REVIEW ARTICLE

Multiple steps in the regulation of transcription-factor level and activity

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This review focuses on the regulation of transcription factors, many of which are DNA-binding proteins that recognize *cis*-regulatory elements of target genes and are the most direct regulators of gene transcription. Transcription factors serve as integration centres of the different signal-transduction pathways affecting a given gene. It is obvious that the regulation of these regulators themselves is of crucial importance for differential gene expression during development and in terminally differentiated cells.Transcription factors can be regulated at two, principally different, levels, namely concentration and activity, each of which can be modulated in a variety of ways. The concentrations of transcription factors, as of intracellular proteins in general, may be regulated at any of the steps leading from DNA to protein, including transcription, RNA processing, mRNA degradation and translation. The activity of a transcription factor is often regulated by (de)phosphorylation, which may affect different functions, e.g. nuclear localization, DNA binding and *trans*-activation. Ligand binding is another mode of transcription-factor activation. It is typical for the large superfamily of nuclear hormone receptors. Heterodimerization between transcription factors adds another dimension to the regulatory diversity and signal integration. Finally, non-DNA-binding (accessory) factors may mediate a diverse range of functions, e.g. serving as a bridge between the transcription factor and the basal transcription machinery, stabilizing the DNA-binding complex or changing the specificity of the target sequence recognition. The present review presents an overview of different modes of transcription-factor regulation, each illustrated by typical examples.

INTRODUCTION

The conversion of abstract coded biological information stored in DNA into concrete physiologically active proteins, called gene expression, is tightly regulated. In a multicellular organism, all cell types with a few exceptions contain the same genetic information. Yet each cell type expresses only a unique subset of the total number of available genes. Differential gene expression is specified by unique epigenetic information which is present in the particular cell and determines its phenotype [1,2]. For many genes, control at the first step of expression, transcription, is paramount. The transcription profile is actually a convenient parameter for the identification of a particular cell type. Some genes are always turned on in all cells; they form the group of so-called 'housekeeping' genes, which encode structural proteins and enzymes catalysing the reactions of basic metabolism. Other genes are only transcribed in one or a few cell types, usually only during a particular stage of development or under the regime of particular extracellular and/or intracellular signals [2]. Differential gene expression is controlled by a complex regulatory network in which specialized transcription factors relay the signals to specific target genes. Many of these transcription factors are DNA-binding proteins that bind to regulatory DNA elements located *cis* to the target genes.

The levels of the DNA-binding transcription factors, or rather their activities, are decisive as to whether their target genes are transcribed and to what extent. This implies that these regulators of gene expression in their turn must be tightly regulated. The question arises as to how this is accomplished without the need of an ever-increasing number of upstream regulatory genes. There are several ways by which cells extend the diversity of their regulatory repertoire. One way is to make use of the combinatorial action of a limited set of transcription factors. Another way is to modulate the activity of a transcription factor once it has been synthesized. The various ways by which transcription factor gene expression can be regulated are depicted in Figure 1. As for genes in general, transcription of regulatory genes is the prime level of control and provides the intermediates at which subsequent steps of control can be exerted. These include splicing, which may occur in alternative modes, transport to the cytoplasm and degradation of mRNA. Translation is another important level of control. Selection of alternative start sites may generate functionally distinct protein isoforms. Once the transcription factor has been synthesized, it has to be transported to the nucleus. Masking of the nuclear localization signal (NLS), e.g. by a sequestering protein or by phosphorylation, may hinder the factor from reaching the nuclear compartment. Finally, the functions that determine the transcription factor's activity, specifically its DNA-binding, dimerization and *trans*-activation functions, may be affected in a variety of ways, including post-translational modification (e.g. phosphorylation), ligand binding and interaction with other proteins. In the following sections of this review, examples of different levels of control will be discussed.

Abbreviations used: NLS, nuclear localization signal; NF-M, nuclear factor-myeloid. PPAR, peroxisome proliferator-activated receptor; HNF, hepatocyte nuclear factor; cAMP, cyclic AMP; CREM, cAMP-response element modulator; CRE, cAMP-responsive promoter element; ICER, inducible cAMP early repressor; 3′-UTR, 3′-untranslated region; ARE, AU-rich element; eIF, eukaryotic initiation factor; MAP kinase, mitogen-activated protein kinase; Met-tRNA$_i^{met}$, methionyl-initiator-tRNA; (u)ORF, (upstream) open reading frame; RARβ2, retinoic acid receptor β2; DBD, DNA-binding domain; HRE, hormone response element; LBD, ligind-binding domain; TR, thyroid receptor; VDR, vitamin D_3 receptor; RXR, retinoid X receptor; DR, direct repeat; 9-*cis*-RA, 9-*cis*-retinoic acid; bZIP, basic zipper; NF-AT, nuclear factor of activated T cells; GR, glucocorticoid receptor; CBP, CREB-binding protein; HTLV-I, human T-cell leukaemia virus type I; DCoH, dimerization cofactor of HNF-1; PCD, pterin-4a-carbinolamine dehydratase; C/EBP, CCAAT/enhancer-binding protein; GRE, glucocorticoid response element; Pit-1, pituitary specific factor 1; (b) HLH protein, (basic) helix–loop–helix protein.

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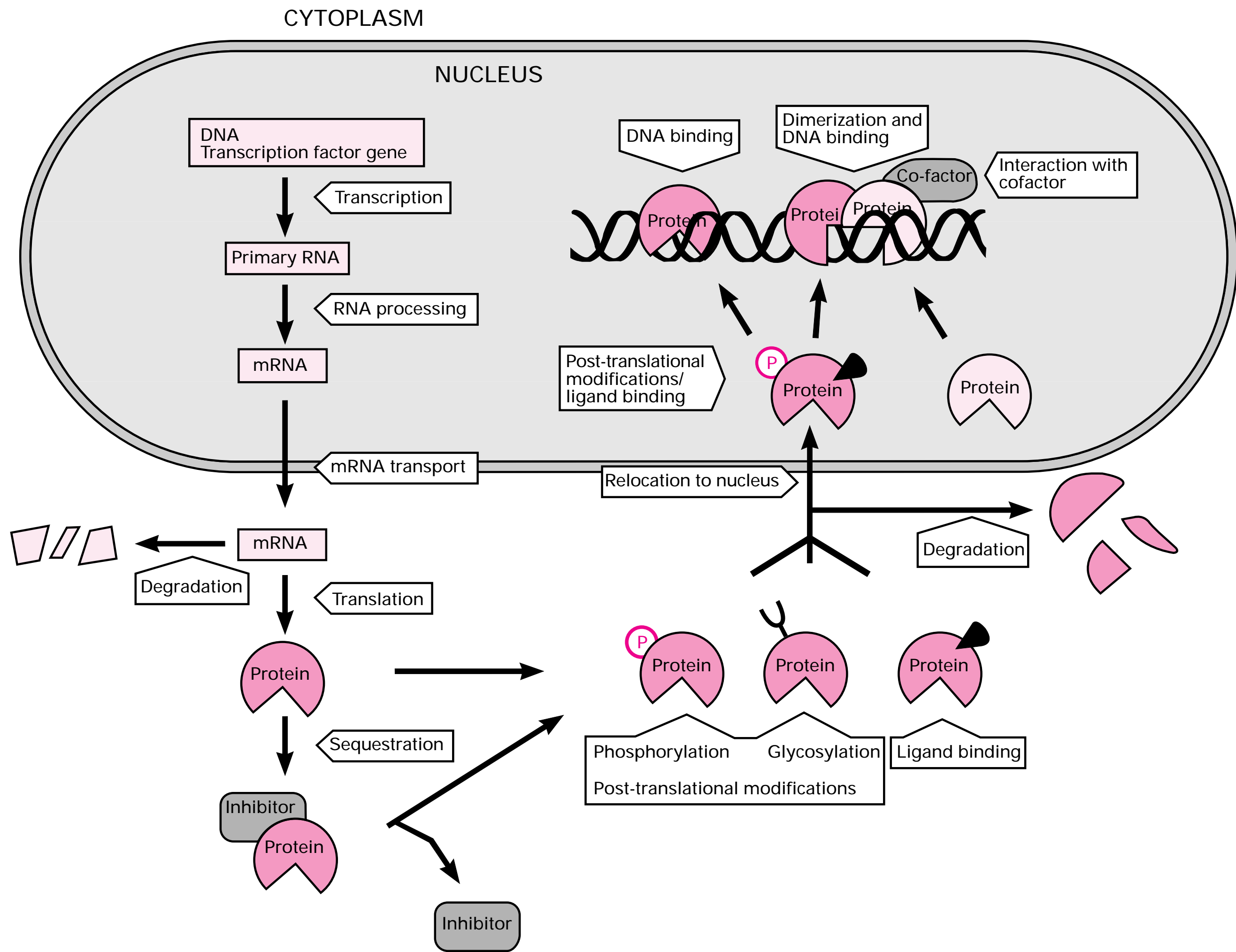

Figure 1 Control levels in the expression and activation of DNA-binding proteins

The scheme depicts crucial steps in the synthesis, activation and action of DNA-binding proteins. Along the pathway potential regulatory points are indicated (white labels). The concentration and activity of a particular DNA-binding protein may be regulated at several points down the pathway to its ultimate action in gene transcription as a transcription factor.

REGULATION OF TRANSCRIPTION-FACTOR LEVELS

Transcription and autoregulation

Synthesis of mRNA is the first level at which regulation can be exercised. Regulation of transcription-factor genes, as that of other genes, is accomplished through the combinatorial action of (other) transcription factors binding to promoter and enhancer sequences. Interestingly, many cell-type specific transcription factors behave as autoregulatory factors, being involved in the transcriptional activation of their own genes [2–12]. Genetic circuits with autoregulatory properties can be of a simple or complex type. A simple autoregulatory circuit consist of one single transcription factor that binds directly to the promoter of its own gene. A complex regulatory circuit comprises several transcription factors that bind to the promoters of one another's genes, thereby indirectly affecting the rate of transcription of their own genes [13]. Autoregulatory positive feedback provides a memory mechanism for maintaining the determined and/or differentiated state associated with a specific cell phenotype. Some examples of autoregulatory transcription factors are described below.

Pit-1

Pituitary specific factor 1 (Pit-1) is a tissue-specific transcription factor obligatory for the development of three cell types in the anterior pituitary gland: lactotrophes, somatotrophes and thyrotrophes. Studies with transgenic mice have revealed that the expression of Pit-1 is governed by a cell-specific enhancer located in the upstream *pit*-1-regulatory region [9]. The action of the enhancer depends on the concerted action of Pit-1-positive autoregulatory sites, a cell-specific element and morphogen response elements. Autoregulation is not required for the initial activation of *pit*-1 gene expression, but it is needed for the maintenance of *pit*-1 gene expression following birth [9].

Myogenic transcription factors

Differentiation of mammalian skeletal-muscle cells is regulated by members of the MyoD family of myogenic transcription factors. These include MyoD [14] and Myf5 [15], which are believed to be responsible for the determined myoblast state, myogenin [16,17], which has a unique role in the transition to the fully differentiated myotube, and MRF4 [18–20], which controls

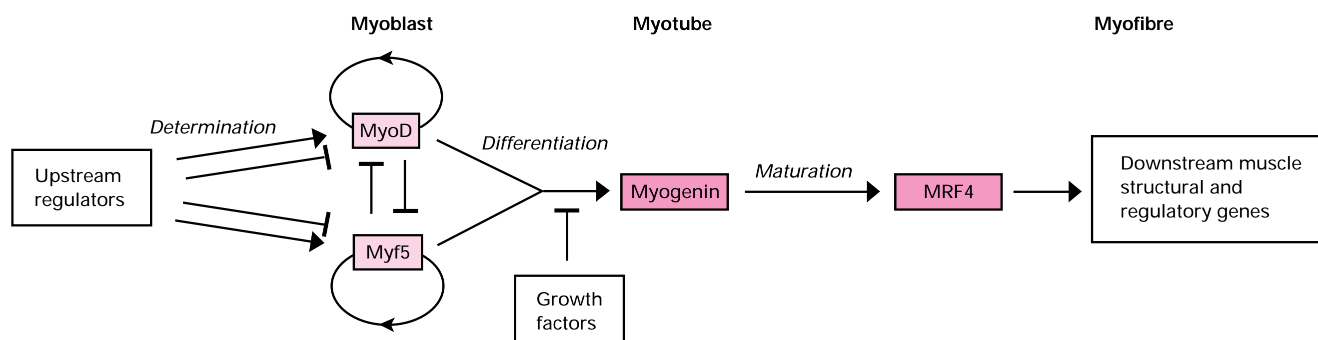

Figure 2 Regulatory circuit of myogenic transcription factors

Hypothetical regulatory circuit for murine myogenic genes comprising a core autoregulatory network of the MyoD transcription-factor family members MyoD, Myf5, myogenin and MRF4. The model implies that Myf5 and MyoD are primarily responsible for defining the myoblast state. Myf5 and MyoD autoregulate their expression, but may negatively regulate one another. Upon depletion of growth factors, MyoD and Myf5 activate myogenin, which induces the differentiated myotube state characterized by the expression myotube-specific genes. During myofibre maturation, MRF4 is up-regulated, which induces the myofibre-specific genes {adapted from Weintraub [21] and Olson and Klein [22]) and reproduced with the permission of the authors and the publishers (copyright Cell Press, 1993, and Cold Spring Harbor Laboraory Press, 1994, respectively)}.

the expression of myofibre-specific genes (reviewed in [21,22]). From experiments with transgenic mice it is inferred that MyoD and Myf5 act by turning on the myogenin gene [23–25]. In fibroblasts and various other cell types, the entire program of muscle differentiation can be turned on by transfection of MyoD or any one of the other members of the family [4,26–28]. Among the endogenous muscle-specific genes activated are the myogenic regulators themselves. These and other experiments have led to the postulation of an auto- and cross-regulatory network [21,22] (Figure 2). The positive-feedback loops tend to make expression of the genes self-sustaining. Maintenance of the determined myoblast in the undifferentiated state for a given length of time appears to be due to negative regulators that have MyoD and Myf5 as their primary targets. One of these inhibitors of differentiation, called Id, is, like MyoD, a helix–loop–helix (HLH) protein, but lacks the basic DNA-binding domain [29–31]. Dimerization with Id would prevent MyoD from binding to DNA. Triggering of the differentiation step may occur via inactivation of Id, allowing MyoD to heterodimerize with ubiquitous basic HLH (bHLH) proteins that potentate its activity, known as E-proteins. This results in activation of the downstream myogenin gene [32–34]. The myogenin gene product, which has been shown to be essential for muscle development *in vivo*, activates muscle-specific genes, inducing the differentiated myotube state [22,24,35]. Finally, upregulation of MRF4 results in the induction of myofibre-specific genes causing the maturation of the myotube into the myofibre [22,36]. Within the myogenic bHLH regulatory network, non-bHLH protein muscle-specific enhancer factors of the MEF2 family [37,38] participate in the autoregulatory circuits and in activation of muscle-specific genes [39–42]. The core regulatory network in which a cell type (myocyte)-specific family of transcriptional regulators (myogenic bHLH proteins) synergize with unrelated factors (MEF2) in the induction of determination and differentiation is strikingly analogous to the regulatory network in adipocyte development, where CCAAT/enhancer-binding proteins (C/EBPs) act in synergy with the unrelated peroxisome proliferator-activated receptors (PPARs) (see below).

C/EBP

The family of C/EBP transcription factors consists of several proteins of which at least one, C/EBPα, appears to be subject to autoregulation [8,11,43]. During cellular differentiation, each of the C/EBPs exhibits a temporal expression pattern which differs between gene types (α, β and δ) and cell type (adipocytes versus myelomonocytes). [44–46]. During hormone-induced differentiation of the 3T3-L1 adipoblast cell line into adipocytes, C/EBPδ and C/EBPβ have early catalytic roles leading to expression of C/EBPα concordant with the acquisition of the differentiated phenotype [44,46]. In differentiating myelomonocytic cells, expression of C/EBPα and C/EBPδ peaks during

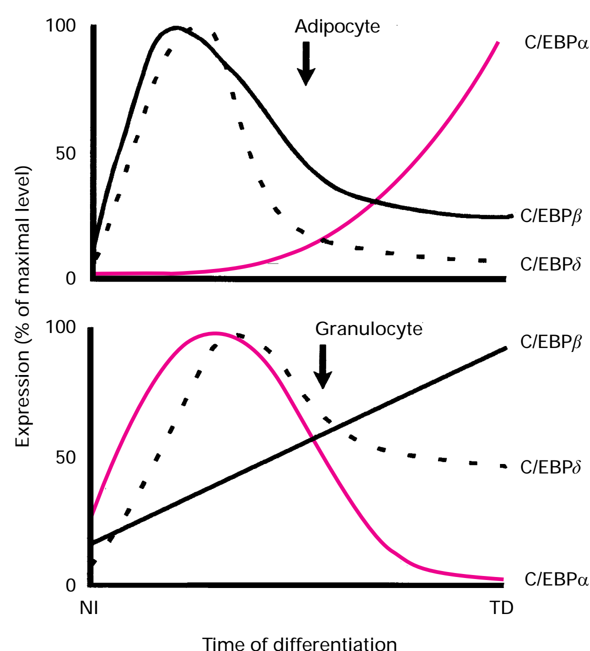

Figure 3 Temporal pattern of C/EBPα, C/EBPβ and C/EBPδ expression

Temporal patterns of C/EBPs expression during induced differentiation of 3T3-L1 preadipocytes (upper) [44] and 32D C13 myeloblasts (lower) [45]. The absolute levels of these proteins are unknown. Abbreviations: NI, non-induced; TD, terminally differentiated. Arrows indicate the approximate time when proliferation ceases in each pathway. This Figure is reproduced with the permission of A. D. Friedman [45] and the publishers (W. B. Saunders Co., Philadelphia, PA, U.S.A.).

the proliferative state and the expression of C/EBPβ increases throughout until the terminally differentiated stage of polymorphonuclear leucocytes (neutrophils) is reached (Figure 3) [45]. The members of the C/EBP family recognize the same DNA targets. The different patterns of C/EBP expression may reflect partial functional redundancy of the related C/EBPα and -β transcription factors, as well as specialization in which the differentiated stage-specific factor in the adipocyte is C/EBPα and that in the myelomonocyte is C/EBPβ. Interestingly, each C/EBPα and C/EBPβ/NF-M (nuclear factor-myeloid) [47], together with the proto-oncogene Myb, can act as a combinatorial switch inducing myeloid-specific gene expression in heterologous cell types [48]. Conditional ectopic expression of C/EBPβ in NIH-3T3 cells initiates adipogenesis, converting these multipotent precursor cells into pre-adipocytes [49]. The concomitant induction of PPARδ by C/EBP, and its activation by ligand, induces the subsequent differentiation into adipocytes. C/EBPα, which together with PPARδ is expressed late in the differentiation process, is thought to be responsible for the establishment of the quiescent, terminally differentiated state [49–55]. C/EBPs and PPARs may be the core of an autoregulatory network responsible for the commitment of the adipocyte phenotype in a way similar to the autoregulatory network of myogenesis. The different levels of the C/EBPs in adipocytes and myelomonocytes may be considered as alternative steady states of an autoregulatory circuit [13] in which an additional synergistic partner is required for the establishment of the specific phenotype.

Lessons from *Drosophila*

Temporal transcriptional regulation of closely related transcription factors and loops of autoregulation appear to be a recurring theme in development and cell differentiation. Cascades of sequential transcriptional control are the basis for early *Drosophila* development. Numerous homeobox-containing genes in *Drosophila* control their own expression by positive autoregulation. It is beyond the scope of this review to describe the complex pattern of transcription-factor expression in this organism. For trancriptional regulation in *Drosophila*, we refer the reader to other publications [2,3,57–59].

RNA splicing

Before the RNA transcript of a eukaryotic gene is translocated to the cytosol to become translated, the non-coding sequences (introns) interrupting the coding sequences (exons) have to be excised from the primary transcript or pre-mRNA by the process of splicing. Alternative splicing may be constitutive, generating always the same isofoms, or regulated, resulting in different isoforms depending on the cell type and circumstances. Both negative-acting protein factors preventing the use of a particular splice site, and positive factors directing the splicing at an unconventional splice site, may play a part [2]. A peculiar mechanism involving inhibition by antisense mRNA of an alternative splice site has been proposed for the thyroid receptor TRα. One of the TRα splicing variants would be specifically suppressed by a partially overlapping antisense transcript [60]. Alternative splicing of transcription factor pre-mRNAs may generate multiple mRNAs that differ in their coding regions, yielding polypeptides with different, often opposing, activities, or in their untranslated regions, affecting mRNA stability, translation efficiency or intracellular localisation [61–64].

Hepatocyte nuclear factor (HNF)

In the case of the HNF homeodomain proteins, HNF1 (=HNF1α) and the closely related, by a different gene encoded variant vHNF1 (=HNF1β), differential use of polyadenylation sites and alternative splicing causes the formation of different isoforms [65]. All these HNF1/vHNF1 isoforms can mutually homo- and heterodimerize to attain the DNA-binding state. The *trans*-activating isoforms HNF1-A, HNF1-B, HNF1-C and vHNF1-A/B differ in the composition of their C-terminal domain, resulting in different *trans*-activating potentials: HNF1-B and HNF1-C > HNF1-A. The vHNF1-C isoform lacks most of the C-terminal amino acid sequences present in the vHNF1-A/B isoforms and behaves as a *trans*-dominant repressor when co-transfected with each of the *trans*-activating isoforms. The isoform mRNA levels vary between cell types and during organ development, suggesting regulation of both differential polyadenylation and splicing [65].

CREM

The CREM [cAMP (cyclic AMP)-response element modulator] gene encodes a family of activating and repressing isoforms binding to cAMP-responsive promoter elements (CREs) of genes involved in neuroendocrine processes and spermatogenesis. The CREM gene is an example of a gene which is very extensively regulated with respect to transcription, RNA processing and post-translational modifications. The gene has a modular structure containing two alternative promoters, two alternative DNA-binding domains and different *trans*-activation domains (Figure 4) [66–68]. Pre-mRNA transcribed from the non-inducible promoter P1 is differentially spliced in a tissue-specific way. This results in the synthesis of activators and repressors with alternative DNA-binding and *trans*-activation domain compositions (Figure 4) [66,69]. For example, during spermatogenesis, a developmental switch from the transcriptional repressors CREMα, CREMβ and CREMδ to the activator CREMτ takes place. CREMτ contains two additional glutamine-rich regions (Q1 and Q2) responsible for *trans*-activation [61,70,71]. The shorter transcript generated from the cAMP-inducible P2 promoter is processed into mRNAs encoding small repressors, so-called ICERs (inducible cAMP early repressors) [68,72]. Rhythmic adrenergic signals sent by the suprachiasmatic nucleus cyclically activate the P2 promoter in the pineal gland by stimulation of the cAMP signal-transduction pathway [72,73].

Other examples of differently acting transcription-factor isoforms generated by alternative splicing are the isoforms of *Bombyx mori* GATAβ [74], the Wilms'-tumour susceptible gene product WT1 [75], the acute-myeloid-leukaemia gene product AML1 [76], the lymphoid transcription factor LyF-1 [77], the upstream stimulatory factor ('USF') [78], the activating transcription factor-3 ('ATF3') [79], I kappa B gamma [80], the octamer motif-binding protein Oct-1 [81], Oct-2 [82], the *Drosophila* chorion transcription factor CF2 [83] and activating protein-2 ('AP-2') [84]. In many of these cases the alternative splicing is developmentally and/or spatially regulated.

Degradation of mRNA

mRNA turnover is an important aspect in the control of gene expression. Eukaryotic mRNAs have different turnover times that range from days to minutes, and often are influenced by environmental signals. Among the less stable mRNAs are those that encode proteins expressed transiently in response to extra-

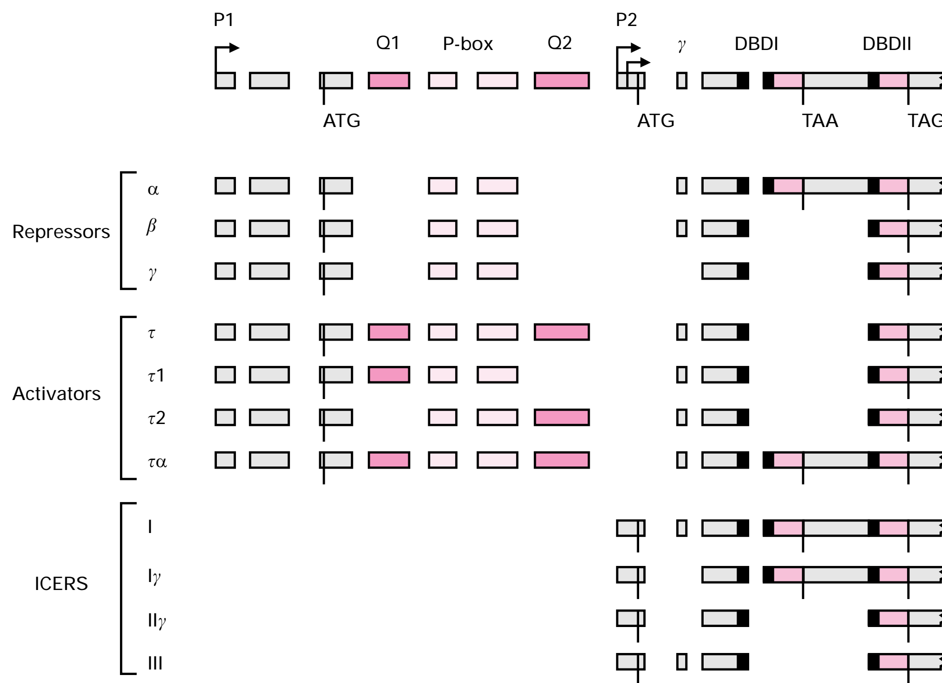

Figure 4 Activators and repressors from the CREM gene

Top: schematic representation of the CREM gene. Exons encoding the glutamine-rich domains (Q1 and Q2), the P-box, the δ-domain (δ) and the two alternative DNA-binding domains (DBDs) (DBDI and DBDII) are shown. Below the various activator and repressor isoforms which have been described to date are represented. The P1 promoter is GC-rich and directs a non-inducible pattern of expression. Also shown is the ICER family. All the ICER transcripts are derived from an internal start-site of transcription (P2) located between the Q2 and δ-exon. A family of four types of ICER transcript is generated by alternative splicing of the DBDs and δ-domain exons ICER-I, ICER-Iδ, ICER-II and ICER-IIδ. This Figure is reproduced with the permission of P. Sassone-Corsi [73] and the publishers (Oxford University Press, Oxford, U.K.).

cellular stimuli like growth factors, cytokines and transcription factors. Rapid mRNA degradation provides an efficient mechanism for transient protein expression because it links protein synthesis directly to the gene transcription rate. Unstable mRNAs may contain one or more specific sequences that stimulate their degradation [85,86].

Of the transcription factors, the proto-oncogene c-*fos* has been most extensively studied with respect to mRNA degradation. c-Fos (the c-*fos* gene product) is required during development and is induced rapidly and transiently by several extracellular stimuli [87,88]. Once synthesized, c-Fos mRNA is transported to the cytoplasm, where it is translated for only a brief period of time because of its rapid degradation [89]. c-Fos mRNA contains in its 3′-untranslated region (3′-UTR) a 75-nucleotide AU-rich element (ARE) containing the nonameric sequence UUAUUUAUU, which has been shown to be critical for mRNA destabilization [90]. The presence of the destabilizing element triggers de-adenylation, which is an early step in mRNA decay. The consensus sequence UUAUUUA(U/A)(U/A) is present within the 3′-UTRs of many labile mRNAs and functions as a transplantable element; when inserted into a stable heterologous mRNA such as β-globin mRNA, it destabilizes the mRNA in a copy-dependent manner [90,91]. The mechanism by which the ARE-containing mRNAs are further degraded is still unknown, but studies in yeast suggest that deadenylation is followed by decapping of the 5′ terminus, making the mRNA accessible to a specific $5' \rightarrow 3'$ exonuclease [85,92]. The short-lived mRNAs of two other transcription factors, c-Myc and c-Jun, which have AREs in their 3′-UTRs, are believed to be subject to the same degradation pathway [90,93]. Besides the determinant in the 3′-UTR, there is an additional instability determinant in the coding region of the c-Fos mRNA. This 0.32 kb Coding Region Determinant of mRNA Instability ('CRDI') also works as an independent mRNA destabilization determinant when incorporated in a heterologous, stable mRNA. The region facilitates mRNA deadenylation and decay by a mechanism coupled to translation [94–96].

Translation

Translational regulation of transcription factors, as of proteins in general, usually occurs at the initiation level, specifically at two steps: (a) selection of the mRNA for translation by the ribosomal complex and (b) linear scanning of the mRNA from the 5′-end by the ribosomal complex to select the translation initiation codon. In both steps several eukaryotic initiation factors (eIFs) are involved [97,98], two of which, eIF-4E and eIF-2, are known to be important players in the regulation of translation.

Initiation factor eIF-4E is the cap-binding protein; it exerts its function as part of a complex termed eIF-4F, which, in addition, contains eIF-4δ of unknown function and eIF4A, an RNA helicase. Initiation factor eIF-4E is sequestered in an inactive complex by the 4E-binding proteins 4E-BP1 (homologous with PHAS-1 [99]) and 4E-BP2 [100], blocking cap-dependent translation. Release of eIF-4E is mediated by mitogen-activated protein (MAP)-kinase-catalysed phosphorylation of 4E-BP1/2, which occurs in response to insulin and/or growth factors [99,100]. An interesting hypothesis, which may have implications for transcription-factor regulation, has been proposed by Proud

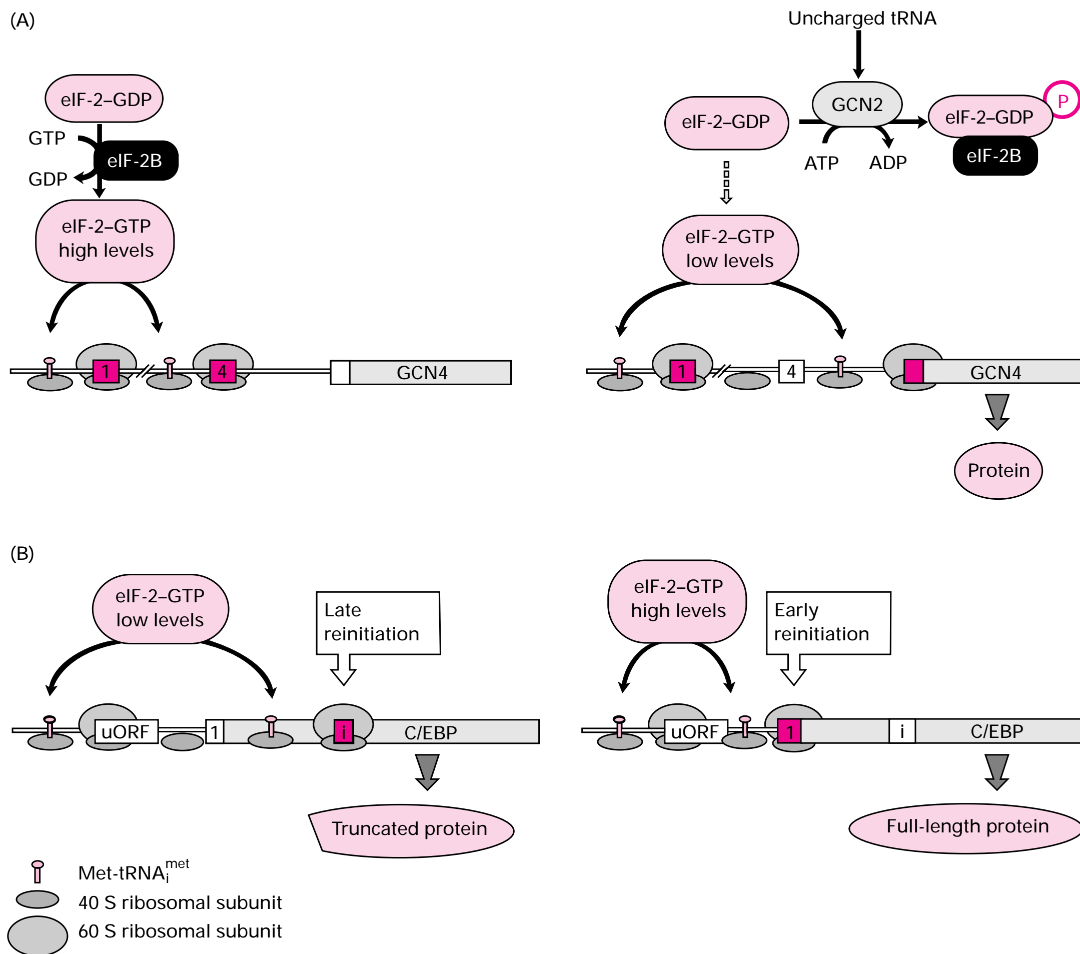

Figure 5 Translational regulation of GCN4 and C/EBP

(**A**) The Figure schematically depicts the action of eIF-2 on translation reinitiation at the GCN4 mRNA. Left: under non-starvation conditions eIF-2 is readily recycled by the guanine nucleotide exchange factor eIF-2B. The high level of eIF-2–GTP ensures frequent reinitiation at the upstream small ORFs with associated concomitant release of the small ribosomal subunit. Translation of the GCN4 ORF is thereby prevented. Right: under starvation conditions uncharged tRNAs accumulate, activating the eIF-2–GDP kinase GCN2. The phosphorylated eIF-2 sequesters the exchange factor eIF-2B in an inactive complex, resulting in low levels of active eIF-2–GTP. Ternary complex (eIF-2–GTP–Met-tRNA$_i^{met}$) assembly is delayed, causing by-passing of the upstream small ORFs and translation reinitiation at the GCN4 ORF {adapted from Hinnebusch [102]) and reproduced with the permission of the author and the publishers (Elsevier Trends Journals)}. (**B**) Hypothetical model for translational regulation of C/EBP mRNA. Analogous to the situation in yeast, high levels of eIF-2–GTP may cause efficient reinitiation after translation of the small upstream ORF, resulting in mainly full-length C/EBP protein (left). When eIF-2–GTP levels are low, delayed translation reinitiation may cause protein synthesis from an internal in-frame start codon (i), resulting in N-terminally truncated C/EBP protein (right).

[101], who stated that higher concentrations of eIF-4F would primarily facilitate the translation of mRNAs whose 5′-UTRs contain significant secondary structure. Interestingly, many mRNAs of regulatory proteins including transcription factors contain such highly structured CG-rich 5′-UTRs.

Modulation of translation initiation via eIF-2 in concert with a specific mRNA sequence is of particular interest because it controls the translation of at least one transcription factor, GCN4 [102], and possibly of the transcription factors C/EBPα and C/EBPβ as well. Initiation factor eIF-2 forms a ternary complex with GTP and methionyl-initiator-tRNA (Met-tRNA$_i^{met}$) and delivers the Met-tRNA$_i^{met}$ to the 40 S ribosomal subunit. Recycling of the eIF–2-GDP formed in the initiation step is inhibited by phosphorylation of eIF-2, sequestering the guanine nucleotide exchange factor GEF (eIF-2B) in an inactive complex with eIF–GDP [97,98,103–105]. Phosphorylation of eIF-2 occurs by specific kinases which are activated under various conditions, including the deprivation of growth factors, amino acid starvation, viral infection, heat-shock, insulin stimulation and entry into the M-phase of the cell cycle [2,102,106–108].

GCN4

The *Saccharomyces cerevisiae* GCN4 gene encodes a transcriptional activator regulating a set of genes engaged in amino acid and purine biosynthesis. In response to starvation, the concentration of GCN4 is up-regulated against an overall reduction of protein synthesis. Regulation occurs at the translational level and acts via the combined action of eIF-2 and four *cis*-regulatory short upstream open reading frames (uORFs) in the 5′ leader sequence of the GCN4 mRNA. Under normal growth conditions the translation of the GCN4 coding region is restricted because the ribosomal subunits scanning the GCN4 mRNA are detached in the translation of the successive uORFs (Figure 5A, left). Under starvation conditions eIF-2 activity is reduced, causing the ribosomes that resume scanning after

translation of the first uORF to ignore the subsequent small uORFs. This effectively enhances the chance of re-initiation at the downstream GCN4 ORF, because at the time the ribosome reaches the distant GCN4 start codon the ternary complex necessary for translation initiation has reassembled (Figure 5A, right) [102,109–112].

C/EBP

C/EBPα and C/EBPβ play a decisive role in the differentiation of a number of cell types, including adipocytes, hepatocytes, enterocytes and myelocytes [44,45,49,51,55,113–115]. The effects of C/EBPα and C/EBPβ proteins appear to be dual: firstly they induce the expression of tissue-specific genes in concert with other transcription factors, and secondly they can evoke growth arrest [51,116,117]. The magnitude of the transcriptional activation by C/EBPα and C/EBPβ transcription factors is modulated by the relative expression of two protein isoforms, a full-length and an N-terminally truncated isoform, both translated from the same mRNA [118–121]. The full-length isoform is a potent transcriptional activator in hepatocytes and adipocytes, and the truncated isoform acts as a repressor, or an activator with low activity, depending on the promoter context [118–122]. The generation of N-terminally truncated isoforms of C/EBPα from internal start codons depends on the presence of a small uORF, conserved in evolution, mediating delayed translation reinitiation [121]. The analogous organization of the C/EBPβ mRNA suggests that the generation of different translational isoforms from this particular mRNA may be governed by a similar mechanism [121].

We hypothesize that the C/EBP isoform ratio may be regulated by translation initiation factor eIF-2. Like the uORFs in GCN4, the C/EBP small uORF may actually sense the activity of eIF-2. Low eIF-2 activity would promote the ribosomes, which resume scanning after having read the uORF, to ignore the proximal C/EBP initiation codon and start at an internal AUG, yielding the truncated C/EBP isoform (Figure 5B, left). High eIF-2 activity would suppress 'leaky' scanning across the first AUG of the C/EBP coding region, as well as the associated internal initiation (Figure 5B, right). Such an eIF-2-sensing system would provide an interesting coupling between, on the one hand, growth and other factors (see above) that effect eIF-2 phosphorylation (and thus activity), and, on the other hand, C/EBP as a regulator of cell-type specific gene expression and growth control. Interestingly, the formation of the full-length C/EBP isoform from a transfected C/EBPα gene in COS cells is promoted by serum addition (C. F. Calkhoven and G. AB, unpublished work), a condition known to increase eIF-2 activity [106]. Moreover, insulin is known to stimulate eIF-2 activity [108] and causes a dramatic and rapid change in the C/EBPβ and a modest change in C/EBPα isoform ratio to the benefit of the full-length products in differentiating adipocytes [123]. C/EBP isoform ratio modulation may provide a mechanism for metabolic and hormone-imposed adaptation of C/EBP target genes. This is interesting in the light of the central roles that C/EBPα and C/EBPβ play in gluconeogenesis and lipogenesis, in liver and fat tissue [44,114,124–127]. Additionally, translational regulation via eIF-2 may be responsible for the temporary down-regulation of the full-length C/EBPα isoform in the M-phase of the cell cycle which is observed during liver regeneration [43].

c-*myc*

The proto-oncogene c-*myc* is a transcription factor having an important role in control of cell growth. The 5′ mRNA leader sequence of murine and mice c-*myc* contains, besides an efficiently used AUG translation start codon, an upstream in-frame non-AUG start codon, CUG [128,129]. In cultured cells upon methionine depletion, the translation of c-*myc* shifts from the AUG codon to the upstream CUG codon. Although the physiological function of the two isoforms is not yet established, it could reflect a switch from cell growth promotion mediated by the smaller isoform to inhibition of cell growth mediated by the larger isoform [129]. Speculation on how the translational shift under methionine deprivation is brought about focus again on a role for eIF-2 in selecting the non-AUG codon [129]. An alternative explanation may be found in the CAP-binding eIF-4F complex, because increased levels of eIF-4F promote CAP-proximal AUG codon usage in cell-free extracts [130].

Retinoic acid receptor β2 (RARβ2)

The complex 5′-untranslated region containing five partially stacked uORFs of the mRNA is responsible for tissue-specific synthesis of its RARβ2 during mouse development. Transgenic mice containing an RARβ2–*lac*Z fusion construct including the 5′-UTR express no protein from the transcribed mRNA in heart and brain. By mutating part or all of the uORFs, the tissue-specific RARβ2 protein synthesis is lost, resulting in expression in heart and brain. The main conclusion drawn from this study is that uORFs in 5′-UTRs in combination with tissue-specific regulation of initiation factor level and/or activity play an important role in regulation of the tissue-specific expression of this regulatory protein [131].

Translation regulation based on start site selection has been implicated in the expression of other critical transcription factors involved in growth and differentiation control [132], e.g. isoforms of the rat hepatic leukaemia factor ('HLF') with different circadian levels, tissue distributions and target preferences [133], and the developmentally-regulated isoforms of the CREM isoforms, the CREMτ activator and the S-CREM repressor [134].

In by-passing the nucleus and relying on *cis*-regulatory elements in the mRNA proper, translation regulation enables fast and co-ordinated responses to external stimuli and provides an additional regulatory checkpoint [132,135–138]. Until now, the role of special structural mRNA features in translation regulation is far from clear. Future experiments should clarify their function and the mechanisms involved.

REGULATION OF TRANSCRIPTION-FACTOR ACTIVITY

Once a transcription factor has been synthesized, its activity can be controlled in a variety of ways, for instance by post-translational modification, for example phosphorylation or by binding of a ligand. These processes induce conformational changes in the transcription factor, exposing, masking or remodelling a particular domain. Another means of activity modulation is by specific protein–protein interaction, either with a member of a related family of transcription factors to form a DNA-binding dimer, or with an unrelated factor.

Post-translational modification by phosphorylation

Transcription factors are important final targets of signal-transduction pathways in which transient signals generated by stimulation of cell-surface receptors are transmitted via phosphorylation cascades to the nucleus. An important facet of this type of modification is that it is reversible.

There are several ways by which phosphorylation can regulate a transcription factor. Firstly, sequestration of the transcription factor in an inactive complex, e.g. by an anchor protein, can be regulated. Phosphorylation or dephosphorylation of the tran-

scription factor or of its anchor protein may result in dissociation of the complex, allowing translocation of the transcription factor to the nucleus. Secondly, phosphorylation may modulate the DNA-binding activity and/or the *trans*-activation potential of the transcription factor (reviewed in [139,140]).

Sequestration

The first example concerns the SW15 protein of *Saccharomyces cerevisiae*, a transcription factor regulating cell-cycle-dependent expression of the specific HO endonuclease involved in mating-type switching. SWI5 localization is thought to be regulated by phosphorylation of three sites within or near the NLS through the cell-cycle regulatory protein kinase Cdc28 in conjunction with an activating cyclin subunit. In the G1-phase, SWI5 is located in the nucleus and activates HO gene transcription. During the other phases of the cell cycle, the SWI5 protein is sequestered in the cytoplasm as a consequence of the phosphorylation of the NLS-proximal Cdc28 sites [141,142].

The second example concerns NF-κB of the Rel-related family of transcription factors [143–145]. NF-κB is ubiquitously expressed, but in most cells it is sequestered in the cytoplasm as an inactive complex with the inhibitory protein IκB, probably by masking of the NLS [146]. The IκB protein contains so-called ankyrin-like ('ANK') repeat motifs which are believed to be involved in protein–protein interactions [147,148] with the p65 subunit of NF-κB. In response to various signals such as mitogens, cytokines, viral double-stranded RNA and oxidative stress, IκB is inactivated by phosphorylation, triggering its degradation by the ubiquitin–proteinase pathway [149–153]. This liberates NF-κB, which then is translocated to the nucleus, where it can activate gene expression by binding to κB enhancer and promoter motifs.

DNA-binding and *trans*-activation

Both the DNA-binding and *trans*-activation functions of a transcription factor may be regulated by phosphorylation, either positively or negatively. An illustrative example is c-Jun, which is phosphorylated at five sites. Three of these are located just N-terminal to the DBD and are phosphorylated by a constitutively active kinase CKII, causing inhibition of DNA binding [154, 155]. Phorbol ester (phorbol 12-myristate 13-acetate), growth factors or expression of transforming oncogenes stimulate an unknown phosphatase which dephosphorylates c-Jun and increases its DNA-binding activity [139,154]. Phosphorylation-dependent DNA-binding activity is observed for many other transcription factors, for example Max [156], Oct1 [157] and SRF [158].

The two other phosphorylation sites of c-Jun, located in the N-terminal *trans*-activation domain, are phosphorylated in response to mitogenic stimulation and stress by distinct MAP kinases, c-Jun N-terminal kinases ('JNKs') and stress-activated protein kinases ('SAPKs'), resulting in elevated *trans*-activation potential [139,159–165].

Whereas c-Jun is activated by phosphorylation of the activation domain proper, C/EBPβ is activated in a different way. [166,167]. In chicken C/EBPβ (NF-M), the N-terminally located *trans*-activation domain is caught in an intramolecular interaction with inhibitory domains. Phosphorylation of these inhibitory domains liberates the *trans*-activation domain, inducing the *trans*-activation function [167]. One of the signal-transduction pathways leading to derepression of C/EBPβ includes MAP kinases that phosphorylate a conserved MAP-kinase site in the C/EBPβ proteins [166]. For murine C/EBPβ a slightly different model was proposed in which both the exposure of the *trans*-activation domain and the DNA-binding domain is regulated by intra-molecular interactions with independent regulatory domains. However, involvement of phosphorylation in this system has not been established [168]. Other conserved phosphorylation sites mapped are a serine in the leucine-zipper dimerization region of C/EBPβ that can be phosphorylated by Ca^{2+}-calmodulin-dependent protein kinase II ('CaMKII') conferring calcium-regulated stimulation of transcriptional activity [169], and a conserved serine in the DNA-contacting region of C/EBPs the phosphorylation of which by protein kinase C attenuates DNA-binding of C/EBPα *in vitro* [170].

It will be interesting to combine these biochemical studies with structural analysis of the differently phosphorylated transcription factors to see what the conformational consequences of these reversible phosphorylations are.

Ligand-dependent activation of nuclear hormone receptors

Lipophilic hormones, such as steroids, retinoids, thyroid hormones, vitamin D_3 and eicosanoids, are potent regulators of transcription. They exert their function within target cells by binding to specific intracellular receptors which function as ligand-activated transcription factors. Although the various ligands are chemically very different, the receptors exhibit a remarkable overall structural unity that permits their classification as one large family, namely the superfamily of nuclear receptors. The family also includes members with no apparent ligand, so-called 'orphan receptors'. Some of these may turn out to interact with novel ligands, while others may be constitutive factors. Excellent reviews on nuclear receptors have appeared recently [171–179]. Before we discuss the mechanism(s) of ligand-dependent activation, the common molecular design of nuclear receptors in terms of structural and functional domains will be described.

The N-terminal domain (A/B) exhibits little sequence similarity across the superfamily and is variable in length. The domain contains a ligand-independent *trans*-activation function with marked cell type and promoter specificity [180].

The most conserved central domain (C), which contains two zinc-co-ordinated modules ('zinc fingers') that fold together to a compact structure [181–185], functions as the DBD that targets the receptor to specific DNA sequences known as hormone response elements (HREs). The minimal target sequence recognized by the DBD consists of a six-base-pair sequence, the core recognition motif PuGGTCA (where Pu is a purine). Naturally occurring response elements frequently contain two copies (half-sites) of the core recognition motif, indicative of the fact that most nuclear receptors function as dimers, i.e. homo- or heterodimers. Receptor-specific differences in the contacts between DBDs permit the formation of a limited number of homodimeric or heterodimeric combinations. The precise sequence, orientation (direct versus inverted repeats) and spacing of the half-sites determine for which dimeric receptor combination an HRE is the target (see [186] and references cited therein). Dimerization between the DBDs results in a co-operative increase in the specificity and affinity of DNA binding. Recently, some orphan receptors recognizing their target DNA sequence in a monomeric mode have been identified [187–190]. In these cases, the affinity and specificity of DNA binding appears to be enhanced by a C-terminal extension of the zinc-finger domain contacting an extension of one to three base-pairs immediately 5′ of the core recognition motif [189,190].

C-terminal to the DBD, connected by a flexible hinge region (D), is the ligand-binding domain (LBD) formed by a large,

hydrophobic, moderately conserved region (E). Together with the ligand-binding function, several other functions are integrated within this domain, including strong dimerization, *trans*-activation, nuclear localization and (in some cases) heat-shock-protein binding. The mechanisms by which ligands induce the receptors appear to be different, depending on the nuclear receptor class.

Steroid-hormone receptors

The steroid-hormone receptors, which function as ligand-induced homodimers (head-to-head configuration) and bind to inverted repeats of the core motif separated by three nucleotides, are associated with a large multiprotein complex of chaperones (Hsp90 and other heat-shock proteins) prior to hormone binding (reviewed in [191]). Binding of the hormone induces a conformational change of the LBD, causing dissociation of the multiprotein complex and exposure of the LBD/dimerization interface. The dimeric receptor can then bind to its palindromic HRE, consisting of AGGTCA or AGAACA half-sites for the oestrogen and the other steroid (glucocorticoids, mineralocorticoids, progesterone, androgen) receptors respectively [192–195]. The role of the chaperones is not simply sterical blocking of DNA binding; they are believed to keep the receptor in a poised, ligand-sensitive state, and help to fold the receptor in its transcriptionally active conformation. The transition exposes a ligand-dependent *trans*-activation function, AF2, which is located in the C-terminus of the LBD and appears to communicate with the transcription initiation complex via co-activators or bridging factors: RIP140 [196], RIP160/ERAP160 [197], TIF1 [198], Trip1 [199,200] and SRC-1 [201].

Non-steroid receptors

Unlike steroid receptors, the non-steroid-hormone receptors do not bind to heat-shock proteins and associate with DNA in the presence and absence of their respective ligands [191,202,203]. Although some of these receptors can also bind as homodimers, high-affinity binding of the RAR, the thyroid receptor (TR), the vitamin D_3 receptor (VDR) and the PPAR to their cognate HREs requires heterodimerization with the retinoid X receptor (RXR) [204–212]. The most potent of these HREs are direct repeats (DRs) of the AGGTCA half-site for which receptor specificity is determined by the so-called '1-to-5 rule' specifying the optimal half-site spacing of 1, 2, 3, 4 and 5 nucleotides for the PPAR, RAR, VDR, TR and RAR response elements respectively [176–205]. Structures of the unliganded human RXRα domain [213] and the liganded domains of the human RARδ [214] and TRα [215] receptors have recently been solved by X-ray crystallography. While the overall folds are similar, the unliganded and liganded domains show interesting differences that may not represent inherent differences between the receptor types but rather reflect ligand-induced conformational changes. Whereas the unliganded hRXRα DBD contains internal hydrophobic cavities, the ligand-bound LBD structures are more compact, with the hormone tightly packed within the core of the domain. In the unliganded receptor, the C-terminal amphipathic α-helix harbouring the transcriptional activation domain AF-2 protrudes from the LBD into the solvent. In the liganded domain, the particular α-helix is packed on to the body of the LBD, with the hydrophobic face contributing part of the hormone-binding cavity. The ligand-induced repositioning of AF-2 may facilitate the formation of transcriptionally active complexes with co-activators like those interacting with the steroid receptors.

The presence of receptor molecules with different ligand specificity within one and the same dimer raises the question of whether a heterodimeric receptor can be activated by both ligands and, if so, whether they act synergistically. Dual ligand-sensitivity of all the RXR-containing heterodimers would create a specificity problem, in the sense that the heterodimeric receptors would be activated by the RXR ligand 9-*cis*-retinoic acid (9-*cis*-RA) in addition to the cognate ligand of the dimerization partner. The actual situation appears to be complex and to be differently dependent on the particular receptor combination [216] (reviewed in [179]). Dual synergistic activation occurs in case of the PPAR/RXR heterodimer. In contrast, ligand-induced transcriptional activity of RXR by 9-*cis*-RA is suppressed when it is complexed with VDR, TR or RAR. The formation of the RXR/RAR heterodimer actually prevents the RXR subunit from binding to its ligand. These observations show that allosteric interactions among heterodimer partners create complexes with unique properties. Also interesting in this respect is the finding that the orphan receptor NGF1-B, which is capable of binding as a monomer to DNA, can form a complex with RXR that is responsive to 9-*cis*-RA. Interestingly, DNA contact by RXR is not required for this effect [216].

In contrast with the steroid receptors, the unliganded TR and RAR bind as heterodimers with RXR to their cognate DNA sites and silence active promoters [217,218]. The suppressing action is mediated by co-repressors which bind to the unliganded receptors [219,220]. Recently two co-repressors, called NCoR and SMRT, have been cloned and further characterized [221–225]. Whether ligand binding is able to trigger the activation process depends on the half-site spacing of the binding site. On a DR5 site ligand binding results in activation, whereas on a DR1 site repression is maintained [226] (Figure 6). This difference finds its cause in the

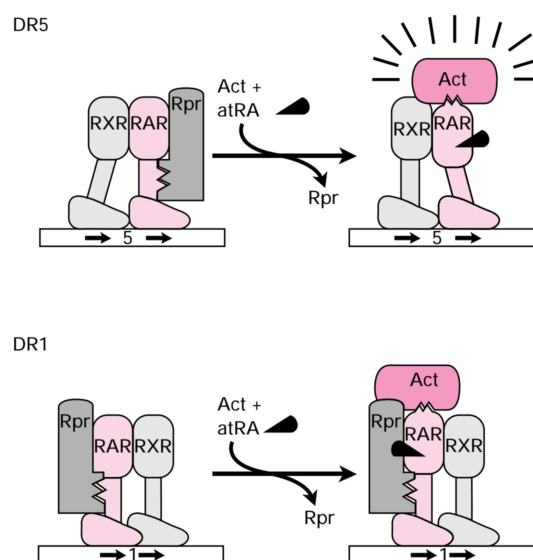

Figure 6 Allosteric control of nuclear receptor activity

The Figure shows a model for allosteric control of the nuclear receptor heterodimer RXR/RAR. Binding of the heterodimers to direct repeats follows strict polarity; if bound to a direct repeat spaced by five nucleotides (DR5), the polarity is 5′-RXR/RAR-3′; at DR1 the polarity is 5′-RAR/RXR-3′. In the absence of the ligand all-*trans* RA (atRA), the co-repressor (Rpr) is associated mediating repression on both DR5 and DR1. Ligand binding induces the recruitment of the co-activator (Act) and on the DR5 dissociation of the co-repressor (Rpr), leading to transcriptional activity. On the DR1, by contrast, the co-repressor is unable to dissociate, keeping the bound complex transcriptionally inactive {adapted from Perlmann and Vennström [225] and reprinted with permission from *Nature* [Copyright (1995) Macmillan Magazines Limited] and the authors}.

different polarities of the receptors in the heterodimer. On a DR5 site the RXR is in the upstream position, whereas on a DR1 site the orientation is reversed. In case of the heterodimer with the RXR/RAR (DR5) polarity, ligand binding results in the release of the co-repressor and binding of the co-activator, whereas with the heterodimer with the reversed polarity the co-repressor stays attached despite the fact that ligand and co-activator binding still takes place [224]. The co-repressor acts dominantly over the co-activator. These observations reveal allosteric interactions between the receptor subunits whose precise effect depends on the particular anisotropic configuration of the heterodimer imposed by the half-site spacing of the binding site. Interestingly the binding site for the co-repressor is located in the hinge region between the DBD and LBD of the RAR or TR; no interaction occurs with the RXR subunit. The hinge region must possess considerable flexibility to accommodate the head-to-tail orientation of the DBDs with the supposed head-to-head orientation of the LBDs on the DNA [213]. It could be envisaged that the binding-associated compaction of the LBD changes the LBD configuration in the dimer, since the DBDs are in a fixed configuration on the DNA. This would result in a conformational change of the hinge region and release of the co-repressor. Such a scheme would imply that the ligand-induced conformational change in the RAR or TR hinge region only occurs when these receptors are in the downstream position.

Protein–protein interactions

The activity of a transcription factor may be affected by the interaction with other proteins, which can be either DNA-binding proteins or non-DNA-binding accessory proteins. Both interactions with transcription factors of the same or a different family of DNA-binding proteins are possible. One example, the dimerization between related members of the nuclear-receptor family has already been discussed in the previous section. Alternatively, interaction takes place with non-DNA factors (accessory factors) that may mediate a divers range of functions, e.g. acting as a 'bridging factor' between the transcription factor and the basal transcription machinery, stabilizing the DNA-binding complex or changing the specificity of the target sequence recognition.

The basic-zipper (bZIP) DNA-binding proteins

The first well-characterized dimerization domain was the leucine zipper, an α-helix characterized by a heptad repeat of leucine residues [227–229]. When two such helices form a parallel coiled coil, the adjacent, positively charged, DNA-contacting regions are positioned in the proper orientation for DNA binding [230–232]. The subfamilies of C/EBP, Fos, Jun and ATF/CREB transcription factors all depend on a bZIP domain for DNA binding [233]. Within the C/EBP subfamily, the bZIP domain is highly conserved; all members can interact with each other, yielding a variety of dimers with very similar DNA-recognition characteristics. A special C/EBP protein called C/EBP-homologous protein CHOP (=C/EBPζ), which dimerizes avidly with other C/EBP proteins, has an unusual amino acid in its DNA-binding motif rendering it unable to bind to the classical C/EBP binding consensus [234]. Since heterodimers containing CHOP cannot bind to C/EBP-sites, CHOP acts as a dominant negative regulator of C/EBP DNA binding. CHOP is induced by a variety of cellular stresses caused by toxins, nutrient deprivation and metabolic inhibitors [234–237]. Its induction inhibits adipogenesis and attenuates C/EBPα and C/EBPβ gene expression, possibly by interference with the autoregulatory C/EBP cascade at an early stage [238].

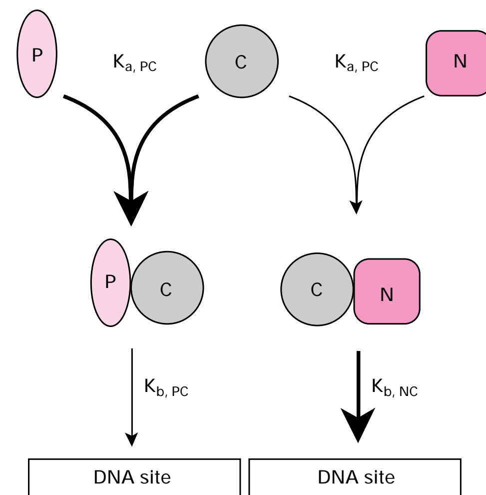

Figure 7 Theoretical model of dimerization-generated ultrasensitivity

The potency of the positive component P and the negative component N as a heterodimeric partner of the central component C for stimulating or repressing transcription is determined by their ability to associate with C and with the DNA target site. Ultrasensitivity can be obtained when, for example, the P–C association constant is higher than the N–C association constant ($K_{a,PC} > K_{a,NC}$) and the DNA–PC binding constant is lower than the DNA–NC binding constant ($K_{b,PC} < K_{b,NC}$). In this case, the P component may easily displace the N component from the C component. Nevertheless, as long as there is still repressor dimer (NC) present, the DNA target cannot be bound by the activator dimer (PC), which exhibits lower affinity for DNA. A near-maximal transcriptional response is induced abruptly, like a molecular switch, only if the majority of C molecules are bound by P in the PC dimer. For example, in the case of a 100-fold difference between $K_{a,NC}$ and $K_{a,PC}$, and between $K_{b,PC}$ and $K_{b,NC}$, a 2-fold increase in P concentration would give a shift from 10 to 90% of the maximum response [239]. (Reproduced with the permission of Swillens [239] and the publishers).

Heterodimerization of transcription factors can provide an explanation for the ultrasensitivity to fluctuations in the effector concentration. In a model outlined by Swillens and Pirson [239] ultrasensitivity can be generated by the reversible coupling of a central component (C) with low affinity to a positive acting (P) and high affinity to a negative acting (N) factor. Ultrasensitivity is obtained if the NC complex has a higher affinity for DNA than the PC complex (Figure 7).

A similar model may help us to understand the observed ultrasensitivity of C/EBP activity to the concentration of small inhibitory C/EBP isoforms [118,121]. Homodimers of small C/EBP isoforms, and probably heterodimers of small/large isoforms too, have higher DNA-binding affinities than homodimers of large isoforms [118,240]. This, with the assumption of a high dimerization constant for dimers containing the large isoform and a lower dimerization constant for the homodimer of the small isoforms, would theoretically explain the ultrasensitivity for small-isoform levels.

Transcription-factor supracomplexes

bZIP protein dimers are often cross-coupled to other transcription-factor dimers in so-called 'transcription-factor supracomplexes' [241]. Supracomplexes between bZIP and Rel transcription factors have physiological functions and DNA-binding characteristics distinct from those of the individual transcription-factor partner [241–243]. C/EBP-Rel supracomplexes do not bind to κB Rel-protein binding sites, but do bind to C/EBP binding sites with the Rel proteins not directly contacting the DNA [244]. The inability of C/EBP–Rel supracomplexes to bind κB sites is consistent with the observed repression of κB-

dependent gene expression by C/EBP [243]. In another bZIP–Rel supracomplex of this kind, AP-1 proteins stabilize the interaction of nuclear factor of activated T cells (NF-AT) with weak NF-AT DNA sites [241,245,246]. Another example of transcription factor cross-coupling in which bZIP proteins play a part is the interaction between AP-1 transcription factors and the glucocorticoid receptor at a 'composite' glucocorticoid response element (GRE) [247]. Interaction between the GR or c-Jun homodimer strongly enhances promoter function, whereas interaction between the GR and the c-Jun–c-Fos heterodimer appears to repress promoter function [247]. Supracomplex formation between c-Jun and MyoD via their respective dimerization domains has been shown to inhibit the function of both proteins [248,249].

Co-activators and -repressors

Most transcription factors mediate diverse effects depending on cell type and the presence or absence of a particular stimulus. The effects depend on accessory proteins or co-factors that are expressed in a tissue-restricted manner and/or interact only with a particular state of the transcription factor. Clear examples of such co-factors are the co-activator and co-repressor proteins interacting with the nuclear receptor dimers as discussed in the previous section 'Ligand-dependent activation of nuclear hormone receptors'. Some other examples of what undoubtedly will become a growing group of transcription factor–cofactor interactions are discussed below.

CREB-binding protein

Signals that increase intracellular concentrations of cAMP activate genes that contain CREs. Target gene activation is mediated by the transcription factor CREB, which is activated by phosphorylation in response to the cAMP signal. The activation is not caused by a change in the intracellular localization, DNA binding or intrinsic *trans*-activation potential of CREB, but by recruitment of a co-activator CREB-binding protein (CBP). CBP only interacts with the phosphorylated CREB and functions as a bridging factor to the basal transcription factor TFIIB and is required for the recruitment of an active polymerase II complex [250–254].

Tax

Human T-cell leukaemia virus type I (HTLV-I) Tax proteins increase the DNA binding of many cellular transcription factors that contain a bZIP DNA-binding domain, including GCN4, ATF, AP-1, CREB and C/EBP. Tax interacts with the basic DNA-contacting region, increasing dimer stability and affinity for DNA. Tax also alters DNA-binding-site selectivity. Both effects are probably important for the ability of Tax to recruit the appropriate cellular bZIP proteins to the HTLV-I long terminal repeat during viral infection. The 'promiscuous' activation of cellular genes by the recruitment of bZIP proteins is probably the basis for Tax's oncogenic activity [255–257].

Dimerization cofactor of HNF-1 (DCoH)

The homeodomain protein HNF-1α regulates the expression of a large number of genes in the liver. For maximal transcriptional activation, the HNF-1α dimer requires the co-activator dimer DCoH. Although DCoH does not alter HNF-1α DNA affinity, it strongly enhances its transcriptional activity. How this is achieved is not known, but one of the effects is that it stabilizes the HNF-1α dimer in solution via interaction with the HNF-1α dimerization domain. The intriguing aspect about DCoH is that it appears to be a bifunctional protein. DCoH was characterized as pterin-4a-carbinolamine dehydratase (PCD), a protein component of the phenylalanine hydroxylation system. PCD deficiency may be correlated with certain hyperphenylalaninaemias in children. Whether the dehydratase activity is also essential for the HNF-1α transcriptional enhancement is not known [258–261].

CONCLUSIONS AND PERSPECTIVES

In this review we have outlined control points in the expression of transcription factors. For these regulatory proteins collectively, any step in the complete sequence leading from the encoding gene to the active transcription factor may potentially be subject to control. For a given transcription factor, regulation is often exerted at more than one step. A clear example is C/EBP, which, during the course of cell differentiation, is primarily controlled at the transciptional level. In addition, phosphorylation of the protein at specific sites provides links to signal-transduction pathways and translation regulation may be important in the response to metabolic signals. Finally, dimerization may affect the specificity and affinity of DNA binding and potentially integrates the regulatory pathways of both partners. Another example of multiple control is provided by the nuclear receptor family, where the restricted presence of the receptor in its target cell is determined at the transcriptional level, the actual activation occurring through the binding of a ligand, and further modulation of the activity may be achieved by the dimerization partner and phosphorylation [262,263]. There is probably a hierarchy in the importance of the control steps for a particular transcription factor, some of which may mainly serve for fine tuning by coupling to other regulatory pathways.

Gene duplication and mutation has generated a great deal of diversity within transcription-factor families, which may serve the purpose of redundancy as well as specialization. Regulatory diversity is further extended by processes like alternative splicing, translation-start-site multiplicity, phosphorylation and protein–protein interaction.

Insight into the action and regulation of transcription factors has been obtained by a variety of techniques. The recent results on transcription factors by X-ray crystallography and NMR methods has allowed us to project earlier, often fragmentary, data on to the three-dimensional structure. In the future more will be learned of how different regulatory pathways involving transcription factors are coupled. Our present knowledge of transcription-factor regulation is probably unbalanced, with a bias against translational regulation. We expect that future attention will be drawn more towards this level of regulation and how it regulates transcription factor activity.

Because of the width of the field we were forced to make a selection of the topics reviewed. Some interesting transcription factors and regulatory pathways are left undiscussed, e.g. proteins with unconventional properties like lactoferrin, which acts as a secretory transcription factor [264], the redox regulation of DNA-binding activity observed for many transcription factors ([265–267] and references therein), the activation of the 'STAT' (signal transducers and activators of transcription) family by cytokines [268] and transcription-factor gradients in embryonic development [59,269].

For a better understanding of the actual potential of a transcription factor in the cell, more detailed quantitative analyses will be necessary. Parameters like dimerization constants, DNA affinity constants, precise concentrations of transcription factors and threshold levels of transcription-factor concen-

trations may be used to generate mathematical models of gene regulation. These models will help to explain the dynamics and kinetics of transcription-factor action in gene expression and to generate new ideas.

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