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A ROLE FOR PIN1 IN MAMMALIAN GERM CELL DEVELOPMENT AND SPERMATOGENESIS

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1. ABSTRACT

The peptidyl-prolyl isomerase Pin1 is proposed to have diverse functions in many vital aspects of the cell. Despite the multitude of proteins targeted by Pin1 and the proposed regulatory role it plays in critical cellular functions, Pin1 is an essential gene in some eukaryotic organisms, but is dispensable in metazoans. In two genetic models, *Candida albicans* and *Drosophila melanogaster*, Pin1 participates in distinct developmental processes regulated by the MAPK pathway. Pin1-deficient mice exhibit decreased primordial germ cell proliferation during embryonic development, along with several degenerative or proliferative defects in the adult testis, retina, mammary gland, and brain. The combination of primordial germ cell deficit and spermatogonial depletion contributes to severe fertility defects in Pin1-null mice. Since growth factor activated MAPK pathways are vital to germ cell proliferation and differentiation, a role for Pin1 in mammalian germ cell development and spermatogenesis is discussed in the context of the Ras/MEK/MAPK pathway.

2. INTRODUCTION

Pin1 participates in various signaling pathways by catalyzing the cis-trans isomerization of phosphorylated serine/threonine-proline (pSer/Thr-Pro) bonds in many important cellular proteins. The resulting conformational change of the phosphoproteins can alter their activity, stability, or function (1). Many crucial signaling proteins have been shown to be regulated by Pin1, including cell cycle regulators such as Cdc25C and cyclin D1, and transcription factors such as c-Jun and c-Myc (2, 3). Multiple regulatory functions have been ascribed to Pin1, including cell cycle regulation, checkpoint control, general transcription, and signal transduction via numerous pathways (4-6). Evolutionally, Pin1 is a well-conserved protein found in all organisms examined to date (7-14). The rescue of the lethal phenotype of *Saccharomyces cerevisiae* pin1/ess1 by Pin1 homologues from *Drosophila*, human, *Schizosaccharomyces pombe*, and even plants, exemplifies the functional interchangeability and conserved nature of Pin1 in eukaryotic organisms (8, 9, 13, 15, 16). Therefore, it is surprising that Pin1 was found to be essential for growth only in some lower eukaryotes, including *S. cerevisiae*, *C. albicans*, and *Aspergillus nidulans* (7, 14, 17, 18). Most other genetic organisms deleted for the Pin1 gene exhibited fairly mild phenotypes, in spite of the numerous critical cellular roles attributed to Pin1 (8, 10, 13, 19, 20). In two genetic models, fly and the fungus *C. albicans*, specific developmental processes regulated by the MAPK pathway were affected in the absence of Pin1 (18, 19). Recently, a role for Pin1 in mammalian developmental signaling has been identified (21). Primordial germ cells (PGCs) displayed defective proliferation in Pin1$^{-/-}$ embryos on an isogenic background (21). In addition, postnatal mitotic germ cells underwent progressive depletion in adult Pin1-deficient testis (22). Interestingly, a principal signaling pathway governing mammalian PGC and spermatogonial proliferation and differentiation is the MAPK pathway, similar to that involved in *Drosophila* dorsal appendage patterning or *C. albicans* filamentation. Here, the proposed function of Pin1 in germ cell proliferation and/or differentiation is discussed, highlighting a vital role for Pin1 in germ cell development, and its potential as an influential regulator of growth factor activated MAPK pathways in mammalian germ cells.

3. PIN1 AND THE MAPK PATHWAY

While an earlier report on the Pin1/Dodo mutant in *Drosophila* described no obvious phenotype, careful analysis of these mutants later revealed developmental defects in dorsal-ventral patterning of the developing egg chamber (8, 19). Dorsal follicle cell fate in the egg chamber is determined by activation of a EGFR/Ras/Raf/MEK/MAPK pathway that leads to MAPK phosphorylation and subsequent degradation of a Kruppel family C2H2-type zinc finger transcriptional repressor, Cf2 (23, 24). The removal of Cf2's repressive influence allows appropriate expression of a crucial dorsalizing gene, rhomboid, thereby establishing dorsal ventral polarity of the developing embryo (23, 24). It was established that

Pin1/Dodo interacts with a MAPK phosphorylated Thr-Pro site on Cf2 and promotes its ubiquitination and proteolytic degradation upon EGFR signaling (19). Importantly, Pin1/Dodo by itself does not determine follicle cell fate, as Pin1 overexpression is not dorsalizing. However, Pin1/Dodo exacerbates the dorsalizing influence of D-Raf (19), thereby implicating Pin1 as a responder to the EGFR/MAPK cascade rather than a direct component of the signaling pathway. Although the mode of regulation by Pin1 on Cf2 degradation is not unique, the recognition of a central role for Pin1 in a MAPK pathway to determine cell fate during normal embryonic development offers new insight into the biological relevance of Pin1 in the context of whole organisms.

Indeed, epistatic analysis in *C. albicans* of Pin1/Ess1 and a transcription activator in the MAPK pathway, Cph1/Ste12, placed Pin1 in the MAPK pathway to enhance morphogenetic switching from yeast to hyphal or pseudohyphal forms of the fungus (18). How Pin1/Ess1 might modulate MAPK mediated fungal cell fate under certain environmental conditions has not been examined. In *S. cerevisiae* where Ste12 performs a similar function in filamentous growth (25), a MAPK Kss1 was found to inhibit filamentation by directly binding to Ste12 in the absence of MEK activation; upon stimulation, the repression on Ste12 was relieved owing to Kss1 phosphorylation, and presumably, Ste12 phosphorylation and altered protein-protein interactions, to permit filamentation (26, 27). Pin1/Ess1 may interact with MAPK phosphorylated Ste12 and augment its transcriptional activity on target genes for filamentous growth to control morphogenetic cell fate in *C. albicans*. Future investigations will shed light on how Pin1 impinges upon the MAPK pathway in this important developmental process of *C. albicans*.

Pin1's involvement in the Ras/MAPK pathway has recently been extended to mammalian cells in the regulation of c-Jun and c-Myc (3, 28). Pin1 has been shown to interact with c-Jun phosphorylated on Ser-63/73 by activated JNK and increase phospho-c-Jun transcriptional activity on the cyclin D1 promoter (28). Another example of Pin1's role in the MAPK pathway is demonstrated in the case of the oncoprotein c-Myc, whose protein accumulation and timely degradation is required for proper progression through the cell cycle. After growth stimulation, c-Myc is phosphorylated by Ras-activated MAPK on Ser-62 which causes Myc protein accumulation during G1 (29). Later in G1/S, the first step triggering a necessary reduction in Myc protein is achieved by GSK3-beta phosphorylating Myc on Thr-58 (29). Dually phosphorylated c-Myc on Thr-58 and Ser-62 was capable of binding Pin1, and Pin1 facilitated dephosphorylation of Ser-62 by PP2A, leading to its subsequent degradation by the ubiquitin-proteasome pathway (3). A stable mutant of Myc, T58A, that cannot bind to Pin1 and cannot be dephosphorylated by PP2A on Ser-62, caused uncontrolled cell proliferation and transformation, attesting to the importance of Pin1 in coordinating this precise succession of phosphorylation and dephosphorylation (3). Although the biological consequence of elevated phospho-c-Myc in the absence of Pin1 remains to be determined, this finding accentuates a critical role for Pin1 in MAPK signaling across a wide spectrum of genetic organisms.

4. GERM CELL PHENOTYPES OF PIN1-DEFICIENT MICE

Mice homozygous for the targeted deletion of the Pin1 gene on a mixed genetic background were initially reported to develop normally without apparent phenotypes (10). A later study uncovered multiple defects in these $Pin1^{-/-}$ mice that resembled cyclin D1 null mice (20). For example, the mammary gland of $Pin1^{-/-}$ pregnant females failed to undergo proliferative expansion, and $Pin1^{-/-}$ retina exhibited degeneration at 16 months of age (20). In addition to the cyclin D1-like phenotypes, $Pin1^{-/-}$ males were reported to display testis atrophy after several months, while $Pin1^{-/-}$ ovaries had normal histology and morphology (20). An age-dependent progressive neuronal degeneration phenotype was also observed in $Pin1^{-/-}$ mice, presumably as a result of aberrant regulation of phosphorylated tau protein in the absence of Pin1 (30-32).

Our laboratory turned to Pin1-deficient mice maintained on an isogenic genetic background in hopes of elucidating additional roles of Pin1 *in vivo*. Indeed, the first evidence of Pin1 functioning during normal mammalian embryonic development came with a recent analysis of these isogenic $Pin1^{-/-}$ animals (21). On a mixed genetic background, $Pin1^{-/-}$ males and females retained reproductive capability, and displayed normal reproductive organ morphology for most of their adulthood until older males developed mild testicular atrophy (20). However, when the null mutation was transferred into an inbred C57BL/6J background, $Pin1^{-/-}$ males and females mated together failed to reproduce a single offspring (21). Detailed examination of the reproductive organs revealed a common deficit at birth, where $Pin1^{-/-}$ males and females were both born with a severely reduced number of germ cells, indicating defects in primordial germ cell (PGC) formation during embryonic development. After tracing the developmental path of PGCs following their allocation at the base of the allantois, it was determined that PGCs in Pin1-deficient embryos failed to proliferate normally during their entire proliferative phase from 8.5 to 13.5 dpc (21). Detailed analysis using markers of apoptosis and different cell cycle phases revealed that PGCs did not arrest in mitosis or G0 phases, nor did they undergo increased apoptosis in the absence of Pin1. Rather, Pin1-deficient PGCs displayed reduced BrdU incorporation when compared to wild-type cells. Taken together, these results suggested a delay in the G1/S phase of the cell cycle in Pin1-null PGCs (21). Furthermore, there was an age-dependent progressive degeneration of spermatogenic cells in adult $Pin1^{-/-}$ testis, owing to depletion of the mitotic (versus meiotic) population of germ cells, suggesting that Pin1 is required to regulate proliferation and/or cell fate of spermatogonia (22). Thus, in our study of Pin1-deficient mice on the C57BL/6J background, severe abnormalities in both embryonic and postnatal germ cells were discovered. In the absence of Pin1, PGCs were allocated properly at the base of the allantois on 8.5 dpc, but displayed profound

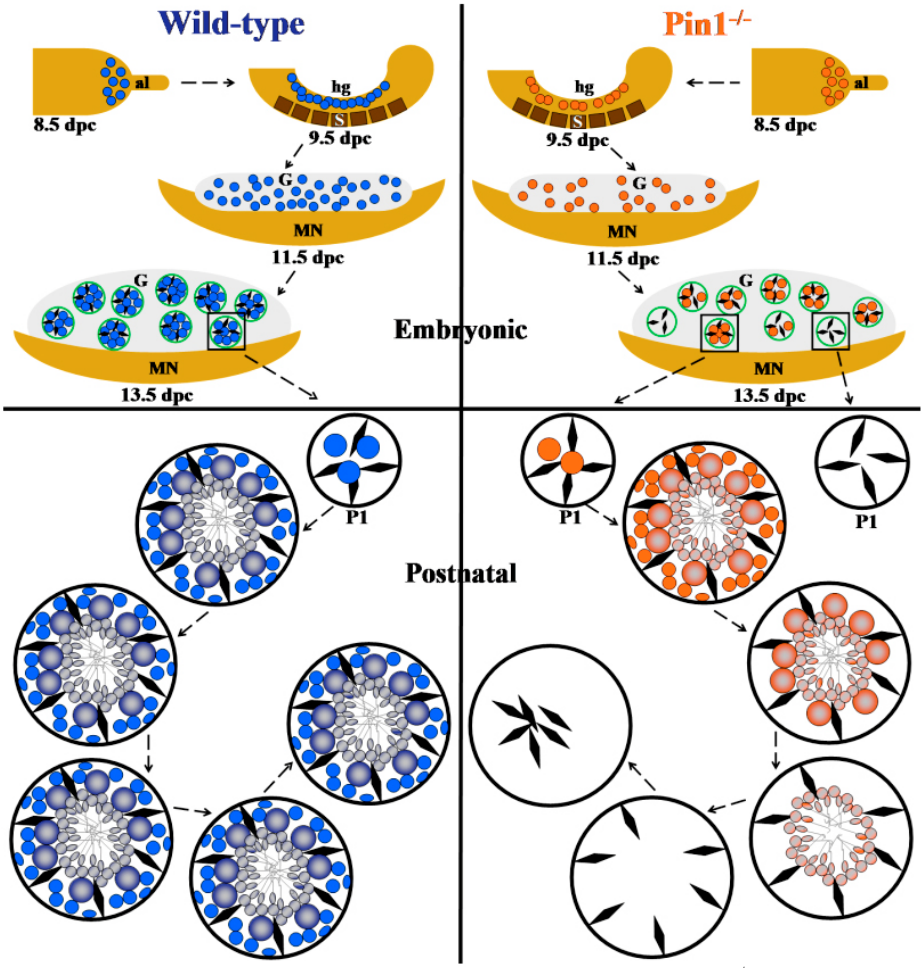

Figure 1. Illustration of PGC development and spermatogenesis in wild-type (left) and Pin1$^{-/-}$ (right) mice. top panels, embryos; bottom panels, postnatal testis tubules. PGCs are properly allocated at the base of the allantois (al) in 8.5 dpc wild-type (blue cells) and Pin1-null (red cells) embryos. At 9.5 dpc, proliferating PGCs migrate through the hindgut (hg), but fewer PGCs are found in Pin1$^{-/-}$ embryos, (s, somite). PGCs have arrived in the gonads (G) by 11.5 dpc, and are organized within immature testis cords (green circles) by 13.5 dpc. Significantly fewer PGCs are seen in the Pin1$^{-/-}$ gonad compared to wild-type, but Sertoli-cells (🔪) formed normal-appearing testis cords in the absence of Pin1 (top panels; MN, mesonephros). Those testis cords without any PGCs in the Pin1$^{-/-}$ gonad remain devoid of germ cells throughout postnatal life (P1, right), but the existing PGCs in some cords develop into gonocytes postnatally (P1, with red cells). Pin1$^{-/-}$ gonocytes, similar to wild-type, are capable of progressing through all stages of mitosis, meiosis and spermiogenesis to form mature spermatozoa. However, at some point during adult life, spermatogonia (🔴) become depleted in the Pin1$^{-/-}$ seminiferous tubule, triggering loss of spermatocytes (🔴) and then spermatids (🔴). Sertoli cells eventually are sloughed off, leading to an empty tubule in Pin1$^{-/-}$ testis. In contrast, dynamic spermatogenesis is maintained in the wild-type tubule throughout the reproductive life of the animal.

defects in proliferation owing to G1/S delays, resulting in fewer germ cells at the end of the proliferative phase at 13.5 dpc (figure 1, tope panels) (21). Existing PGCs gave rise to postnatal gonocytes in Pin1-null males, which were capable of the entire process of meiosis to produce functional spermatozoa (figure 1, bottom panels). However, the mitotic population of spermatogenic cells (spermatogonia) was gradually depleted in Pin1-null testis, possibly due to decreased proliferation or increased differentiation, leading to progressive degeneration of the seminiferous epithelium (figure 1, bottom panels) (22). The molecular target(s) of Pin1 in germ cell development and spermatogenesis remains to be determined.

5. MAPK SIGNALING IN MAMMALIAN GERM CELLS

Unlike the mammary gland and retinal degeneration phenotypes in Pin1$^{-/-}$ mice reported by Liou *et al* (20), the fertility defects of Pin1$^{-/-}$ mice cannot be attributed to a deficiency in cyclin D1 protein, as complete lack of cyclin D1 did not affect fertility in cyclin D1 null mice (33, 34). Another reported target of Pin1 during G1/S is the transcription factor c-Jun, but again, transgenic mice with the endogenous Jun allele replaced by a mutant Jun allele with the critical Pin1 binding residues Ser-63/73 mutated to Ala were viable and fertile (35). Although

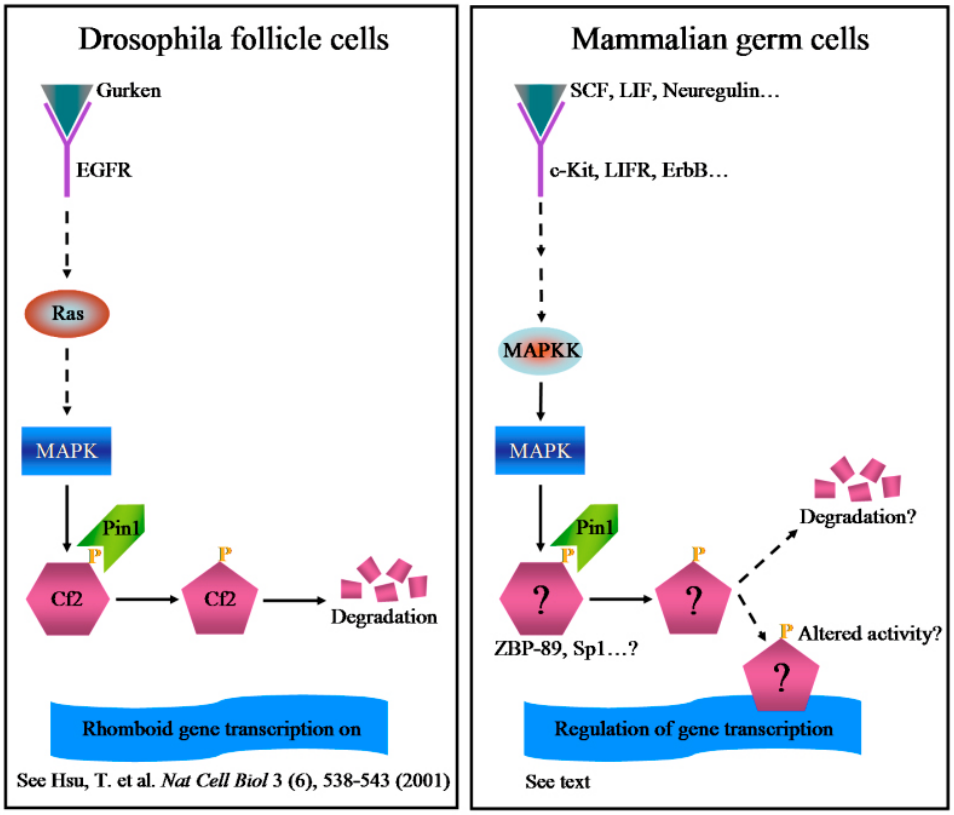

Figure 2. Hypothesized model of Pin1 function in MAPK pathway(s) in mammalian germ cells. In *Drosophila* follicle cells during embryogenesis, EGFR-activated MAPK phosphorylates the transcriptional repressor, Cf2, thus allowing Pin1 to bind and isomerize Cf2. The conformational change of Cf2 triggers its degradation by the ubiquitin-mediated proteasomal pathway, thereby relieving repression of the rhomboid gene for dorsalization (19). A similar mode of Pin1 regulation may exist in mammalian germ cells. Factors such as SCF, LIF, or Neuregulin can activate their respective receptors, c-Kit, LIFR, or ErbB, leading to activation of the MAPK pathway(s). Potential transcription factors, including ZBP-89 and Sp1, are phosphorylated by MAP kinases and represent attractive targets for Pin1 regulation. Pin1 could alter the stability or transcriptional activity of these downstream transcription factors of the MAPK cascade and regulate gene expression in germ cells during growth and/or differentiation (see text).

primary fibroblasts from Pin1$^{-/-}$ embryos on either mixed or inbred genetic background grew more slowly than wild-type cells, the molecular mechanism of this growth defect is unknown (3, 10, 36). Multiple proposed targets of Pin1, including p53, NF-kappaB, and c-Myc, have been identified from studies utilizing MEFs from Pin1$^{-/-}$ mice, but only after those cells were manipulated *ex vivo* in some way to expose the defects, such as treatment with DNA damaging agents, stimulation with exogenous cytokines, or serum starvation (3, 37-39). Therefore, Pin1 can function in multiple signaling pathways, depending on the context of the cellular environment. Thus, in our evaluation of important factors controlling both PGC and spermatogonial development, and in consideration of Pin1's function in the MAPK pathway during *Drosophila* embryogenesis and fungal morphogenesis, we focused on growth factor-activated MAPK signaling as a potential pathway for Pin1 participation (figure 2).

Although the molecular mechanisms of germ cell proliferation and differentiation remain poorly understood, a ligand-receptor system, stem cell factor (SCF; steel; kit ligand) and its receptor c-Kit, is widely recognized as essential for normal PGC development and spermatogenesis (40-44). Many natural mutations in both SCF and c-Kit, at the mouse steel and white spotting loci, respectively, caused severe abnormalities in gametogenesis, hematopoiesis, and melanogenesis (45, 46). It is well established that SCF and c-Kit promote PGC survival and proliferation, and spermatogonial proliferation and differentiation (40, 41, 44, 47-50). Upon activation, c-Kit, a receptor tyrosine kinase of the platelet-derived growth factor receptor (PDGFR) family, is capable of initiating several intracellular signaling cascades, including the PI3K/AKT/mTOR/p70S6K, Ras/MEK/MAPK, and JAK/STAT pathways (48-53). Although the specific intracellular pathways in the regulation of mitogenesis, survival, and differentiation via c-Kit are largely unknown, recent evidence suggests that various signaling pathways may perform different functions in a cell selective manner (50, 53, 54). The most compelling example came from studies of mutant mice with the c-Kit receptor mutated at Tyr-719 to Phe, the critical PI3K binding site (54). Surprisingly, these mice did not develop hematopoietic or pigmentation defects like those seen in loss-of-function c-

Kit mutants; rather, they displayed male specific infertility as a result of decreased spermatogonial proliferation/differentiation at an early postnatal stage. In addition, PGC development was normal in these mice, as was female fertility (54). These results provided elegant proof that a specific pathway, namely the PI3K signaling pathway, was required for c-Kit regulation of early postnatal spermatogonial proliferation/differentiation, but dispensable for c-Kit function in PGCs, melanocytes, and erythroid cells. Whether the PI3K pathway is required or dispensable for adult spermatogonial proliferation/differentiation remains an open question, since recent evidence showed that some differences exist in niche and properties of postnatal gonocytes/spermatogonia and adult spermatogonia, suggesting that the regulatory signals governing the initiation of spermatogenesis and the maintenance of spermatogenesis may differ (55-58). *In vitro* culture studies supported that the function of PI3K in PGCs was unnecessary, and suggested that the c-Kit mediated Ras/MEK/MAPK pathway promoted PGC proliferation as well as spermatogonial proliferation (49, 50). Furthermore, a number of extracellular factors and cytokines capable of signaling through the MAPK pathway, such as leukemia inhibitory factor (LIF), fibroblast growth factor (FGF) and Neuregulin, have been demonstrated to promote PGC survival and proliferation in culture (44, 59-62). As phosphorylation on SP or TP residues by MAPK creates potential Pin1 binding sites, Pin1 may modulate critical downstream target(s) of activated MAPKK/MAPK signaling in PGCs and spermatogonia (figure 2).

6. ZBP-89 AND SP1 AS POTENTIAL PIN1 TARGETS IN THE MAPK PATHWAY

Among the few identified intracellular molecules that have been shown to be involved in PGC development, a Kruppel family C2H2-type zinc finger transcriptional repressor, ZBP-89, seemed to represent an attractive target for Pin1 (63). Embryos heterozygous for the ZBP-89 gene (Zfp148 in mouse) initially possessed PGCs, but they were mostly lost by 13.5 dpc through a process suspected to be apoptosis (63). Although the process by which the number of PGCs was reduced was dissimilar between $Pin1^{-/-}$ and $ZBP\text{-}89^{+/-}$ embryos, one being decreased proliferation, the other being apoptosis, we were prompted to examine the potential relationship between ZBP-89 and Pin1 for several reasons. The haploinsufficiency of ZBP-89 suggests that a delicate balance of its protein level, (and by inference its transcriptional activity), is essential for normal PGC development. Indeed, overexpression of ZBP-89 protein in cultured cells was shown to cause decreased proliferation, evidenced by an increased G1 cell population and reduced BrdU incorporation, indicating a role for ZBP-89 in the G1/S transition (64, 65). Thus, a precise level of ZBP-89 protein and/or ZBP-89 activity might be tightly regulated in cells. As Pin1 has been demonstrated to have the ability to regulate another Kruppel family C2H2-type zinc finger transcriptional repressor, Cf2, in a manner dependent on Ras/MEK/MAPK signaling during *Drosophila* embryonic development (19), it seemed possible that ZBP-89 was a downstream component of a growth factor-activated MAPK cascade in PGCs and was modulated by Pin1. To our knowledge, nothing was known about whether ZBP-89 is post-translationally modified by phosphorylation. Our preliminary data showed that ZBP-89 is an *in vitro* substrate of Erk, and an *in vivo* substrate of activated MEK1/MAP kinase. ZBP-89 binds to the WW domain of Pin1, and this interaction depends on ZBP-89 phosphorylation (Atchison *et al*, unpublished data). Since ZBP-89 exerts its effect on gene expression mainly by competing with another Kruppel family zinc finger transcription factor, Sp1, a potential role for Sp1 in germ cell proliferation is also plausible. Unfortunately, mice null for the Sp1 gene showed severe developmental retardation and died prior to 11.5 dpc, so a function for Sp1 in germ cell proliferation *in vivo* cannot be evaluated without generation of conditional mutants (66). However, among other kinases, MAPK can phosphorylate Sp1 on several S/T-P residues, and Sp1 phosphorylation by MEK/MAPK signaling can activate or repress transcription depending on the promoter (67-69). We recently found that MEK/MAPK-phosphorylated Sp1 also interacted with Pin1. Transcriptional studies of ZBP-89 and Sp1 activity on a promoter where they exert opposing functions revealed significantly higher promoter activity in $Pin1^{-/-}$ cells compared to wild-type cells, but only in response to MEK/MAPK stimulation (Atchison *et al*, unpublished data). Thus, two mammalian Kruppel-type zinc finger transcription factors have been identified as Pin1 interactors dependent on MAPK phosphorylation. The biological consequence of these interactions is currently under investigation (figure 2).

Future efforts to identify potential molecular targets of Pin1 will benefit from discovery of additional cellular proteins that affect PGC development in genetic mouse models. In principle, *in vitro* experiments can then be carried out to ascertain their potential regulation by Pin1. It is imperative to note that there may be factors that normally participate in PGC development but whose deletion results in embryonic or early postnatal lethality and therefore remain unidentified. For example, deletion of beta-catenin, a critical signaling protein during G1/S and a target of Pin1 (70), resulted in lethality at gastrulation prior to PGC allocation (71). Any effects of beta-catenin and its proposed regulation by Pin1 on PGC development will only be apparent upon analysis of a PGC specific knockout of beta-catenin. Thus, conditional genetic mouse models should prove invaluable in discovering new regulators of PGC proliferation. At present, excision of genes in PGCs is potentially possible by utilizing a targeting construct with tissue non-specific alkaline phosphatase (TNAP) driving Cre expression (72). A more direct approach to address specific effects of Pin1 in PGCs would be purification and *in vitro* culture of PGCs. However, very few PGCs can be isolated, and currently severe technical limitations exist in our ability to manipulate these cells *in vitro* (50, 73). Although several laboratories have reported isolation of PGCs and have placed them in short-term culture on feeder cell layers, they had to pool PGCs, frequently not sorted as a pure population during isolation, from many normal embryos to do so (41, 42, 59, 73, 74). The infertility of $Pin1^{-/-}$ mice precludes large scale pooling of PGCs from Pin1-deficient embryos for cell culture studies. One reported method of fluorescence-activated cell sorting of

PGCs by TNAP driven beta-galactosidase expression offers a more convenient and reliable way to highly enrich PGCs (75, 76). Utilizing this procedure to obtain sufficient quantities of pure populations of wild-type and Pin1$^{-/-}$ PGCs for mRNA isolation in gene expression profiling may offer insight into the spectrum of genes affected by the absence of Pin1.

7. PERSPECTIVE

With increasing attention on Pin1 and correlation of Pin1 levels with the degree of tumorigenesity of some cancers (77-79), efforts are being made to synthesize selective Pin1 inhibitors as anti-cancer drugs (5). In this regard, the importance of thorough analyses of genetic mammalian models at the whole organism level cannot be overemphasized. Phenotypes of Pin1-null mice serve as valuable guides for clinical monitoring of drug toxicity. Long-term usage of potential anti-Pin1 drugs might produce irreversible retinal, testicular, and neuronal degeneration, while its administration to pregnant women may cause failure of mammary gland proliferation. The primordial germ cell phenotype of Pin1-null embryos raises the caution of teratogenic effects of potential anti-Pin1 drugs, as such agents might cross the placenta during pregnancy and contribute to fertility problems in the offspring. Understanding the molecular nature of Pin1 regulation in germ cells contributes to the knowledge required for future therapeutic intervention.

We hypothesize that Pin1 regulates germ cell proliferation and/or differentiation by exerting an influence on the MAPK pathway, in a manner similar to that seen in *Drosophila* embryogenesis and fungal morphogenesis. However, unlike mammalian PGCs, the *Drosophila* follicle cells at the stages of dorsal ventral patterning are not proliferative, and Pin1 participates in cell fate decision rather than cell cycle progression (19). This is not completely surprising given that a central tenant of Pin1 action is that it facilitates, rather than directly transduces, signaling. This notion is accentuated by the observation that Pin1 alone does not alter cell fate (dorsalize), but merely responds to a signal to facilitate dorsalization (19). Thus, while the phenotypic outcome of Pin1 deletion in genetic organisms such as the fungus, the fly, and the mouse, is different, the concept that Pin1 is an important modulator of the MAPK pathway during growth and differentiation presents a cohesive theme for future investigation. The reason for such dramatic effects of Pin1 during some developmental stages but not others in fungus and fly and mouse is not well understood, given that MAPK signaling is pervasive in many biological processes ongoing in these organisms. Progression through some developmental stages may be more susceptible to disturbances in equilibrium of interconnecting signaling networks, and reactions catalyzed by Pin1 are essential for the orderly timing of biological events. This view complements the usual inference of genetic redundancy and/or functional degeneracy in genetic studies when expected phenotypes fail to emerge, and is consistent with the multi-functional behavior of Pin1 in cell-based studies and the limited spectrum of its biological impact in whole organisms.

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