CHAPTER 1

1 Haemopoiesis

This first chapter is concerned with the general aspects of blood cell formation (haemopoiesis). The processes that regulate haemopoiesis and the early stages of formation of red cells (erythropoiesis), granulocytes and monocytes (myelopoiesis) and platelets (thrombopoiesis) are also discussed.

Site of haemopoiesis

In the first few weeks of gestation the yolk sac is the main site of haemopoiesis. However, definitive haemopoiesis derives from a population of stem cells first observed on the dorsal aorta termed the AGM (aorta-gonads-mesonephros) region. These common precursor of endothelial and haemopoietic cells (haemangloblasts) are believed to seed the liver, spleen and bone marrow and from 6 weeks until 6–7 months of fetal life the liver and spleen are the major haemopoietic organs and continue to produce blood cells until about 2 weeks after birth (Table 1.1) (see Fig. 6.1b). The bone marrow is the most important site from 6 to 7 months of fetal life. During normal childhood and adult life the marrow is the only source of new blood cells. The developing cells are situated outside the bone marrow sinuses and mature cells are released into the sinus spaces, the marrow microcirculation and so into the general circulation.

Table 1.1 Sites of haemopoiesis.

Fetus	0–2 months (yolk sac) 2–7 months (liver, spleen) 5–9 months (bone marrow)
Infants	Bone marrow (practically all bones)
Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

In infancy all the bone marrow is haemopoietic but during childhood there is progressive fatty replacement of marrow throughout the long bones so that in adult life haemopoietic marrow is confined to the central skeleton and proximal ends of the femurs and humeri (Table 1.1). Even in these haemopoietic areas, approximately 50% of the marrow consists of fat (Fig. 1.1). The remaining fatty marrow is capable of reversion to haemopoiesis and in many diseases there is also expansion of haemopoiesis down the long bones. Moreover, the liver and spleen can resume their fetal haemopoietic role ('extramedullary haemopoiesis').

Haemopoietic stem and progenitor cells

Haemopoiesis starts with a pluripotential stem cell that can give rise to the separate cell lineages.

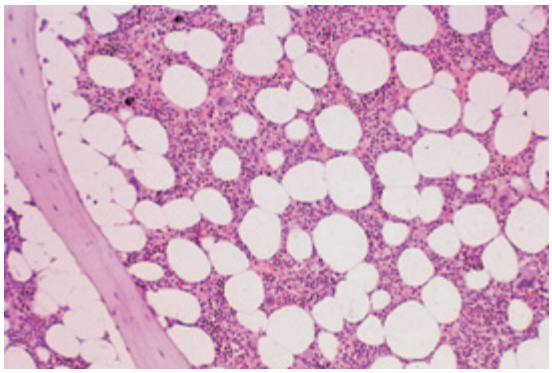

Fig. 1.1 A normal bone marrow trephine biopsy (posterior iliac crest). Haematoxylin and eosin stain; approximately 50% of the intertrabecular tissue is haemopoietic tissue and 50% is fat.

This ***haemopoietic stem cell*** is rare, perhaps 1 in every 20 million nucleated cells in bone marrow. Although its exact phenotype is unknown, on immunological testing it is $CD34^+$ $CD38^-$ and has the appearance of a small or medium-sized lymphocyte (Fig. 21.3). Cell differentiation occurs from the stem cell via the committed ***haemopoietic progenitors*** which are restricted in their developmental potential (Fig. 1.2). The existence of the separate progenitor cells can be demonstrated by *in vitro* culture techniques. Very early progenitors are assayed by culture on bone marrow stroma as long-term culture initiating cells whereas late progenitors are generally assayed in semi-solid media. An example is the earliest detectable mixed myeloid precursor which gives rise to granulocytes, erythrocytes, monocytes and megakaryocytes and is termed

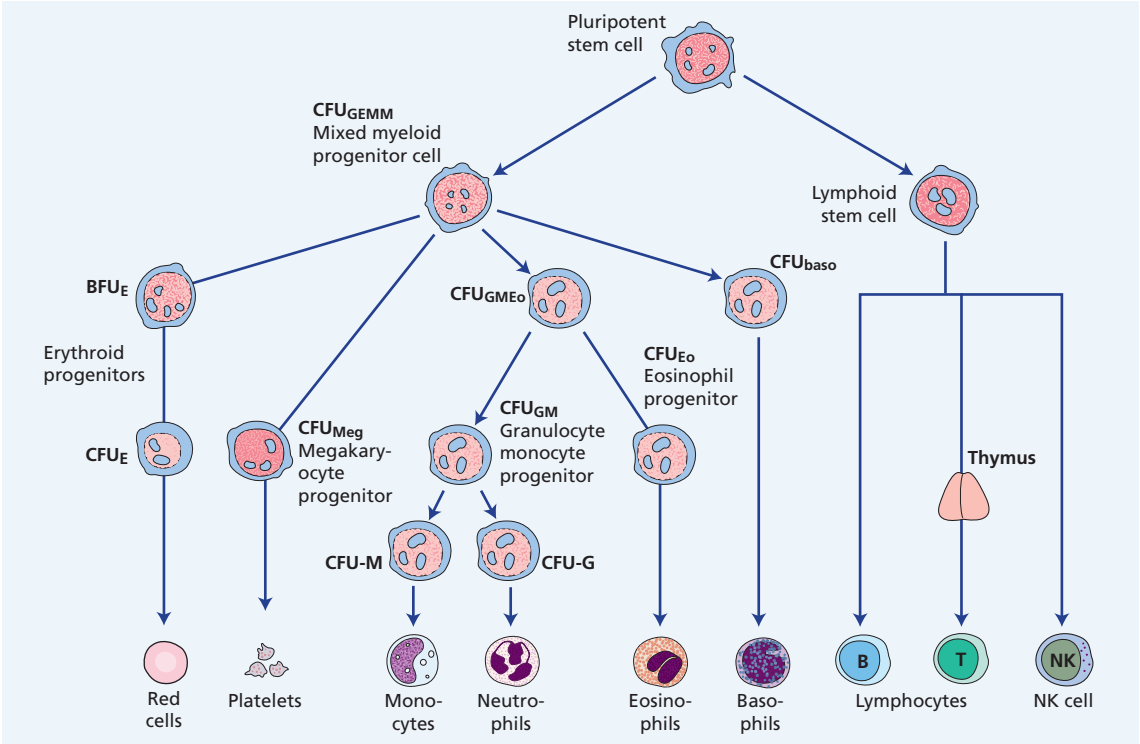

Fig. 1.2 Diagrammatic representation of the bone marrow pluripotent stem cell and the cell lines that arise from it. Various progenitor cells can be identified by culture in semi-solid medium by the type of colony they form. Baso, basophil; BFU, burst-forming unit; CFU, colony-forming unit; E, erythroid; Eo, eosinophil; GEMM, granulocyte, erythroid, monocyte and megakaryocyte; GM, granulocyte, monocyte; Meg, megakaryocyte; NK, natural killer.

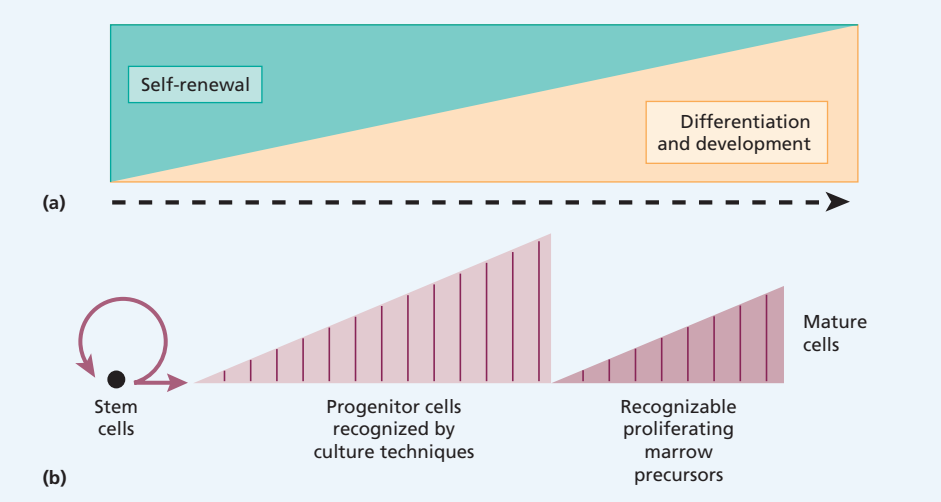

Fig. 1.3 **(a)** Bone marrow cells are increasingly differentiated and lose the capacity for self-renewal as they mature. **(b)** A single stem cell gives rise, after multiple cell divisions (shown by vertical lines), to >10^6 mature cells.

CFU (colony-forming unit)-GEMM (Fig. 1.2). The bone marrow is also the primary site of origin of lymphocytes (Chapter 8) which differentiate from a common lymphoid precursor.

The stem cell has the capability for ***self-renewal*** (Fig. 1.3) so that marrow cellularity remains constant in a normal healthy steady state. There is considerable amplification in the system: one stem cell is capable of producing about 10^6 mature blood cells after 20 cell divisions (Fig. 1.3). The precursor cells are, however, capable of responding to haemopoietic growth factors with increased production of one or other cell line when the need arises. The development of the ***mature cells*** (red cells, granulocytes, monocytes, megakaryocytes and lymphocytes) is considered further in other sections of this book.

Bone marrow stroma

The bone marrow forms a suitable environment for stem cell survival, growth and development. It is composed of stromal cells and a microvascular network (Fig. 1.4). The stromal cells include adipocytes, fibroblasts, endothelial cells and macrophages and

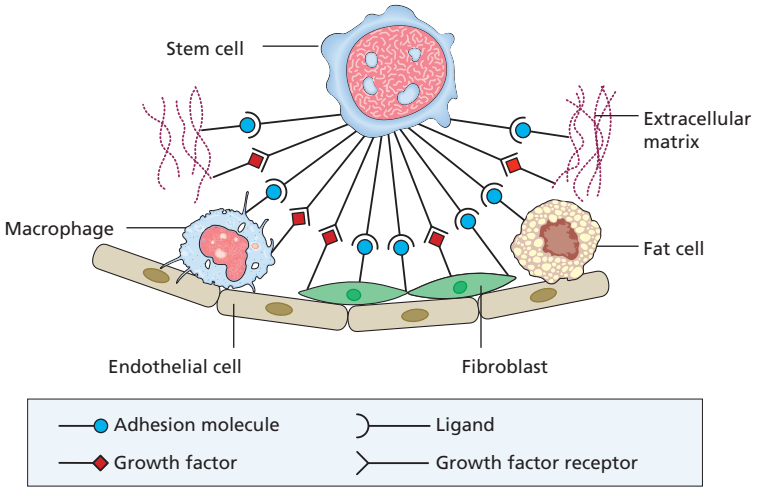

Fig. 1.4 Haemopoiesis occurs in a suitable microenvironment provided by a stromal matrix on which stem cells grow and divide. There are probably specific recognition and adhesion sites (p. 11); extracellular glycoproteins and other compounds are involved in the binding.

they secrete extracellular molecules such as collagen, glycoproteins (fibronectin and thrombospondin) and glycosaminoglycans (hyaluronic acid and chondroitin derivatives) to form an extracellular matrix. In addition, stromal cells secrete several growth factors necessary for stem cell survival. ***Mesenchymal stem cells*** are thought to be critical in stromal cell formation.

Stem cells are able to traffic around the body and are found in peripheral blood in low numbers. In order to exit the bone marrow, cells must cross the blood vessel endothelium and this process of ***mobilization*** is enhanced by administration of cytokines such as granulocyte colony-stimulating factor (G-CSF) or granulocyte–macrophage colony-stimulating factor (GM-CSF) (p. 97). The reverse process of stem cell ***homing*** appears to depend on a chemokine gradient in which the stromal-derived factor (SDF-1) is critical. Several critical interactions maintain stem cell viability and production in the stroma including stem cell factor (SCF) and Jagged proteins expressed on stroma and their respective receptors c-Kit and Notch expressed on stem cell.

Stem cell plasticity

There is some evidence that adult stem cells in different organs are ***pluripotent*** and can generate various types of tissue (Fig. 1.5). Studies in patients and animals who have received haemopoietic stem cell transplants (Chapter 21) have suggested that donor cells may contribute to tissues such as neurons, liver and muscle. The contribution of adult donor bone marrow cells to non-haemopoietic tissues is at most small. The persistence of pluripotential stem cells in postnatal life, organ-specific stem cells and fusion of transplanted cells with host cells

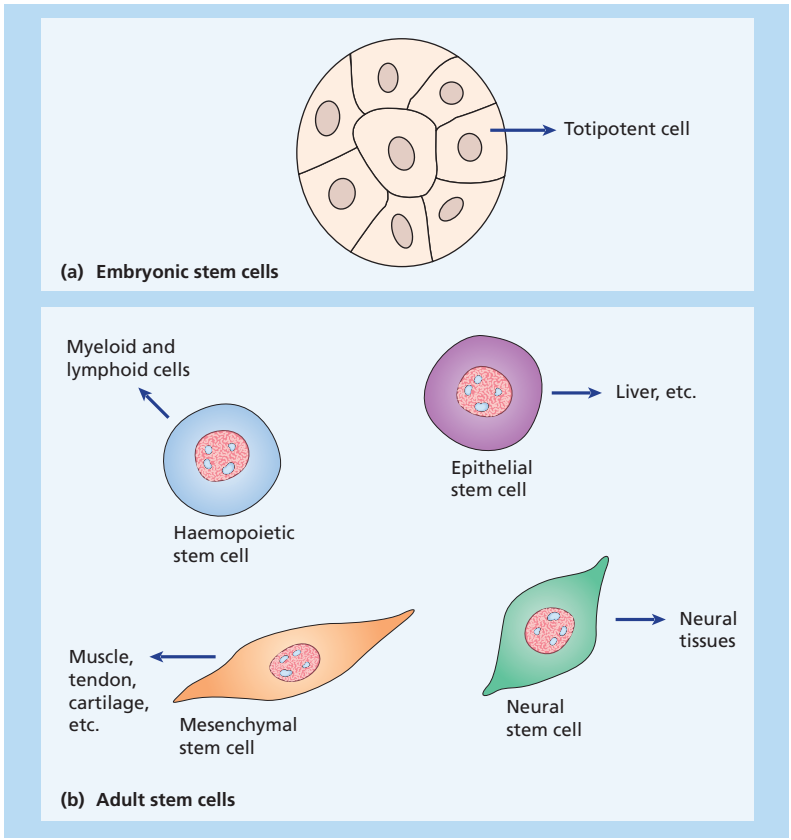

Fig. 1.5 **(a)** Cells in the early embryo are able to generate all the tissues of the body and are known as totipotent. **(b)** Specialized adult stem cells of the bone marrow, nervous tissue, epithelial and other tissues give rise to differentiated cells of the same tissue and possibly to other tissues (see text).

have all been proposed, however, to explain many of the findings suggesting stem cell plasticity.

The regulation of haemopoiesis

Haemopoiesis starts with stem cell division in which one cell replaces the stem cell (***self-renewal***) and the other is committed to differentiation. These early committed progenitors express low levels of transcription factors that may commit them to discrete cell lineages. Which cell lineage is selected for differentiation may depend both on chance and on the external signals received by progenitor cells. Several transcription factors have been isolated that regulate differentiation along the major cell lineages. For instance, PU.1 commits cells to the myeloid lineage whereas GATA-1 has an essential role in erythropoietic and megakaryocytic differentiation.

Haemopoietic growth factors

The haemopoietic growth factors are glycoprotein hormones that regulate the proliferation and differentiation of haemopoietic progenitor cells and the function of mature blood cells. They may act locally at the site where they are produced by cell–cell contact or circulate in plasma. They also bind to the extracellular matrix to form niches to which stem and progenitor cells adhere. The growth factors may cause cell proliferation but can also stimulate differentiation, maturation, prevent apoptosis and affect the function of mature cells (Fig. 1.6).

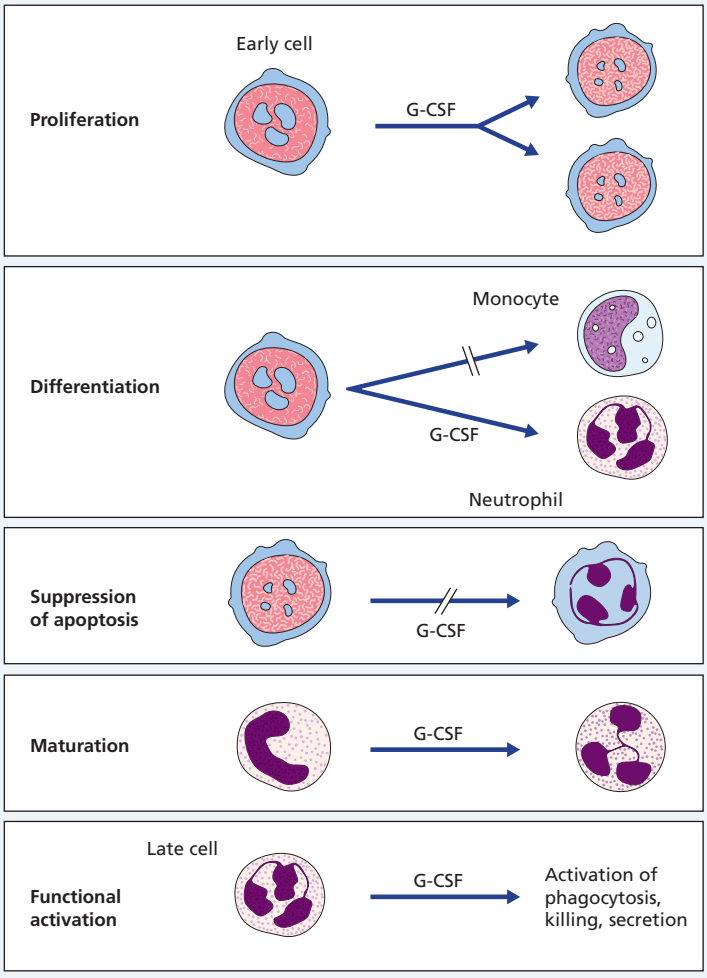

Fig. 1.6 Growth factors may stimulate proliferation of early bone marrow cells, direct differentiation to one or other cell type, stimulate cell maturation, suppress apoptosis or affect the function of mature non-dividing cells, as illustrated here for granulocyte colony-stimulating factor (G-CSF) for an early myeloid progenitor and a neutrophil.

Table 1.2 General characteristics of myeloid and lymphoid growth factors.

Glycoproteins that act at very low concentrations
Act hierarchically
Usually produced by many cell types
Usually affect more than one lineage
Usually active on stem/progenitor cells and on functional end cells
Usually show synergistic or additive interactions with other growth factors
Often act on the neoplastic equivalent of a normal cell
Multiple actions: proliferation, differentiation, maturation, functional activation, prevention of apoptosis of progenitor cells

Table 1.3 Haemopoietic growth factors.

Act on stromal cells
IL-1
TNF
Act on pluripotential stem cells
SCF
Flt-L
Act on multipotential progenitor cells
IL-3
GM-CSF
IL-6
G-CSF
Thrombopoietin
Act on committed progenitor cells
G-CSF*
M-CSF
IL-5 (eosinophil-CSF)
Erythropoietin
Thrombopoietin*

Flt-L, Flt ligand; G- and GM-CSF, granulocyte and granulocyte–macrophage colony-stimulating factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; SCF, stem cell factor; TNF, tumour necrosis factor.
* These also act synergistically with early acting factors on pluripotential progenitors.

They share a number of common properties (Table 1.2) and act at different stages of haemopoiesis (Table 1.3; Fig. 1.7). Stromal cells are the major source of growth factors except for erythropoietin, 90% of which is synthesized in the kidney, and thrombopoietin, made largely in the liver. An important feature of growth factor action is that two or more factors may synergize in stimulating a particular cell to proliferate or differentiate. Moreover, the action of one growth factor on a cell may stimulate production of another growth factor or growth factor receptor. SCF and Flt ligand (Flt-L) act locally on the pluripotential stem cells and on early myeloid and lymphoid progenitors (Fig. 1.7). Interleukin 3 (IL-3) and GM-CSF are multipotential growth factors with overlapping activities. G-CSF and thrombopoietin enhance the effects of SCF, Flt-L, IL-3 and GM-CSF on survival and differentiation of the early haemopoietic cells.

These factors maintain a pool of haemopoietic stem and progenitor cells on which later acting factors erythropoietin, G-CSF, M-CSF, IL-5 and thrombopoietin act to increase production of one or other cell lineage in response to the body's need. Granulocyte and monocyte formation, for example, can be stimulated by infection or inflammation through release of IL-1 and tumour necrosis factor (TNF) which then stimulate stromal cells to produce growth factors in an interacting network (Fig. 7.4). In contrast, cytokines such as transforming growth factor-β (TGF-β) and γ-interferon (IFN-γ) can exert a negative effect on haemopoiesis and may have a role in the development of aplastic anaemia (p. 244).

Growth factor receptors and signal transduction

The biological effects of growth factors are mediated through specific receptors on target cells. Many receptors (e.g. erythropoietin (epo) receptor (R), GM-CSF-R) are from the ***haematopoietin receptor superfamily*** which dimerize after binding their ligand.

Dimerization of the receptor leads to activation of a complex series of intracellular signal transduction pathways of which the three major ones are the JAK/STAT, the mitogen activated protein (MAP) kinase and the phosphatidylinositol 3 (PI3) kinase pathways (Figs 1.8, 19.2). The Janus associated kinase (JAK) proteins are a family of four tyrosine-specific protein kinases that associate with the intracellular domains of the growth factor receptors (Fig. 1.8). A growth factor molecule binds simultaneously to the extracellular domains of two or three receptor

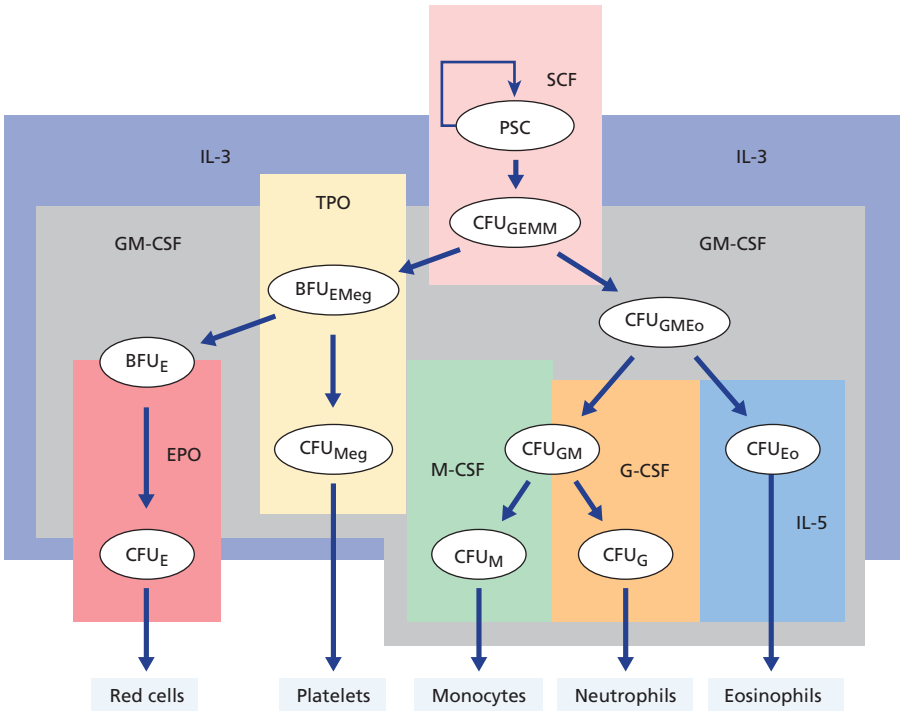

Fig. 1.7 A diagram of the role of growth factors in normal haemopoiesis. Multiple growth factors act on the earlier marrow stem and progenitor cells. EPO, erythropoietin; PSC, pluripotential stem cell; SCF, stem cell factor; TPO, thrombopoietin. For other abbreviations see Fig. 1.2.

molecules, resulting in their aggregation. Receptor aggregation induces activation of the JAKs which now phosphorylate members of the signal transducer and activator of transcription (STAT) family of transcription factors. This results in their dimerization and translocation from the cell cytoplasm across the nuclear membrane to the cell nucleus. Within the nucleus STAT dimers activate transcription of specific genes. A model for control of gene expression by a transcription factor is shown in Fig. 1.9. The clinical importance of this pathway is revealed by the finding of an activating mutation of the *JAK2* gene as the cause of polycythaemia rubra vera (p. 230).

JAK can also activate the MAPK pathway which is regulated by Ras and controls proliferation. PI3 kinases phophorylate inositol lipids which have a wide range of downstream effects including activation of AKT (protein kinase) B leading to block of apoptosis and other actions (Fig. 1.8, 19.2). Different domains of the intracellular receptor protein may signal for the different processes (e.g. proliferation or suppression of apoptosis) mediated by growth factors.

A second smaller group of growth factors, including SCF, Flt-3L and macrophage colony-stimulating factor (M-CSF) (Table 1.3), bind to receptors that have an extracellular immunoglobulin-like domain linked via a transmembrane bridge to a cytoplasmic tyrosine kinase domain. Growth factor binding results in dimerization of these receptors and consequent activation of the tyrosine kinase domain. Phosphorylation of tyrosine residues in the receptor itself generates binding sites for signalling proteins which initiate complex cascades of biochemical events resulting in changes in gene expression, cell proliferation and prevention of apoptosis.

The cell cycle

The cell division cycle, generally known simply as the ***cell cycle***, is a complex process that lies at

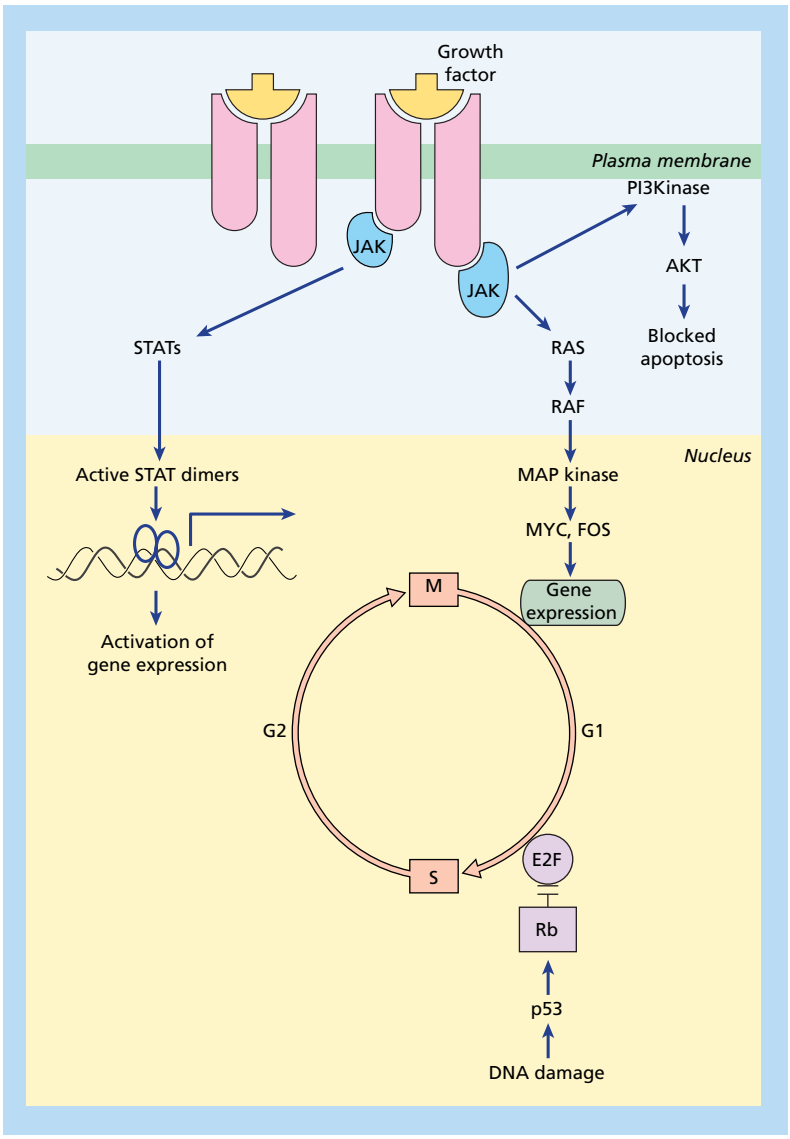

Fig. 1.8 Control of haemopoiesis by growth factors. The factors act on cells expressing the corresponding receptors. Binding of a growth factor to its receptor activates the JAK/STAT, MAPK and hosphatidyl-inositol3-kinase (PI3K) pathways (Fig. 19.2) which leads to transcriptional activation of specific genes. E2F is a transcription factor needed for cell transition from G1 to S phase. E2F is inhibited by the tumour suppressor gene *Rb* (retinoblastoma) which can be indirectly activated by p53. The synthesis and degradation of different cyclins (not shown) stimulates the cell to pass through the different phases of the cell cycle. The growth factors may also suppress apoptosis by activating protein kinase B.

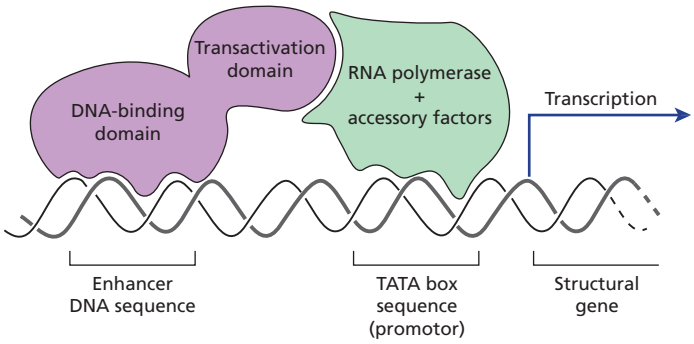

Fig. 1.9 Model for control of gene expression by a transcription factor. The DNA-binding domain of a transcription factor binds a specific enhancer sequence adjacent to a structural gene. The transactivation domain then binds a molecule of RNA polymerase, thus augmenting its binding to the TATA box. The RNA polymerase now initiates transcription of the structural gene to form mRNA. Translation of the mRNA by the ribosomes generates the protein encoded by the gene.

the heart of haemopoiesis. Dysregulation of cell proliferation is also the key to the development of malignant disease. The duration of the cell cycle is variable between different tissues but the basic principles remain constant. The cycle is divided in to the mitotic phase (***M phase***), during which the cell physically divides, and ***interphase*** during which the chromosomes are duplicated and cell growth occurs prior to division (Fig. 1.10). The M phase is further partitioned into classical ***mitosis*** in which nuclear division is accomplished, and ***cytokinesis*** in which cell fission occurs.

Interphase is divided into three main stages: a ***G_1 phase*** in which the cell begins to commit to replication, an ***S phase*** during which DNA content doubles (Fig. 1.10b) and the chromosomes replicate and the ***G_2 phase*** in which the cell organelles are copied and cytoplasmic volume is increased. If cells rest prior to division they enter a G_0 state where they can remain for long periods of time. The number of cells at each stage of the cell cycle can be assessed by exposing cells to a chemical or radiolabel that gets incorporated into newly generated DNA or by flow cytometry.

The cell cycle is controlled by two ***checkpoints*** which act as brakes to coordinate the division process at the end of the G_1 and G_2 phases. Two major classes of molecules control these checkpoints, ***cyclin dependent protein kinases*** (Cdk) which phosophorylate downstream protein targets and ***cyclins*** which bind to Cdks and regulate their activity. An example of the importance of these systems is demonstrated by mantle cell lymphoma which results from the constitutive activation of cyclin D1 as a result of a chromosomal translocation (p. 212).

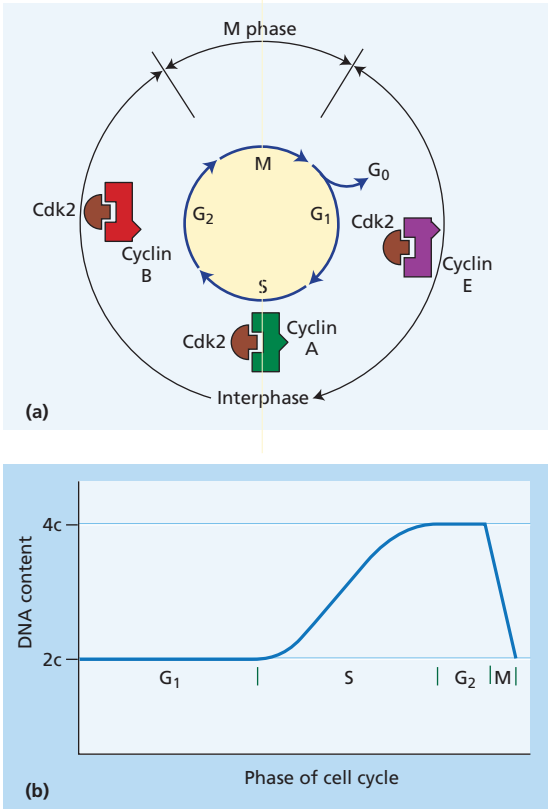

Fig. 1.10 **(a)** The stages of the cell cycle. Progression through cell cycle is regulated by specific combinations of cyclin dependent protein kinase (Cdk) and cyclin proteins. The synthesis and degradation of different cyclins stimulates the cell to pass through the different phases of the cell cycle although the exact role of each heterodimer is currently uncertain. **(b)** Relationship between the DNA content of a cell expressed in arbitrary units as 2c increasing to 4c and its position in the cell cycle. (Adapted from Wickramasinghe S.N. (1975) *Human Bone Marrow*, Blackwell Scientific, Oxford, p. 13.)

Apoptosis

Apoptosis is a regulated process of physiological cell death in which cells are triggered to activate intracellular proteins that lead to the death of the cell. Morphologically it is characterized by cell shrinkage, condensation of the nuclear chromatin, fragmentation of the nucleus and cleavage of DNA at internucleosomal sites. It is an important process for maintaining tissue homoeostasis in haemopoiesis and lymphocyte development.

Apoptosis results from the action of intracellular cysteine proteases called ***caspases*** which are activated following cleavage and lead to endonuclease digestion of DNA and disintegration of the cell skeleton (Fig. 1.11). There are two major pathways by which caspases can be activated. The first is by signalling through membrane proteins such as Fas or TNF receptor via their intracellular death domain. An example of this mechanism is shown by activated cytotoxic T cells expressing Fas ligand which induce apoptosis in target cells. The second

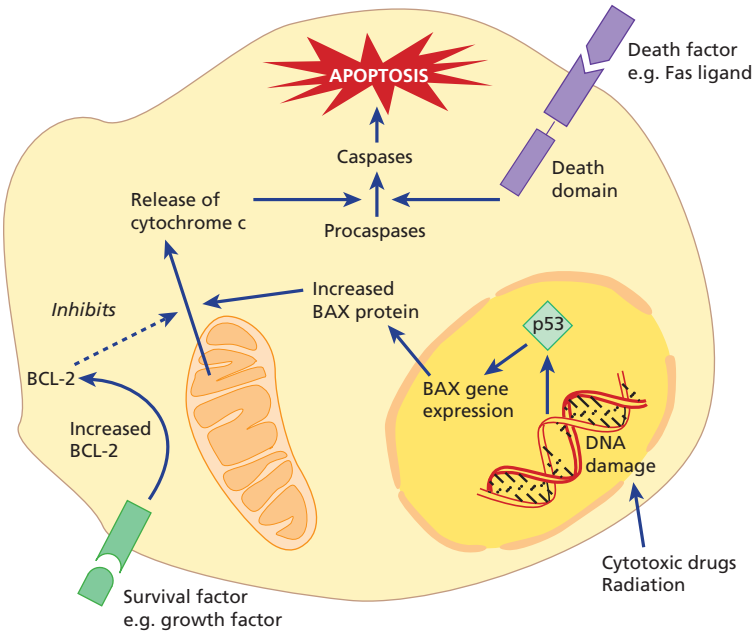

Fig. 1.11 Representation of apoptosis. Apoptosis is initiated via two main stimuli: (i) signalling through cell membrane receptors such as FAS or tumour necrosis factor (TNF) receptor; or (ii) release of cytochrome c from mitochondria. Membrane receptors signal apoptosis through an intracellular death domain leading to activation of caspases which digest DNA. Cytochrome c binds to the cytoplasmic protein Apaf-1 leading to activation of caspases. The intracellular ratio of pro- (e.g. BAX) or anti-apoptotic (e.g. BCL-2) members of the BCL-2 family may influence mitochondrial cytochrome c release. Growth factors raise the level of BCL-2 inhibiting cytochrome c release whereas DNA damage, by activating p53, raises the level of BAX which enhances cytochrome c release.

pathway is via the release of cytochrome c from mitochondria. Cytochrome c binds to Apaf-1 which then activates caspases. DNA damage induced by irradiation or chemotherapy may act through this pathway. The protein p53 has an important role in sensing DNA damage. It activates apoptosis by raising the cell level of BAX which then increases cytochrome c release (Fig. 1.11). P53 also shuts down the cell cycle to stop the damaged cell from dividing (Fig. 1.8). The cellular level of p53 is rigidly controlled by a second protein MDM2. Following death, apoptotic cells display molecules that lead to their ingestion by macrophages.

As well as molecules that mediate apoptosis there are several intracellular proteins that protect cells from apoptosis. The best characterized example is BCL-2. BCL-2 is the prototype of a family of related proteins, some of which are anti-apoptotic and some, like BAX, pro-apoptotic. The intracellular ratio of BAX and BCL-2 determines the relative susceptibility of cells to apoptosis and may act through regulation of cytochrome c release from mitochondria.

Many of the genetic changes associated with malignant disease lead to a reduced rate of apoptosis and hence prolonged cell survival. The clearest example is the translocation of the *BCL-2* gene to the immunoglobulin heavy chain locus in the t(14; 18) translocation in follicle centre lymphoma. Overexpression of the BCL-2 protein makes the malignant B cells less susceptible to apoptosis. Apoptosis is the normal fate for most B cells undergoing selection in the lymphoid germinal centres.

Several translocations leading to the generation of fusion proteins such as t(9; 22), t(1; 14) and t(15; 17) also result in inhibition of apoptosis (Chapter 10). In addition, genes encoding proteins that are involved in mediating apoptosis following DNA damage, such as p53 and ATM, are also frequently mutated and therefore inactivated in haemopoietic malignancies.

Transcription factors

Transcription factors regulate gene expression by controlling the transcription of specific genes or gene families. Typically, they contain at least two domains: a ***DNA-binding domain*** such as a leucine zipper or helix-loop-helix motif which binds to a specific DNA sequence, and an ***activation domain*** which contributes to assembly of the transcription complex at a gene promoter.

Adhesion molecules

A large family of glycoprotein molecules termed adhesion molecules mediate the attachment of marrow precursors, leucocytes and platelets to various components of the extracellular matrix, to endothelium, to other surfaces and to each other. The adhesion molecules on the surface of leucocytes are termed receptors and these interact with molecules (termed ligands) on the surface of potential target cells. Three main families exist:

1 ***Immunoglobulin superfamily*** This includes receptors that react with antigens (the T-cell receptors and the immunoglobulins) and antigen-independent surface adhesion molecules.

2 ***Selectins*** These are mainly involved in leucocyte and platelet adhesion to endothelium during inflammation and coagulation.

3 ***Integrins*** These are involved in cell adhesion to extracellular matrix (e.g. to collagen in wound healing and in leucocyte and platelet adhesion).

The adhesion molecules are thus important in the development and maintenance of inflammatory and immune responses, and in platelet–vessel wall and leucocyte–vessel wall interactions. Expression of adhesion molecules can be modifed by extracellular and intracellular factors and this alteration of expression may be quantitative or functional. IL-1, TNF, IFN-γ, T-cell activation, adhesion to extracellular proteins and viral infection may all up-regulate expression of these molecules.

The pattern of expression of adhesion molecules on tumour cells may determine their mode of spread and tissue localization (e.g. the pattern of metastasis of carcinoma cells or non-Hodgkin's lymphoma cells into a follicular or diffuse pattern). The adhesion molecules may also determine whether or not cells circulate in the bloodstream or remain fixed in tissues. They may also partly determine whether or not tumour cells are susceptible to the body's immune defences.

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