Gerhard Obermeyer
José Feijó *Editors*

Pollen Tip Growth

From Biophysical Aspects to Systems Biology

Pollen Tip Growth

Gerhard Obermeyer • José Feijó
Editors

Pollen Tip Growth

From Biophysical Aspects to Systems Biology

Springer

Editors
Gerhard Obermeyer
Department of Molecular Biology
University of Salzburg
Salzburg, Austria

José Feijó
Cell Biology Molecular Genetics
University of Maryland
College Park
Maryland, USA

ISBN 978-3-319-56644-3 ISBN 978-3-319-56645-0 (eBook)
DOI 10.1007/978-3-319-56645-0

Library of Congress Control Number: 2017942819

© Springer International Publishing AG 2017
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

Part I Introduction

1 Pollen Tubes and Tip Growth: of Biophysics and Tipomics 3
Gerhard Obermeyer and José Feijó

Part II Biophysics of Tip Growth

2 Water Transport in Pollen 13
Gerhard Obermeyer

3 The Cytoskeleton of Pollen Tubes and How It Determines the Physico-mechanical Properties of Cell Wall 35
Giampiero Cai, Luigi Parrotta, and Mauro Cresti

Part III Technical Improvements to Study Tip Growth

4 Measuring Cytomechanical Forces on Growing Pollen Tubes 65
Hannes Vogler, Naveen Shamsudhin, Bradley J. Nelson, and Ueli Grossniklaus

5 Microfluidic- and Microelectromechanical System (MEMS)-Based Platforms for Experimental Analysis of Pollen Tube Growth Behavior and Quantification of Cell Mechanical Properties 87
Anja Geitmann

Part IV Subcellular Processes

6 Polar Protein Exocytosis: Lessons from Plant Pollen Tube 107
Hao Wang and Liwen Jiang

7 Pollen Tip Growth: Control of Cellular Morphogenesis Through Intracellular Trafficking 129
Hana Rakusová and Anja Geitmann

8 Directional Growth for Sperm Delivery 149
Subramanian Sankaranarayanan and Tetsuya Higashiyama

9 Molecular Mechanisms Regulating Root Hair Tip Growth: A Comparison with Pollen Tubes 167
Sébastjen Schoenaers, Daria Balcerowicz, and Kris Vissenberg

Part V Tipomics: Omic Approaches in Tip Growth

10 When Simple Meets Complex: Pollen and the -Omics 247
Jan Fíla, Lenka Záveská Drábková, Antónia Gibalová, and David Honys

11 The Pollen Membrane Proteome 293
Heidi Pertl-Obermeyer

12 Pollen Metabolome Dynamics: Biochemistry, Regulation and Analysis 319
Thomas Nägele, Lena Fragner, Palak Chaturvedi, Arindam Ghatak, and Wolfram Weckwerth

Part VI Modeling Tip Growth

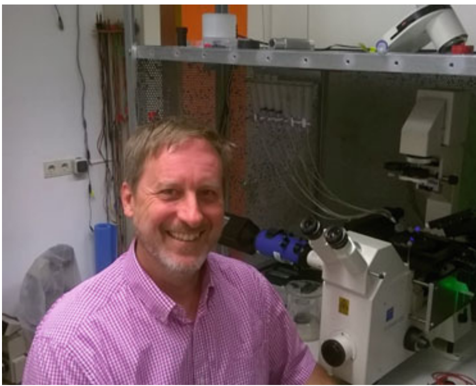
13 Derivation and Use of Mathematical Models in Systems Biology 339
Robert W. Smith and Christian Fleck

14 A Fresh Look at Growth Oscillations in Pollen Tubes: Kinematic and Mechanistic Descriptions 369
Milenka Van Hemelryck, Roberto Bernal, Enrique Rojas, Jacques Dumais, and Jens H. Kroeger

15 One Thousand and One Oscillators at the Pollen Tube Tip: The Quest for a Central Pacemaker Revisited 391
Daniel S.C. Damineli, Maria Teresa Portes, and José A. Feijó

Index 415

Editors' Biographies



Gerhard Obermeyer born in 1961, studied biology at the University of Konstanz, Germany, with majors in membrane and cell biophysics. His Ph.D. thesis at the Karlsruhe Institute of Technology (Germany) included the imaging of tip-localized Ca^{2+} gradients in pollen tubes and first patch-clamp experiments to characterize pollen ion channels. As a postdoc, he worked at Wye College (now part of Imperial College, London, UK) on ion channels from guard cells and pollen grains and continued pollen research at the Institute of Plant Physiology (University of Salzburg, Austria) with intermediate fellowships to visit labs at the University of Adelaide (South Australia), in Cuernavaca (Mexico), and in Oxford (UK) to work on symbiosome membranes and intracellular pH measurements. Finally, he became an associate professor at the University of Salzburg where he established molecular plant physiology in research and teaching.

Prof. Obermeyer's research focuses mainly on pollen physiology. The growth of pollen tubes through the style tissue is a prerequisite for a successful fertilization which guarantees high crop yields for human nutrition. Problems caused by global warming like drought and temperature stress, can disturb pollen function and are studied using several single-molecule/single-cell techniques in combination with -omics approaches to reveal functional protein complexes in the plasma membrane and their role in osmosensing and osmoregulation as well as in tip growth.

José Feijó studied biology at the University of Lisbon, Portugal, specializing in cell biology of orchid pollen, and obtained his master's in plant biotechnology. During his Ph.D. he enlarged his focus into development, progressively introducing electrophysiology and mathematical modelling as routine approaches to the study of pollen tubes. A Fulbright fellowship brought him to Peter Hepler's lab at the University of Massachusetts in Amherst to further deepen his skills on ion imaging. His return to the University of Lisbon in 1996 marked the beginning of his professorship and independent research career; from 1999 to 2013, he ran in parallel with an independent lab at the Gulbenkian Institute for Science. Along this path, he has served as a director of the Imaging Unit, organized over a dozen EMBO practical courses on plant development and imaging, acted as a curator, and collaborated with numerous educational projects; he was also responsible for a number of initiatives to commemorate Darwin's bicentenary in 2009. In late 2013, he moved to the University of Maryland, College Park, and lives in Washington, DC.

The research of Feijó's group is focused on the development of integrated models of apical cell growth and morphogenesis, using the pollen tube as a biological model, ion dynamics as an experimental paradigm, and theoretical modeling as an integrative tool. The group uses Arabidopsis, lily, tobacco, and tomato as model species for higher plants and the moss Physcomitrella as an evolutionary correlate of apical growth evolution. On the path to develop models by which ion dynamics choreographies integrate spatial and temporal cues to coordinate cell biology, the group contributed to novel ion channels and sensors involved in pollen tube biology and their regulation mechanisms. Most results involve a combination of imaging, electrophysiology, genetics, and molecular biology. Feijó's group further pioneered transcriptomics of plant male gametes and its consequences for plant reproduction and improvement, namely, at the epigenetics level. These activities were routinely grounded in collaborations with groups in over 12 countries in the 4 continents.

The authors met at the Sexual Plant Reproduction meeting in Vienna, Austria, in 1998, and collaborated; they became good friends ever since.

Part I
Introduction

Chapter 1
Pollen Tubes and Tip Growth: of Biophysics and Tipomics

Gerhard Obermeyer and José Feijó

Keywords Biophysics • Molecular mechanisms • Omics techniques • Pollen • Systems pollen biology • Tip growth • Tipomics

1.1 Introduction

Pollen is the male gametophyte of higher plants and is responsible for the successful transport of the sperm cells to the ovules. After landing on a stigma, pollen grains will germinate and grow a pollen tube through the stigma and style tissue towards the ovules, where fertilization takes place. In terms of cell biology, the elongation of the pollen tube is characterized by a dramatically polarized growth process, tip growth, which is common to root hairs, fungal hyphae and some developing neurites. Due to their simple morphology and function, growing pollen tubes became the most well-established model system to study tip growth. Both the editors of this book have been enthusiastic paladins of this trend, even since they first met in 1994 during the 13th International Congress on Sexual Plant Reproduction in Vienna. Our intellectual enthusiasm was first materialized in an essay in which a naive and simple yet forward theoretical model was set forth, implying a set of electrochemistry rules to be at the core of a minimal set of mechanism underlying cell polarity establishment and maintenance during pollen tube growth (Feijó et al. 1995). In short, subcellular biophysical processes, like ion transport, endogenous electrical fields and a tip-focussed Ca^{2+} gradient, would regulate the shape and growth rate in an essentially self-organizing process. Ever since then, the multitude of nuts and bolts and genes and pathways and their biological consequences have amounted to a vast literature in practically every aspect of the biology of pollen.

G. Obermeyer (✉)
Molecular Plant Biophysics and Biochemistry, Department of Molecular Biology, University of Salzburg, Billrothstrasse 11, 5020 Salzburg, Austria
e-mail: gerhard.obermeyer@sbg.ac.at

J. Feijó
Department of Cell Biology and Molecular Genetics, University of Maryland, Bioscience Research Building, College Park, MD 20742, USA
e-mail: jfeijo@umd.edu

© Springer International Publishing AG 2017

G. Obermeyer, J. Feijó (eds.), *Pollen Tip Growth*,
DOI 10.1007/978-3-319-56645-0_1

And yet, some of the essential parts of this naive biophysical view of the pollen tube remained elusive, namely the existence and features of the channels responsible for the unique ion biology of pollen (Michard et al. 2017).

By necessity, the views on tip growth in pollen tubes have changed immensely ever since this fortunate encounter of the editors, and a multitude of functional and theoretical models have been proposed, reflecting as many facets as the expertise area of the different groups that since then have been pushing the field forward, be it cytoskeleton, biomechanics, membrane trafficking, signalling, protein sorting, etc. [reviewed by Feijó et al. (2001), Holdaway-Clarke and Hepler (2003), Bibikova et al. (2004) and Certal et al. (2008)]. And from a systems biological view, the emergence of transcriptomics had a tremendous impact, with pollen and fertilization emerging as some of the best described biological processes in terms of specific transcriptional profiles, with databases in practically every facet of its biology.

And yet a generalized accepted model for cell apical growth is lacking. Of notice, from our initial biophysical perspective of the pollen tube, the relationships between the activity of ion channels and pumps in the plasma membrane of the growing pollen tube, the ion fluxes and cytosolic gradients of K^+, Cl^-, Ca^{2+} and H^+ they generate and growth rate, determinants of growth direction and oscillatory growth of pollen tubes are still elusive and subject to multiple interpretation. Even a simple hypothesis, like the heterogeneous distribution of different ion channel types and pumps is responsible for the localized, specific ion fluxes along a growing pollen tube (Feijó et al. 1995) that could be easily tested by following expression chimeras of GFP, is largely missing for practically every transporter identified either by physiology, by genetics or even less by transcriptomics (Michard et al. 2017). Usually, the spatial distribution of these ion fluxes around a germinating pollen grain or along a growing pollen tube can be measured with ion-sensitive vibrating electrodes (Kunkel et al. 2006). Although this technique allows a characterization of the currents and current pattern, it cannot identify the transport protein responsible for the fluxes. In addition, a number of signal transduction pathways are already known as components of a regulatory network that contribute to activation or inactivation of cellular processes involved in tip growth. The signal transduction pathways include the cytosolic free Ca^{2+} concentration, the cytosolic pH, protein phosphorylation and 14-3-3 proteins, G-proteins, Ca^{2+} activated kinases, CBL/CIPK pairs and phospholipids (Kost et al. 1999; Pertl et al. 2001; Potocky et al. 2003; Monteiro et al. 2005; Michard et al. 2008; Steinhorst et al. 2015). All mentioned components were found to be essential for the growth process of pollen tubes, and one may assume that they also interact with each other thus forming a complex, spatially and temporally regulated network coordinating the dynamic events necessary for pollen tube growth. In accordance with these results, the molecular and cellular processes do not form a linear causality chain, but pollen tube growth can be seen as a complex system of interacting networks formed by nodes connected with edges (see Klipp et al. (2009) and Coruzzi and Gutiérrez (2009) for an introduction to "Systems Biology"). Such type of systems studies for pollen tube tip growth could plausibly be achieved by combining information coming from the different omic levels, if properly framed with specific hypothesis-driven approaches. While any such model

will by necessity face enormous challenges for validation because of elevated degree of spatial and temporal dynamics characteristic of pollen tube growth, even coarse approaches could be tremendously influential to characterize the information flow carried by different networks that collectively contribute to the major outcome of pollen tubes: growth and delivery of the sperm.

This was essentially the mind-set of the authors while embarking the edition of this book. The invited authors contributed chapters that highlight many important aspects of pollen physiology and tube tip growth and other conceptual fields which are needed to better understand it. Hopefully our book will provide an updated and balanced overview of the current knowledge and present future research perspectives and will contribute to fill the many gaps obviously still existing in our fundamental understanding on how pollen tubes growth is driven and regulated by molecular interactions underlying the cellular processes. Chapters range from molecular biophysical concepts to comprehensive omic studies and computational modelling of the tip growth process. In addition, a chapter on root hair cells is included to provide, although almost similar in many aspects, an alternative view on the underlying molecular principles of the tip growth process.

1.2 Some Unsolved (Bio)physical Aspects of Tip Growth

It's very simple: the pollen tube and all other tip-growing cells grow by increasing their volume and, in the case of plant cells, mostly through the uptake and accumulation of water in the vacuole. Although simple, this process causes many difficulties and problems for pollen tubes. For example, net water uptake will cause an increase in turgor pressure and, thus, will exert pressures yet to be precisely determined at the nascent cell wall at the tube tip. This means that the stability of the newly formed cell wall has to be adapted to the growth speed and to the water uptake rates, and one might expect receptors for turgor pressure and/or cell wall strain, which sense the actual osmotic state and the stability of the cell wall. On a different perspective, in growing pollen tubes, the water potential inside the tube has to be lower than in the surrounding tissue to enable an influx of water. This can be achieved by regulating the cytosolic osmotic and turgor pressure of the pollen, but also of the surrounding style tissue. Of relevance, these two issues bring to the board two major aspects of an essential biophysics approach, biomechanics and electrochemistry, that can and should be properly quantifiable and modelled.

Although these considerations are prerequisites for every fast growing plant cell, not much is known about the molecules, processes and signalling pathways involved in the regulation of water transport. Biophysical concepts, recent results and future perspectives are summarized in the first chapters of this book with Chap. 2 describing aspects of water transport in pollen tubes and Chaps. 3, 4 and 5 covering subjects of cell wall stability and mechanical forces during tube expansion. Readers will notice that open questions are in most cases related to still unknown physical parameters. Other than just turgor pressure, also the viscosity and elastic modulus

of cell walls are important for the stability and flexibility of the cell wall to adapt to new micro-environments along the path to the ovule.

1.3 Technical Improvements and a Wish List for the Future

To answer some of these unsolved biophysical questions, new techniques must be developed or adapted to the fast growing pollen tube. For instance, our understanding will immensely increase if one could monitor the dynamic changes of cell wall elasticity in an elongating tube. In a dramatic turn of page into nano-techniques, Chaps. 4 and 5 propose original approaches based on micromachined mazes, which incorporate microfluidics capacity allowing the use of non-invasive cellular force microscopy to reveal changes in cell wall elasticity, live cell imaging and the challenge of the growing tubes with a number of microfluidic treatments that can physically alter the forces involved in growth. The promise that these approaches can incorporate higher level of resolution in terms of live cell imaging could be of paramount importance to understand the flexibility of the pollen tube growth process.

In fact, starting from the early 1990s, which have seen some, still coarse, videos of tip-localized Ca^{2+} gradients determining the pollen tube growth direction, the techniques of fluorescent reporters are becoming more and more sophisticated. By adapting the use of genetically engineered nanosensors almost every ion (Ca^{2+}, pH, phosphate), metabolite (glucose, sucrose, ATP) or physical parameter (membrane voltage) can be monitored in living plant cells and, therefore, also in pollen tubes (Swanson et al. 2011; Okumoto et al. 2012). So far, only Ca^{2+}, pH and chloride sensors have been used in pollen tubes, but looking at the still increasing availability of nanosensor constructs (Hamers et al. 2014), we might be able to watch combinations of parameters simultaneously in the future, maybe in real time and under physiological conditions, namely, in pollen tubes growing through the style tissue.

So far, most experiments have been performed with in vitro growing pollen tubes in liquid or on solidified media. An increasing number of studies have shown that pollen tubes are conditioned during their journey through the stigma and style tissue (see Chap. 8). Using microfluidic chambers or lab-on-chip devices for pollen tube growth, the physiological conditions of a surrounding tissue can be mimicked, and, furthermore, semi-in vivo pollen cultures can be set up that allow pollen grains to germinate on the stigma and grow a few microns through the style tissue before entering a microchannel, which then allows a detailed and high-resolution view of the growing tip. The use of various microfluidic devices is described and discussed in Chaps. 4, 5 and 8.

Also in the front of better and improved imaging methods, Chap. 6 pushes the envelope in terms of microscopic observations of membrane trafficking and referred to a number of essential publications on state-of-the-art microscopy, for instance, light sheet microscopy (Maizel et al. 2011; Kumar et al. 2014), fluorescence

lifetime FRET (Schleifenbaum et al. 2009), 2-photon confocal microscopy (Feijó and Moreno 2004) or TIRF (Wang et al. 2016).

1.4 Tipomics: Pollen Systems Biology and Modelling Tip Growth

A major step towards a comprehensive view of the tip growth process in pollen tubes was the application of various omics techniques to reveal the molecular details of as many cellular processes as possible. In addition to the pollen transcriptome, the data from proteome and metabolome allow the modelling of metabolic pathways during different states of the pollen's life, whereas studies of the protein interactome and the phosphoproteome show the dynamics of signalling events and signal transduction pathways in pollen (see Chaps. 10, 11 and 12). Hereby, the system can be restricted to a single-cell type like the pollen grain, but may also span different tissue and organs of the whole organism. By considering the time dependence of signal propagation, a time-resolved map of information flux inside a cell or inside an organism can be studied. The spatial resolution of cellular dynamics is getting more and more refined as e.g. with the studies of distribution of proteins and metabolites in cell organelles. Integration can be achieved by information on protein localization derived from databases, literature or immunolocalization experiments and be used to reveal the dynamics of protein re-localization during biological processes in regular proteomics approaches (Dunkley et al. 2006; Pertl-Obermeyer et al. 2016). Excellent examples of the technical feasibility with omic methods are given in recent studies that investigated the phosphoproteome dynamics of in vivo and in vitro insulin signalling with a highly time-resolved, spatial distribution of signalling information (Humphrey et al. 2015), showed a high spatial resolution of cellular organelles (Christoforou et al. 2016) or gave a comprehensive protein interaction map for human cells (Liu et al. 2015) and enabled the detection of the spatial distribution of metabolites in cell organelles (Krueger et al. 2011; Arrivault et al. 2014). In pollen, the experimental challenge will be to apply the recently developed omics techniques to the fast growing pollen tube with a suitable time and spatial resolution. A major limitation will be the material that can be collected at each time point and the preparation methods to avoid loss of material and to extract all components of interest. These problems are addressed by the authors of Chaps. 10, 11 and 12. However, recent improvements in mass spectrometry allow an optimistic view that the amount of experimental material can be decreased even further. Due to the development of more sensitive and faster mass spectrometers (Michalski et al. 2011, 2012) and open source analysis software, e.g. MaxQuant (Tyanova et al. 2016) or PatternLab (Carvalho et al. 2016), proteome data can be sampled with only low amounts of starting material and analysed in a flexible way, thus enabling also proteome analyses with *Arabidopsis* pollen during signalling events at higher time resolution than just ungerminated and germinated. While the transcriptome can be

studied already in single cells (Ntranos et al. 2016), the single pollen proteomics might be possible in the near future, too.

Another experimental challenge for omics approaches is the application to in vivo situations. Pollen tubes growing through the style towards the egg cells are conditioned to respond to the female tissue signals (see Chap. 8). In addition, as most processes and molecules responsible for growth of pollen tubes can be observed in the tube tip and this tip responds fast to signals, a detailed view to the proteome, metabolome, ionome, interactome, etc. of the tube tip with a high time and spatial resolution might push our knowledge on tip growth dynamics further beyond the present horizon. In combination with physiological parameters, nonlinear statistics of the large omics data sets can be used in up-to-date mathematical modelling to generate unbiased computer models of tip growth (Klipp et al. 2009; Ingalls 2013). The last three Chaps. 13, 14 and 15 give an impression on how mathematical models can be derived from the present data and which considerations have to be made to generate a valuable model that allows testing our hypotheses and can make predictions that can be tested experimentally. In fact many such theoretical models have been proposed to explain the essential features of pollen tube, and a critical view of their essential features, potential and limitations is offered in Chap. 15.

1.5 Conclusion, Open Questions and Perspective

The last decades of pollen tip growth research have shown a vigorous increase in processes “important for tube growth”. Almost every cellular process or molecule under investigation could be shown to be important, responsible, essential or crucial for pollen tubes. Have they all a similar importance or is there a master regulator? This will be a challenge to test mathematical models that describe pollen tip growth and also a challenge to design unbiased data collection in future experiments. Probably, we have to disband our own way of thinking in linear causation chains and have to imagine complex networks which provide numerous alternative ways to achieve an aim. This might be exactly the case for pollen tip growth. Why should such an important and robust but on the same hand delicate process, which creates the progeny of a population, be so vulnerably relying on a single event cascade or causality chain where a single failing chain link can cause the abortion of the process, namely, final growth stop? On the other hand, one may argue that pollen tube growth is highly competitive and selective, so that a single mistake is enough to kick this individual out of the gene pool ensuring a healthy next generation. As almost always in biology, a combination of both extremes is very likely: a complex network with a major route along all nodes but with several alternative ways and bypasses to achieve the goal.

All authors contributing to this book have pinpointed important gaps in the data of their research field. We are grateful for their generous effort in putting thorough texts of high quality that gain a special corpus we set together in the form of a reference book. We hope our collective effort as editors and authors may offer

other colleagues and young starting researchers solutions, either experimental or theoretical, to fill our perceived gaps of knowledge in this area or to solve some of the still many mysterious behaviour of pollen tube tips.

References

Arrivault S, Guenther M, Florian A, Encke B, Feil R, Vosloh D, Lunn JE, Sulpice R, Fernie AR, Stitt M, Schulze WX (2014) Dissecting the subcellular compartmentation of proteins and metabolites in Arabidopsis leaves using non-aqueous fractionation. Mol Cell Proteomics 13:2246–2259

Bibikova TN, Assmann S, Gilroy S (2004) Ca^{2+} and pH as integrated signals in transport control. In: Blatt MR (ed) Membrane transport in plants, Annual plant reviews, vol 15. Blackwell, Oxford, pp 252–278

Carvalho PC, Lima DB, Leprevost FV, Santos MDM, Fischer JSG, Aquino PF, Moresco JJ, Yates JR III, Barbosa VC (2016) Integrated analysis of shotgun proteomic data with PatternLab for proteomics 4.0. Nat Protoc 11:102–117

Certal AC, Almeida RB, Carvalho LM, Wong E, Moreno N, Michard E, Carneiro J, Rodriguez-Leon J, Wu H-M, Cheung AY, Feijo J (2008) Exclusion of a proton ATPase from the apical membrane is associated with cell polarity and tip growth in *Nicotiana tabacum* pollen tubes. Plant Cell 20:614–634

Christoforou A, Mulvey CM, Breckels LM, Geladaki A, Hurrell T, Hayward PC, Naake T, Gatto L, Viner R, Martinez Arias A, Lilley KS (2016) A draft map of the mouse pluripotent stem cell spatial proteome. Nat Commun 7. doi:10.1038/ncomms9992

Coruzzi GM, Gutiérrez RA (2009) Plant systems biology. In: Annual plant reviews, vol 35. Wiley-Blackwell, Oxford

Dunkley TPJ, Hester S, Shadford IP, Runions J, Hanton SL, Griffin JL, Bessant C, Brandizzi F, Hawes C, Watson RB, Dupree P, Lilley KS (2006) Mapping the *Arabidopsis* organelle proteome. Proc Natl Acad Sci USA 103:6518–6523

Feijó JA, Moreno N (2004) Imaging plant cells by two-photon excitation. Protoplasma 223:1–32

Feijó JA, Malhó R, Obermeyer G (1995) Ion dynamics and its possible role during *in vitro* pollen germination and tube growth. Protoplasma 187:155–167

Feijó JA, Sainhas J, Holdaway-Clarke T, Cordeiro S, Kunkel JG, Hepler PK (2001) Cellular oscillations and the regulation of growth: the pollen tube paradigm. Bioessays 23:86–94

Hamers D, van Voorst VL, Borst JW, Goedhart J (2014) Development of FRET biosensors for mammalian and plant systems. Protoplasma 251:333–347

Holdaway-Clarke T, Hepler PK (2003) Control of pollen tube growth: role of ion gradients and fluxes. New Phytol 159:539–563

Humphrey SJ, Azimifar SB, Mann M (2015) High-throughput phosphoproteomics reveals *in vivo* insulin signaling dynamics. Nat Biotechnol 33:990–995

Ingalls BP (2013) Mathematical modeling in systems biology. MIT Press, Cambridge

Klipp E, Liebermeister W, Wierling C, Kowald A, Lehrach H, Herwig R (2009) Systems biology. A textbook. Wiley Blackwell, Weinheim

Kost B, Lemichez E, Spielhofer P, Hong Y, Tolias K, Carpenter C, Chua N-H (1999) Rac homologues and compartmentalized phosphatidylinositol 4, 5-bisphosphate act in a common pathway to regulate polar pollen tube growth. J Cell Biol 145:317–330

Krueger S, Giavalisco P, Krall L, Steinhauser M-C, Büssis D, Usadel B, Flügge U-I, Fernie AR, Willmitzer L, Steinhauser D (2011) A topological map of the compartmentalized *Arabidopsis thaliana* leaf metabolome. PLoS One 6:e17806

Kumar A, Wu Y, Christensen R, Chandris P, Gandler W, McCreedy E, Bokinsky A, Colón-Ramos DA, Bao Z, McAuliffe M, Rondeau G, Shroff H (2014) Dual-view plane illumination microscopy for rapid and spatially isotropic imaging. Nat Protoc 9:2555–2573

Kunkel JG, Cordeiro S, Xu Y, Shipley AM, Feijó JA (2006) Use of non-invasive ion-selective microelectrode techniques for the study of plant development. In: Volkov AG (ed) Plant electrophysiology. Theory and methods. Springer, Berlin, pp 110–137

Liu F, Rijkers DTS, Post H, Heck AJR (2015) Proteome-wide profiling of protein assemblies by cross-linking mass spectrometry. Nat Methods. doi:10.1038/NMETH.3603

Maizel A, von Wangenheim D, Federici F, Haseloff J, Stelzer EHK (2011) High-resolution live imaging of plant growth in near physiological bright conditions using light sheet fluorescence microscopy. Plant J 68:377–385

Michalski A, Damoc E, Hauschild J-P, Lange O, Wieghaus A, Makarov A, Nagaraj N, Cox J, Mann M, Horning S (2011) Mass spectrometry-based proteomics using Q exactive, a high-performance benchtop quadrupole orbitrap mass spectrometer. Mol Cell Proteomics 10. doi:10.1074/mcp.M111.011015-1

Michalski A, Damoc E, Lange O, Denisov E, Nolting D, Müller M, Viner R, Schwartz J, Remes P, Belford M, Dunyach J-J, Cox J, Horning S, Mann M, Makarov A (2012) Ultra high resolution linear ion trap orbitrap mass spectrometer (Orbitrap Elite) facilitates top down LC MS/MS and versatile peptide fragmentation modes. Mol Cell Proteomics 11. doi:10.1074/mcp.O111.013698–1

Michard E, Dias P, Feijo JA (2008) Tobacco pollen tubes as cellular models for ion dynamics: improved spatial and temporal resolution of extracellular flux and free cytosolic concentration of calcium and protons using pHluorin and YC3.1 CaMeleon. Sex Plant Reprod 21:169–181

Michard E, Simon AA, Tavares B, Wudick MM, Feijo JA (2017) Signaling with ions: the keystone for apical cell growth and morphogenesis in pollen tubes. Plant Physiol 173:91–111

Monteiro D, Liu Q, Lisboa S, Scherer GEF, Quader H, Malhó R (2005) Phosphoinositides and phosphatidic acid regulate pollen tube growth and reorientation through modulation of $[Ca^{2+}]_c$ and membrane secretion. J Exp Bot 56:1665–1674

Ntranos V, Kamath GM, Zhang JM, Pachter L, Tse DN (2016) Fast and accurate single-cell RNA-seq analysis by clustering of transcript-compatibility counts. Genome Biol 17:112. doi:10.1186/s13059-016-0970-8

Okumoto S, Jones A, Frommer WB (2012) Quantitative imaging with fluorescent biosensors. Annu Rev Plant Biol 63:663–706

Pertl H, Himly M, Gehwolf R, Kriechbaumer R, Strasser D, Michalke W, Richter K, Ferreira F, Obermeyer G (2001) Molecular and physiological characterisation of a 14-3-3 protein from lily pollen grains regulating the activity of the plasma membrane H^+ ATPase during pollen grain germination and tube growth. Planta 213:132–141

Pertl-Obermeyer H, Wu XN, Schrodt J, Müdsam C, Obermeyer G, Schulze WX (2016) Identification of cargo for adaptor protein complexes AP-3 and AP-4 by sucrose gradient profiling. Mol Cell Proteomics 15. doi:10.1074/mcp.M116.060129

Potocky M, Elias M, Profotova B, Novotna Z, Valentova O, Zarsky V (2003) Phosphatidic acid produced by phospholipase D is required for tobacco pollen tube growth. Planta 217:122–130

Schleifenbaum F, Elgass K, Sackrow M, Caesar K, Berendzen K, Meixner AJ, Harter K (2009) Fluorescence intensity decay shape analysis microscopy (FIDSAM) for quantitative and sensitive live-cell imaging: a novel technique for fluorescence microscopy of endogenously expressed fusion proteins. Mol Plant 3:555–562

Steinhorst L, Mähs A, Ischebeck T, Zhang C, TZhang X, Arendt S, Schültke S, Heilmann I, Kudla J (2015) Vacuolar CBL-CIPK12 Ca^{2+}-sensor-kinase complexes are required for polarized pollen tube growth. Curr Biol 25:475–482

Swanson SJ, Choi W-G, Chanoca A, Gilroy S (2011) *In vivo* imaging of Ca^{2+}, pH, and reactive oxygen species using fluorescent probes in plants. Annu Rev Plant Biol 62:273–297

Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, Mann M, Cox J (2016) The Perseus computational platform for comprehensive analysis of (prote)omics data. Nat Methods. doi:10.1038/nmeth.3901

Wang H, Zhuang X, Wang X, Law AHY, Zhao T, Du S, Loy MMT, Jiang L (2016) A distinct pathway for polar exocytosis in plant cell wall formation. Plant Physiol 172:1003–1018

Part II
Biophysics of Tip Growth

Chapter 2
Water Transport in Pollen

Gerhard Obermeyer

Abstract Pollen is the male gametophyte of plants, and its major function is to deliver sperm cells to the ovules to ensure a successful fertilisation. Sperm cells are carried inside the pollen tube, a growth extension of the vegetative cell of pollen that grows through the stigma and style tissue. The elongation/growth of pollen tubes is a highly regulated biological process, and the uptake of water is thought to account for the enormous increase in cell volume. Water transport into the pollen grain initiates the transition from the quiescent mature to the active, rehydrated pollen grain and subsequently allows the large increase in volume during tube growth. To maintain the water uptake, internal pollen water potential has to be lower than the external water potential which can be achieved by lowering the turgor pressure, by increasing the internal osmotic pressure or by decreasing the external osmotic pressure. Uptake or synthesis of osmotic active compounds may be necessary to adapt the internal osmotic pressure due to changes in osmotic conditions during the tube's journey through the stigma tissue. Especially in periods of severe drought stress, the water potential of the stigma cells may drop, and thus water uptake into the pollen tube is distorted. The ability to sense and to adapt to osmotic conditions is therefore an important feature of the pollen to warrant a successful fertilisation and in consequence to ensure high crop yields.

Keywords Aquaporin • Turgor pressure • Water potential • Water transport

Abbreviations

AQP Aquaporin
L_P Hydraulic conductivity
MIP Major intrinsic protein

G. Obermeyer (✉)
Molecular Plant Biophysics and Biochemistry, Department of Molecular Biology, University of Salzburg, Billrothstrasse 11, 5020 Salzburg, Austria
e-mail: gerhard.obermeyer@sbg.ac.at

© Springer International Publishing AG 2017

G. Obermeyer, J. Feijó (eds.), *Pollen Tip Growth*,
DOI 10.1007/978-3-319-56645-0_2

PIP Plasma membrane intrinsic protein
PM Plasma membrane
P_{os} Osmotic permeability

2.1 Introduction

No water, no life. This simple statement counts for all biological organisms and life itself. Particularly in plants, water uptake and transport through the entire plant fuels a number of physiological processes that rely on constant water supply. For instance, uptake of nutrients by roots, transport of ions and sugars by the xylem and phloem system, respectively, and finally plant growth which among other factors is driven by elongation of single cells, which is supported by taking up water. Fast-growing plant organs need a high water uptake rate to maintain their turgor pressure despite volume increases. More than other cells, pollen tubes, which are among the fastest growing organisms in biology with a speed of up to 30 μm min^{-1}, need high water uptake rates to ensure the fastest possible growth through the stigma tissue. On the other hand, water uptake has to be regulated and well synchronised with other growth-related cellular processes like synthesis and transport of cell wall material, vesicle transport and the cytoskeleton to optimise fast growth to ensure fertilisation.

2.2 Water Potential, Water Transport and Pollen Tube Growth

The physical concept of the water potential is very useful especially on the level of single cells to describe and predict water fluxes between cells and the environment. It has to be noted that the common equations used are an approximation as emphasised by Jack Dainty (1963) more than 50 years ago: 'I am afraid that the only correct theory is that based on the theory of irreversible thermodynamics and this must be faced by all workers in this field'. Nevertheless, the water potential concept for everyday use is practical and allows a comprehensive modelling and prediction of water transport. Basic concepts of water relations in plant cells are described in a number of review article and book chapters (Dainty 1963; Steudle 1989; Zimmermann 1989; Nobel 2009). In analogy to Ohm's law $I = U \bullet R^{-1}$, the water (volume) flow J_V can be described as

$$J_V = L_P \bullet \Delta\Psi \tag{2.1}$$

with L_P as the hydraulic conductivity, and the water potential difference $\Delta\Psi$ acts as the driving force for water flux. The water potential itself derives from the chemical

potential of water and at the cellular level is defined as

$$\Psi = \frac{\mu_W - \mu_W^o}{\overline{V}_W} = P - \pi = \Psi_P + \Psi_\pi \tag{2.2}$$

where μ_W and μ_W^o are the chemical potential of water and the standard chemical potential for water, respectively, and $\overline{V}$ is the molar volume of water ($18.05 \cdot 10^{-6}$ m^{3}mol^{-1}), P ($= \Psi_P$) is the hydrostatic pressure (or pressure potential) and π ($= -\Psi_\pi$) is the osmotic pressure (or osmotic potential). The water potential is expressed in units of pressure (1 MPa = 10 bar) with 0 MPa as the highest value ($\mu_W = \mu_W^o$), and thus, water is always flowing in the direction to the more negative Ψ_W value.

In the presence of nonpermeable solutes only, the water (volume) flow density (j_V) across the plasma membrane separating the plant cell interior (i) from the medium (o), Eq. (2.1), can be written as

$$j_V = -\frac{1}{A}\frac{dV}{dt} = L_P\left(P - \Delta\pi\right) = L_P\left[P - \left(\pi^i - \pi^o\right)\right] \tag{2.3}$$

with A as the surface area of the cell; V, cell volume; L_P, the hydraulic conductivity; P, turgor pressure; R, gas constant; T, absolute temperature; and c^i and c^o, concentration of internal and external nonpermeable solutes, respectively. By considering the van't Hoff relation ($\pi \cong RT \bullet c$), Eq. (2.3) can be written as

$$j_V = -\frac{1}{A}\frac{dV}{dt} = L_P\left[P - RT\left(c^i - c^o\right)\right] \tag{2.4}$$

It has to be noted that only the case for nonpermeable solutes was considered in these equations. For a more general description of water transport including permeable solutes and simultaneous fluxes of water and solutes, please consult the literature (Kedem and Katchalsky 1958; Dainty 1963; Kramer and Boyer 1995; Murphy and Smith 1998).

A simplified relation between water fluxes and pollen tube growth is illustrated in Fig. 2.1. Growth of pollen tubes, namely, the increase in volume (dV/dt), is based on influx of water which according to Eq. (2.1) occurs only if $\Psi_i < \Psi_o$ or if $\Delta\pi > P$ (Eq. 2.3). To reduce the internal water potential, pollen tubes have to decrease their turgor pressure P or increase the internal osmolyte concentration c^i by uptake of solutes from the surrounding medium or by synthesis of osmotic active solutes. The movement of water into or out of a cell is always passive, and water follows the changes in water potential, which can be actively influenced by cells via solute transport or synthesis. The cell, or in this case the pollen tube, may reach an equilibrium when the turgor pressure equals the cytosolic osmotic pressure ($P = \Delta\pi$), and water uptake ceases as well as tube growth.

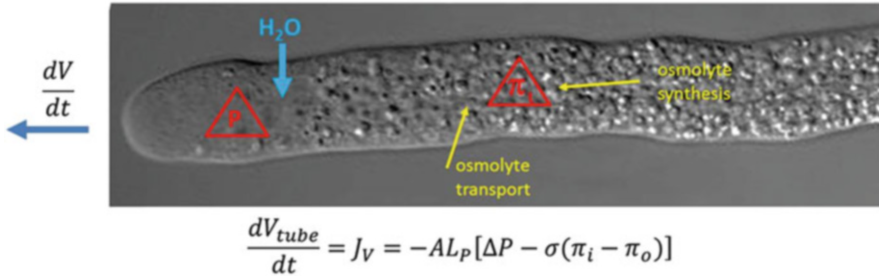


Fig. 2.1 Simplified scheme of water uptake in pollen tubes. Uptake of water changes the pollen tube's volume (V_{tube}), thus leading to tube elongation with a growth rate of $\frac{dV_{\text{tube}}}{dt}$. The volume flux ($J_V$) depends on the pollen surface (A), the hydraulic conductivity (L_P), the turgor pressure (ΔP) and the difference in internal and external osmotic pressure, π_i and π_o, respectively. The σ denotes the reflection coefficient that is 1 for nonpermeable solutes

2.3 Measuring Water Potential Parameters and Water Transport in Pollen

A simple way to estimate the minimal net water uptake in pollen tubes that is the difference between water uptake and water release is to measure the tube growth rate. Because pollen tubes grow with an almost constant diameter and the increase in tube volume is mainly caused by water uptake, the growth rate correlates well with the volume increase per time unit (dV/dt). For instance, a pollen tube with a diameter of 12 μm that grows with a speed of 10 $\mu\text{m min}^{-1}$ adds a volume of 1.131 pl each minute ($V = r^2 \cdot \pi \cdot h = 1131 \times 10^{-18}\ \text{m}^3$). This equals a minimal water flux of 1 pmol s^{-1}. This simple estimation already predicts that the growing pollen tube has to be capable to transport at least 1 pl water per minute across its plasma membrane to reach the observed tube growth speed. The parameters, which describe the water permeability of biological membranes, are the hydraulic conductivity (L_P) or the osmotic permeability (P_{os}) that are related as:

$$P_{\text{os}} = RT\frac{L_P}{\overline{V}_w} \tag{2.5}$$

For pollen grains of *Lilium longiflorum*, both parameters have been determined experimentally by using either the pressure probe (Pertl et al. 2010) or a protoplast swell assay (Sommer et al. 2007) under isovolumetric and isobarometric conditions, respectively. It has to be noted that the water permeability differs slightly between intact pollen grains and pollen grain protoplasts due to the contribution of the cell wall to the water permeability. Furthermore, one may also expect different water permeabilities in pollen grains and tubes that are also caused by the differences in cell wall composition between grains and tubes.

2.3.1 Protoplast Swell Assays

Swell assays can be performed with every protoplast originated from plant cells to determine the osmotic permeability of the plasma membrane. There are numerous examples with protoplasts prepared from different plant tissues and plant species with slight variations in methods (Ramahaleo et al. 1999; Moshelion et al. 2004; Murai-Hatano and Kuwagata 2007; Sommer et al. 2007). In principle, the cell wall is digested enzymatically, and the resulting protoplasts are challenged with media of varying osmolyte concentrations. The increase or decrease in volume of the spherical protoplast is monitored with a video-equipped microscope. Although this set-up faces some experimental and theoretical problems, it can be easily applied. One experimental challenge is the fixation of the protoplasts in the measuring chamber without modifying its ball-like geometry. Another major complication results from 'theory-demanded' instantaneous change of the external medium when the transition between two equilibrium states is studied experimentally. This needs fast solution fluxes and can hardly be realised without washing away the observed protoplast. An additional biological problem results from the exocytosis capacity of each cell which brings about a modification of the plasma membrane area during the experiment. However, bearing these limitations in mind, swell assays produce reliable data for the osmotic permeability. Even if the absolute values of P_{os} might not be entirely correct, they can reflect changes in treatments, mutants or growth conditions (Wu et al. 2013). In pollen, protoplast swell assays have only been applied to *Lilium* pollen, giving values for P_{os} of 6.60 $\mu m\ s^{-1}$ and 13.24 $\mu m\ s^{-1}$ for grain and tube protoplasts, respectively (Sommer et al. 2008).

In addition to the direct determination of the osmotic permeability from time-dependent volume changes, swell assays can be used to calculate the water flux densities (Fig. 2.2). In accordance with electrophysiological experiments, the data may be plotted like current–voltage curves, resulting in graphs of the water flux (density) plotted against the osmotic potential difference (driving force) with a slope corresponding to P_{os}. The data in Fig. 2.2c clearly show that water influx into pollen grain protoplasts (swelling, endo-osmosis) is faster than water efflux (shrinking, exo-osmosis). These observations in water transport were also made in other plant cells (Steudle and Tyerman 1983; Murai-Hatano and Kuwagata 2007).

2.3.2 Pressure Probe Technique

The pressure probe was developed by Ulrich Zimmermann and Ernst Steudle to measure the turgor pressure of plant cells (Hüsken et al. 1978), and its use is well described (Boyer 1995; Zimmermann 1989; Steudle 1993; Tomos and Leigh 1999). In comparison with other techniques, the turgor pressure is directly recorded, whereas other techniques like ball tonometry, micro-indentation or cellular force microscopy (Linthilhac et al. 2000; Routier-Kierzkowska et al. 2012; Milani et al.

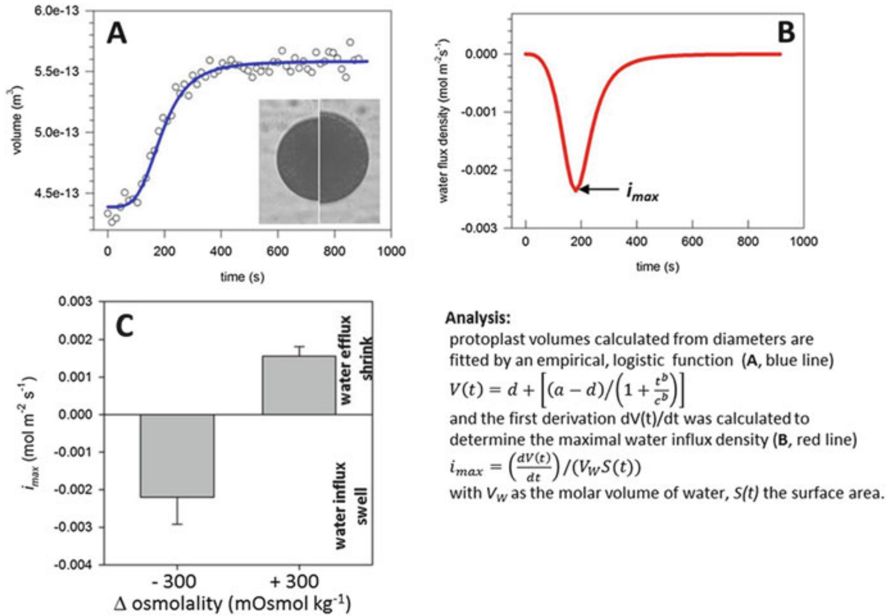


Fig. 2.2 Analysis of pollen grain protoplast swell assays. (**a**) *Lilium* pollen protoplasts are transferred from an iso-osmotic (680 mOsmol kg^{-1}) to a hypoosmotic medium (380 mOsmol kg^{-1}) giving an osmolality difference of ca. 300 mOsmol kg^{-1}. The protoplast's diameter is measured every 10 s and calculated to volume. *Inset* shows a protoplast in iso-osmotic (*left*) and after reaching the equilibrium in hypoosmotic medium (*right*). The volume data were fitted with an empirical, logistic function $V(t)$ similar to Richards (1959). (**b**) The first derivation $dV(t)/dt$ is used to determine the maximal water flux density i_{max}. (**c**) Treatment with hypo- and hyperosmotic conditions shows that swelling of lily pollen grain protoplasts is faster than shrinking despite the same size of the driving force *Δ* osmolality

2013) measure the cellular micromechanics of a plant cell and extrapolate the turgor pressure value. Although the pressure probe technique needs some experimental skills, it also enables to determine the hydraulic conductivity and the elastic modulus in addition to turgor pressure values in a single experiment (Fig. 2.3). So far, the pressure probe, micro-indentation and cellular force microscopy were used to determine turgor pressure and cellular mechanics of *Lilium* and *Nicotiana* pollen tubes (Benkert et al. 1997; Zerzour et al. 2009; Vogler et al. 2013). The mean turgor pressure of growing lily pollen tubes was about 2.1 bar ranging between 1 and 4 bar (Benkert et al. 1997), while lily pollen grains had a turgor pressure of 3.2 bar (Pertl et al. 2010); both were measured in standard germination medium with an osmolality of ca. 300 mOsmol kg^{-1}. Although the turgor pressure of lily pollen tubes could be deduced by cellular micro-force microscopy after plasmolysis (3 bar, Vogler et al. 2013), the pressure probe might be the only technique, so far, to determine the turgor pressure in the rigid, double-walled pollen grain.

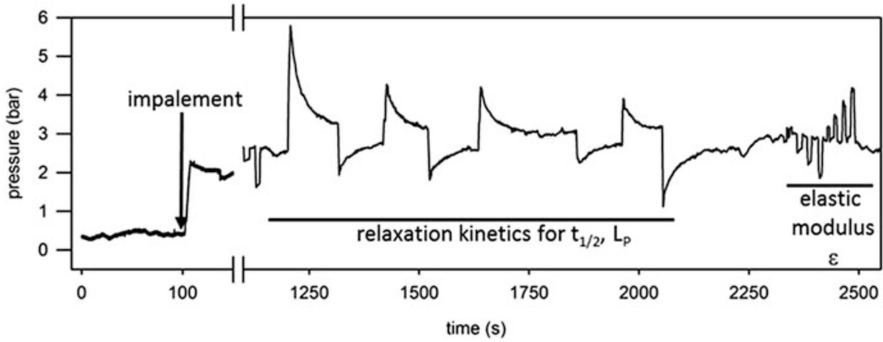


Fig. 2.3 Turgor pressure measurement in *Lilium* pollen grains. An example for a typical pressure experiment is shown. The first 150 s are presented at a higher time resolution. After impalement with the micro-capillary, a sudden increase in pressure is noticed. In addition to turgor pressure, the half-time of the pressure relaxation curves allows determination of the hydraulic conductivity, and known volume changes causing a pressure response can be used to calculate the elastic modulus ε

Hydraulic conductivity, which was also calculated from pressure relaxation curves after application of sudden pressure increases or pressure decreases (Fig. 2.3), gave an average L_P value of 4.9×10^{-8} m $\text{MPa}^{-1}\text{s}^{-1}$ (Pertl et al. 2010) that corresponds according to Eq. (2.5) to a P_{os} value of approximately 7.4 μm s^{-1}. Although both techniques, the pressure probe and the protoplast swell assay, are sometimes scrutinised due to the experimental manipulation during the measurements, e.g. micro-capillary impalement and cell wall digestion, respectively, the measured values for P_{os} were similar (6.6 μm s^{-1} for swell assays, see above). Nevertheless, a disadvantage of the pressure probe technique is the need of sophisticated experimental skills due to micromanipulation during impalement and balancing of the oil-cytosol meniscus in the micro-capillary. Single, individual cells or small organisms like pollen grains need to be fixed somehow while forcing the micro-capillary through the cell wall. While pollen grains are fixed with a second holding pipette, the more fragile pollen tubes can be immobilised by agarose or poly-L-lysine-coated cover glasses.

In addition to hydrodynamic parameters like the turgor pressure and the hydraulic conductivity, the pressure probe allows determination of the Young's or elastic modulus (ε) which describes the stiffness of the cell wall. Measurements in lily pollen grains with the pressure probe gave an elastic modulus of 3.9 MPa (Pertl et al. 2010), while cellular force microscopy in combination with a mechanical model based on the finite element method estimated the cell wall elasticity of lily pollen tubes between 20 and 90 MPa (Vogler et al. 2013). Recently, a microfluidic chip technique was tested (Bending Lab-On Chip, Nezhad et al. 2013), and values of 350 MPa were recorded for the longitudinal direction of *Camellia* pollen tubes. These are, so far, the only values from pollen and, thus, do not allow any conclusion before more data are available.

2.4 Pollen Water Transport

In pollen, water transport is an important feature during several stages of flower development. The life of pollen can be classified in five major stages in which water status plays an important role (Firon et al. 2012). In the first phase (microsporogenesis), pollen grains develop inside the anther and are surrounded by the locular fluid which contains sufficient nutrient and water provided by the vegetative tissue. In the dehydration phase, the locular fluid disappears, while the pollen grains mature and dehydrate. The anther opens and the mature, dehydrated pollen is presented for dispersal (presentation phase) followed by the dispersal phase in which the mature pollen grains are exposed to the environment while transported to a stigma. Finally, if pollen grains land on a compatible stigma, they start to rehydrate, germinate and grow pollen tubes towards the egg cells (pollen-stigma interaction phase). This review focusses on water transport during the last phase taking place on the stigma, the rehydration and the pollen tube elongation. The water status during flowering and pollen development is described elsewhere (Firon et al. 2012; Beauzamy et al. 2014).

For both processes, rehydration and tube elongation, a more negative water potential has to be postulated for the pollen interior than in the external medium to allow an influx of water. Although, active water transport against its potential gradient is thermodynamically possible (Onsager 1931; Zeuthen 1995), its role and contribution to water uptake and transport in plants was suggested only recently (Wegner 2015).

2.4.1 Aquaporins in Pollen

Water may enter a cell by three different molecular pathways: (a) diffusion through the lipid bilayer, (b) facilitated transport through aquaporins (AQPs) or (c) cotransport with ions and nutrients via channel or carrier proteins. The latter pathway is very particular since it also enables an ‘uphill’ transport of water against its potential gradient and might be very interesting in the aspect of osmoregulation and concerted transport of water and ions during tube elongation.

Compared to ions or small solutes with permeability coefficients between 0.1 pm s^{-1} and 0.1 nm s^{-1}, respectively, the permeability of lipid bilayers is quite high for water (1 μm s^{-1}, Hill et al. 2004) which is ten times less than P_{os} values measured for pollen grains. According to Eq. (2.4), the water influx into a 1000 μm long pollen tube with a diameter of 12 μm can be calculated as 0.17 pl min^{-1} when assuming a water permeability of the lipid bilayer alone (1 μm s^{-1}), a turgor pressure of 2 bar and an osmotic potential difference of 380 mOsmol kg^{-1}. This water transport capacity is not sufficient to account for the required water uptake at tube growth rates of 10 μm min^{-1}. However, increasing the osmotic permeability to 20 μm s^{-1}, as measured in lily pollen tubes, allows water uptake of 3.4 pl min^{-1},

which is close to the 11 pl min^{-1} estimated for growth rates of 10 μm min^{-1}. Nevertheless, the question remains: does the pollen need aquaporins for water uptake, and if not which role have the aquaporins known to be expressed in pollen (e.g. Wudick et al. 2014)? If water transport is restricted to a small area and/or is tightly regulated to prevent tube bursting, specific transport proteins for water could play an important role or regulatory function in water homeostasis.

Aquaporins (AQPs) are water-conducting pores formed by four protein subunits of the *m*ajor *i*ntrinsic *p*rotein (MIP) family. One may further distinguish proteins from the plasma membrane intrinsic protein (PIP) and the tonoplast intrinsic protein (TIP) subfamilies which are completed by the SIP and the NIP subfamilies (*s*mall basic and *n*odulin26-like *i*ntrinsic *p*rotein, respectively; for reviews see Chaumont et al. 2005; Ludewig and Dynowski 2009; Maurel et al. 2015). Water channels are mainly built from subunits of the PIP or the TIP subfamilies and, as already implicated by their names, are primarily localised in the plasma membrane and in vacuolar membranes, respectively. One may expect that PIPs are highly expressed in the pollen due to the fast tube growth correlating with high water uptake rates. Evidence for an involvement of aquaporins (PIPs) came from experiments that used mercury ions to inhibit and reducing agents to restore pollen tube growth (Shachar-Hill et al. 2013 and Fig. 2.4), which was taken as an indication for a physiological role of aquaporins because of the reversible effect on a Cys residue close at the permeation pore (Niemietz and Tyerman 2002; Hirano et al. 2010). However, mercury ions have also dramatic effects on all other transport processes as reflected by a large depolarisation of the plasma membrane after addition of mercury compounds (Schütz and Tyerman 1997 and Fig. 2.4) thus questioning the conclusions from such inhibitor experiments.

In opposition to physiological data, no expression of PIP genes could be detected in *Arabidopsis* pollen in various expression and transcriptome studies (Becker et al. 2003; Honys and Twell 2003; Qin et al. 2009). An in silico analysis using *Arabidopsis* microarray data (www.genvestigator.com) revealed almost no detectable PIP-mRNAs in pollen which was confirm by detailed studies (Soto et al. 2008; Wudick et al. 2014). Although PIPs were not detected, 6 out of 35 members of the MIP/aquaporin family were repeatedly reported as expressed in *Arabidopsis* pollen: AtNIP7;1, AtNIP4;1, AtSIP1;1, AtSIP2;1, AtTIP1;3 and AtTIP5;1 (Ishikawa et al. 2005; Bock et al. 2006; Borges et al. 2008; Qin et al. 2009; Loraine et al. 2013). Expression analysis of *Brassica* pollen AQPs was contradictory, showing low expression levels of PIPs in immunoblots, whereas PIP-mRNA could not be detected (Marin-Olivier et al. 2000; Dixit et al. 2001). At least in tobacco pollen, NtPIP1;1 and NtPIP2;1 could be detected at a low level by RT-PCR (Bots et al. 2005), whereas in *Lilium* pollen, partial sequences of PIPs as well as TIP, NIP and SIP sequences were reported in proteome and transcriptome studies (Pertl et al. 2009; Pertl-Obermeyer et al. 2014; Lang et al. 2014).

In summary, a very contradictory image about the presence and possible role of aquaporins in pollen is presented. It might depend on the pollen species, the state of pollen development or the difference in the pollination process itself whether the

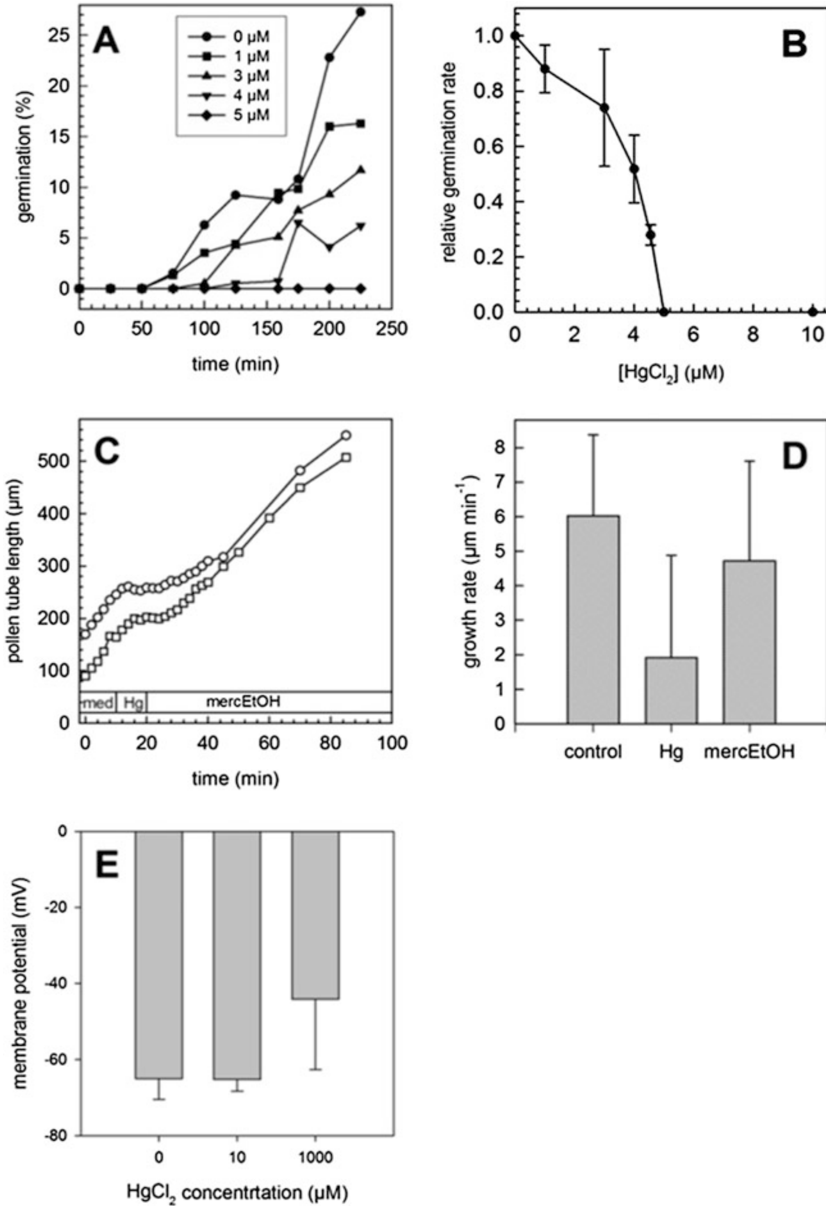


Fig. 2.4 Effect of mercury ions on *Lilium* pollen. (**a**) $HgCl_2$ inhibits pollen germination with a total inhibition at 5 μM. (**b**) Concentration-dependent germination of lily pollen grains. The relative germination rate was calculated from the slopes of germination curves and divided by the control data ($n = 5$) (**c**) Inhibition and recovery of growth of two individual pollen tubes by mercury chloride (5 μM) and mercaptoethanol (10 mM), respectively. Pollen tubes were growing in standard germination medium (med) in a chamber which was perfused with germination medium plus $HgCl_2$ or with mercaptoethanol (mercEtOH). (**d**) Average growth rate of pollen tubes during incubation with 5 μM $HgCl_2$ and recovery in 10 mM mercEtOH ($n = 4$–6). (**e**) Effect on $HgCl_2$ on the membrane potential of pollen grains ($n = 5$)

pollen or the stigma tissue express aquaporins. Pollination involving the so-called 'wet' stigmas might prefer aquaporins in the pollen, while dry stigmas (*Arabidopsis*, *Brassica*) express AQPs in the stigma tissue and, thus, controlling water transport at the stigma site. The lack of large amounts of plasma membrane-localised aquaporins also implies that the water conductance of the lipid bilayer might be sufficient for water uptake to provide the observed tube growth rates, and TIP aquaporins in the tonoplast are adequate to facilitate cell volume enlargements as has been detected during emergence of lateral root primordia in *Arabidopsis* (Reinhard et al. 2016). In pollen, much more research is necessary to obtain a clear and reliable image on the expression and role of aquaporins and PIPs, especially, during pollination.

2.4.2 Pollen Grain Rehydration

Experimental data on the first events during pollen grain rehydration are difficult to monitor, and many observations were collected from studies on different pollen types and species emphasising various aspects during pollen grain water transport. The first synopsis on the hydrodynamics of pollen grains considering the differences in water potential was given by Jack Heslop-Harrison (1979). However, a sound description of water movements seems to be complicated by the state of the plasma membrane bilayer in the dehydrated pollen grain: transition from the lamellar/bilayer structure to a hexagonal (HII) phase occurs under specific conditions, e.g. when the water content of the pollen grains decreases during dehydration, and a lamellar structure becomes thermodynamically instable and turns into a less water-requiring hexagonal or cubic state (Gordon-Kamm and Steponkus 1984; Jouhet 2013). During the first minute, if not the first seconds of the rehydration phase, the cytoplasmic organisation of pollen membranes changes from a micellar (hexagonal) form to a hydrated, continuous bilayer structure (Elleman and Dickinson 1986; Tiwari et al. 1990). A phase transition of phospholipids from the gel to the liquid-crystalline structure was detected during imbibition of dry pollen grains (Crowe et al. 1989; Hoekstra et al. 1991). The reformation of the plasma membrane bilayer implies that a short time period exists during which pollen grains are leaky and might lose cytosolic contents or take up membrane impermeable compounds. This fact might be utilised experimentally to manipulate pollen grains from plants that cannot easily be transformed, e.g. to introduce fluorescent sensor molecules or antisense RNA (Moutinho et al. 2001; Potocky et al. 2007; Mizuta and Higashijima 2014).

After the formation of a continuous lipid bilayer, additional water is taken up across the new-built plasma membrane to allow adjustment of cytoplasmic ion concentrations (pH, Ca^{2+}, K^{+}, etc.), turgor pressure and metabolite concentrations. For instance, the cytoplasmic K^{+} concentration has to be adjusted to allow protein translation (reviewed by Mascarenhas 1993). This early water uptake was postulated as being an initial step in pollen grain germination (Feijó et al. 1995). Although no data on these early events of water uptake are available, one may postulate that water potential inside the pollen grain is much lower than the water potential

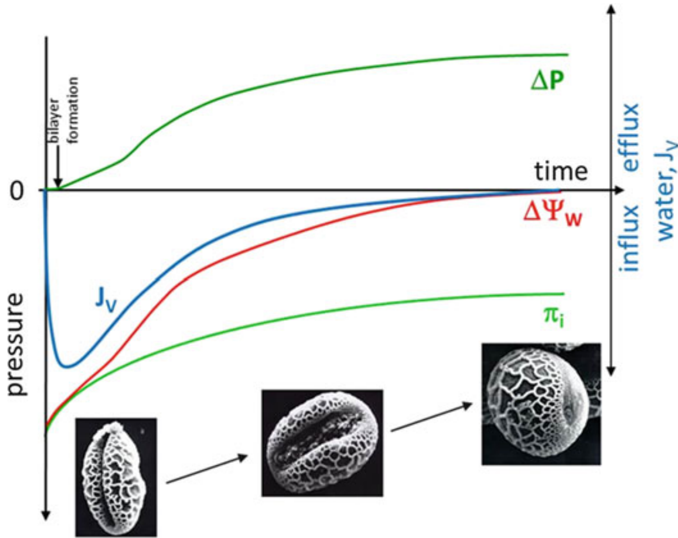


Fig. 2.5 A simple scheme of the water potential relations and parameters during pollen grain rehydration. See text for detailed explanation

in the environment or in the stigma cells which have to supply the dehydrated pollen grain with water. The following scenario might describe the events during pollen rehydration (Fig. 2.5): the mature, dry pollen grain possesses a high osmotic pressure (π_i) caused by ions and metabolites which were concentrated previously during pollen development by the dehydration process. The plasma membrane is not in its bilayer but hexagonal state. Subsequently, no turgor pressure is expected. While water is flowing into the pollen grain following the water potential difference ($\Delta\Psi$), it swells and enlarges its volume; the osmotic pressure decreases due to dilution of the cytosolic and organelle compounds. The plasma membrane transforms into a lipid bilayer, and the turgor pressure ΔP slowly increases as more and more water flows into the grain, enlarging the volume and expands the pollen wall (Katifori et al. 2010). The pollen wall limits the volume of the pollen grain, and the turgor pressure increases faster as more water flows into the grain. Water is taken up until $\Delta P = \Delta\pi$, and any further uptake water needs a decrease in turgor pressure ΔP or an increase in $\Delta\pi$. The osmotic pressure difference ($\Delta\pi$) can either be changed by increasing π_{i} or decreasing π_{o}, with both changes leading to water influx. In barley pollen, rapid swelling was observed upon contact with germination medium, and the fast water uptake was correlated with an accumulation of K^+ at the aperture inside mature pollen grains (high π_{i}) which was suggested to cause the fast swelling in a fraction of a second (Rehman et al. 2004). In addition, volume changes of barley pollen were also observed during pollen development at the stage of anther dehiscence which were probably caused by changes in K^+ concentrations, too (Matsui et al. 2000; Rehman and Yun 2006).