Immediate-early gene activation by the MAPK pathways: what do and don’t we know?

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Abstract
The study of IE (immediate-early) gene activation mechanisms has provided numerous paradigms for how transcription is controlled in response to extracellular signalling. Many of the findings have been derived from investigating one of the IE genes, FOS, and the models extrapolated to regulatory mechanisms for other IE genes. However, whereas the overall principles of activation appear similar, recent evidence suggests that the underlying mechanistic details may differ depending on cell type, cellular stimulus and IE gene under investigation. In the present paper, we review recent advances in our understanding of IE gene transcription, chiefly focusing on FOS and its activation by ERK (extracellular-signal-regulated kinase) MAPK (mitogen-activated protein kinase) pathway signalling. We highlight important fundamental regulatory principles, but also illustrate the gaps in our current knowledge and the potential danger in making assumptions based on extrapolation from disparate studies.

FOS and the IE (immediate-early) genes
FOS was initially identified as a gene that is very rapidly induced upon treatment of mammalian cells with serum or growth factors without the need for new protein synthesis [1–3]. The latter property is particularly important as it is indicative of direct modification of transcriptional regulators in response to cellular signalling rather than the production of new regulators to control FOS expression. The identification of FOS was followed by the discovery of a larger group of genes that share the same basic regulatory properties [4], and includes well-studied members such as EGR1-3, FOSB and FOSL1. Owing to their rapid activation kinetics, these genes are collectively known as IE genes. The proteins encoded by IE genes are themselves very important, as many encode transcription factors or signalling pathway regulators which can further affect cellular gene expression profiles and hence promote phenotypic changes. Indeed, as part of the AP1 (activator protein 1) transcription factor, FOS plays a key role in controlling invasion during both normal development and also in disease states such as cancer [5].

Since these early discoveries, we have learnt a lot about how IE genes are regulated, and a great deal of the focus has centred on FOS. In particular, much attention has been paid to how the MAPK (mitogen-activated protein kinase) pathways signal from receptor tyrosine kinases through to transcriptional regulators that affect FOS regulation (reviewed in [6,7]). However, despite extensive studies, we arguably only know a rough framework of how FOS is regulated and even less about other IE genes. Moreover, as a lot of the studies are conducted in different cell types under different signalling conditions, the models we have for FOS regulation are amalgamations of facts from a disparate set of studies. It is therefore not clear whether the current picture of FOS regulatory mechanisms is generally applicable in all circumstances and, moreover, whether it can be extrapolated to other IE genes. In the present review, we provide an outline of what we know about the regulation of FOS and relate this to other IE genes. Perhaps more importantly, we also highlight what is currently ambiguous or what we simply do not know. A recent review provides an alternative view on aspects of IE gene regulation [8].

FOS control: chromatin and transcriptional changes
The control of transcription can be conceptually separated into two distinct steps: chromatin modification, which changes promoter architecture, and RNA polymerase engagement and activation, which allows gene transcription to proceed (reviewed in [9,10]). In practice, however, these steps are more interdigitated, with chromatin changes also affecting the post initiation steps of RNA polymerase action. What makes IE genes distinctive is their very rapid induction from low basal levels with high amplitude activation (Figure 1). Thus the series of activation steps for IE genes takes a matter of minutes rather than hours or days, which might be crucial for genes that are transiently activated during cellular differentiation and development. In
Figure 1 | The three transcriptional states of the FOS gene

A typical mRNA activation profile (real-time reverse transcription–PCR analysis) of the FOS gene following stimulation with the potent ERK pathway activator PMA is shown in the centre. This is split into three different phases: basal expression, an activation phase, and a repression phase. The status of ERK pathway activity, the chromatin modifications around the TSS and the transcriptional regulators associated with the promoter region are also shown. P, Ac, S, and Me³ represent H3S10 phosphorylation, H3K14 acetylation, SUMOylation and H3K4 trimethylation respectively. CRE, cAMP-response element; HDAC, histone deacetylase; SIE, Sis-inducible element.

In this regard, IE gene transcription is akin to gene regulation by nuclear hormone receptors where a multitude of molecular events also takes place over a timescale of minutes, leading to potent and rapid transcriptional activation ([11] and reviewed in [12]).

FOS is probably the best studied IE gene, and numerous studies over the last 27 years since its initial discovery have contributed to our current understanding of its regulation. In terms of chromatin-based alterations, it is firmly established that histone modification by phosphorylation and acetylation contributes significantly to FOS activation; in particular histone H3S10 (H3 Ser¹⁰) phosphorylation, which promotes H3K14 (H3 Lys³⁴) acetylation [13]. Molecularly H3S10 phosphorylation permits the recruitment of 14-3-3 family members, but it is unclear what role 14-3-3 proteins subsequently play in the context of FOS regulation, although one suggested activity is to provide a binding platform for additional regulatory proteins [14]. Indeed, in the context of other IE genes, 14-3-3 proteins have been shown to be important for the recruitment of the chromatin-remodelling complex component BRG1 (Brahma-related gene 1) [15]. Both H3S10 and H3K14 histone marks are inducible in response to growth factor signalling, with phosphorylation promoted directly by MSK (mitogen- and stress-activated kinase) 1/2 [16]. Furthermore, dynamic histone acetylation/deacetylation, rather than merely a static increase in acetylation levels, has been shown to be important for FOS activation; this process appears to be dependent on
the presence of H3K4me3 (H3 trimethylated Lys4), which promotes histone acetylation turnover [17]. The underlying mechanism(s) linking these processes is still unclear, and there is currently no clear mechanistic explanation of why acetylation turnover is important.

The biggest changes in dynamic histone modifications following growth factor stimulation take place on promoter-proximal nucleosomes, especially those located downstream from the TSS (transcriptional start site) [17]. However, in FOS, there is a key nucleosome located upstream from the TSS (the ‘−1 nucleosome’), which is thought to play a crucial role in promoter activation [18] (Figure 1). Some of the earliest events in FOS activation take place in and around this nucleosome. In the case of growth factor signalling, a key transcription factor complex consisting of SRF (serum-response factor) and a member of the TCF (ternary complex factor) family of ETS transcription factors is responsible for transducing the signal from the ERK (extracellular-signal-regulated kinase) MAPK pathway. The TCF component is a major recipient of this signal due to being a direct MAPK phosphorylation target (reviewed in [19–21]). SRF, in contrast, appears to act as a platform for TCF recruitment in this context. Indeed, through the elegant use of chimaeric proteins, where SRF was fused to the C-terminal region of ELK (E26-like kinase) 1 (containing the MAPK-controlled transcriptional activation domain), it was recently shown that signalling through the TCF component is sufficient to couple ERK pathway signalling through SRF to T-cell development in vivo [22]. In other signalling situations, SRF can recruit and co-operate with other co-regulatory factors, such as members of the MRTF (myocardin-related transcription factor) family [23,24], which can affect the regulation of FOS expression in some contexts [25]. MRTFs can themselves be phosphorylated by ERK MAPK and hence potentially act as direct recipients of signals from this pathway and thereby contribute to the output of MAPK pathway signalling to IE genes [26]. Importantly, FOS expression is reliant not only on signalling through the TCF–SRF complex, but also on a whole series of other transcription factors that bind upstream and down-stream from the TCF–SRF-binding site. This was illustrated convincingly by analysing a series of transgenic animals harbouring a reporter gene driven by various mutated versions of the FOS promoter, where only the wild-type promoter functioned correctly [27]. Thus the TCF–SRF complex does not function in isolation; it is unclear what the other transcription factors and co-regulators contribute to activation by ERK MAPK signalling, but one potential role would be to create a structural enhanceosome-like structure [28].

The binding site for the TCF–SRF complex (known as the SRE (serum-response element)) is located just upstream of the positioned −1 nucleosome. There are three TCF proteins, and one of these, ELK1, is SUMO (small ubiquitin-related modifier)-modified in the absence of growth factor signalling. This in turn can recruit HDAC (histone deacetylase)-containing co-repressor complexes to the FOS promoter to maintain its low basal expression levels (reviewed in [20]). Upon growth factor-mediated activation of the ERK MAPK pathway, a series of molecular events are triggered around the −1 nucleosome [29] (Figures 1 and 2). First, histone acetylation levels are increased in a p300-dependent manner. This is thought to be due to allosteric activation of p300 by ELK1 following phosphorylation-induced conformational changes in ELK1 [30]. However, it remains to be proved whether such changes occur in the context of SRF–ELK1 complexes, as the former study only examined ELK1 functioning in an SRF-independent manner in a different promoter context. ELK1 has been implicated in recruiting MSKs to the promoter and hence promoting H3S10 phosphorylation [31] (Figure 1). The histone acetylation changes then allow access of NF1 (nuclear factor 1) to a binding site occluded by the −1 nucleosome. Only after NF1 binding can subsequent transcriptional activation be achieved. This observation does, however, raise new questions such as how acetylation promotes NF1 binding and whether other chromatin-modifying activities are required to remodel the −1 nucleosome and other downstream nucleosomes. PARP1 (poly(ADP-ribose) polymerase 1) appears to be one such activity that is recruited to the FOS promoter and can then trigger the binding of additional regulators (A. O’Donnell and A.D. Sharrocks, unpublished work). An alternative, more indirect, role for PARP1 in FOS regulation has been suggested, and, in this role, it functions through directly enhancing ERK-mediated ELK1 phosphorylation [32]. Surprisingly, despite these intricate regulatory steps centred on this nucleosome, removal of the nucleosome from engineered episomal constructs has little overall effect on FOS activation levels by the potent MAPK pathway activator PMA [33]. This suggests that this nucleosome might play a role in affecting the basal promoter activity levels or might affect the timing of responses depending on the signals encountered, rather than being absolutely required for promoter activation. Detailed kinetic analysis of FOS induction was not, however, analysed on these engineered constructs.

Finally, once these chromatin-remodelling/modification steps have been completed, then Mediator can be recruited and RNA polymerase activity at the promoter can be increased. Here, Mediator appears to be important for both RNA polymerase recruitment and subsequent transcriptional initiation [34]. ELK1 again plays a key role in this process by undergoing a phosphorylation-dependent interaction with MED23 [35], although caution should be applied to these results as they were performed on a different promoter (EGR1) and the same events have not been documented on the FOS promoter.

In addition to transcriptional initiation, a major control point for FOS transcription is at the elongation stage. In quiescent cells, poised RNA polymerase II is already associated with the FOS promoter region. Thus there is an intrinsic block to transcriptional elongation which must be overcome. This is achieved through the inducible recruitment of a group of transcriptional elongation factors which is triggered by the recruitment of the CDK8 (cyclin-dependent kinase 8)-containing submodule of the Mediator complex [36].
Figure 2 | Molecular events during FOS promoter activation

Model of the molecular events involved in FOS promoter activation following activation of the ERK MAPK pathway. The SIE (Sis-inducible element), SRE and CRE (cAMP-response element) binding elements are indicated in I, as are the identities of ELK1 (light grey oval) and SRF (dark grey oval). The TSS is indicated by a grey arrow. Under quiescent conditions (I), the promoter is in a poised position with pre-bound SRF, ELK1 and p300. Following ERK pathway activation, the −1 nucleosome (striped oval) becomes acetylated, and thereby allows NFI recruitment (II). Subsequently, NFI recruits PARP1 (III) which in turn can potentially facilitate recruitment of other chromatin-remodelling complexes (IV), leading to further structural changes in the −1 nucleosome. Next, Mediator can be recruited via ELK1 binding (V) and this can then facilitate basal transcription factor assembly, RNA polymerase recruitment and transcriptional initiation (VI). Note, however, that it is currently unclear at which stage Mediator is initially recruited. Finally, the elongator complex can be recruited, and transcriptional elongation is promoted in a Mediator-dependent manner (VII). Note, however, that in addition to de novo RNA polymerase recruitment, there is also evidence for poised polymerase at the FOS promoter, and this would be activated at step VII. See the text for further details of the mechanisms involved. Broken curved arrows indicate that a factor/complex is being recruited. Ac, acetylation.

(Figure 3). Interestingly, IE genes can be classified into two different categories on the basis of expression kinetics: very rapid activation as seen with FOS, and genes with apparent slower activation. In fact, transcriptional activation of both classes is initiated on a similar timescale, but the delay in accumulation of transcripts of the second class is due, at least in part, to their genomic structure [37]. The ‘delayed’ IE genes tend to produce longer primary transcripts and hence provide more opportunity for differential control at the transcriptional elongation stage.

Whereas a lot of studies have focused on the chromatin changes associated with promoter-proximal nucleosomes, one study has examined the transcriptional consequences and histone acetylation status of genes that are near-neighbours to IE genes [38]. This study demonstrated that, upon growth factor stimulation, genes flanking IE genes, even as far away as 100 kb, showed up-regulation (albeit to a lower level than IE genes) and a concomitant increase in histone acetylation levels. This was termed the ‘transcriptional ripple effect’ and suggests that events on the promoters of IE genes can be propagated to nearby gene promoters, presumably due to their close physical proximity in the genome. It is tempting to speculate that these promoters might be, at least transiently, associated with transcription factories or other higher-order chromatin-organizing centres (reviewed in [39]), thereby facilitating this cross-regulation. Whereas a large proportion of these IE gene-neighbouring transcripts mapped to annotated genes, the majority were intergenic transcripts. Some of these intergenic transcripts may be explained by two studies in mammals that found that genes with an initiated, but paused, RNA polymerase II located just downstream of the transcription start site (as is the case for inducible genes, such as IE genes) also possess transcriptionally engaged RNA polymerase II upstream of the promoter which proceeds in the antisense direction [40,41]. Polymerases bound in both locations are flanked by ‘active’ nucleosomes bearing H3K4me3 and H3 and H4 acetylation. Although this study may account for promoter-proximal antisense ‘ripples’, divergent upstream transcription terminates approximately 2 kb away from the TSS due to a weak RNA polymerase elongation activity. Thus
alternative mechanisms for generating the ripple effect are likely. Whatever the underlying mechanism, the physiological significance of the ripple effect remains unclear, and it is likely that this is an unwanted secondary and unavoidable consequence of the high-level transcription initiated at IE gene promoters.

**FOS control: other mechanisms**

In addition to their rapid activation kinetics, the transcriptional response of IE genes is often very transient, and, for FOS, peak expression is typically attained 30–60 min after stimulation, with a subsequent return to baseline expression by 60–90 min (Figure 1). This observation has two important implications. The first is that mRNA stability has to be low to allow rapid clearance of the mRNA; this is mediated by the RNA-binding protein ZFP36 (zinc-finger protein 36) which promotes FOS (and other IE gene) transcript degradation [42]. ZFP36 is itself part of a negative-feedback loop generated by MAPK signalling and is encoded by an IE gene. Importantly, a number of additional negative-feedback regulators were also identified, such as the transcriptional regulators KLF2 (Krüppel-like factor 2) and MAFF and a number of DUSP (dual-specificity phosphatase) family members [42]. The function of the DUSPs in dephosphorylating and inactivating MAPKs is well established (reviewed in [43]) and partly explains how the input signal is attenuated, but the molecular role of the negatively acting transcription factors is unclear. However, both types of regulators might affect the second important implication of the transient expression kinetics, namely that the transcriptional activation signals must be extinguished. At the level of the FOS promoter, it is not clear how this is achieved, as the chromatin state must be reset and the regulatory proteins engaged with the promoter returned to their pre-stimulation state. The simplest explanation is that once the input signal from the MAPK pathways is lost, then things naturally return to their basal levels through processes such as dephosphorylation and deacetylation, with this being the default setting. However, it is more tempting to believe that this is likely to occur in a more regulated manner and we have a few clues as to how this can occur. The first is the observation that Id (inhibitor of DNA-binding) proteins (encoded by delayed-response genes) can displace TCF proteins from DNA and hence might facilitate the exchange of modified TCFs for unmodified versions [44]. Secondly, the TCF ELK1 can recruit the Sin3 corepressor complex to the FOS promoter, and this might then participate in reducing acetylation back to basal levels (Figure 1). However, despite these findings, we probably have lots to learn about the inactivation process and how it is controlled.

Another interesting aspect of IE genes is their generally very low basal level expression. Part of the reason for this can be explained by mechanisms such as the SUMO-dependent corepressor complex recruitment discussed above. However, miRNAs (microRNAs) have also been implicated in this process, and a group of miRNAs have been identified that act on IE gene transcripts to keep their basal levels low [45]. This is important, as it suggests that the mechanisms in place to turn off basal expression are not watertight and therefore provides a failsafe mechanism. Indeed, poised RNA polymerase is found at the FOS promoter, and it is possible that this can occasionally escape from the elongation block to provide such background expression [46]. Moreover, these miRNAs are intimately connected to MAPK pathway signalling as their expression is rapidly extinguished in response to growth factor stimulation, thereby permitting the rapid accumulation of IE gene transcripts. Furthermore, these miRNAs are down-regulated in breast and brain tumours that
are driven by mutant receptor tyrosine kinase receptors such as EGFR (epidermal growth factor receptor) and HER2 and hence might contribute to deregulated IE gene transcription in such cancers.

**The FOS regulators: do they all function in the same way?**

Given that the fact the TCF–SRF complex plays a central role in FOS regulation, it is tempting to speculate that the identity of the TCF component is largely irrelevant as all of the TCFs [ELK1, ELK3/SAP (SRF accessory protein) 2, ELK4/SAP1] can act as recipients of signals from the ERK signalling pathway. On a superficial level, this might be true, and indeed there is evidence for functional redundancy of these factors *in vivo* in T-cells [47] and at least at the level of chromatin association in cultured cells [48,49]. However, whereas ELK1 and ELK4 are both SUMO-modified [50–52], the modification is not in the same region of the protein and it is not known whether ELK4 SUMOylation is reversed by ERK pathway activation. Furthermore, whereas ELK1 and ELK4 both recruit the Mediator complex, they probably do so through different subunits and the relative contribution of ELK1 and ELK4 to IE gene transcription changes according to cell type [53]. Superimposed on this, is a differential response to the MAPK signalling cascades. Both ELK1 and ELK3 respond to ERK and JNK (c-Jun N-terminal kinase) signalling and ERK binds directly to the same region of both proteins; however, the JNK-binding sites differ and, in the case of ELK3, JNK triggers its nuclear export [54], whereas ELK1 transactivation capacity is activated [20]. Focusing more on ELK1, differential responses to ERK and p38 MAPK signalling can at least partially be explained by the intervention of PIA3xox [protein inhibitor of activated STAT (signal transducer and activator of transcription) xo], which promotes SUMO loss in the case of ERK signalling, but aids its retention in the presence of p38 signalling [55]. This results in lower amplitude activation of ELK1 by the p38 pathway. Thus it is dangerous to assume that even highly similar regulatory proteins function in exactly the same manner, and depending on which family member is present in a given cell type, a different molecular mechanism might be employed. The same caution should be applied when considering other FOS and IE gene regulators.

**Are all IE genes controlled in the same manner?**

One of the common assumptions made in deriving transcriptional control mechanisms is that clusters of genes that show the same regulatory pattern will be controlled by the same transcriptional regulators using the same molecular mechanisms. This theory is based on work in simple organisms such as the bacterium *Escherichia coli*, where transcriptional activators such as CRP (cAMP receptor protein) or FNR (fumarate and nitrate reductase regulatory protein) promote the activation of subsets of target genes in very similar manners in response to particular growth conditions (high cAMP levels or anaerobic growth conditions respectively) [56]. However, in higher eukaryotes, including mammalian systems, transcriptional control is much more complex and has to deal with DNA-regulatory regions that contain an array of different recognition sites for transcription factors and these regions are embedded in different chromatin contexts. Thus, potentially, each gene might present a unique set of challenges to the transcriptional regulatory machinery.

The question therefore arises as to whether we can ever decipher a regulatory code which can apply to all IE genes and whether we can extrapolate from one system to another. For example, 14-3-3 proteins are inducibly recruited to FOS by H3S10 phosphorylation [14] and, equally, the same seems to occur on other IE genes such as FOSL1 [15]. In the case of FOSLI, a consequence of H3S10 phosphorylation is the recruitment of the chromatin remodeler BRG1 [15]. Can we therefore conclude that BRG1 will also be recruited to FOS in response to MAPK pathway activation? This appears likely as other studies have shown that BRG1 can control FOS expression, albeit in a negative manner [57] and BRG1 is inducibly recruited to the FOS promoter in stimulated helper T-cells [58]. However, without definitive proof, it could be dangerous to make unsubstantiated predictions of this sort. On many occasions, however, such extrapolations turn out to be correct and, more importantly, do so in cases where there is even less circumstantial evidence than in this example.

Another case which illustrates the potential pitfalls of extrapolating from disparate studies, is the role of the CDK8 Mediator submodule in transcriptional regulation (Figure 3). This has been documented as a key regulatory step in controlling transcriptional regulation of a large number of IE genes, including FOS [36]. These studies were performed in human HCT116 cells, but it is not entirely clear how these results relate to other studies performed in other cell types, such as the role of Mediator recruitment by ELK1, which was uncovered in mouse embryonic stem cells [35]. Furthermore, another study provided an alternative molecular link to transcription elongation complexes, whereby P-TEFb (positive transcription elongation factor b) was not recruited via Mediator, but was instead recruited by BRD4 (bromodomain-containing protein 4), which itself was recruited by H4K5/8/12 acetylation [46]. These studies were performed in mouse bone-marrow-derived macrophages stimulated with LPS (lipopolysaccharide) (rather than serum for the HCT116-based studies). This mechanism therefore differs, but is this due to the stimulus used, the different cell types or is it a redundant mechanism? This situation is complicated further by another study performed in HEK (human embryonic kidney)-293 cells using serum stimulation [59]. This study made the interesting observation that H3S10 phosphorylation promotes 14-3-3 protein binding, the recruitment of the histone acetyltransferase MOF and the subsequent acetylation of H4K16. This in turn triggers BRD4 recruitment and P-TEFb binding and hence leads to enhanced transcriptional elongation. However, the gene studied was FOSL1 and not FOS, and the initial kinase involved was PIM1 rather than MSK1/2. The acetylation event appears to
be different, but the downstream consequence, i.e. BRD4 and P-TEFB binding, is the same. Thus here again we have mixed cell types, alternative stimuli and different genes studied, and we have to ask whether one mechanism can be readily extrapolated from one study to another?

**Perspectives**

What then do we know about FOS regulation and other IE genes? It is clear that one of the major signalling routes to the FOS promoter is through the TCF component of the TCF–SRF complex. This then triggers an intricate series of molecular events that change the chromatin structure around the FOS promoter and prime the gene for transcription. It appears that both RNA polymerase recruitment and activation through to elongation are then stimulated, and a series of feedback loops kick in to shut down transcription after the initial burst of activity. Many of the molecular details of how these events are linked still need to be determined. For example, how are the molecular events at the −1 nucleosome coupled to transcriptional elongation control? Is this merely a consequence of Mediator recruitment or is there another novel twist to this mechanism? In terms of other IE genes, many of the same basic principles apply, but the molecular details vary. For example, not all IE genes are controlled by the TCF–SRF complex, and those that are probably follow activation regimes that differ in their details from those exhibited by FOS. Thus here too there is much to be learnt about how transcriptional control is exerted in the context of different promoter architectures.

Given the ambiguities arising from trying to combine information from different studies using differing stimuli and different cell types, attempts at building a model for how a single gene is regulated should really be performed in a single cell type. This is especially important as most cultured cells harbour important genetic abnormalities of varying severity. Moreover, comparing mouse and human systems is also potentially very dangerous as the promoter structure of orthologous genes is often very different, even though many of the same transcription factors might be involved in the control process [60]. The second important consideration is attempting to extrapolate from one IE to another. Many IE genes are controlled by the ELK1–SRF complex. Equally, however, many are not, and ChIP-seq (chromatin immunoprecipitation sequencing) studies indicate that many are not even bound by ELK1 (Z.A. Odrowaz and A.D. Sharrocks, unpublished work). Thus the regulatory modes of IE genes are clearly not universal, and we have to ask whether one mechanism can be readily extrapolated from one study to another?

There are therefore two paths that need to be followed. Given the advent of genome-wide approaches [chiefly ChIP-chip (chromatin immunoprecipitation on a chip)/ChIP-seq and expression microarray/RNA-seq analysis], it is now feasible to interrogate a wide range of molecular events occurring on all IE genes over a time course of growth factor stimulation. This will provide a broad-brush picture of the similarities and differences in control mechanisms among IE genes. Follow-up studies should then be performed on individual genes to dissect the mechanisms further. In all cases, a single stimulus and single cell line should initially be used, and the findings then extrapolated to other cell lines and more complex signalling scenarios. Only then will we have a detailed, coherent and accurate picture of how the IE genes are controlled. Finally, we should ask whether this is worth the effort; however, considering the fundamental discoveries that have been uncovered previously from IE gene model systems (reviewed in [8]), these genes likely represent fertile ground for uncovering future key findings.

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**References**


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