PREFACE

This book summarizes some of the major processes involved in the production of daughter cells as they have been reported from work mainly with synchronous cultures of microalgae over the 50 years or so since that technique was first developed. It highlights the key findings that have led to our present understanding of cell cycle processes in microalgae with particular reference to those that control daughter-cell production. The authors of this book also systematize the data on molecular genetic system controlling drosophila bristle morphogenesis and proposes an integral scheme of its functioning. In addition, the current understanding of kidney regeneration after injury are examined from the perspective of renotropic factors, renal stem/progenitor cells, and stem cell therapies. In unicellular and multicellular organisms, asymmetric division enables segregation of damaged molecules into one daughter cell. The authors suggest that partition of damaged proteins and organelles and segregation of template DNA may function together to produce long-lived stem cells. Daughter cells' behavior of the chlorarachniophytes are also described and the evolution and biological implications are discussed. Lastly, recent advances in stem cell biology are summarized, and ways in which clinical medicine could take advantage of this fascinating field of biology are examined.

Chapter 1 - This review summarises some of the major processes involved in the production of daughter cells as they have been reported from work mainly with synchronous cultures of microalgae over the 50 or so years since that technique was first developed. It highlights the key findings that have led to our present understanding of cell cycle processes in microalgae with particular reference to those that control daughter-cell production. Among the microalgal species that have been studied are those that divide by simple binary fission, like the diatoms (Bacillariophyceae), dinoflagellates (Pyrrophyta) and the euglenoids (Euglenophyta) as well as those, mainly represented by the green microalgae (Chlorophyta), that divide by various more complex processes of multiple fission. In the latter, the cell cycle includes multiple rounds of duplication yielding 2^n daughter cells per mother cell (n = 2-5). Some examples of cell cycle processes in colonial microalgal forms are included for comparison and, for the purpose of this review, the unicellular prokaryotic cyanobacteria (blue-green algae) are also included because they allow comparisons between prokaryotic and eukaryotic cell cycles.

It will be shown that although certain details of the cell cycle will vary between the microalgal species, as affected by morphological and physiological features of the adult cell, the basic control and regulatory mechanisms have much in common not only among the microalgae themselves but between them and the cell cycles of other micro-organisms, animals and plants. The case studies described will highlight the flexibility of the microalgal cell cycle under different conditions and the range of processes involved in the progression of the cell through its natural cycle culminating in daughter cell production and release. They will also address the concept of commitment to divide, the role of cell cycle regulatory proteins and their genetic control and possible links of cell cycle processes to certain endogenous circadian rhythms known to exist in a number of microalgal species. It will be clear that despite the large amount of information now available on various microalgal cell cycles there is much yet to be revealed, especially in relation to the control mechanisms involved.

Chapter 2 - Today's actual question is *creation of the science on stem cells* and development of its theoretical bases, taking into account comparative evolutionary cell biology and the *cardinal problem of the developmental biology – the problem of wholeness* for representatives of various kingdoms. This allows revealing correlations and studying correlative dependences of various structures at different levels of biohierarchy. The creation of every science is impossible without the application of methodology.

In current work the system of nontraditional ideas about the nature and role of stem cells in ontogenesis, reproduction and evolution of plants is proposed.

The main properties of plant stem cells have been developed by us, which has shown the integrity of morphogenous and reproductive processes at all stages of plant's life cycle. A special attention should be paid to the dynamics of the stem cells' pool and its correlative dependence with niche.

System approach and analysis of data on male and female generative structures – egg cell at different stages of development, double fertilization in model objects: syngamy, triple fusion, plasmogamy and karyogamy taking into consideration the cell cycle, zygote, embryogenesis of model objects in representatives of a lot of flowering plants taxa allowed us to express nontraditional idea, that from the embryological point of view zygote is proximate stem cell and gives a rise for all other stem cells in plant ontogenesis, particularly those of shoot and root apices.

Zygote contains information about architectonics and development of organization of an individual, which is connected with gradients and correlations between its parts, and consequently with heterogeneity of different axes of a future organism.

The data of developmental biology and especially comparative embryology of representatives of various taxa of *flowering* plants enable to consider that *formation of stem cells derived from zygote, are probably typical for all organs – flower, stem, leaf, root – and at all stages of the life cycle – sporophyte, gametophyte. Their localization is depending, first of all, on their functioning.*

Not every cell possesses the totipotency and not every totipotent cell is able to become the stem cell, and not every stem cell can produce an individual.

One has to pay espesial attention to the role of somatic cells in organization of embryo's body, in reproduction, and particularly, in changing of morphogenetic programs in

ontogenesis and evolution. This view of the role of somatic cells also corresponds to the data of discoveries made in the middle of the XXth century: stem cells, growing plants out of somatic cells, haploidy, parasexual hybridization, and embryoidogeny. These categories are for the first rime considered by us from the position of stem cells.

A new phenomenon, embryoidogeny, was for the first time revealed by us in 1977 – a new type of vegetative propagation, where elementary structural unit is an embryoid (somatic embryo) forming out of somatic cells.

Thus, not only zygote, but somatic cell as well is able to be the initial cell – stem cell of a plant, playing a big role in ontogenesis and evolution.

The diversity of morphogenetic processes comprehends two components: potentially infinite somatic cells activity that is conditioned by the functioning of dormant centres' cells, being in intermediate differentiated state, and loss of stemness – senescence and apoptosis.

The plasticity and polyvariety of these processes are conditioned by the diversity of the morphogenesis and origin of vegetative and generative structures in which the peculiar reserve of cells, possessing the stemness property, is situated. Hence in plant morphogenetic processes even third component is present – restoring of stemness in many respects depending on stemness degree.

The arsenal of the data received in the XX-XXI centuries on biology of development including stem cells biology has allowed us to propose and discuss the three main perspective trends in study of stem cells – traditional and nontraditional.

Nontraditional trends:

- gametes – zygote – embryo – individual – population – coenosis;
- different types of somatic cells considering their localization and function in the organism; possibility of homologization of some somatic cells with the zygote;

Traditional trend:

- apical meristem of shoot apex and root apex in which organization somatic cells take part.

Chapter III - Formation of specialized spatial structures comprising various cell types is most important in the ontogenesis of multicellular organisms. An example is the *D. melanogaster* bristle organs. Bristles (micro- and macrochaetes) are external sensory organs, elements of the peripheral nervous system, playing the role of mechanoreceptors. Their comparatively simple organization comprising only four specialized cells and a common origin of these cells make macrochaetes a convenient model for studying cell differentiation.

The four cells forming bristle organ result from two successive divisions of a single cell, sensory organ precursor (SOP) cell. The number of macrochaetes on drosophila body corresponds to the number of SOP cells.

The morphogenesis of macrochaetes comprises three stages, the first two determining a neural fate of the cells. The third stage is cell specialization into components of the bristle organ—neuron, thecogen, tormogen, and trichogen.

Development of each bristle commences from segregation of proneural clusters, of 20–30 cells, from the massif of undifferentiated cells of the wing imaginal disc. At this stage, each cluster cell can potentially become a SOP cell. At the second stage, the only SOP cell and its position are determined within each cluster. Finally, two asymmetric divisions of the SOP cell with subsequent differentiation of the daughter cells gives the bristle organ.

Several dozens genes are involved in the control of macrochaete morphogenesis. The main component of this system is the proneural genes of *achaete-scute* complex (*AS-C*). An increased content of proneural proteins fundamentally distinguished the cells that will follow the neural developmental pathway from the disc epidermal cells.

A local *AS-C* expression, initiated at specified disc sites by specific transcription factors, determines the number and topology of proneural clusters. The expression of *AS-C* genes, continuing in the cells of the cluster, increases the difference in proneural protein content, first, between the cluster cells and then, between the cluster cells and the single SOP cell, where it reaches the maximum level. This process is provided by both the intracellular regulation of *AS-C* gene activity and intercellular events mediated via the EGFR and Notch signaling pathways.

The third stage in macrochaete morphogenesis comprises two successive asymmetric SOP cell divisions, determining the final specialization. The selector genes, in particular, *numb*, *neuralized*, *tramtrack*, and *musashi*, play the key role in cell type specification.

This review systematizes the data on molecular genetic system controlling drosophila bristle morphogenesis and proposes an integral scheme of its functioning.

Chapter IV - Stem cells (SCs) are thought to reside in many tissues within a niche formed by a group of surrounding cells and their extracellular matrices, which provide an optimal microenvironment for the SCs function. In general, the niche consists of a highly organized microenvironment in which various factors, such as signals coming from secreted cytokines, extracellular matrix (ECM), and intercellular adhesions are thought to work cooperatively to maintain the undifferentiated stem cell phenotype. Among SCs, adult SCs are often localized into specific niches where they utilize many, but not necessarily all, the external and intrinsic factors used by the embryonal counterparts to select a specific fate. Within the niche, SCs can maintain their ability for self-renewal as well as their potential so that, consequently, their detachment from the niche compartment induces their differentiation and loss of self-renewal capacity. Thus, when a stem cell begins to divide, it is thought that one daughter cell remains in a niche to replace the original stem cell, whereas another daughter cell is expelled out of the niche and starts its process of differentiation. In this process, a cell retains self-renewal properties and differentiation inhibitory factors, so that it stays as SC, whereas another daughter cell is destined to proliferate during a certain number of divisions for final differentiation along a particular lineage. This latter daughter cell will receive too few *stemness* factors to maintain its identity as a stem cell, and/or will inherit differentiation factors that can overcome its stem cell phenotype. To maintain tissue homeostasis and correct functioning of an organism, the number of daughter cells that retain stem cell identity must be strictly controlled such that differentiated cells can be generated in response to any injury. Likewise, the rate of division of SCs into a niche must be tightly controlled, since an overproduction of daughter cells destined to be differentiated may be harmful because this may result in cancer generation. In the present chapter, the authors will analyze distinct types

of niches for distinct tissues as well as the mechanisms that influence the generation of daughter cells from each niche.

Chapter V - Kidney has a capacity to regenerate itself after a variety of insults. For the past few decades, the search for the factor(s) that can promote regeneration of the injured kidney has been a principal and fundamental theme in nephrology. Using various kidney injury models, the role of growth factors in kidney regeneration has been extensively examined and several factors such as hepatocyte growth factor (HGF) have been demonstrated to act as renotropic factor(s). Lately, along with other organs, the identification of adult tissue stem cell is one of the most exciting subjects in this area. A number of cell populations expressing putative stem cell markers or possessing stem cell property has been found within the kidney. Using BrdU labeling methods, it was shown that a slow-cycling tubular cell population was actively engaged in the regeneration processes of the kidney after ischemia and function as an origin of regenerating cells. Like in other tissues, side population cells have been identified in the kidney. Several groups have reported that cell therapies with bone marrow-derived cells (hematopoietic stem cell or mesenchymal stem cell) are highly effective for the treatment of acute or chronic renal failure in animals, which seems to be via the production of regenerative factor(s) rather than direct differentiation into a component of renal cells. In the near future, therapeutic strategy with stem cells might be possible for the treatment of various kidney diseases.

In this chapter, the authors summarize and discuss the current understanding of kidney regeneration after injury from the perspective of renotropic factors, renal stem/progenitor cells, and stem cell therapies.

Chapter VI - Exfoliation is a loss of cellular material retaining the basic cytological features of typical cells (plasma membrane, cytoplasm and nucleus). However, the set of physiological parameters leading to exfoliation is complex and difficult to measure. Exfoliated epithelial cells can be obtained from a wide range of mucosa like mammary glands, oral mucosa by scraping, bronchial mucosa by expectoration and gastric or colonic epithelia by extensive rinsings during routine procedures of endoscopic examination. From an histological point of view, epithelia are organized in functional units containing different cellular compartments (stem, proliferative, mature or functional and senescent cells). Exfoliation is either (1) a natural loss of body cells implying a molecular signal related to the turn-over of terminally differentiated cells and to the progressive mobilization of proliferative as well as stem cells or (2) the result of manual exfoliation by applying mechanical constraints like gentle swabbing or scraping. Exfoliated epithelial cells are believed to be either in apoptosis or in anoïkis. In the latter group, cells may survive by autophagy retaining their growth potential if proper matrix is rapidly provided after exfoliation. Most studies are based on cytological proof using microscopic examination along with a limited number of biomarkers. Only few studies using proteomics or transcriptomics are available and they open discussion about tissue references and normalization. Exfoliated cells may represent valuable source of information on the physiopathological status of the mucosa. In translational research, ethical reasons as well as legal ones are limiting the use of biopsies in favor of measures realized on epithelial cells isolated from biological fluids (milk, urines, gastric aspirates, stools) collected by way of non-invasive technology. Unambiguous knowledge on

the advantages and the limitations of exfoliation methodologies could have far reaching impact on chrononutrition or chronopharmacology.

Chapter VII - Plants can show flexible capacity of coping with the impairing factors of their abiotic and biotic environment and their high developmental plasticity, and easily forced cellular totipotency is a general feature. Plants have a notable potential to maintain separate cell groups in their apical meristems to assure growing. In addition to these proembryonic cell division centers, plants posses various manners (e.g., dormant meristemoids and residual stem cells) to acquire cell division capability in their bodies and, as a result, the daughter cells achieve an altered differentiation status there. The initiated growth and regeneration might also be based on newly formed dividing cells entering again into the cell cycle, and the different cell fate of unequal daughter cells is usually fundamental in the realization of altered development. In the latter case, a precondition of the subsequent events is dedifferentiation of mature, specialized cells and remodeling of their genetic program.

The authors results demonstrate that cells may attain disparate dedifferentiation statuses depending on their ancestry, and the initiated redifferentiation processes might be arrested even before entering cell divisions or continued up to complete root formation. Transfer cells of leaf veins share function in short-distance transport under normal conditions and stem cell formation under forced organogenesis. The *Rubia tinctorum* in vitro model system allowed us to detect the main symptoms of the rapid morphological changes during dedifferentiation. Modification of cell wall structure, enhanced autophagic activity, asymmetric plastid fission and unequal cell division seem to be basic steps of meristemoid (stem cell) formation. The theoretical background and the importance of the cytological characteristics mentioned above are also discussed.

Chapter VIII - To escape senescence and dying, cells endure continual proliferation, as only dividing cells can self-renew. In the course of evolution, asymmetric division was used by multicellular organisms to solve the problem of replicative aging of stem cells. There are data showing that aged tissue-specific stem cells retain their intrinsic proliferative potential and that age-related changes in the systemic environment and niche in which stem cells reside can modulate the molecular signaling pathways critical to the function of stem cells. In unicellular and multicellular organisms, asymmetric division enables segregation of damaged molecules (biological garbage) into one daughter cell. This appears to be an evolutionarily conserved mechanism preventing transmission of damaged proteins to the longest-lived daughter cell. Thus, dividing stem cells have a simple physiological mechanism to free themselves of toxic proteins destined for degradation during asymmetric cell division. The authors suggest that partition of damaged proteins and organelles and segregation of template DNA may function together to produce long-lived stem cells.

Chapter IX - Stem cells (SC) research is an important part of biotechnology studies that could lead to the development of new therapeutic strategies in different fields of medicine.

It is known that stem cells exist in early embryos as well as in some individual adult tissues. A lot of efforts have been made to understand biology of stem cells, particularly the features concerning their proliferation, self-renewal and differentiation abilities (the three major characteristics of SC) and subsequently to find out the role of the genes and proteins involved in these processes.

Research on stem cells is advancing knowledge on how healthy cells replace damaged cells in adult organisms. This promising area of science is also leading scientists to investigate the possibility of cell-based therapies to treat disease, which is often referred to as regenerative or reparative medicine.

Stem-cell biology is in a phase of dynamic expansion and is forming connections with a broad range of basic and applied disciplines. Stem cell research has offered the promise of effective cell-based therapies in treating many debilitating diseases such as diabetes, cardiovascular and neurodegenerative diseases, and cancer. In the last few years, the therapeutic potential of stem cells has inspired the imagination, intense interest, and targeted investment of scientists, clinicians, and the general public toward this area of biology.

Thus, in this chapter the authors would like to summarize recent advances in stem cell biology, as well as to discuss how clinical medicine could take advantage from this fascinating field of biology.

Chapter X - Chlorarachniophytes constitute a group of photosynthetic cercozoans (one of the ecologically significant protists) living in marine environments. They possess plastids of secondary endosymbiotic origin, which have a remnant nucleus of the green algal endosymbiont (nucleomorph) located in the periplastidial compartment, between the inner and the outer pairs of the plastid membranes. With respect to the plastid evolution, the chlorarachniophytes constitute therefore one of the most interesting unicellular algal groups. Since this algal group was found in 1930, little is known about their cell cycle as well as their taxonomy and geographical distribution. During the authors taxonomic studies of the chlorarachniophytes, an interesting difference in behavior between the daughter cells has been observed: once the cell has divided, one of the amoeboid daughter cells constricts till it is as narrow as one of its pseudopodia; then, the cytoplasm of this new cell flows in a thin trickle along the pseudopodium, collecting to form another cell body some distance from the stay-at-home nucleus. This recently-discovered phenomenon raises some questions about the mechanism and the evolution of the unique behavior. Here I describe in detail the daughter cells' behavior of the chlorarachniophytes, and discuss about the evolution and biological implications.