Preface

The pronounced developmental and metabolic capacities of plant cells provide the base of agriculture and green biotechnology and have been studied very intensively with respect to its genetic aspects. However, it becomes increasingly clear that the potential of applied plant science can only be fully exploited when we grasp the underlying cellular mechanisms such as compartmentalisation, intracellular transport, cell differentiation, and communication. To understand biotechnologically relevant molecular mechanisms for stress tolerance and accumulation of interesting compounds, we need a strong background in molecular cell biology.

The so-called Green Revolution has ensured a reliable and cost-efficient supply of a growing population with food resources. A major part of this success story is based on three factors: advances in plant nutrition, advances in plant protection, and advances in plant genetics. The challenge of the coming years will be to reconcile increased agricultural productivity with sustainability. The potential of plant nutrition and chemical plant protection has been basically exploited; what remains, are advances in generating new genotypes with improved productivity, tolerance to biotic and abiotic stresses, and improved or even novel metabolic potential. The book is therefore divided into four parts that deal with the control of development, the control of stress tolerance, the control of metabolic activity, and novel additions to the toolbox of modern plant cell biology.

A general theme subtending all fields of contemporary plant biotechnology, often in an implicit manner, is the regenerative ability of plant cells, which is much more pronounced as compared to animals. The book will therefore begin with a *prologue* describing how the current concepts on the "totipotency" of plant cells has evolved in the historical context. Interestingly, already the beginnings of plant cell biology were linked with application, namely the intention to understand the pronounced regenerative ability of plants and the mechanisms of their vegetative propagation. The prologue of this volume will therefore recapitulate this development that began in the second half of the nineteenth century in Central Europe and has been extremely fruitful since. From the plant-specific concepts created at that time, meanwhile a plant-specific concept of stem cells has emerged that is progressively supported by detailed molecular mechanisms. In the field of application, these concepts have helped to lead plant regeneration as central strategy for green biotechnology from the realm of pure empiry into a technology that can be controlled and designed on a scientific base. This is of particular importance for so-called recalcitrant systems such as particular cell strains, organs or organism that do not respond to conventional techniques of vegetative propagation.

Control of Growth and Development

The first part of the book will address the cellular base of growth and development. The highly plastic plant cells can respond to biotic and abiotic factors and integrate information over the state of their environment with developmental programs through signalling pathways converging at the chromatin level. Signal-dependent changes of gene expression, in turn, will be expressed as adaptive changes of growth and morphogenesis that help to cope with environmental challenges.

One of the central players for this adaptive morphogenesis is the plant cytoskeleton. A plant-specific theme is the so-called cortical cytoskeleton that, in interphase cells, is intimately linked to the cell wall, whose physical properties define the turgor-driven growth of plant cells, providing versatile tools for biotechnological manipulation of plant architecture. However, during the last years, a novel function of this cortical cytoskeleton has emerged: it not only acts as downstream effector for the environmental response, but conveys a second function situated upstream in the sensory process itself. Cortical microtubules participate in the sensing of abiotic and biotic stress factors, which opens new possibilities for application, for instance, in the context of cold tolerance of crop plants.

The plasticity of plant development is organised and balanced by a dynamic "hormonal status," which is a process integrating the current cellular content of a phytohormone (depending on synthesis, transport and metabolic inactivation), and the activity of perception and signalling in response to this cellular hormonal content. The central players are auxins and cytokinins, and, for both hormones there is a long tradition of practical application – it is not exaggerated to say that green biotechnology would have been impossible without the discovery of auxins and cytokinins. Two chapters therefore summarize the current state of the art, but also applications based on new synthetic analogues as well as transgenic approaches targeting hormonal transport, metabolism, and local maxima/minima providing a whole plethora of tools and strategies for biotechnological manipulation.

A further plant-specific target for application is the manipulation of programmed cell death for development, addressed in the two final chapters of the first part. It seems that specific cell lineages have to be assigned for cell death in order to activate the developmental potency of other lineages to turn into stem cells. To get control of the astounding regenerative capacities of plants will therefore require cell-type specific manipulation of programmed cell death. The signalling underlying this fatal decision of individual cells between "Life" and "Death" is described in one of the two chapters in the context of somatic embryogenesis, a field that provides the technological choice for industrial propagation of economically important species, especially conifers. Breeding has been the crucial factor for the success of the Green Revolution. The final chapter is therefore dedicated to pollen development as key target for breeders. It is the pollen that, in nature, ensures the sexual motility required to sustain efficient gene flux supporting the genetic diversity of a species. Because many crop plants are self-fertile, breeders need to suppress selfing in order to maintain heterozygosity. Since the days of Gregor Mendel, this is achieved by manual removal of the stamina in the receiving flower of a cross – a technique as cumbersome as it is expensive. A comprehensive overview on the cellular events regulating pollen genesis, maturation and development, and their underlying molecular mechanisms has opened numerous venues to control and manipulate male fertility in numerous important crop plants which are of high relevance for breeders.

Stress Tolerance

Since plants cannot run away, they have to adapt to environmental challenges to a much stronger degree as compared to animals. Stress tolerance is progressively seen as the major factor for the future of agriculture and its sustainability. Desertification, urbanisation and the ongoing increase of the world population require agriculture to be extended to so-called marginal lands that are often challenged by undesired chemical elements released from acidic soils. To safeguard yields, at present, extensive plant protection is required, progressively arising public concern. Thus, not only the tolerance to abiotic stress, but also cellular mechanisms of innate immunity have shifted into the focus of application.

The part starts with a chapter on the cellular responses to Zn and Cd as central heavy metals in polluted soils. Interestingly, the role of Zn is a bit scintillating since it is also required as trace element, however, in very low concentrations. To assess the toxicity of these elements, it is relevant to understand, not only on a cellular but on a systemic level, how Zn and Cd actually enter the root of a plant, and which barriers have to be crossed on their path from the root to the aerial organs. The detailed knowledge of the molecular and cellular mechanisms relevant for uptake have, again, stimulated novel applications to manipulate this uptake, whereby the aim can be diverse – on the one hand, it is beneficial to prevent these metals from accumulation in those parts of a crop plant that actually will enter the food chain. On the other hand, it can also be rewarding to stimulate uptake, when heavily contaminated soils are to be sanitized by so-called phytoremediation.

Also the second chapter deals with an element that can be either noxious or beneficial depending on its dose: Uptake and metabolism of selenium is closely related with the compartmentalisation of sulphur. Again, molecular information on transporters and the mechanisms of intracellular sequestrations likewise allows to prevent excessive accumulation of Se in crop plants, or to safeguard a minimal daily uptake of Se in regions depleted from this exotic element. The synthesis of sulphur-rich secondary compounds, which is also reviewed in this chapter, not only bears on redox homeostasis crucial to control otherwise destructive oxidative burst triggered in response to abiotic stress, but also connects with the field of secondary metabolism not only highly relevant for biomedical applications of plant products, but also for the field of defence against pathogen attack.

Tolerance against biotic stress is in the focus of the third chapter of this part: the plant flagellin receptor as central player of plant innate immunity has been identified as crosspoint that allows to tune defence with signalling from hormonal or developmental signalling. Defence represents a considerable investment that impinges on the resources available for growth, development and agronomical yield. As the human immune system must be strictly controlled in time and amplitude, also plant immunity has to be confined. The flagellin receptor, as worked out in this chapter, undergoes endocytosis, and this phenomenon emerges as a switch that not only allows the plant to tune the amplitude of the defence response with the amplitude of the pathogen challenge, but also to decide which type of defence response is selected. No wonder that this event is also target for microbial effectors that have evolved to quell plant immunity. Again, detailed cellular and molecular insights into defence signalling have allowed to design novel strategies to manipulate plant defence as central element of more sustainable approaches to plant protection.

Plant Metabolism

Plants produce a vast array of natural products (primary and secondary metabolites), many of which have evolved to confer adaptive advantages against biotic and abiotic stresses in natural environments. Often, certain species produce and accumulate particular metabolites. The transcription factors controlling plant metabolic pathways leading to biosynthesis of flavonoids, glucosinolates, lignins, and of terpenoid indole alkaloids have been isolated and characterised. This information can now be used to decipher the molecular mechanisms responsible for coordinate induction of transcriptional networks in particular cell types. But it can also be used to engineer plant metabolism. This is exemplarily demonstrated in the three chapters of the third part.

The cytoskeleton represents an attractive target for therapeutical compounds, because via the cytoskeleton, unregulated cell divisions underlying the growth of cancer can be controlled. Classical anti-cancer drugs such as colchicines, vinca alkaloids, taxanes, and podophyllotoxins are of plant origin. Due to their complex chemical structure, they cannot be synthetised technically, but have to be extracted directly from plant cells. The market for anti-cancer compounds is tremendous, and the need for more specific compounds has stimulated high-throughput screening of secondary plant products based on bioassays with animal cells. The first chapter not only surveys the fascinating field of biomedically active plant compounds, but also describes a very innovative strategy, how new anticytoskeletal plant compounds can be isolated and identified.

In addition to the search for novel compounds, also the existing knowledge on the medical effect of traditional plant ailments is progressively mined by comprehensive approaches. Especially India and China have established very elaborated traditional healing systems that are based on the experience from several thousands of years and await to be merged with contemporary science on molecular modes of action and cellular responses triggered by specific secondary compounds. In one of the probably most comprehensive approaches available so far, reviewed in the second chapter of the third part, the traditional medical plant Ashwagandha (*Withania somnifera*) has been thoroughly analysed with respect to the developmental and tissue-dependent profile of medically active metabolites, the chemistry of the underlying pathways, the enzymes driving the steps of these pathways, the regulation of the genes coding for these enzymes, and the diversity of chemically different genotypes (so-called chemovars) within the species.

Wood represents the most important biomaterial on this planet, and this biomaterial comes in a huge multitude of versions differing in mechanical, esthetical and biological properties. In contrast to other plants used by mankind, domestication of wood plants is still in an early phase. Humans exploit wood as if they still were in the hunter-gathering phase prior to the neolithic revolution. This cannot go on for long, since non-sustainable exploitation of wood has wiped out most of the rain forests on our planet. However, the secondary metabolism of lignin is quite well understood, and the last chapter of the third part describes novel strategies for genetical engineering wood with preset properties. It is to be hoped that a smart biotechnological use of the molecular and cellular mechanisms defining wood quality will allow for sustainable alternatives to the wild deforestation in developing countries.

The Cell Biology Toolbox: New Approaches

The last two decades were mainly shaped by breakthrough technologies of molecular biology, often linked to high throughput "-omics" approaches. The renewed interest in epigenetic phenomena has reconfirmed the importance of spatial organisation, for instance, when gene activity is linked with the spatial organisation of chromatin. Stimulated by this shift in concept, several techniques and experimental models have experienced a renaissance, which is now firmly rooted to the molecular base of epigenesis. It is to be expected that the postgenomic era will see a rising impact of cell biology. However, the toolbox for cellular manipulation is still to be developed – here it is not sufficient to make specific molecules appear or disappear in a global manner. In cell biology, it is space and time that matter. Therefore, the last part of the book is dedicated to the methodological base of applied plant cell biology.

Flow sorting, widely used in medicine, has been successfully transferred to plant cells and developed into a very powerful approach to collect and purify specific chromosomes. The success of this technology, as pointed out in the first chapter of the final part, depends on the methodological details – the secrets behind the success story of this technology are revealed and discussed as well as the versatile application of this tool. By far the most important application in plant science is the sorting of nuclei to determine nuclear DNA content. The information derived from this approach is of crucial importance for the design and management of genome projects, but also has advanced diverse fields of plant research as from understanding regulation of the cell cycle till detection of incipient speciation in evolutionary studies.

Concepts of the cell have been shaped by the available technologies: the advent of biochemistry and enzymology in the 1950s and the concept of the cell as a "bag of enzymes" was followed by a fascination for the internal structure of cells made visible by the brand-new technology of electron microscopy, and the concerted effect of green fluorescent proteins and advanced fluorescence microscopy revealed a surprisingly dynamic cellular structure – the term "cytoskeleton" coined from (fixed and sectioned) electron microscopical images was replaced by a dynamic equilibrium of rapidly cycling microtubules and actin filaments. As shown by the second chapter of the final part, photoconvertible reporters might become the next methodological advance with prospective conceptual consequences: These reporters not only allow to see specific molecules in living plant cells, but to visualise their history and compartment-specific reporters allow to get valuable insight into the dynamic compartmentalisation characteristic and essential for the metabolic proficiency of plants.

Cell biology has profited tremendously from cellular models. As pointed out in the prologue chapter, the race for immortal cell lines was initially won by the animal field. However, after auxins and cytokinins had been identified as reprogramming factors for the developmental potency of plant cells, the plant fields caught up immediately. Plant cell strains, reviewed in the final chapter, have become a unique experimental model to study the cellular aspects of cell division, cell expansion, cell morphogenesis, senescence and cell death, which are often not experimentally accessible in the context of an organ composed of complex tissues. Especially for the widespread tobacco cell line BY-2, fluorescently tagged markers for different organelles and proteins have been established such that non-invasive life-cell imaging has been thoroughly integrated providing powerful tools for molecular cell biology. However, against the conventional prejudice that cell lines are just chaotic masses of rapidly dividing cells, a closer look reveals that these cell strains undergo a defined, albeit strongly reduced, developmental programme. By this property they turn into unique experimental models for plant cell phenomics allowing integrated molecular, biochemical, cytological and morphological analysis in living, intact plant cells in the absence of the correlative (especially mechanical) constraints of the complex organism, such that the primordial, basal morphogenetic potential of individual plant cells becomes accessible to analysis. Thanks to their enormous reproductive ability, some cell strains (with tobacco BY-2 being the most prominent example) have been successfully adapted to molecular farming. Especially for products tailored to specific medical applications, where diversity and versatility of molecular farming is more relevant as compared to large yield, plant molecular farming is economically superior as compared to animal or bacterial models. The considerable potential of this accession to the cell biology toolbox still provides space for exploitation.

Cell biology has been traditionally perceived as a "pure", "fundamental" field of science striving to understand, the building blocks of all living beings. It has been overlooked that cell biology, by its very essence, subtends and supports all biotechnological applications. This is prominently true for plants, where organisation is much deeper routed in the versatility of individual cells. The mission of this book is to show that plant cell biology evolved from the continuous and intensive dialogue between fundamental research and application – a non-interrupted line of tradition that has been successfully pursued over almost more than 150 years. The next step for this fruitful liaison will be to integrate the challenge to develop sustainable solutions for the agriculture of the twenty-first century.

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