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Gene regulation in the immediate-early response process

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ABSTRACT

Immediate-early genes (IEGs) can be activated and transcribed within minutes after stimulation, without the need for *de novo* protein synthesis, and they are stimulated in response to both cell-extrinsic and cell-intrinsic signals. Extracellular signals are transduced from the cell surface, through receptors activating a chain of proteins in the cell, in particular extracellular-signal-regulated kinases (ERKs), mitogen-activated protein kinases (MAPKs) and members of the RhoA-actin pathway. These communicate through a signaling cascade by adding phosphate groups to neighboring proteins, and this will eventually activate and translocate TFs to the nucleus and thereby induce gene expression. The gene activation also involves proximal and distal enhancers that interact with promoters to simulate gene expression. The immediate-early genes have essential biological roles, in particular in stress response, like the immune system, and in differentiation. Therefore they also have important roles in various diseases, including cancer development. In this paper we summarize some recent advances on key aspects of the activation and regulation of immediate-early genes.

Contents

Abbreviations: IER, immediate-early response; IEG, immediate-early gene; PRG, primary response gene; SRG, secondary response genes; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; SRF, serum-response factor; NF-kB, nuclear factor-kB; CREB, cyclic AMP response element-binding protein; AP-1, activator protein-1; TCF, ternary complex factor; ERK, extracellular signal-regulated kinase; MAPK, Mitogen-activated protein kinases; ELK1, E26-like kinase; MRTF, myocardin related transcription factor; NF1, nuclear factor 1; PARP1, Poly (ADP-ribose) polymerase 1; RSK, p90 ribosomal S6 kinase; JNK, c-Jun N- terminal kinase; ERK5, extracellular signal regulated kinase-5; BMK1, Big MAP kinase-1; MSK, Mitogen/stress activated protein kinase; RNA Pol II, RNA polymerase II; GO, Gene Ontology; TBP, TATA binding protein; TSS, Transcription Start Site; HAT, histone acetyl transferase; IRF3, interferon regulatory factor 3; TLR, Toll-like receptor; NGF, nerve growth factor; G protein, guanine nucleotide binding protein; TF, Transcription Factor; MKL, megakaryoblastic leukemia; ESC, embryonic stem cells; DSIF, DRB sensitivity-inducing factor; NELF, negative elongation factor; P-TEFb, positive transcription elongation factor; CTD, C-terminal domain; eRNA, enhancer RNA.

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1. Introduction

Regulation of gene transcription is one of the main mechanisms that are used by cells to increase or decrease the concentration of specific gene products (RNA and protein) (Lewin, 2004). Gene transcription is controlled through many layers of regulation, where the choice of specific pathways affects the timing of induced gene expression as a response to an external signal. A specific group of genes seems to be able to respond very quickly to regulatory signals, for example in immune responses or cellular stress. Such processes are often known as immediate-early response (IER) processes, and the genes involved are therefore known as immediate-early genes (IEGs).

There are many relevant questions regarding IEGs. For example, how are IEGs activated, since they are able to respond very rapidly to external signals? What are the key aspects of their promoters? Do they interact with enhancers? How important is the epigenetic profile of the IEGs? This paper tries to summarize and provide updated information on some of these questions.

2. Early gene responses

2.1. Primary responses

Several genes respond rapidly to cellular signals, and such signal-responsive primary response genes (PRGs) are expressed following a wide range of different stimuli, linked to diverse signaling pathways. They can be divided into two main classes; the immediate-early response genes, and the delayed primary response genes.

2.1.1. Immediate-early response genes

The mRNA for IEGs may appear in cells within minutes after stimulation. Even more important, cells can transcribe mRNA for IEGs in the presence of protein synthesis inhibitors, indicating that the proteins required for their synthesis (including e.g. the transcription factors) are already available in the cell, and not synthesized as part of the activation process (Herschman, 1991; Morgan and Curran, 1991). These genes respond to a wide variety of extrinsic stimuli and in multiple cell types (Fowler et al., 2011), indicating a very general response mechanism. There are probably a few hundred genes in this group. These genes were first identified in cells exposed to mitogens, and have an important role in the regulation of the cell cycle (Greenberg and Ziff, 1984). Many IEGs are proto-oncogenes and their sustained expression can have profound effects on cellular growth.

2.1.2. Delayed primary response genes

Many of the primary response genes encode transcription factors, which again regulate secondary response genes (Winkles, 1998) (see subsection Secondary responses). However, it has been shown that some of the delayed inductions do not require protein synthesis, and therefore represent delayed induction of primary response genes rather than induction of secondary response genes. This group of genes is called delayed primary response genes, and they are different from IEGs both in function and in genomic architecture (Tullai et al., 2007).

2.2. Secondary responses

This group of genes is also expressed in response to signaling, but requires *de novo* protein synthesis. These genes are much more abundant than the genes in the first group, and are called secondary response genes (SRGs) (Herschman, 1991; Serrat et al., 2014).

2.3. General properties of IEGs

Expression of IEGs is quick and mainly transient, it does not require protein synthesis, and therefore translational inhibitors have no effect on their expression. Their expression in interphasic cells is initiated by an extracellular signal, such as growth factors (e.g. platelet-derived growth factor (PDGF) and epidermal growth factor (EGF)), mitogens and phorbol esters, immunological and neurological signals, developmental, and stress (e.g. UV, toxins) (Herschman, 1991; Morgan and Curran, 1991; O'Donnell et al., 2012). For example, expression of the FOS gene peaks 30–60 min after stimulation, and returns to basal expression after 90 min (Greenberg and Ziff, 1984). IEG protein products are usually unstable and they are sometimes targeted for proteolytic degradation by the proteasome without prior ubiquitination (Gomard et al., 2008). For IEG transcripts, downregulation is suggested to follow an additional mechanism through the actions of targeted microRNAs (Aitken et al., 2015; Avraham et al., 2010), where a family of microRNAs target the 3′ UTR region of several transcripts. Multiple microRNAs may target multiple IEGs, which provides some redundancy. After stimulation of IEG expression the production of these microRNAs is blocked, but then comes quickly back to normal levels (Aitken et al., 2015; Avraham et al., 2010). The combination of several mechanisms for rapid degradation and inactivation enables very transient signaling after IEG activation.

IEGs have on average shorter length than other genes (19 kb versus 58 kb), and they have significantly fewer exons. They have a high prevalence of TATA boxes and CpG islands. There is an enrichment for some specific transcription factor binding sites within regulatory regions of IEGs, including serum-response factor (SRF), nuclear factor kappa B (NF-kB) and cyclic AMP response element-binding protein (CREB) binding sites. This suggests a consistent and maybe redundant mechanism of transcription regulation (Healy et al., 2013).

3. Important IEGs and pathways

Our current knowledge about IEGs and how they are activated is to a large extent based on studies of individual genes and pathways. Here we describe some representative examples.

3.1. Important immediate early genes

Two of the most famous and well-characterized immediate-early genes are FOS and JUN (Healy et al., 2013; O'Donnell et al., 2012). They can be rapidly and transiently induced by a variety of stimuli, including serum, growth factors, cytokines, tumor promoters, and UV radiation. FOS plays a key role in cellular events, including proliferation, differentiation and survival, and is also regulated by posttranslational modification such as phosphorylation by different kinases like MAP kinases, which influences protein stability, DNA-binding activity and the trans-activating potential of the transcription factors (O'Donnell et al., 2012).

The FOS and JUN proteins have a leucine zipper-containing domain (Pfam bZIP_1) used for dimerization and DNA-binding. The JUN protein also includes a JUN domain, which can be modified by posttranslational modifications such as phosphorylation and acetylation (Bahrami et al., 2015; Finn et al., 2014). The FOS transcription factor is not independently active, and must form a heterodimer with a member of the JUN family to form the active transcription factor activator protein (AP-1). This interaction happens via the leucine zipper motif, forming a bipartite DNA-binding domain (Healy et al., 2013). AP-1 regulates the expression of target genes by binding DNA at the consensus sequence known as the TPA responsive element (TRE), which is found within the upstream promoter region of AP-1 target genes (Healy et al., 2013). This transcription factor plays an important role during both normal development and disease states such as cancer (Ozanne et al., 2006).

Early Growth Response gene 1 (EGR-1) is another member of the immediate early genes family. EGR-1 is also known as Zif268 and encodes a nuclear phosphoprotein, also known as Krox24 (Kukushkin et al., 2005). EGR-1 has both DNA-binding and non-DNA-binding domains (Bahrami et al., 2015), it has interaction with CEBPB, PSMA3 and P53 and is involved in the regulation of cell growth and differentiation in response to signals such as mitogens, growth factors, and stress stimuli (Bae et al., 2002; Liu et al., 2001; Zhang et al., 2003).

3.2. Signaling pathways for immediate early genes

Extracellular signals will promote activation of an assortment of pathways within the cell, leading to activation of transcription factors and induction of gene expression, in particular IEGs. There are several pathways that lead to the activation of regulatory proteins involved in IEG expression, such as the RhoA-actin, ERK and p38 MAPK and PI3K pathways. Here we will mainly focus on the RhoA-actin pathway and the ERK and p38 MAPK pathways (Fig. 1). These pathways lead to phosphorylation and activation of regulatory proteins involved in IEG expression, such as members of the ETS-domain family, for example transcription factors ELK1 and ETS1/2, which bind to the promoter of relevant genes and form complex with lysine

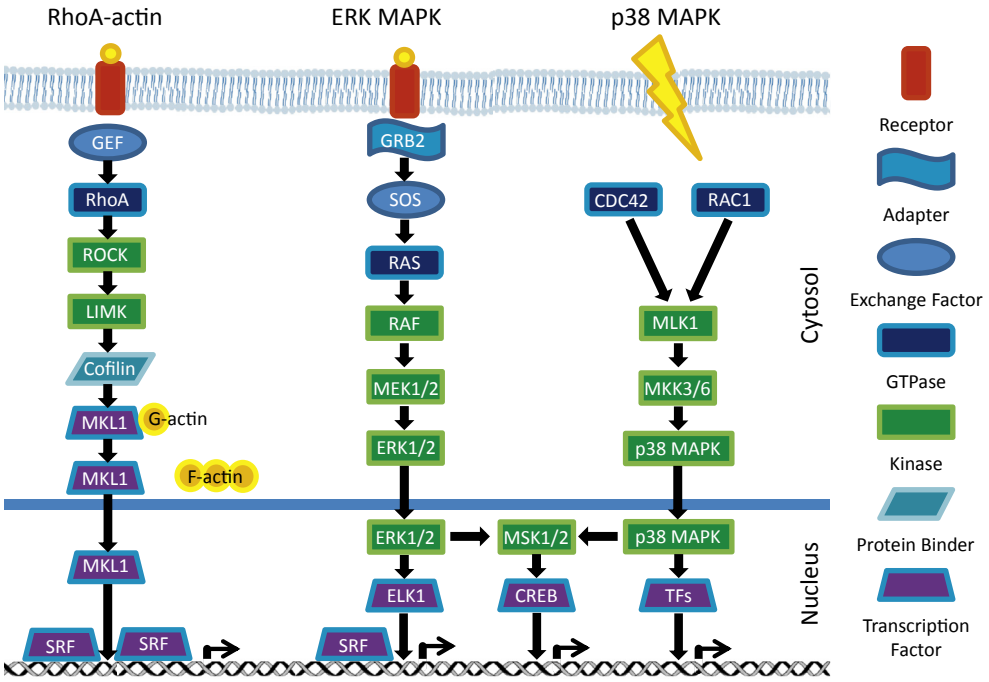

Fig. 1. Important signaling pathways. The RhoA-actin, ERK-MAPK and p38-MAPK pathways initiated by different external stimuli are shown. RhoA-actin and ERK are in particular activated by mitogenic stimuli such as growth factors and hormones while p38 is activated by stress stimuli. These pathways will also initiate chromatin modifications. The pathways are simplified, and only selected components are shown. The figure is based on data from several sources, in particular Healy et al. (2013).

acetyltransferases. Also, these pathways lead to activation of other regulatory factors that are essential for induction, such as the SRF and the Mediator complex (Fowler et al., 2011). They will also initiate changes in post-translational modifications of histones, leading to changes in the chromatin structure (Ciccarelli and Giustetto, 2014; Flouriot et al., 2014; Sawicka et al., 2014). Multiple pathways may be activated in parallel for a given signal (Bebien et al., 2003).

Rho GTPases regulate the activity of SRF, one of the transcription factors that regulate many immediate-early genes, through their ability to induce actin polymerization. The Rho GTPases is a family of small signaling G proteins, and one of the major Rho GTPases involved in for example spine morphogenesis is RhoA, which modulates the regulation and timing of cell division. The major receptors of RhoA are GPCR (G-Protein Coupled Receptor), EphA (Ephrin A), IGF (Insulin-like Growth Factor) and Ktn1 (Kinectin-1).

There is a cycle between an active GTP-bound state and an inactive GDP-bound state for Rho proteins. Their activation state is controlled by regulatory proteins such as GEFs (guanine exchange factors), which catalyze the exchange of GDP for GTP and thereby activates Rho, as well as GDIs (guanine dissociation inhibitors) and GAPs (GTPase activating proteins).

The Rho-associated kinases (ROCKs) are principal mediators of RhoA activity. ROCK leads to the stimulation of LIMK (LIM-kinase). Both LIMK1 and 2 phosphorylate and inactivate Cofilin, an actin-depolymerizing factor, and Cofilin reorganizes the actin cytoskeleton of the cell, leading to polymerization of G-actin into F-actin. G-actin binds MKL1 through N-terminal RPEL motifs (Miralles et al., 2003; Vartiainen et al., 2007), and a reduction in G-actin therefore leads to more free MKL1 in the nucleus (Vartiainen et al., 2007). MKL1/2 forms a complex with SRF and activates SRF target gene expression in the nucleus, including the SRF gene itself (Cen et al., 2003; Miralles et al., 2003).

IEG expression can also be induced by one of the MAPK (mitogen-activated protein kinase) effector cascades. There are different MAPK cascades, with five major groups of MAPKs in mammalian cells, including ERK (extracellular signal regulated kinase), RSK (p90 ribosomal S6 kinase), JNK (c-Jun N-terminal kinase), p38, and ERK5 (extracellular signal regulated kinase-5, also called Big MAP kinase-1 (BMK1)) (Raman et al., 2007; Yang et al., 2003; Yasuda and Kurosaki, 2008). JNK and p38 are activated by UV or stress stimuli, ERK and RSK are mainly activated by mitogenic stimuli such as growth factors and hormones, whereas ERK5 is activated by both stress stimuli and growth factors (Yang et al., 2003).

Here we will focus on two important MAPK cascades; the ERK-MAPK and the p38-MAPK pathways (Fig. 1). In the ERK-MAPK pathway, signals lead to phosphorylation of ELK-1 by ERK1/2, and ELK-1, which is a ternary complex factor (TCF), acts as a co-factor for SRF (Yang et al., 2003). Phosphorylation of ELK-1 leads to alternation of the complex with p300 and facilitates transcriptional activation (Li et al., 2003). Phosphorylated ELK-1 binds to SRE target sites and is associated with transcriptional co-activators like CREB-binding protein and/or p300 (Hazzalin and Mahadevan, 2005; Li et al., 2003). The p38-MAPK pathway can be stimulated by both growth factors and general stress, and leads to activation of the p38 MAPK kinase,

which subsequently activates several transcription factors, including ELK1. MSK1/2 (mitogen- and stress-activated protein kinase 1and 2) are downstream targets that can be phosphorylated by both ERK1/2 and p38 MAPK, and therefore this represents a link between these two pathways. MSK1/2 phosphorylates several proteins such as transcription factors of CREB and NF-kB, which regulate IEG expression, and also histone H3 at serine 10 and serine 28 at the upstream promoter region of IEGs. It has been shown that these kinases are active as negative regulators of acute inflammation, and for example MSK1/2 is involved in the activation of feedback mechanisms that dampen oxazolone-induced skin inflammation (Bertelsen et al., 2011; Soloaga et al., 2003).

A binding site for the phosphoserine binding protein 14-3-3 is created by MSK1/2 (Macdonald et al., 2005), and this protein connects components of the transcription activation machinery, such as the lysine acetyltransferase PCAF and the SWI/SNF ATPase BRG1 (Drobic et al., 2010). These components produce an open promoter complex which allows transcription to proceed. Extracellular signaling via activation of MSK1/2 leads to direct chromatin modification, and this regulation is called the nucleosomal response. If MSK1/2 is knocked out or blocked the expression of IEGs is reduced (Soloaga et al., 2003). It has on the other hand been observed that phosphorylated histone H3 at serine10 (H3S10ph) has a significant role in transcription initiation. For example, following induction and MSK1/2-induced phosphorylation of histone H3, this modification site acts towards the lysine acetyltransferase MOF. This transferase acetylates lysine 16 on histone H4 (H4K16), which is bound by the bromodomain of BRD4. BRD4 recruits the kinase PTEF-b. Then the kinase PTEF-b phosphorylates and releases stalled RNA polymerase from the proximal promoter region, which finally results in transcription elongation (Zippo et al., 2009).

The molecular events during FOS expression can be used as an example of IEG regulation. A key transcription factor complex consisting of SRF and a member of the TCF family of ETS transcription factors is responsible for transduction of a signal from the ERK-associated MAPK pathway. The TCF component is a receptor of this signal, being a direct MAPK phosphorylation target (Selvaraj et al., 2015; Shaw and Saxton, 2003; Yang and Sharrocks, 2006). TCFs can be multiple phosphorylated (Bahrami et al., 2015), although the exact role of this is unclear. ELK1 is example of an ETS/TCF-type transcription factor containing a carboxy-terminal MAPK-controlled transcriptional activation domain activated by MAPKs (Mylona et al., 2011). TCFs have a high affinity to DNA (Bahrami et al., 2015), and the affinity of TCFs for the binary SRF-DNA complex increases upon phosphorylation by MAPKs and decreases markedly upon treatment with phosphatases (Price et al., 1995).

SRF acts as a platform for TCF. SRF has been fused to the C-terminal region of ELK1, which has been used to show that the TCF component signaling through SRF is enough to couple ERK pathway signaling *in vivo* to T-cell development (Mylona et al., 2011). In other signaling situations, SRF can co-operate with other co-regulatory factors such as members of the MRTF (myocardin-related transcription factor) family, and thereby affect the regulation of FOS expression (Cen et al., 2003; Knoll and Nordheim, 2009; Posern and Treisman, 2006).

Several other transcription factors that bind up- and downstream from the TCF-SRF binding site may play a potential role in FOS expression. ELK1 is one the TCF proteins that is located upstream of the positioned −1 nucleosome where there is a binding site for the TCF-SRF complex. The transcription factor is modified through sumoylation in the absence of growth factor signaling. This can recruit histone deacetylase (HDAC)-containing co-repressor complexes to the FOS promoter to maintain a low basal expression level (Khan and Davie, 2013; Yang and Sharrocks, 2006). Upon growth factor-mediated activation of the ERK MAPK pathway, a p300-dependent pathway leads to increased histone acetylation levels. This occurs through allosteric activation of p300 by ELK1 phosphorylation (Li et al., 2003). ELK1 leads to recruitment of MSKs to the promoter and thereby H3S10 phosphorylation (Zhang et al., 2008). The changes in histone acetylation lead to access of NF1 (nuclear factor 1) to a binding site occluded by the −1 nucleosome, and thereby transcriptional activation can take place. Also PARP1 (poly (ADP-ribose) polymerase 1) is recruited and can trigger the binding of additional regulators to the FOS promoter. PARP1 in FOS regulation functions through directly enhancing ERK-mediated ELK1 phosphorylation (Cohen-Armon et al., 2007).

Once the chromatin remodeling (modification) steps are completed, Mediator can be added by undergoing a phosphorylation-dependent interaction with ELK1, and finally RNA polymerase activity can increase at the FOS promoter (Wang et al., 2005). This has been shown for the EGR1 promoter, and it has been indicated that the process for the FOS promoter is similar (O'Donnell et al., 2012). The main components in transcriptional activation of FOS as an IEG are shown in Fig. 2.

3.3. The immune system as a model

The immune system is a well-studied system where rapid response is essential, and many IEGs have an important role there. The activation of B and T lymphocytes is generally initiated by signaling through the antigen receptor, and it is often regulated by other cell surface proteins such as adhesion molecules, co-stimulatory molecules, and cytokine receptors. The transcription factor products of IEGs play an important role in dictating patterns of expression of downstream, function-related genes. Several studies indicate that a well-known IEG such as EGR1 may be of particular importance in response of the immune system (McMahon and Monroe, 1996), but many other genes are also involved. It has for example been shown that stimulation of airway epithelial cells with house dust mite extract leads to rapid up-regulation of ATF3, EGR1, DUSP1 and FOS, and a later strong up-regulation of JUN (Golebski et al., 2014). Stimulation with a viral double stranded RNA analog leads to a similar response. Stimulation of mouse bone marrow derived macrophages with LPS, which will activate genes via Toll-like receptors, leads to strong induction of e.g. NR4A1, EGR1, EGR2, JUN, JUNB, FOS and FOSB (Ramirez-Carrozzi et al., 2009).

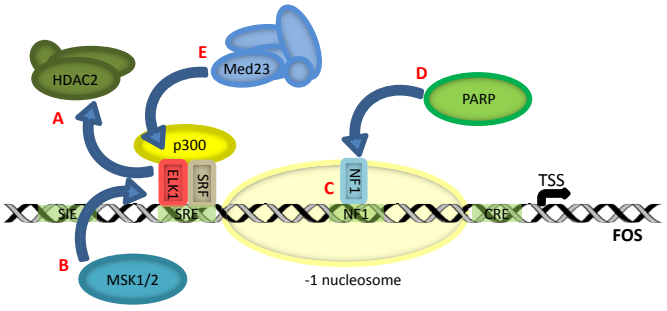

Fig. 2. Molecular events during FOS promoter activation. The promoter with pre-bound SRF, EKL1 and p300 is in a poised condition. ELK1 is maintained in an inactive form via SUMO-modification, and this permits recruitment of the repressive modifier HDAC2. During ERK pathway activation loss of SUMO-modification and HDAC2 from ELK1 (A) leads to recruitment of MSKs to the promoter (B). This promotes histone modification and the −1 nucleosome becomes acetylated, which facilitates NF1 recruitment (C). The NF1 then recruits PARP, which will open up for recruitment of other chromatin remodeling complexes (D). Then ELK1 recruits the Mediator complex. This enables basal transcription factors and RNA polymerase, and initiation of transcription (E). See the text for more details. The figure is adapted from O'Donnell et al. (2012).

However, the actual picture is sensitive both to the cellular system, how it is stimulated, and how rapid the measurement is done. For example, activation of lymphocytes with concanavalin A and measurement after 4 h identifies e.g. EGR1, EGR2, EGR3 and ATF3 as up-regulated, but FOS and JUNB as down-regulated (Ellisen et al., 2001). Infection of human epithelial lung cells with influenza virus leads to a strong down-regulation of e.g. FOS, EGR1, EGR2, FOSB, JUN, NR4A1 and NR4A2 after 8 and 24 h (Tatebe et al., 2010). This shows that the identification of IEGs is sensitive to the experimental conditions.

4. Characterization of IER gene sets

Although there are both general IEGs that are expressed in almost all cell types, and more cell-type specific IEGs, they are likely to share some key properties. It may be useful to have a good understanding of these properties as general principles of IEG activation and regulation. Shared properties of IEGs can be identified from collections (lists) of genes displaying IEG behavior in various contexts. Most analyses of IEG properties have focused on identified IEGs from experiments for specific processes or pathways, and we will first present such a study in some detail (subsection Identification and analysis of IEGs). This is followed by a more general overview of IEG properties, also largely based on studies of individual systems (subsection General properties of IEG-like genes).

4.1. Identification and analysis of IEGs

Several studies have characterized IEGs based on experimental data for specific cell types and conditions. For example, Tullai et al. (2007) have done an extensive analysis of genes induced within four hours after growth factor stimulation, using T98G human glioblastoma cells and PDGF (platelet-derived growth factor). They identified 49 IEGs, 58 delayed primary response genes, and 26 secondary response genes. An analysis of gene ontology showed that the IEGs were enriched in terms for molecular function related to transcriptional regulation, in particular “transcription factor activity” and “DNA binding”. However, these terms were not significantly enriched in either delayed primary response or secondary response genes. The immediate-early genes were also highly enriched in the cellular component term “nucleus”, but again the term was not enriched in the delayed primary response or secondary response genes. On the other hand, both the delayed primary response and secondary response genes were highly enriched in the cellular component term “extracellular region”, but this was not seen for the IEGs. This is consistent with the assumption that many IEGs encode transcription factors that in turn regulate the secondary response genes (Tullai et al., 2007).

Analysis of promoters and upstream regions of the genes showed that the difference in induction between the IEGs and the delayed primary response genes could be caused by a variety of factors, including differences in transcription initiation, elongation, pre-mRNA processing, or mRNA stability. The analysis of human sequences showed that in upstream sequences of the IEGs, four transcription factors were significantly overrepresented; SRF, NF-κB, PAX-3 and KROX. However, for delayed primary response genes no transcription factor was found to be overrepresented (Tullai et al., 2007). Also the analysis was extended with phylogenetic footprinting to identify over-represented binding sites that were conserved in orthologous genomic regions, and this showed that conserved occurrences of binding sites of SRF, NF-κB, CREB (cyclic AMP response element-binding protein) and AP-1 were significantly overrepresented in the upstream regions of IEGs (Tullai et al., 2007).

Comparison of the core promoter sequences of IEGs and the delayed primary response genes with respect to binding sites for general transcription factors indicated that there on average is a significantly higher score for a TATA box (subsection The promoter structure - CpG and TATA) for the IEGs in comparison to delayed primary response genes. Also, it was shown that the IEGs may have a greater tendency to initiate transcription from an initiation site than the delayed primary response genes, indicating that the lag in delayed primary response gene expression could be caused by RNA Pol II (RNA polymerase II)

abundance and/or recruitment at target gene promoters, and that the delay in mRNA induction for these genes occurs after the recruitment of RNA Pol II (Tullai et al., 2007).

Comparison of mRNA processing of IEGs and delayed primary response genes showed that there was no significant difference between the splice site characteristics of these groups of genes. But there was a significant difference in both the primary transcript length and exon frequency; the primary transcripts of the IEGs were significantly shorter than the primary transcripts of the delayed primary response genes and contained significantly fewer exons (Tullai et al., 2007).

4.2. General properties of IEG-like genes

4.2.1. The promoter structure – CpG and TATA

Many genes in mammalian genomes start transcription from regions of the genome with an elevated content of CpG dinucleotides and G + C base pairs referred to as 'CpG islands'. CpG islands have a high frequency of CpG sites and are typically 300–3000 base pairs long. They have been found within or close to almost 40% of all promoters of mammalian genes (Deaton and Bird, 2011; Fatemi et al., 2005). Also, the core promoter of eukaryotic genes often includes a short motif around 30 nucleotides before transcription start, known as the TATA-box. During transcription the TATA binding protein (TBP) normally binds to the TATA-box sequence, and this unwinds the DNA. The AT-rich sequence of the TATA-box facilitates easy unwinding (Kutyavin et al., 2000; Yang et al., 2007).

A major class of IEGs has been associated with CpG-island promoters. The promoters of these genes assemble into unstable nucleosomes, and therefore they do not need nucleosome remodeling complexes to facilitate induction from active chromatin. There is also another major class of IEGs with non-CpG-island promoters and stable nucleosomes, which results in dependence on nucleosome remodeling and transcription factors that promote this. However, both classes are induced by the same signaling cascade initiated from Toll-like receptors (Ramirez-Carrozzi et al., 2009).

As already mentioned, promotors of IEGs have more high-affinity TATA boxes than other gene classes. This can play an important role in transcriptional activity at the promoter of IEGs, and high affinity of the TBP binding site may also lead to rapid re-initiation.

4.2.2. Chromatin structure

IEGs have a special chromatin structure which seems to contribute to the rapid activation of transcription. A genome-wide mapping of repressed intergenic and intragenic transcription start sites (TSSs) enriched with active chromatin marks and RNA polymerase II showed strong association with IEGs (Rye et al., 2014). Such promoters are often bivalent, which means that they have both repressive and activating histone modifications. They are therefore silenced, but still poised for rapid activation. An important repressive mark is methylation at histone H3 lysine 27 (H3K27me3), whereas methylation at histone H3 lysine 4 (H3K4me3) is an important activating mark (Bernstein et al., 2006; Spaapen et al., 2013).

It has been shown that histone acetylation remains consistently present both prior to and after stimulation of gene expression, and this generates a constitutively permissive and open promoter structure (Healy et al., 2012; Soloaga et al., 2003). There is a high level of H3K4me3 marks across the promoter region of IEGs, a mark normally found around the transcription start site of actively transcribed genes, as well as H3K36me3 in the coding region, indicating actively transcribed gene bodies. The promoter regions are also enriched in the repressive H3K27me3 mark, creating a bivalent promoter. However, this is different from a silenced promoter with inactive chromatin marks. These are enriched in H3K9me3 and H3K27me2/me3 and are correlated with transcriptional repression (Bernstein et al., 2006; Rosenfeld et al., 2009). It has also been shown that there is a dynamic turnover of histone acetylation by the action of histone acetyl transferases (HATs) and histone deacetylases (HDACs), which affects all K4me3-modified H3s. This is detectable also in the absence of signaling (Edmunds et al., 2008), and it has been shown that a specific HAT (p300/CBP) mediates the dynamic acetylation of IEG regions (Crump et al., 2011). Lysine acetyltransferase p300 transfers an acetyl group to specific histone lysines, and bookmarks the proximal promoter region of IEGs when the transcription is finished, and reactivates it again following gene induction. Also RNA polymerase II is accumulated and "poised" at the proximal promoter region of IEGs (Byun et al., 2009; Tullai et al., 2007).

Maintenance of histone acetylation seems to be important for IEGs. Crump et al. (2011) have shown that fibroblasts taken from a p300/CBP double knockout mouse display inhibition of signal-induced acetylation of H4K5, K8, K12 and K16 at IEGs. However, for efficient expression of IEGs a high level of acetylation is not enough, and reduction in transcription of such genes as a result of p300 ablation cannot be overcome by pre-acetylating nucleosomes before inhibition.

Also other histone modifications are important. PIM1 kinase phosphorylates H3 at serine 10 (H3S10ph) at the FOSL1 enhancer, and recruits the HAT protein MOF (Zippo et al., 2009). Then MOF promotes H4K16Ac by generating a histone crosstalk and increased recruitment of bromodomain-containing protein BRD4 via interaction with P-TEFb. Enhanced recruitment of P-TEFb is accompanied by release of paused RNA Pol II and continuation of elongation. So H3S10ph stimulates a relay switch, which connects changes in chromatin landscape with transcriptional elongation via P-TEFb (Zippo et al., 2009). Also the modification H3S28ph has been linked to this process (Lau and Cheung, 2011).

It has been shown that poly(ADP-ribosyl)ation is required to modulate chromatin changes, for example at the MYC promoter during emergence from quiescence. Poly(ADP-ribosyl)ation is a post-translational modification found in several types of proteins, and it has an important role in the regulation of chromatin structure and transcription. PARP-1 is a major family member of poly(ADP-ribose)polymerases, participate in the cell cycle reactivation of resting cells by regulating the expression of several IEGs, such as MYC, FOS, JUNB and EGR-1 (Mostocotto et al., 2014). Inhibition of PARP activity along with

serum stimulation, by preventing the accumulation of histone H3 phosphoacetylation, damages MYC induction, and this can be a specific chromatin mark for the activation of IEGs (Mostocotto et al., 2014).

4.2.3. Chromatin remodeling

Chromatin can exist in different structural states, and dynamic modification of chromatin structure through 'chromatin remodeling' can be accomplished by covalent histone modifications, utilization of histone variants, DNA methylation and/or by the action of ATP-dependent remodeling complexes. Chromatin remodeling allows proteins of the regulatory transcription machinery access to condensed genomic DNA, and thereby control of gene expression (Teif and Rippe, 2009).

An important factor in chromatin remodeling is remodeling complexes. These use ATP hydrolysis to alter the state of chromatin by moving, ejecting, or restructuring the nucleosome. There are four important families of chromatin remodeling complexes, including the SWI/SNF family, ISWI family, CHD family, and INO80 family remodelers (Clapier and Cairns, 2009).

The assembly of CpG-island promoters into unstable nucleosomes contributes to their independence of chromatin remodeling complexes (SWI/SNF). The unstable nucleosomes, in the absence of transcription factor targeting, are sensitive to acetylation and methylation, although it is possible that expressed transcription factors play an important role in targeting histone modifications (Ramirez-Carrozzi et al., 2009). SWI/SNF-independent genes are in general induced more quickly than SWI/SNF-dependent genes (Ramirez-Carrozzi et al., 2006). It has also been shown that nucleosomes associated with inducible CpG-island promoters are structurally different from nucleosomes associated with non-CpG-island promoters in unstimulated cells. It is possible that the CpG-island sequence is responsible for the low nucleosome occupancy (Ramirez-Carrozzi et al., 2009).

Most LPS-induced primary response genes are SWI/SNF independent, but some of them show a substantial SWI/SNF dependence. Some of these genes, for activation in LPS-stimulated macrophages, require IRF3 (interferon regulatory factor 3), which is induced by a subset of TLRs (Toll-like receptors) such as TLR4. It has been shown that most primary response genes that require IRF3 for expression in LPS inducted macrophages are SWI/SNF dependent, and these IRF3-dependent primary response genes do in general not have CpG-island promoters (Ramirez-Carrozzi et al., 2009).

There are also SWI/SNF-dependent primary response genes that do not require IRF3 for expression. It has been hypothesized that one or more specialized LPS-induced transcription factors other than IRF3 promote nucleosome remodeling at promoters within this class, contributing to their selective activation (Ramirez-Carrozzi et al., 2009). Ramirez et al. have shown that TNFα signaling does not induce IRF3, and may also not directly induce any other transcription factors for nucleosome remodeling in macrophages (Ramirez-Carrozzi et al., 2009), which limits activation to SWI/SNF-independent primary response genes. Therefore, IFN-induced factors might be suitable for the selective activation of SWI/SNF-dependent genes assembled into stable nucleosomes. On the other hand, IFNβ induces transcription via IRFs and STAT proteins, and both of these protein families promote nucleosome remodeling by SWI/SNF complexes. This shows that perhaps some stimuli preferentially induce SWI/SNF independent CpG-island genes during a primary response, but that these stimuli cannot activate transcription factors capable of promoting nucleosome remodeling (Ramirez-Carrozzi et al., 2009).

4.2.4. Initiation of transcription – transient and sustained signals

Transcription of IEGs is initiated by signaling cascades, and such signals can be either short-term (transient) or long-term (sustained). Depending on the kind of cell type and the duration of signaling, the biological outcome may be different (Murphy and Blenis, 2006). For example, studies with PC12 cells showed that sustained signaling with nerve growth factor (NGF) led to neurite outgrowth in tissue culture, while transient signaling in these cells resulted in proliferation (Marshall, 1995). Both transient and sustained signaling leads to ERK activation in PC12 cells, but corresponding nuclear translocation is associated only with sustained signaling. Nuclear accumulation of active ERK will result in phosphorylation of transcription factors, leading to different outcomes of transient and sustained signaling (Marshall, 1995). ERK-dependent phosphorylation of the FOS protein protects it from degradation and results in cell cycle entry (Fowler et al., 2011; Murphy and Blenis, 2006; Yamamoto et al., 2006).

ERKs in transient versus sustained signaling can regulate PRGs and affect cell fate choices in several ways. For example, angiotensin II-mediated signaling involves heteromeric guanine nucleotide binding protein (G-protein) and β-arrestin. The G-protein dependent pathway produces a transient ERK activation, nuclear accumulation, and activation of IEGs. However, the β-arrestin-dependent pathway results in a sustained ERK activation and restricts localization to cytosol and endosomes (Shenoy and Lefkowitz, 2005).

Glauser and Schlegel have shown that almost 90% of the genes regulated by sustained signaling were not regulated by transient signaling (Glauser and Schlegel, 2006). Indeed, only a few genes were regulated by transient signaling, while many genes were regulated by sustained signaling, and some genes were regulated by both mechanisms. There were several IEGs (e.g., FOS and EGR1), which were rapidly induced by transient signaling. Both the duration of signaling and cell type context are important for biological responses, and the levels of expression of IEGs might have distinct effects in determining these responses (Damdinsuren et al., 2010; Fowler et al., 2011; Spaapen et al., 2013).

4.2.5. Transcription factors

Regulation of gene expression includes the binding of multiple transcription factors to the regulatory regions of a given gene (Gill, 2001). However, in IEGs the role of TFs is somewhat more unclear. There is no need for *de novo* synthesis of TFs to activate IEGs. On the other hand there are some specific transcription factors such as serum-response factor (SRF), nuclear

factor κB (NFκB), cyclic AMP response element-binding protein (CREB) and Zeste-like that are frequently found in the upstream promoter region of IEGs (Fowler et al., 2011; Pintchovski et al., 2009; Tullai et al., 2007). Serum response factor (SRF) belongs to the MADS family of transcription factors, and it is essential for the induction of many IEGs through signaling cascades such as the RAS-MAPK signaling pathway (Yang et al., 2003) and the RhoA actin pathway (Hill et al., 1995).

Selvaraj and Prywes (2004) suggested that TCF and MKL/MRTF family factors might function in an antagonistic fashion, so that SRF target gene regulation and cell fate choices are likely to be determined by the specificity of these cofactors (Lee et al., 2010; Selvaraj and Prywes, 2004). Also some of the IEGs that are SRF targets (e.g., FOS, EGR1 and EGR2) are MKL1 independent, while others like JUNB and FOSL1 (FOS-like 1) are MKL1-dependent targets (Lee et al., 2010; Selvaraj and Prywes, 2004). Lee et al. (2010) showed that some IEGs need just MKLs for serum induction, while other IEGs could be activated by either the TCFs or MKLs (Lee et al., 2010).

The importance of the control of MKL1 activation by TCFs or other factors is clear in megakaryoblastic leukemia, where MKL1 is fused to the RBM15 protein and activated due to constitutive nuclear localization (Cen et al., 2003; Guettler et al., 2008). Phosphorylation of MKL1 inhibits its activity, while SUMO-modification of MKL1 and myocardin has the opposite effect (Nakagawa and Kuzumaki, 2005; Wang et al., 2007).

4.2.6. The role of enhancers and the mediator complex in regulation of IEGs

An enhancer is a short region of DNA that can be bound by transcription factors to activate gene transcription. Pintchovski et al. (2009) showed that there are both distal and proximal enhancer regions for IEGs. The proximal enhancer contains one or more DNA elements. For example the Zeste-like factor binds to such sites and plays a key role for some IEGs, such as the Arc gene (Pintchovski et al., 2009). Here the distal enhancer has a functional and conserved serum response element (SRE), this binds SRF and ELK-1, which are important transcription factors for the induction of many IEGs through the ERK signaling pathway (Pintchovski et al., 2009).

It has also been shown that most IEGs are in an epigenetically poised state (Bahrami and Drabløs, 2015). They may be activated through interaction with enhancers, and it has been hypothesized that such enhancers may produce eRNA, which may play a key role in active elongation of transcription as described below.

Mediator is a multi-protein complex that is evolutionarily conserved, and it is an important transcriptional regulator of protein-coding genes by forming an interface between gene-specific activator proteins and the preinitiation complex with RNA Pol II (Malik and Roeder, 2010). In particular, it may mediate long-range interactions between promoters and enhancers, together with cohesin. The Mediator subunit MED23 is very important for regulation of EGR1 in the context of ERK/MAPK signaling through the serum response pathway (Balamotis et al., 2009). MED23 knockout leads to elimination of EGR1 expression in embryonic stem cells (ESCs) with paused RNA Pol II at the promoter, while the same effect was not observed in differentiated fibroblasts (Balamotis et al., 2009). This shows that the mechanism of regulation of IEGs in embryonic stem cells might differ from differentiated cells in a cell type specific manner (Balamotis et al., 2009). A missense mutation in MED23 leads to change in interaction of the Mediator complex with ELK1 and TCF4 and altered regulation of IEGs FOS and JUN. Deregulation of these IEGs was also observed in neurocognitive deficits. This shows that MED23 is important for regulation of IEGs (Hashimoto et al., 2011). Also the CDK8 subunit of Mediator regulates IEGs in response to serum stimulation by enhancing transcription elongation (Galbraith and Espinosa, 2011). After stimulation a CDK8-containing Mediator subcomplex is recruited to the IEG promoters where it functions as a co-activator (Donner et al., 2010). Positive transcription elongation factor, P-TEFb, plays an essential role in the regulation of transcription by pausing of RNA Pol II soon after transcription initiation in eukaryotes (Cheng et al., 2012; Zhou et al., 2012). Signal-dependent CDK8 recruitment to IEGs increases ultimately the recruitment of P-TEFb, so damage to CDK8 results in a decrease of induction of these genes by impacting both RNA Pol II and P-TEFb recruitment (Donner et al., 2010).

4.2.7. The elongation step of transcription

Eukaryote transcription consists of a series of steps. First a preinitiation complex assembles at the promoter, leading to DNA separation and initiation of transcription. After a short initial transcript has formed the process may move into elongation. This elongation continues until the final step, termination, where the transcript and the polymerase are released. However, there may also be pausing of the transcription at the start of the elongation step.

The elongation step of IEGs, and thereby also transcription, seems to be controlled by transcription elongation factors (Fujita et al., 2009). This includes factors such as DSIF (DRB sensitivity-inducing factor), NELF (negative elongation factor) and P-TEFb (positive transcription elongation factor). DRB is a nucleoside analog that inhibits transcription elongation by RNA Pol II. DSIF is a heterodimeric protein complex consisting of the Spt4 and Spt5 subunits, and is essential for cell growth and survival at the single-cell level. DSIF may act as a negative or positive elongation factor according to the phosphorylation state of Spt5 (Komori et al., 2009; Wada et al., 1998; Yamada et al., 2006). NELF is a DSIF cofactor that consists of four subunits (A, B, C/D and E). P-TEFb is a protein kinase composed of Cdk9 and Cyclin T, and it phosphorylates the C-terminal domain (CTD) of the largest RNA Pol II subunit in a DRB-sensitive manner (Peng et al., 1998).

Transcription elongation factors are necessary for development in higher eukaryotes, and many of the IEGs, such as FOS and JUNB, are controlled by these elongation factors (Aida et al., 2006).

As noted above, NELF and DSIF may pause RNA Pol II at the promoter-proximal regions by binding directly to it, and Spt5 of DSIF binds to the clamp domain of RNA Pol II (Hirtreiter et al., 2010; Martinez-Rucobo et al., 2011). Since the clamp is a flexible domain that tightly holds DNA and RNA (Cramer et al., 2001), any structural changes in this region are likely to have an

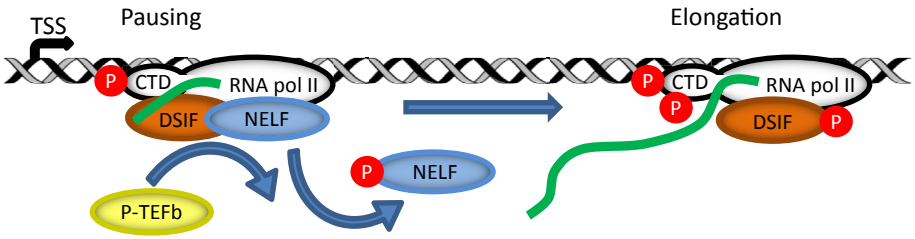

Fig. 3. A model of stimulation-specific activation of IEG transcription. Transcription starts with initiation at the transcription start site (TSS). The DSIF/NELF complex then directly stalls RNA Pol II at the promoter-proximal regions of IEGs. After stimulation, P-TEFb activates DSIF as an accelerative elongation factor and NELF to detach from the promoter, and this reactivates the transcription. NELF also stimulates directly or indirectly the expression of genes coding for factors which maintain TRH-dependent activation of the ERK1/2 MAP kinase pathway. The figure is adapted from Fujita et al. (2009).

important influence on elongation kinetics, possibly by affecting the translocation step of the elongation cycle. NELF is also likely to bind to the RNA Pol II clamp (Yamaguchi et al., 2001).

But how do transcription elongation factors regulate overall transcription elongation of IEGs during a specific stimulus? The complex of DSIF/NELF directly acts as a negative regulator complex to pause RNA Pol II at the promoter-proximal regions of IEGs. But during stimulation, RNA Pol II elongation proceeds together with the continuous association of P-TEFb and DSIF as a positive regulator, where P-TEFb allows DSIF to function as an accelerative elongation factor, and NELF to separate from the IEGs (Fujita et al., 2009; Rogatsky and Adelman, 2014).

DSIF requires NELF to induce promoter-proximal pausing. On the other hand, NELF probably requires DSIF to repress transcription fully because NELF only binds to RNA Pol II with low affinity (Yamaguchi et al., 1999). Within the paused RNA Pol II complex, CTD Ser-2 of RNA Pol II is hypophosphorylated, and then P-TEFb phosphorylates CTD Ser-2 of RNA Pol II to repress transcriptional pausing. So, CTD Ser-2 phosphorylation results in dissociation of NELF and the transcription to leave from pausing (Rogatsky and Adelman, 2014; Yamada et al., 2006). The mechanism is illustrated in Fig. 3. However, the role of NELF seems to depend upon the type of stimulation. Stable knock-down of NELF by RNAi showed very little effect on activation by EGF, whereas THR-induced activation of the MAP kinase pathway was clearly down-regulated (Fujita et al., 2009).

Thus stable NELF knock-down affects transcription of IEGs both directly via RNA Pol II elongation on IEGs as well as indirectly via activation of the ERK1/2 MAP kinase pathway after stimulations such as by TRH. This shows that the regulation of transcription of IEGs by the NELF is both direct and indirect and that it is stimulation-specific (Fujita et al., 2009).

Enhancer RNAs (eRNAs) seem to play an important role in the early transcription elongation step that involves RNA Pol II pausing and release in the IEGs. The eRNAs probably destabilize the association of the DSIF-NELF complex with RNA Pol II and facilitate the transition of paused RNA Pol II into productive elongation by interaction with the NELF complex upon induction of IEGs (Schaukowitch et al., 2014).

5. Conclusions

IEGs have an important role in several essential cellular systems, for example the immune system, and they are also important in serious diseases like cancer. It is therefore highly relevant to have a good understanding of the properties of IEGs, including gene structure, how they are activated and regulated, and how they affect downstream processes. In this paper we have summarized some key elements of our current understanding of IEGs, including the importance of genetic and epigenetic structure, and the role of poised genes and how IEGs may interact with strong enhancers.

Conflicts of interest

The authors declare no conflict of interest.

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