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Multiple facets of junD gene expression are atypical among AP-1 family members

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Abstract

JunD is a versatile AP-1 transcription factor that can activate or repress a diverse collection of target genes. Precise control of *junD* expression and JunD protein–protein interactions modulate tumor angiogenesis, cellular differentiation, proliferation and apoptosis. Molecular and clinical knowledge of two decades has revealed that precise JunD activity is elaborated by interrelated layers of constitutive transcriptional control, complex post-transcriptional regulation and a collection of post-translational modifications and protein–protein interactions. The stakes are high, as inappropriate JunD activity contributes to neoplastic, metabolic and viral diseases. This article deconvolutes multiple layers of control that safeguard *junD* gene expression and functional activity. The activity of JunD in transcriptional activation and repression is integrated into a regulatory network by which JunD exerts a pivotal role in cellular growth control. Our discussion of the JunD regulatory network integrates important open issues and posits new therapeutic targets for the neoplastic, metabolic and viral diseases associated with JunD/AP-1 expression.

Keywords

JunD; AP-1; RNA helicase A and post-transcriptional control element; HTLV-1; cancer; heart failure

Introduction

The activating protein-1 (AP-1) transcription factor is a collection of dimeric complexes composed of members of three families of DNA-binding proteins: Jun (c-Jun, JunB, v-Jun, JunD), Fos (Fra-1 and Fra-2, c-Fos, FosB) and ATF/CREB (ATF1 through 4, ATF-6, β-ATF, ATFx) (Hai and Curran, 1991; Persengiev and Green, 2003; Milde-Langosch, 2005). The AP-1 component proteins are characterized structurally by their leucine-zipper dimerization motif and basic DNA-binding domain. They can either activate or repress transcription and this versatile functional activity is dependent on the specific components of the dimeric complex and the cellular environment (Eferl and Wagner, 2003; Hess *et al.*, 2004). AP-1 figures prominently in transcriptional regulation of early response genes (reviewed by Jochum *et al.*,

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2001; Mechta-Grigoriou *et al.*, 2001; Eferl and Wagner, 2003). A feature that characterizes the typical jun family members (*junB* and *c-jun*) is their dramatic transcriptional induction by cell growth factors. Their protein products (a) are regulated post-translationally by phosphorylation, (b) bind as heterodimers (some can also bind as homodimers) to the palindromic DNA sequence TGAC/GTCA, also known as the 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA)-response element (TRE), (c) transmit Ras-mediated transformation signals and (d) participate in the control of apoptosis (Mechta-Grigoriou *et al.*, 2001). Tight control of their gene expression fits hand-in-hand with programmed cell growth.

The *junD* gene is the most recent addition to the *jun* family and was identified in a screen of a mouse 3T3 cell cDNA library (Hirai *et al.*, 1989). Hirai *et al.* (1989) demonstrated JunD binds to the TRE *in vitro* by DNA electrophoretic mobility-shift assays. JunD behaved similarly to previously identified Jun proteins to transactivate an AP-1-responsive promoter in conjunction with c-Fos. The domain structure of JunD matches other AP-1 component proteins. However, the expression pattern of *junD* diverges from the well-characterized growth factor-inducible pattern of the *c-jun* and *junB* early response genes (Hirai *et al.*, 1989; Pfarr *et al.*, 1994). Transcription of *junD* is constitutive in quiescent cells and is not induced by addition of serum. In addition, junD mRNA exhibits a divergent expression pattern across tissues. Transgenic mice studies demonstrated c-Jun and JunB are essential for embryonic development, whereas JunD is dispensable and *junD*−/− mice are viable (Thepot *et al.*, 2000). Together, these features implicated a distinct gene regulation profile and possible function for JunD in relation to c-Jun and JunB (Pfarr *et al.*, 1994). Experimental results over the past two decades have validated these predictions and provided appreciable molecular and clinical knowledge of JunD. This information has yet to be integrated into a cohesive model of the JunD-regulatory network.

This article reviews the significant body of molecular and clinical knowledge of (a) control of *junD* gene expression at the transcriptional and post-transcriptional levels and (b) post-translational modifications and alternative protein–protein interactions of JunD and their effect on the functional activity of JunD. We integrate JunD transcriptional activation and repression of a diverse collection of target genes into a regulatory network that is pivotal to cellular growth control.

Regulation of *JUND* gene expression

junD is not regulated by a typical immediate-early gene transcriptional mechanism

The mRNA template of typical AP-1 component proteins is undetectable in quiescent cells but robustly induced by serum stimulation (Herschman, 1991). By contrast, junD mRNA is detectable in quiescent cells, and neither serum stimulation nor TPA treatment significantly increase steady state expression (Hirai *et al.*, 1989; Herschman, 1991). In contrast to typical AP-1 proteins, JunD protein is degraded within the first 30 min following serum stimulation of a quiescent cell population (Pfarr *et al.*, 1994). Subsequently, JunD protein reemerges and steadily increases as cells progress to G1 (Pfarr *et al.*, 1994). This opposite trend in comparison to other AP-1 family members implicates unique post-transcriptional and/or post-translational control mechanisms in the regulation of *junD* expression.

Given the drastic differences in the transcriptional regulation between *junD* and the other *jun* gene family members, the *junD* promoter/enhancer sequence may be expected to lack *cis*-elements that mediate the transcriptional induction characteristic of *c-jun* and other AP-1 genes. However, *junD* contains a conserved TRE in the *junD* enhancer region (Figure 1). Experiments in a heterologous reporter system determined that the TRE is TPA-inducible and recognized by AP-1 (de Groot *et al.*, 1991). However, this TRE is not induced by AP-1 in the natural context of the *junD* promoter/enhancer. Instead, this transcription unit is rendered constitutive by an octamer-binding transcription factor 1 site adjacent to the TRE site (Figure 1). Two

explanations for the lack of TPA induction of this TRE are that (1) octamer-binding transcription factor 1 binding sterically precludes AP-1 binding to the TRE site or (2) octamer-binding transcription factor 1 binding to the octamer site maximally activates the promoter (de Groot *et al.*, 1991). However, conservation of the TRE in the *junD* promoter argues for a functional role. Two possible roles for the TRE include binding of JunD homodimers, which generates a positive autoregulatory loop during serum starvation (Figure 1;Berger and Shaul, 1991) and serum stimulation of c-Fos, which leads to downregulation of *junD* transcription by JunD-cFos heterodimeric AP-1 recruited to this TRE (Figure 1;Berger and Shaul, 1991, 1994).

junD is regulated by a unique post-transcriptional control mechanism

Given the constitutive activity of the *junD* promoter, the main changes in the abundance of JunD protein are regulated prominently downstream of transcription. Interrogation of *junD* has revealed complex features of the junD transcript that implicate a specialized post-transcriptional regulatory mechanism. First, the *junD* transcription product is intronless, which circumvents the process of intron removal from pre-mRNA in the nucleus. Intron removal has been shown to facilitate mRNA translation in the cytoplasm and presently is attributed to the activity of a multi-component exon junction complex that is deposited near exon–exon junctions (Braddock *et al.*, 1994; Matsumoto *et al.*, 1998; Wiegand *et al.*, 2003; Nott *et al.*, 2004; Tange *et al.*, 2004; Gudikote *et al.*, 2005). Second, the *junD* gene encodes a G/C-rich (86%) and relatively long 5′ untranslated region (UTR; Figure 1; Short and Pfarr, 2002). Third, initiation of translation at alternative AUG codons results in two biochemically distinct isoforms of JunD that orchestrate different protein–protein interactions (Okazaki *et al.*, 1998; Short and Pfarr, 2002). The two isoforms of JunD are a full-length, 39-kDa protein, and a 34-kDa protein that lacks 43 amino acids at the N-terminus (ΔJunD in Figure 1; Short and Pfarr, 2002). Efficient cap-dependent translation initiation is favored by relatively short (less than 100 nt) and unstructured 5′ UTR and by single AUG translation initiation codon embedded in a robust consensus Kozak sequence (5′-GCC(A/G)CCAUGG-3′) (Kozak, 1984a, 1984b; Merrick and Hershey, 1996; Yilmaz *et al.*, 2006). The longer and structured 5′ UTR and alternative initiation codons in junD are features discordant with efficient cap-dependent translation initiation.

Short and Pfarr (2002) investigated the possibility that the complex features of the junD 5′ UTR promote internal initiation by ribosome recruitment to an internal ribosome entry site. Cap-independent internal ribosome entry is utilized by some viruses and cellular RNAs that exhibit a long and structured 5′ UTR (reviewed by Baird *et al.*, 2006). Bicistronic reporter assays in HeLa, COS-1 and CHO cells determined that the 5′ UTR of rat junD mRNA does not function as an internal ribosome entry site to promote internal initiation (Short and Pfarr, 2002). These results indicated that translation initiation of the junD mRNA is dependent on ribosome scanning. Efficient translation initiation of junD RNA was predicted to involve RNA–protein interactions that neutralize structural barriers within the 5′ UTR to derepress ribosome scanning and promote efficient translation (Short and Pfarr, 2002). A clue in deciphering junD translational control derived from parallels drawn from the retrovirus model system.

Interaction between RNA helicase A and the 5′ terminal PCE is necessary for efficient translation of *junD*

Similar to junD, the complex 5′ UTR of retrovirus mRNA templates poses structural barriers to robust cap-dependent translation (Yilmaz *et al.*, 2006). Highly conserved structural *cis*-acting replication motifs conserved among all retrovirus 5′ UTRs impede ribosome scanning (Yilmaz *et al.*, 2006). Similar to junD, internal ribosome entry site activity in the 5′ UTR has been ruled out for spleen necrosis virus, reticuloendotheliosis virus A and human T-cell

leukemia virus type 1 (HTLV-1) (Bolinger *et al.*, 2007). Instead, they contain a unique post-transcriptional control element (PCE) that promotes efficient cap-dependent translation (Butsch *et al.*, 1999; Roberts and Boris-Lawrie, 2003; Hartman *et al.*, 2006; Bolinger *et al.*, 2007). PCE is an orientation-dependent (Butsch *et al.*, 1999), ~150-nt structural element located adjacent to the RNA cap site (+ 1) comprised of two functionally redundant stem-loop structures (Roberts and Boris-Lawrie, 2000, 2003). Proteomic analysis identified that PCE interacts specifically with RNA helicase A (RHA) (Hartman *et al.*, 2006). RHA is a ubiquitous nucleocytoplasmic shuttle protein that also is known as the DEIH (Asp–Glu–Ile–His) box polypeptide 9 and nuclear helicase II (Zhang and Grosse, 2004). The RHA/PCE interaction is necessary for efficient retrovirus translation (Hartman *et al.*, 2006; Bolinger *et al.*, 2007). Given the related characteristics of the junD 5′ UTR, *de novo* JunD protein synthesis was investigated. First, rat junD 5′ UTR exhibited PCE activity in reporter assays (Hartman *et al.*, 2006). Second, the rate of *de novo* junD protein synthesis was drastically reduced upon downregulation of RHA in simian cells (Hartman *et al.*, 2006). Third, subcellular fractionation experiments determined that RHA does not affect cytoplasmic accumulation of junD, but is necessary for polyribo-some association of junD. Co-immunoprecipitation studies determined that junD mRNA interacts with RHA in both the nucleus and cytoplasm, positing the hypothesis that RHA is recruited co-transcriptionally (Hartman *et al.*, 2006). The current model is that RHA-junD PCE RNA interaction neutralizes structural barriers within the 5′ UTR to derepress ribosome scanning and promote efficient translation.

An important open issue is whether RHA translational enhancement on the junD PCE is constitutively active or inducible during the course of JunD biology. The steady increase in JunD protein level that starts 30 min after serum stimulation is proportional to the increase in the RHA protein level (C Bolinger, A Sharma and K Boris-Lawrie, unpublished data). By contrast, caspase 3 activity on RHA is a possible negative regulator of *de novo* JunD synthesis during induction of apoptosis (Takeda *et al.*, 1999; Myohanen and Baylin, 2001; Abdelhaleem, 2003) and during serum starvation (A Sharma, C Bolinger and K Boris-Lawrie, unpublished data). The comprehensive role of RHA/JunD PCE activity in junD post-transcriptional control remains to be fully elucidated.

We speculate that induction of JunD translation governed by the RHA–PCE interaction represents a potent regulatory point for JunD protein production and would robustly amplify JunD transcriptional target genes (Figure 2). By influencing the expression of growth control genes such as p21 (CDKN1), which controls cell-cycle progression at G1 (Li *et al.*, 1994), and $p19^{ARF}$ (CDKN2A), a regulator of the p53 pathway via MDM2 (Kamijo *et al.*, 1998;Pomerantz *et al.*, 1998;Zhang *et al.*, 1998), RHA–PCE regulation of JunD is likely to have important consequences for control of cell growth and proliferation. The fact that JunD regulation of these important cellular processes has been conserved among mammalian systems and the conservation of junD PCE activity in primate and rodent supports the importance of RHA–PCE regulation in JunD biology.

The microRNA pathway may contribute to post-transcriptional regulation of *junD*

MicroRNA regulation of human genes is estimated to be prominent (≥30% of human genes) (Willingham and Gingeras, 2006). The junD 681-nt 3′ UTR harbors several microRNA target sequences predicted by the Sanger Institute database miRBase (JM Hernandez and K Boris-Lawrie, unpublished data). The observation that RHA interacts with the RNA-induced signaling complex in human cells and acts as an small interfering RNA-loading factor (Robb and Rana, 2007) may provide another link between RHA and *junD* gene expression. We speculate that RHA is a gatekeeper that associates with PCE to facilitate efficient JunD protein synthesis, or associates with a complementary microRNA to enact translation suppression. We currently are testing these possibilities.

Post-translational modification and alternative protein–protein interactions modulate JunD

JunD diverges from c-Jun and JunB and is not subject to ubiquitin-mediated degradation

Post-translational modification and alternative viral and cellular protein–protein interactions contribute to the versatility of JunD in effecting change in cellular environments. The observed alteration in JunD protein stability during quiescence (Pfarr *et al.*, 1994) has yet to be elucidated in detail. Interestingly, the initial decrease in steady-state JunD protein levels observed upon serum stimulation correlates with a small increase in molecular weight (Pfarr *et al.*, 1994). One unresolved possibility is that JunD is post-translationally modified by factors that target JunD for proteolytic cleavage and/or degradation. Indeed, phosphorylation has been shown to precede ubiquitination of many transcription factors (reviewed by Harper and Elledge, 1999; Punga *et al.*, 2006).

Ubiquitination and proteasome degradation prominently govern levels of c-Jun and JunB (Treier *et al.*, 1994; Fuchs *et al.*, 1997). Their ubiquitination is mediated through the δ-domain (Treier *et al.*, 1994; Fuchs *et al.*, 1997), which first was defined as 27 residues (amino acids 30–57 in c-Jun) present in the N-terminal region of c-Jun, JunB, and is deleted in v-Jun (Morgan *et al.*, 1993; Fuchs *et al.*, 1997). JunD is ubiquitinated inefficiently in spite of the presence of a δ-like domain comprising amino acids 43–69 (Musti *et al.*, 1996). This difference could contribute to the longer half-life of JunD relative to c-Jun (360 and 90 min, respectively) (Treier *et al.*, 1994; Musti *et al.*, 1996). Treier *et al.* (1994) have shown that deletion of the first seven amino acids of the δ-domain severely reduces ubiquitination of c-Jun. In addition, ubiquitination of the Jun proteins is dependent on physical interaction between the Jun N-terminal kinase (JNK) and a binding region present in c-Jun and JunB (Fuchs *et al.*, 1997). This region is absent in JunD and ΔJunD, which further compromises ubiquitination (Kallunki *et al.*, 1996; Musti *et al.*, 1996).

Pfarr *et al.* (1994) hypothesized that the stability of JunD is increased during serum starvation, which suggests that JunD protein degradation is subject to negative regulation under this condition. However, the degradation mechanism for JunD has yet to be elucidated as indicated by the question mark in Figure 1.

JunD activity is positively regulated by mitogen activated protein kinase

Phosphorylation of JunD at the N-terminus by mitogen activated protein kinase JNK positively regulates trans-activation activity (Adler *et al.*, 1994; Yazgan and Pfarr, 2002). As highlighted in Figure 1, JunD is phosphorylated by JNK at serines 90 and 100, which are conserved phosphorylation sites in c-Jun, and at threonine 117 (Yazgan and Pfarr, 2002). Downregulation of JNK by estrogen decreases JunD phosphorylation, which in turn decreases expression of the junD mRNA. These observations indicate that phosphorylation is necessary for JunD transcriptional trans-activation of the *junD* promoter and that JNK stimulates the autoregulatory loop that modulates *junD* expression (Srivastava *et al.*, 1999). Although the 43 N-terminal residues of JunD do not bind JNK (and do not promote ubiquitination, as discussed above), residues 49–59 of JunD provide a low-affinity docking domain for JNK that is necessary for phosphorylation (Figure 1, efficient phosphorylation is designated by the red squares labeled Pi) (Kallunki *et al.*, 1996; Fuchs *et al.*, 1997; Yazgan and Pfarr, 2002). Although these phosphorylation sites are retained in the ΔJunD isoform, JNK binding and phosphorylation are less efficient *in vitro* compared with JunD (Figure 1, inefficient phosphorylation is designated by the pink circles labeled Pi) (Yazgan and Pfarr, 2002). Yazgan and Pfarr (2002) speculated that ΔJunD is a less efficient phosphorylation substrate because of conformational changes attributable to the N-terminal truncation of ΔJunD. Results of co-

transfection experiments support the conclusion that the less efficient phosphorylation of ΔJunD results in weaker transcriptional activity (Yazgan and Pfarr, 2002).

JunD also is regulated by the extracellular signal-regulated kinase (ERK)–mitogen-activated protein kinase pathway, also known as the MEK–ERK kinase cascade. The evidence is that MEK1–ERK1/2 specific inhibitors PD98059 and UO126 reduce the phosphorylated form of JunD, and that constitutively active forms of the ERK2 enhance JunD phosphorylation (presumably at the same positions as JNK) (Gallo *et al.*, 2002). Figure 2 summarizes the outcome of Ras signaling of ERK2 and JNK/ERK2 on JunD. Phosphorylated JunD exhibits versatile activity in repressing transcription of target genes involved in cell proliferation, apoptosis and tumor angiogenesis, and activating target genes involved in cell differentiation. The role of JunD in differentiation of osteoblast and keratinocyte lineages is described in more detail below.

MEN1 represses JunD activity by two mechanisms

Multiple endocrine neoplasia type-1 (MEN1) is a broadly expressed tumor suppressor and mutations in this gene contribute to tumorigenesis in a diverse range of tissues (reviewed by Agarwal *et al.*, 2003; Dreijerink *et al.*, 2006). The interaction of JunD with MEN1 is mediated by N-terminal residues 1–120 (Figure 1; Agarwal *et al.*, 1999). Both of N- and C-terminal regions of MEN1 are needed for efficient JunD binding (Gobl *et al.*, 1999). MEN1 negatively effects ERK- and JNK-dependent phosphorylation of JunD, without affecting the activation of either kinase (Gallo *et al.*, 2002). The observation that a deletion mutant of MEN1 interferes with ERK2-dependent phosphorylation of JunD, but does not suppress phosphorylation by JNK, indicates that MEN1 affects each of these pathways by a distinct mechanism (Gallo *et al.*, 2002).

As highlighted in Figure 1, MEN1 interaction represses JunD transcriptional activity by inhibition of JunD phosphorylation by JNK and ERK2 (Agarwal *et al.*, 1999;Gobl *et al.*, 1999, 2002; Naito *et al.*, 2005), and also by recruitment of the mSin3A–histone deacetylase (HDAC) complex (Kim *et al.*, 2003). This effect of MEN1 on JunD function involves changes in chromatin structure in the promoter regions of JunD-MEN1 transcriptional targets. MEN1-mediated repression is sensitive to the HDAC inhibitor trichostatin A (Gobl *et al.*, 1999;Kim *et al.*, 2003). One possible mechanism is that the recruitment of the HDAC complex is dependent on sumoylation of JunD that occurs upon formation of the JunD–MEN1 complex; a similar mechanism has been described for ELK1 (Yang and Sharrocks, 2004). A regulatory domain motif that mediates sumoylation, which first was identified in four members of the C/EBP family (C/EBPα, C/EBPβ, C/EBPδ and C/EBPε), is present in JunD (Figure 1;Kim *et al.*, 2002). The JunD-regulatory domain motif corresponds to the sequence LKDEP (amino acids 233–237), which is a putative sumoylation site. The regulatory domain motif is well conserved in c-Jun and JunB, and was determined to have a negative effect on transcription (Kim *et al.*, 2002).

Downregulation of MEN1 in osteoblasts increases JunD transcriptional activity via the *junD* transcription autoregulatory loop, which in turn increases steady-state JunD protein level (Naito *et al.*, 2005). Since MEN1 interaction is mediated by the JunD N-terminus, ΔJunD does not bind MEN1 and is not susceptible to MEN1 repression (Yazgan and Pfarr, 2002). The expectation that ΔJunD is constitutively active could be offset by reduced transcriptional activity that is attributed to inefficient phosphorylation (Yazgan and Pfarr, 2001, 2002).

MEN1 repression of JunD activity in T cells reduces transcription of the Nur77 locus by recruitment of mSin3A–HDAC (Kim *et al.*, 2003, 2005). During T-cell receptor-mediated thymocyte apoptosis, T-cell receptor activation can derepress Nur77 transcription via the protein kinase C pathway (Kim *et al.*, 2005). Protein kinase C mediates phosphorylation of

serine 100 residue of JunD by activating JNK and ERK2, which in turn may mediate the recruitment of p300 to cooperatively activate transcription of Nur77 (Kim *et al.*, 2005). The recruitment of p300 derepresses JunD activity, presumably by reversing HDAC-mediated silencing of the Nur77 promoter (Kim *et al.*, 2005). Repression of MEN1 also allows ERK2-mediated JunD phosphorylation, thus providing a pleiotropic effect (Gallo *et al.*, 2002). The phosphorylation of JunD is dependent on repression of MEN1, but not on recruitment of p300.

JunD activity is altered in heterodimers with human retrovirus leucine zipper protein

Decade-long persistent infection with HTLV-1 can progress to an aggressive, chemotherapy-refractive infectious adult T-cell leukemia (Matsuoka and Jeang, 2007). JunD is postulated to contribute to this process by forming active heterodimers with the HTLV-1 basic leucine zipper factor (HBZ) (Thebault *et al.*, 2004). HBZ also forms heterodimers with c-Jun and JunB (Larocca *et al.*, 1989; Basbous *et al.*, 2003; Cavanagh *et al.*, 2006). HBZ represses c-Jun activity by impairing DNA binding and decreasing c-Jun protein stability, which culminates in reduced expression of c-Jun target genes (Matsumoto *et al.*, 2005). By contrast, HBZ heterodimerizes with either JunD (Figure 1) or JunB and trans-activates expression of AP-1 target genes (Figure 1; Thebault *et al.*, 2004). Studies in a rabbit model system have demonstrated HBZ plays an important role in HTLV-1 infectivity and persistence (Arnold *et al.*, 2006).

HBZ contains an as yet-to-be understood modulatory domain that upregulates JunD transcriptional activity (Hivin *et al.*, 2006). Hivin *et al.* (2006) have shown that the modulatory activity is not attributable to changes in HBZ–JunD interaction or DNA binding, and they speculated this domain inhibits recruitment of MEN1 or another negative regulator of JunD (Figure 1). A possible scenario is that the HBZ interaction with JunD induces a conformational change that eliminates efficient interaction with MEN1.

JunD activity may figure prominently in the model for HTLV-1 persistence. JunD/HBZ heterodimers are postulated to induce downregulation of cellular proliferation, lymphocyte activation and viral transcription to favor viral latency and persistence and coordinately slow outgrowth of transformed cells. Specifically, HBZ is postulated to enhance JunD activity on target genes (Kuhlmann *et al.*, 2007) that inhibit cell growth (Thebault *et al.*, 2004) and coordinately downregulate c-Jun activity on cellular growth genes and the HTLV-1 genes (Thebault *et al.*, 2004; Matsumoto *et al.*, 2005). However, if the HBZ–JunD interaction interferes with MEN1-mediated JunD transcriptional repression of cellular genes, dysregulation of cellular signaling pathways could occur and drive the cell fate toward neoplastic transformation.

An important caveat of the majority of published studies is the reliance on overexpression of HBZ, rather than physiologic expression from provirus. In authentic infection, HBZ expression is expected to be significantly lower than in standard overexpression systems (Arnold *et al.*, 2006; Li and Green, 2007). This critical difference will undoubtedly affect studies of the physiological significance of HBZ in dysregulation of cellular gene expression. Another important consideration toward understanding HBZ activity is the elucidation of the stoichiometry of HBZ-JunD heterodimers in relation to other AP-1 complexes in infected cells.

JunD is a central molecule in an intricate regulatory network

The ultimate function of JunD is to regulate transcription of target genes that help the cell cope with environmental signals perceived from the environment. As summarized in Table 1, JunD can act as an activator or a repressor of transcription of diverse cell type-specific genes involved in oxidative stress, cell proliferation and differentiation. As a consequence, JunD is associated with a broad array of clinical scenarios including neoplasia, bone, cardiac and epithelial cell biology. The wide spectrum of JunD disease associations is not surprising given its broad

expression pattern (Hirai *et al.*, 1989). Accordingly, the role that JunD plays in a given cell depends on the identity of its partner in the AP-1 dimer, the presence or absence of JunD post-transcriptional and post-translational regulators, and the presence of other transcription factors that combinatorially regulate JunD transcriptional target genes.

As highlighted in Table 2, downregulation of JunD affects normal progression of cell maturation and differentiation and contributes to uncontrolled cell proliferation. Accordingly, JunD generally is considered to be a negative regulator of cell proliferation. For instance, JunD exhibits a cell-dependent role in apoptosis and prevents cell death in adult mouse heart cells (Hilfiker-Kleiner *et al.*, 2005) and in UV/H_2O_2-stressed mouse embryonic fibroblasts (Zhou *et al.*, 2007), while enhancing UV-induced increases in caspase-3 activity and apoptosis in human myeloblastic leukemia ML-1 cells (Li *et al.*, 2002b). Given its pivotal role in balancing cell proliferation, differentiation and apoptosis, and the association of JunD dysregulation with several neoplasms and metabolic diseases, JunD is an attractive target molecule for therapeutic intervention.

Abnormal levels of steady state junD mRNA and JunD protein have been reported in many types of cancer cells and in response to hormones and other factors (Table 2; Neyns *et al.*, 1996;Pollack *et al.*, 1997;Li *et al.*, 2002a,b;Troen *et al.*, 2004). Some studies solely measured the abundance of JunD protein. Given the prominent influence that post-transcriptional and post-translational regulation can exert in balancing JunD abundance and activity, an important consideration is to dissect the relative roles of transcriptional and post-transcriptional regulation in examples of JunD dysregulation. In at least one case, a variation in the stability of the JunD mRNA was identified as the reason for the phenotype (Table 2;Li *et al.*, 2002a).

JunD negatively regulates Ras-mediated transformation

Ras regulates cell proliferation, apoptosis, tumor angiogenesis and accumulation of reactive oxygen species (reviewed by Finkel, 2006). Contrary to c-Jun, JunD acts as a negative regulator of Ras-mediated transformation by downregulating cell growth in response to Ras signal transduction (reviewed by Jochum *et al.*, 2001; Mechta-Grigoriou *et al.*, 2001; Eferl and Wagner, 2003, and summarized in Figure 2). The initial clue that JunD is a negative regulator of Ras was that overexpression of JunD caused a reduction in Ras-induced tumor growth (Pfarr *et al.*, 1994). Consistently, immortalized *JunD*−/− mouse cell lines exhibit increased proliferation. However primary *JunD*−/− mouse embryonic fibroblasts exhibit upregulation of $p19^{ARF}$, which causes early senescence, and p53-dependent apoptosis upon stress (Weitzman *et al.*, 2000). These observations indicate that JunD protects against Ras-induced apoptosis and neutralizes Ras transformation. Contrary to c-Jun, JunD protects cells from oxidative stress by contributing to the downregulation of angiogenic transcription factors like hypoxia-inducible factor α (Gerald *et al.*, 2004). A possible scenario is JunD trans-activation of *ferritin* (Figure 2), which may be attributable to JunD recruitment at two copies of a bidirectional AP-1 site. This site is located within an antioxidant/electrophile response element 4.5 kb upstream of the human *ferritin H* transcription start site (Figure 2; Table 1; Tsuji, 2005).

JunD as a positive regulator of cellular maturation

JunD plays a role in cellular maturation of several cell types. JunD positively regulates the expression of osteoblast-specific proteins, type I collagen, osteocalcin and alkaline phosphatase (Table 1; Naito *et al.*, 2005;Akhouayri and St Arnaud, 2007). JunD is coexpressed at high levels with Fra-2 in fully differentiated osteoblasts (McCabe *et al.*, 1996). Loss of function of the negative JunD regulator, MEN1, correlates with increased JunD expression in osteoblasts and upregulation of osteoblast markers (Hendy *et al.*, 2005;Naito *et al.*, 2005). A clue that JunD acts after the commitment of the cells to the osteoblast lineage is the observation that JunD regulates critical osteoblast genes, although *JunD*−/− mice have not been reported

to demonstrate severe skeletal deformities characteristic of other osteoblast lineage genes. Since JunD regulates critical osteoblast genes, an important open issue is the potential for a bone phenotype in JunD-deficient mice under basal versus stress conditions.

JunD plays a role in additional cell types that undergo continuous renewal, differentiation and maturation: keratinocytes, spermatocytes and hematopoeitic cells. Keratinocytes express JunD in all layers of the dermis (Mehic *et al.*, 2005). Jun proteins play a positive role in keratinocyte differentiation in part through positive effects on epidermal growth factor receptor expression (reviewed by Zenz and Wagner, 2006). *JunD* −/− mice do not exhibit an obvious skin phenotype, although this needs to be evaluated further. The potent function of c-Jun and the redundancy of other AP-1 complex members could contribute to the apparent dispensability of JunD in skin renewal.

JunD is the only AP-1 family member that is expressed at high levels in post-meiotic spermatocytes; the other AP-1 family members are expressed throughout early spermatocyte development (Alcivar *et al.*, 1991). Male *junD* − /− mice are sterile and exhibit loss of expression of late markers of spermatogenesis, such as calspermin, BMP8 and RT7. However, the open issues remain as to whether or not these markers are direct transcriptional targets of JunD (Thepot *et al.*, 2000). JunD-transgenic mice have decreased numbers of lymphocytes, indicating that JunD may play a negative role in lymphocyte maturation (Meixner *et al.*, 2004).

As highlighted in Table 1, other cell developmental pathways for which JunD is important are maturation of ovarian cells and chondrocytes (Gunthert *et al.*, 2002). Additional studies of the transcriptional regulation by JunD of late markers of cell type maturation will be important to establish the exact role of JunD in this context. The possible role of RHA/PCE translational control among the cell developmental pathways remains an open issue.

Perspectives

These transcriptional, post-transcriptional and post-translational control mechanisms represent interrelated conduits to ensure tight control of JunD functional activity (Figure 1). The outcome is JunD activation and repression of transcription of a diverse collection of target genes, which in turn operate a regulatory network that exerts a pivotal role in cellular growth control (Figure 2). The JunD-regulatory pathways that safeguard precise JunD activity are, in addition to *junD* gene sequence, potential loci for genetic mutations that dysregulate *junD* biology. Examples are MEN1 and other proteins that interaction with JunD. These pathways also offer therapeutic targets to treat *junD* dysregulation. For instance, a JunD transdominant mutation that prevents functional interaction with HTLV-1 HBZ may stall neoplastic transformation by HTLV-1. Continued investigation of the role of JunD in controlling proper growth of cells that undergo continuous renewal, differentiation and maturation (for example, keratinocytes, spermatocytes and hematopoeitic) is expected to identify selective therapeutic targeting of cancers and metabolic diseases.

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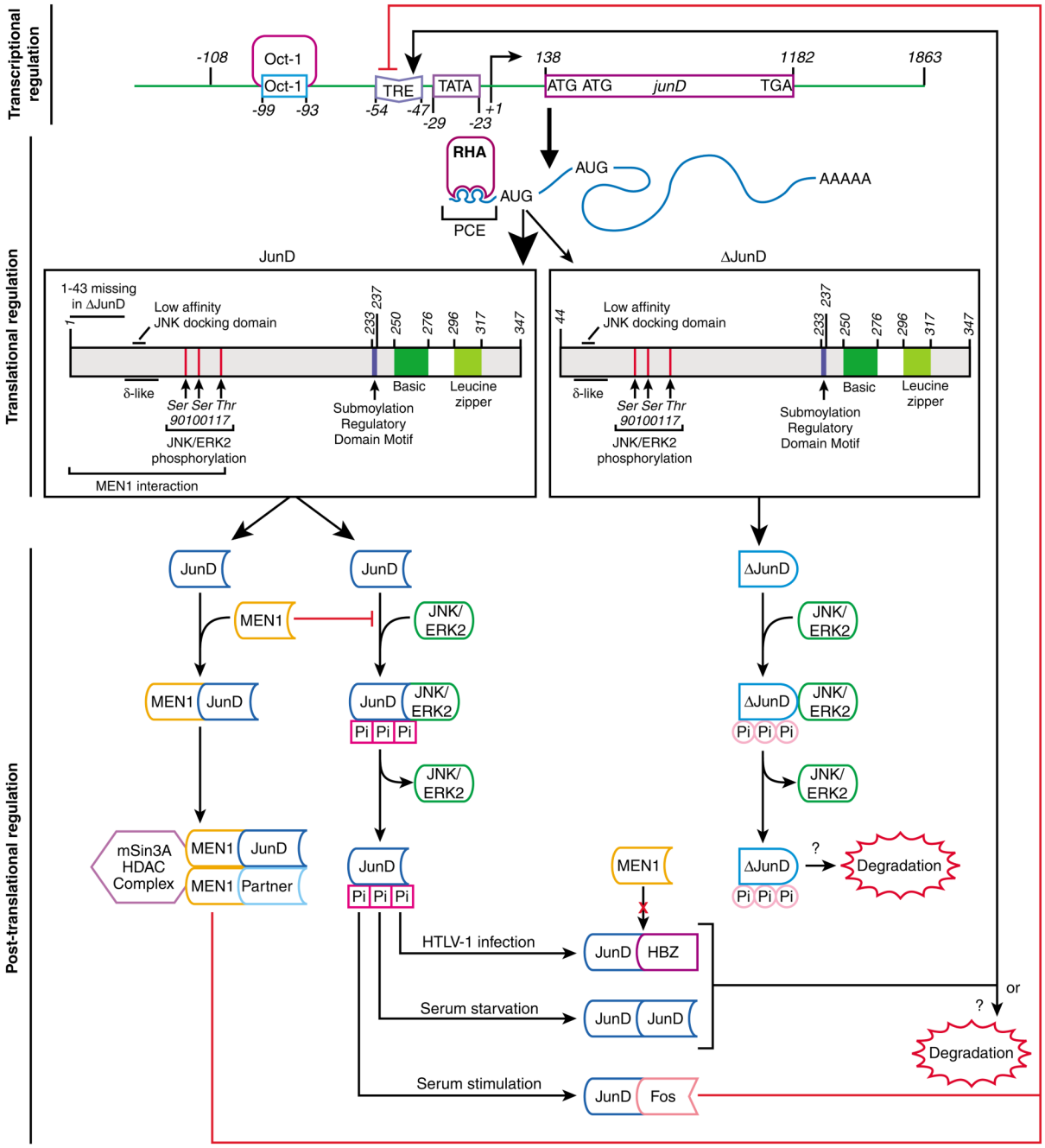

Figure 1.

The regulation of JunD activity is operated by interrelated layers of transcriptional, post-transcriptional and post-translational control. The top panel summarizes general features of the human *junD* gene structure and its transcriptional regulation. Positions within the promoter region of the octamer-binding transcription factor 1, TPA response element (TRE) and TATA-binding sites are indicated with sequence numbering relative to the transcription start site (+1). The transcription of *junD* is constitutive and subject to an autoregulatory loop. The middle panel summarizes the structure of the primary transcription product and two translation products. JunD is more abundant than the N-terminal truncated ΔJunD, as indicated by the thicker arrow. The mRNA contains one exon and no introns. Two alternative translation

initiation codons are utilized and these mRNA templates contain either a 138-nt (JunD) or a 681-nt (ΔJunD) 5′ UTR. The 5′ UTR forms stem loop structures that that act as a 5′ terminal PCE. PCE interaction with RHA derepresses mRNA translation and is necessary for junD translation. Domains of the JunD protein are indicated: basic and leucine zipper domains, MEN1 interaction region, the δ-like domain, JNK phosphorylation sites and the sumoylation regulatory domain motif. The N-terminus of JunD is truncated in ΔJunD, which profoundly alters the profile of protein–protein interactions. The lower panel summarizes protein–protein interactions that modulate the versatile functional activity of JunD. The N-terminal low-affinity docking site is important for efficient JunD phosphorylation by JNK, as indicated with red squares labeled Pi. ΔJunD, which lacks this domain is not efficiently phosphorylated by JNK, as indicated by the pink circles labeled Pi. Also, ΔJunD does not interact with MEN1. Interaction of JunD with MEN1 inhibits transcription of JunD target genes. MEN1 interaction with JunD has been proposed to be inhibited for JunD-HBZ heterodimers. MEN1 recruits an HDAC complex that renders the JunD dimer a repressor. Phosphorylation by JNK/ERK2 also is inhibited by MEN1. The identity of the dimerization partner of JunD is determined by the cell type and growth conditions. Little is known about the mechanism of JunD protein degradation. ERK, extracellular signal-regulated kinase; HDAC, histone deacetylase; JNK, Jun N-terminal kinase; MEN1, multiple endocrine neoplasia type-1; PCE, post-transcriptional control element; RHA, RNA helicase A; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate; TRE, TPA-response element.

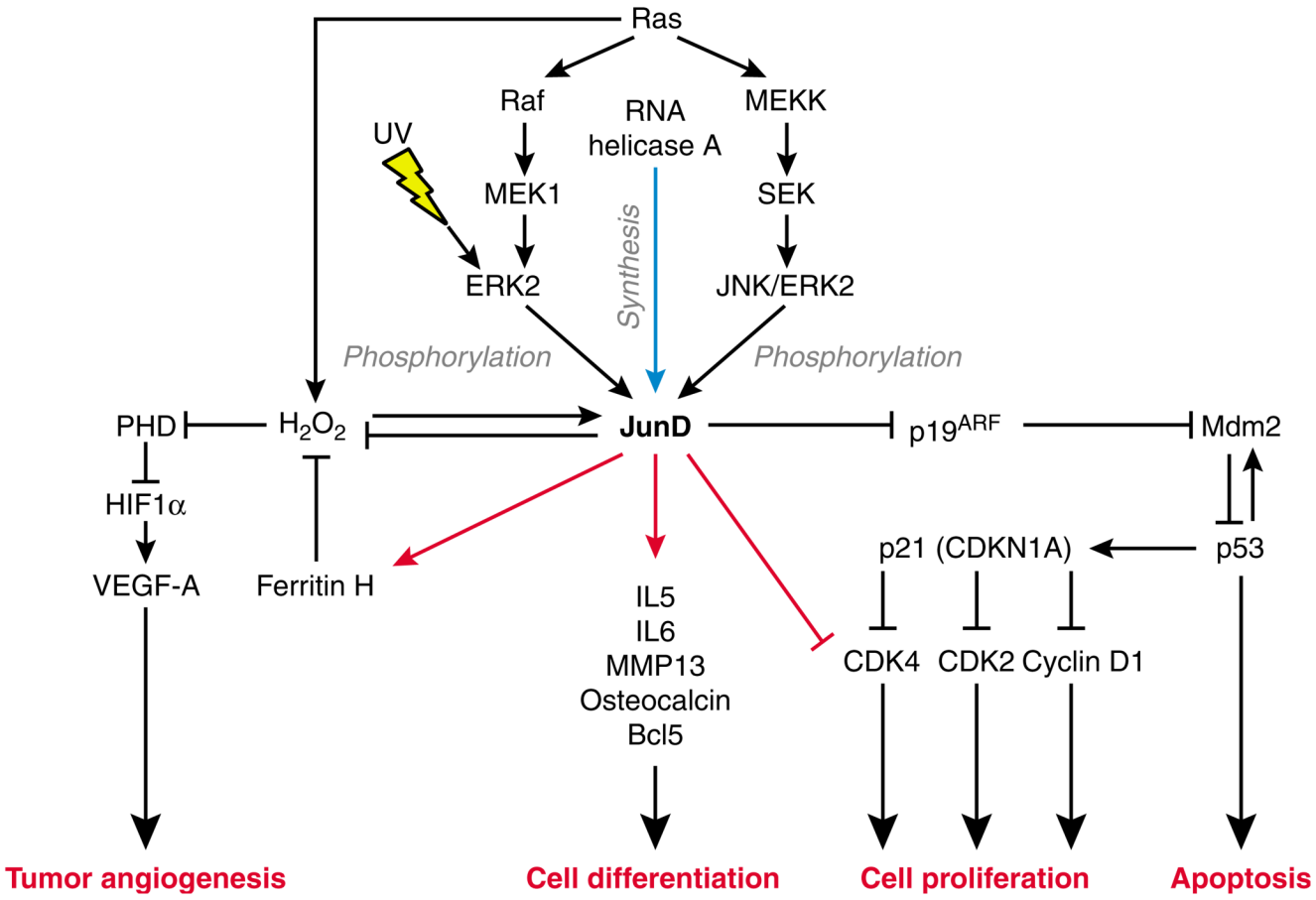

Figure 2.

Proposed network operated by JunD activation and repression of a diverse collection of target genes modulates tumor angiogenesis, cellular differentiation, proliferation and apoptosis. Increased JunD protein synthesis occurs upon derepression of efficient junD mRNA translation by RNA helicase A (indicated by blue arrow). The Ras signal transduction cascade activates phosphorylation of JunD. Phosphorylated JunD exhibits versatile activity to selectively repress or activate transcription selected direct target genes (indicated by red lines). Protein–protein interactions, post-translational modification and/or other molecular interactions are indicated by black lines. RHA.

Table 1

Features of selected JunD transcriptional targets

Gene	Cell system	JunD activity	Pathway	References
Ferritin H	Oxidative stress in human hepatoma cells	Activator	Antioxidant response during oxidative stress	Tsuji (2005)
CDK4	Polyamine depletion in human intestinal epithelial cells	Repressor	Cell proliferation	Xiao *et al.* (2007)
IL-5	Human primary T cells	Activator	Cell differentiation	Schwenger *et al.* (2002)
IL-6	Human androgen independent prostate cancer cells	Activator	Cell differentiation	Zerbini *et al.* (2003)
Bcl6	Mouse germinal center B cells	Activator	Cell differentiation	Arguni *et al.* (2006)
Osteocalcin	Mouse osteoblasts	Activator	Cell differentiation	Naito *et al.* (2005); Akhouayri and St Arnaud (2007)
MMP13	Human chondrocytes and fibroblasts	Activator	Cell differentiation	Uria *et al.* (1998); Ijiri *et al.* (2005)
C4.4A	Rat tumor cell lines	Activator	Cell proliferation	Fries *et al.* (2007)

Abbreviations: CDK, cyclin-dependent kinase; IL, interleukin; MMP, matrix metalloprotease.

Table 2

JunD modulates a broad array of cell types and clinical scenarios

Cellular process	Treatment	Effect on JunD expression	Outcome	References
Human ovarian cell differentiation	LHRH and FSH/LH	JunD protein levels increase	JunD is upregulated during cell maturation to arrest the cells at G0	Gunthert *et al.* (2002)
Human epithelial ovarian cancer	Disease	JunD mRNA levels decrease	Downregulation of *junD* may contribute to the malignant phenotype	Neyns *et al.* (1996)
Adult mouse hearts under chronic moderate pressure overload	Moderate thoracic aortic constriction	JunD protein levels decrease	JunD protects from cardiac hypertrophy, apoptosis, and angiogenesis under pressure overload	Hilfiker-Kleiner *et al.* (2005)
Failing human myocardium	Hearts obtained at the time of transplantation	*junD* mRNA levels decrease by factor of four	*junD* downregulation in myopathic heart	Pollack *et al.* (1997)
Chicken chondrocyte differentiation	Parathyroid hormone	JunD protein levels increase	JunD suppresses chondrocyte maturation	Kameda *et al.* (1997)
Mouse T-cell differentiation	Ubi-junD^{m} *junD*−/− transgenic cells	Not applicable	JunD suppresses lymphocyte proliferation and has a negative effect on Th cell differentiation	Meixner *et al.* (2004)
Human myeloblastic leukemia	UV radiation and TPA	*junD* mRNA levels increase	UV upregulates *junD* in ML1 cells via PKC-coupled Erk-signaling pathway and plays a role in UV-induced cell death	Li *et al.* (2002b)
Splenic marginal zone lymphoma (SMZL)	Analysis of genome-wide gene expression	*junD* mRNA levels increase	*junD* as well as other AP-1 genes may to be autoregulated by a MAP kinase-independent mechanism in SMZL	Troen *et al.* (2004)
Human intestinal epithelial cells	Polyamine depletion	*junD* mRNA is stabilized	Polyamines downregulates *junD* mRNA post-transcriptionally	Li *et al.* (2002a)
Mouse embryonic fibroblasts	V-C or H_2O_2	JunD protein level and transcriptional activity increase	JunD plays an antagonistic role to UV-induced and H_2O_2-induced apoptosis	Zhou *et al.* (2007)

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; LHRH, luteinizing hormone-releasing hormone; TPA, TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate.