A mitogen-activated protein kinase (MAPK) has been cloned and sequenced from a Drosophila neoplastic l(2)mbn cell line. The cDNA sequence analysis showed that this Drosophila kinase is a homologue of mammalian p38 MAPK and the yeast HOG1 gene and thus was referred to as Dp38. A distinguishing feature of all MAPKs is the conserved sequence TGY in the activation domain. Dp38 was rapidly tyrosine 186-phosphorylated in response to osmotic stress, heat shock, serum starvation, and \( \text{H}_2\text{O}_2 \) in Drosophila l(2)mbn and Schneider cell lines. However, unlike mammalian p38 MAPK, the addition of lipopolysaccharide (LPS) did not significantly affect the phosphorylation of Dp38 in the LPS-responsive l(2)mbn cell line. Following osmotic stress, tyrosine 186-phosphorylated forms of Dp38 MAPK were detected exclusively in nuclear regions of Schneider cells. Yeast complementation studies demonstrated that the Saccharomyces cerevisiae HOG1 mutant strain JBY10 (hog1-Δ1) was functionally complemented by Dp38 cDNA in hyperosmolar medium. These findings demonstrate that similar osmotic stress-responsive signal transduction pathways are conserved in yeast, Drosophila, and mammalian cells, whereas LPS signal transduction pathways appear to be different.

Various forms of cellular stress are known to activate a distinct subfamily of mammalian mitogen-activated protein kinases (MAPKs), termed p38 MAPK/reactivating kinase (RK) (1, 2). This kinase was found to be most homologous to the Saccharomyces cerevisiae HOG1 kinase that is involved in host protection from hyperosmotic stress (3). In mammals, p38 MAPK is activated by osmotic stress, inflammatory cytokines, and UV irradiation (4). Once activated, p38 is known to phosphorylate mitogen-activated protein kinase-activated protein kinase-2, which in turn phosphorylates a small heat shock protein (2, 5). Other substrates such as activating transcription factor-2 and Max have also been identified as targets of p38 kinase (4, 6). Furthermore, p38 MAPK was also discovered as an intracellular target for molecules that act as cytokine-suppressive anti-inflammatory drugs (7). The activation of p38 MAPK requires phosphorylation of both threonine and tyrosine residues in subdomain VIII of the activation domain. The p38/HOG1 subfamily enzymes are distinguished by the dual phosphorylation site motif (TGY) in the activation domain (1), which differs from TPY for c-Jun amino-terminal kinase (JNK) (8) and TEY for growth factor-activated MAPK, termed extra-cellular signal-regulated kinase (ERK) (9). JNK is activated in response to stimuli similar to those for p38 MAPK and phosphorylates a c-Jun NH\(_2\)-terminal domain. The third subgroup of the MAPKs is ERK, which is activated by epidermal growth factor and nerve growth factor. The ERK subgroup plays a central role in growth and differentiation (9, 10). Several upstream kinases that are responsible for MAPK activation have been recently characterized and were shown to have specificity for the three subfamilies of MAPKs. MKK1 and MKK2 activate ERK; MKK3, MKK4, and MKK6 activate p38; and MKK4 activates JNK (11–14).

In Drosophila, ERK and JNK have been cloned, and their functional roles in development have been well studied (15–23). Drosophila ERK-A, encoded by \textit{rolled} was found to mediate receptor kinase signal transduction pathways including torso, DER, and sevenless pathways (16, 17). Drosophila JNK was shown to participate in dorsal closure and LPS signal transduction (18–23). A partial deduced amino acid sequence of a Drosophila p38 MAPK from a PCR product has been previously reported by Biggs and Zipursky (15). However, no information is available with regard to Drosophila p38 cDNA(s), and its function in Drosophila cell physiology and development remains to be elucidated. In this work, we describe the full cDNA of a Drosophila homologue of p38 MAPK and certain functional roles in Drosophila cells.

**EXPERIMENTAL PROCEDURES**

Materials—Reagents were purchased from following sources: \textit{Thermus aquaticus} polymerase (Takara); Mega prime DNA labeling system (Amersham); Cy5 Autocycle sequencing kit and Cy5 Autoread sequencing kit (Pharmacia Biotech Inc.); Immobilon-P membrane (Millipore Corp.); Nylon filter (Amersham Hybond-N+); PCR machine (Perkin-Elmer); A.L.F. express DNA sequencer (Pharmacia); enhanced chemiluminescence (ECL) reagent (Amersham Corp.); phosphospecific p38 MAPK antibody (New England Biolabs). Water was of Milli Q grade. All other chemicals were of the highest commercial grade.

**Insect Cell Cultures**—Gatell’s Drosophila l(2)mbn hemocyte cell line (24) and Schneider cell line (25) were grown at 23 °C in Corning flasks in Schneider medium (Life Technologies, Inc.) supplemented with 5 or

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10% fetal bovine serum (Biowhittaker) heat-inactivated at 56 °C for 30 min.

PCR Amplification of Dp38 MAPK—Except when specially mentioned, all DNA and RNA manipulations were carried out using standard techniques (26). Two pairs of degenerate oligonucleotide primers were synthesized, sense primer-1 (SP-1; 5′-GTN GCN ATH AAR AAR YT-3′) and sense primer-2 (SP-2; 5′-GTN GCN ATH AAR AAR AT-3′), which included all possible codons specifying conserved subdomain II sequences VAIKKL and VAIKKL, respectively. Antisense primers were synthesized; antisense-1 (AP-1; 5′-GCN-ACR-TAN-CCN-GTC-AT-3′) and antisense-2 (AP-2; 5′-GCN ACR-TAN CCN GTN AR-3′) correspond to the complementary sequences MTGYVA and LTGYVA, respectively, both of which contain the conserved phosphorylation sites located in subdomain VIII. An aliquot of the Drosophila l(2)mbn cDNA library (2.5 ml) was amplified with 1 μM of each primer in pairs (four combinations: SP-1/AP-1, SP-2/AP-1, SP-1/AP-2, and SP-2/AP-2) with the following thermal cycle profile: 94 °C for 3 min; 35 cycles of 94 °C for 30 s, 45 °C for 30 s, and 72 °C for 1 min. The amplified mixtures were separated on 8% polyacrylamide gels, and each band was purified and cloned into pBlueScript SK(−).

Screening of the Drosophila l(2)mbn cDNA Library—A Drosophila cDNA library in λ Zap II (Stratagene) was made as described (27) and kindly provided by Dr. Dan Hultmark (University of Stockholm, Sweden). A full-length Dp38 MAPK cDNA was isolated from 2.5 × 10^5 independent clones previously transferred onto Hybond N+ membranes (Amer sham) using a specific 420-bp PCR product as a probe. The 420-bp PCR product was random-primed using fluorescein-11-dUTP. The membranes were prehybridized at 42 °C for 30 min with a solution containing 5 × SSC, 5 × Denhardt's solution, 0.1% SDS, 5% dextran sulfate at 60 °C for 2 h. Hybridization was carried out overnight at 60 °C in this solution containing fluorescein-11-dUTP-labeled probe, washed, and detected using anti-fluorescein-horseradish peroxidase-conjugated antibody according to the manufacturer's instructions (Amersham). Positive plaques were purified by two rounds of screening, and the cDNA inserts were cloned into pBlueScript SK(−) plasmid by in vivo excision from the recombinant λ Zap II as described by Stratagene. The cDNA inserts were sequenced across both strands using an ALF express automatic sequencer (Pharmacia).

Western Blot Analysis—Insect cells (~10^6), exposed to various stress conditions, were solubilized in SDS-PAGE sample buffer. The total cellular proteins (100 μg/lane) were separated by SDS-PAGE (28) and transferred onto polyvinylidene difluoride membrane (29). The antibody used for this study was rabbit anti-human p38 phosphospecific antibody, purchased from New England Biolabs. This antibody specifically recognizes the phosphorylated active form of Tyr182 of human p38 MAPK and cross-reacts with Drosophila p38 and murine p38 MAPKs. According to the manufacturer's information, it does not cross-react with either inactive p38 or other MAPKs (JNK and ERK). The anti-p38 phosphospecific antibody was diluted to 1:1000 and incubated with the membrane for 1 h at room temperature (25 °C). After washing, the blot was subsequently incubated for 1 h with goat anti-rabbit IgG conjugated to horseradish peroxidase diluted 1:3000. Detection was carried out using the ECL Western blot detection kit (Amersham).

Northern Blot Analysis—Total RNA from Drosophila l(2)mbn cells and Schneider cells was extracted (30) and separated on 1% formaldehyde agarose gels, transferred to nylon membranes, and hybridized with the cDNA insert labeled with fluorescein-11-dUTP. The membranes were washed, and the probe was detected using anti-fluorescein-horseradish peroxidase-conjugated antibody according to the manufacturer's instructions (Amersham). Positive spots were purified by two rounds of screening, and the cDNA inserts were cloned into pBlueScript SK(−) plasmid by in vivo excision from the recombinant λ Zap II (Stratagene). The cDNA inserts were sequenced across both strands using an ALF express automatic sequencer (Pharmacia).

FIG. 1. Nucleotide and deduced amino acid sequence of the Drosophila p38 MAPK. The complete cDNA sequence of Dp38 MAPK has been deposited in GenBank under accession number of U86867.
electrophoresis on a denaturating formamide-agarose gel. The RNA was capillary-transferred to a Hybond-N membrane, baked at 80 °C for 2 h, and cross-linked by UV irradiation. The membrane was prehybridized at 42 °C for 1 h in 50% formamide, 0.1% SDS, 100 μg/ml of sonicated salmon sperm DNA. Hybridization was carried out in this solution containing [α-32P]dCTP random-primed Dp38 cDNA insert (106 cpm/ml). The blot was briefly washed at 50 °C in 2× SSC, 0.1% SDS at room temperature and then washed twice at 50 °C in 2× SSC, 0.1% SDS for 20 min and twice at 55 °C in 2× SSC, 0.1% SDS for 5 min and autoradiographed at −70 °C using intensifying screens.

Complementation of the hog1 Mutant by Drosophila p38 MAPK—The S. cerevisiae hog1 mutant (JBY10) and HOG1 plasmid (pJB30; HOG1 URA3 CEN) as described by Brewter et al. (3) were kindly provided by Dr. C. Gustin (Rice University, Houston, TX). The yeast strain was transformed with Dp38-sense (Dp38-sense URA3 CEN), Dp38-anti-sense (Dp38-antisense URA3 CEN), or pJB30 (HOG1 URA3 CEN) plasmid as described previously (31). The structures of all plasmids were confirmed by DNA sequencing. The transformants were selected on synthetic complete (SC)-Ura medium (32), and osmotic cell growth was tested on a YPD plate containing 0.6 or 0.9 M NaCl (32).

Cell Growth and Preparation of Yeast Extracts—Yeast transformants were cultured 15 h in 5 ml of SC-Ura medium at 30 °C with vigorous shaking. The cultured cells were inoculated into 80 ml of fresh SC-Ura medium, and cells were grown further under the same condition. When A600 reached 1.0, the cells were harvested and resuspended into 200 ml of the same medium containing 0.6 M NaCl. The cells were further incubated for 1.5 h at 30 °C with vigorous shaking. The cells were harvested by centrifugation at 2,000 rpm for 10 min at 4 °C. The cell pellets were stored at −70 °C for later use.

Protein preparation was modified by using a modified H buffer (33) containing 10% glycerol (25 mM Tris-HCl, pH 7.4, 15 mM MgCl2, 1 mM dithiothreitol, 0.1% Triton X-100, 0.25 mM each meta- and orthovanadate, 0.1 mg/ml phenylmethylsulfonyl fluoride, 5 μg/ml each pepstatin A, chymostatin, leupeptin, and pepstatin). An equal volume of acid-washed glass beads (425–600 μm; Sigma) were added, and cells were broken by vortexing six times for 30 min at 4 °C. After transferring supernatants into new tubes, samples were recentrifuged at 15,000 rpm for 10 min in microcentrifuge tubes at 4 °C. The supernatants were collected, stored at −70 °C, and later used for protein determination and Western blot analysis.

Transfection Experiment—Epitope (6× His) tag was added to the amino-terminal region of Dp38 by subcloning of Dp38 cDNA into His-tagged bacterial expression vector PQE-30 (Qiagen). The His-tagged Dp38 insert was subsequently subcloned into Drosophila transfection vector, pPacPL that contains the actin 5C promotor (34). The His-tagged Dp38 insert was subsequently subcloned into Drosophila transfection vector, pPacPL that contains the actin 5C promoter (34).
tion vector pPacPL-luciferase was constructed to control transfection efficiency. Cells were transfected by using the calcium phosphate precipitation method (35). The cells were used 24 h post-transfection.

**Immunoprecipitation and Kinase Assay—** Schneider cells expressing His-tagged proteins were activated with 300 mM NaCl for 0, 5, 15, and 60 min. The cells were washed twice in Tris-buffered saline and solubilized in lysis buffer (20 mM HEPEs, pH 7.4, 2 mM EDTA, 50 mM β-glycerophosphate, 1 mM dithiothreitol, 1 mM sodium orthovanadate, 1% Triton X-100, 10% glycerol, 25 μg/ml aprotinin, and 40 μg/ml phenylmethylsulfonyl fluoride) on ice for 30 min and subsequently centrifuged at 15,000 × g for 15 min at 4 °C. The epitope-tagged protein kinases were immunoprecipitated by incubation for 1 h at 4 °C with the monoclonal anti-His antibody (Qiagen) prebound to protein G-agarose for 15 min at room temperature. The immunoprecipitates were washed twice successively with decreasing concentrations of NETF buffer, i.e., 6, 3, and 0% NETF (6, 3, and 0% Nonidet P-40, respectively, in 100 mM Tris-HCl, pH 7.4, 50 mM NaCl, and 0.1 mM sodium orthovanadate). The immunocomplexes were resuspended in 25 μl of kinase buffer containing 20 μM ATP and 10 μg of myelin basic protein, and the kinase assays were initiated by the addition of 1 μl of γ[32P]ATP (3000 Ci/mmol). The reactions were terminated after 30 min at 30 °C by the addition of Laemmli sample buffer. The phosphorylation of the substrate proteins was examined by SDS-PAGE by autoradiography and phosphor-imaging (Fuji BAS 2500) analysis.

**Immunocytochemistry—** Cells were grown in 24-well culture dishes at a density of 2 × 10^5 cells/ml and then were fixed for 30 min in 3% formaldehyde, washed, and blocked in a Tris-buffered saline solution containing 0.1% Triton X-100 and 5% normal goat antibody for 1 h at room temperature. Cells were incubated at 4 °C overnight in a Tris-buffered saline solution containing Triton X-100, 5% bovine serum albumin, and a 1:2000 dilution of p38 phosphospecific antibody. The cells were washed with Tris-buffered saline plus 0.1% Triton X-100 and incubated with biotinylated anti-rabbit antibody for 1 h. Detection was carried out with the ABC reagent kit using diaminobenzidine tetrahydrochloride according to the manufacturer's instructions. The cells were mounted in glycerol and examined with a Olympus BX microscope.

**Computer Analysis for Sequence Homology—** The Fasta, TFASTA, WORDSEARCH, and BLAST algorithms were used to determine nucleic acid and protein sequence homology between Dp38 and other known proteins (36).

**RESULTS AND DISCUSSION**

**Cloning, Sequence Analysis, and Expression of Dp38 MAPK in Drosophila Cells—** To isolate a Drosophila homologue of p38 MAPK, primers were synthesized based on conserved subdomains II and VIII of known MAPKs. One of the primer pairs (sense primer-1 and antisense primer-1) gave a specific 420-bp PCR product that was subsequently cloned, reamplified, and used to screen a Drosophila l(2)mbn cDNA library. From approximately 2.5 × 10^6 clones, one positive plaque was obtained. This plaque was purified, and the insert was excised into pBlueScript SK(-) and sequenced. The insert of this clone contained a 3584-bp contig and an open reading frame of 1098 bp corresponding to 366 amino acid residues (Fig. 1). An untranslated flanking sequence of 187 bp was present between the terminal codon and the poly(A) site. The calculated molecular mass of the deduced protein was 42,151 Da.

Comparative sequence analysis showed Dp38 to be most homologous to human p38 MAPK (37) followed by Xenopus Mpk2 (2), Drosophila JNK (18), human JNK1 (8), human ERK1 (38), and Drosophila ERK-A (15). The Thr^384 and Tyr^386 residues in subdomain VIII are in an equivalent position to TOY, TID, and TIE sequences in the members of the ERK family (Fig. 2A). It is interesting to note that Dp38 is more closely related to other p38 MAPKs than to Drosophila ERK and Drosophila JNK (Fig. 2B).

A recent crystallography study of p38 MAPK comparing its structure with that of ERK2 demonstrated that the phosphorylation “Lip” (a regulatory loop near the active site) of p38 consists of 13 residues, 6 residues shorter than that of ERK2, and the conformation of the Lip of unphosphorylated p38 is unique in that it plays a role in activator specificity (39). Like mammalian p38, the phosphorylation Lips of Dp38 and HOG1 are also 6 residues shorter than that of Drosophila ERK (Fig. 2A). These observations suggest that Dp38, together with HOG1 and mammalian p38, falls into the same structural MAPK subgroup.

The dual phosphorylation of Thr and Tyr by upstream kinases is necessary for full MAPK enzymatic activity (40). In the case of mammalian p38 MAPK, the activation is mediated by phosphorylation on Thr and Tyr by MAPK kinase 3 (MKK3) and MKK6, whereas SEK/MKK4 may activate both p38 and JNK (11–14). A recent study demonstrated that HEP, a Drosophila homologue of MKK3, MKK4, and MKK6, phosphorylated and activated Drosophila JNK in vitro (15). Whether HEP is also an upstream activator for Dp38 MAPK remains to be determined.

To verify the expression of Dp38 MAPK in Drosophila cell lines, total RNA from l(2)mbn and Schneider cells was hybridized with a random-primed cDNA fragment. In a Northern blot, high expression of a single RNA band appeared below the 18 S ribosomal RNA in both Drosophila cell lines (data not shown).

**Induction of Tyr^386 Phosphorylation of Dp38 by Various Stress Stimuli—** To address stimulus specificity of Dp38 kinase, Tyr^386 phosphorylation of Dp38 was examined using p38 Tyr^386-specific antibody following various forms of extracellular stress. This antibody is specific for the Tyr^386-phosphorylated “active” form of Dp38 and does not recognize unphosphorylated Dp38. Fig. 3A shows that Tyr^386 of Dp38 was intensely phosphorylated in both Drosophila Schneider and l(2)mbn cells in response to osmotic shock, heat shock, and H2O2, whereas unstimulated cells contained only trace levels of Tyr^386 phosphorylation. In addition, we also observed that when l(2)mbn cells were cultured at the recommended concentration of 5% fetal bovine serum (24), Dp38 (Tyr^386) was constitutively and intensely phosphorylated; however, if the concentration of fetal bovine serum in the culture medium was increased to 10%, as in the present study, the phosphorylated state was not detectable without stimulus (Fig. 3B). We find this result very interesting, since this is the first time a p38 MAPK has been shown to be phosphorylated under...
conditions of serum starvation. This result suggests the involvement of Dp38 in a wide range of physiological stress conditions. It is important to note that up until now, almost all studies carried out with l(2)mbn cells cultured in Schneider medium were supplemented with 5% fetal bovine serum according to Gateff’s instructions (24). The fact that Dp38 is constitutively activated when cells are cultured in 5% fetal bovine serum reflects their state of cellular stress. Hence, one should take into consideration this physiological state when conducting experiments on these cells.

Interestingly, LPS, which is known to activate p38 kinase in mammalian cells (1), does not cause significant phosphorylation of Dp38 in either Schneider cells or immunocompetent Drosophila l(2)mbn cells (Fig. 3A). This result contrasts with the observations reported for Drosophila JNK. Drosophila JNK is activated in response to LPS treatment in both l(2)mbn and S2* Schneider cells (18). It is well established that l(2)mbn cells when treated with even infinitesimal concentrations of LPS respond by inducing a rapid up-regulation of antibacterial peptide genes (41).

In addition, Tyr186 phosphorylation of Dp38 was examined in Schneider cells following a 15-min treatment with increasing concentrations of NaCl (0–500 mM NaCl). Our results suggest that the amount of Tyr186 phosphorylation of Dp38 was proportional to the NaCl concentration with maximal phosphorylation at 300 mM (Fig. 3C). We also examined the time course of Tyr186 phosphorylation from 0 to 120 min in the presence of 300 mM NaCl. Tyr186 phosphorylation was detected within 2 min, reached its maximum level after 5 min, and returned to its basal level by 60 min (Fig. 3D). This result suggests that tyrosine phosphorylation of Dp38 is one of the earliest events following osmotic shock. The kinetics of Tyr186 phosphorylation in response to osmotic shock are similar to those observed for HOG1 in yeast (3).

Tyr186 Phosphorylation Causes Increased Dp38 Enzymatic Activity—To confirm that the Tyr186 phosphorylation status is directly proportional to the Dp38 MAPK activity, Dp38 kinase activity was assayed using myelin basic protein as a substrate. Schneider cells transfected with epitope (His)-tagged Dp38 were treated with 300 mM NaCl from 0 to 60 min and then immunoprecipitated with monoclonal anti-His antibody bound to protein G agarose. The kinase assay was performed as described under “Experimental Procedures.” Fig. 4 shows that there is a good correlation between kinase activity and the Tyr186 phosphorylation status shown in Fig. 3D. While at time 0 cells simply showed background levels of activity, the osmotic stressed Dp38-transfected cells showed highest MAPK activity after 5 min, which then gradually decreased (Fig. 4). However, no kinase activity was detected after osmotic shock in the untransfected control cells (Fig. 4). These results demonstrate that Dp38 Tyr186 phosphorylation is necessary for kinase activity. Furthermore, this result is consistent with the observation that mammalian p38 with mutations in the dual phosphorylation motif (Ala-Gly-Phe instead of Thr-Gly-Tyr) could not be phosphorylated on Tyr and Thr after a stress stimulus and was catalytically inactive (4).

Dp38 Partially Complemented the Function of HOG1 in HOG1-Δ1 Mutant Cells—The high sequence homology and similar activation mechanism induced by hyperosmolality between Dp38 and HOG1 led us to investigate whether Dp38 is a functional homologue of the HOG1 gene. HOG1-Δ1 mutant yeasts were transformed with HOG1, Dp38-sense, or Dp38-antisense constructs. The viability of transformed yeast cells was then tested on medium containing 0.6 M NaCl and 0.9 M NaCl. The results demonstrated that while Dp38-antisense-transformed cells failed to grow on 0.6 M NaCl medium, whereas HOG1 and Dp38-sense-transformed cells grew well (Fig. 5). However, Dp38-sense-transformed cells failed to grow on the medium containing 0.9 M NaCl (data not shown). These results demonstrate that Dp38 partially complemented the function of HOG1 in a hyperosmotic environment. Han et al. (1) reported that murine p38 MAPK could complement the HOG1-Δ1 mutant strain on 0.9 M medium with lower efficacy.
Furthermore, Dp38 could complement a number of stresses such as osmotic shock, heat shock, serum starvation, and phosphorylation in response to various extracellular stress signals, which is well conserved in yeast cell survival under hyperosmotic media. 

MAPK—HOG1 gene. To verify the Tyr186 phosphorylation status of Dp38 under hyperosmotic conditions, overnight cultures of Dp38-sense- and Dp38-antisense-transformed yeast cells were harvested and treated with medium containing 0.6 M NaCl for 1.5 h at 30 °C. An aliquot of each lysate (Dp38-sense- and Dp38-antisense-transformed cells) was subjected to Western blot analysis using Tyr186 phosphospecific antibody (Fig. 6). The results demonstrate that Dp38-sense-transformed cells contain the Tyr186-phosphorylated form of Dp38 in the presence of 0.6 M NaCl. However, no phosphorylation band was detected in Dp38-antisense-transformed cells (Fig. 6). This result leads us to believe that the Tyr186-phosphorylated active form of Dp38 in complemented cells is an important event for yeast cell survival under hyperosmotic media.

**Subcellular Distribution of Tyr186-phosphorylated Dp38 MAPK**—The subcellular distribution of Tyr186-phosphorylated Dp38 MAPK was examined by immunocytochemistry. In the control experiment, no reliable staining was detected, which indicates that the level of the Tyr186-phosphorylated form of Dp38 is not significant (data not shown). However, very specific staining was detected in the nuclear region of hyperosmotically stimulated cells (Fig. 7). These data suggest that Dp38 was specifically Tyr186-phosphorylated in response to hyperosmotic stress and exclusively located in the nucleus.

In conclusion, we have cloned and sequenced the Drosophila p38 MAPK from a 1/2 mbn cDNA library and demonstrated its phosphorylation in response to various extracellular stress signals such as osmotic shock, heat shock, serum starvation, and H2O2. Furthermore, Dp38 could complement a HOG1-Δ mutant strain in medium containing 0.6 M NaCl. The Tyr186 phosphorylation of Dp38 was very rapid following stress, and the phosphorylated Dp38 was exclusively detected in the nuclear zone of Schneider cells. To our knowledge, this is the first report demonstrating that the p38 MAPK stress signal pathway is well conserved in Drosophila. The availability of the Dp38 cDNA will allow us to better understand the role of this kinase in the physiology and development of Drosophila.