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The GCK II and III subfamilies of the STE20 group kinases

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1. ABSTRACT

The Ste20 (sterile 20) proteins are a large family of serine/treonine kinases. Since their discovery a growing body of evidence has implicated them in the regulation of signaling pathways governing cell growth, cell differentiation cell death and cell volume. Approximately 30 human members have been identified based on the high degree of homology of their catalytic domain to that of the Ste20p from *Saccharomyces cerevisiae*. In addition to the conserved regions, there are also regions of sequence that make each of them unique. In this review we will focus on two subfamilies of the group, GCK-II and GCK-III, families that are closely related but, again, unique in their structural features and biological functions. Herein, we will present what we hope will be the current state of knowledge about these kinases, and discuss what remains to be done in order to better understand their activity and regulation.

2. INTRODUCTION

The Ste20p (sterile 20 protein) was first described in the early 1990s as the upstream ser/thr kinase required to link G-protein beta-gamma subunits within the pheromone response pathway to downstream signaling components in *Saccharomyces cerevisiae* (1). The Ste20p is 44% identical in its catalytic domain to the kinase domain of sporulation protein 1 (Sps1) from *Saccharomyces cerevisiae*. Sps1 was identified on the basis of its requirement for normal progression of transcriptional, biochemical and morphological events during the later portion of the sporulation program (2). Structurally, Ste20p has its kinase domain located in the C-terminus of the protein whereas for Sps1 the catalityc region is in its N-terminus. Soon after the description of these two yeast proteins the first Ste20-like kinases in vertebrates were identified. The groups of Louis Lim and John Kehrl characterized the two mammalian homologues, PAK1

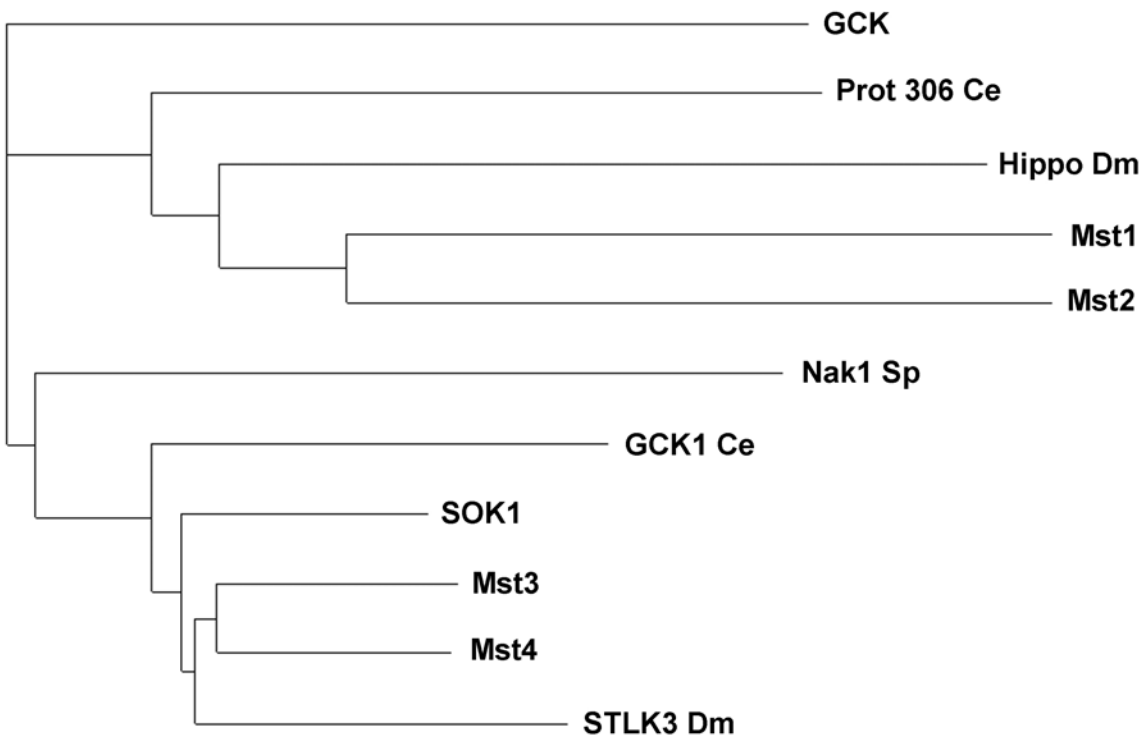

Figure 1. Phylogenetic relationship of GCKII and III subfamily of kinases. Shown are human proteins, and proteins from *D. melanogaster* (Dm), *C. elegans* (Ce), *D. discoideum* (Dd), and *S. pombe* (Sp). The sequences were aligned with the clustalw package, and a guide tree constructed using the neighbour joining method. GCK (a GCK-I subfamily kinase) was used as an outgroup to generate a root for the phylogenetic tree.

(p21-activated kinase 1)(3) and GCK (human germinal center kinase) (4), after which were named the two presently-defined Ste20 families. As described elsewhere (5) the two families are distinguished by the location of their kinase domains: those of the PAKs are located within the carboxy-terminal region as with Ste20p, whereas those of the GCKs are at the N-termini as with Sps1. Since then, a large number of proteins have been identified as members of the Ste20 group. The work done by Ippetia Dan and co-workers established the phylogenetic relationships among the different members of the group and allowed them to recognize 2 distinct subfamilies within the PAK family, and 8 subfamilies within the GCK family (6). Their physiological roles are at present under investigation, they have been implicated in developmental processes as well as in the regulation of systemic osmotic homeostasis (7). At the cellular level there is a growing number of publications that validate a role of these proteins in the regulation of various cellular processes such as cell divison and cell death in response to a variety of different factors and different cellular stresses (6). In this review we will discuss what is known about two closely related subfamilies of the GCK family, namely the GCK-II and the GCK-III subfamilies, and will attempt to clarify their cellular/physiological roles. All members of these two subfamilies share a common ancestral gene and are evolutionary most closely related to each other than to other members of the GCK family, which from a phylogenetical point of view makes them more susceptible to act or function alike (8). In fact and different from some other GCKs, all of the GCK-II and GCK-III subfamilies members have been functionally related with apoptotic processes. Furthermore, they also share some structural characteristics, as the presence of a putative nuclear localization sequence domain and a putative caspase cleavage site, proven functional for some of them, that are not found in other members of the GCK family. We will also discuss orthologs from *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae, Caenorhabditis elegans*, *Drosophila melanogaster* and *Dictyostelium discoideum* since they may provide additional clues to the roles played by mammalian GCKs.

First we will analyze the structure of their kinase domains and the putative key regulatory elements within their regulatory domains. Then we will focus on the stimuli that activate these kinases, the signal transduction pathways that they activate and the proteins with which they interact, focusing on the biological responses resulting from the activation of these proteins. In addition, we will review the somewhat limited data obtained from animal models in which a given GCK is either over-expressed or deleted.

3. GCK-II AND GCK-III PROTEINS: STRUCTURE AND FUNCTIONAL PROPERTIES

The GCK-II subfamily of Ste20 kinases as defined by Dan et al, encompass Mst1 and Mst2 from

Homo sapiens and other vertebrates (Figure 1). Orthologs in other organisms include Hippo in *D. melanogaster* and C24A8.4 in *C. elegans*. The vertebrate members of the GCK-III subfamily are Mst3, Mst4/MASK (Mst3 and SOK1-related kinase), and SOK-1 (Ste20/oxidant stress response kinase-1)/YSK1 (Yeast Sps1/Ste20-related kinase 1). There is a putative *D. melanogaster* ortholog, STLK3 (also known as CG5169), and a *C. elegans* GCK-1 has also been described. The protein severin kinase from *Dictyostelum discoideum* is also related to this GCK-III subfamily of kinases, as it bears a 75% homology to the human proteins in its kinase domain. Nak1/Orb3 kinase from *S. pombe* and Kic1p from *S. cerevisiae* are the closest yeast orthologs of the GCK-III subfamily.

As four of the five human members of the two subfamilies are named with the Mst acronym (Mammalian Ste20 like kinases), henceforth we will refer to the subfamilies collectively as Mst kinases.

As with the other members of the GCK family, GCK-II and GCK-III subfamily members have N-terminal kinase domains and C-terminal putative regulatory domains. The kinase domain is subdivided into the 11 conserved subdomains within the protein ser/thr kinases. The subdomain VIII contains a conserved sequence, (v/i)GTPyWMAPEv (a small letter indicates less conservation), termed the Ste20 signature sequence (6).

The mechanisms that regulate activity of the Mst kinases are beginning to be defined. As described for other protein kinases, phosphorylation seems to play a major role in the regulation of Mst kinases. In fact, all of the studied Mst kinases can undergo autophosphorylation at least in vitro. In most cases where the relevant residues have been identified, they map to the T (or activation) loop, and the phosphorylation event is proposed to open the kinase active site and increase the enzymatic activity of the protein. Authophosphorylation of SOK1 is triggered by binding to the Golgi matrix protein GM130 (9) but the precise events that lead to the authophosphorylation of the other Msts have not been clearly defined. By far, the best studied of the Mst proteins is Mst1. The phosphorylation of Mst1 and the effects of this on its biochemistry have been extensively studied. However, proposed effects are complex and sometimes contradictory. As stated above, Mst1 can be phosphorylated in its T loop leading to increased activity. On the other hand, authophosphorylation of Mst1 at Ser327 (a residue that is not within the T loop) does not increase its kinase activity toward exogenous substrates, but apparently plays a role in the inhibition of the proteolysis of the kinase by caspases (see below) (10). Other data suggest that Mst1 is negatively regulated by phosphorylation (11). In contrast, others conclude that full activation of the kinase is only achieved when the protein is phosphorylated and cleaved, and that phosphorylation itself may influence the susceptibility of the kinase to cleavage (10). More recently Rezka and co-workers investigated the role of 4 putative phosphorylation sites and found that two of these residues, Thr183 and Thr187 within the activation loop, are essential for kinase activation. They further determined that phosphorylation at Thr183 is achieved by intermolecular autophosphorylation within an Mst1 dimer (12), findings that were later confirmed by Avruch and co-workers (13).

Extensive work has also been done to define the mechanisms of regulation by phosphorylation of the closest homologue of Mst1, Mst2. Both the native form and the caspase-3-cleaved form of Mst2 depend on autophosphorylation of a single residue, Thr180 (Thr 183 of Mst1), for kinase activity (14).This work also describes a novel molecular mechanism that makes possible the discrimination by protein phosphatases between the native Mst2 and the truncated phosphorylated Mst2, the latter being resistant to dephosphorylation by protein phosphatases. The residues regulating activity of the other Mst family members remain to be defined.

Dimerization also plays a role in the regulation of function of these kinases as exemplified by Mst1. The region required for Mst1 homo-dimerization has been precisely defined, and it encompasses an α-helical structure in the extreme C-terminal 56 amino acids that has been called the SARAH domain (for Sav/Rassf/Hpo) (15) (Figure 2). Furthermore, the alfa-helical structure is required for homo-dimerization, as a single aminoacid substitution that disrupts it alters the ability of Mst1 to multimerize (16). The functional relevance of dimerization was unveiled 6 years after its description by Lee and Yonehara who showed that dimerization enhances cytoplasmic localization of the protein (17). Despite the fact that the C-termini of GCK-II and GCK-III kinases are not highly homologous, both Mst4 and SOK1 can homodimerize through this region (5,9,18).

As noted above, caspase-mediated proteolysis is also important in the activation of these proteins. Again Mst1 was the first of the Mst kinases found to be cleaved and activated by caspases. In 1998 Krebs and co-workers identified a 36 kDa in-gel kinase activity to be a cleavage fragment of a 63 kDa kinase that was specifically recognized using an antibody against Mst1 (19). Soon thereafter Lee et al corroborated this finding (20). Both groups identified the putative cleavage site as being between aspartic acid 326 and serine 327 in the sequence DEMDS. They further determined that caspase-mediated cleavage during apoptosis results in an increase in the catalytic activity of Mst1. Further experiments defined a second caspase cleavage site, this one flanked by aspartic acid 349 and glycine 350 (TMTDG) (10). Since then, others employing model systems such as osteoclasts and eosinophils have also shown a direct relation between caspase cleavage of the kinase and cell death (21,22). Similar to Mst1 and Mst2 (14), Mst3 can also be cleaved by caspases during apoptosis, although the caspase cleavage site in Mst3 is mapped to AETD^{313}G. Again, catalytic cleavage of Mst3 activates its intrinsic kinase activity (23). In contrast, the results reported for Mst4 are contradictory, with some groups reporting that Mst4 cannot be cleaved by caspases (24) and others suggesting that the protein is at least partially susceptible to caspase cleavage (18). Interestingly, the C-terminal domain of SOK-1 is inhibitory for its kinase activity (25). Whether SOK-1 is cleaved and activated under any circumstances in vivo however,

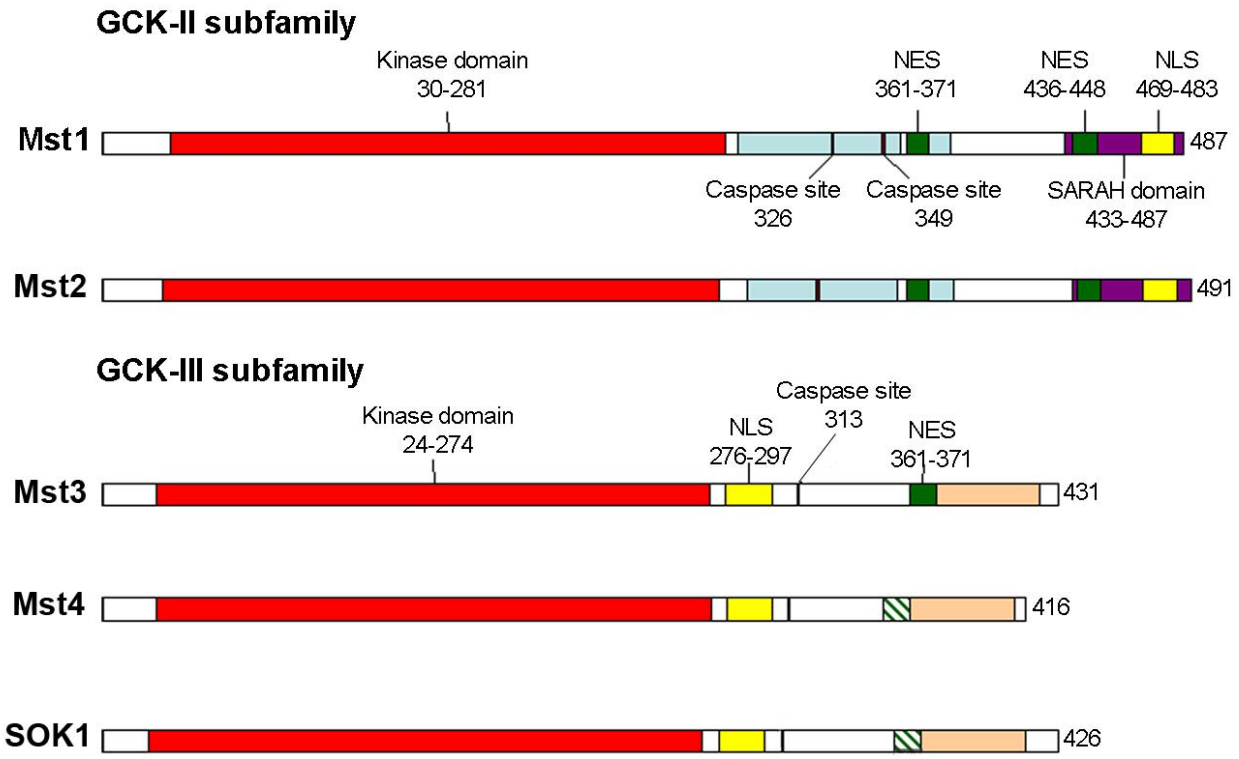

Figure 2. Structure of the mammalian GCKII and GCKIII proteins. The name and location of the most prominent features have been specified for the best studied protein of subfamily II (Mst1), and subfamily III (Mst3). The nuclear export sequence (NES), nuclear localization signal (NLS), and caspase cleavage site have not been identified for Mst4 and SOK, and are included in the scheme based only on homology with Mst3. The unnamed colored segments are regions homologous within GCKII (light blue), or GCKII (rose) subfamilies with an as yet unassigned function.

remains to be seen. (Figure 2 identifies caspase cleavage sites on the different Mst proteins).

The cellular distribution also appears to be an important determinant of the cell biological effects elicited by these proteins, and this also appears to be structure dependent. The cleavage of Mst1 changes its cellular localization from the cytoplasm to the nucleus. As noted, dimerization appears to lead to retention of the kinase in the cytoplasm. In addition to these mechanisms, two functional nuclear export sequences (NES) have been described in Mst1 (Figure 2). These sequences, located in the C-terminal domain, are separated from the N-terminal kinase domain upon caspase- mediated cleavage, allowing nuclear translocation of the kinase domain (26). Loss of the NES seems not to be the only reason for nuclear translocation of Mst1, as the protein also has a nuclear localization sequence (NLS, Figure 2) that may be masked in the inactive state and exposed after activation (17).

Whether GCK-III kinases also translocate to the nucleus and what sequences are important for this feature are less clear. To date, the only protein of this subfamily proposed to translocate to the nucleus is Mst3. Both an NLS in the C-terminus of its kinase domain and an NES in the C-terminus of the protein have been described (27). The latter is a remarkable functional conservation with respect to GCK-II subfamily proteins, since otherwise there is very low homology between the two subfamilies in the C-terminal region. The proteolytic cleavage of Mst3 during apoptosis may expose the NLS signal and subsequently mediate the translocation of truncated Mst3 to the nucleus.

Surprisingly, both SOK-1 and Mst4 have been found in the *cis*-Golgi network. Given that these proteins share a high degree of homology with Mst-3 in the NLS and NES motifs, there is the possibility that they are translocated to the nucleus under special circumstances (e.g. during apoptosis, when caspases might cleave the C-terminus).

In summary regulation of Msts is a multilayered process that we are beginning to understand. Even more levels of regulation are possible. For instance, the regulation of the closely related GCK protein depends on its stabilization, rather than on the rise of the specific activity of the enzyme (28). Further experiments are needed to asses whether more levels of regulation could also apply for the Mst kinases.

4. ACTIVATING STIMULI

What remains undefined at this time are the specific physiological/pathological roles of the various

kinases of the GCK-II and GCK-III subfamilies. Although they are clearly homologous in their kinase domains they differ in an unambiguous manner in their regulatory domains, and these differences may determine specific physiological roles of each of the kinases. The pattern of expression of the different subtypes is also not well defined. However, most of them are ubiquitously expressed with no clearly defined differences in expression between tissues that may provide clues to function. To begin to understand biological functions, we can first examine the different stimuli that activate these proteins. Relatively little is known, however, about the primary stimuli that activate these kinases, although some appear to be activated by different stress stimuli and at the least inhibited by treatment with growth factors. Specifically, Mst1 and 2 were shown to be inhibited by EGF treatment (11) and stimulated by the protein phosphatase 2a inhibitor, okadaic acid (OA), and by extreme forms of stress such as heat shock, sodium arsenite, and staurosporine (29). Subsequently, activation of Mst1 by Fas was reported, and this was the first time that a physiological stimulus was shown to activate an Mst (19). SOK1, as its name implies, is activated by oxidative stress and even more potently by chemical anoxia, a cell culture model of ischemia that leads to pronounced production of reactive oxygen species. To date no stimuli have been found to consistently activate Mst3 (30), even the extreme stresses that activate SOK1 (25,31). Mst4 is the only Mst reported to be activated by growth factors, specifically treatment of serum-starved cells with EGF and TGF-alfa (32).

5. PARTNERS AND SIGNAL TRANDUCTION PATHWAYS ACTIVATED/REGULATED BY THE GCK-II AND GCK-III PROTEINS

Based on sequence similarity with yeast Ste20p, it would be logical to propose that the proteins of the GCK-II and GCK-III subfamilies could act as MAP kinase kinase kinase kinases (MAP4K) that directly phosphorylate and activate a MAP3K, leading to the activation of a MAPK cascade. This has been reported to be the case for some of the mammalian Ste20s such as HPK1, which belongs to the GCK-I subfamily, or PAK2, that belongs to the PAK-I subfamily (6). For other GCK family members, including some of the Msts, they can bind and/or modulate the activity of MAP3Ks but not by direct phosphorylation. This leads us to state, at least for the moment, that the Msts are not MAP4Ks.

Mst2 binds to Raf-1, a MAP3K, although there is no evidence that Mst2 regulates Raf-1. However Raf-1 does interfere with Mst2 activity at several levels. Indeed, Raf-1 can cause disassembly of Mst2 dimers, abrogating Mst2 phosphorylation and impairing kinase activity. Since catalytically-inactive Raf-1 has the same effects, Raf-1-mediated inhibition of Mst2 does not depend on its kinase activity (33). The biological consequences of the interaction are that in certain settings, Raf-1 may protect against apoptosis by negatively regulating the proapototic kinase, Mst2 (34).

Mst4 may also be functionally related to Raf-1 since it was cloned using a screen for Raf-interacting proteins. Although the authors were not able to confirm the interaction in mammalian cells, they argued that Mst4 might modulate MAPK signaling in a tissue-or cell specific manner (24). Subsequently, Hsiu-Ming Shih and co-workers reported that Erk-MAPK was activated in Phoenix cells overexpresing Mst4, although in a Ras/Raf independent fashion (35). They also showed that the activation of Erk was specific since neither JNK nor p38 were activated.

In contrast to Mst4, Mst1 does not activate Erk (11,19) but it does activate p38 and JNK (19). Mst1 appears to function upstream of the MAPKK MKK6 to activate p38 and upstream of the MAPKK MKK7 to activate JNK, in the latter case likely acting through the MAPKKK, MEKK1 (10). This led the authors to conclude that Mst1 functions as a MAPKKKK, although Mst1 was not shown to directly phosphorylate MEKK1. As with Mst1, Mst2, its closest homolog, also activates p38 and JNK. On the contrary, neither Mst3 nor SOK1 appear to activate the Erk, p38 or JNK pathways, raising the possibility that these kinases could be part of novel signal transduction pathways (25,30). Although no report has contradicted this statement for SOK1, Zhou et al. reported that overexpresion of Mst3 in HEK 293 cells activated endogenous Erk1/2 (36). In the same report, the authors published the sequence of a novel brain isoform, Mst3b, that can also activate the Erk1/2 pathway and is negatively regulated by protein kinase A (PKA), suggesting for the first time cross-talk between an Mst and PKA, and also providing a novel mechanism for PKA to modulate MAPK pathways.

Apart from their contribution to the regulation of the described MAPK pathways, the Msts have also been implicated in the binding to and/or phosphorylation of other proteins. The implication of these interactions for a functional cellular response regulated by Msts and their cross-talk with the already described pathways opens new avenues to explore and may contribute to better understanding of the function of Msts. For example, Mst1 has been shown to interact with components of the ras pathway. Mst1 binds to the non-catalytic peptides NORE1 (novel Ras effector) and RASSF1A, members of the RASSF (Ras association domain family) family. The complex of NORE1-Mst1 is constitutive, and Mst1 associates with ras through NORE1 upon activation of ras by serum stimulation or oncogenic mutation. Despite the fact that NORE1 can inhibit Mst1 activating autophosphorylation at Thr183 in vitro, NORE1 also targets Mst1 to the plasma membrane, where it seems to interact with its activators. This recruitment of Mst1 may explain the proapoptotic activity of ras in certain scenarios, such as overexpression of constitutively active K-ras V12 in 293 cells. Whether Mst1 mediates apoptosis induced by endogenous ras in other settings, such as following trophic factor withdrawal, TCR-induced expression of FasL, or TNF-induced apoptosis, remains to be proven.

Another protein that is an Mst1 binding partner is the death-associated protein 4 (DAP4). The apoptotic response caused by overexpression of Mst1 was shown to be enhanced by coexpresion with DAP4. The authors

propose that DAP4 brings together Mst1 and p53, enabling colocalization of Mst1 with p53, thereby enhancing the proapoptotic efficacy of p53 in what appears to be a new pathway of Mst1-mediated cell death (37). Although NORE1 and DAP4 have been shown to bind to and somehow regulate Mst1-induced apoptotic cell death, neither of them is a substrate or a modulator of Mst1 kinase activity.

In 2003, Cheung et al. (38) identified a physiological Mst1 substrate, histone H2B. They reported the phosphorylation of H2B on Ser 14 by caspase-cleaved Mst1, an event that correlates with programmed cell death in vertebrates. Soon thereafter they extended these results to unicellular eukaryotes by demonstrating that H2B is specifically phosphorylated at Ser 10 by the Ste20 kinase in a hydrogen peroxide-induced cell death pathway in *S. cerevisiae*. This phosphorylation was found to be essential for cell death and the condensation of chromatin. Leading them to conclude that the mechanism of chromatin remodeling, a critical feature of mammalian apoptosis, developed very early on in evolution (39).

The interaction found by Barr´s group between both Mst4 and SOK1/YSK1 and the Golgi matrix protein GM130 opens new avenues to explore as it places two of the Mst kinases in a new location for the Msts, the Golgi apparatus. The localization of these two Msts at this organelle depends on GM130. Not only is the presence of GM130 necessary for these proteins to be located at the Golgi but it is also necessary for catalytic activity of the kinases. The binding of GM130 to Mst4 or SOK1 appears to force their homodimerization and autoactivation (9) although with different outcomes. Blocking SOK1 activity in stationary cells causes the Golgi to disperse from the microtubule organizing center, whereas a dominant-negative form of Mst4 has no such effect. Furthermore, migration is blocked by a dominant-negative form of SOK1, but also by active Mst4, reaffirming that the kinases have opposing actions. The authors also identified 14-3-3zeta as a specific substrate for SOK1 at the Golgi apparatus. Moreover, their data suggest that 14-3-3zeta may act downstream of SOK1 and is probably the key substrate that mediates the effects of SOK1 on cell migration.

Recently two closely related serine/threonine kinases, Lats1 and NDR, known to participate in the regulation of cell cycle progression and cell morphology, have been found to be phosphorylated and regulated by Mst2/1 and Mst3 respectively (40,41). Although further studies are needed to define the biological consequences of what appears to be a new pathway in humans, in *Drosophila*, as we will discuss below, the pathway has been linked to development and tissue homeostasis.

6. CELL RESPONSES ELICITED BY THE GCK-II AND GCK-III PROTEINS. LESSONS TO LEARN FROM ANIMAL MODELS AND UNICELLULAR ORGANISMS

As discussed above, most Mst kinases are activated by stress stimuli, sometimes, as in the case of SOK1, by extreme stresses. Others can also be activated by caspase-mediated cleavage. In addition, some members of these subfamilies can activate proapoptotic MAPK pathways. All of this suggests that Mst kinases may be involved in the process of apoptotic cell death. This is consistent with the ability of at least some Mst kinases to induce apoptosis when overexpressed. Specifically, overexpression of Mst1 can induce apoptosis and nuclear condensation in BJAB, 293T and COS-1 cells (10,26,42), whereas it promotes nuclear condensation without apparent chromosomal cleavage in HeLa cells (43) (for a review see (44). It seems then that Mst1 forms part of an apoptotic cell pathway in which its caspase-cleaved form translocates to the nucleus where it mediates its effects, probably through nuclear proteins such as DAP4 and H2B, and with the necessary presence of CAD (caspase-activated DNAse). The latter was proven true by the group of Yukiko Gotoh. It is well known that in non-apoptotic cells CAD forms a complex with ICAD which inhibits its nuclease activity. Upon an apoptotic stimulus caspase cleavage of ICAD allows the release of CAD as an active form and consequently the DNA is fragmented. Overexpressing either a wild type or a noncleavable (dominant-negative) form of ICAD, to block the activation of endogenous CAD by caspases, they observed that Mst1-induced nuclear fragmentation is prevented (42). The study of the role of Mst1 in cell death has also been extended and confirmed in primary cells and mouse models (for a review see (44). Briefly, it was shown that bisphosphonate-induced apoptosis of bone-reabsorbing murine cells is mediated by caspase-mediated cleavage of Mst1 (21). Furthermore, an active cleaved form of Mst1 was identified in apoptotic eosinophils from human peripheral blood, although it remains to be confirmed whether cleavage of Mst1 is essential for eosinophil apoptosis or is merely a by-product of this process (22). In vivo, cardiac-specific overexpression of Mst1 in transgenic mice results in activation of caspases, increased apoptosis, and dilated cardiomyopathy. Conversely cardiac-specific overexpression of dominant negative Mst1 in transgenic mice prevents myocyte death induced by ischemia/reperfusion injury (45). Although these studies identify a potential role for Msts in ischemic injury, it is important to note that over-expression of several GCK family members can lead to cell death though it remains uncertain whether this is a true biological function of the kinases or an artefact of over-expression.

Although not as extensively studied, the other Mst kinases also seem to be involved in apoptosis. Like Mst1, overexpression of Mst2 leads to apoptotic cell death. This proapoptotic activity has been shown to be inhibited by Raf-1 as noted above. In fact, Raf-1 -/- fibroblasts are especially prone to apoptosis, and this can be rescued by downregulation of Mst2 by siRNA. The effects of Mst3 on cell death have been documented in Huang et al., since overexpression of the wild type protein or a truncated kinase-active form in 293 cells induces apoptotic-like features (23). It has also been reported that overexpression of Mst4/MASK in MCF-7 breast carcinoma cells leads to apoptotic death (18). Finally, unpublished data from our laboratory show that SOK1 when overexpresed in a variety

of cell lines drives cell death (Nogueira et al, unpublished results).

A role for Mst4 in the regulation of cell proliferation and transformation has also been proposed, as its overexpression leads to an increase in cell number and anchorage-independent growth of Phoenix cells (35). These data were reinforced by those of Sung et al who demonstrated that Mst4 can also alter the morphology and confer anchorage-independent growth of prostate cancer cells *in vitro*, and promote tumor xenograft growth *in vivo* (32). Further work needs to be done to reconcile the observationthat Mst4 participates in cell responses as different as proliferation and apoptosis. The participation of Mst4 in proliferative responses contrasts also with the fact that its closest relatives Mst3 and SOK1 are involved in apoptotic processes. Once again as in the case of its role in Golgi migration, Mst4 and SOK1 seem to have contrasting functions.

Mst kinases have also been involved in processes of cell migration(46). The recent work of Barr and colleagues on SOK1/YSK1 demonstrated that migration is blocked by a dominant-negative form of SOK1/YSK1 and also by an active form of Mst4 (9). The mechanism depends on the interaction of the Golgi protein GM130 with SOK1 and its subsequent phosphorylation of 14-3-3zeta, events that may be important for the redirection of the Golgi apparatus towards the leading edge of the motile cell which is necessary for migration in some cell types (46).

Finally we will review some of the work done with the Mst orthologues from *Drosophila*, *Caenorhabditis* and yeast, since this may allow us to gain further insights into the function of Mst kinases in mammals.

The *Drosophila* GCK-II ortholog, Hippo (Hpo) has been implicated in the regulation of cellular homeostasis, as it appears to coordinate both the regulation of cell division and cell death. Five papers published in 2003 reported that loss of function mutations of the Hpo gene caused tissues to proliferate more than normal as a result of an increase in tissue growth and a decrease in apoptosis (47,48,49,50,51). The molecular events that determine these phenotypes were explored and were found to be elevated levels of the cell cycle regulator, cyclin E, and DIAP1 (*Drosophila* inhibitor of apoptosis protein 1). What remains controversial at this point is the regulation of DIAP by Hpo. The groups of Pan and Halder propose that Hpo represses the transcription of *diap1*, but those of Tapon and Hariharan provide evidence for a post-transcriptional inactivation of DIAP1 (52).

Hpo also interacts with two other Drosophila proteins, Warts (wts), a protein kinase, and Salvador (Sav), a scaffold protein containing WW and coiled-coil domains, and Sav loss of funtion mutations develop a similar phenotype to that of Hpo. Hpo associates with and phosphorylates Sav, and association with Sav promotes Wts phosphorylation by Hpo. More recently, Mats, a Mob (Mps one binder) 1A- family protein, has been shown to physically associate with Wts and also to stimulate its kinase activity (53,54). Together these four proteins form part of what seems to be a new pathway that regulates cell homeostasis. Recently the discovery of Yorkie as a downstream target of Wts, and the finding that Merlin the protein product of the Neurofibromatosis type-2 gene, and Expanded are upstream regulators of Hpo, further delineates this pathway. Yorkie is the *Drosophila* ortholog of the transcripition factor YAP (yes-associated protein) and it has been shown that its phosphorylation by Wts impairs its transcriptional induction of *diap1* (55). Merlin and Expanded are members of the ezrin/radixin/moesin (ERM) family of FERM domain-containing proteins. Epistatic analysis places them upstream of Hpo and Wts, although biochemical tests indicate that Merlin and Expanded do not bind directely to Hpo, Sav or Wts. Further experiments are needed to explain the missing link and the molecular mechanism of Hpo and Wts activation by Merlin and Expanded (for a more detaliled review see (56). The various components of the pathway, and its relevance in mammals are just being discerned. At least two components of this pathway, WW45 (the human Sav ortholog) and Lats-1/2(the two human Wts orthologs) can be considered tumor suppressors (15,57). It also seems apparent that the pathway has been evolutionary conserved since Mst2 and hWW45 interact with each other in human cells, and both Mst1 and Mst2 are able to phosphorylate Lats-1 and Lats-2, thereby stimulating Lats kinase activity (40).

As for other Ste20 kinases, GCK-III subfamily kinases may have an important role in regulating the cytoskeleton. Several pieces of evidence point to this possibility. STLK3, the Drosophila GCK-III ortholog, has been implicated in cytoskeletal organization since knock-down of the protein by RNAi results in the formation of actin-rich protusions (58). The nematode *C. elegans* GCK-1, which also falls into the GCK-III subfamily, is located in the mitotic cleavage furrow in embryonic somatic cells. Severin kinase, from *D. discoideum* (the most closely related human ortholog is SOK1) has been implicated in cytokeletal reorganization through the phosphorylation of severin, a protein that belongs to the class of F-actin fragmenting and capping proteins which are especially well suited for causing rapid rearrangements in the filamentous actin network (59). In the fission yeast *S. pombe*, the Ste20/GCK kinase Nak1/Orb3 has been shown to be required to polarize the actin cytoskeleton at the tips of the cells and for cell separation, and so has been proposed to be involved in controlling both cell shape and the late stages of cytokinesis. The phosphorylation of Nak1/Orb3 is periodic during the cell cycle and could be part of a mechanism that relocalizes a constitutively active kinase from the cell tips to the middle of the cell, thereby coordinating reorganization of the actin cytoskeleton and regulation of cell separation with cell-cycle progression (60). In the budding yeast *S. cerevisiae*, Kic1p mutants are defective for actin localization, cell integrity and morphogenesis (61). Because all the evidence cited above is derived from non vertebrate orthologs of the GCK-III subfamily, future research will be required to confirm a role in vertebrates.

7. PERSPECTIVE

An enormous amount of data has been generated in the past 10 years concerning the GCK-II and GCK-III subfamilies of Ste20 kinases, but many questions remain. One of the issues that will have to be addressed for this family of proteins, as with many others, is that of specificity. Some of the regulators and substrates described thus far seem to be quite specific. For instance, only SOK-1 seems to phosphorylate 14-3-3zeta, and Lats1 is phosphorylated by Mst1 and Mst2 but not by Mst4. This leads us to propose that at least for some Msts, the role acquired during evolution may be more specific than redundant. However, concluding this will require an intense focus on unveiling further regulators and substrates, and to untangle the signaling pathways and physiological processes they regulate. To achieve this goal, new experimental approaches will have to be taken. Specifically, overexpression experiments that have been used widely up to now may be misleading because of the high sequence conservation among the different family members and because of non-physiological interactions with proteins driven by over-expression. In this respect, the development of mice with targeted deletion of specific Msts, especially if they are conditional, making it possible to inactivate a specific kinase in a specific tissue or cell type (better avoiding compensation by other family members that might be seen with traditional knockouts), should help us to answer many of these and other important questions.

With the data we have at present, we propose that at least some of the Mst kinases may be part of the cellular response to certain types of stress, as they are activated by specific stressors, and that they could drive the cell to apoptotic death. Identifying the specific physiological or pathological circumstances in which each Mst is active will allow a determination of their precise role in the response to those stresses.

More work is also needed to reconcile the fact that these kinases participate not only in apoptotic responses but also in general cellular processes such as the regulation of Golgi function and of the cytoskeleton. We need to answer the question of whether this is part of the same action as the stress-response activity (e.g regulation of basic cellular processes vs induction of apoptosis may be due to quantitative, as opposed to qualitative differences in downstream targets), or that they are able to perform different functions in response to different stimuli or settings due to different targets.

Regarding their regulation, Mst kinases certainly have elements in common in the mechanisms that regulate their activity, both among them and with other families of kinases, but more studies are needed to determine the specific regulation of each Mst.

The answers to these questions will help us to better understand the role of these kinses in the complex biology of cells and tissues, and may allow us to develop better strategies to treat pathophysiological disorders that are triggered by the cellular stresses that activate Mst kinases.

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