Stem Cells

Thomas C. G. Bosch
Editor

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From *Hydra* to Man

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Editor
Prof. Dr. Thomas C. G. Bosch
Zoological Institute
Christian-Albrechts-University
Kiel, Germany

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Preface

There are two strategies to maintain a complex structure over a long period of time: Repair and preserve as good as you can; or re-build the identical complex continuously. To let our heritage go into a state of disrepair is not acceptable for most of us. Man-made buildings, therefore, need to be repaired and preserved continuously and lots of efforts are devoted to it. The Ise Shrine in the Mie Prefecture on the South East coast of Japan is maintained over hundreds of year by the alternative strategy: instead of repairing it continuously, the shrine is just rebuilt every 20 years. According to popular belief, in this way the site is purified and building materials renewed while preserving the original design from the third and fourth centuries A.D. The new shrines, however identical with the old ones, are not considered a replica of Ise, but are "Ise re-created." The recreation process reveals Shinto's understanding of nature which does not make monuments, but lives and dies, always renewed and reborn. Similar to house constructions, the adult body needs efforts, tools and methods to maintain its tissue and organs. That is what stem cells are used for in the adult body. Only stem cells have the ability to self-renew and to generate progeny capable of differentiating into one or more cell types. Numerous stem cell types are located in various depository sites and wait for demand, due to tissue maintenance in replacement of damaged or aged cells.

Much of our knowledge of stem cells has been inferred from studies of remarkable few species. The ability to manipulate stem cells in "model" organisms such as the mouse and a few other vertebrate species has driven our understanding of basic biology of stem cells. Data obtained suggest that a constellation of intrinsic and extrinsic cellular mechanisms regulates the balance of self-renewal and differentiation in all stem cells; the transcription factors Oct4 and Nanog, as well as the LIF-gp130-Stat3, BMP-TGF-b-Smad, MAPK-ERK and possibly the WNT signaling pathways, all have important roles in this process. Thus, an emerging theme is the implementation of the same signaling pathways in distinct stem cell systems. Questions about where stem cells are located, how they are maintained, what they can become, and the interchangeable nature of the stem and transit amplifying state are central topics of current research in vertebrate stem cell biology.

Organisms become models when they support sustainable opportunities with uncompromising experimental rigor and ease of use. The power and efficiency of studying model organisms, however, comes at a cost since a few species, obviously,

do not reflect nature's true diversity. Unfortunately, although all multicellular organisms seem to rely on stem cells, and although this seems to be a question of key importance for understanding the evolution of animal life, little is known about stem cells in early-branching taxa.

Therefore, welcome to ***Stem Cells: From* Hydra *to Man***. The title reflects an enormous growth in the knowledge of stem cells in various organisms over the past few decades. Large scale species comparisons at the genome and EST level have revealed that early-branching metazoans such as sponges and cnidarians share many if not most of their genes with the allegedly advanced vertebrates including man. The ancestor of all animals may thus have been much more complex than anticipated. Does that hold true also for the stem cells? Do all stem cells in the animal kingdom follow the same rules? Or are there real differences between stem cells in different organisms? The purpose of the book is to illustrate that here is more than human and mouse stem cells to learn from. The book presents the conceptual language and the nature of questions, as well as a summary of the advances in our understanding of stem cells from a comparative point of view that has resulted from the development of new technology and the development of novel model organisms over the past decade. As such this book is largely a horizon analysis of a frontier rather than a retrospective. It presents an integrative approach to animal stem cells and covers the major contributions, tools and trends in a newly emerging field: comparative stem cell biology.

We begin by considering stem cells in plants. Plants and animals may have evolved in quite different fashions. However, there is no doubt that plants and animals have evolved from a common eukaryotic ancestor, as for example indicated by the clear homology of genes that control the chromatin level of gene regulation. In plants, the shoot apical meristem can initiate organs and secondary meristems throughout the life of a plant. A few cells located in the central zone of the meristem act as pluripotent stem cells: They divide slowly, thereby displacing daughter cells outwards to the periphery where they eventually become incorporated into organ primordia and differentiate. **Jan U. Lohmann** reviews the latest information on plant stem cell regulation and how transcription factors and hormones control cell proliferation and maintenance of stemness, as well as how this ultimately leads to appropriate differentiation during plant development. We have included a chapter on plant stem cells in a book describing mostly stem cells in animals, since only by fully understanding the differences between plants and animals can we distinguish between those features of developmental pattern formation and cellular signaling that are necessary aspects of complex organisms, and those that are accidents of evolutionary history.

Although understanding stem cells in early-branching metazoan animals in general is a field in its infancy, we start to get interesting insights from one of the oldest multicellular organisms, the sponges (phylum Porifera). Based on her studies in the freshwater sponge *Ephydatia fluviatilis*, **Noriko Funayama** summarizes recent studies on the sponge stem cell system. She emphasizes that the stem cell system includes two types of pluripotent stem cells, archaeocytes and choanocytes and describes some of the molecular markers which recently came to light.

An important step during animal phylogeny was the invention of a tissue layer construction and nervous system within the phylum Cnidaria. Cnidarians, therefore, are an informative animal group separating from other metazoans prior to the origin of the bilaterian assemblage. Early work in hydrozoan cnidarians such as the freshwater polyp *Hydra* has shown that there are three distinct stem cell lineages. In Chapter 3, I summarize the current state of knowledge and the significant progress that has been made in recent years towards understanding the molecular control mechanisms involved in the *Hydra* stem cell system.

Macroscopic flatworms known as freshwater planarians (Platyhelminthes) possess an extraordinary stem cell system. A single cell type – pluripotent stem cells (neoblasts) – is responsible for cell renewal during growth, development, homeostasis and regeneration; neoblasts are also responsible for germ line formation. Since 1897 and appearance of a classic paper (H Randolph's "Observations and experiments on regeneration in planarians") these organisms, therefore, became attractive models to study stem cells. Similar to *Hydra*, research in Platyhelminthes has been revitalized by the recent application of the tools of molecular and cellar biology. Two papers in this volume describe that Plathelminthes are unique animals which we can utilize to understand fundamental mechanisms of stem cell systems. **Kiyokazu Agata** summarizes recent work on the planarian stem cell system at the cellular and molecular level. **Peter Ladurner** and colleagues introduce a rather novel model to study platyhelminth stem cell biology, the free-living flatworm *Macrostomum lignano*.

As an approximation of ancestral chordates, ascidians (Urochordata) can provide insight into the link between non-chordate deuterostomes and chordates, as well as the origination of vertebrates. Urochordates in fact are now considered the closest living relatives of vertebrates and because of their unique evolutionary position very valuable models. **Anthony W. De Tomaso** and colleagues summarize the renewing cell populations in adult ascidians and their role in regeneration and asexual proliferation. He shows that stem cells in ascidians are excellent models to study the biology of both embryonic and adult stem cells.

Can stem cells functionally contribute to human tissue repair? The answer is already known and is "yes". Three chapters in this volume show that both animal data and observations in humans indicate that stem cells may restore damaged organ function. **Makoto Asashima** and colleagues uses two types of undifferentiated cells, amphibian animal caps and mouse embryonic stem cells, to identify and characterize factors involved *in vitro* in maintenance of the undifferentiated state as well as the mechanisms regulating differentiation and control of organogenesisusing amphibian and stem cells.

Emerging evidence from stem cell research has strengthened the idea that stem cell fate is determined by a specialized environment, known as the stem cell niche. **Masatake Osawa** and colleagues summarizes studies in melanocyte stem cells (MSCs), which not only allowed the identification of individual stem cells in the niche, but also to obtain the molecular signature of individual MSCs in the niche. Since loss-of-function mutations in the genes responsible for MSC regulation in mice are readily identifiable by a prematuring hair greying phenotype, the MSC system appears to be an excellent model to study stem cell biology.

Embryonic stem cells are also a cornerstone of Singapore's National Biomedical Science Strategy. **William L. Rust** from Singapore's Institute of Medical Biology summarizes the state of the art of human embryonic stem cell research (hESC) aimed at generating tissues suitable for clinical use. Giving an outlook on the future directions of hESC research, the chapter provides a fascinating link between stem cells and their use in clinical settings.

Finally, there is emerging evidence that some blood cell cancers and solid tumors may contain a cancer cell hierarchy reminiscent of the normal tissue in which the malignancies first arose, with a cancer stem cell producing progeny with limited replication potential. The discovery of tumor cells that behave like stem cells suggests why cancer may be so hard to eradicate – and how new therapies might be targeted. Given the possible importance of cancer stem cells as therapeutic targets, **Holger Kalthoff** and **Ibrahim Alkatout** reviews recent advances in understanding the development of cancer and the role of cancer stem cells.

Taken together, the chapters in ***Stem Cells: From* Hydra *to Man*** reveal many common themes utilized in the maintenance and differentiation of stem cells of apparently disparate organs in animal and plant models. From an experimental point of view, each stem cell model system has its advantages and disadvantages. The book chapters show that although the molecular players controlling stem cell behavior are different in plants and animals, the overriding theme remains that signals and transcription factors are utilized to control pattern formation and differentiation from stem cell precursors. Across the animal kingdom there is a striking conservation of signalling and transcriptional mechanisms utilized in diverse stem cell differentiation processes. A comparative analysis of stem cells in diverse organisms, therefore, promises new insights into how stem cells act to construct and maintain tissues, and to reveals how the diverse stem cell systems may have evolved. Although applying genomic approaches to non-models is still challenging, the dichotomy between models and non-models is diminishing.

In conclusion, as the Ise shrine illustrates that death and constant renewal is the essence of nature, the work summarized in this book emphasizes the central role of stem cells as well as the mechanisms underlying their renewal capacity.

Thomas C. G. Bosch

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Contents

Contributors

Kiyokazu Agata
Department of Biophysics, Graduate School of Science,
Kyoto University Kitashirakawa-Oiwake, Sakyo-ku, Kyoto 606-8502,
Japan, agata@mdb.biophys.kyoto-u.ac.jp

Ibrahim Alkatout
Department of General and Thoracic Surgery, Division of Molecular Oncology,
University Hospital Schleswig-Holstein, Campus Kiel, Christian-Albrechts-
University Kiel, Arnold-Heller-Str. 7, D-24105 Kiel, ialkatout@path.uni-kiel.de

Makoto Asashima
Department of Life Sciences (Biology), Graduate School
of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku,
Tokyo 153-8902, Japan, asashi@bio.c.u-tokyo.ac.jp

Thomas C. G. Bosch
Zoological Institute, Christian-Albrechts-University Kiel, Olshausenstrasse 40,
D-24098 Kiel, Germany, tbosch@zoologie.uni-kiel.de

Federico D. Brown
Institute for Stem Cell Biology and Regenerative Medicine, Stanford University,
Hopkins Marine Station, Pacific Grove, California 93950, USA

Katrien De Mulder
Institute of Zoology, University of Innsbruck, Technikerstrasse 25, A-6020
Innsbruck, Austria, Katrien.DeMulder@ugent.de

Anthony W. De Tomaso
Institute for Stem Cell Biology and Regenerative Medicine, Stanford University,
Hopkins Marine Station, Pacific Grove, California 93950, USA,
tdet@stanford.edu

Bernhard Egger
Institute of Zoology, University of Innsbruck, Technikerstrasse 25, A-6020
Innsbruck, Austria, Bernhard.Egger@uibk.ac.at

Noriko Funayama
Department of Biophysics, Graduate School of Science,
Kyoto University Kitashirakawa-Oiwake, Sakyo-ku, Kyoto 606-8502, Japan,
funayama@mdb.biophys.kyoto-u.ac.jp

Kiyotaka Hasegawa
Cutaneous Biology Research Center, Massachusetts General Hospital,
MGH East, Building 149, 13th Street, Charlestown, Massachusetts 02129, USA

Holger Kalthoff and Ibrahim Alkatout
Department of General and Thoracic Surgery, Division of Molecular Oncology,
University Hospital Schleswig-Holstein, Campus Kiel, Christian-Albrechts-
University Kiel, Arnold-Heller-Str. 7, D-24105 Kiel, hkalthoff@email.uni-kiel.de

Georg Kuales
Institute of Zoology, University of Innsbruck, Technikerstrasse 25, A-6020
Innsbruck, Austria, Georg.Kuales@uibk.ac.at

Akira Kurisaki
Department of Life Sciences (Biology), Graduate School
of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku,
Tokyo 153-8902, Japan

Peter Ladurner
Institute of Zoology, University of Innsbruck, Technikerstrasse 25, A-6020
Innsbruck, Austria, peter.ladurner@uibk.ac.at

Jan U. Lohmann
Max Planck Institute for Developmental Biology, Spemannstrasse 37-39,
D-72076 Tübingen, Germany, jlohmann@tuebingen.mpg.de

Mariko Moriyama
Cutaneous Biology Research Center, Massachusetts General Hospital, MGH East,
Building 149, 13th Street, Charlestown, Massachusetts 02129, USA

Tatsuo Muchiue
Department of Life Sciences (Biology), Graduate School
of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku,
Tokyo 153-8902, Japan

Shin-Ichi Nishikawa
Center for Developmental Biology
2-2-3 Minatojima, Minami-machi, Chuo-ku, Kobe 650-0047, Japan

Masatake Osawa
Cutaneous Biology Research Center, Massachusetts General Hospital, MGH East,
Building 149, 13th Street, Charlestown, Massachusetts 02129, USA, masatake.
osawa@cbrc2.mgh.harvard.edu

Daniela Pfister
Institute of Zoology, University of Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria, Daniela.Pfister@uibk.ac.at

William Rust
Institute of Medical Biology, A STAR (Agency for Science, Technology, and Research), 8A Biomedical Grove, #06-06 Immunos, Singapore 138648. wllmrust@yahoo.com.sg

Willi Salvenmoser
Institute of Zoology, University of Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria, Willi.Salvenmoser@uibk.ac.at

Lukas Schärer
Institute of Zoology, University of Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria, Lukas.Scharer@uibk.ac.at

Stefano Tiozzo
Institute of Medical Biology A*STAR (Agency for Science, Technology, and Research), 8A Biomedical Grove, #06-06 Immunos, Singapore 138648. wllmrust@yahoo.com.sg

Chapter 1
Plant Stem Cells: Divide et Impera

Jan U. Lohmann

Abstract Stem cells are an essential and defining feature of multicellular organisms. Since multicellularity arose independently in the plant and animal kingdom, it follows that also the stem cell concept has evolved independently in the two linages. Nevertheless, there are striking similarities in the way plants and animals organize their stem cell pools, suggesting that there might have been strong evolutionary constraints that shaped the path for the development of stem cell systems. This is illustrated by the fact that in both worlds, stem cell promoting signals are usually absent from the stem cells themselves, but rather found in neighboring cells, which provide an inductive cellular environment, also called niche. While there are perplexing similarities with regards to the overall stem cell concept, there are also profound differences. Arguably the most important disparity lies in the capacity of plants to maintain totipotent stem cells throughout their entire lives and that these cells are directly responsible for giving rise to the vast majority of the cellular mass of the adult plant body. Another fundamental difference between plants and animals lies in the dramatic developmental plasticity of plant cells, which allows them to take on multiple fates during their life. Therefore, plants have evolved a complex regulatory network, which allows for a precise control of stem cell proliferation and cell fate specification.

Keywords ant stem cell, meristem, WUSCHEL, CLAVATA, Plant hormore

1.1 Introduction

When a baby is born we marvel at its perfection: Eyes, hands, feet – all body structures are elaborated with stunning precision and in miniature size. Thus, while it will take years for the baby to grow, mature, and learn to become an adult, the basic body

Center for Organismal Studies, University of Heidelberg,
im Neuenheimer Feld 230, D-69120 Heidelberg, Germany.
E-mail: jlohmann@tuebingen.mpg.de

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plan is already laid down at birth. This so-called embryonic mode of development is not only limited to humans, but is in fact employed by most animals. In many species, immature offspring are even capable of life in the absence of further parental input.

While seedlings of plants can also survive without their parents, their mode of development differs dramatically from that of animals. Despite the fact that their embryos remotely resemble the adult structure, organs of embryonic origin do not contribute substantially to the adult plant. Instead, proliferative tissues called meristems, which harbor stem cells, continuously generate cells during the adult life phase to support growth and development of the plant (Fig. 1.1). Because plant cells are surrounded by a rigid, yet permeable, cell wall, they are unable to move within the organism by migration. Rather, cells are displaced passively by tissue streaming, which is driven by both cell proliferation and cell expansion. Cell proliferation is limited to meristems and to those cells that have recently been displaced from them. Most visible plant growth, however, is due to cell elongation and enlargement in differentiating organs and tissues. Thus, the body structure of a plant is elaborated and refined over the entire lifespan of the individual and is subject to constant change until senescence. This mode of development, generally referred to as postembryonic, allows plants not only to form new organs of various kind more or less continuously over their entire lifecycle, but also to regenerate

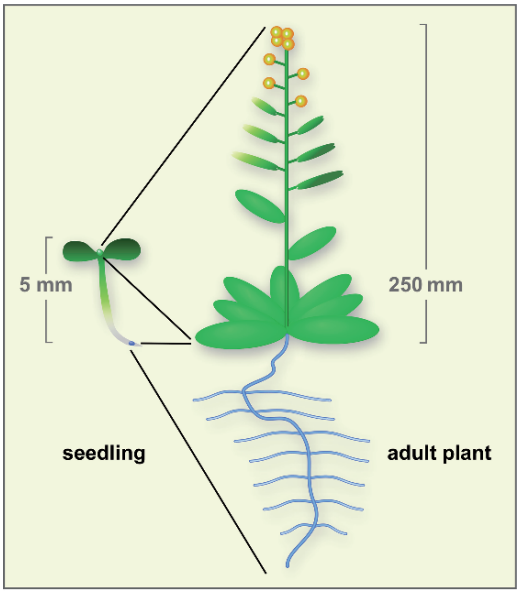

Fig. 1.1 Contribution of apical meristems to the plant body. The entire shoot system is derived from cells of the shoot apical meristem, while the root is derived from the root apical meristem. The contribution of embryonic tissues to the adult plant is marginal. Adapted by permission from Macmillan: Nature, Weigel and Jürgens, copyright 2002

lost structures with ease. A major challenge for plant development arises from their sessile lifestyle. While most animals can avoid harsh environmental conditions by seeking shelter or by migration, plants have to cope with diurnal as well as seasonal oscillations *in situ*. The adaptation to changes in light intensity, or to stresses such as heat, cold, or drought, not only require the plant to implement mechanisms to protect itself, but also to integrate these cues with a continuously active developmental program. As a result, plants have evolved a complex regulatory network to efficiently control the balance between stem cell proliferation and differentiation that functions over time frames lasting as long as thousands of years (e.g., for some tree species).

In this chapter I describe the structure and origin of the stem cell harboring tissue of the shoot, summarize what is known about the regulatory mechanisms underlying shoot stem cell homeostasis, and discuss some of the remarkable properties of plant stem cell regulation.

1.1.1 Structure and Function of the Shoot Apical Meristem

During embryogenesis two populations of totipotent stem cells are initiated that are dedicated to producing the shoot and the root, respectively (reviewed in Jürgens, 2001). These stem cell pools are embedded into specialized tissues called meristems. Despite the fact that the root system is of equal importance to the shoot and much is known about the root meristem, here I will focus on the stem cells of the shoot for the sake of simplicity.

The shoot apical meristem (SAM) is the source of all above ground tissues of a plant. It is one of the first structures to be initiated during embryogenesis (Fig. 1.2) and molecular markers for the SAM become expressed in a localized fashion by the 16-cell stage (Mayer et al., 1998). The presumptive SAM remains small and inactive throughout embryogenesis and acquires its full function during germination, when the embryo emerges from the seed coat (Jürgens et al., 1995; Laux and Jürgens, 1997; Jürgens, 2003).

In angiosperms, or flowering plants, the SAM of an adult plant is a dome shaped structure with a diameter of about 250 µm that contains a few hundred undifferentiated and dividing cells (Fig. 1.3). In contrast to cells in mature plant organs, which are large and vacuolated, meristematic cells are small and rich in cytoplasm. Despite the fact that cellular morphology is remarkably uniform across the meristem, not all cells in the meristem are stem cells – this fate is restricted to about 30 cells in the upper center of the meristematic dome. Cells that are born in the meristem are displaced to the periphery where they differentiate to form new organs, such as leaves and flowers.

Not unlike the germ layers in animals, the body of dicotyledonous plants is build from three clonally distinct tissue layers, termed L1, L2 and L3 (Fig. 1.3). L1 and L2 together form the superficial tunica and are both limited to a single layer of cells. The L3 layer in turn makes up the internal corpus. These layers are established

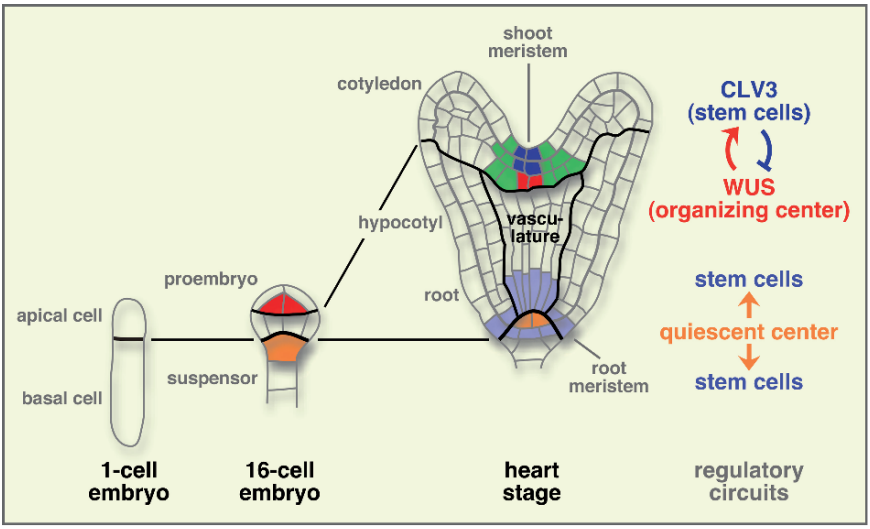

Fig. 1.2 Embryonic origin and molecular organization of apical meristems. The first marker for the identity of the shoot apical meristem is *WUSCHEL*, which is expressed at the 16 cell embryo and remains confined to a restricted domain throughout embryogenesis. Adapted by permission from Macmillan: Nature, Weigel and Jürgens, copyright 2002

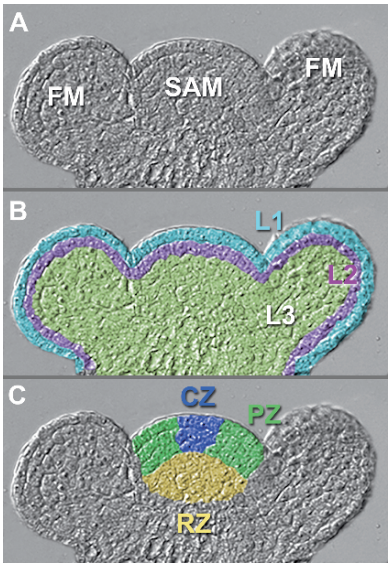

Fig. 1.3 Organization of the shoot apical meristem (SAM) during the reproductive phase. Longitudinal section through the center of the meristem are shown. (A) From the central SAM, which is home to the totipotent stem cells, floral meristems (FM) are formed, which will differentiate into mature flowers. (B) Tissue layer organization as observed at the SAM. Layers L1 and L2 form the superficial tunica, while L3 makes up the internal corpus. The layers remain clonally distinct by anticlinal cell divisions in L1 and L2. (C) Domain structure of the SAM. Totipotent and slowly dividing stem cells make up the central zone (CZ), while the cells of the peripheral zone (PZ), remain undifferentiated and divide more rapidly. The rib zone (RB) contains the stem cell inducing cells of the organizing center, which coincides with *WUSCHEL* expression

early during embryogenesis and remain separated over the entire course of plant development by anticlinal cell divisions in the L1 and L2 layers, while L3 cells divide in all planes. This layered organization is well reflected in the structure of the SAM and requires that cell proliferation and differentiation are coordinated by extensive cell-cell communication.

The organization into distinct tissue layers requires the presence of stem cells in all three layers and, consequently, cell behavior in the SAM is also highly regulated at the level of meristematic domains that include cells of different clonal origin (Fig. 1.3). About 20 to 30 slowly dividing and self-renewing totipotent stem cells make up the central zone, which spans the L1, L2 and L3 in the center of the meristem dome. Analysis of mericlinal chimeras, which can be induced by X-ray mutagenesis or transposon activation in living plant embryos of various species, has shown that the majority of cells in an adult plant are descendents of these stem cells. In *Arabidopsis thaliana*, for example, the cells of the entire embryonic meristem give rise to the first six leaves, while the remainder of the cell mass is clonally related to the stem cells (Irish, 1991). This was a remarkable finding, not only because it showed the important contribution of the true stem cells, but also because it highlighted the extent of cell proliferation in the meristem outside the stem cell domain. Indeed, adjacent to the central zone that harbors stem cells is a domain marked by rapid cell proliferation, which has been termed the peripheral zone (Grandjean et al., 2004; Reddy et al., 2004; Traas and Bohn-Courseau, 2005; Vernoux et al., 2000). Stem cell daughters that are themselves displaced from the central zone into the peripheral zone, loose stem cell identity and start to divide rapidly before they are incorporated into forming organs and subsequently differentiate. Thus, cells of the peripheral zone are similar to the so-called transit-amplifying cells of animal stem cell systems. Another feature of plant stem cells that bears striking similarity to animal stem cell systems is that they acquire their identity through signals emanating from neighboring cells. In the SAM these cells are embedded in the deeper layers of the meristem, known as the rib-zone. Only a small group of about 20–30 cells spanning three cell files below the central zone has stem cell inducing capacity. This group of cells is known as the organizing center (Mayer et al., 1998). Interestingly, while the organization of stem cell induction by neighboring cells is equivalent to the niche concept well established in animals, there are striking differences in how the stem cell niche is organized in the SAM. The most obvious difference is that not all stem cells have direct contact with the cells of the organizing center. Stem cells of the L1 and the cells in the lowest file of the organizing center are separated by six cell diameters and stem cells of the L1 and L2 are never in contact with the organizing center. This effectively rules out that molecules displayed at the cell surface constitute a stem cell inductive signal. Another major difference of the SAM stem cell system is the continuous turnover of the organizing center. Because the cells of the organizing center divide at very low rates, they are regularly displaced by cells leaving the stem cell domain during the growth of the plant. Thus, a plant cell is able to switch from stem cell fate to organizing fate and finally to a differentiation program purely based on its position in the organism. Elegant cell ablation studies in the root meristem have confirmed

this experimentally (van den Berg et al., 1995; van den Berg et al., 1997) and support the notion that plant cells have an extraordinary developmental plasticity. Recent studies using laser ablations of entire meristematic domains have shown that the SAM is able to recover from the loss of all stem cells within a relatively short period of time (Reinhardt et al., 2003a). Moreover, these experiments revealed that even the ablation of the organizing center in addition to the stem cells can be overcome by regulatory processes. Thus, the SAM is a highly plastic stem cell niche that provides an extremely robust environment for the long-term maintenance of totipotent stem cells.

1.1.2 Stem Cell Regulation at the SAM

In the following section I will discuss the molecular mechanisms of stem cell control in the SAM. Again, for the sake of clarity, I will not give a comprehensive overview, but rather focus on the key players.

Since the SAM is laid down in the embryo, genetic screens aimed at finding regulators of plant embryogenesis were very successful in identifying mutations affecting meristem regulation (Clark et al., 1993; Clark et al., 1995; Kayes and Clark, 1998; Long et al., 1996; Mayer et al., 1998). However, only a handful of genes with mutant phenotypes resulting in severe meristematic defects were isolated, which essentially fall into two classes: mutants in which the SAM is arrested and non-functional on the one hand, and those in which the SAM is enlarged and marked by overproliferation of cells on the other hand.

The first gene that was cloned from the former class was *SHOOTMERISTEM-LESS (STM)*, which codes for a homeodomain transcription factor of the plant specific KNOX class (Long et al., 1996). *STM* shows a SAM specific expression with *STM* transcripts excluded from cells that are part of an organ primordium. Thus, the absence of *STM* RNA is one of the earliest markers for differentiation of meristematic cells that morphologically are indistinguishable from other cells in the SAM. Consistent with this, inactivation of *STM* causes premature cell differentiation within the SAM and, consequently, a breakdown of stem cell maintenance. Thus, while *STM* plays a central role for SAM function by allowing cells to proliferate, it does not seem to be directly involved in setting up stem cell fate (Clark et al., 1996; Long et al., 1996).

The second mutant from the arrested meristem class is called *wuschel* (*wus*) (Laux et al., 1996), which is the German word for bushy. As usual, the mutant name reflects the phenotype and *wus* mutants suffer from repeated meristem termination and reinitiation, which produces plants with disorganized and bushy shoots. The *WUS* gene codes for a homeodomain transcription factor, but in contrast to *STM* it is not a member of the KNOX class, but rather the founding member of the WOX class of transcription factors (Mayer et al., 1998). *WUS* is expressed in the organizing center of the SAM, and acts in a non-cell autonomous fashion to induce stem cell

fate in the central zone. In plants lacking *WUS* activity, the SAM is depleted of stem cells, the meritematic dome collapses into a flat structure and differentiation occurs. Conversely, ectopic expression of *WUS* within the meristem causes massive overproliferation of cells (Schoof et al., 2000).

A similar overproliferation phenotype is also observed in the *clavata* (*clv*) mutants that fall into the second class of meristematic mutations (Clark et al., 1993; Clark et al., 1995; Kayes and Clark, 1998). In plants lacking *clv* function, the meristematic dome is expanded and frequently transformed into an elongated oval shape to accommodate the proliferating cell mass, a process known as fasciation. Genetic analysis has revealed that mutations in three independent loci produce *clv* phenotypes, with *clv1* and *clv3* having the most dramatic effects. Cloning of the corresponding genes revealed that all *CLV* genes are most likely part of a single signaling pathway. *CLV1* codes for a Leucine Rich Repeat (LRR) transmembrane protein with an intracellular kinase domain (Clark et al., 1997). These proteins frequently function as plasmamembrane receptors and constitute a large family in the Arabidopsis genome. CLV2 has a similar molecular nature, but while it is also a LRR transmembrane protein, it lacks the intracellular kinase domain (Jeong et al., 1999). In contrast, the CLV3 protein is of very different structure (Fletcher et al., 1999). The active form of CLV3 is a short secreted peptide of 12 amino acids, which is processed from a longer precursor (Ito et al., 2006; Kondo et al., 2006; Rojo et al., 2002). Expression analysis of the *CLV* genes revealed that they are directly involved in plant stem cell control. *CLV3* transcripts can be found exclusively in the stem cells of the central zone, while *CLV1* RNA is restricted to a domain overlapping with the organizing center but extending partially into the stem cell domain (Clark et al., 1997; Fletcher et al., 1999). In contrast, *CLV2* is expressed more widely (Jeong et al., 1999). Double mutant analysis of *clv1* and *clv3* have indicated that both genes function in the same pathway. Further, the molecular nature of the CLV proteins indicates that CLV3 might act as a ligand for the CLV1 and CLV2 receptors. Consistent with the ligand-receptor hypothesis for the CLV pathway, CLV1 and CLV2 are known to associate in the plasma membrane (Jeong et al., 1999). So far, however, no experimental evidence for a direct interaction between CLV3 and the CLV1/2 receptors has been reported.

Taking the molecular nature and expression patterns of the *CLV* genes into account it is attractive to hypothesize that their biological function is to relay information from the stem cells to the organizing center. Based on the mutant phenotypes of all meristem regulators described above, the *CLV* pathway would relay a negative signal from the stem cells to the organizing center, while unknown molecules downstream of *WUS* would carry a positive signal in the other direction. The finding that a mutation in *WUS* is fully epistatic to all *clv* mutants has provided strong genetic support for this idea (Laux et al., 1996). Indeed, it was shown by a series of elegant experiments that *WUS* and *CLV3* are connected by a negative feedback loop (Brand et al., 2000; Schoof et al., 2000) While overexpression of *WUS* leads to ectopic formation of stem cells accompanied