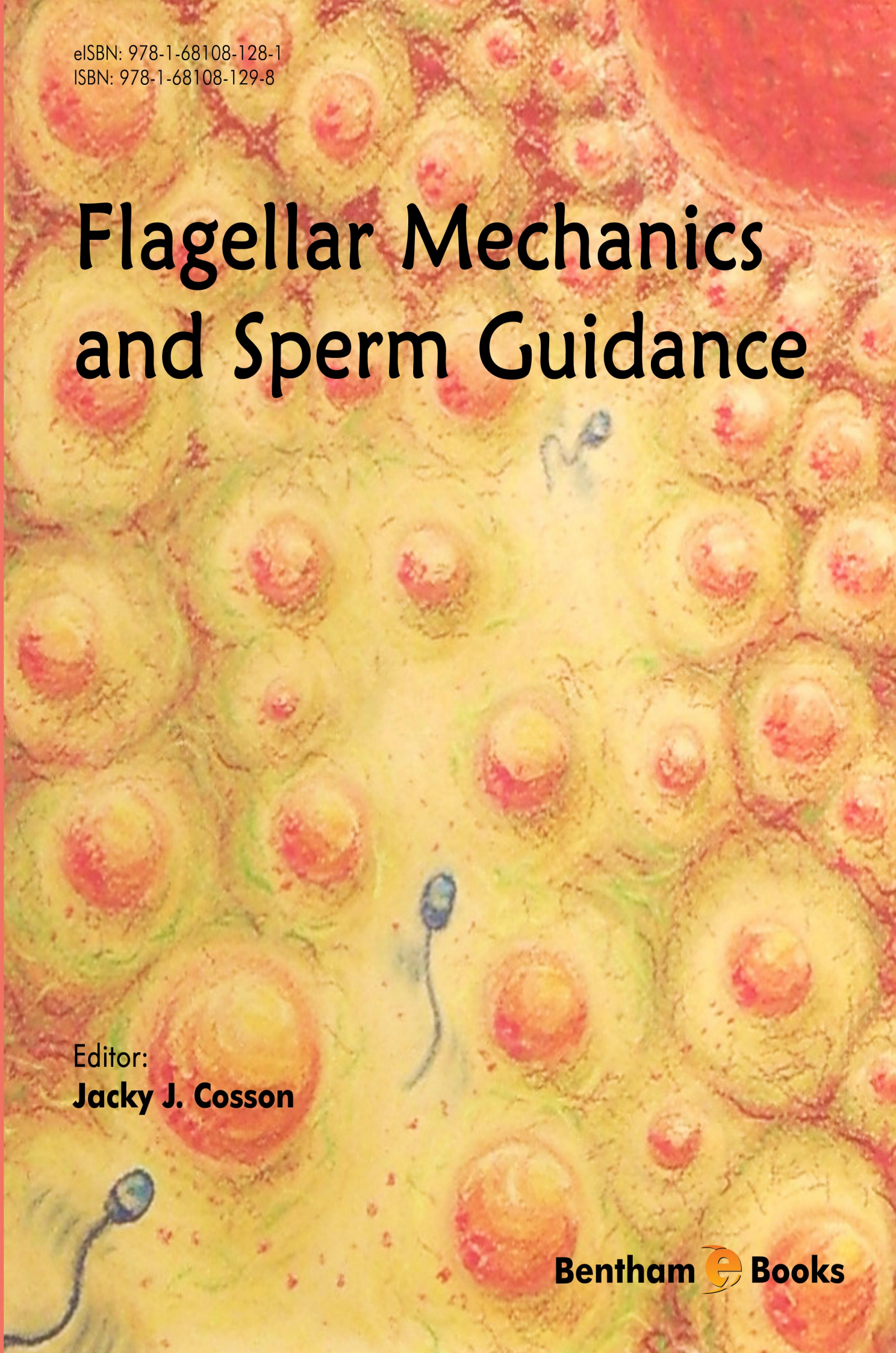
Flagellar Mechanics and Sperm Guidance



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CONTENTS

Book Cover: "Encuentro", pastel, 2006, by Laura C. Giojalas

FOREWORD

Experimental research using spermatozoa, a highly specialized cell with cell organelles involved in transfer of genetic information, energy supply and motility, has always occupied the forefront of studies in cell biology, as well as reproductive biology. Among trend variety of research carried out on sperm cells, attraction of sperm toward the female gamete, so-called sperm chemotaxis or sperm guidance, which ubiquitously occurs in reproductive system of many animals and plants, plays an important role in fertilization success. Sperm chemotaxis in plant and animal species was discovered in the late part of 19th century and in the middle of 20th century, respectively, and thereafter, detailed fundamental mechanisms of the sperm behavior were developed. Application of research in sperm science to the medical and agricultural fields has become very important in more recent years. Structure of molecules relevant to sperm chemotaxis has been recently determined in several species of invertebrates and vertebrates, further remarkable progress for understanding events relevant to reproductive system. Reflecting these trends in the long history concerning sperm chemotaxis, the chapters of the book *"Flagellar Mechanics, its Contribution to Sperm Guidance",* result from an assembly of the world-leading scientists in the field, who focused on sperm chemotaxis toward female gamete with chemical cue in protists, marine invertebrates and vertebrates including mammals. Topics relevant to sperm behavior complementary to chemotaxis such as initiation and activation of sperm motility at spawning, sperm thermotaxis, mathematical modeling for micro-swimming, *etc*. are also included in the book. Basic knowledge presented in this book will be of great help to stimulate the interest of research in a broad variety of readers.

Masaaki Morisawa
The University of Tokyo,
Japan

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DEDICATION

This book is dedicated to the memory of the late Marie-Paule COSSON, who passed away in 1991 after many years of studies on flagella motility and sperm chemotaxis.

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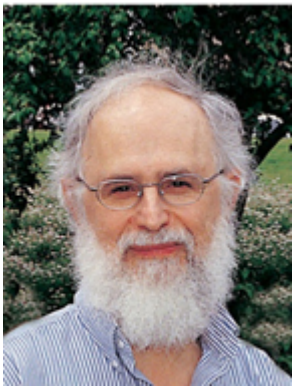
Gary N. Cherr: Received his B.A. from Sonoma State University, California in 1979 and his Ph.D. in Zoology from University of California Davis in 1984. Gary has focused his research on fish and mammalian sperm-egg interactions, including induction of the acrosome reaction, sperm motility initiation, and the sperm glycocalyx. His laboratory has investigated ionic regulation and signaling in sperm as well as the role of sperm surface molecules in immunoprotection and interaction with the female reproductive tract. His research on herring has focused on environmental factors regulating fertilization and development, including understanding how different stressors affect these reproductive processes. Gary is Professor of Environmental Toxicology and Nutrition and is presently the Director of the UC Davis Bodega Marine Laboratory.

Jacky J. Cosson: Born in France in 1944, J. Cosson graduated in the Univ. of Paris-South (France) with a B.Sc in Chemistry and a M.Sc in Biophysics followed by a Ph.D in Biochemical Genetics defended in 1976 on yeast mitochondrial genes expression. He stayed as a two years PostDoc in USA, during 1976 in Univ. of Hawaii, on mitochondrial ATP-synthase and in 1977 in Columbia Univ. (New York) on sequencing of mitochondrial ATP-synthase genes. He obtained a permanent position in the French CNRS (National Research Agency) in 1969 and retired from CNRS as Research Director in 2010. Since he moved to the Marine Station (P. & M. Curie/Paris 6 Univ.) located close to Nice in 1981, his main topics of interest are focused on studies of the mechanics and regulation of the movement of cilia and flagella in many species (fishes and other aquatic animals, mammalian, *etc*) and cell types such as spermatozoa, protists, embryonic cells *etc*. On these topics, he collaborated with and visited many colleagues all over the world and attended many International Conferences. He also developed several techniques for recording and studying in details flagellar beating characteristics. After a one-year stay in the Univ. of French Polynesia as a visiting professor in 2009, he received in 2010 Honoris Causa Doctor distivtion by the University of South Bohemia (Czech Rep.) as an award for his long-term collaboration with this University on studies on fish spermatozoa. Since his retirement, he is half-time present in this University where he is, nowadays, in charge of several Ph.D. students and received several Research Grants.

Alberto Darszon is Full Professor in Cell Biology at the Instituto de Biotecnología (IBT), Departamento de Genética del Desarrollo y Fisiología Molecular of the National Autonomous University of Mexico (UNAM). He did his Bachelor's degree in organic chemistry at the Universidad Iberoamericana, his Ph. D. at the Center for Research and Advanced Studies of the National Polytechnical Institute (CINVESTAV), and his postdoctoral work at the University of California in San Diego. As sperm are tiny and their electrophysiological characterization difficult, his group has combined planar bilayer techniques, *in vivo* patch clamp and membrane potential (Em), intracellular Ca^{2+} ($[Ca^{2+}]_i$ and intracellular pH (pHi) measurements with Molecular Biology and Biochemistry to study how sperm ion channels participate in the main functions of this cell and in fertilization. Sperm are attracted by diffusible chemical factors (che-moattractants) released from the egg, which redirect their swimming paths towards their source. This redirection is driven by increases in flagellar curvature that correlate with transient flagellar Ca^{2+} increases regulated by Em and pHi changes. Our recent experimental and modeling results provide insights into the signal flow underlying the translation of an external chemical gradient into an intracellular molecular and motor response. The ability of sperm to suppress Ca^{2+}-mediated increases in flagellar curvature while experiencing an increasing chemo-attractant gradient is fundamental for sea-urchin sperm chemotaxis.



Michael Eisenbach: Born in Tel Aviv, Michael Eisenbach graduated from Tel Aviv University with a BSc in chemistry, and an MSc and a PhD in biochemistry. He did his postdoctoral research at the Weizmann Institute of Science and at the University of Wisconsin, Madison. He returned to the Weizmann Institute in 1980, where he is a Professor in the Department of Biological Chemistry. He has served as the Departmental Chair, Director of the Josef Cohn Minerva Center for Biomembrane Research, Chairman of the Israel National Committee for Microbiology of the Israel Academy of Sciences and Humanities, Chairman of Weizmann Institute's Scientific Council, President of the Israel Society for Biochemistry and Molecular Biology, and President of the Pasteur-Weizmann Scientific Council. He is the incumbent of the Jack and Simon Djanogly Professorial Chair in Biochemistry. Eisenbach's research focuses on cell guidance in bacteria and sperm cells. He opened the field of mammalian sperm guidance by demonstrating that sperm cells of mammals are equipped with machineries that enable them to respond to gradients of attractive chemicals and temperature similar to those existing at ovulation in the female genital tract. In 2010, he was awarded the Sarov Prize in Mic-robiology from the Israel Society for Microbiology.

Daniel Alejandro Priego Espinosa obtained his bachelor's degree in the interdisciplinary Undergraduate Program on Genomic Sciences of the National Autonomous University of Mexico (UNAM). Thereafter, he became interested in developmental biology and systems biology. He is currently associated to the Institute of Physical Sciences, UNAM, as a Ph.D. student of the Graduate Program on Biomedical Sciences. My research is focused on mathematical modeling of the signaling network responsible of steering the swimming of sea urchin sperm.

Eamonn A. Gaffney Following a Ph.D. in Theoretical High Energy Physics at Cambridge UK, EAG subsequently pursued a Wellcome Trust Post-Doctoral Fellowship at The Wolfson Centre for Mathematical Biology Oxford, UK, studying biological and biomedical applications of mathematics. This was followed by a faculty position at The School of Mathematics, University of Birmingham, before returning to Oxford and the Wolfson Centre for Mathematical Biology in 2006. His research is mainly in the field of physiological fluid dynamics and transport, especially cellular microswimming; ciliary and ocular fluid flows; the transport of oxygen, metabolites and waste products in skeletal and cardiac muscle; and the transport of water and ions across epithelia. Further interests include mechanisms of cellular self-organisation.

Laura Cecilia Giojalas is Biologist. She is Full Professor of Cell Biology at the University of Cordoba and Principal Investigator of the National Council of Research in Argentina. She is the Director of the Center for Cell and Molecular Biology of the same University. She has been postdoctoral fellow of the Weizmann Institute (Israel) in 1995. In the last 20 years, her laboratory has been focused on understanding the chemical interaction between mammalian gametes before fertilization, with particular interest in sperm guiding navigation (mainly chemotaxis) and its potential application for Assisted Reproduction.

Frederick J. Griffin: Born in Palo Alto, California in 1947. Received an M.S degree in Biology from Sonoma State University in 1981, a Ph.D. in Zoology from University of California, Davis in 1987, and was a Research Scientist at the Bodega Marine Laboratory until his retirement. His research has paralleled those of the Reproductive Toxicology Group (Gary N. Cherr, PI) at the University of California Davis Bodega Marine Laboratory, and have include research into: 1) the mechanisms of gamete interaction and embryonic development; 2) the mechanisms by which natural and anthropogenic stressors affect fertilization and early life stages of a variety of aquatic organisms; and 3) the consequences of such stresses to organisms in the natural environment. Much of the research has in the past and continues in the present to involve multi-disciplinary collaborations with other investigators at both the marine laboratory and at other institutions and as such has provided a broad basis for scientific investigation. Since his retirement he has also devoted time to teaching undergraduate biology courses at Sonoma State University, California.

Adán Guerrero is a researcher at the Laboratorio Nacional de Microscopía Avanzada hosted by the Instituto de Biotecnología, Universidad Nacional Autónoma de México (LNMA/IBt-UNAM), Cuernavaca, Mexico. He carried out his Ph.D. with Alberto Darszon at the IBt-UNAM where he contributed to the understanding of sperm chemotaxis studying marine invertebrates; his postdoctoral research with Mónica Bettencourt-Dias at the Instituto Gulbenkian de Ciência (IGC; Oeiras, Portugal) was focused on understanding how centriole biogenesis is regulated in space and time. He actually continues studying centriole biogenesis and sperm chemotaxis form a quantitative perspective through the developing of image processing tools and computational models in combination with newer experimental assays.

Héctor Alejandro Guidobaldi is Biologist. He is Assistant Professor of Cell Biology at the University of Córdoba and Assistant Investigator of the National Council of Research in Argentina. In 2008 he obtained his Ph.D., by studying sperm transport mechanisms in mammalian spermatozoa, under the supervision of Dr. Giojalas. Since then, he continued working in the same field now also focused on the development of microdevices for sperm selection.

Kazuo Inaba: Born in Yamanashi, Japan in 1962. B. Sc, Biology, Faculty of Science, Shizuoka University in1985. M.Sc, Graduate School of Science, University of Tokyo in 1987. D. Sc, Graduate School of Science, University of Tokyo in 1990. Assistant professor, Faculty of Science, University of Tokyo (Misaki Marine Biological Station) in 1990-1996. Visiting Scientist, Worcester Foundation, MA, USA in 1996. Assistant professor, Graduate School of Science, University of Tokyo in 1996-1998. Associate professor, Faculty of Science, Tohoku University in 1998-1999. Associate professor, Graduate School of Science, Tohoku University in 1999-2004. Visiting associate professor, National Institute for Basic Biology in 2002. Professor, Shimoda Marine Research Center, University of Tsukuba in 2004-present. Director, Shimoda Marine Research Center, University of Tsukuba in 2005-present. President, Japanese Association for Marine Biology, 2009-present. K. Inaba received Ph.D. to the study on conformational changes of dyneins from sea urchin sperm. Since 1990, he has been studying the structure, function, regulation and evolution of eukaryotic cilia and flagella using variety of organisms, including sperm from marine invertebrates, fish and mouse; embryos and larvae of marine invertebrates; fungi; Chlamydomonas and other algal species.

Kenta Ishimoto After receiving his Ph.D. from Kyoto University in 2015, KI is appointed as an assistant professor at The Hakubi Center for Advanced Research, Kyoto University, Japan, and is concurrently a project assistant professor at Research Institute for Mathematical Sciences (RIMS), Kyoto University. His research is mainly on theoretical hydrodynamics of swimming cells with cilia and flagella.

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Gustavo Martínez-Mekler obtained a degree in physics from the National Autonomous University of México (UNAM), an M.Sc in mathematics from Warwick University and a Ph.D. in physics from the University of Manchester. He has been visiting professor at the University of Illinois, Urbana Champagne and the University of Florence. Presently he is full professor at the Institute of Physical Sciences (UNAM) and member of the governing board of the Center for Complexity Sciences (UNAM). Based on a background in statistical physics and dynamical systems he has looked through a complex systems perspective into physical (phase transitions and criticality in condensed matter), geophysical (volcanism and earthquakes) and biological phenomena. In the latter, mostly with a systems biology approach, he has addressed ecological succession, origin of the genetic code, HIV genetic sequence evolution, embryology, immunology and fertilization issues. Recently, his trans-disciplinary interests have also lead him to the study of complexity in the arts.

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Murali C. Pillai: Received his B.Sc. in Zoology from University of Kerala, India in 1977, his M.Sc. in Zoology from University of Poona, India in 1980, and his Ph.D. in Reproductive Biology from University of California Davis in 1988. Murali worked on crustacean sperm-egg interaction and later the control of the mammalian sperm acrosome reaction and sperm hyaluronidases. He has investigated herring sperm motility and gamete interaction for many years and is presently Professor of Biology at Sonoma State University, California where he also serves as Department Chair.

Galina Prokopchuk obtained a Master degree in biophysics at the chair of Biological and Medical Physics, V.N. Karazin Kharkiv National University (Kharkiv, Ukraine) in 2011. She joined the Laboratory of Reproductive Physiology at the Research Institute of Fish Culture and Hydrobiology in Vodnany, Faculty of Fisheries and Protection of Waters, University of South Bohemia (Czech Republic) for doctoral study in October 2011. Her thesis subject is on description of fish spermatozoa flagellar movement in various physiological situations, establishing the interrelationships between physical and biochemical controls of fish sperm motility, mechanistic aspects of the flagellum functioning as well as description of the initiation and regulation of flagellar motility in various fish species.

Raúl Sánchez is Gynecologist and Andrologist. He is Full Professor of Preclinical Sciences in the Faculty of Medicine, and Director of the Center for Biotechnology of Reproduction at the University of La Frontera in Chile. He became Vice Dean of the same university. He was doctoral and postdoctoral fellow of the Alexander von Humboldt Foundation. He has mainly investigated sperm physiology and gamete preservation methods in several mammalian and non- mammalian species, and its clinical applications in health and disease.

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Carol A. Vines: Received her Ph.D. in Pharmacology and Toxicology from the University of California at Davis in 1999 where her thesis focused on sperm motility in Pacific herring and the effects of creosote pilings on development of Pacific herring larvae. She is currently a project scientist in Dr. Gary N. Cherr's laboratory at the Bodega Marine Laboratory, where she has continued to investigate sperm motility and function in fish and mammals, as well as fertilization and early development in fish and invertebrates. Research interests have also included the effects of polycyclic aromatic hydrocarbons on fish and invertebrate embryos (including oil spill effects) and endocrine disruption in estuarine fish. She is currently investigating the effects of nanomaterials on physiological processes in invertebrates.

Chris Wood is an optical microscopist and cell biologist, and Director of the Laboratorio Nacional de Microscopía Avanzada (National Laboratory for Advanced Microscopy), incorporated into the Instituto de Biotecnología, UNAM. He graduated from the University of Oxford with a Bachelor's degree in Biochemistry, and in 2000 was awarded his Ph.D. by the University of Liverpool, during which he developed a novel photon-counting assay for measuring luciferase activity in single cells. While undertaking postgraduate studies in Ca^{2+} signalling at fertilization, he met Dr. Alberto Darszon of the Instituto de Biotecnología, UNAM, who persuaded him to tackle the far more challenging problem of determining the role of Ca^{2+} transients in swimming spermatozoa. These studies continued for 5 years after his arrival in Mexico in 2002, whereupon he took an Associate Researcher position in the laboratory of Dr. Luis Covarrubias, developing macro-bioluminescence imaging techniques for studying embryonic development of the midbrain in mice. Since 2008 he has worked on establishing Mexico's first open-access microscopy core facility, and in after receiving funding from Conacyt and UNAM in 2011, the Laboratorio Nacional de Microscopía Avanzada opened its doors to scientific researchers in any discipline or Institution in January 2013.

Ryuzo Yanagimachi: Received his B.Sc. from Hokkaido University, Japan in 1952 and his D.Sc. from Hokkaido University in 1960 where he conducted research on herring fertilization and reproduction of parasitic cirripeds. He was a postdoctoral fellow at Worcester Foundation for Experimental Biology from 1960-64 to begin mammalian fertilization studies and a Professor at the University of Hawaii Medical School since 1966. Yana has been emeritus since 2004. For nearly four decades, his research has focused on gametes and fertilization biology. His work has advanced not only basic knowledge of mammalian fertilization, but also contributed to the development of assisted fertilization technologies, such as in vitro fertilization and intra-cytoplasmic sperm injection (ICSI), which are widely used in human infertility clinics. He also contributed to the field of transgenesis and cloning. In 2000, he founded and was the first Director of the Institute for Biogenesis Research, housed in the Department of Anatomy, Biochemistry and Physiology at the University of Hawaii. Under his leadership, research in the institute expanded to include stem cell biology, genome structure and its role in embryogenesis, transgenesis technology, and the elucidation of molecular mechanisms during normal and abnormal embryo development. Yana was elected as a member of the National Academy of Sciences in 2001.

Manabu Yoshida: Is Associate Professor of Misaki Marine Biological Station, School of Science, University of Tokyo. He was born in Chiba, Japan, graduated from University of Tokyo with a BSc in Biology, and was awarded a PhD in Zoology in 1995. He did hid postdoctoral research at RIKEN Brain Science Institute. In 1998 he returned to Misaki Marine Biological Station as a Assistant Professor. In 2001 he moved to Japan Science and Technology Agency, and studied on the Ca^{2+} oscillations of eggs after fertilization. Since 2003 he has worked at Misaki Marine Biological Station, and focused on understanding the regulation of sperm function by female and male factors in the process of fertilization, especially chemotaxis and capacitation. He was awarded the Awards for Young Scientist from Zoological Society Japan.

CHAPTER 1

The Flagellar Mechanics of Spermatozoa and its Regulation

Jacky J. Cosson[1,*], Galina Prokopchuk[1], Kazuo Inaba[2]

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Abstract: Motility is a characteristic function of the male gamete, which allows spermatozoa to actively reach and penetrate the female gamete in organisms with internal and external fertilization. Sperm motility is acquired under the control of many extrinsic and intrinsic factors and is based on the specialized structure of the sperm flagellum. An overview of how the sperm flagellum is organized and works to support cell motility is presented, with special focus on the molecular mechanisms and factors involved in the development and maintenance of sperm motility. Data obtained both in organisms with external fertilization, such as sea urchins, ascidians or fishes as well as those relying on internal fertilization, such as mammals, are critically analyzed.

In most animal species, sperm motility is dependent on a long appendage called flagellum. Flagella are essential organelles found in most eukaryotic cells: their basic structure is the axoneme, built of a scaffold of microtubules and responsible for movement generation in an autonomous manner provided energy in the form of ATP is present. Beating of flagella allows movement, using thrust on the milieu surrounding sperm cells and is responsible of the translational drive of spermatozoa either in the fluid or by contact with structures cells or tissues. The present paper aims to describe:

1. The biochemical and structural elements of the "9+2" flagellar structure, so called axoneme, a complicated arrangement of at least 250 different protein subunits which sustains motility.
2. The mechanisms of wave generation and propagation along the

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axoneme of flagella, stating that in paradigms of wave propagation, a clear distinction is made between the dynein dependent microtubule sliding (oscillatory motor) and the bending mechanism (including regulator of wave propagation). The waves propagation is supported by a bending/relaxing cyclic mechanism which propagates in register, but frame-shifted with the powering action of the dynein-ATPase motors all along the axoneme. While knowledge has been largely accumulated on the motor components, little is known about the elements regulating the bending processes.

3. Guidance of spermatozoa is closely dependent on flagellar behavior. An overview of the various ways by which a spermatozoon can orient itself or be oriented to the corresponding egg in order to improve fertilization success is presented. As specific guidance mechanisms occur in response to chemicals such as Ca^{2+} ions controlling asymmetry of flagella beating special emphasis is devoted to such regulatory aspects. A discussion is also devoted to the way a cell elaborates its own flagellum and details possible hypothesis able to explain the origin of the axoneme, recognized as an ancestral structure with a high degree of conservation, as well as phylological aspects of this unique mechanical device used in an extremely large variety of situations to insure efficient cell movements of the male gametes, but also many unicells, such as the green algae Chlamydomonas, used as models to better understand the properties of this ubiquitous organelle due to its high degree of structure and function conservation all along evolutionary tree.

Keywords: Axoneme, Calcium ions, Dynein, Flagella, Membrane, Microtubules, Molecular motors, Motility, Motility activation, Regulation, Phosphorylation, Osmolarity, Energy, Viscosity.

INTRODUCTION

The male germ cell is the only individual cell (with the exception of ovocyte) of metazoan organisms, including human, that leaves the body and achieves its final and highly sophisticated structure and properties. The spermatozoon is designed

for one purpose: to reach the female gamete and to fertilize it so as to contribute its genome. The various stages in the development of the male germ cell are characterized by proliferative phases, by the recombination of the maternal and paternal chromosomes and by the differentiation and development of a specialized transportation vehicle, the spermatozoon equipped with a highly efficient motor/rudder, its flagellum.

In almost all animal species investigated, it is usual for spermatozoa to become motile either during, or immediately following, their release from storage within the male. In species utilizing external fertilization, such as many echinoderms (Trimmer and Vacquier, 1986), some species of fish (Scheuring, 1924; Stoss, 1983) and amphibians (Hardy and Dent, 1986), spermatozoa become motile once they are diluted into the surrounding water column at spawning. Only in a very few documented cases do spermatozoa remain immotile following release; most notable among these exceptions are the herring *Clupea sp*. (Yanagimachi, 1953) and the horseshoe crab *Limullus polyphelus* (Clapper and Brown, 1980 a & b). In these species, spermatozoa become motile only after interaction with specific chemical substances derived from the egg. Sperm motility prior to the release of sperm from storage within the male is not the case in species utilizing external fertilization and has only been recorded for one species, the rabbit, which utilizes internal fertilization (Turner and Reich, 1985). In any case, activation as well as propulsive translation of spermatozoa is relying on flagellar movement.

Among the propulsive organs present in living organisms, flagella and cilia (F/C), also collectively called "oscillopodia" (Holwill, 1974) or "undulipodia", as proposed by Barth *et al.* (1991) and by Margulis *et al.* (2006) as well, but rarely employed, present an extreme case of mechanical miniaturization. Many cell types in the animal and vegetal kingdom possess indeed cilia (for example, bronchial tissue) or flagella (spermatozoa, protozoa): these organelles are all very ancient and with evolutionaly very stable (Mitchell, 2004; 2007). One should emphasize that phylogenetic data lead to divide eukaryotes into unikonts and bikonts, a distinction based on the number of flagella per cell and their direction of movement (Cavalier-Smith, 2002). All animals belong to Opistokonta, a super group that, with Amoebozoa and Fungi, constitutes unikonts, while bikonts contain organisms themselves classified (see more details in chapter 10) as Plantae

CHAPTER 2

Sea Urchin Sperm Chemotaxis

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Abstract: A variety of broadcast-spawning marine organisms require sophisticated sperm guidance mechanisms, named chemotaxis, to locate the egg and fertilize it. In sea urchins, oocytes release small peptides from the egg outer envelope that bind to their sperm flagellar receptors and trigger a signaling pathway that results in intracellular Ca^{2+} concentration fluctuations. Each transient Ca^{2+} increase leads to a momentary elevation of flagellar bending asymmetry which results in a pronounced turn essential for chemotaxis. In addition, this process needs a precise spatiotemporal coordination between the Ca^{2+}-dependent turns, the form of chemoattractant gradient and periods of straighter swimming. Chemotaxis results when spermatozoa are able to undergo Ca^{2+}-dependent turns when swimming down the chemoattractant gradient, while they suppress turning events when swimming up the gradient. This chapter summarizes the sequence of events and known components of the signaling pathway leading to chemotaxis in sea urchin spermatozoa, and the strategies that are being employed to unravel this fascinating and fundamental process.

Keywords: Axoneme, Chemotaxis, Cell-cell communication, Chemoattractant signalling, Calcium signalling, Mathematical modelling of signal transduction, Sperm motility.

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1. INTRODUCTION

Broadcast spawners, commonly marine invertebrates, release their gametes into their environment and thus have to contend with difficulties of unlimited dilution which decrease their encounter probability (Lotterhos & Levitan, 2010). Fertilization efficiency in these species may be enhanced by sperm chemotaxis towards the egg. In many marine organisms, female gametes release diffusible molecules which attract their homologous spermatozoa (Lillie 1913; Miller 1985; Suzuki 1995). Propelled by their beating flagella, spermatozoa are able to rapidly and precisely detect chemoattractant gradients and steer their swimming toward the gradient source (the egg), an amazing feat involving the regulation of intracellular Ca^{2+} ($[Ca^{2+}]_i$) and other ions (Darszon *et al*. 2008; Kaupp *et al*. 2008). Though it was in bracken ferns where the phenomenon of sperm chemotaxis was first identified (Pfeffer 1884), sea urchins have become the best-characterized model system for understanding sperm chemotaxis at a molecular level (Cook *et al*. 1994; Darszon *et al*. 2008; Guerrero *et al*. 2010; Kaupp *et al*. 2008).

The egg-jelly, an extracellular matrix surrounding the sea urchin egg, contains polysaccharides and small peptides that regulate sperm motility (sperm-activating peptides, SAPs; (Suzuki 1995); see section 2.1)). In addition it contains a fucose-polymer that species-specifically triggers the acrosome reaction (AR) (Neill & Vacquier 2004). SAPs induce rapid changes in sperm cyclic nucleotide levels, membrane potential (Em), pHi, $[Ca^{2+}]_i$ and other ionic permeabilities (Darszon *et al*. 2008; Kaupp *et al*. 2008).

Our understanding of the signaling pathways triggered by SAPs has notably advanced in recent years due to significant advances in Ca^{2+} imaging (Böhmer *et al*. 2005; Guerrero *et al*. 2010; Wood *et al*. 2003; Wood *et al*. 2005; Wood *et al*. 2007), time-resolved fluorescence spectroscopy (Kaupp *et al*. 2003; Matsumoto *et al*. 2003; Nishigaki *et al*. 2004; Nishigaki *et al*. 2001) and molecular identification (Bönigk *et al*. 2009; Galindo *et al*. 2007; Gauss *et al*. 1998; Granados-Gonzalez *et al*. 2005; Su & Vacquier 2002; Su & Vacquier 2006; Chung, Shim, R. Everley, *et al*. 2014). The probability of sperm-egg encounter is enhanced by the timely translation of the biochemical and permeability changes triggered by SAPs into redirection of the sperm trajectory. Sea urchin spermatozoa swim in circles close

to surfaces (Gray 1955; Gray & Hancock 1955) and SAP gradients induce periodic increases in $[Ca^{2+}]_i$ that are associated with sharp turning events (high path curvature) interspersed with periods of straighter swimming episodes (low path curvature). A wide variety of organisms with external fertilization display a similar swimming pattern (Miller 1985).

This chapter will summarize our current knowledge on sea urchin sperm chemotaxis and present our inklings regarding a model of this remarkable signaling pathway that orchestrates spatiotemporal egg cues to sperm swimming, enhancing the probability of fertilization in the sea.

2. GAMETE COMMUNICATION, SENSING OF SPATIAL CUES

To ensure reproductive success, marine invertebrates release billions of gametes that navigate into a complex media containing various chemical cues shaped by the hydrodynamics of their ecological niche. Echinoderm spermatozoa are attracted to their con-specific female gamete by diffusible peptides released from the egg investments upon spawning into the open sea. These SAPs trigger the synthesis of cGMP through irreversible binding to a plasma membrane guanylyl cyclase (mGC) located at the sperm flagella in high concentrations (~10^5 copies/cell). The elegant work of Suzuki's group performed in the 1990s regarding the diversity of SAPs found in the echinoderm phylum will be recapitulated. In addition, the pioneering work during the 1980s and 1990s by David Garbers, Victor Vacquier and colleagues, that resulted in the discovery and characterization of the mGC is summarized. These molecular aspects combined with physical principles of chemosensation, mainly developed by Berg and Purcell in the 70s, encompass an elegant theory that explains how spermatozoa know where they are and where to find the egg.

2.1. Sperm-Activating Peptides and the Taxonomy of Echinoids

The discovery of animal sperm chemoattractants was made about a century ago, when it was shown that a soluble factor associated with eggs of certain sea urchin species stimulate the respiration and motility of their con-specific spermatozoa (Lillie 1913; Gray 1928; Carter 1931). This factor turned out to be alcohol-soluble, heat stable, diffusible during dialysis, and non-volatile (Hathaway 1963).

CHAPTER 3

Sperm Chemotaxis in Urochordates

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Abstract: Urochordates are marine animals, and spermatozoa of many urochordates show chemotactic behavior toward conspecific eggs during fertilization. Sperm chemotaxis of the ascidian Ciona intestinalis has been particularly well investigated. The sperm-activating and sperm-attracting factor (SAAF) of the phlebobranchian ascidians are secreted from the egg cells, and identified as polyhydroxylsterolsulfates. The molecular structure of SAAFs differs in different species, and the differences may cause species-specific chemotactic responses. Ascidian spermatozoa appear to sense a SAAF concentration decrease, resulting in a transient increases in intracellular Ca^{2+} in the flagellum and quick changes in the swimming direction of the sperm. In this chapter, we will introduce the features and molecular mechanisms of sperm chemotaxis in urochordates, and particularly those in ascidians.

Keywords: Ascidian, Ascidia sydneiensis, Calaxin, Ciona intestinalis, Fertilization, Flagellar beating, SAAF, Sperm chemotaxis.

INTRODUCTION

Urochordates (tunicates, sea squirts) are a group of primary chordates, and currently many Urochordate species are used as experimental models; particularly in developmental biology. Urochordates comprise ascidians, salps, and larvaceans. The genome of the ascidian *Ciona intestinalis* was the first among marine invertebrates to be completely decoded (Dehal *et al.* 2002). Subsequent transcriptome (Satou *et al.* 2002) and proteome (Endo *et al.* 2011) analyses of the species produced many molecular techniques for the study of physiological

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functions. Furthermore, *Ciona intestinalis* is a species in which sperm attractants have been identified (Yoshida *et al.* 2002), and it has thus become one of the most investigated species regarding sperm chemotaxis.

In common with many marine invertebrates, the spermatozoa of Urochordata species show chemotactic behavior toward conspecific eggs. Conklin observed that spermatozoa of the ascidian, *Styela*, gather at the vegetal pole of the egg and seem to enter the egg from there (Conklin, 1905). Sperm activation and agglutination in the ascidians was first described in 1951 (Minganti, 1951). The chemotaxis of ascidian sperm was subsequently investigated by Miller (Miller, 1975). Sperm chemotaxis of Urochordata species other than ascidians has also been observed (Miller & King, 1983).

In this chapter, we will focus on ascidian sperm chemotaxis, particularly in *Ciona intestinalis*.

FEATURES OF ASCIDIAN SPERM CHEMOTAXIS

Sperm motility activation in Phallusia mammillata (ascidian) are almost quiescent or of low motility when they are spawned into seawater (Yoshida *et al.* 1993). *Ciona* spermatozoa, initially inactive when suspended in seawater, become active and are attracted toward an egg when one is present nearby (Miller, 1982). Sperm attraction and activation of motility are mediated by some egg-derived factor (Miller, 1982; Yoshida *et al.* 1993).

In general, an animal ovum consists of an oocyte, extracellular matrix such as jelly layer, and accessory cells. The question arises as to which component releases the sperm attractant. In echinoderm eggs, sperm attractants are present in the jelly layer (Ward *et al.* 1985), and in the hydrozoan siphonophore, a cupule, the extracellular structure of the egg releases the sperm attractant (Carré & Sardet, 1981). Therefore, in these species the egg accessory components release their sperm attractants. On the other hand, in the ascidians *Ciona intestinalis* and *C. savignyi*, sperm-attracting activity is not observed in the egg's accessory components, the vitelline membrane, follicle cells, and test cells, but sperm of Ciona intestinalis (Ascidian) swimming in the vicinity of egg (Yoshida *et al.* 1993), although ethanol extracts of follicle cells also have sperm-attracting

activity (Miller, 1975). This indicates that, in the ascidian, the eggs themselves release the sperm attractant and that possibly the sperm-attracting substance is in an impermeable form in the follicle cells.

During chemotaxis, spermatozoa show characteristic features. *Ciona intestinalis* spermatozoa are initially activated, chemotactic behavior is induced, and then spermatozoa approach the eggs with circular or "turn" movements (Miller, 1975, 1982; Yoshida *et al.* 1993). When a micropipette including the sperm attractant is inserted into a sperm suspension, Sperm motility attraction by a micro-pipet in Phallusia mammillata (ascidian) around tip of the micropipette often show quick turning: the swimming-path curvature suddenly increases and the waveform of flagellar beats becomes highly asymmetric ("turning" state) (Shiba *et al.* 2008) (Fig. **1**). After a short delay, the spermatozoa change their swimming patterns: swimming straight with low path curvatures and symmetric flagellar waveforms ("straight swimming" state) (Shiba *et al.* 2008) (Fig. **1**). After the response, the spermatozoa are again in a "resting movement" state (Fig. **1**). These dynamic changes in sperm swimming patterns are generally repeated, and the spermatozoa finally approach the attractant source. The "turn" and "straight swimming" movements are generally triggered when the spermatozoon swims down-gradient of the attractant (Shiba *et al.* 2008). Thus, if sperm move farther from the egg, they change swimming direction. This trial and error process of sperm swimming direction results in approach to the egg.

SPECIFICITY OF ASCIDIAN SPERM CHEMOTAXIS

The specificity of sperm attraction toward eggs may prevent crossbreeding, and ensure species-specific fertilization. Sperm chemotaxis specificity was described in hydrozoans (Miller, 1979), echinoderms (Miller, 1985, 1997), and abalone (Riffell *et al.* 2004). On the other hand, there seems to be a lack of sperm chemotaxis species-specificity among chitons (Miller, 1977), and in mammals (Sun *et al.* 2003; Teves *et al.* 2006; Guidobaldi *et al.* 2008). In ascidians, specificity of sperm agglutination using five Mediterranean ascidian species was observed in 1951 (Minganti, 1951), and precise examinations of species-specific sperm attraction have been performed in many ascidian species (Miller, 1975, 1982). These studies show many interspecific chemotactic responses, particularly

CHAPTER 4

Sperm Motility Initiation in Pacific Herring

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Abstract: Pacific herring *(Clupea pallasi)* are estuarine fish that spawn in reduced salinity water. The spermatozoa are virtually motionless upon spawning into water of varying salinities and only initiate motility upon contact with a chorion-bound glycoprotein. Sperm can remain in the water column for up to 24 hrs, yet are still capable of fertilizing eggs. Immotility in the environment is maintained as a result of herring sperm utilizing reverse sodium (Na)-calcium (Ca) exchange (Ca^{2+} in, Na^{+} out) as a mechanism for increasing intracellular calcium at motility initiation. The primary initiator of motility, Sperm Motility Initiation Factor (SMIF) requires protein kinase C activation that in turn appears to increase the reverse Na-Ca exchanger. A non-diffusible chemoattractant, Micropylar Sperm Attractant (MSA) is also present on the chorion immediately surrounding the micropyle opening in herring (as well as other fish and insects) that induces a rapid increase in intracellular Ca^{2+} when sperm come in contact with it and this causes sperm to make sudden turns toward the canal opening. As such, herring sperm appear to undergo at least two increases in intracellular Ca^{2+}: one at motility initiation by SMIF, and a further increase as they contact MSA at the micropylar opening.

Keywords: Circular motility, Flagellar bending, Herring, Micropylar Sperm Attractant, Micropyle, Motility initiation, PKC, Sodium-calcium exchange, Sperm Motility Initiation Factor.

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INTRODUCTION

In external spawning aquatic organisms, spermatozoa must be motile in order to fertilize the egg. Sperm are typically nonmotile within the testes and seminal fluid prior to release, and initiate motility after dilution into the external environment where spawning occurs. This quiescence within the testes is thought to be a mechanism to conserve limited metabolic resources until such time as they are needed (reviewed by Morisawa, 1994). The extracellular triggers and the cellular mechanisms by which sperm motility is initiated at spawning have been well characterized in only a few aquatic systems (*e.g.* sea urchins and some teleost fishes). Teleost fish provide an excellent system for investigating sperm motility because this process is not complicated with other physiological changes that can be associated with sperm activation, *e.g.* an acrosome reaction, since teleost sperm do not possess an acrosome (see details in chapter 5 paragraph 3). Sperm of the Pacific herring, *Clupea pallasi*, like those of other teleosts are immotile in seminal fluid. They differ from most other fish, however, in that they are virtually motionless after spawning (in diluted seawater) and become vigorously motile only after contact with motility initiating factors, sperm motility initiation factor (SMIF; Yanagimachi *et al.* 1992; Griffin *et al.* 1996; Cherr *et al.* 2008) or herring sperm activating peptides (HSAPs; Morisawa, *et al.* 1992; Oda *et al.* 1995; Yoshida *et al.* 1999). Thus herring sperm motility initiation provides the simplicity of teleost sperm with the added benefit that cell activation is in response to a specific biological factor or inducer.

In teleost sperm, an increase in intracellular calcium ($[Ca^{2+}]_i$) caused by different signaling mechanisms among species, occurs upon motility initiation (reviewed by Darszon *et al.* 1999, and this issue; Kho *et al.* 2005; Alavi and Cosson, 2006). Four primary mechanisms of sperm motility initiation in fish have been reported: 1) a reduction in extracellular K^+ upon dilution of milt initiates motility in salmonids (Morisawa, 1985); 2) hypotonic exposure following dilution into freshwater in non-salmonid freshwater fishes (Morisawa and Suzuki, 1980; Morisawa *et al.* 1983b; Krasznai *et al.* 2003; Morita *et al.* 2003); 3) hypertonic exposure in marine fish (Morisawa and Suzuki, 1980; Oda and Morisawa, 1993; Detweiler and Thomas, 1998); and 4) egg-associated molecules in only two species (Yanagimachi and Kanoh, 1953; Yanagimachi, 1957a,b; Suzuki, 1958).

This chapter will focus on this latter mechanism of motility initiation in the Pacific herring (*Clupea pallasi*).

Herring Sperm Motility: Molecules that Initiate Motility

The sperm of Pacific herring is immotile at spawning (regardless of osmotic strength of the medium) and only become vigorously motile upon interaction with the egg micropyle region (Yanagimachi and Kanoh, 1953;Yanagimachi, 1957a; Yanagimachi *et al.* 1992; Griffin *et al.* 1996; Cherr *et al.* 2008; see Video 1). The natural initiation of sperm motility, leading directly to successful fertilization, occurs when sperm contact the micropylar region of the egg and interact with the chorion-bound molecule SMIF (Yanagimachi *et al.* 1992; Pillai *et al.* 1993; Griffin *et al.* 1996). Herring sperm continues to swarm to the opening of the micropyle for several hours after the canal is filled with sperm (or after sperm are expelled from the canal) following fertilization (Yanagimachi *et al.* 1992; see Video 2). In contrast to SMIF, HSAPs (Morsiawa *et al.* 1992; Oda *et al.* 1995; 1998; Ohtake, 2003) are released from the egg at spawning, can be concentrated, and induce linear motility *in vitro*. However during spawning and subsequent sperm-egg interaction, HSAPs rapidly become diluted and have a minimal effect and thus are not required for fertilization (Cherr *et al.* 2008). SMIF is a 105 kDa, basic (pI 8.1) glycoprotein that is non-diffusible and tightly bound to the micropylar region of the chorion (Griffin *et al.* 1996). When SMIF is extracted from the egg using acidic ½ strength seawater, eggs are rendered unfertilizable yet viable (Yanagimachi *et al.* 1992; Pillai *et al.* 1994; Griffin *et al.* 1996; Cherr *et al.* 2008). Isolated SMIF can be added back to eggs where SMIF has been removed and they once again become fertilizable (Cherr *et al.* 2008). The majority of SMIF binds to the sperm midpiece, but some are associated with the entire sperm surface (Griffin *et al.* 1996).

Herring Sperm Motility: Extracellular Ion Requirements

Herring are marine fish that migrate into estuaries or coastal regions where there are reduced salinities (Hay, 1985); as such, spawning usually occurs during the wet winter months or the spring ice melt at more northern latitudes. Sperm motility, fertilization, and embryo development in Pacific herring are optimal at ½

CHAPTER 5

Sperm Guidance in Other Animals and Phylla (Such as Other Fish, Jelly-Fish, or Amphibian)

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Abstract: The specific guidance mechanisms occurring in response to chemical substances (often *via* Ca^{2+} controlled asymmetry of beating) or using guides developed in various species because of special structures or tissues as well as by eggs in the vicinity of a unique entrance point on egg surface, the micropyle present in some species, are explored in this chapter. In order to avoid an excess of overlap with other chapters, several situations are described either, in the case of "simple spermatozoa" in species with external fertilisation. Part of this review chapter presents a tentative synthesis of the distribution of chemotaxis during the course of evolution.

Keywords: Allomycetes, Brown algeas, Batracian, Calcium, Chemical gradient, Cnidarian, Corals, Evolution, Echinoderms, Flagella, Fish, Ferns, Hydromedusa, Insects, Jelly-fish, Mollusks, Salps, Siphonophores, Urochordates.

INTRODUCTION

Earliest studies regarding movement and biochemical composition of flagella were devoted to "simple" spermatozoa, mainly those of sea urchins (Gray, 1955, Gibbons, 1995, 1996) or ascidians (Brokaw, 1997), which behave in a much more homogeneous manner as compared to mammalian spermatozoa (Jouannet and Serres, 1998) and where organelles are in very restricted number: the nucleus, the acrosome, the basal body prolonged by the axoneme and one or several mitochondria (see chapter 1, Figs. **1** & **2**). Species used as models for flagellar motility studies (Gibbons, 1995; 1996) rapidly revealed to be typical examples on

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which to study sperm chemotaxis towards eggs. But in early literature, there are observations in numerous other species where author described several aspects of sperm chemoattraction towards eggs.

As summarized in a quite exhaustive review by Miller (1985), data available in the literature show that chemotaxis seems to be distributed among most phyla of Metazoa (Fig. **1**). In this respect, readers could also consult also Cosson (1990) and Eisenbach (2004). A review, dealing with molecules used by gametes for their mutual recognition, was published by Vacquier (1995).

It is worth to remark that the physiological role of sperm chemotaxis in non-mammalian species appears to differ from that in mammals. In non-mammalian species, sperm chemotaxis strives to bring as many spermatozoa as possible to the egg. However, in mammals, the role appears to be rather the recruitment of a selective population of capacitated ('ripe') spermatozoa to fertilize the egg (Eisenbach, 2009a).

Below in Fig. (**1**), a brief overview of most species or group of species where indications of sperm chemotaxis or guidance were reported, is presented.

In the Cnideria (mostly in the Hydrozoa order), in the Mollusca (such as abalones and mostly in Polyplacophora and Gastropoda classes), in the Echinodermata (in classes such as Asteroidea, Holothuroidia and Ophiuroidea) and in Urochordata (in sessile, solitary tunicates as well as in planktonix larvacean) chemotaxis is established without ambiguity; in addition, there are some evidences which need better experimental support in Nematoda, Chaetognata, Lophophora (Bryozoa) and Annelida. Some weak evidences are present in the classes of Cephallopoda (belonging to Mollusca phyllum), of Calcarea (phyllum Porifera) and of Echinoidea (phyllum Echinodermata). The most salient examples are briefly presented below.

Since the pionnering studies of Lillie (1914), a major milestone in sperm chemotaxis understanding comes from studies on egg/sperm relationships in several sea urchin species. But the occurrence of a sperm chemotactic response was demonstrated in five species of tunicates (Miller, 1975) and more than 20 species of Cnidarians (Miller, 1973). Apart from Hydromedusae (see below),

chemataxis was also observed in simple animals (Miller, 1976) such as calciferous sponges (Porifers), bryozoans (Lophohorata) and chaetognates with various degrees of details.

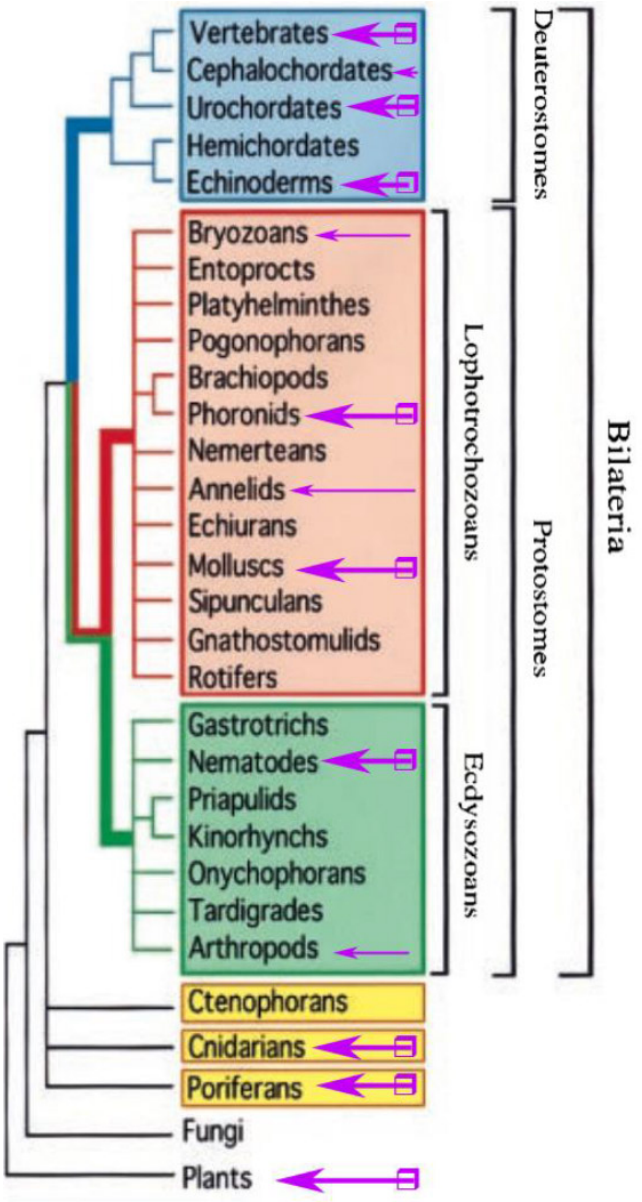

Fig. (1). Main classes of animal species are presented: squared arrows indicate groups of species where sperm guidance (mostly chemo-attraction) was observed and described at different levels of details.

CHAPTER 6

Mammalian Sperm Guidance — An Overview

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Abstract: In the recent two decades mammalian spermatozoa were demonstrated to perform chemotaxis, thermotaxis and rheotaxis. It is believed that in the Fallopian tube spermatozoa are first guided to the fertilization site by long-range mechanisms, thermotaxis and rheotaxis, and there they are guided to the egg by two processes of chemotaxis, considered a short-range mechanism. The occurrence of chemotaxis and thermotaxis in additional locations along the female genital tract cannot be excluded.

Keywords: Capacitation, Hyperactivation, Sperm chemotaxis, Sperm rheotaxis, Sperm thermotaxis.

The preceding chapters dealt with sperm guidance in marine species. In such species, where fertilization is mostly external, the need for sperm guidance is obvious. The female spawns its eggs into seawater and the male's spermatozoa find their way to the egg by chemotaxis mainly. In mammals, where fertilization is internal, the need for sperm guidance is not obvious, because very large numbers of spermatozoa are ejaculated directly into the female genital tract. This, however, does not mean that large numbers of spermatozoa reach the egg by coincidence. As a matter of fact, very few of the ejaculated spermatozoa succeed in entering the Fallopian tubes. In humans, only ~1 of every million enters the tube on average, and of these few no more than ~10% are capacitated (Cohen-Dayag *et al.* 1995) and, therefore, capable of fertilizing the egg (Jaiswal & Eisenbach, 2002). Given the ~200 average number of spermatozoa that get into the Fallopian tube in humans (Harper, 1982), this means that the number of capacitated spermatozoa in each tube is at the order of 10–20 cells only. These very few cells have to swim a relatively long way, full of obstacles, before

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Jacky J. Cosson (Ed)

reaching the egg. Taken with the small dimensions of the gametes relative to the tube, these imply that the probability of coincidental sperm arrival to the egg is extremely low. One might expect that this information would challenge the scientific community to look for a mechanism that assists spermatozoa to reach the egg. Surprisingly, however, this was not the case, and the dogma of coincidental sperm arrival to the egg persisted.

Some two decades ago, believing that sperm guidance must occur in mammals and knowing that chemotaxis is the most common cell guidance mechanism, my group looked for the occurrence of sperm chemotaxis in humans. With our finding that sperm chemotaxis indeed occurs (Ralt *et al.* 1994) the research field of mammalian sperm guidance started. It later turned out that there are at least two processes of chemotaxis, one to the cumulus cells and one to the egg within the cumulus matrix (Sun *et al.* 2005). Subsequent studies revealed the identity of the chemoattractant secreted from the cumulus cells (Teves *et al.* 2006; Oren-Benaroya *et al.* 2008) and the nature of the more potent one secreted from the egg (Armon *et al.* 2014) (Chapter 7).

Like in any other highly essential system in biology, mammalian sperm guidance is expected to involve redundancy. Indeed, additional potential sperm guidance mechanisms have been discovered in humans and other mammals. The finding of the formation of an ovulation-dependent temperature gradient in the oviduct (David *et al.* 1972; Bahat *et al.* 2005) led to the discovery of sperm thermotaxis (Bahat *et al.* 2003), which is characterized by its extremely high temperature sensitivity (Bahat *et al.* 2012). The observation of post-coitus flow of oviductal fluid secretion let to the finding of rheotaxis (Miki & Clapham, 2013). These two potential guidance mechanisms are considered to be long-range mechanisms. The notion today is that mammalian spermatozoa are guided in the tube by at least three mechanisms: two long-range mechanisms, thermotaxis (Chapter 8) and rheotaxis (Chapter 9), and a short-range two-step mechanism, chemotaxis (Chapter 7) (Fig. **1**). Due to the redundancy, when one of these mechanisms is not functional for any reason, guidance is not expected to be lost and the cells should still be able to navigate to the egg. This is similar to the case of guidance of migrating birds, where the birds' navigation is unaffected when one of the guidance mechanisms is not functional (Mouritsen *et al.* 2003).

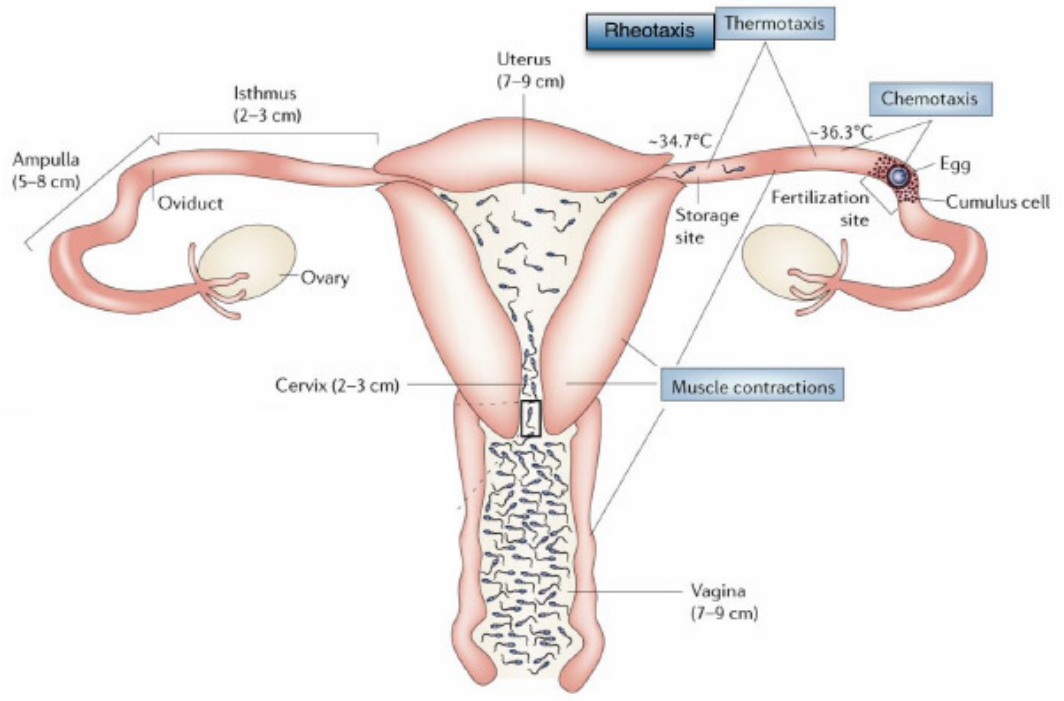

Fig. (1). The mammalian female genital tract and sperm guidance mechanisms. The diagram, which is not drawn to scale, and the dimensions shown (Harper, 1982), are derived from studies in humans, whereas the temperature values shown are those measured in rabbits (Bahat *et al.*, 2005) (there are no published measurements in humans). The dimensions in parentheses indicate the length of the respective organ. The spermatozoa in the vagina use both active swimming and passive drag by female genital tract muscular contraction to reach the storage site in the oviduct. Once here, a small fraction of spermatozoa undergoes ripening, or capacitation, which enables them to fertilize the egg at the fertilization site. Capacitated spermatozoa are guided from the storage site to the egg by a combination of rheotaxis, thermotaxis and chemotaxis. Oviductal contractions may also play a role. An ovulation-dependent temperature gradient between the storage site and the fertilization site provides the thermotactic stimulus. Post-coitus oviductal fluid flow provides the stimulus for rheotaxis. Chemoattractants are secreted from the egg and the surrounding cumulus cells. [Modified with permission from (Eisenbach & Giojalas, 2006)].

The behavioral mechanisms of these three guidance mechanisms have been revealed to a large extent (Gakamsky *et al.* 2009; Armon & Eisenbach, 2011; Blengini *et al.* 2011; Boryshpolets *et al.* 2015; Miki & Clapham, 2013; Kantsler *et al.* 2014). Progress has also been made towards the understanding of the molecular mechanisms underlying chemotaxis (Gakamsky *et al.* 2009; Teves *et al.* 2009; Strunker *et al.* 2011; Lishko *et al.* 2011; Brenker *et al.* 2012) and thermotaxis (Bahat & Eisenbach, 2010).

CHAPTER 7

Sperm Chemotaxis in Mammals

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Abstract: Sperm cells evolved to acquire a sophisticated motility structure called a flagellum which gives spermatozoa a self-propelling force. Nonetheless, in internal fertilizing species such as mammals, the length and complexity of the female genital tract mean that sperm motility is insufficient to accomplish the sperm's mission of transferring genetic material to the oocyte. Thus, a long-standing question in the reproductive biology field has been how spermatozoa are delivered to the oocyte surface. Several mechanisms have been proposed to help sperm transport, such as oviduct peristalsis, chemotaxis, thermotaxis and rheotaxis. In this chapter we will discuss the state of the art of sperm chemotaxis.

Keywords: Chemoattractants, Chemoattractant sources, Methods for chemotaxis evaluation, Sperm chemotaxis.

INTRODUCTION

Fertilization in mammals takes place inside the female reproductive tract, which involves an adaptation of gamete physiology to the female environment. In most mammal species, to reach the fertilization site in the oviduct, spermatozoa must traverse several parts of the female reproductive tract, such as the cervix, uterus and the first half of the oviduct (Harper *et al.* 1982; Suarez and Pacey, 2006).This route presents several barriers. In species in which spermatozoa are deposited in the vagina, they have to cross the cervical mucus, which extracts dead and abnormal spermatozoa (Harper *et al.* 1982; Suarez and Pacey, 2006), while others are stored in the cervical crypts whose function is still unknown (Suarez and

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Pacey, 2006). The transit through the uterus is relatively fast, taking a few minutes (Overstreet and Cooper, 1978; Harper *et al.* 1982; Suarez and Pacey, 2006), and there the interaction with the uterine fluids prompts sperm capacitation (Hunter and Rodriguez-Martinez, 2004), which is their physiological preparation to fertilize the egg (Austin, 1951; Chang, 1951). They reach the utero-tubal junction, which connects the uterus with the isthmus (lower oviduct), where they have to pass through a narrow channel, the diameter of which is enlarged around ovulation (Suarez and Pacey, 2006). There, spermatozoa are stored in close association with the epithelium, completing sperm capacitation (Suarez, 2002; Hunter and Rodriguez-Martinez, 2004; Suarez and Pacey, 2006).

Once capacitated, spermatozoa are detached from the isthmus epithelium (Suarez and Pacey, 2006), but they still have to travel several centimeters to the fertilization site through an intricate architecture and physiological environment. The oviduct mucosa has numerous longitudinal folds with increasing complexity towards the ampulla (Fig. **1**; Yániz *et al.* 2000, 2006; Burkitt *et al.* 2011). The hormonal changes during the female cycle induce modifications in the epithelium and fluid composition; for instance, the proportion of ciliated and secretory cells varies all along the oviduct regions (Yániz *et al.* 2000). In addition, the oviduct displays peristalsis, with a frequency and amplitude that depend on the hormone levels throughout the cycle (Salomy and Harper, 1971; Bourdage and Halbert, 1980). As a consequence of oviduct contractions, at the time of ovulation the tubal fluid is moved forward and backward with a net fluid progression towards the peritoneal cavity (Maia and Coutinho, 1970; Ito *et al.* 1991; Menezo and Guerin, 1997; Guidobaldi *et al.* 2012).

Meanwhile, the epithelium cilia synchronously beat towards the uterus, generating the mechanical transport of the egg to the fertilization site (Halbert *et al.* 1976, 1989) and a current of fluid which moves spermatozoa out of the depth of the epithelium folds toward the middle of the oviduct channel (Kölle *et al.* 2009).

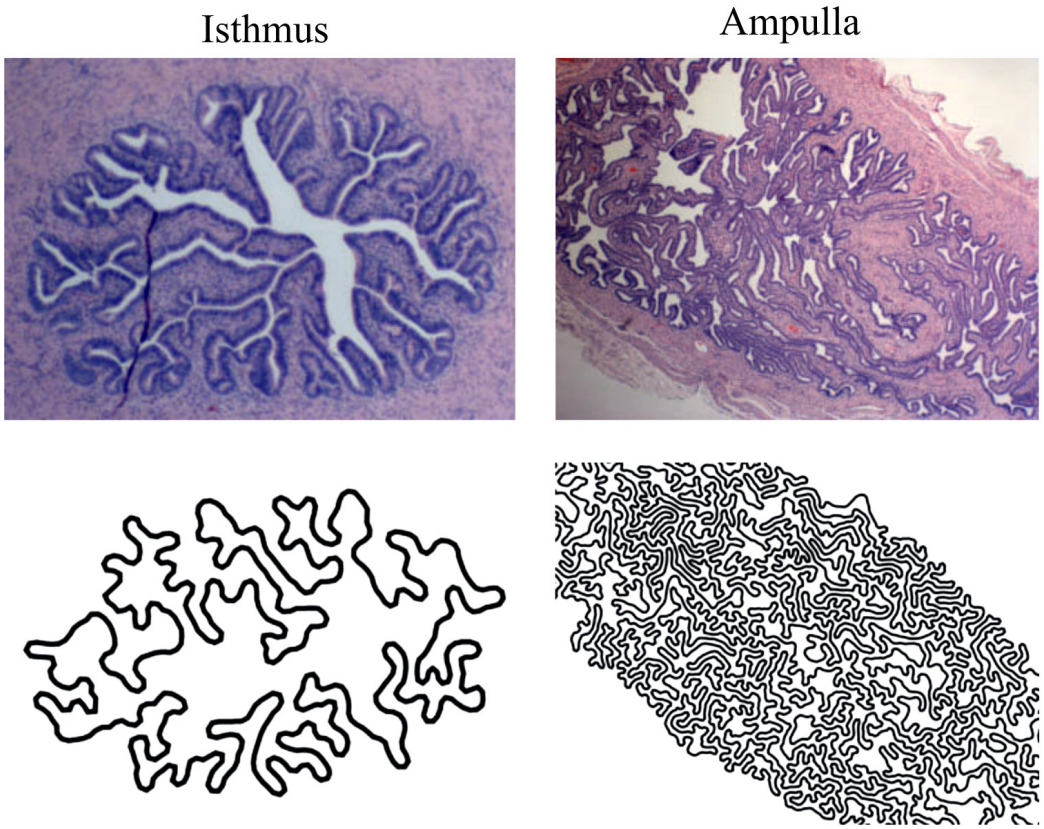

Fig. (1). Microscopic section of the oviduct isthmus and ampulla with the respective computer simulation of the lumen, showing the high complexity of the oviduct. The figure was adapted from Burkitt *et al.* (2011) with permission of CSIRO publishing group.

Once the spermatozoon arrives in the vicinity of the egg, it still has to traverse several layers to get to the oocyte surface: the cumulus oophorus (cells immersed in an extracellular matrix) as the outer coat, and the zona pellucida (a glycoprotein envelope that covers the oocyte surface) (Cardullo and Thaler, 2002). The oviduct thus becomes a crossroads for gamete encounter, where finding the oocyte's location is apparently not an easy task for the spermatozoon.

In this context, apart from sperm self-motility, several transport mechanisms have been proposed, such as oviduct muscle contractions (Ito *et al.* 1991; Kölle *et al.* 2009; Guidobaldi *et al.* 2012), chemotaxis (Harper, 1973; Guidobaldi *et al.* 2012),

CHAPTER 8

Mammalian Sperm Behavior During Thermotaxis

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Abstract: Sperm thermotaxis, the active orientation of sperm swimming according to a temperature gradient has been suggested to act as a long-range guidance mechanism in the oviduct during fertilization, between the cooler sperm storage site and the warmer fertilization site. In this process capacitated spermatozoa can sense even very shallow temperature gradients. They respond to the changing temperature by modulating their flagellar beating. The outcome is a higher frequency of turns and hyperactivation events when the temperature drops, and a rather linear swimming when they sense a temperature increase. In this way they are guided towards the warmer temperature.

Keywords: Flagellar shape, Hyperactivation, Sperm guidance, Sperm velocity.

INTRODUCTION

The investigation of mammalian sperm thermotaxis started following the surprising finding that, at ovulation, even though the body temperature goes up, the temperature at the isthmus (the utero-proximal part of the oviduct, considered to be the sperm storage site) goes down, creating a temperature gradient in the oviduct between the storage and fertilization sites (David *et al.*, 1972; Bahat *et al.*, 2005). Considering the possibility that such a temperature gradient can serve as a cue for guiding spermatozoa in the oviduct (Hunter, 1998), *in vitro* studies were initiated to determine whether spermatozoa can indeed sense such a gradient and respond to it. These studies not only revealed that spermatozoa can respond to a temperature gradient by thermotaxis, changing their swimming direction towards the higher temperature (Bahat *et al.*, 2003), but that they can respond to very shallow temperature gradients — shallower than 0.014°C/mm equivalent to a temperature difference of 0.0006°C as a spermatozoon swims its body-length distance (Bahat *et al.*, 2012). Even though measurements of sperm thermotaxis

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cannot be performed *in vivo* for technical reasons, it is believed that sperm thermotaxis is a long-range guidance mechanism (Bahat *et al.*, 2003; Eisenbach & Giojalas, 2006) (Chapter 6).

What do we know about sperm thermotaxis? What are its molecular and behavioral mechanisms? A few signaling molecules were identified as being involved in human sperm thermotaxis, pointing to the phospholipase C signaling pathway as one of the pathways underlying the molecular mechanism of sperm thermotaxis (Bahat & Eisenbach, 2010). Yet, the complete molecular mechanism is still obscure, especially the mechanism that confers on sperm such extreme temperature sensitivity. As to the behavioral mechanism, this was a subject of a recent study (Boryshpolets *et al.*, 2015), demonstrating that a temperature shift results in both a kinetic response and a directional response. The probable outcome of these responses is thermotaxis.

KINETIC RESPONSE OF HUMAN SPERMATOZOA TO A TEMPERATURE SHIFT

Human spermatozoa respond to an upward temperature shift by increasing their swimming velocity, and to a temperature drop by slowing down (Fig. **1**). The response in either case is fully reversible. As a result of the increased velocity reflected in the above parameters, the values characterizing sperm linearity increase with the temperature as well, meaning that the trajectories of their motility become smoother and more linear.

FLAGELLAR RESPONSE AND DIRECTIONAL CHANGES IN SPERM SWIMMING

The changes in sperm head trajectories, mentioned just above, are the outcome of changes in flagellar movement. Thus, when the temperature rises, the flagellar wave amplitude close to the head is reduced with consequent more linear swimming (Fig. **2**; Movie 1 at https://www.youtube.com/watch?v=Xjq66vS-Cj4), similar to the swimming in a viscous medium. This mode of swimming is probably a more efficient way of propagation, especially when coupled with velocity increase. In contrast, when the temperature is shifted down, the amplitude of the flagellar wave increases close to the sperm head (Fig. **2**). The outcome of

this is larger side-to-side head displacement, which in turn may lead to changes in the direction of swimming (Fig. **3A**). In other words, when spermatozoa sense a temperature drop they turn more.

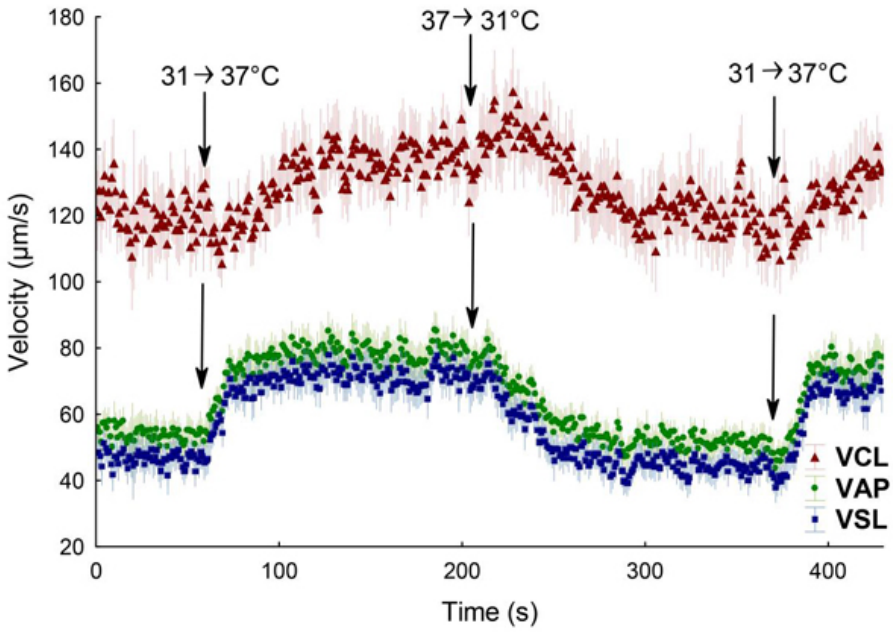

Fig. (1). Motility parameters of human spermatozoa during temperature changes. Spermatozoa were exposed to the indicated temperature changes and their motility was recorded and analyzed. The motility parameters shown are curvilinear velocity (VCL, time-averaged velocity of a sperm head along its actual curvilinear path), average pass velocity (VAP, velocity over an average path) and straight-line velocity (VSL, time-average velocity of the sperm head along a straight line from its first position to its last position). Each experimental point is an average of 10–50 spermatozoa measured for 1 s each. The bars represent 0.95 confidence intervals. [Taken with permission from (Boryshpolets *et al*., 2015)].

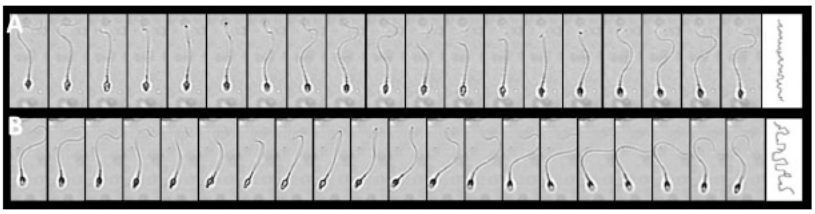

Fig. (2). Representative photographs of temperature-jump stimulated changes in flagellar wave propagation. The photographs are shown at 0.005 s intervals. The last frames (frames with white background at the right side of each row) show typical head trajectories for these flagellar motions. **A**, A spermatozoon at 37°C just prior to the temperature shift to 31°C. **B**, A spermatozoon at 31°C just after the temperature shift from 37°C. [Taken with permission from (Boryshpolets *et al*., 2015)].

CHAPTER 9

Modelling Spermatozoan Swimming: Its Capabilities and Limitations for Contributing to the Understanding of Sperm Guidance

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Abstract: Spermatozoa face the Herculean task of finding an egg and are beset with numerous challenges, not least reaching their target without getting lost. Furthermore, the sperm flagellum not only allows rapid swimming but also an ability to steer and thus navigate, invariably coupling sperm motility and guidance cue response. Thus in the following we briefly review the mechanics of how sperm swim, together with modelling strategies for developing computational simulations of swimming sperm. We proceed to consider how these simulations can inform our understanding of sperm guidance together with the limitations of modelling approaches, as well as perspectives of future studies that can be tackled with present modelling frameworks and where fundamental advances in our biological understanding are required for further progress.

Keywords: Boundary element method, Chemotaxis, Dynein regulation, Flagellar waveform, Hydrodynamics, Low Reynolds number, Mechanics, Modeling, Thigmotaxis, Numerical simulation, Regularized Stokeslet method, Resistive force theory, Rheotaxis, Slender-body theory, Stokes flow, Sperm swimming.

1. INTRODUCTION

All of us who have been conceived without the use of *in-vitro* fertilisation technologies are the result of an Olympian spermatozoan prevailing in a remarkable journey to the egg. A core question across all species is how did such

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a fertilising sperm navigate to reach the egg? There are numerous guidance cues which various sperm have been reported to respond to, including: thigmotaxis, that is the influence of surfaces and, by extension, confined geometries (Rothschild 1965, Woolley 2003); chemotaxis and thermotaxis, where sperm respond to chemical or thermal gradients (Kaupp *et al.*, 2008 and Bahat *et al.* 2003); and rheotaxis, where sperm are directed by fluid flows (Lott 1872, Miki and Clapham 2013). Furthermore the mechanism by which such cues influence sperm behaviour is complex and may generally be classified either as mechanical or biological. With the former mechanical cue, the local microenvironment induces external forces and torques that guide sperm, whereas with the latter biological cue, signal transduction ultimately alters the regulation of molecular motors, dyneins, in the flagellum inducing a change in the flagellar beat pattern, which in turn alters sperm trajectories. In addition, there is also the prospect of population effects, for instance with the external motions driven by multiple sperm flagella altering the magnitude of an external chemotactic gradient (Cisneros *et al*. 2007), and thus sperm should not always be considered just in isolation.

Furthermore, recent developments in the videomicroscopy of sperm and their computational simulation have advanced the study of sperm dynamics from fundamental mechanical and biophysical principles, at least once the flagellar waveform is known (Gaffney *et al.*, 2011). However, the details of how the flagellar beat is generated are currently subject to numerous competing schools of thought and thus our understanding in this field is less mature in terms of validated and tested computational simulation, though it is developing at a rapid rate (Lindemann, 2014a; Mukundan *et al.* 2014). Despite such limitations, computational simulation and modelling can nonetheless contribute to testing our ideas and improving our understanding for at least a selection of sperm guidance cues, especially given guidance arises from regulating swimming directionality. Hence, in this chapter, we briefly summarize both classical and recent studies modelling sperm swimming with a discussion of the extent to which, and how, such frameworks can be utilized in studying sperm guidance. Ultrastructural and biochemical features of spermatozoa underlying the modeling aspects developed below are detailed in chapter 1.

2. AN OVERVIEW OF SPERM MODELLING

2.1. The Fundamental Mechanics of Swimming

A breakthrough study on modelling sperm swimming was pursued by Gray and Hancock (1955) in which hydrodynamics was applied to approximately determine the forces exerted on the flagellum by a surrounding simple fluid, such as water with isotonic electrolytes, which was then utilized to understand how a sperm swims. In summarising how Gray and Hancock's work illustrated the mechanism of sperm swimming, we also restrict ourselves to such a simple fluid which is commonly referred to as Newtonian. Thus, we neglect the complex properties of numerous physiological media such as elasticity and shear thinning, which arise due to the stretching, tangling and interactions of long-chain molecules in solution.

While these complexities are highly relevant for aspects of sperm motility, especially in mammals, they are also poorly understood and do not contribute to understanding fundamental mechanisms.

2.1.1. Navier-Stokes Equations. A Qualitative Summary

Thus our starting point is the dynamics of a Newtonian fluid, as governed by the Navier-Stokes equations, which represent the principles of fluid incompressibility and momentum conservation. One consequence of incompressibility is that the fluid density is constant (if it is constant to start with, which is always assumed). The conservation of momentum is essentially Newton's second law for any material element of fluid: the product of the element's mass and acceleration is equal to the total force it experiences. This is equivalent to the statement

[density of fluid] x [acceleration of the element of fluid] =
[force acting on the element of fluid per unit of its volume].

The upper line, involving acceleration, is the inertial term and a measure of the tendency of the fluid to keep moving once a driving force is removed. The forces per unit volume on the lower line are due to pressure gradients and the fluid viscosity, with the latter removing energy from the system by viscous heating,

CHAPTER 10

Sperm Guidance: Comparison with Motility Regulation in Bikont Species

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Abstract: Sperm chemotactic behavior is based on the control of swimming direction. Transient conversion of the asymmetry in the flagellar waveform is the most used regulatory mechanism to change the swimming direction. Direct regulation of outer arm dynein by a neuronal calcium sensor type of Ca^{2+}-binding protein, calaxin, is a prerequisite for the regulation of chemotaxis. Bikont species, such as green algae, brown algae, dinoflagellates, ciliates and excavates, also show similar directional movements, including phototaxis, chemotaxis, and responses to mechanical stimuli. These behaviors depend on changes in flagellar motility in response to the gradient or direction of chemical or physical stimuli. However calaxin is not present in bikont species; instead they appear to use another Ca^{2+}-sensor similar to the outer arm dynein light chain LC4 in *Chlamydomonas*. In this chapter, we briefly describe the mechanism of sperm chemotaxis, compare it with flagellar regulation seen in several taxis of bikont species, many of them model species for understanding flagellar mechanics, and discuss the common and divergent strategies tuning the control of flagellar response during eukaryotic taxis.

Keywords: Algae, Bikont, Calaxin, Calcium, Chemotaxis, *Chlamydomonas*, Ciliate, Dinoflagellate, *Euglena*, Opisthokont, Phototaxis, *Trypanosoma*.

INTRODUCTION

Sperms are male gametes that are highly differentiated for delivering paternal DNA for fertilization. Flagellar motility is the main mode used for the

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transportation for delivery. The movement is achieved by a microtubule-based structure called the axoneme. Microtubules are arranged in a 9+2 lattice in flagella. The motor units for motility are outer and inner arm dyneins that are anchored on each outer doublet microtubule. The central apparatus (the central pair microtubule and its associated structure) and radial spokes regulate dyneins and are thereby involved in planar flagellar movement or in the conversion of symmetry/asymmetry of the flagellar waveform (Inaba, 2003, Inaba, 2011). More details about structure and function of axonemes are found in chapter 1.

To accurately arrive at the egg, sperms use a gradient of chemo-attractant diffusing from the egg, change the flagellar waveform and move toward the egg. This phenomenon, called sperm chemotaxis, is seen in the sperm of most animals and plants. Ca^{2+} is an important signaling ion in the transduction of the chemo-attractant cue into the modulation of flagellar motility. In particular, Ca^{2+} ion concentration regulates the formation of asymmetric waveforms through the central apparatus/radial spokes and inner arm dyneins (Smith and Yang, 2004). However, sperm movement with an asymmetric waveform needs the operation of outer arm dynein. A neuronal calcium sensor protein called calaxin regulates outer arm dynein activity to complete the asymmetric flagellar movement of the sperm (Mizuno *et al.* 2009, Mizuno *et al*., 2012).

Eukaryotic flagella, as well as cilia, are one of the organelles with their structure highly conserved throughout evolution (Mitchell, 2007). Cilia and flagella are distinctly named but these structures are almost identical (conventionally named according to the number per cell or motility pattern) and are considered as ancient structures that characterize eukaryotes. The components of cilia and flagella are mostly common between them but show heterogeneity among cells or tissues (Konno *et al*., 2015). Examples of ciliary beating such as Ciliary beating on individual cells of sea urchin embryo and Cilia beating at the surface of sea urchin embryo are presented in two accompanying videos: in this case, each individual cell presents a unique cilium. Phylogenetic analyses demonstrate that eukaryotes are divided into unikonts and bikonts, from the point of view of number of flagella per cell and the direction of movement (Cavalier-Smith, 2002).

All animals belong to the Opisthokonta, one of the supergroups in the unikonts

(Opisthokonta and Amoebozoa). Bikonts are organisms other than unikonts. Bikont groups include the Archaeplastida, Alveolata, Stramenopiles, Rhizaria, Haptophytes, Cryptophytes and Excavates. Although the eukaryotes include this great diversity of organisms, the regulatory mechanisms for ciliary/flagellar movements are common and shared in most cases. It is an intriguing feature, however, that diversities are seen in the numbers of flagella per cell, the mechanism for changing direction of a cell, and the response to a change in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Inaba, 2015).

Sperm guidance is essentially a phenomenon of producing directional movement by modulating flagellar waveforms. This is not only observed in spermatozoa, but is one of the reactions commonly observed in eukaryotic cells. In this chapter, we briefly describe the mechanism of sperm guidance, in particular how the regulation of the flagellar waveform occurs in a Ca^{2+}-dependent manner, survey homologous behavior seen in other eukaryotic unicellular organisms, mostly bikont species, and discuss how eukaryotes have developed a mechanism leading to changes in the direction of movement of the cell in a concentration gradient of molecules.

1. FLAGELLAR REGULATION IN SPERM CHEMOTAXIS

1-1. Pattern of Flagellar Movement in Chemotaxis

Sperm chemotactic behavior is widely conserved in most organisms, and especially marine invertebrates (Miller, 1985) (Fig. **1**). Sperm in most marine invertebrates shows thigmotactic behavior at the interface between the slide glass (or air) and water needed for microscopic observation. This feature allows us to easily observe changes in swimming trajectory toward the source of an attractant. When sperms swim away from the attractant, a turning movement occurs to redirect the sperm towards the source. The repetitive turning movement results in sperm approaching, and staying around, the attractant source.

To develop such turning movement, sperm cells transiently swim in small circles, and alternatively switch to a swimming pattern closer to a straight path. Usually sperm swim in a steady circle of constant curvature. When the curvature of the swimming trajectory increases, sperm cells exhibit the turning movement. The

CHAPTER 11

Sperm Guidance: Chemotactic Features Common to Sperm in Various Species

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Abstract: This chapter comes in continuity with chapter 10 in a sense that it tentatively aims to provide a synthetic overview of problematic posed by sperm guidance in a large variety of species and summarize globally the diversity of mechanisms individually adopted in each group where sperm attraction was characterized, while discerning common strategies.

Keywords: CyclicAMP, Ca^{2+} ions, Directionality, Motility activation, Movement, Phosphorylation, Regulation, Sperm.

INTRODUCTION

The flagellum appears to be a complex machinery built as an assembly of a very large number of components, many of them being potentially devoted to all kind of regulations, as predicted by proteomics (Diniz *et al.*, 2012). Main ways by which flagella operate regulation pathways are Calcium- and/or phosphorylation-dependent processes or an association of both.

1. THE KEY ROLES OF Ca^{2+} IONS, CAMP AND PROTEIN PHOSPHORYLATION

In animals of sexual reproduction, the appropriate communication between mature and competent male and female gametes determines the generation of a new individual. Two signaling pathways have emerged as central to normal

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mammalian as well as non-mammalian sperm motility: the cAMP/PKA pathway and calcium signaling. According to Chung *et al.* (2014), the two pathways (one being tyrosine-dependent and second being Ca^{2+}-dependent) represent structurally separated domains of flagella. In case of ionic exchanges such as Ca^{2+}, ion channels are key elements in the dialogue between sperm, its environment, and the egg (Darszon *et al.*, 1999; 2001; 2011). An impressive mass of data regarding a very large number of species has been accumulated and relates intracellular Ca^{2+} concentration to sperm behavior (Darzon *et al.*, 2005; 2011; Publicover *et al*., 2007; 2008) and more specially to asymmetry of flagellar beating (Brokaw, 1974; 1979); this feature constitutes a common step shared by most of the chemotaxis mechanisms so far understood (Cosson, 1990; Darzon *et al.*, 2011). All along the present book, many results confirm this fact.

External calcium concentration is known to regulate the beating asymmetry of axonemes, either *in situ* inside flagella or in situations resulting from membrane removal (Gibbons and Gibbons, 1980). This asymmetry regulation is definitely of biological importance, as shown for example in transient sperm resting situations (Gibbons, 1986; Pacey *et al.*, 1994b) or as a response to chemotaxis signal. Flagella can change their beating pattern (asymmetric or symmetric) in response to internal concentration perceived by the axoneme (Brokaw, 1979) as a result of external modification of Ca^{2+} concentration; the axoneme contains several calcium binding proteins (see list in Table 1 of Chapter 1): among those, it is worth to mention calmodulin (Yang *et al.*, 2001), centrin/caltractin (Yanagisawa *et al.*, 2001) at the attachment point of the I2 and I3 inner dynein heavy chains (DRC, Dynein Regulatory Complex ; LeDizet & Piperno, 1995), calaxin (Padma *et al*., 2001; 2003): all these elements appear to control the internal Ca^{2+}concentration, thus contributing to asymmetry of flagellar beating (Darszon *et al.,* 2011).

As stated by King (2010), calcium ions concentration is used to control several distinct axonemal responses (Publicover *et al.*, 2008) and there are multiple Ca^{2+}- and/or Ca^{2+}/calmodulin-binding proteins located in various axonemal substructures located mainly in the central pair complex (Wargo *et al.*, 2005; DiPetrillo & Smith, 2009) and the radial spokes (Yang *et al.*, 2001, 2006; Patel-King *et al.*, 2004). In addition, the outer dynein arm is associated with two Ca-binding calmodulin homologues: LC4 interacts with the gamma-HC N-terminal

domain, (Sakato *et al.*, 2007) and DC3 is a component of the docking complex required for outer arm assembly at appropriate locations on the axoneme (Casey *et al.*, 2003a). These proteins bind Ca^{2+} ions (one to DC3 or 1–2 to LC4) with affinities of 1.10^{-5} and 3.10^{-5} M, respectively; for LC4, binding is Ca^{2+} specific (King & Patel-King, 1995; Casey *et al.*, 2003b). A third Ca^{2+}-binding protein, centrin, is present in monomeric inner arm species b, e, and g (Piperno *et al.*, 1990; Kagami & Kamiya, 1992) and binds two Ca^{2+} ions with high affinity ($1.2.10^{-6}$ M) and two others with significantly lower affinity ($1.6x10^{-4}$ M) (Weber *et al.*, 1994).

A possible mechanism tentatively explaining how the Ca^{2+} signaling could be integrated by axoneme at the level of the outer arm dynein was recently proposed by King (2010). Numerous studies using Chlamydomonas mutants suggest that the gamma heavy chain (HC) acts as a key regulatory node for controlling outer arm function in response to alterations in curvature and ligand binding. The molecular mechanism by which altered Ca^{2+} levels might lead to a change in flagellar/ciliary waveform would be by controlling whether one heavy chain of outer arm dynein acts as a microtubule translocase or as an ATP-dependent brake that limits the amount of inter-doublet sliding. Depending on Ca^{2+} concentration the gamma HC could act either as an active lever (low Ca^{2+}) responsible of efficient sliding or as a Ca^{2+}-activated tethered winch: indeed, under high Ca^{2+} conditions, the gamma HC may act as an ATP-dependent brake able to limit and thus inhibit local sliding. The latter situation would improve value to the possibility that the HC functions as one of the springs (in this case, one whose compliance is tunable in a Ca^{2+}-dependent manner) that have been hypothesized to be necessary for "self-organized oscillation" in axoneme (Mitchison and Mitchison, 2010).

Path curvature is intuitively related to flagellar curvature or asymmetry. It was shown that the proportionality faction ranges values of 1.5 - 2 and it increases to values >3 when the waves amplitude increases (Friedrich *et al.*, 2010).

According to literature review (Turner, 2013), some indirect evidence for a role of calcium ions in mammalian sperm motility is that several calcium channels of alpha-1 type (ie, pore-forming) subunits have been identified in sperm (Lievano

CHAPTER 12

Conclusions on Sperm Guidance Features

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Abstract: This book is aiming to present a compilation of most data presently explaining how a flagellum operates and how it governs the direction of the sperm cell that it propels. Sum of knowledge in these fields and general laws that can be formulated are briefly developed in this conclusion.

Keyword: Sperm guidance.

In 1903, a paper published by Buller was entitled: "Is chemotaxis a factor in the fertilization of the eggs of animals?" The attempted answer to this question was first reported by Lillie in 1912, who observed the existence of sperm chemotaxis in the animal kingdom. However, sperm chemotaxis was just confirmed in marine invertebrate and mammalian species after a 50 and 70 years gap, respectively (Kaupp, 2012; Eisenbach and Giojalas, 2006). Since the pioneering period, sperm chemotaxis was further characterized in a lot of animal species, especially in external fertilizers by Miller (1985), and a few in mammals (Eisenbach and Giojalas, 2006). The diversity in reproductive strategies between species is evident. Internal reproduction allows participants such as female genital tract to intervene. Conversely, external fertilizing species liberate sperm "in the wild" at spawning, limiting the female role to the vicinity of the egg. In any event, it is clear the need for sperm guidance towards the egg surface. Even though several sperm orienting mechanisms have been proposed, chemotaxis is the most widely

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studied phenomenon. Thus, several features appear to be shared among the so many species where chemotaxis was revealed, which are summarized below.

The **source of attractants** is, in most cases, the egg itself, sometimes egg structures/cells surrounding the egg, or fluids from the female genital tract. Nevertheless, a question remains open in many species: do all eggs in a batch produce the attractant? Is there a specific stage of maturation at which attractant molecules are specifically produced?

The **chemical nature of attractants** is highly diversified, even though a small number have been so far identified. Diversity may be related to the necessity of a high species-specificity (*e.g.* in case of external fertilization). However, attractant diversity might also ensure the occurrence of chemotaxis in case of failure of one attractant, or even to sustain a step-by-step guidance (*e.g.* in mammals). In any event the latter possibilities needs further investigation.

The constitution of a **concentration gradient** of attracting molecules is a must for the occurrence of chemotaxis. The environment of the source of the attractant should guarantee the availability and stability of the attractant gradient in the vicinity of the eggs as long as they are viable. For that, biological system evolved for adaptations to resist or take advantage, from gradient disrupting agents like fluid movement (for instance, water currents and oviduct peristalsis).

The optimum **attractant concentration** seems to be associated to sperm adaptations which evolved towards a very high sensitivity for the attractant sensor, leading even to fentomolar detection thresholds.

In the few cases where the biological response is understood in details, it is frequently associated to a **temporal gradient** preferentially to a spatial one, meaning that the attractant gradient is sensed over time. Nevertheless, the influence of effective distance for attractant distribution along the oocyte and surrounding layers is still an opened question.

In mammals spermatozoa needs a **physiological preparation** in order to elicit the chemotactic response, which gives to the chemical orientation a “sperm selective” aptitude. This fact refer to the possibility of cell to cell variability within sperm

population, which might differ from male to male or with age of the sperm sample. However, the lack of observations about physiological aptitude for invertebrate sperm chemotaxis, does not necessary discard this possibility in this group of animals.

In all cases so far well documented, the chemotaxis molecular mechanism appears to be regulated by **Ca^{2+} mobilization**, which becomes an unavoidable step in the cascade of events leading to turns of the sperm paths due to an increase of the asymmetry of flagellar waves. The latter events are pronounced in invertebrate marine and smoother in mammals, facilitating the approach towards the source of attractants.

Though the occurrence of sperm chemotaxis seems to enhance **gamete encounter** inside complex environments, other mechanisms (for instance, thermotaxis, rheotaxis) may complement the sperm transport to ensure an efficient sperm delivery to the egg surface or serve as alternative strategies to pursue the same goal. The complementary aspects between chemo-, thermo- and rheo-taxis were very recently discussed by Cerezales *et al*. (2015). It should be emphasized that additional purposes of sperm chemotaxis cannot be discarded. For instance, high specificity of gametes recognition in a same species contributes to minimize cross-fertilization between close species. Also, sperm-to-sperm attraction looks like an adaptation that evolved in sneaker sperm to increase their chance to fertilize the egg (Hiroashi *et al*., 2013). Sperm chemo-attraction might also be a way to find out a sperm reservoir such as the cervix or the lower isthmus. In any event, it is clear that eggs also developed "**sex appeal**" towards sperm partners. Even though a large amount of knowledge about sperm chemotaxis has been accumulated along a hundred year (as summarized in this book), a lot of details in the mechanistic still remain to be revealed.

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Appendix

CHAPTER 1

Drosophila spermatozoa
http://youtu.be/kDB2ATUTwKg

Sea urchin spermatozoon under stroboscopic illuminations
http://youtu.be/d0hnYf4YOqI

Sea urchin sperm: flagellar waves
http://youtu.be/34MGU72OBno

Human spermatozoa swimming
http://youtu.be/S3tUcSIUz2s

Human spermatozoa at high magnification
http://youtu.be/oUqzIzdDudY

Goat sperm motility
http://youtu.be/UWbrho_Wc4M

Sea urchin sperm flagellum: 3D distortion
http://youtu.be/aQRp0e2g2Bo

Sea urchin spermatozoon swimming in egg jelly
http://youtu.be/B12c7jexgts

Oyster sperm maturation
http://youtu.be/9zIuQgGYV94

Oyster sperm motility
http://youtu.be/rpjtQGR3m3U

Oyster sperm movement: effect of viscosity
http://youtu.be/N9GbeZIyTTU

Sturgeon sperm flagella recorded with a high-speed camera
http://youtu.be/ZNewjimkRCw

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Flagella microtubules recorded while sliding
http://youtu.be/rMrZn8SjKS4

Effect of Ca^{2+} ions on flagella shape in sea urchin sperm
http://youtu.be/6VAyycXgukM

Demembranation and movement reactivation of sea urchin sperm flagella
http://youtu.be/YmnWW38hZCc

Effects of CO_2 on turbot sperm motility
http://youtu.be/YmnWW38hZCc

Demembranation and motility reactivation by ATP of turbot (fish) spermatozoa
http://youtu.be/QkjDOuxWZNs

Turbot (fish) spermatozoa swimming in the vicinity of the egg micropyle
http://youtu.be/pYJU6eq8LMw

Normal swim of Turbot (fish) spermatozoa
http://youtu.be/RFJgRjsZmrw

Motility of Turbot (fish) spermatozoa activated by sea water
http://youtu.be/xOY-2p5U5oE

Swimming period of turbot (fish) spermatozoa
http://youtu.be/NqXEszPXmFM

Xenopus spermatozoa: helical waves
http://youtu.be/MFnWwhCMENU

CHAPTER 3

Sperm motility activation in *Phallusia mammillata* (ascidian)
http://youtu.be/Yg5g8W6z3Ig

Sperm motility attraction by a micro-pipet in *Phallusia mammillata* (ascidian)
http://youtu.be/5yB1kGnkfDc

Sperm motility attraction by micro-beads in *Phallusia mammillata* (ascidian)
http://youtu.be/s6g7VE5Ww-w

CHAPTER 5

Sperm attraction by an oyster egg
http://youtu.be/i-yYsy3k1Co

Attraction of siphonophore (jelly-fish) spermatozoa to an egg structure (Cupule)
http://youtu.be/XuwkJC8452M

Description of the sperm attractant in a siphonophore (jelly-fish)
http://youtu.be/odYJZDI182Y

Sperm of siphonophore (jelly-fish) attracted to a micro-pipet tip
http://youtu.be/-qTM8_E6AeE

Sperm accumulation to Turbot (fish) egg micropyle
http://youtu.be/pYJU6eq8LMw

CHAPTER 8

https://www.youtube.com/watch?v=Xjq66vS-Cj4
https://www.youtube.com/watch?v=_K3hG5VLzZo

CHAPTER 9

Virtual-Sperm Rheotaxis
https://www.youtube.com/watch?v=G10ItBO55BM&feature=youtu.be

CHAPTER 10

Contraction of the posterior flagellum of a dinoflagellate (*Ceratium*)
http://youtu.be/WAyc0-dfL7s

Intra-flagellar particles movement in Chlamydomonas (IFT)
http://youtu.be/uHHLVLHKMME

The flagellate Chlamydomonas movement: native and demembranated cells
http://youtu.be/LjgGktCSjqE

Dinoflagellates: General description
http://youtu.be/g8t0UEcsoDk

A dinoflagellate (Erythropsis) with an unusual type of contraction/retraction organelle
http://youtu.be/12W34KmtSDQ

SUBJECT INDEX

E

F

G

H

I

J

L

M

N

O

P

R