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The multicellular organism and cancer

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Introduction

Cancer has a lot to do with the way life has developed on our planet and the successful evolution of multicellular organisms. Cells are the smallest unit containing all the features necessary and sufficient to life, the viruses occupying a special place. In 1863, the German pathologist Rudolf Virchow introduced the concept of cellular pathology (Virchow, 1863) stating that diseases are due to the occurrence of a pathological process at cellular level. This is very much the case with cancer that is definitively a disease of a cell belonging to a multicellular organism.

A brief history of the cell: Eubacteria (Bacteria), Archaea, Eukaryotes, and the last unknown common ancestors

The defining moment for the appearance of the cell has been the formation of what we now call the cell membrane. This is a complex structure able to form vesicles allowing the segregation inside of genetic material (the Genotype) plus the molecular machinery (the Phenotype) needed for this new structure to grow and reproduce copies of itself, through the cell cycle.

Cells are divided into two taxons, Prokaryota and Eukaryota, a taxon being formed by organisms included in a particular entity (e.g. in a family or in a genus; (Thain and Hickman, 2004). This distinction is based on the structure and organization of the cell: in the Eukaryotes (Composite), cell membranes are present also inside the cells delimiting discrete internal structures such as, for example, nucleus and mitochondria, while no such division can be found in the prokaryotes (non-composite; Fig. 1.1). All the cells share a set of common features: they contain their genetic information, replicate throughout the cell cycle, their activity is governed through cell signalling and can produce energy through a metabolic apparatus. Approximately 200 gene families are common to the two taxons.

The introduction of genomic studies, as a tool to investigate the evolutionary correlations between organisms, has unveiled within the prokaryotes two distinct groups or domains, the Bacteria and the Archaea, as distant from one other as they are from the Eukaryotes. It has therefore been proposed that, above the division into animal Kingdoms, exists a division into three domains: the Eubacteria (or Bacteria), the Archaea, and the Eukaryotes (Woese et al., 1990), each domain comprising a variety of kingdoms (Fig. 1.2). Molecular studies have demonstrated that the two domains Bacteria and Archaea derive from the last unknown common ancestors (LUCA), while the Eukaryotes evolved from the Archaea (Fig. 1.2). LUCA is defined as the last organism preceding, in the evolutionary tree, the division into the two domains of Bacteria and Archaea and it is assumed to be the living organism from which all present living organisms descend. It is estimated that LUCA lived between some 3.5 to 3.8 billion years ago (Fig. 1.3).

The genetic division of cells into these three domains is reflected by their biological characteristics, some of which are summarized in Table 1.1. The mechanism of transcription, translation, and splicing in the Archaea is close to that of the Eukarya and both differ from the one found in the Prokaryota. Crucially, although the Archaea do not have a nucleus, they have histone proteins that bind to DNA double strand, compacting it into nucleosome-related structures, and Archaea RNA polymerases have the multisubunit complexity of Eukarya RNA polymerases. On the other side, the metabolism of the Archaea is more similar to Eubacteria than to Eukarya (Olsen and Woese, 1997). Despite the closer similarity in metabolic functions of Eubacteria to Archaea, there is one exception: the use of photosynthesis that can be found both in Eubacteria and Eukarya but is absent in Archaea. This is due to the fact that, although genetic evidences show that the Eukarya evolved from the Archaea, horizontal transfer of genes has happened between Eubacteria and Eukarya (Hedges, 2002).

Basic anatomy of the eukaryotic cell in Metazoa

In the cytological classification dividing the prokaryote from the eukaryotic cell the latter is distinguished as it is composed by several organelles, some possibly reminding a more primitive cell, which have learned to live in symbiosis. Each of these organelles contributes to specific need(s) of the eukaryotic cell. It is now believed that the crucial moment to the transition from a simpler cell to the more complex eukaryote was when different cells started to live inside others. Crucial to all this was the formation of the nucleus and the appearance of mitochondria. The main anatomical

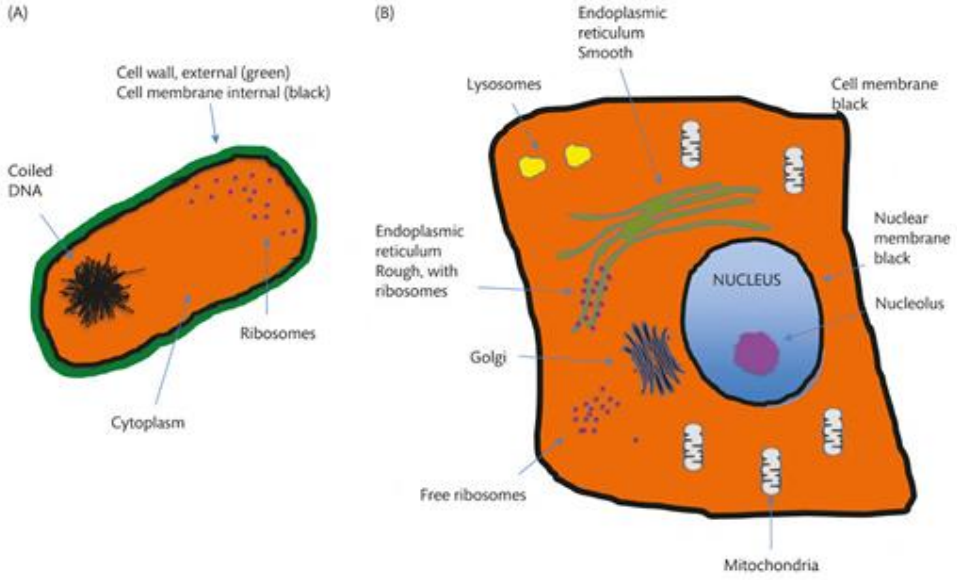

Fig. 1.1 The prokaryotic and the eukaryotic cells. (A) The prokaryotic cell is defined by the cell membrane. Inside the space delimitated by this membrane is the cytoplasm in which all the molecules are contained in one unique space. (B) The eukaryotic cell is also defined by the cell membrane, however the cell membrane is also present inside the cells where defines different organelles. The most prominent is the nucleus, in which the genetic material, the DNA, is segregated. Other cell membrane-defined organelles are the mitochondria, the Golgi apparatus, lysosomes, and the endoplasmic reticulum (ER). The former is divided into the ER rough, when ribosomes are attached to its membrane, and smooth, when ribosomes are not present.

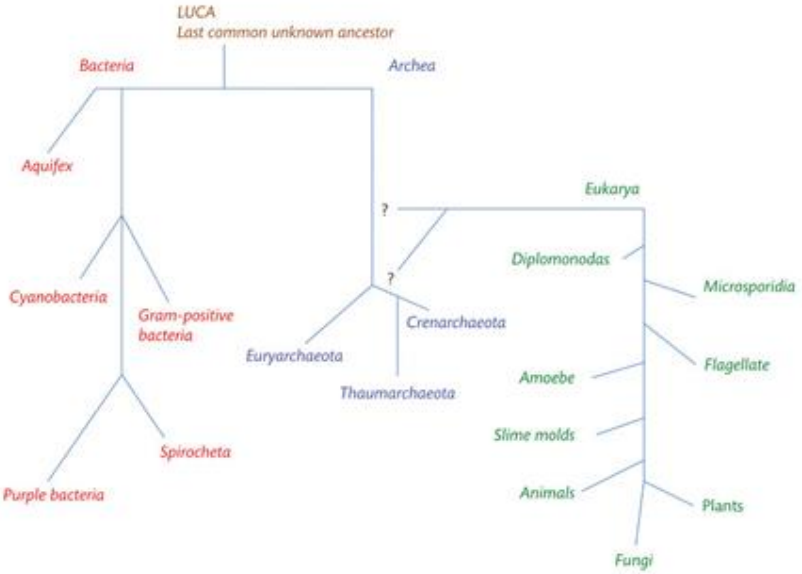

Fig. 1.2 The three domains: Bacteria, Archaea, and Eukaryota. The last unknown common ancestor (LUCA) evolved into the first two domains, Bacteria and Archaea, which, as far as the anatomical structure is concerned, are prokaryotic cells. Subsequently from the Archaea, the third domain evolved: the Eukaryota. Each of these three domains evolved into several kingdoms.

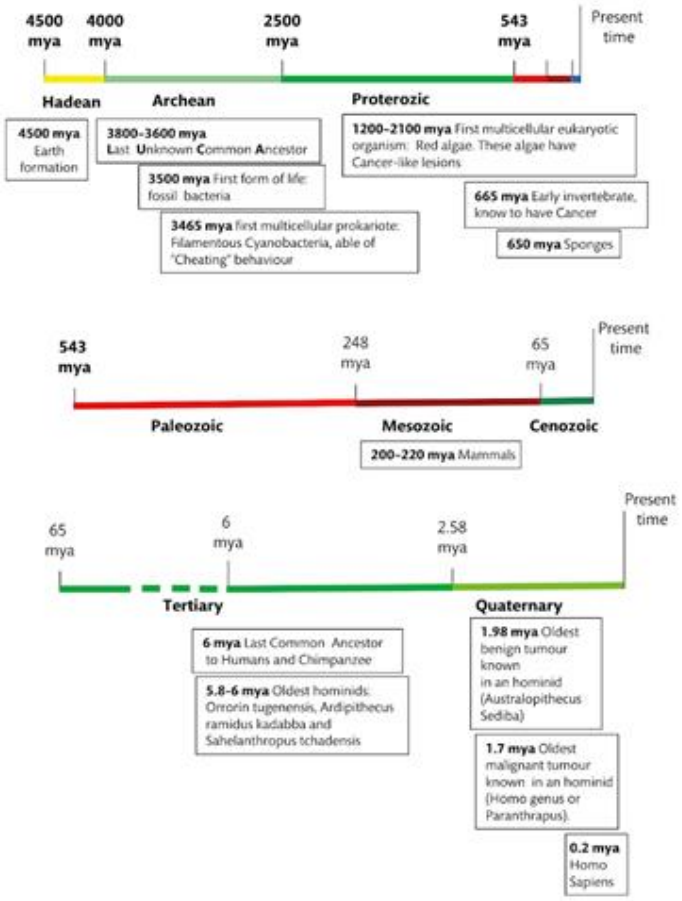

Fig. 1.3 Timeline. Mya, millions of years ago.

characteristics of the eukaryotic cells, when not dividing, are represented in Fig. 1.1.

The cell membrane

The cell membrane is a bilayer of phospholipids, each with a hydrophilic head and a hydrophobic tail. In aqueous environments, the phospholipids spontaneously organize themselves as a double layer with the hydrophobic tails inside and the head outside, so originating the cell bio membrane (Fig. 1.4). In a cell, the membranous network is divided into two main components: the cell surface membrane, the plasma membrane delimiting the actual cell and representing the border with the extracellular world, and those membranes delimiting the internal cellular compartments. In the plasma membrane within the scaffolding formed by the phospholipid bilayer are numerous different structures. These are made by proteins, 500 (five hundred) types of lipid molecules and 10,000 (ten thousand) proteins are involved in the making of the cell membrane. The main structures formed by intramembranous proteins are channels (e.g. ion pumps) and receptors (e.g. epidermal growth factor receptor). Some molecules however, like oxygen, can diffuse through the membrane without needing a specific pump.

The plasma membrane is a highly dynamic fluid structure and all the protein complexes are 'floating' wtihin it and also the very same lipid molecules are continuing moving within the membrane. Groups of lipids can also form units called 'rafts', which move among the other lipids. This dynamic nature of the plasma membrane was firstly described in 1972 as the fluid mosaic model (Singer and Nicolson, 1972; Edidin, 2003). The external cell membrane is in continuity with the internal membranes that not only defines the internal organelles of the cells, but also provides a framework for countless biochemical reactions and trafficking of molecules.

Table 1.1 Comparison of the main biological characteristics of Eubacteria, Archaea, and Eukaryota

	Eubacteria	Archaea	Eukaryota
Cell membrane	Yes	Yes	Yes
Transcription and translation	Yes	Yes	Yes
Signal transduction	Yes	Yes	Yes
Epigenetic change	Yes	Yes	Yes
Protein chaperons	Yes	Yes	Yes
Nucleus	No	No	Yes
Cytoskeleton	No	No	Yes
Organelles	No	No	Yes
DNA	Circular	Circular	Linear
Operons	Yes	Yes	No
Ribosome	70 s	70 s	80 s
Grow above 80°C	Yes	Yes	No
Number of genes	1,000–6,000	1,000–6,000	6,000–50,000
Operons	Yes	Yes	No
Multicellularity	No	No	Yes
Introns	No	Yes	Yes
Histone proteins	No	Yes	Yes
DNA-dependent RNA polymerase	Simple subunit	Complex subunit	Complex subunit
tRNA initiator	Formylmethionine	Methionine	Methionine
Transcription factors	Yes	No	Yes
Spore formation	Yes	No	No
Photosynthesis	Yes	No	Yes

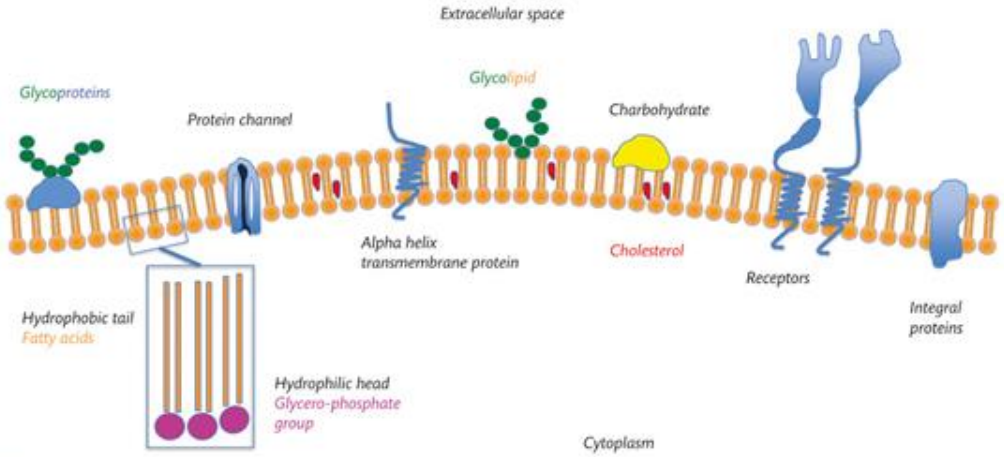

Fig. 1.4 The cell membrane. The cell membrane is made up by phospholipid molecules with a hydrophilic head (orange) and a hydrophobic tail (yellow). Within the membrane are several different structures that can 'float' across the membrane, which has fluid property. Transmembrane proteins span all thickness of the membrane and the main types are the protein channels, the integral protein, and the alpha-helix proteins. Glycoproteins and carbohydrates are present on the external surface.

The two major compartments inside the cells are the nucleus and the cytoplasm; the latter includes all the intracellular volume which is not nucleus.

The cytoplasm

The cytoplasm is occupied by cytosol, an aqueous medium rich in proteins and salts accounting for approximately 50% of the cytoplasm. The main site of protein synthesis and degradation and of intermediate metabolism, forms the cytosol that permeates all the organelles. The main reason for different organelles is the need for keeping apart different biochemical reactions. A single membrane delimits all the organelles, with the only exceptions being the mitochondria and the nucleus, which have an outer and one inner membrane.

Mitochondria are organelles formed by an external and one internal membrane filled with matrix. It is where the oxidative phosphorylation (i.e. respiration), occurs and where adenosine triphosphate (ATP) is produced. ATP is the source of energy for all cellular functions: such an energy is liberated when an ATP molecule is hydrolysed producing adenosine diphosphate (ADP), phosphate, and energy. The endoplasmic reticulum, or ER, forms the major cytoplasmic network, most of which is Rough ER where ribosomes are located and protein synthesis occurs. The remaining one is called the Smooth ER.

Another prominent function of the ER is lipid synthesis. The Golgi apparatus is another system of cisterns where simpler molecules are packaged into more complex ones: it is also where lysosomes are built. The lysosomes are spherical membrane vesicles containing a wide range of hydrolytic enzymes able to degrade many molecules and their purpose is to eliminate any damaged or unwanted molecules. Such molecules are transported to the lysosome by specialized vesicles called endosomes. Peroxisomes are instead involved in several metabolic and catabolic functions. The most important are catabolism of very long chain fatty acids into branched chain fatty acids, D-amino acids, and polyamines with reduction of reactive oxygen species. They are also the place where phospholipids are synthesized and the pentose phosphate pathway, critical for the energy metabolism, is located. Finally, free ribosomes are also present within the cytoplasmic matrix.

The nucleus

The nucleus is the largest organelle. A double bilayer membrane, the nuclear membrane or nuclear envelope, in which numerous pores (nuclear pore complexes) are present, thus allowing communication with the cytoplasm, which delimits it. Within the nucleus is the genome (with only the exception of mitochondrial DNA) and its transcriptional machinery. It can be divided into two main structures: the nuclear membrane and the nuclear interior (Lammerding, 2011).

Between the two layers of the nuclear membrane is the perinuclear space. Under the nuclear membrane is the nuclear lamina, mainly made up by laminin filaments. This membrane is perforated by the nuclear pore complexes which cause the inner and outer membranes to fuse. Nuclear pore are large complexes made of approximately 50 nucleoporins and regulate the trafficking between nucleus and cytoplasm. Nuclear pore complexes are not the only protein structure within the nuclear membrane, with some spanning the whole thickness (Lammerding, 2011).

When not dividing, approximately half of the nuclear volume is occupied by chromatin made of the unfolded DNA packed around histone proteins. There are two types of chromatin: heterochromatin, more packed and less transcriptionally active, and euchromatin, which is not so condensed and in which most of the transcription occurs (Lammerding, 2011). The aggregates of DNA and histones form structures called nucleosomes: packaged DNA from different chromosomes occupy distinct areas in the non-dividing nucleus. Nucleoli are discrete bodies formed by proteins and nucleic acids and are the production site of the ribosome. Cajal bodies are located in the proximity of the nucleoli and contain different formations: for example, the snurposome and spliceosome are involved in the processing of the recently transcribed mRNA. Finally, there is the nucleoskeleton, a protein scaffolding supporting the different nuclear components. All these structures are immersed in the nucleoplasm, a very protein rich aqueous medium equivalent to the cytosol.

The life of the single cell

All the unicellular organisms tend to grow without limitation according to the availability of resources. The life cycle of each of these organisms therefore coincides with the time required to duplicate (i.e. to complete the cell cycle), the cell cycle being a complex of events that brings one cell to divide into two. Prokaryotes do not age: their life cycle is very simple. They die when conditions became adverse and food is scanty, although this is not always the case: in determinate conditions, some cells can become 'dormant' and resume growth when the environment becomes permissive again. In most eukaryotic cells ageing does appear: their lifespan is regulated by an internal clock made up by telomeres and telomerase. The main physiological events in the life cycle of a single cell are reproduction through mitosis, response to damage, cell death, and movement. Cells divide through mitosis, a process in which the genetic code is duplicated and then the cells divide into equal new cells. As cells are exposed to external insults, either chemical or physical, repair mechanisms are present that can block the further division until the necessary corrections are made. Should the repairs fail, the cells can trigger their own death through apoptosis. Apoptosis does not only follow damage within a multicellular organism but can also be triggered at appropriate moments during the organism's development or life.

Multicellular organisms and the development of cancer

During the evolution of life, multicellularity has appeared independently at several different times, exploiting different strategies (Kaiser, 2001). There are therefore several mechanisms leading to the formation of multicellular organisms. For example, while plants relied on the formation of a rigid cell wall which brings different cells into one organism, the animal cells, which do not have cell walls, had to rely on membrane proteins called adhesion molecules to provide a mechanism allowing the cells to stick to each other (Bonner, 1998).

When confronted by an aggregate of cells, the first issue is how to differentiate a multicellular organism from a colony. The most commonly used, and the broadest criteria, is the existence of a spatial division of work in multicellular organisms, compared

to the colony in which each unicellular member performs the same tasks. According to this definition, the oldest known unambiguous multicellular organisms belong to the Bacteria domain and are the filamentous cyanobacteria. These emerged, as suggested by fossil dating, 3,465 million years ago (mya), approximately 1,000 million years after the Earth's formation, which is estimated at 4,500 mya (Fig. 1.3).

Cyanobacteria were the first organisms to develop photosynthesis and release oxygen. However, these bacteria also rely on the enzyme nitrogenase to convert nitrogen gas into ammonia, necessary to build their proteins and other essential structural components when combined nitrogen (i.e. reactive molecules containing nitrogen), like nitrate, nitrite, ammonium, urea, and amino acids, are not available (Fig. 1.5). However, nitrogenase is irreversibly destroyed in the presence of oxygen and therefore cyanobacteria had to find a way to be able to perform oxygen-producing photosynthesis and, at the same time, to maintain nitrogenase function. This problem has been solved by multicellularity: formation of filaments, made up by a line of cyanobacteria in which two differently evolved cyanobacteria can be found. Those containing chlorophyll, performing photosynthesis, and releasing oxygen, are more numerous. These differentiated into a form called heterocysts, in which nitrogenase function is maintained in the absence of photosynthesis, as they do not contain chlorophyll (Fig. 1.3). Therefore, a simple prokaryotic multicellular organism containing two types of cells was formed (Bonner, 1998; Adams, 2000; Flores and Herrero, 2010).

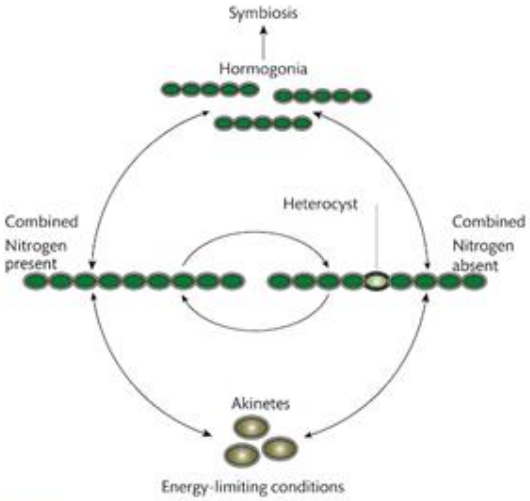

Fig. 1.5 The filamentous cyanobacteria. Cyanobacteria exists more commonly as vegetative form but can differentiate into three further forms: heterocyst, akinetes, and hormogonial cells. In absence of 'combined nitrogen', like nitrate, nitrite, ammonium, urea, and amino acids which easily react and combine with other molecules and can be used for protein production, cyanobacteria needs to 'fixate' the poorly reactive nitrogen and transform it into the more reactive ammonia according to the following reaction $N_2 + 8\,H^+ + 8\,e^- \rightarrow 2\,NH_3 + H_2$ catalysed by a nitrogenase enzyme. Vegetative cells cannot do that as they produce oxygen which is toxic for the nitrogenase enzyme. Therefore, some vegetative cells differentiate into heterocysts, cells that do not produce oxygen, but are able to fixate N_2 into ammonia in response to deprivation of combined nitrogen. Subsequently, the filamentous cyanobacteria acquires its new structure, characterized by a number of vegetative cells regularly interrupted by one heterocyst. When nutrients and energy are scanty some vegetative cells differentiate into akinetes, which can than start to proliferate again and produce vegetative cells when nutrients became available again. Vegetative cells from some filamentous cyanobacteria can also differentiate into hormogonia, which are than dispersed and can subsequently originate new filamentous cyanobacteria growing in symbiosis with plants.

The time of the emergence of eukaryotic multicellular organisms as assessed today is still a broad estimate, possibly sometime between 2,100 (Donoghue and Antcliffe, 2010) and 1,200 (Rokas, 2008) mya. Red algae are so far considered as the first eukaryotic multicellular organisms, appearing 1,200 mya (Fig. 1.3). The main strategies employed by eukaryotic cells to build a multicellular entity include: lack of cell separation after mitosis; mostly found in aquatic organisms; and aggregation of single cells prevalent in terrestrial creatures. A final fundamental characteristic in the classification of multicellular organisms is complexity. The easiest and most practical approach to 'measure' complexity is the number of cells making up the organism (Rokas, 2008).

Hallmarks of multicellularity

Multicellularity required the acquisition of functions not present or diversely utilized in single cell organisms. Up to seven hallmarks of multicellularity have been described (Rokas, 2008; Srivastava et al., 2010; Aktipis et al., 2015).

Regulation and control of the cell cycle

A strict control of proliferation is essential to the development and survival of a multicellular organism. For the organism to maintain itself, proliferation can occur only in well-defined circumstances and is regulated by a series of positive signals, inducing it, and suppressive signals, blocking it. Furthermore, these control rules are different from tissue to tissue (e.g. the neurons do not enter proliferation ever), while, on the other extreme, bone marrow stem cells are continuously proliferating to provide new blood cells, which have a very high turnover. To guarantee this strict control, redundant mechanisms are present.

Apoptosis (programmed cell death)

While unicellular organisms just proliferate, within multicellular organisms remodelling takes place, mostly during development when some embryonic structures are temporary and need to be eliminated as the fetus develops. Apoptosis is also required in adulthood (e.g. after immune stimulations, only some of the immune cells specifically responding will survive; the others, responding in a non-specific way, will undergo apoptosis and disappear). This is possible thanks to the appearance of apoptosis, or programmed cell death, which causes, when necessary, the death of selected cells according to the organism's blueprint.

The interaction with the extracellular environment

The extracellular matrix is essential for cells to maintain their physiological functions. Furthermore, it is where cells come into