Review

Growth-inhibiting Activity of Transcription Factor C/EBPα, Its Role in Haematopoiesis and Its Tumour Suppressor or Oncogenic Properties in Leukaemias

(C/EBPα / cell cycle / proliferation / differentiation / haematopoiesis / leukaemia / tumour suppressor)

O. FUCHS

Institute of Haematology and Blood Transfusion, Prague, Czech Republic

Abstract. The CCAAT/enhancer binding protein alpha (C/EBPα or CEBPA) is the founding member of a family of related leucine zipper transcription factors that play important roles in myeloid differentiation. Targeted inactivation of C/EBPα in mice demonstrates its importance in the proper development and function of liver, adipose tissue, lung and haematopoietic tissues. C/EBPα is highly expressed in these differentiated tissues where it controls differentiation-dependent gene expression and inhibits cell proliferation. Learning more about the precise molecular functions of the C/EBPα protein and how these are affected by leukaemogenic mutations should lead to an improved understanding of the cellular functions that are disrupted in patients with AML. Decreased expression of C/EBPα but not *C/EBPα* mutation has been shown in patients with granulocytic leukaemias that are associated with translocations t(8;21), inv (16) or t(15;17). Derived fusion proteins repress C/EBPα expression. Differentiation therapy of some AML types is based on restoring C/EBPα function. However, apparently normal C/EBPα is overexpressed in BCP-ALL harbouring the translocation t(14; 19)(q32; q13). C/EBPα may exhibit oncogenic as well as tumour suppressor properties in human leukaemogenesis. *C/EBPα* mutations were not found in non-haematopoietic cancers. DNA hypermethylation of the upstream *C/EBPα* promoter region is responsible for very low C/EBPα expression in human lung and endometrial cancer. C/EBPα expression may be a biomarker for early detection of these cancers and DNA-modifying drugs such as demethylating agents and/or histone deacetylase inhibitors could be used in the treatment of these malignancies.

Introduction

The CCAAT/enhancer binding protein alpha (C/EBPα or CEBPA) transcription factor regulates the balance between cell proliferation and differentiation in haematopoietic and non-haematopoietic tissues (Hendricks-Taylor and Darlington, 1995; Zhang et al., 1997; Johansen et al., 2001; McKnight, 2001; Sugahara et al.,

Abbreviations: AME – AML1-MDS1-EVI1 fusion protein, AML – acute myeloid leukaemia, ATP – adenosine triphosphate, ATPases – enzymes involved in ATP hydrolysis, ATRA – all-trans retinoic acid, BaP – basophil progenitor, bcl – B-cell leukaemia/lymphoma, BCP-ALL – B-cell precursor-acute lymphoblastic leukaemia, Bcr-Abl – a constitutively activated tyrosine kinase resulting from the t(9;22) chromosomal translocation in CML, BMCP – basophil/mast cell progenitor, brm – brahma, bZIP – basic region and leucine zipper region, C/EBPα or CEBPA - CCAAT/enhancer binding protein alpha, Cdk – cyclin-dependent kinase, CLL - chronic lymphocytic leukaemia, CLP – common lymphoid progenitor, CMP – common myeloid progenitor, EoP – eosinophil progenitor, E2F – transcription factor involved in cell-cycle regulation and synthesis of DNA in mammalian cells, ETO – eight-twenty-one- according to the gene located on chromosome 8 which is involved in translocation with the AML1 gene, also named RUNX1 on chromosome 21, Ets transcription factors – family named according to first member discovered as part of avian E26 (E-twenty-six) retrovirus genome, EVI1 – ecotropic viral integration site 1 transcription factor, Flt3 - Fms-like tyrosine kinase 3, *fms* – oncogene of the McDonough strain of feline sarcoma virus, G-CSF – granulocyte-colony stimulating factor, GM-CSF – granulocyte-macrophage-colony stimulating factor, GMP – granulocyte/monocyte progenitor, GSK-3 - glycogen synthase kinase-3, HSC – haematopoietic stem cell, IGH- immunoglobulin heavy chain locus, IL – interleukin, IκB – protein family of inhibitors of nuclear factor kappa B, Mad – transcription factor, an antagonist of the c-Myc transcription factor, Max – a member of the basic region-helix-loop-helix-leucine zipper protein family, MCP – mast cell progenitor, MDS1 – myelodysplasia syndrome 1, MEP – megakaryocyte/erythroid progenitor, NF-κB – nuclear factor kappa B, Pax – paired box family of transcription factors, PI3K – phosphatidylinositol 3-kinase, PP2A – protein phosphatase 2A, PU.1 – a member of the Ets transcription factors that is expressed specifically in myeloid and B cells, Rb – retinoblastoma protein, SUMO-1 – small ubiquitin related modifier-1, SWI/SNF – a nucleosome remodelling complex composed of several proteins-products of the SWI and SNF genes as well as several other polypeptides, TBP – TATA box-binding protein, TFIIB – transcription factor which binds directly to TBP and recruits RNA polymerase II.

2001). C/EBPα is a central regulator of energy metabolism as it directly activates the transcription of many metabolically important genes (McKnight et al., 1989; Ramji and Foka, 2002). Several human tumour types display reduction in the levels of C/EBPα, suggesting that C/EBPα is a tumour suppressor (Takai et al., 2005; Tada et al., 2006). However, genetic evidence supporting the tumour suppressor function of C/EBPα has been only obtained for myeloid leukaemias (Nerlov, 2004; Mueller and Pabst, 2006; Schuster and Porse, 2006). On the other hand, overexpression of apparently normal C/EBPα RNA or protein was observed in six patients with B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) harbouring the translocation t(14; 19)(q32; q13). C/EBPα is activated in these BCP-ALL cells by juxtaposition to the immunoglobulin gene enhancer upon this rearrangement and exhibits oncogenic properties (Chapiro et al., 2006). Mice lacking C/EBPα die within 8 hours after birth, presumably from impaired glucose metabolism and altered fat metabolism with a failure of adipocytes to accumulate lipids (Wang et al., 1995; Flodby et al., 1996; Timchenko et al., 1997; Kimura et al., 1998). They show signs of hyperplasia in both the developing lung and liver. The lung shows hyperproliferation of type II pneumocytes and abnormal alveolar structure. Histopathology of the liver displays a structure resembling hepatocellular carcinoma. The C/EBPα null mice also display impaired neutrophil development intrinsic to the haematopoietic tissue.

C/EBP form a subgroup within the basic region/leucine zipper superfamily of transcription factors (bZIP). Members of this family of transcription factors consist of an N-terminal transactivation domain, a DNA-binding basic region, and a leucine rich dimerization domain (Vinson et al., 1989; Kerppola and Curran, 1991). The dimerization domain, termed the "leucine zipper", contains leucine repeats that intercalate with leucine repeats of the dimer partner forming a coiled coil of α helices in parallel orientation. There are six members of the C/EBP family (C/EBPα, C/EBPβ, C/EBPγ, C/EBPδ, C/EBPε, and C/EBPζ). C/EBPα forms homodimers or heterodimers with other C/EBP proteins as well as with transcription factors of other families to precisely modulate the transcription of target genes (Lekstrom-Himes and Xanthopoulos, 1998). C/EBP dimerization is a prerequisite to DNA binding. However, DNA binding specificity is determined by the DNA contact surface of C/EBP, the "basic" region, specifically by three aminoacids upstream of the leucine zipper. Domains responsible for transcriptional activation and/or repression are located in the N-terminal end of C/EBPs.

Similarities between C/EBP family members suggest an evolutionary history of genetic duplications with subsequent pressure to diversify. The resulting proteins vary in tissue specificity and transactivating ability. The pleiotropic effects of C/EBPs are caused by tissue- and stage-specific expression, leaky ribosomal reading, post-transcriptional modifications, and variable DNA binding specificities. Experiments with mice genetically altered to abolish the expression of C/EBPs underscores the role these factors play in normal tissue development and cellular function, cellular proliferation and functional differentiation (Wang et al., 1995; Flodby et al., 1996; Zhang et al., 1997; Martis et al., 2006).

C/EBPα gene, mRNA and protein

C/EBPα is encoded by an intronless gene that is 2783 bp long and maps to human chromosome 19q13.1 (Hendricks-Taylor et al., 1992). Expression patterns of C/EBPα mRNA are similar in the mouse and human with measurable levels in liver, adipose tissue, intestine, lung, adrenal gland, skeletal muscle, pancreas, placenta, prostate gland, mammary glands and peripheral blood mononuclear cells. However, the expression was undetectable or very low in brain, kidney, thymus, testis and ovary (Birkenmeier et al., 1989). In liver and adipose tissue, highest levels of C/EBPα mRNA are detected only in differentiated tissue.

C/EBPα mRNA is translated into two major proteins, C/EBPα p42 (42 kDa) and C/EBPα p30 (30 kDa) by a ribosome-scanning mechanism in which a fraction of ribosomes ignore the first two AUG codons and initiate translation at the third AUG codon located 351 nucleotides downstream of the first one (Fig. 1). Translation start site multiplicity of the C/EBPα mRNA is dictated by a small 5' open reading frame (Calkhoven et al., 1994, 2000). The translation product C/EBPα p30 initiated at the third AUG codon is devoid of the potent transcription-regulation domain contained in C/EBPα p42 and stimulates transcription of the target gene (e.g. albumin gene) much less efficiently than the C/EBPα p42 (Ossipow et al., 1993).

The full-length, 42 kDa form of C/EBPα contains three transactivation domains (TE-I, TE-II and TE-III) as well as the basic region/leucine zipper (bZIP) (Nerlov and Ziff, 1994). This bZIP domain contains a basic region (BR), which mediates DNA binding, and a leucine zipper region (LZ) for homodimerization and heterodimerization of C/EBPα with other C/EBP and different proteins (Fig. 1). TE-I and TE-II domains mediate cooperative binding of C/EBPα to TBP (TATA box-binding protein) and another basal transcription initiation factor TFIIB (Nerlov and Ziff, 1995). Both, TBP and TFIIB are essential components of the RNA polymerase II basal transcription apparatus. The TE-III domain contains a negative regulatory subdomain (Pei and Shih, 1991; Nerlov and Ziff, 1994). This negative regulatory region or transcriptional attenuator domain also inhibits transcriptional synergy of multiple DNA-binding regulators and was named synergy control (SC) motif (Iniguez-Lluhí and Pearce, 2000).

The truncated C/EBPα p30 isoform acts as an inhibitor of C/EBPα p42-mediated transactivation of transcription of target genes. This inhibition occurs by formation of heterodimers of both C/EBPα isoforms. These heterodimers have impaired DNA-binding ability and transcription transactivation capacity compared with C/EBPα p42 homodimers (Ossipow et al., 1993, Cleaves et al., 2004). There are examples where the C/EBPα p30 isoform has some transcriptional activity (Calkhoven et al., 1997). In these cases the truncated C/EBPα p30 isoform might stabilize binding of other transcription factors and activate transcription. This 30 kDa product also lacks the antimiotic activity exhibited by the full-length C/EBPα p42 (Lin et al., 1993).

The basic region (BR) of C/EBP, which mediates DNA binding, preferentially recognizes the palindromic DNA sequence 5'-ATTGCGCAAT-3' (Johnson, 1993; Koldin et al., 1995). Although the C/EBPs possess similar DNA binding specificities and dimerization properties, each protein exhibits unique functional properties *in vivo.* The appearance of specific phenotypes in each C/EBP-deficient mouse shows that these proteins do not have fully redundant functions. Direct evidence for specific functions has come from experiments where the coding sequence for C/EBPα was replaced with the coding sequence C/EBPβ. This gene replacement strategy to generate a viable and fertile C/EBPα-null mouse line rescued hepatic-specific function to maintain normal blood glucose levels, but could not rescue function in white adipose tissue to regulate fat storage (Chen et al., 2000).

Post-translational modifications of C/EBPα

Phosphorylation and sumoylation of C/EBPα are important regulatory mechanisms which cause changes in function of this protein. Phosphorylation and sumoylation sites in the C/EBPα protein are shown in Fig. 1.

C/EBPα protein phosphorylation

The McDonough strain of feline sarcoma virus contains an oncogene called v-*fms* with tyrosine kinase activity. Fms-like tyrosine kinase 3 (Flt3) encodes a receptor tyrosine kinase for which activating mutations have been identified in a proportion of acute myelogenous leukemia (AML) patients. These mutations activate the Flt3 kinase activity constitutively, and result in increased cellular proliferation and viability. Activation of Flt3 inhibits C/EBPα function by extracellular signal receptor kinase (ERK)1/2-mediated phosphorylation on serine 21, which affects the ability of C/EBPα to induce granulocytic differentiation and may explain the differentiation block of leukaemic blasts (Radomska et al., 2006).

Glycogen synthase kinase-3 (GSK3), an insulin-inhibited protein kinase phosphorylates C/EBPα on two threonine residues (T222 and T226, refer to rodent sequence) and on serine (S230). The functional importance of these phosphorylation events in the regulation of C/EBPα activity is not yet clear but plays some role in preadipocyte diferentiation (Liu et al., 2006).

Ras signalling phosphorylates C/EBPα on serine 248 of the transactivation domain, resulting in an enhancement of the ability of C/EBPα to transactivate the granulocyte-colony stimulating factor (G-CSF) receptor promoter, which contributes to the induction of granulocyte differentiation (Behre et al., 2002).

The biological function of C/EBPα in liver cells also depends on phosphorylation-dephosphorylation of a single serine 193 (S193) residue within the C/EBPα growth-inhibitory region (Wang and Timchenko, 2005). S193-phosphorylated C/EBPα binds to cyclin-dependent kinase 2 (Cdk2) and to brahma (Brm, named according to the ATPase of the Drosophila SWI/SNF complex involved in chromatin remodelling during transcription) and inhibits proliferation. S193-dephosphorylated C/EBPα accelerates proliferation by neutralization growth-inhibitory activity of retinoblastoma protein (Rb) through sequestering Rb from E2F-Rb complex repressors. The E2F transcription factor plays a crucial role in the control of cell cycle progression and regulates the expression of genes required for G1/S transition (Fig. 2). E2F activity is modulated by multiple mechanisms including negative regulation by interaction with the product of the Rb tumour suppressor gene expression. Binding of Rb to E2F results in active transcriptional repression of E2F-regulated genes and growth suppression.

The activation of phospatidylinositol 3-kinase (PI3K)/Akt (protein kinase B) in liver tumours leads to accumulation of protein phosphatase 2A (PP2A) in the nuclei, where PP2A dephosphorylates C/EBPα on S193 and blocks its growth-inhibitory activity (Wang et al., 2004). This PI3K/Akt-mediated block of C/EBPα inhibition leads to the lack of negative control of proliferation in the liver and to development of tumours.

C/EBPα protein sumoylation

C/EBPα can be sumoylated at the lysine residue of SC motif within the transcriptional attenuator domain (Subramanian et al., 2003; Sato et al., 2006). Sumoylation of this motif can affect the inhibitory function by influencing protein-protein interactions, a mechanism by which sumoylation probably regulates the activity of the transcription factor. Sato et al. (2006) investigated the level and functional roles of sumoylated C/EBPα during the differentiation of hepatocytes. SUMO-1 (small ubiquitin-related modifier-1) masks BRG1 (product of expression of brahma-related gene 1)-binding site of C/EBPα. BRG-1 is the core subunit of an ATP-dependent chromatin remodelling complex. Sumoylation of C/EBPα dramatically decreases the stimulation of C/EBPα-mediated transactivation of the liver-specific albumin gene by BRG1. Sumoylated C/EBPα failed to
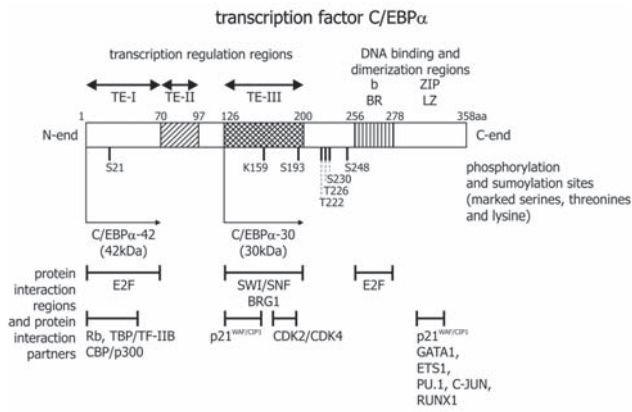
Fig. 1. Schematic representation of the domain structure of C/EBPα protein, phosphorylation and sumoylation sites, protein interaction regions and protein interaction partners. Numbers directly above the schema of protein indicate the amino acids of the rat C/EBPα protein. The full-length, 42 kDa form of C/EBPα protein and the shorter 30 kDa form of the protein are also shown. Other details are described in the text.

induce proliferation arrest because its interaction with BRG1 was inhibited.

Growth-inhibiting activity of C/EBPα

C/EBPα is a strong inhibitor of cell proliferation when overexpressed in cultured cells (Hendricks-Taylor and Darlington, 1995; Schuster and Porse, 2006). C/EBPα mediates differentiation in several organ systems, including liver, adipose, lung and the haematopoietic tissue. C/EBPα promotes differentiation by the up-regulation of lineage-specific gene products and by the exit from cell cycle that means proliferation arrest. The capacity of C/EBPα to promote growth arrest has been studied *in vitro*, by analysis of knockout mice and by examination of leukaemic cells (Johnson, 2005; Schuster and Porse, 2006). Several models of C/EBPα-induced growth arrest have been described (Johnson, 2005; Schuster and Porse, 2006). These include C/EBPα-mediated (1) stabilization of the cyclin-dependent kinase 2 (Cdk2) inhibitor, p21WAF1/CIP1 (Timchenko et al., 1997), (2) regulation of growth-inhibiting Rb-E2F complexes (Timchenko et al., 1999), (3) interaction with free E2F, inhibition of E2F activity and down-regulation of the E2F target gene c-*myc* (Johansen et al., 2001, Porse et al., 2001), (4) interaction with Max, a member of the basic region-helix-loop-helix-leucine zipper proteins, that belongs to a network of transcription factors including the Myc and Mad families of proteins (Grandori et al., 2000, Zada et al., 2006), (5) inhibition of Cdk2 and Cdk4 activity (Wang et al., 2001), and (6) interaction with the SWI/SNF chromatin remodelling complexes (Muller et al., 2004). Models (1) and (2) have been questioned by experiments performed
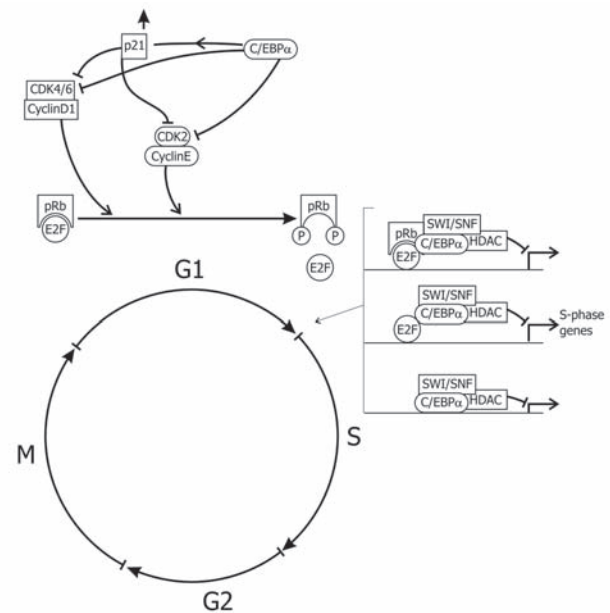

Fig. 2. Regulation of G1-S progression during cell cycle by C/EBPα protein. Retinoblastoma protein (Rb) is phosphorylated by Cdks that are inhibited by C/EBPα protein and p21WAF1/CIP1 Cdk inhibitor induced by C/EBPα protein. Phosphorylation of Rb and its release from transcription factor E2F derepresses S-phase genes, which are otherwise inhibited by the pRb-E2F complex through recruitment of the SWI/SNF chromatin-remodelling complex and histone deacetylases (HDACs). C/EBPα protein may be bound to promoters of target S-phase genes indirectly by E2F or it could bind directly to target S-phase gene promoters.

in p21WAF1/CIP1 (Muller et al., 1999) and Rb (Hendricks-Taylor and Darlington, 1995) null cell lines. Model (5) has also been questioned by control experiments with mice homozygous for the deletion of the 15-amino acid proline- and histidine-rich region (PHR) located in the central part of C/EBPα (Porse et al., 2006). C/EBPα PHR is responsible for interaction of C/EBPα with Cdk2/4. Mice homozygous for the ΔPHR allele did not display any phenotype that could be related to the role of C/EBPα as a growth repressor (Porse et al., 2006). C/EBPα may promote growth arrest by cell-specific mechanisms. This means that different models of C/EBPα-mediated cell-cycle arrest (Fig. 2) operate in different cell types.

Different regions of C/EBPα are involved in growth inhibition (Fig. 1). According to the third model, C/EBPα interacts with free E2F through the non-DNA binding surface of its basic region and the N-terminal region of the C/EBPα also plays a role in growth inhibition (Johansen et al., 2001). The basic DNA-binding region of the C/EBPα is also involved in the C/EBPα and Max interaction, likely through C/EBPα R297, K298 and/or K302 in the fourth model (Zada et al., 2006). C/EBPα R297 is known to participate in the interaction between

C/EBPα and E2F (Porse et al., 2001). In the Cdk2/Cdk4 inhibition model, C/EBPα interacts with and inhibits the activity of Cdk2/Cdk4 through PHR (Wang et al., 2001). Finally, according to the SWI/SNF recruitment model, C/EBPα interacts with SWI/SNF components through a centrally located 75-amino acid region overlapping with the Cdk2/Cdk4 binding region (Pedersen et al., 2001; Muller et al., 2004).

Although over-expression experiments and analysis of cells lacking known cell-cycle regulators are useful for identifying the pathways in which C/EBPα functions, these approaches do not necessarily reveal the primary target.

Fig. 3. Schematic presentation of roles of the C/EBPα protein and other transcription factors in lineage specification.

Functional role of CEBPα in hierarchical specification of haematopoietic lineages and in the development of granulocytes

C/EBPα and the commitment of self-renewing haematopoietic stem cells and downstream progenitors

Haematopoiesis is a life-long, highly regulated multistage process wherein a pluripotent self-renewing haematopoietic stem cell (HSC) differentiates into more committed progenitor cells that give rise to all blood cell lineages. Sequential lineage specification processes are called commitment. Transcription factors have emerged as key regulators of lineage determination and differentiation during haematopoiesis (Sieweke and Graf, 1998; Orkin, 2000; Rosmarin et al., 2005). C/EBPα affects haematopoietic cell fate decisions by inducing myeloid differentiation and inhibiting erythroid differentiation in progenitors more primitive than GMPs (Suh et al., 2006). C/EBPα also plays a regulatory role in maintenance of the HSC population, since both C/EBPα-deficient foetal liver cells and adult bone marrow cells display a competitive advantage over wild-type bone marrow cells in transplantation experiments (Zhang et al., 2004).

It has been thought that the commitment was an irreversible process, and cells differentiated into a certain lineage would not change their own fate. However, recent evidence suggests that many immature progenitors still sustain latent differentiation programmes to other lineages than their own. Lymphoid lineage-committed progenitors (CLPs, "common lymphoid progenitors", see Fig. 3) maintain a latent myeloid differentiation potential, which can be initiated through exogenously expressed interleukin-2 (IL-2) receptors. Transcription factor C/EBPα is promptly up-regulated in CLPs after ectopic IL-2 stimulation. This C/EBPα up-regulation initiates myeloid differentiation from CLPs and decreases expression of a B lymphoid-specific transcription factor, Pax5, which belongs to the paired box family of transcription factors (Hsu et al., 2006). Using transgenic mice expressing a conditional form of C/EBPα whose activity can be regulated, Fukuchi et al. (2006) tested
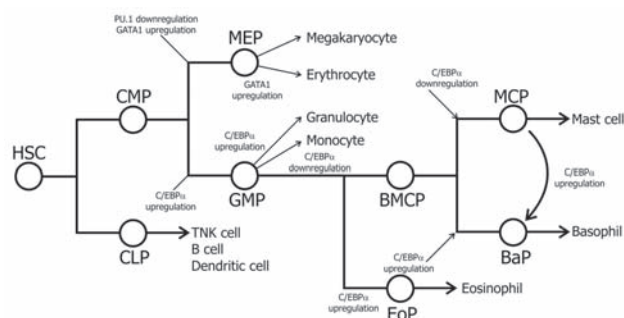
megakaryocyte/erythroid progenitors (MEPs) and CLPs whether they could be redirected to myeloid lineage by C/EBPα activation. Lineage conversion was accomplished in both cases by short-term activation of C/EBPα (Fukuchi et al., 2006). These data establish a critical role of C/EBPα not only in the myeloid lineage, but also in the whole haematopoietic system.

C/EBPα is highly expressed in granulocyte/monocyte progenitors (GMPs) but significantly decreased in basophil/mast cell progenitors (BMCPs) or in mast cell progenitors (MCPs), suggesting that the down-regulation of C/EBPα is critical for the development of basophil and mast cell lineages (Iwasaki et al., 2006). C/EBPα needs to be suppressed at the GMP stage for both basophil and mast cell development (Fig. 3). C/EBPα is expressed in a biphasic manner for basophil development from GMPs through BMCPs. The down-regulation of C/EBPα at the GMP stage proceeds into BMCPs and its reactivation at the BMCP stage gives rise to mature basophils (Iwasaki et al., 2006). Transcription factor GATA1 is important for the megakaryocyte/erythrocyte lineage commitment and the transcription factor GATA-2 instructs GMPs to exclusively select the eosinophil fate (Iwasaki et al., 2006).

C/EBPα function in granulopoiesis

The main role of C/EBPα in haematopoiesis is in the development of granulocytes (Tenen et al., 1997, Keeshan et al., 2003, Rosmarin et al. 2005, Suh et al. 2006). A critical role for the function of C/EBPα in granulopoiesis was demonstrated in mice harbouring a disruption of the *C/EBPα* gene (Wang et al., 1995). These mice show a selective early block in granulopoiesis, with the appearance of many myeloid blasts in foetal liver and peripheral blood (Zhang et al., 1997). Other lineages, including macrophages, were not affected. These mice had a selective loss of granulocyte colony-forming units and IL-6 responsive colony-forming units, which could be explained by the loss of expression of the granulocyte-colony-stimulating factor (G-CSF) receptor and IL-6 receptor (Iwama et al., 1998; Zhang et al., 1998).

Haematopoietic cells from these mice failed to express mRNAs for primary or secondary neutrophil granule proteins, such as major primary granule protein (MPO) or lactoferrin (Iwama et al., 1998). Transcription activation function of C/EBPα is required for induction of granulocytic differentiation (Fig. 3) (Keeshan et al., 2003). Further studies demonstrated that at least *in vitro,* restoration of granulocytic differentiation could be effected by administration of the cytokines IL-3 and granulocyte-macrophage-colony stimulating factor (GM-CSF), but not with all-*trans* retinoic acid (ATRA) (Zhang et al., 1997; Zhang et al., 2002). These studies support a model of at least two pathways leading to the differentiation of myeloid progenitors to granulocytes, one involving C/EBPα and one involving IL-3 and GM-CSF (Zhang et al., 2002).

Cooperation of PU.1 and C/EBPα transcription factors in lineage decision

C/EBPα can cooperate with additional factors to direct monocytic commitment of primary myeloid progenitors (Wang et al., 2006). C/EBPα induces transcription factor PU.1 mRNA 2-fold in normal myeloid progenitors. C/EBPα binds and activates the endogenous *PU.1* gene in myeloid cells. Induction of PU.1 by C/EBPα may account for increased levels of PU.1 in myeloid as compared with B-lymphoid cells, and in this way contribute to the specification of myeloid progenitors (Kummalue and Friedman, 2003). Genetic analyses suggest that elevation of PU.1 supports monocytic over granulocytic development. Lack of one *PU.1* allele favours neutrophil development from embryonic stem cells *in vitro* and favours neutrophil development *in vivo* in the absence of G-CSF receptor (Dahl et al., 2003). Cre recombinase-mediated deletion of *PU.1* in adult mice preserves granulocytes at the expense of monocytes (Dakic et al., 2005). C/EBPα and PU.1 are expressed in HSC and are up-regulated in GMPs during granulocyte and macrophage development. However, both these transcription factors are down-regulated in megakaryocyte-erythrocyte progenitors (Suh et al., 2006).

Regulation of microRNA-223 involved in granulocytic differentiation by C/EBPα

C/EBPα regulates not only growth factor receptors and other myeloid-specific gene products, but also microRNA-223 (miR223), whose expression is confined to haematopoietic cells (Chen et al., 2004; Fazi et al., 2005). Two transcriptional factors, NFI-A and C/EBPα, compete for binding to the miR-223 promoter (Fazi et al., 2005). NFI-A maintains miR-223 at low levels, whereas its replacement by C/EBPα, following retinoic acid-induced differentiation, up-regulates miR-223 expression (Fig. 4). The granulocytic differentiation is also favoured by a negative-feedback loop in which miR-223 represses NFI-A translation.
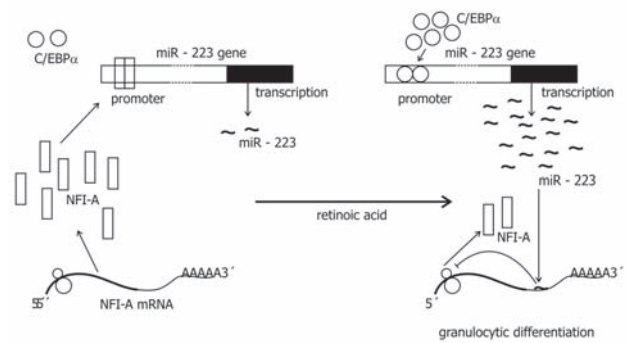

Fig. 4. Important roles of microRNA-223 and two transcription factors (C/EBPα and NFI-A) in undifferentiated acute promyelocytic NB4 cells (left panel) and after induction of granulocytic differentiation by retinoic acid (right panel). Two transcriptional factors, NFI-A and C/EBPα, compete for binding to the miR-223 promoter. NFI-A maintains miR-223 at low levels (left panel), whereas its replacement by C/EBPα, following retinoic acid-induced differentiation, up-regulates miR-223 expression (right panel).

C/EBPα and the pathophysiology of acute leukaemias

C/EBPα mutations

Acute myeloid leukaemias (AMLs) are clonal disorders that are characterized by a block in differentiation along one or more haematopoietic lineages. Molecular abnormalities are frequently detected in AML. The genetic alterations in AML often affect transcription factors that also have an important role in normal haematopoiesis. C/EBPα mutations have been observed in AML patients with the approximate frequency of 5–14% (Pabst et al., 2001b; Gombart et al., 2002; Preudhomme et al., 2002; Barjesteh van Waalvijk van Doorn-Khosrovani et al., 2003; Kaeferstein et al., 2003; Snaddon et al., 2003; Fröhling et al., 2005; Leroy et al., 2005; Liang et al., 2005; Shih et al., 2005, 2006). The mutations can be largely divided into two common types. First, carboxy-terminal in-frame mutations disrupt the basic zipper region, thus affecting DNA binding as well as homo- and heterodimerization with other C/EBP family members. These mutations are in most AML cases in one of the two *C/EBPα* alleles and are often associated with a second mutation in the other allele, which usually leads to loss of C/EBPα function. Second, amino-terminal frame shift mutations result in premature termination of the normal 42 kDa form of the C/EBPα protein while preserving the 30 kDa form leading to the induction of proliferation. A striking feature of C/EBPα mutations in AML patients is that no bi-allelic null mutations have been reported thus far. It therefore seems most likely that some residual C/EBPα activity has to be present in order for malignant transformation to occur. This observation correlates with the observation that C/EBPα (-/-)

mice never become truly leukaemic (Zhang et al., 2004).

Inherited AML associated with an identical mutation in *C/EBPα* has been reported in only a few families (Smith et al., 2004, Sellick et al., 2005). No additional chromosomal aberrations were detected in these cases. The long latency of the development of the disease (10 to 30 years) may reflect secondary mutations that are not detectable. One possibility is that carriers of a *C/EBPα* mutation have a large population of poorly differentiated myeloid cells associated with an increased risk of "second genetic hit" that would lead to AML.

Decreased expression of C/EBPα by transcriptional regulation

In the absence of specific *C/EBPα* mutations, decreased exptression may serve as an alternative mechanism that disrupts C/EBPα function. AML with the t(8;21) translocation gives rise to the fusion gene *RUNX1-CBF2T1* (also known as *AML1-ETO*) encoding the AML1-ETO fusion protein. In AML patient samples with this translocation as well as in cell lines (AML1-ETO-positive Kasumi-1 cells) derived from these patients, C/EBPα is undetectable. The specific depletion of AML1-ETO (also known as AML1-MTG8) in Kasumi-1 cells by AML1-ETO small interfering RNAs (siRNAs) led to an approximately 15-fold increase in C/EBPα mRNA expression, whereas electroporation with control siRNAs had no effect (Heidenreich et al., 2003). AML1-ETO appears to suppress C/EBPα expression indirectly by inhibiting positive autoregulation of the *C/EBPα* promoter. Moreover, the application of AML1-ETO siRNAs followed by stimulation with inducers of differentiation (transforming growth factor β1 and vitamin D$_3$) caused a higher expression of C/EBPα in comparison to these inducers alone. In addition, conditional expression of C/EBPα overcomes the block of differentiation caused by AML1-ETO and is sufficient to trigger terminal neutrophilic differentiation. Restoring C/EBPα expression will have therapeutic applications in *AML1-ETO*-positive leukaemias (Pabst et al., 2001a).

Decreased expression of C/EBPα by posttranscriptional regulation

Posttranscriptional regulation of C/EBPα in myeloid leukaemias was demonstrated in AML with t(3;21)(q26;q22) translocation encoding the *AML1-MDS1-EVI1* (*AME*) fusion gene (Helbling et al., 2004). The RNA-binding protein calreticulin was strongly activated in AML patient samples with AME fusion protein. Calreticulin binds strongly to the GC-rich stem structure in the stem loop within the coding region of C/EBPα mRNA and inhibits translation of this mRNA (Helbling et al., 2004). The same mechanism of inhibition of C/EBPα mRNA translation was described for CBFB-MYH11 ("core binding factor ß-smooth muscle myosin heavy chain") leukaemic fusion protein, expressed as a result of inv(16)(p13q22), that activates calreticulin binding to C/EBPα mRNA (Helbling et al., 2005).

Decreased or increased expression of C/EBPα by posttranslational regulation

Posttranslational regulation of C/EBPα activity in myeloid leukaemias is based on phosphorylation and probably also on sumoylation of the C/EBPα protein, described in the paragraph about the posttranscriptional modifications of C/EBPα. The further mechanism of C/EBPα protein inactivation is its proteasomal degradation after association with Tribbles homologue 2 (Trib2) (Keeshan et al., 2006). Analysis of 285 AMLs showed that elevated *Trib2* expression preferentially associated with a cluster of AMLs characterized by C/EBPα deficiency. Trib2 is an oncoprotein that contributes to the pathogenesis of AML through the inhibition of C/EBPα function. On the other hand, a mass spectrometry-based proteomic approach to systematically identify putative co-activator proteins interacting with the DNA-binding domain (DBD) of C/EBPα identified c-Jun N-terminal kinase (JNK) 1 among others proteins as proteins interacting with DBD of C/EBPα from nuclear extract of myelomonocytic U937 cells (Trivedi et al., 2007). Kinase JNK1 physically interacts with DBD of C/EBPα *in vitro* and *in vivo*. Active JNK1 inhibits ubiquitination of C/EBPα possibly by phosphorylating in its DBD (Trivedi et al., 2007). Consequently, JNK1 prolongs C/EBPα protein half-life, leading to its enhanced transactivation and DNA-binding capacity. In certain AML patients, however, the JNK1 mRNA expression and its kinase activity is decreased, which suggests a possible reason for C/EBPα inactivation in AML. JNK1 is a positive regulator of C/EBPα (Trivedi et al., 2007).

Oncogenic properties of C/EBPα

C/EBPα is activated in human precursor-B lymphoblastic acute leukaemia (BCP-ALL) cells by juxtaposition to the immunoglobulin gene enhancer upon the t(14;19)(q32;q13) chromosomal rearrangement. Translocations involving the immunoglobulin heavy chain locus (*IGH*) at chromosomal band 14q32 in BCP-ALL cells is a rare but recurrent event. These translocations can be connected with the translocation of the *C/EBPα* gene on chromosome 19q13.1. This t(14;19)(q32;q13) chromosomal rearrangement leads to overexpression of C/EBPα protein, usually of normal sequence, which exhibits oncogenic properties (Chapiro et al., 2006). Thus it appears that either loss of function of C/EBPα or gain of function of C/EBPα has leukaemogenic potential (Fig. 5). In these BCP-ALL patients, the breakpoint on chromosome 19 differs from the t(14;19)(q32;q13) identified in patients with diagnosis of atypical chronic lymphocytic leukaemia (CLL) (Robinson et al., 2004). This translocation in atypical CLL and in B-cell lymhoma
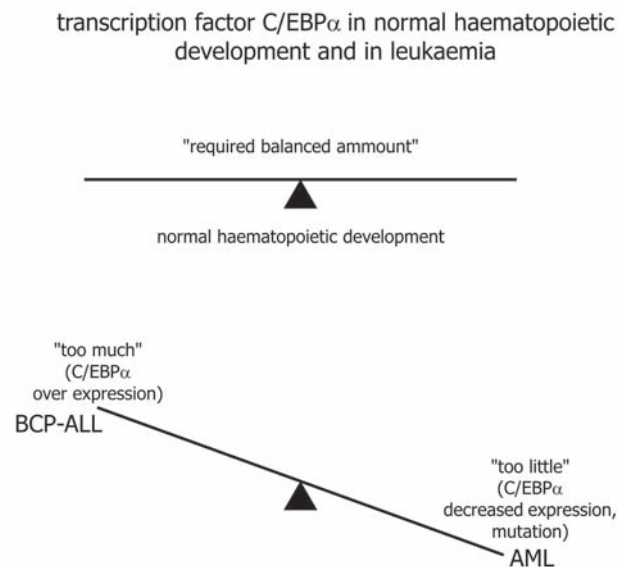
transcription factor C/EBPα in normal haematopoietic
development and in leukaemia

Fig. 5. The effect of the amount of transcription factor C/EBPα in leukaemogenesis. "Just the right" amount of C/EBPα is needed for maintenance of normal haematopoiesis. "Too much or too little" of C/EBPα can contribute to leukaemogenesis. Apparently normal C/EBPα is over-expressed in BCP-ALL harbouring the translocation t(14; 19)(q32; q13). Mutations or decreased expression of C/EBPα cause AML.

patients juxtaposes the *bcl-3* (B-cell CLL/lymphoma 3 gene) at chromosome 19q13 with the immunoglobulin heavy chain gene locus at 14q32 (Huh et al., 2007). The *bcl-3* gene encodes a member of the IκB protein family of inhibitors of nuclear factor kappa B (NF-κB). Bcl-3 expression also suppresses p53 activation and inhibits p53-induced apoptosis.

Suppression of the C/EBPα expression by Bcr-Abl oncoproteins

Bcr-Abl is a constitutively activated tyrosine kinase resulting from the t(9;22) chromosomal translocation in chronic myelogenous leukaemia (CML). In human CML, Bcr-Abl causes a chronic phase characterized by an increased production of granulocytic cells with normal maturation. In most cases of human CML, the disease ultimately undergoes a transition to blast crisis. In myeloid blast crisis, granulocyte differentiation is blocked. Down-regulation of the C/EBPα protein has been implicated in this transition (Perrotti et al., 2002). In primary bone marrow cells taken from patients with CML in blast crisis, C/EBPα mRNA is clearly present but C/EBPα protein is undetectable. Bcr-Abl regulates the expression of C/EBPα by inducing the poly(rC)-binding protein hnRNP E2, which inhibits the translation of C/EBPα mRNA (Perrotti et al., 2002). The effect of the absence of C/EBPα was tested in Bcr-Abl-induced murine leukaemia (Wagner et al., 2006). In the absence

of C/EBPα, Bcr-Abl induces an immature erythroleukaemia, and no myeloid cells are detected. These results indicate that in malignant haematopoiesis residual C/EBPα function is also required for myeloid lineage commitment (Wagner et al., 2006). The concept of reduced rather than loss of transcription factor C/EBPα expression might be important for future attempts at modelling leukaemia.

Conclusion

Transcription factor C/EBPα plays an important role in numerous cellular processes including proliferation, differentiation, apoptosis, control of metabolism and other specific functions. The antiproliferative role of C/EBPα and the underlying mechanisms were described as well as its function in cell differentiation, mainly in myeloid cell differentiation. C/EBPα levels correlate with Bcl-2 (B-cell leukemia/lymphoma-2) in a defined subset of AML cases. C/EBPα induces endogenous *bcl-2* expression in myeloid and lymphoid cell lines and normal cells by cooperation with NF-κB p50 and inhibits apoptosis (Paz-Priel et al., 2005). In addition, cooperation of C/EBPα or C/EBPβ with NF-κB induces multiple genes involved in the inflammatory response, a process defective in C/EBPα (-/-) mice (Burgess-Beusse and Darlington, 1998). C/EBPα is a critical tumour suppressor in the haematopoietic tissue but the genetic evidence for this role in non-haematopoietic tissue has not been obtained until now. DNA hypermethylation of the upstream *C/EBPα* promoter region is responsible for very low C/EBPα expression in human endometrial and lung cancer (Takai et al., 2005, Tada et al., 2006). C/EBPα expression may be a biomarker for early detection of these cancers. DNA-modifying drugs such as demethylating agents (5-azacytidine or 5-aza-2'-deoxycytidine) and/or histone deacetylase inhibitors (depsipeptide, trichostatin A, valproic acid, suberoylanilide hydroxamic acid, butyrate) could be used in the treatment of these malignancies. On the other hand, C/EBPα can act as an oncogene in B-cell precursor ALL cases. New research is targeted to a better description of C/EBPα targets and biological activities. The new tools to identify target genes, binding sites in chromosomal DNA, protein-protein interactions and protein modifications, as well as powerful genetic methods should soon bring new results in this field.

References

Barjesteh van Waalwijk van Doorn-Khosrovani, S., Erpelinck, C., Meijer, J., van Oosterhoud, S., van Putten, W. L., Valk, P. J., Beverloo, H., Tenen, D. G., Löwenberg, B., Delwel, R. (2003) Biallelic mutations in the CEBPA gene and low CEBPA expression levels as prognostic markers in intermediate-risk AML. *Hematol. J.* **4**, 31-40.

Behre, G., Singh, S. M., Liu, H., Bortolin, L. T., Christopei, M., Radomska, H. S., Rangatia, J., Hiddemann, W., Friedman,

A. D., Tenen, D. G. (2002) Ras signaling enhances the activity of C/EBPα to induce granulocytic differentiation by phosphorylation of serine 248. *J. Biol. Chem.* **277**, 26293-26299.

Birkenmeier, E. H., Gwynn, B., Howard, S., Jerry, J., Gordon, J. I., Landschulz, W. H., McKnight, S. L. (1989) Tissue-specific expression, developmental regulation and genetic mapping of the gene encoding CCAAT/enhancer binding protein. *Genes Dev.* **3**, 1146-1156.

Burgess-Beuse, B. L., Darlington, G. J. (1998) C/EBPα is critical for neonatal acute-phase response to inflammation. *Mol. Cell. Biol.* **18**, 7269-7277.

Calkhoven, C. F., Bouwman, P. R. J., Snippe, L., Geert, A. B. (1994) Translation start site multiplicity of the CCAAT/enhancer binding protein α mRNA is dictated by a small 5′ open reading frame. *Nucleic Acids Res.* **22**, 5540-5547.

Calkhoven, C. F., Snippe, L., Ab, G. (1997) Differential stimulation by CCAAT/enhancer-binding protein α isoforms of the estrogen-activated promoter of the very-low-density apolipoprotein II gene. *Eur. J. Biochem.* **249**, 113-120.

Calkhoven, C. F., Muller, C., Leutz, A. (2000) Translational control of C/EBPα and C/EBPβ isoform expression. *Genes Dev.* **14**, 1920-1932.

Chapiro, E., Russell, L., Radford-Weiss, I., Bastard, C., Lessard, M., Struski, S., Cave, H., Fert-Ferrer, S., Barin, C., Maarek, O., Della-Valle, V., Strefford, J. C., Berger, R., Harrison, C. J., Bernard, O. A., Nguyen-Khac, F., for the Groupe Francophone de Cytogénétique Hématologique (2006) Overexpression of *CEBPA* resulting from the translocation t(14; 19)(q32; q13) of human precursor-B-cell acute lymphoblastic leukaemia. *Blood* **108**, 3560-3563.

Chen, C. Z., Li, L., Lodish, H. F., Bartel, D. P. (2004) Micro RNAs modulate hematopoietic lineage differentiation. *Science* **303**, 83-86.

Chen, S.-S., Chen, J.-F., Johnson, P. F., Muppala, V., Lee, Y.-H. (2000) C/EBPβ, when expressed from the *C/ebpα* gene locus, can functionally replace C/EBPα in liver but not in adipose tissue. *Mol. Cell. Biol.* **20**, 7292-7299.

Cleaves, R., Wang Q. F., Friedman A. D. (2004) C/EBPα p30, a myeloid leukemia oncoprotein, limits G-CSF receptor expression but not terminal granulopoiesis via site-selective inhibition of C/EBP DNA binding. *Oncogene* **23**, 716-725.

Dahl, R., Walsh, J. C., Lancki, D., Laslo, P., Iyer, S. R., Singh, H., Simon, M. C. (2003) Regulation of macrophage and neutrophil cell fates by the PU.1: C/EBPα ratio and granulocyte colony-stimulating factor. *Nat. Immunol.* **4**, 1029-1036.

Dakic, A., Metcalf, D., Di Rago, L., Mifsud, S., Wu, L., Nutt, S. L. (2005) PU.1 regulates the commitment of adult hematopoietic progenitors and restricts granulopoiesis. *J. Exp. Med.* **201**, 1487-1502.

Fazi, F., Rosa, A., Fatica, A., Gelmetti, V., De Marchis, M. L., Nervi, C., Bozzoni, I. (2005) A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBPα regulates human granulopoiesis. *Cell* **123**, 819-831.

Flodby, P., Barlow, C., Kylefjord, H., Ährlund-Richter, L., Xanthopoulos, K. G. (1996) Increased hepatic cell proliferation and lung abnormalities in mice deficient in CCAAT/enhancer binding protein α. *J. Biol. Chem.* **271**, 24753-760.

Fröhlig, S., Schlenk, R. F., Krauter, J., Thiede, C., Ehninger, G., Haase, D., Harder, L., Kreitmeier, S., Scholl, C., Caligiuri, M. A., Bloomfield, C. D., Döhner, H., Döhner, K. (2005) Acute myeloid leukemia with deletion 9q within a noncomplex karyotype is associated with *CEBPA* loss-of-function mutations. *Genes Chromosomes Cancer* **42**, 427- 432.

Fukuchi, Y., Shibata, F., Miyuki, I., Goto-Koshino, Y., Sotomaru, Y., Ito, M., Kitamura, T., Nakajima, H. (2006) Comprehensive analysis of myeloid lineage conversion using mice expressing an inducible form of C/EBPα. *EMBO J.* **25**, 3398-3410.

Gombart, A. F., Hofmann, W.-K., Kawano, S., Takeuchi, S., Krug, U., Kwok, S. H., Larsen, S. J., Asou, H., Miller, C. W., Hoelzer, D., Koeffler, H. P. (2002) Mutations in the gene encoding the transcription factor CCAAT/enhancer binding protein α in myelodysplastic syndromes and acute myeloid leukemias. *Blood* **99**, 1332-1340.

Grandori, C., Cowley, S. M., James, L. P., Eisenman, R. N. (2000) The Myc/Max/Mad network and the transcriptional control of cell behavior. *Annu. Rev. Cell. Dev. Biol.* **16**, 653-699.

Heidenreich, O., Krauter, J., Riehle, H., Hadwiger, P., John, M., Heil, G., Vornlocher, H.-P., Nordheim, A. (2003) AML1/MTG8 oncogene suppression by small interfering RNAs supports myeloid differentiation of t(8;21)-positive leukemic cells. *Blood* **101**, 3157-3163.

Helbling, D., Mueller, B. U., Timchenko, N. A., Hagemeijer, A., Jotterand, M., Meyer-Monard, S., Lister, A., Rowley, J. D., Huegli, B., Fey, M. F., Pabst, T. (2004) The leukemic fusion gene AML1-MDS1-EVI1 suppresses CEBPA in acute myeloid leukemia by activation of Calreticulin. *Proc. Natl. Acad. Sci. USA* **101**, 13312-13317.

Helbling, D., Mueller, B. U., Timchenko, N. A., Schardt, J., Eyer, M., Betts, D. R., Jotterand, M., Meyer-Monard, Fey, M. F., Pabst, T. (2005) CBFB-SMMHC is correlated with increased calreticulin expression and suppresses the granulocytic differentiation factor CEBPA in AML with inv(16). *Blood* **106**, 1369-1375.

Hendricks-Taylor, L. R., Darlington, G. J. (1995) Inhibition of cell proliferation by C/EBP α occurs in many cell types, does not require the presence of p53 or Rb, and is not affected by large T-antigen. *Nucleic Acids Res.* **23**, 426-433.

Hendricks-Taylor, L. R., Bachinski, L. L., Sicilliano, M. J., Fertitta, A., Trask, B., deJong, P. J., Ledbetter, D. H., Darlington, G. J. (1992) The CCAAT/enhancer binding protein (C/EBPα) gene (CEBPA) maps to human chromosome 19q13.1 and the related nuclear factor NF-IL6 (C/EBP ß) gene (CEBPB) maps to human chromosome 20q13.1. *Genomics* **14**, 12-17.

Hsu, C.-L., King-Fleischman, A. G., Lai, A. Y., Matsumoto, Y., Weissman, I. L., Kondo, M. (2006) Antagonistic effect of CCAAT enhancer-binding protein-α and Pax5 in myeloid or lymphoid lineage choice in common lymphoid progenitors. *Proc. Natl. Acad. Sci. USA* **103**, 672-677.

Huh, Y. O., Abruzzo, L. V., Rassidakis, G. Z., Parry-Jones, N., Schlette, E., Brito-Bapabulle, V., Matutes, E., Wotherspoon, A., Keating, M. J., Medeiros, J., Catovsky, D. (2007) The t(14;19)(q32;q13)-positive small B-cell leukaemia: a clinicopathologic and cytogenetic study of seven cases. *Br. J. Haematol.* **136**, 220-228.

Iniguez-Lluhí, J., Pearce, D. (2000) A common motif within the negative regulatory regions of multiple factors inhibit their transcriptional synergy. *Mol. Cell. Biol.* **20**, 6040-6050.

Iwama, A., Zhang, P., Darlington, G. J., McKercher, S. R., Maki, R. A., Tenen, D. G. (1998) Use of RDA analysis of knockout mice to identify myeloid genes regulated in vivo by PU.1 and C/EBPα. *Nucleic Acids Res.* **26**, 3034-3043.

Iwasaki, H., Mizuno, S., Arinobu, Y., Ozawa, H., Mori, Y., Shigematsu, H., Takatsu, K., Tenen, D. G., Akashi, K. (2006) The order of expression of transcription factors directs hierarchical specification of hematopoietic lineages. *Genes Dev.* **20**, 3010-3021.

Johansen, L. M., Iwama, A., Lodie, T. A., Sasaki, K., Felsher, D. W., Golub, T. R., Tenen D. G. (2001) c-Myc is a critical target for C/EBPα in granulopoiesis. *Mol. Cell. Biol.* **21**, 389-3806.

Johnson, P. F. (1993) Identification of C/EBP basic region residues involved in DNA sequence recognition and half-site spacing preference. *Mol. Cell. Biol.* **13**, 6916-6930.

Johnson, P. F. (2005) Molecular stop signs: regulation of cell cycle arrest by C/EBP transcription factors. *J. Cell Sci.* **118**, 2545-2555.

Kaeferstein, A., Krug, U., Tiesmeier, J., Aivado, M., Faulhaber, M., Stadler, M., Krauter, J., Germing, U., Hofmann, W. K., Koeffler, H. P., Ganser, A., Verbeek, W. (2003) The emergence of a C/EBPα mutation in the clonal evolution of MDS towards secondary AML. *Leukemia* **17**, 343-349.

Keeshan, K., Santilli, G., Corradini, F., Perrotti, D., Calabretta, B. (2003) Transcription activation function of C/EBPα is required for induction of granulocyte differentiation. *Blood* **102**, 1267-1275.

Keeshan, K., He, Y., Wouters, B. J., Shestova, O., Xu, L., Sai, H., Rodriguez, C. G., Mailard, I., Tobias, J. W., Valk, P., Carrol, M., Aster, J. C., Delwel, R., Pear, W. S. (2006) Tribbles homolog 2 inactivates C/EBPα and causes acute myelogenous leukemia. *Cancer Cell* **10**, 401-411.

Kerppola, T. K., Curran, T. (1991) Transcription factors interactions: basics on zippers. *Curr. Opin. Struct. Biol.* **1**, 71-79.

Kimura, T., Christoffels, V. M., Chowdhury, S., Iwase, K., Matsuzaki, H., Mori, M., Lamers, W. H., Darlington, G. J., Takiguchi, M. (1998) Hypoglycemia-associated hyperammonemia caused by impaired expression of ornithine cycle enzyme genes in C/EBPα knockout mice. *J. Biol. Chem.* **273**, 27505-27510.

Koldin, B., Suckow, M., Seydel, A., von Wilcken-Bergmann, B., Müller-Hill, B. (1995) A comparison of the different DNA binding specificities of the bZIP proteins C/EBP and GCN4. *Nucleic Acids Res* **23**, 4162-4169.

Kummalue, T., Friedman, A. D. (2003) Cross-talk between regulators of myeloid development: C/EBPα binds and activates the promoter of the PU.1 gene. *J. Leukoc. Biol.* **74**, 464-470.

Lekstrom-Himes, J., Xanthopoulos, K. G. (1998) Biological role of the CCAAT/enhancer-binding protein family of transcription factors. *J. Biol. Chem.* **273**, 28545-28548.

Leroy, H., Roumier, C., Huyghe, P., Biggio, V., Fenaux, P., Preudhomme, C. (2005) *CEBPA* point mutations in hematological malignancies. *Leukemia* **19**, 329-334.

Liang, D.-C., Shih, L.-Y., Huang, C.-F., Hung, I.-J., Yang, C. P., Liu, H. C., Jaing, T. H., Wang, L. Y., Chang, W.-H. (2005) *CEBPα* mutations in childhood acute myeloid leukemia. *Leukemia* **19**, 410-414.

Lin, F. T., McDougold, C. A., Diehl, A. M., Lane, M. D. (1993) A 30-kDa alternative translation product of the CCAAT/ enhancer binding protein α message: transcriptional activator lacking antimiotic activity. *Proc. Natl. Acad. Sci. USA* **90**, 9606-9610.

Liu, H.-K., Perrier, S., Lipina, C., Finlay, D., McLauchlan, H., Hastie, C. J., Hundal, H. S., Sutherland, C. (2006) Functional characterisation of the regulation of CAAT enhancer binding protein α by GSK-3 phosphorylation of threonines 222/226. *BMC Mol. Biol.* **7**, 14, 1-12.

Martis, P. C., Whitsett, J. A., Xu, Y., Perl, A.-K. T., Wan, H., Ikegami, M. (2006) C/EBPα is required for lung maturation at birth. *Development* **133**, 1155-1164.

McKnight, S. L. (2001) McBindall – a better name for CCAAT/enhancer binding proteins? *Cell* **107**, 259-261.

McKnight, S. L., Lane, M. D., Gluecksohn-Waelsch, S. (1989) Is CCAAT/enhancer binding protein a central regulator of energy metabolism? *Genes Dev.* **3**, 2021-2024.

Mueller, B. U., Pabst, T. (2006) C/EBPα and the pathophysiology of acute myeloid leukemia. *Curr. Opin. Hematol.* **13**, 7-14.

Muller, C., Alunni-Fabbroni, M., Kowenz-Leutz, E., Mo, X., Tommasino, M., Leutz, A. (1999) Separation of C/EBPα-mediated proliferation arrest and differentiation pathways. *Proc. Natl. Acad. Sci. USA* **96**, 7276-7281.

Muller, C., Calkhoven, C. F., Sha, X., Leutz, A. (2004) The CCAAT enhancer-binding protein α (C/EBPα) requires a SWI/SNF complex for proliferation arrest. *J. Biol. Chem.* **279**, 7353-7358.

Nerlov, C. (2004) C/EBPα mutations in acute myeloid leukaemias. *Nat. Rev. Cancer* **4**, 394-400.

Nerlov, C., Ziff, E. B. (1994) Three levels of functional interaction determine the activity of CCAAT/enhancer binding protein-α on the serum albumin promoter. *Genes Dev.* **8**, 350-362.

Nerlov, C., Ziff, E. B. (1995) CCAAT/enhancer binding protein-α amino acid motifs with dual TBP and TFIIB binding ability co-operate to activate transcription in both yeast and mammalian cells. *EMBO J.* **14**, 4318-4328.

Orkin S. H. (2000) Diversification of haematopoietic stem cells to specific lineages. *Nat. Rev. Genet.* **1**, 57-64.

Ossipow, V., Decombes, P., Schibler, U. (1993) CCAAT/ enhancer-binding protein mRNA is translated into multiple proteins with different transcription activation potentials. *Proc. Natl. Acad. Sci. USA* **90**, 8219-8223.

Pabst, T., Mueller, B. U., Harakawa, N., Schoch, C., Haferlach, Behre, G., T., Hiddemann, W., Zhang, D.-E., Tenen, D. G. (2001a) AML1-ETO downregulates the granulocytic differentiation factor C/EBPα in t(8;21) myeloid leukemia. *Nat. Med.* **7**, 444-451.

Pabst, T., Mueller, B. U., Zhang, P., Radomska, H. S., Narravula, S., Schnittger, S., Behre, G., Hiddemann, W., Tenen, D. G. (2001b) Dominant negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-α (C/EBPα) in acute myeloid leukemia. *Nat. Genet.* **27**, 263-270.

Paz-Priel, I., Cai, D. H., Wang, D., Kowalski, J., Blackford, A., Liu, H., Heckman, C. A., Gombart, A. F., Koeffler, H. P., Boxer, L. M., Friedman, A. D. (2005) CCAAT/enhancer binding protein α (C/EBPα) myeloid oncoproteins induce bcl-2 via interaction of their basic regions with nuclear factor-κB p50. *Mol. Cancer Res.* **3**, 585-596.

Pedersen, T. A., Kowenz-Leutz, E., Leutz, A., Nerlov, C. (2001) Cooperation between C/EBPα, TBP/TFIIB and SWI/SNF recruiting domains is required for adipocyte differentiation. *Genes Dev.* **15**, 3208-3216.

Pei, D. O., Shih, C. H. (1991) An "attenuator domain" is sandwiched by two distinct transactivation domains in the transcription factor C/EBP. *Mol. Cell. Biol.* 11, 1480-1487.

Perrotti, D., Cesi, V., Trotta, R., Guerzoni, C., Santilli, G., Campbell, K., Iervolino, A., Condorelli, F., Gambacorti-Passerini, C., caligiuri, M. A., Calabretta, B. (2002) BCR-ABL suppresses C/EBPα expression through inhibitory action of hnRNP E2. *Nat. Genet.* **30**, 48-58.

Porse, B. T., Pedersen, T. A., Xu, X., Lindberg, B., Wewer, U. M., Friis-Hansen, L., Nerlov, C. (2001) E2F repression by C/EBPα is required for adipogenesis and granulopoiesis in vivo. *Cell* **107**, 247-258.

Porse, B. T., Pedersen, T. A., Hasemann, M. S., Schuster, M. B., Kirstetter, P., Luedde, T., Damgaard, I., Kurz, E., Karlskov Schjerling, C., Nerlov, C. (2006) The proline-histidine-rich CDK2/CDK4 interaction region of C/EBPα is dispensable for C/EBPα-mediated growth regulation in vivo. *Mol. Cell. Biol.* **26**, 1028-1037.

Preudhomme, C., Sagot, C., Boissel, N., Cayuela, J.-M., Tigaud, I., de Botton, S., Thomas, X., Raffoux, E., Lamandin, C., Castaigne, S., Fenaux, P., Dombret, H. (20002) Favorable prognostic significance of *CEBPA* mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French association (ALFA). *Blood* **100**, 2717-2723.

Radomska, H. S., Basseres, D. S., Zheng, R., Zhang, P., Dayaram, T., Yamamoto, Y., Sternberg, D. W., Lokker, N., Giese, N. A., Bohlander, S. K., Schnittger, S., Delmotte M.-H., Davis, R. J., Small, D., Hiddemann, W., Gilliland, D. G., Tenen, D. G. (2006) Block of C/EBPα function by phosphorylation in acute myeloid leukemia with FLT3 activating mutations. *J. Exp. Med.* **203**, 371-381.

Ramji, D. P., Foka, P. (2002) CCAAT/enhancer-binding proteins: structure, function and regulation. *Biochem. J.* **365**, 561-575.

Robinson, H. M., Taylor, K. E., Jalali, G. R., Cheung, K. L., Harrison, C. J., Moorman, A. V. (2004) t(14;19)(q32;q13): A recurrent translocation in B-cell precursor acute lympho-blastic leukemia. *Genes Chromosomes Cancer* **39**, 88-92.

Rosmarin, A. G., Yang, Z., Resendes, K. K. (2005) Transcriptional regulation in myelopoiesis: Hematopoietic fate choice, myeloid differentiation, and leukemogenesis. *Exp. Hematol.* **33**, 131-143.

Sato, Y., Miyake, K., Kaneoka, H., Iijima, S. (2006) Sumoylation of CCAAT/enhancer-binding protein α and its functional roles in hepatocyte differentiation. *J. Biol. Chem.* **281**, 21629-21639.

Schuster, M. B., Porse, B. T. (2006) C/EBPα: a tumour suppressor in multiple tissues? *Biochim. Biophys. Acta* **1766**, 88-103.

Sellick, G. S., Spendlove, H. E., Catovsky, D., Pritchard-Jones, K., Houlston, R. S. (2005) Further evidence that germline *CEBPA* mutations cause dominant inheritance of acute myeloid leukaemia. *Leukemia* **19**, 1276-1278.

Shih, L. Y., Huang, C.-F., Lin, T.-L., Wu, J.-H., Wang, P.-N., Dunn, P., Kuo, M.-C., Tang, T.-C. (2005) Heterogeneous patterns of *CEBPα* mutation status in the progression of myelodysplastic syndrome and chronic myelomonocytic leukemia to acute myelogenous leukemia. *Clin. Cancer Res.* **11**, 1821-1826.

Sieweke, M. H., Graf, T. (1998) A transcription factor party during blood cell differentiation. *Curr. Opin. Genet. Dev.* **8,** 545-551.

Shih, L. Y., Liang, D.-C., Huang, C.-F.,Wu, J.-H., Lin, T.-L., Wang, P.-N., Dunn, P., Kuo, M.-C., Tang, T.-C. (2006) AML patients with *CEBPα* mutations mostly retain identical mutant patterns but frequently change in allelic distribution at relapse: a comparative analysis on paired diagnosis and relapse samples. *Leukemia* **20**, 604-609.

Smith, M. L., Cavenagh, J. D., Lister, T. A., Fitzgibbon, J. (2004) Mutation of *CEBPA* in familial acute myeloid leukemia. *N. Engl. J. Med.* **351**, 2403-2407.

Snaddon, J., Smith, M. L., Neat, M., Cambal-Parrales, M., Dixon-McIver, A., Arch, R., Amess, J. A., Rohatiner, A. Z., Lister, T. A., Titzgibbon, J. (2003) Mutations of C/EBPα in acute myeloid leukemia FAB types M1 and M2. *Genes Chromosomes Cancer* **37**, 72-78.

Subramanian, L., Benson, M. D., Iniguez-Lluhí, J. A. (2003) A synergy control motif within the attenuator domain of CCAAT/enhancer-binding protein α inhibits transcriptional synergy through its PIASy-enhanced modification by SUMO-1 or SUMO-3. *J. Biol. Chem.* **278**, 9134-9141.

Sugahara, K., Iyama, K. I., Kimura, T., Sano, K., Darlington, G. J., Akiba, T., Takiguchi, M. (2001) Mice lacking CCAAT/enhancer-binding protein-α show hyperproliferation of alveolar type II cells and increased surfactant protein mRNAs. *Cell Tissue Res.* **306**, 57-63.

Suh, H. C., Goya, J., Renn, K., Friedman, A. D., Johnson, P. F., Keller, J. R. (2006) C/EBPα determines hematopoietic cell fate in multipotential progenitor cells by inhibiting erythroid differentiation and inducing myeloid differentiation. *Blood* **107**, 4308-4316.

Tada, Y., Brena, R. M., Hackanson, B., Morrison, C., Otterson, G. A., Plass, C. (2006) Epigenetic modulation of tumor suppressor CCAAT/enhancer binding protein α activity in lung cancer. *J. Natl. Cancer Inst.* **98**, 396-406.

Takai, N., Kawamata, N., Walsh, C. S., Gery, S., Desmond, J. C., Whittaker, S., Said, J. W., Popoviciu, L. M., Jones, P. A., Miyakawa, I., Koeffler, H. P. (2005) Discovery of epigenetically masked tumor suppressor genes in endometrial cancer. *Mol. Cancer Res.* **3**, 261-269.

Tenen, D. G., Hromas, R., Licht, J. D., Zhang, D. E. (1997) Transcription factors, normal myeloid development, and leukemia. *Blood* **90**, 489-519.

Timchenko, N. A., Harris, T. E., Wilde, M., Bilyeu, T. A., Burgess-Beusse, B. L., Finegold, M. J., Darlington, G. J. (1997) CCAAT/enhancer-binding protein α regulates p21 protein and hepatocyte proliferation in newborn mice. *Mol. Cell. Biol.* **17**, 7353-7361.

Timchenko, N. A., Wilde, M., Darlington, G. J. (1999) C/EBPα regulates formation of S-phase -specific E2F-p107 complexes in livers of newborn mice. *Mol. Cell. Biol.* **19**, 2966-2945.

Trivedi, A. K., Bararia, D., Christopeit, M., Peerzada, A. A., Singh, S. M., Kieser, A., Hiddemann, W., Behre, H. M., Behre, G. (2007) Proteomic identification of C/EBP-DBD multiprotein complex: JNK1 activates stem cell regulator C/EBPα by inhibiting its ubiquitination. *Oncogene* **26,** 1789-1801.

Vinson, C. R., Sigler, P. B., McKnight, S. L. (1989) Scissors-grip model for DNA recognition by a family of leucine zipper proteins. *Science* **246**, 911-916.

Wagner, K., Zhang, P., Rosenbauer, F., Drescher, B., Kobayashi, S., Radomska, H. S., Kutok, J. L., Gilliland, G., Krauter, J., Tenen, D. G. (2006) Absence of the transcription factor CCAAT enhancer binding protein α results in loss of myeloid identity in bcr/abl-induced malignancy. *Proc. Natl. Acad. Sci. USA* **103**, 6338-6343.

Wang, D., D'Costa, J., Civin, C. I., Friedman, A. D. (2006) C/EBPα directs monocytic commitment of primary myeloid progenitors. *Blood* **108**, 1223-1229.

Wang, G.-L., Timchenko, N. A. (2005) Dephosphorylated C/EBPα accelerates cell proliferation through sequestering retinoblastoma protein. *Mol. Cell. Biol.* **25**, 1325-1338.

Wang, G.-L., Iakova, P., Wilde, M., Awad, S., Timchenko, N. A. (2004) Liver tumors escape negative control of proliferation via PI3K/Akt-mediated block of C/EBPα growth inhibitory activity. *Genes Dev.* **18**, 912-925.

Wang, H., Iakova, P., Wilde, M., Welm, A., Goode, T., Roesler, W. J., Timchenko, N. A. (2001) C/EBPα arrests cell proliferation through direct inhibition of Cdk2 and Cdk4. *Mol. Cell* **8**, 817-828.

Wang, N. D., Finegold, M. J., Bradley, A., Ou, C. N., Abdelsayed, S. V., Wilde, M. D., Taylor, R. J., Wilson, D. R., Darlington, G. J. (1995) Impaired energy homeostasis in C/EBP α knockout mice. *Science* **269**, 1108-1112.

Zada, A. A., Pulikkan, J. A., Bararia, D., Geletu, M., Trivedi, A. K., Balkhi, M. Y., Hiddemann, W. D., Tenen, D. G., Behre, H. M., Behre, G. (2006) Proteomic discovery of Max as a novel interacting partner of C/EBPα: a Myc/Max/Mad link. *Leukemia* **20**, 2137-2146.

Zhang, D. E., Zhang, P., Wang, N. D., Hetherington, C. J., Darlington, G. J., Tenen, D. G. (1997) Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein α-deficient mice. *Proc. Natl. Acad. Sci. USA* **94**, 569-574.

Zhang, P., Iwama, A., Datta, M. W., Darlington, G. J., Link, D. C., Tenen, D. G. (1998) Upregulation of interleukin 6 and granulocyte colony-stimulating factor receptors by transcription factor CCAAT enhancer binding protein α (C/EBPα) is critical for granulopoiesis. *J. Exp. Med.* **188**, 1173-1184.

Zhang, P., Nelson, E., Radomska, H. S., Iwasaki-Arai, J., Akashi, K., Friedman, A. D., Tenen, D. G. (2002) Induction of granulocytic differentiation by 2 pathways. *Blood* **99**, 4406-4412.

Zhang, P., Iwasaki-Arai, J., Iwasaki, H., Fenyus, M. L., Dayaram, T., Owens, B. M., Shigematsu, H., Levantini, E., Huettner, C. S., Lekstrom-Himes, J. A., Akashi , K., Tenen, D. G. (2004) Enhancement of hematopoietic stem cell repopulating capacity and self-renewal in the absence of the transcription factor C/EBPα. *Immunity* **21**, 853-863.