[Cell Cycle 5:8, 824-828, 15 April 2006]; ©2006 Landes Bioscience

Perspective

p38 MAPK in Development and Cancer

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Original manuscript submitted: 03/06/06
Manuscript accepted: 03/08/06

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=2685

KEY WORDS

p38 MAPK, embryo, development, cancer, review

ACKNOWLEDGEMENTS

We thank Jenifer Croce and Wendy Beane for critical review of this manuscript. C.A.B. and D.R.M. were funded by NIH grants 61464, HD14483 and DOE grant DE-FG02-03ER63584.

ABSTRACT

p38 is a MAPK that has been shown to induce a wide variety of biological effects in cell culture in response to a wide range of stimuli. These effects are dependent not only on the stimuli, but also on the cellular context, resulting in a bewildering array of possibilites. For example, p38 was shown to induce apoptosis in some cells, but prevent apoptosis in others. Similarly opposed effects had been observed with respect to cell cycle regulation. The role of p38 in inflammatory disease has been appreciated from the beginning, since it was initially identified as an cytokine inducer.[3] More recently, p38 function has been evaluated in vivo, and through these studies p38 has emerged as an important regulator of both embryonic development and cancer progression. This review will focus on these in vivo studies in an effort to provide perspective on p38 biologically and as a pharmacological target.

INTRODUCTION

p38 is a mitogen-activated protein kinase (MAPK) that is known as a stress-activated MAPK, since it is responsive to cellular stress and cytokines. However, p38 has much broader functions physiologically. As a MAPK, p38 is activated by dual phosphorylation as the terminal kinase in various signaling cascades. Figure 1 illustrates some of these pathways, the details of which have been well-reviewed elsewhere.[1,2] Most p38-activating pathways include a MAPK kinase (MKK) and MKK kinase (MKKK or MEKK), although the TAK1 > TAB > p38 pathway does not. In that case, TAB induces p38 to auto-phosphorylate and thus to self-activate.[4] p38 activation downstream from the T cell receptor also occurs via autophosphorylation in an MKK-independent manner, possibly through the promotion of dimerization.[5] The study of p38 has been facilitated by the use of p38 inhibitors, such as the SB family of drugs.[6] Since these drugs inhibit the kinase function of p38, they can be used to discriminate auto-activation of p38, which is drug sensitive, vs. activation by a MKK, which is not.[4,5]

How is specificity achieved by these pathways? Part of the answer is scaffold proteins, which bind and organize other proteins but (usually) lack intrinsic enzymatic activity. Scaffolds sequester and arrange sequential signaling proteins, insulating them from phosphorylating alternate cytoplasmic targets and speeding signaling through the pathway. For example, the dual phosphorylation of ERK (another well-studied MAPK) by its upstream MKK is a nonprocessive process in solution, but becomes highly processive in the scaffold, dramatically changing the kinetics of activation[7] (Fig. 2A). Several different types of scaffolds have been observed. In yeast, Ste5 is the scaffold protein for the pathway signaling through Fus3 (homologous to ERK). In contrast, the yeast Hog1 (p38) pathway does not have a separate scaffold; instead, the upstream MKK Pbs additionally functions as the pathway scaffold[8] (Fig. 2B). A mammalian p38 scaffold has recently been identified that binds Rac, MEKK3, and MKK3 in response to osmolarity challenges, and has been named OSM (osmolarity scaffold for MEKK3).[9] In this example, the scaffold does not bind the MAPK directly.

Upon activation, MAPKs generally detach from the scaffold and translocate to the nucleus, suggesting that the specificity for their targets must be an inherent property.[8] However, differing MAPKs frequently activate an overlapping set of transcription factors, again raising the question of how specificity arises. Kinase-independent inhibitory functions of yeast MAPKs contribute to the specificity of the response;[10,11] kinase-independent functions for mammalian p38 have also been described.[12] A recent study in yeast has addressed issue of downstream specificity by analyzing the transcription factor Ste12.

Ste12 activated by two distinct MAPKs, Fus3 and Kss1, during two distinct events, mating and filamentous growth, respectively. The authors performed a genome-wide survey of Ste12 targets during these two events, and found that Ste12 binds to distinct sets of genes depending on the stimuli and the upstream MAPK.[13] Filamentous growth required the concurrent binding of Tec1, a transcription factor and binding partner that appears to direct site selection.[13] Thus, in yeast, two MAPKs dictate that the same transcription factor controls two distinct sets of target genes by differentially regulating the requirement for a binding partner.

p38 can also affect transcription by modulating chromatin structure. The yeast p38 homolog Hog1 is known to respond to osmotic stress by inducing osmoresponsive genes. A recent study in has shown that Hog1 binds to rpd3-sin3, a histone deacetylase (HDAC).[14] During osmotic stress, Hog1 then recruits this HDAC to specific genes involved in the osmotic stress response, enabling their transcription.[14] Loss of rpd3-sin3 impairs the cellular response to osmotic stress,[14] demonstrating the importance of this mechanism. p38 has also been shown to modulate chromatin structure during inflammatory responses in mammalian systems[15] (see below).

Besides its effects on transcription, p38 also regulates gene expression post-transcriptionally, via the control of both mRNA stability and by translational access. Early observations showed that p38 is required for the expression of the cytokines TNFα and IL-1β during inflammatory responses.[16,17] However, in the absence of p38 activity, although cytokine proteins were not expressed, cytokine mRNA levels were unaffected, indicating that p38 was specifically required for their translation.[16,17] This translational control occurs through the p38 target MAPKAPK2 (MK2), since targeted deletion of MK2 similarly interferes with TNFα and IL1β translation. In addition, specific RNA sequences in the 3'UTR of TNFα and IL1β, known as AU-rich elements (AREs), mediate this translational control in vivo.[18,19] AREs regulate both translational control (through unknown mechanisms) and transcript stability. AREs are recognized by ARE binding proteins (AREBPs), which can, for example, recruit a multi-protein exosome to an mRNA to hasten its destruction.[20] Interestingly, in vitro studies indicate that sensitivity to this post-transcriptional control can be linked to the differentiation state of the cell. In monocytes, for example, TNFα mRNA stability was sensitive to p38 inhibition in differentiated cells but not in undifferentiated cells.[21] Thus, sensitivity to p38 is acquired as the cells develop. This post-transcriptional sensitivity to p38 also applies to other inflammatory proteins, including COX-2 and IL-8, and is dependent on both MK2 activity and specific ARE sequences in each case.[7]

p38 is a well-appreciated convergence point for the expression of inflammatory mediators, since it also promotes iNOS expression, PGE2 production, and the induction of secondary mediators such as MMPs and VCAM.[22] In addition, p38 primes inflammatory cytokines and chemokines for NF-κB-mediated transcription by phosphorylating histone H3 at specific sites to expose NF-κB promoters that would otherwise be hidden.[15] p38 has therefore become a highly sought pharmacological target for inflammatory diseases, such as Crohn's and rheumatoid arthritis, both of which are currently treated by blocking either TNFα or IL-1β action using expensive protein-based strategies.[22] The best clinical results are obtained when both signals are blocked, explaining the interest in p38, as it is upstream from both proteins and inhibitable with small molecule drugs that can be delivered orally. Several p38 inhibitors have recently reached stage I clinical trials.[22]

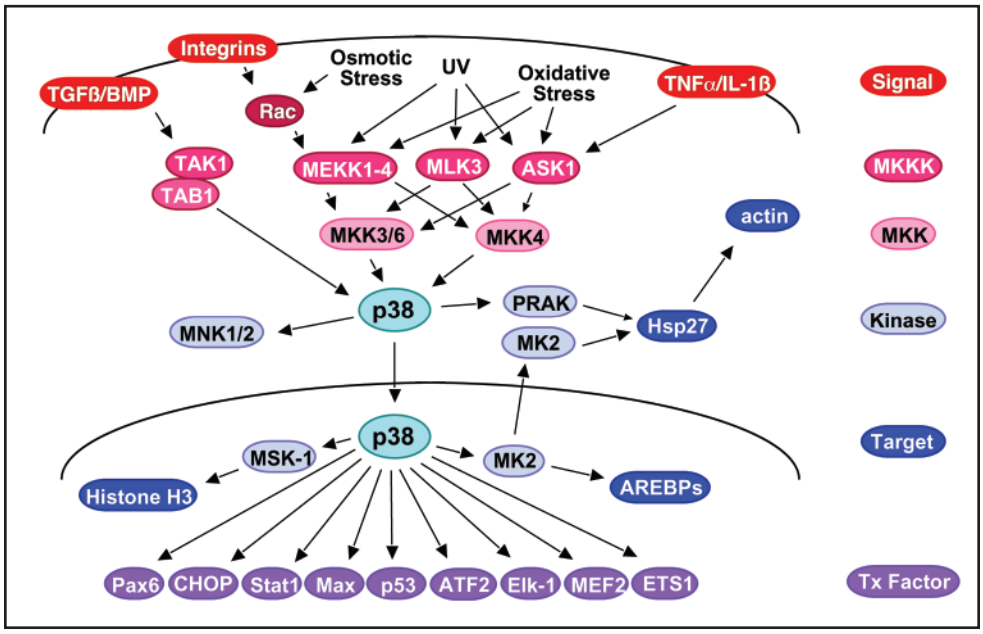

Figure 1. A schematic of p38 signaling interactions. Adapted from Cell Signaling Technologies, Inc. The right panel is a hierarchy key. For details, see the text and references 1 and 2.

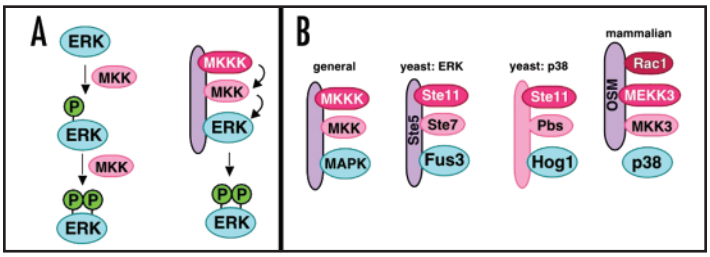

Figure 2. (A) MAPK activation by upstream MKKs requires 2 phosphorylation events. In solution, only one phosphorylation occurs with each interaction since the MKKs are not processive (left). However, in the scaffold, both phosphorylation events occur much more efficiently, with kinetics equivelent to processivity. (B) Schematics illustrating various scaffolding arrangements, in general (left), in yeast (center), and in mammals (right).

p38 IN DEVELOPMENT

In the late 90s the role of the p38 pathway was probed in *Drosophila* embryos by deleting licorne (lic), the MKK for *Drosophila* p38.[23] Lic$^{-/-}$ embryos have a ventralized egg shell and loss of abdominal segments, as well as no pole cells. Suzanne et al. showed that oskar mRNA, normally anchored at the posterior pole, was initially normal in lic$^{-/-}$ mutants, but then diffused broadly throughout the embryo.[23] Gurken (grk), a signaling ligand, is required

during oogenesis for both oskar localization and dorsal specification. The authors showed that although grk mRNA levels were normal in lic$^{-/-}$ embryos, protein levels were reduced and mislocalized[23] indicating that the p38 pathway is required for the post-transcriptional regulation of grk. Thus, in *Drosophila* embryos, the p38 pathway functions maternally to position the future anterior-posterior (AP) and dorsal-ventral (DV) axes.[23]

In the mouse, the situation is more complicated because of gene duplications. There are 4 mammalian p38 isoforms: α, β, γ and δ. Only p38α and β are sensitive to SB inhibitors,[24] and so p38γ and p38δ are much less well characterized. However, p38δ is expressed in specific sets of tissues throughout embryogenesis, including the foregut and pharynx, liver, lung, kidney, adrenal gland, small and large intestine, and epidermis,[25] suggesting a potential and as yet unexplored developmental role for this isoform. p38α is also strongly expressed in specific tissues during embryogenesis, namely the somites, brachial arches, limb buds, heart, and placenta.[26] Mouse embryos that are homozygous null for p38α fail to develop beginning at d10.5.[26-28] However, this is because p38α is specifically required for normal placental development and p38α$^{-/-}$ embryos die of suffocation and starvation.[26] This was demonstrated by fusing wild type tetraploid blastocysts with p38α$^{-/-}$ blastocysts, since the tetraploid cells can contribute to extraembryonic structures but not to the embryo proper. In p38α$^{-/-}$ tetraploid fusions, the placenta was composed almost exclusively of tetraploid cells, and embryonic development was fully rescued,[26] indicating that p38α is required for normal placental development. Consistent with this, a role for p38 in human trophoblast differentiation has also been shown in culture models.[29] Thus, p38α appears to be dispensable for embryonic development. However, a more important role for p38 is likely to be masked by functional redundancies provided by the other p38 isoforms in the embryo but not the placenta.

A more recent study tested the role of p38 signaling in preimplantation embryos. Embryos were treated with the p38 inhibitors SB203580 or SB220025, each of which inhibit both p38α and p38β.[24] Drug treatment caused the embryos to halt at the 8 cell stage and impaired embryonic compaction,[30] revealing an early requirement for p38α/β during cleavage. This effect of p38 inhibition was completely reversible since removal from the drug restored normal embryonic development.[30] The study shows that p38 inhibition completely blocks the accumulation of filamentous actin,[30] which appears beginning at the 8 cell stage[31] and depends on the induction of Hsp27 downstream from MK2.[30] This effect on actin is echoed by a study of p38 function in rat granulosa cells, an oocyte-supporting population. In these cells, follicle stimulating hormone (FSH) promotes maturation and survival, typified by the induction of a dramatic cell shape change. The results show that this cell shape change is dependent on SB203580-inhibitable p38 activity.[32] FSH activates p38 via cAMP and protein kinase A, while p38 in turn activates Hsp27.[32] Hsp27 can effect actin remodeling by functioning as an F-actin cap binding protein, dependent on its phosphorylation state.[33] These data show that p38 also plays a role in oocyte development, in a loose parallel to Drosophila. Thus, mammalian p38 plays important roles during oocyte maturation and embryonic cleavage, as well as being indispensable for normal placental function. However, it is likely that several important functions have not yet been revealed. Ultimately this will require targeted disruption of each of the p38 isoforms individually and in combination, as evidenced by gene targeting experiments with JNK, a related MAPK. Targeted disruption of either JNK1 or JNK2 did not impair embryonic development,[34,35] but the embryos lacking both JNK1 and JNK2 displayed profound neural tube closure defects due to failed neuronal apoptosis.[36,37]

We have recently evaluated the role of p38 signaling during sea urchin embryonic development. Our study shows that p38, a single isoform in the urchin, is uniformly active and nuclearized during cleavage stages, then is transiently inactivated and cleared from nuclei on one side of the embryo at late blastula stage, resulting in a functional asymmetry.[38] We demonstrate that this asymmetry coincides with and is required for the specification of the larval DV axis, such that the active side gives rise to ventral fates.[38] This finding provides an intriguing parallel to the asymmetric nuclearization of transcriptional activators associated with the initiation of axis specification in *Drosophila*, sea urchin, *Xenopus* and zebrafish.[39-43] In MAPK signaling pathways, the MAPK translocates to the nucleus where it modulates gene expression by activating (or inhibiting) transcription factors (Fig. 1). In other pathways a transcriptional activator, such as NF-κB or β-catenin, typically translocates to the nucleus in response to an inducing signal. An asymmetry of the *Drosophila* NF-κB homolog Dorsal initiates DV axis specification,[41-43] while asymmetric β-catenin nuclearization initiates the DV axis in *Xenopus* and zebrafish.[40] In the urchin embryo, asymmetric β-catenin initiates the primary animal-vegetal axis,[39] while asymmetric nuclearization of p38 drives the secondary DV axis,[38] suggesting that asymmetric p38 nuclearization may also occur in other embryonic contexts. We further demonstrate that p38 activity is upstream from Nodal, a TGFβ family member previously shown to specify the ventral territory,[44] and that p38 drives a signaling and gene regulatory network to specify the ventral territory.[38] Thus, we find that p38 activity is important during cleavage stages, as in mouse embryos, and is required for axial specification, as in *Drosophila* embryos. Since there is only a single p38 gene in the sea urchin genome,[45] the results are not ambiguous with respect to possible redundancies. We speculate that axial defects may be revealed by combined gene targeting of p38 isoforms in murine embryos.

What about other systems? In zebrafish embryos, p38 is activated asymmetrically during cleavage, and is required for cell division in a correspondingly asymmetric manner,[46] such that embryos injected with either dominant negative p38 or treated with SB203580 fail to cleave on one side. This side does not correlate with the first cleavage plane, but rather with the future dorsal side of the embryo, based on the expression pattern of dorsal markers. Finally, treatments such as UV irradiation and vegetal yolk mass removal which block dorsal specification also inhibit the asymmetric activation of p38.[46] Thus, zebrafish embryos also utilize p38 during cleavage stages, where it potentially plays a role in DV axis specification.

In a recent study in *Xenopus* embryos, inhibition of p38α with an antisense strategy was used to probe the role of p38 in development.[47] The results show that p38α is required for the expression of Myf5. Knockdown of p38α resulted in extended proliferation of the presomitic mesoderm and delayed somitogenesis, as well as a blockade to convergent extension of the presomitic mesoderm. Although somites were formed, the boundaries between them disappeared coincident with apoptosis throughout the somite region.[47] These effects were rescued by exogenous Myf5,[47] indicating that this muscle-specific transcription factor is the physiologically relevant target for p38α. Thus in *Xenopus*, p38α plays a role distinct from what has been observed in other model systems. This may reflect genuine divergence; alternatively, *Xenopus* p38α may turn out to have functional parallels to mammalian p38γ, which is specifically enriched in

muscle.[48] Xenopus has a distinct p38 isoform reported to function during egg activation.[49] However, simultaneous inhibition of both isoforms has not been reported. In an earlier study using *Xenopus* animal caps (but not intact embryos), SB203580-inhibitable p38 was shown to function downstream from BMP in the specification of the epidermal (vs. neural) territory.[50] In this study, BMP signals to p38 via TAK1.[50] SB-sensitive *Xenopus* p38 thus functions asymmetrically to partition the ectoderm, roughly analogous to its role in sea urchin embryos.[38]

p38 IN CANCER

p38 has recently gained attention as a tumor suppressor. This effect was first demonstrated by the simultaneous targeted deletion of the p38-specific MKKs 3 and 6.[51] Embryos lacking both MKK3 and MKK6 have a phenotype similar to the p38$\alpha^{-/-}$ mice,[51] in that the embryos die due to a placental defect. MKK3/6$^{-/-}$ fibroblasts, but not control fibroblasts, produced tumors in nude mice,[51] demonstrating a previously unsuspected tumor suppressor function for p38. An interesting in vivo study compared a tumorigenic HEp3 line and a derivative dormant cell line.[52] The authors devised a strategy to monitor p38 activity in vivo using a GFP reporter system, and studied the behavior of these cell lines in tumorigenic and metastatic models in both chick embryos and nude mice. They observed that both lines initially activate p38 in a strong and transient manner on chorioallantoic membranes of chick embryos and in nude mice. p38 was quickly inactivated concomitant with robust tumor growth and metastatic behavior in the tumorigenic cells.[52] The dormant line, in contrast, sustained p38 activity at low levels for several weeks, during which the cells failed to proliferate and metastasize.[52] Interestingly, the two lines grew with identical kinetics in vitro;[52] thus, this growth 'defect' in the dormant cells is context-dependent. The authors tested the relevance of the differing p38 activity profiles by pretreating the dormant line with SB203580 prior to the in vivo assays, and this pretreatment reversed the dormancy state, allowing the line to proliferate in vivo,[52] demonstrating that p38 activity is required for dormancy. These results corroborate the tumor suppressor function for p38. A comparison of 20 human hepatocellular carcinomas with adjacent nonneoplastic tissue showed that p38 was significantly less active in tumors; additionally, the larger the tumor, the less p38 activity was detected.[53] Other studies showed that oncogenic Ras cannot induce transformation unless p38 is silenced.[54,55] Overall, these studies indicate that sustained p38 activity is consistent with dormancy behavior and inconsistent with proliferation and growth of primary tumors in vivo. Another way to interpret this is that p38 activity induces responsiveness to the cellular context, suggested by the differential proliferation of the dormant HEp3 cells in vitro and in vivo.[52] In fact, this cell line was made dormant by reversing constitutive integrin activation.[52]

Thus it appears that p38 activation ought to suppress oncogenesis. Indeed, when p38 activity was forced in rhabdomyosarcoma cells, it induced terminal differentiation.[56] Similarly, deletion of a p38-inhibitory phosphatase blocked Hras1- and erbB2-induced carcinogenesis in vivo, whereas inhibition of p38 promoted tumor formation.[57] Imposed p38 activation in HeLa cells specifically suppressed their tumor-forming ability in vivo.[58] Other studies have evaluated p38 activity in the response to chemotherapy. The results indicate that diverse chemotherapeutic agents stimulate apoptosis in a p38-dependent manner. For example, microtubule-perturbing drugs such as vinblastine, vincristine and taxol induce tumor cell apoptosis that is inhibited by treatment with SB203580.[59] In these cells, the basal level of apoptosis is 2%, but can be raised to 54% by activating p38.[59] Cisplatinin, a DNA damaging agent, also induced p38-dependent apoptosis in several cells lines.[60] Thus, p38 appears to promote tumor cell apoptosis, at least in some cases. However, this should not be construed as a universal relationship, since p38 activity can also block apoptosis. For example, the anthrax lethal factor promotes macrophage apoptosis by inhibiting p38 activity.[61]

Dendritic cells exposed to tumor cell-conditioned media (TCCM) become poor immune response stimulators.[62] The authors show that TCCM induces p38 activation, whereas p38 inhibition restores normal dendritic cell function.[62] The authors suggest that tumors activate p38 in dendritic cell to avoid provoking an immune response. Thus it seems again that p38 activity corresponds with dormancy, and while that effect is desirable in tumor cells, it is deleterious in antigen presenting cells. Activation of p38 is associated with disease states in the brain, including Parkinson's disease, amyotrophic sclerosis, multiple sclerosis, and Alzheimer's disease.[63] Further, in animal models of stroke, inhibition of p38 is protective, resulting in decreased infarct size, reduced neurological deficits, and reduced expression of the inflammatory mediators TNFα and IL-1β.[64] Thus, as for inflammatory disease, activation of p38 in the adult brain is strongly associated with negative consequences.

Consideration of these studies suggests that clinical approaches toward modulating p38 will need to be something of a balancing act. If p38 is inhibited to treat arthritis or Crohn's disease, or to protect stroke victims from massive injury, the patients will be at risk for tumorigenesis. On the other hand, if p38 is activated to inhibit tumor growth and metastasis in cancer patients, this treatment will come with the risk of inducing inflammatory or neurodegenerative disease. Thus, modulation of p38 activity would be best accomplished with approaches that provide some tissue targeting specificity, for example restricting p38 activators from crossing the blood-brain barrier, or targeting p38 inhibitors specifically to the immune system or brain. Targeted gene therapy may ultimately be necessary to fine-tune clinical modulation of p38 function.

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