Oncogene (1999) 18, 6172–6182
© 1999 Stockton Press All rights reserved 0950–9232/99 $15.00
http://www.stockton-press.co.uk/onc

Stress signaling in *Drosophila*

Beth E Stronach*,[1] and Norbert Perrimon[1]

[1]*Howard Hughes Medical Institute, Harvard Medical School, Department of Genetics, 200 Longwood Avenue, Boston, Massachusetts, MA 02115, USA*

Cells commonly use multiprotein kinase cascades to signal information from the cell membrane to the nucleus. Several conserved signaling pathways related to the mitogen activated protein kinase (MAPK) pathway allow cells to respond to normal developmental signals as well as signals produced under stressful conditions. Genetic and molecular studies in *Drosophila melanogaster* over the last several years have related that components of stress signaling pathways, namely the Jun kinase (JNK) and p38 kinase signaling modules, are functionally conserved and participate in numerous processes during normal development. Specifically, the JNK pathway is required for morphogenetic movements in embryogenesis and generation of tissue polarity in the adult. The role of the p38 pathway in generation of axial polarity during oogenesis has been inferred from phenotypic analysis of mutations in the *Drosophila* homolog of DMKK3. In addition to their requirement for normal development, cell culture and genetic investigations point to a role for both the JNK and p38 pathways in regulation of the immune response in the fly. This review details the known components of stress signaling pathways in *Drosophila* and recent insights into how these pathways are used and regulated during development and homeostasis.

Keywords: dorsal closure; morphogenesis; tissue polarity; immunity; JNK; p38

Introduction

Eukaryotic cells detect and respond to a variety of external signals and stresses by utilizing evolutionarily conserved protein kinase signaling modules, such as the mitogen-activated protein kinase (MAPK) cascades. These multiprotein modules afford versatility to cells at several levels. First, a cascade of consecutive kinases allows for signal amplification and multiple sites of regulation. Second, the kinase module can couple to diverse receptors for signal detection and integration. Finally, interactions with different effectors can elicit a diverse array of cellular responses.

Reflecting the large variety of signals that cells encounter, numerous related but functionally distinct MAPK cascades have evolved (Widmann *et al*., 1999) (Table 1). Signals from growth factors or mitogens are typically transduced by the MAPK/ERK (extracellular signal-responsive kinase) signaling pathway. Two other related pathways are activated in response to stress stimuli, such as mechanical deformation, UV irradiation and heat shock, as well as to hormones, and cytokine ligands (Kyriakis, 1999). These stimuli signal activation of the stress-activated protein kinases (SAPKs), exemplified by the Jun amino (N)-terminal kinase (JNK), and the p38 kinases. Like MAPK/ERK kinases, JNK and p38 catalytic activity is stimulated in response to stress signals by phosphorylation and activation of several upstream kinases, JNK kinase (JNKK or MKK) and JNK kinase kinase (JNKKK, MKKK or MEKK). Kinase cascades of this sort are typically linked to regulation of gene expression, because transcription factors are often the targets of regulatory phosphorylation events. In the stress response pathway, one target of JNK phosphorylation is the c-Jun transcription factor, that mediates transcriptional regulation of gene expression when in complex with c-Fos to form the activator protein-1 (AP-1) transcriptional regulator; ATF2 (activated transcription factor-2) is another common substrate of both the JNK and p38 pathways (Kyriakis and Avruch, 1996; Paul *et al*., 1997).

Stress response outputs in mammalian cells mediated by the JNK and p38 pathways are equally as diverse as the inputs and include cell cycle regulation, inflammatory response, cell growth, differentiation, and apoptosis (Kyriakis and Avruch, 1996; Paul *et al*., 1997). In unicellular yeast cells, the more distantly related MAPK cascades also regulate cell cycle, but in addition, function in the mating response, osmotic homeostasis and cell wall remodeling (Gustin *et al*., 1998; Herskowitz, 1995; Toone and Jones, 1998). Common features of these diverse behavioral responses from yeast to man involve not only transcriptional regulation of gene expression but also reorganization of cytoskeletal elements and thus cell shape, adhesion, and orientation. It is, therefore, becoming increasingly important to recognize and understand the role of regulatory interplay between the cellular signal transduction machinery and the cellular structural machinery. In fact, accumulating evidence suggests that specificity in signaling can be achieved by spatially restricting activation of signaling components through interactions with scaffolding proteins localized to the cytoskeleton or membrane (Garrington and Johnson, 1999; Madhani and Fink, 1998).

Components of all three prototypical MAPK cascades have now been identified and characterized in *Drosophila melanogaster*. Genetic studies in the fly emphasize the versatility of MAPK signal transduction pathways for normal developmental processes. In the past several years in particular, we have witnessed a bounty of new findings about the regulation and function of stress signaling pathways. Mutations in the *Drosophila* homologs of JNK pathway components result in a common embryonic phenotype implicating their combined function in the morphogenetic process of dorsal closure (DC) during embryogenesis. Further

*Correspondence: BE Stronach

Table 1 Evolutionarily conserved MAPK signaling pathways

	Yeast mating response		*Mammals*		*Fly dorsal closure*
Input	Mating pheromone	Mitogens	Stress, inflammatory cytokines		?
MEKKK	Ste20		PAK	NIK,GCK, HPK	Msn
MEKK (MKKK)	Ste11	Raf	ASK,TAK	MEKK1,4	?
MEK (MKK)	Ste7	MEK1,2	MKK3,6	MKK4,7	Hep
MAPK	Fus3	ERK1,2	p38	JNK	Bsk
Target	Far1	Elk1	ATF2	AP-1	AP-1

analysis has revealed additional requirements for several components of the JNK pathway in immunity and the generation of cellular polarity. Components of the p38 pathway have also been identified, but to date, the phenotypic consequences of mutations in the genes have only been reported for *DMKK3/licorne* defining its role in establishment of axial polarity during oogenesis. Though mutations in the two p38 kinases have not yet been characterized, genetic, biochemical, and cell culture studies have set the stage for characterization of p38 kinase cascades in immunity and morphogenesis.

The focus of this review is an update on stress signaling cascades in *Drosophila*. The roles of the JNK and p38 pathways are being defined largely in the context of normal development. Though we know surprisingly little about the molecular nature of cellular responses to physiological stresses on the fly in its natural environment, it is likely that the stress response involves inducible activity of the JNK and p38 cascades. Investigation of JNK and p38 activity during an immune challenge may shed light on the regulation of this inducible response. In this review, we detail recent observations about newly identified components within these two pathways and discuss their utilization during development and homeostasis. Several recent reports have brought new insights as well as new questions to the table with respect to how JNK pathway activity is regulated and how it may interface with the cytoskeleton.

The JNK signaling pathway is required for morphogenesis in *Drosophila*

Elements of the stress signaling pathway, specifically DJun, JNK, and JNKK, have been cloned and characterized in *Drosophila* over the last several years (Glise *et al*., 1995; Hou *et al*., 1997; Kockel *et al*., 1997; Riesgo-Escovar *et al*., 1996; Sluss *et al*., 1996). These investigations have shown not only that the genes are present in the fly and highly conserved at the sequence level in comparison to their mammalian counterparts, but also that they function in a concerted signal transduction pathway to regulate gene expression and cytoskeletal remodeling during embryogenesis. In particular, mutations in JNK pathway components disrupt a morphogenetic process referred to as dorsal closure (DC).

DC is the movement of the dorsolateral epidermis on either side of the embryo toward the dorsal midline to cover a degenerative squamous epithelium, the amnioserosa, and to enclose the embryo in a continuous epithelium (Figure 1). Closure relies predominantly on cell shape changes within the postmitotic epidermis, instead of cell division or cell intercalation (Campos-Ortega and Hartenstein, 1985; Young *et al*., 1993). Initially, just after retraction of the embryonic germband is complete, cells at the leading edge (LE) of the dorsal epidermis, which abut the amnioserosa, begin to elongate dorsally. At the dorsalmost membrane of these cells adjacent to the amnioserosa, there is an obvious enrichment of cytoskeletal proteins such as nonmuscle myosin and actin (Young *et al*., 1993). Concomitant with cytoskeletal remodeling and changes in cell shape from polygonal to elongated, LE cells upregulate expression of at least two genes including *decapentaplegic (dpp)*, a TGF-β-like molecule, and *puckered (puc)*, a phosphatase (Ring and Martinez-Arias, 1993; St. Johnston and Gelbart, 1987). After the inital elongation of LE cells, a spreading phase ensues. LE cells become markedly more narrow and stretched, presumably due to contraction of the actin-myosin network, resulting in

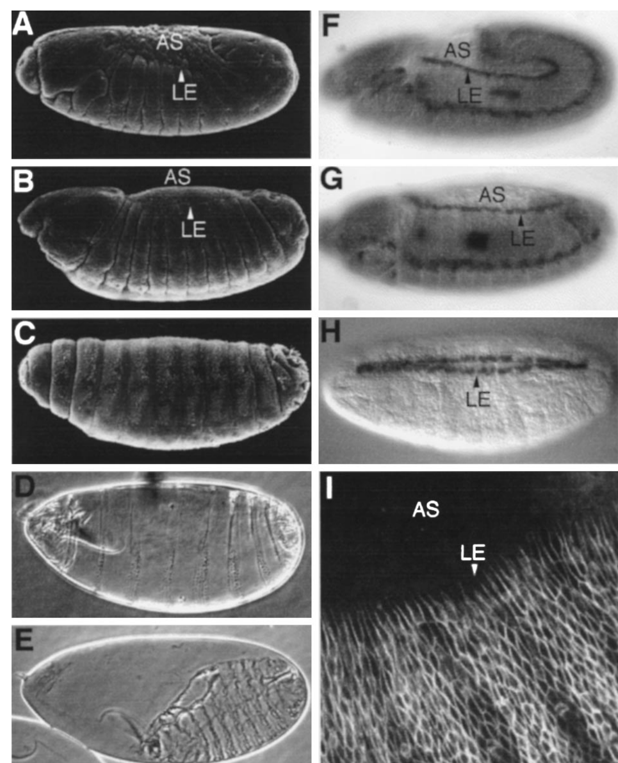

Figure 1 Movements, phenotypes, molecular markers, and cell shape changes associated with dorsal closure in *Drosophila*. Scanning electron micrographs of embryos reveal the morphogenetic movements of dorsal closure (**a–c**). Just after germband retraction (**a**), the leading edge, LE, of the epidermis begins to move dorsally toward the midline to cover a degenerative squamous epithelium, the amnioserosa, AS (**b**). At the end of embryogenesis, the embryo is completely surrounded by epidermis (**c**) which secretes a continuous patterned cuticle (**d**). Mutant embryos in which dorsal closure fails, produce cuticles with dorsal holes (**e**). During closure, JNK pathway signaling results in the expression of two transcriptional targets in LE cells, *dpp* and *puc* (see text for details). *dpp* transcripts are observed in several tissues including the LE during germband retraction (**f**) and dorsal closure (**g**). The phosphatase, Puc, is detected by expression of a *LacZ* transgene inserted in the *puc* locus. Puc expression is restricted to LE cells, shown here late in dorsal closure when they meet at the dorsal midline (**h**). Immunofluorescent labeling of cell membranes in the epidermis show the dramatic stretching of LE and lateral cells during closure (**i**). In all panels, anterior is to the left and dorsal is up

the dorsalward movement of the whole epithelium toward the midline (Young *et al*., 1993). Also during this phase, a wave of cellular elongation proceeds ventrally away from the LE to include more lateral cells. These dramatic shape changes are eventually sufficient to allow the entire dorsolateral epithelium to cover the region previously occupied by the amnioserosa (Young *et al*., 1993). DC ends with suturing or fusion, in which the epithelia on both sides of the embryo finally meet at the dorsal midline and adhere to one another without continuing to move over the midline. The entire process takes approximately 2 h to complete.

Embryos that fail to complete DC because of mutations in genes required for the process exhibit a characteristic dorsal hole in the cuticles they secrete (Figure 1). This is due to the fact that amnioserosa cells normally degenerate when enclosed inside the epidermis and do not contribute to the cuticle (Campos-Ortega and Hartenstein, 1985). Therefore, if amnioserosa cells are left exposed in embryos which fail to close, the cuticle is not complete. The 'dorsal open' cuticle has been used successfully in genetic screens as a criterion for isolating genes which function during the process of DC (Chou and Perrimon, 1996; Nusslein-Volhard *et al*., 1984; Perrimon *et al*., 1989). In this way, more than two dozen genes have been characterized that when mutant either zygotically, or maternally, or both, give rise to varying degrees of open cuticles. Most of the genes fall into several groups based on the proposed functions of each gene product (Table 2). For instance, one class of dorsal open mutants define genes encoding components of the cytoskeleton or cell adhesion machinery. A second group of genes implicated in DC are members of the JNK signal transduction pathway. A third group is represented by genes encoding components of the Dpp signal transduction pathway. Finally, additional molecules are thought to participate in DC based not on mutant phenotype but on overexpression studies or suggestive expression patterns. Most notable are the small GTPases of the Ras superfamily, Rho, Rac and Cdc42 (among which *RhoA* mutants have been reported) and their associated proteins such as DPAK, the *Drosophila* homolog of p21-activated kinase (Harden *et al*., 1996, 1999; Strutt *et al*., 1997).

Extensive genetic epistasis and interaction studies coupled with biochemical and molecular data have led to an integrated model describing the regulatory control and cellular mechanisms driving DC (Figure 2). One of the first events is thought to be activation of the JNK signaling pathway in the LE cells. Members of the core JNK signaling module that have been cloned in *Drosophila* include *hemipterous (hep)*, a JNKK and functional homolog of mammalian MKK7, *basket (bsk)*, a JNK, and *DJun* (Glise *et al*., 1995; Holland *et al*., 1997; Hou *et al*., 1997; Kockel *et al*., 1997; Riesgo-Escovar *et al*., 1996; Sluss *et al*., 1996). Mutations in each gene lead to embryos with severe dorsal and anterior open cuticles indicative of a failure of DC and head involution. Loss of JNK pathway activity has

Table 2 Genes involved in DC

Gene	*Protein structure*	*Reference*
Cytoskeleton/adhesion		
myospheroid	Integrin β subunit	(Brown, 1994)
scab	Integrin α subunit	(Stark *et al*., 1997)
coracle	Band 4.1	(Fehon *et al*., 1994)
zipper	Nonmuscle myosin heavy chain	(Young *et al*., 1993)
collagen	Extracellular matrix	(Borchiellini *et al*., 1996)
canoe	PDZ protein	(Jurgens *et al*., 1984; Takahashi *et al*., 1998)
ZO-1	PDZ and guanylate kinase	(Takahashi *et al*., 1998)
discs large	PDZ and guanylate kinase	(Perrimon, 1988)
Notch	Transmembrane receptor	(Zecchini *et al*., 1999)
raw	Unknown	(Blake *et al*., 1998)
ribbon	Unknown	(Blake *et al*., 1998)
GTPases and effectors		
Drac1	GTPase	(Glise & Noselli, 1997; Harden *et al*., 1995; 1999)
Dcdc42	GTPase	(Glise & Noselli, 1997; Harden *et al*., 1995; 1999)
DRho1	GTPase	(Harden *et al*., 1999; Strutt *et al*., 1997)
Pkn	Protein kinase C-related kinase	(Lu and Settleman, 1999)
myoblast city	DOCK180/ced-5 homolog	(Erickson *et al*., 1997; Nolan *et al*., 1998)
DPAK	p21-associated kinase	(Harden *et al*., 1996)
JNK pathway		
misshapen	Ste20 family kinase	(Su *et al*., 1998)
hemipterous	Jun N-terminal kinase kinase	(Glise *et al*., 1995)
basket	Jun N-terminal kinase	(Riesgo-Escovar *et al*., 1996; Sluss *et al*., 1996)
DJun	Jun transcription factor	(Glise and Noselli, 1997; Hou *et al*., 1997; Kockel *et al*., 1997; Riesgo-Escovar and Hafen, 1997)
kayak	Fos transcription factor	(Zeitlinger *et al*., 1997)
anterior open/yan	ETS domain	(Lai and Rubin, 1992; Riesgo-Escovar and Hafen, 1997)
puckered	VH-1 family phosphatase	(Martin-Blanco *et al*., 1998; Ring and Martinez-Arias, 1993)
DPP pathway		
decapentaplegic	Transforming growth factor β	(Padgett *et al*., 1987; St. Johnston and Gelbart, 1987)
thick veins	TGFβ type I receptor	(Affolter *et al*., 1994; Brummel *et al*., 1994; Nellen *et al*., 1994; Penton *et al*., 1994)
punt	TGFβ type II receptor	(Letsou *et al*., 1995; Ruberte *et al*., 1995)
mothers against dpp	R-smad	(Hudson *et al*., 1998)
medea	Co-smad	(Das *et al*., 1998; Hudson *et al*., 1998; Wisotzkey *et al*., 1998)
schnurri	Nuclear zinc-finger	(Arora *et al*., 1995; Grieder *et al*., 1995; Staehling-Hampton *et al*., 1995)

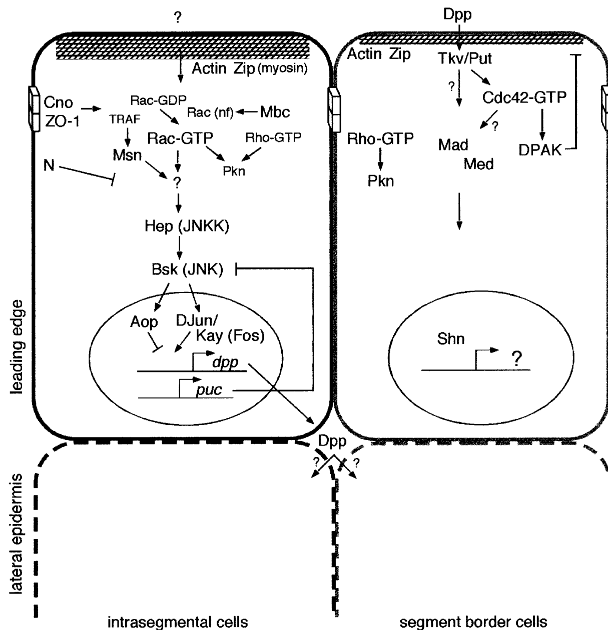

Figure 2 The JNK pathway signaling cascade regulates dorsal closure. This model takes into consideration many observations reported over the last few years, however, it is not inclusive of every molecule that has been described to play a role in dorsal closure. Prior to dorsal closure, the JNK pathway is activated by an unknown mechanism in leading edge cells via the action of the GTPase, Rac. Many proteins contribute to regulation of the JNK pathway including components of cell junctions, Cno and ZO-1, a Rac-interacting protein, Mbc and a ste20 family member, Msn, all of which can exert a positive effect on signaling. Membrane-associated N appears to negatively regulate the JNK cascade. Signaling through the Rac/JNK pathway leads to accumulation of an actomyosin cytoskeletal network at the dorsal membrane of LE cells and also activates the Jun/Fos AP-1 transcription factor resulting in upregulation of two transcriptional targets, *dpp* and *puc*. Puc, a phosphatase for Bsk, participates in negative feedback regulation of JNK signaling in the leading edge. Dpp is secreted and stimulates a conserved signaling pathway via the receptors, Tkv and Put, and the effectors, Mad and Med. Recent data supports the view that Dpp signal transduction predominates in the LE, at or near segment borders, however Dpp may still signal to the lateral epidermis to mediate the spread of cell shape changes more ventrally. Dpp signaling appears to require the GTPase Cdc42, and through its effector, DPAK, regulates the extent of cytoskeletal accumulation along the LE and thus mechanics of closure across the epidermis. The GTPase, Rho, and its effector kinase Pkn, have also been implicated in closure; this pathway may be restricted to a subpopulation of cells in the LE. Refer to text for additional details

several consequences that contribute to a failure of DC. Mutant embryos exhibit a loss of *dpp* expression in LE cells without perturbing other elements of the *dpp* expression pattern (Glise and Noselli, 1997; Hou *et al*., 1997; Riesgo-Escovar and Hafen, 1997). *puc* expression is similarly affected. JNK pathway mutants also fail to accumulate the proper cytoskeletal network in LE cells and thus LE cells do not fully elongate dorsally (Hou *et al*., 1997; Ricos *et al*., 1999). Lateral cells never elongate when the JNK signaling cascade is blocked by mutation suggesting that Dpp, a JNK pathway transcriptional target and secreted factor, may mediate the ventrally directed wave of cellular elongation observed in wild-type embryos during DC. LE expression of a constitutively activated form of c-Jun, *Jun*^{act}, is capable of rescuing not only the cuticle phenotype caused by mutations in either *bsk* or *hep* but also of restoring *dpp* expression at the LE and cell elongation in the lateral epidermis (Hou *et al*., 1997; Riesgo-Escovar and Hafen, 1997). These results emphasize several important facets of the JNK signaling cascade and the DC system in general. First, signaling through the JNK pathway is required only in the LE and results in activation of the Jun/Fos AP-1 complex. Second, a critical outcome of signaling via Jun/Fos appears to be transcriptional upregulation of *dpp* in the LE and subsequent signaling via the Dpp pathway to cells in more lateral positions in the epithelium. A prediction of this model linking the two signaling pathways is that restoration of Dpp signaling should alleviate the block to DC caused by mutations in positively acting components of the JNK pathway. Indeed, targeted expression in lateral epidermal cells of an activated Dpp receptor, *tkv*^{act}, rescues partially the loss of *bsk* or *DJun* function presumably by mediating the effects of Dpp in the lateral cells (Hou *et al*., 1997; Riesgo-Escovar and Hafen, 1997). Taken together with biochemical experiments demonstrating the catalytic activity and substrate specificity of Hep (JNKK) and Bsk (JNK) (Glise *et al*., 1995; Sluss *et al*., 1996), these results confirm that the components of the JNK signaling cascade in *Drosophila* functionally resemble the stress signaling kinase cascades defined in yeast and vertebrates.

Using similar criteria, it has been possible to establish the position of the Rac and Cdc42 GTPases within the signaling hierarchy. Dominant negative versions of Rac and Cdc42, when expressed in the dorsolateral epidermis, can block DC, *dpp* transcript accumulation, and enrichment of cytoskeletal components at the LE (Harden *et al*., 1995; Hou *et al*., 1997). On the other hand, ectopic expression of gain-of-function or activating mutants of Rac and Cdc42 provoke a strong ectopic upregulation of *dpp*, consistent with a positive role in regulating DC and a hyperactivation of the pathway (Glise and Noselli, 1997). These effects are suppressed in embryos additionally mutant for *hep* (Glise and Noselli, 1997). Together, these observations place Rac and Cdc42 function upstream of the JNK cassette consistent with the ability of vertebrate Rho family members to activate the JNK pathway (Coso *et al*., 1995; Minden *et al*., 1995).

Several negative regulators of DC have also been identified genetically. *puc* encodes a dual-specificity protein phosphatase of the VH-1 family of MAP kinase phosphatases (MKPs), that is localized specifically in LE cells (Martin-Blanco *et al*., 1998; Ring and Martinez-Arias, 1993). Puc functions to moderate signaling through the JNK pathway by antagonizing the kinase activity of Bsk. In fact *puc* loss-of-function mutant embryo lysates have increased JNK activity in kinase assays, and enhanced *dpp* expression in embryos (Martin-Blanco *et al*., 1998). In contrast, ubiquitous expression in embryos of wildtype Puc leads to dorsal cuticle holes and a loss of *dpp* expression indicating a substantial downregulation of JNK pathway activity (Martin-Blanco *et al*., 1998). Therefore, Puc serves a key regulatory role in JNK signaling as a transcriptional target of the pathway that builds up and feeds back negatively on the activity of Bsk to moderate signaling through the pathway. Another negative component is encoded by the *anterior open/yan (aop/yan)* gene (Lai and Rubin, 1992). Whereas an activated form of Jun rescues the phenotypes associated with mutations in upstream components, *aop* loss-of-function mutations dominantly suppress *bsk* mutations (Riesgo-Escovar

and Hafen, 1997). *aop* codes for a nuclear ETS domain protein that acts downstream of *hep* and *bsk* as a transcriptional repressor (Lai and Rubin, 1992; Riesgo-Escovar and Hafen, 1997). Phosphorylation by the Bsk kinase of Aop and DJun has the net effect of strong *dpp* expression because repression by Aop is relieved and activation by DJun is promoted.

The intricate nature of morphogenetic movements that take place during DC is reflected in the underlying molecular complexity given the large number of genes that have been identified to act in the process. Although much progress has been made in defining a fundamental signaling mechanism linking the JNK cascade to Dpp signaling to coordinate epithelial movement, additional components are still undefined. It remains unclear how the LE is determined and organized, how the JNK pathway is activated initially in these cells, and what role Dpp signaling plays in the lateral epidermis.

New components and complex regulation of JNK signaling during DC: the role of the GTPases

Although the dorsal open cuticle phenotype has allowed identification of many genes that participate in DC, the cuticle itself may be a rather crude indicator of the specific requirements of gene products during closure. Recent studies to elucidate the individual role of each GTPase, Rho, Rac, and Cdc42 in DC are beginning to suggest that not all cells of the LE are created equal. Even though expression of dominant negative versions of all three GTPases can result in cuticles with holes, distinct phenotypes are observed at the cellular and molecular level (Harden *et al*., 1999; Ricos *et al*., 1999). For instance, segmental or ubiquitous expression of dominant negative (DN)-Rho, DN-Cdc42, and DN-Rac can cause varying degrees of dorsal openness. However, while ubiquitous expression of DN-Rac and to a lesser extent DN-Cdc42 results in loss of cytoskeletal elements (actin, myosin, phosphotyrosine) in all LE cells, expression of DN-Rho leads to disruptions of LE cytoskeleton only in cells at the segment border (Harden *et al*., 1999). Additionally, there appears to be a more robust effect of Cdc42, than the other GTPases, on the levels and activity of a presumed effector, DPAK (p21-activated kinase) (Harden *et al*., 1999). Taking into account the presence or absence of LE cytoskeleton and the morphology of the epithelium, Ricos and colleagues have extended the analysis and show that DN-Rac shares phenotypes with JNK pathway mutants while DN-Cdc42-mediated effects more closely resemble the phenotypes associated with Dpp signaling components (Ricos *et al*., 1999). These observations point out that there are likely to be differential GTPase requirements across the row of LE cells and uncovers for the first time in this system differences between segment border and intrasegmental cells. Utilization of the different GTPases in distinct subpopulations of LE cells may be a way to generate distinct cytoskeletal configurations, or to regulate differential adhesiveness, contractility, or signaling activities. In turn, cell type specific GTPase effectors may be required to coordinate movement of the segmented epithelium as a whole to ensure proper meeting of the contralateral cells at the midline. It will be interesting to see if any newly discovered genes required for DC show a segmental expression pattern.

GTPase modulators, Mbc and Pkn

In the last year, several new regulators and effectors specific to certain GTPases have been reported and thus serve as candidates to mediate the unique GTPase functions. One modulator of Rac function was identified in a search for dominant suppressors of a Rac-induced rough-eye phenotype (Nolan *et al*., 1998). This suppressor, *Su(rac)1*, is allelic to *myoblast city (mbc)*, a gene previously isolated for its role in myoblast fusion during muscle development (Rushton *et al*., 1995). *mbc* mutant embryos are defective not only in myoblast fusion, but in DC and nervous system wiring (Erickson *et al*., 1997; Nolan *et al*., 1998). These phenotypes are reminiscent of those associated with overexpression of activated or antimorphic forms of Rac, and together with the genetic suppression data suggest involvement of Mbc in a common pathway with Rac (Erickson *et al*., 1997; Luo *et al*., 1994; Nolan *et al*., 1998). Mutations in *mbc* suppress both Rac- and Cdc42-, but not Rho-induced eye defects (Nolan *et al*., 1998). However, in cotransfection assays in vertebrate cells, the human homolog of Mbc, DOCK180, interacts exclusively with Rac in a nucleotide-independent manner (Nolan *et al*., 1998). Human DOCK180 has been implicated in regulating cell morphology when targeted to the cell membrane (Hasegawa *et al*., 1996). In addition, biochemical data revealed a physical interaction between DOCK180 and Crk, an adaptor protein localized to sites of cell adhesion (Hasegawa *et al*., 1996). Taken together, a plausible functional model consistent with the reported data is that Mbc/DOCK180 may influence Rac-mediated changes in membrane morphology or cytoskeleton through recruitment to specific subcellular sites or through localized activation of Rac at the membrane.

What is the role, if any, of Mbc in Rac-dependent transcriptional control? Although activated Rac and DOCK180 can both stimulate JNK activity in transfected cells as measured by an increase in Jun phosphorylation, *mbc* mutant embryos show limited effects on *dpp* expression in the LE (Nolan *et al*., 1998). These results raise the possibilities that there are alternative ways to activate Rac and thus the JNK pathway during DC or that Rac may not be the sole input to activate the JNK pathway. Furthermore, Rac may participate in some LE functions independent of its role in transcriptional control. Epistasis analysis revealed that DN-Rac cotransfected with DOCK180 could inhibit the DOCK180-dependent stimulation of JNK catalytic activity, placing Mbc/DOCK180 upstream of Rac (Nolan *et al*., 1998). In conclusion, Mbc appears to be a specific upstream modulator of Rac function in several developmental processes that require extensive cytoskeletal remodeling and changes in membrane morphology. Questions still remain over the exact nature of Rac regulation by Mbc and whether *mbc* mutant defects are mediated solely through the cytoskeleton or via transcriptional control using the JNK signaling pathway.

Another GTPase-interacting protein, *Drosophila* protein kinase N (Pkn), has been implicated in DC

(Lu and Settleman, 1999). Pkn is a member of the protein kinase C-related serine/threonine kinases and is the predominant kinase associated with Rho in mammalian tissues (Vincent and Settleman, 1997). In tissue culture cells, transfected *Drosophila* Pkn specifically associates with the activated GTP-bound forms of Rho and Rac in pull-down assays and its catalytic activity is enhanced slightly in response to these interactions (Lu and Settleman, 1999). *pkn* mutant embryos have a nearly identical dorsal open cuticle phenotype as embryos zygotically mutant for *rhoA*; however, *pkn* does not suppress Rho- or Rac-induced defects in the eye or perturb any gastrulation processes for which Rho has been implicated. Therefore, Pkn appears to be a specific GTPase effector for DC. Interestingly, similar to *mbc* mutants and embryos expressing DN-Rho, but in contrast to JNK pathway components, *pkn* mutations do not significantly perturb *dpp* expression in the LE (Lu and Settleman, 1999). This is one of the first GTPase effectors that does not seem to influence JNK transcriptional targets during DC, while still producing a strong cuticle phenotype and defects in the LE cytoskeleton. Thus, Yu and Settleman (1999) propose that Pkn acts predominantly in a Rho-specific pathway in parallel with the Rac-JNK pathway to control LE behavior and cytoskeletal remodeling, though their data does not rule out the possibility that Pkn may mediate some Rac functions independent of the downstream JNK cascade. Nonetheless, it is likely that the two pathways, Rho-Pkn and Rac-JNK, eventually converge on some common function because both are clearly required for LE cell stretching and ultimately DC. It is formally possible, however, that the two pathways literally function independently because they are restricted to distinct cell types within the LE. Finally, characterization of *pkn* mutants begs the question of the role of Dpp in lateral cell elongation because lateral cells do not elongate in *pkn* mutants even though *dpp* is still expressed. One explanation is that the Rho-Pkn pathway generates a second signal required for lateral cell stretching that acts coordinately with Dpp. Alternatively the function of the Dpp signaling pathway may not be to promote lateral cell stretching per se but is permissive for those cell shape changes. Perhaps the answer simply involves the biomechanics of the system, that cells in the lateral epidermis cannot elongate unless or until LE cells have already done so despite the presence of all the correct signals.

MKKKKs and TRAF

In an effort to determine the mechanism by which the JNK pathway is activated and DC is initiated, many groups have sought to identify upstream components in the pathway through characterization of new dorsal open mutants or cloning functional candidates by analogy with signaling pathways elucidated in other organisms. These methods have successfully converged to implicate *misshapen (msn)* in the regulation of DC and JNK signaling (Su *et al*., 1998). Msn was originally identified as a target of an eye-specific transcription factor and msn mutant clones in the eye lead to abnormal photoreceptor morphology (Treisman *et al*., 1997). Embryos zygotically mutant for *msn* show a low penetrance dorsal open phenotype (Su *et al*., 1998). Cloning the *msn* gene revealed that it is a member of the growing superfamily of protein kinases related to mammalian PAK and yeast sterile 20 (ste20), a kinase required for the mating response (Herskowitz, 1995). These kinases could be considered as MKKKKs (or MEKKKs), which phosphorylate and activate MKKKs (JNKKKs or MEKKs). Thus, by analogy Msn could presumably activate an as yet unknown kinase for which Hep, the *Drosophila* JNKK, would be the substrate. To address whether Msn indeed impinges on the JNK cascade in *Drosophila*, cellular and genetic analysis was undertaken. Both Msn and its mammalian homolog, NIK (NCK-interacting kinase), can stimulate JNK activity in transfected cells (Su *et al*., 1998). This activation is markedly decreased by simultaneous cotransfection of DN-Rac, suggesting that Msn may be upstream of Rac. Although Msn may lie in a signaling pathway with Rac, it does not interact directly with Rac (by two-hybrid or overlay assay), consistent with its lacking a defined GTPase binding domain (Su *et al*., 1998). Unlike typical ste20 kinases, such as PAK, which exhibit Rac and Cdc42 binding domains coupled to C terminal kinase domains, SPS-1 subfamily ste20-related kinases, for which Msn is highly related, have no GTPase binding sites and have N-terminal kinase domains (Kyriakis, 1999). Thus currently, just where Msn fits into the JNK signaling hierarchy is still somewhat unclear but perhaps Msn and Rac cooperate to activate a kinase upstream of Hep similar to the way Ras and an as yet unidentified kinase converge to activate Raf and subsequently the MAPK cascade (Morrison and Cutler, 1997).

In vivo genetic analysis lends further support to the notion that Msn is required for signaling through the JNK pathway. For example, in *msn* mutant embryos, there is a substantial reduction in *dpp* transcript accumulation in the LE (Su *et al*., 1998). Additionally, expression of an activated form of c-Jun or an activated Tkv receptor can partially rescue *msn* mutant embryos. To address how Msn, and ste20 kinases in general, are activated and regulated, Msn sequences were used in the yeast two-hybrid assay to identify interacting protein partners. One protein identified was *Drosophila* TRAF1 (TNF-receptor associated factor) (Liu *et al*., 1999). Extensive analysis of TRAF family members in mammalian tissues has shown that TRAFs serve as adaptor proteins to link cell surface receptors to both the NF-κB pathway and the JNK pathway (Arch *et al*., 1998). *In vitro* kinase assays demonstrate that both *Drosophila* Msn and TRAF1 can stimulate JNK activity in transfected cells (Liu *et al*., 1999). Stimulation of Jun phosphorylation by TRAF1 can be blocked, however, by cotransfection of a dominant negative form of Msn, lacking the kinase domain, suggesting that TRAF1 is likely to be upstream of Msn in signaling, consistent with the placement of TRAFs in vertebrate signaling pathways (Arch *et al*., 1998; Kyriakis, 1999; Liu *et al*., 1999). Thus, Msn and perhaps TRAF1 could be the most upstream components yet identified in the regulatory signaling cascades controlling DC, but confirmation awaits a genetic analysis of TRAF1 in *Drosophila* development.

Notch

As an old player with another new function, Notch (N) may contribute to the regulation of morphogenesis, independent of its better known nuclear pathway. Zygotic *N* mutant embryos have a neurogenic phenotype due to the failure of lateral inhibition during the specification of neural versus epidermal tissue (Artavanis-Tsakonas *et al*., 1999). Thus, neural tissue hypertrophy on the ventral side of the embryo, at the expense of epidermis, leaves just a scrap of dorsal cuticle. Loss of both maternal and zygotic *N* function increases the severity of the cuticle phenotype such that cuticles are even further reduced in size and exhibit dorsal defects, including what appears to be a dorsal midline hole (Zecchini *et al*., 1999). This phenotype suggests that N may have a function in dorsal tissue during the process of DC. To establish a potential mechanism for the effect of N on DC, JNK target gene expression was examined. Although the morphology of the embryos mutant for N is severely distorted, it appears that both *dpp* and *puc* are detected ectopically; *dpp* then fades earlier than normal (Zecchini *et al*., 1999). Although this may be due to hyperactivation of the JNK pathway, similar to what is observed in embryos programmed to express activated Rac or in embryos mutant for negative regulators of the JNK pathway such as Puc, it seems feasible that the N effect could be due not to ectopic expression via JNK activation but by 'hypertrophy' of the LE. In other words, if N functions in LE cell specification at the boundary of two cell sheets, the amnioserosa and the dorsal epidermis, then the increased expression of LE specific molecules may reflect an increased number of LE cells that have differentiated due to failure in lateral inhibition. The function of N in lateral inhibition is mediated by an intracellular pathway involving cleavage of the N cytoplasmic domain, translocation to the nucleus, and transcriptional upregulation of genes in cooperation with the Suppressor of Hairless (Su(H)) protein (Artavanis-Tsakonas *et al*., 1999). The effects of N in DC appear not to require this pathway, though, for several reasons. First, mutations in *Su(H)* do not confer dorsal defects nor changes in *dpp* expression. In addition, DC defects mediated by N require N to be at the cell surface and can be manifested in embryos expressing various N constructs that cannot be proteolytically processed (Zecchini *et al*., 1999). Thus, it is unlikely that the requirement of N in DC works through a pathway involving lateral inhibition on the dorsal side of the embryo but rather uses a mechanism that moderates JNK activity more directly. Consistent with this notion, extracts from *N* mutant embryos show increased JNK activity and Jun phosphorylation, which can be suppressed by mutations in *hep* (Zecchini *et al*., 1999). Thus, collectively, it appears that N is a new negative regulator of the JNK pathway in DC and serves to antagonize JNK activation presumably through a position at the cell membrane.

Cell junction proteins

An emerging theme in *Drosophila* DC, as well as many other systems, is that signaling pathways are subject to regulation by cellular junctions. This is likely to be an essential way that cells limit or extend responses to secreted factors by the integration of signals from cell-cell or cell-matrix receptors poised to monitor cell adhesion, polarity, and spatial position. It is probably at or near the membrane that this signal integration takes place. With this in mind, a recent report characterizing *canoe (cno)*, a gene previously identified as a DC mutant (Jurgens *et al*., 1984), emphasizes the importance of cellular junctions for JNK signaling and the regulation of DC movements (Takahashi *et al*., 1998). Genetic studies reveal an interaction between *cno* and components of the JNK pathway. Specifically, loss of one copy of *hep* or *bsk* can enhance an embryonic viable *cno* allele combination whereas in a complimentary experiment, overexpression of wildtype *bsk* can suppress or rescue a slightly stronger *cno* allele combination. Examination of JNK target genes reveals that indeed *dpp* and *puc* are significantly reduced in *cno* mutant embryos in comparison with wildtype embryos. Thus, like other positively acting components of the JNK pathway, Cno permits or stimulates signaling. So where might Cno fit into the JNK pathway? Since *cno* mutations do not block the stimulation of target genes induced by expression of activated Rac, it appears that Cno is required upstream of or in parallel with the GTPase, Rac. Further genetic and biochemical evidence indicates that Cno interacts with *Drosophila* ZO-1, whose mammalian homolog is a known component of cellular junctions and member of the MAGUK family of proteins containing protein-binding PDZ and guanylate kinase domains (Itoh *et al*., 1993; Stevenson *et al*., 1986). Interestingly, *cno* encodes a protein that also contains a PDZ domain and, in addition, contains motifs that resemble kinesin, myosin V, and Ras binding domains (Miyamoto *et al*., 1995; Ponting, 1995). Examination of Cno protein distribution in fly embryos reveals broad tissue distribution but restricted subcellular localization to the adherens junctions of epithelial cells (Takahashi *et al*., 1998). ZO-1 is similarly distributed but shows a broader membrane localization encompassing both adherens and septate junctions of epithelial cells. It is provocative that JNK signaling and DC are defective in *cno* and *ZO-1* mutants whose wildtype functions presumably contribute to junction assembly, maintenance, or regulation and suggest that there is crosstalk between sites of cell adhesion and the proteins involved in initial activation the JNK pathway. This scenario has been demonstrated elegantly in the nematode *C. elegans*. Genetic studies have shown that vulval induction requires the proper spatial distribution of the EGF receptor, which subsequently signals through a Ras-Raf- MAPK cassette (Kim, 1995). Mislocalization of the receptor in mutants which disrupt epithelial polarity or junctional integrity or both, causes abnormal vulval induction, due to a failure to receive the signal properly. Now, in *Drosophila*, characterization of the role of junctional proteins in DC provides another insight into the regulation of the JNK signaling pathway and may provide new ways to assay for potential ligands and receptors that to date have been elusive in this system.

Remaining questions

As new molecules are shown to interface with the components of the Rac-JNK signaling cascade, certain

gaps in our understanding are filled but many more arise with the increasing complexity of interactions. Several compelling problems surrounding the current paradigm for regulation of DC need to be addressed in future studies.

First, the nature of the stimulus for JNK activation during DC is unknown. Direct experimental evidence suggests that active signaling through the JNK pathway is required throughout the progression of DC to maintain the movements, but the observation that LE cells are able to stretch to some degree, although not to the full extent, calls into question the requirement of active JNK signaling for initiation of DC. It is intriguing to speculate that tension, or adhesion, sustained by cell junctions, or contractility itself, mediated by the cytoskeleton, might stimulate the JNK pathway, which then maintains the progression of cellular elongation and movement through regulated feedback mechanisms such as Puc. This so-called mechanotransduction, or coupling of cellular deformation and stretching to the signal transduction machinery, has been documented in mammalian muscle and bone cells and often leads to stimulation of stress signaling cascades (Hamada *et al*., 1998; Komuro *et al*., 1996; MacKenna *et al*., 1998). The source of the proposed signal may also be from tissue neighboring the LE, such as the amnioserosa, but to date, little evidence has accumulated addressing the role of the amnioserosa in DC. Perhaps contraction of the amnioserosa itself triggers LE elongation and DC initiation without the use of a secreted ligand. This may prove to be an area of active research for the future.

Second, and related to the previous issue, there is a disparity in the temporal relationship between JNK pathway activation, using *dpp* as a readout, and the movements of DC. Clearly JNK signaling is crucial for the movements of DC because mutations in JNK pathway components lead to a severe dorsal open cuticle. But in elaborating the role of the JNK pathway, studies have focused on events that occur post-germband retraction, because this is when cellular elongation and morphogenetic movements take place. The events that occur earlier during germband extension and retraction may hold a key to some of the unanswered questions, particularly about the specification of the cell types (LE) and the origin of 'signals' that activate the JNK pathway. The *dpp* expression pattern is refined during stages 11/12 from an earlier broader pattern to a LE-restricted pattern, at about the same time as the germband retracts. This fact emphasizes that the JNK pathway must be active several hours before cellular elongation in the dorsal epidermis commences. It will be important to consider what is happening prior to and during this period of *dpp* pattern refinement and also what takes place just after germ band retraction and the onset of DC. Continuing investigation is necessary to understand how the temporal and spatial confinement of signaling elements is regulated.

Third, how do we make sense of the role of the various Rho family GTPases in DC and in the activation of the JNK pathway? Characterization of Pkn and Mbc begin to raise the possibility that there may be a functional branch in the pathway from Rac to the cytoskeleton independent of the JNK pathway and transcriptional control. The key evidence for this notion is that transcriptional targets of the JNK pathway are minimally affected in *pkn* and *mbc* mutant embryos but LE cell elongation is blocked and DC still fails (Lu and Settleman, 1999; Nolan *et al*., 1998). In contrast, multiple lines of evidence suggest that the essential function of Rac is the ultimate activation of Jun/Fos AP-1 and transcriptional regulation mediated through the JNK pathway. For example, mutations in *hep* can suppress activated Rac, and Puc phosphatase overexpression mimics the effects on the LE cytoskeleton of both JNK loss-of-function and expression of DN-Rac (Glise and Noselli, 1997; Martin-Blanco *et al*., 1998). These data call into question the role of each GTPase in DC and the extent of branching signals upstream of the JNK pathway.

JNK signaling in tissue polarity

Although DC has provided an excellent system for teasing apart the regulation JNK signaling, additional requirements for *bsk* have been noted in postembryonic developmental processes, suggesting reuse of JNK signaling components in distinct developmental scenarios. For instance, Bsk and several other molecules described in this review contribute to proper development of the adult eye. Comprehensive genetic studies of eye development have elucidated the essential role of the Ras-Raf-MAPK pathway, linked to two receptor tyrosine kinases, sevenless and DER (*Drosophila* EGF receptor), in regulating photoreceptor specification and differentiation (Hafen *et al*., 1993; Wassarman *et al*., 1995). Although the role of the JNK pathway in photoreceptor development has been questioned because of lack of phenotypes of *bsk* or *DJun* mutant clones in the eye (Hou *et al*., 1997; Kockel *et al*., 1997; Riesgo-Escovar *et al*., 1996), this interpretation may be confounded by redundancy with the MAPK pathway. Alternatively, JNK signaling may not be required for photoreceptor differentiation *per se*, but for a distinct developmental event such as rotation of the eye facets. Indeed, genetic epistasis has placed *bsk*, *hep*, *rhoA* and *dishevelled (dsh*) in a pathway downstream of the *Frizzled (Fz*) serpentine receptor in generating proper tissue polarity based on dominant suppression, by mutations in these genes, of Fz- or dsh-induced ommatidial rotation defects (Boutros *et al*., 1998; Strutt *et al*., 1997). The connection between dsh and bsk is further supported by experiments in tissue culture cells which revealed that transfected dsh is capable of stimulating JNK activity (Boutros *et al*., 1998). It remains to be determined whether the linkage between Fz/dsh and the JNK signaling cassette has any implications for DC, though it seems unlikely since *dsh* mutations lead to segmentation defects during embryogenesis and do not cause dorsal holes. It is still intriguing to speculate that perhaps DC also relies on proper planar polarity of the embryonic dorsal epidermis in order to coordinate the directed cellular movements of the epithelium toward the midline.

p38 signaling in development

In the last year and a half several groups have reported the identification of *Drosophila* homologs of components in the p38 MAPK signaling cascade. For

example, two laboratories isolated *DMKK3/licorne (lic)*, by homology-based screening using a degenerate PCR approach (Han *et al*., 1998b), and also by functional complementation of the *polymyxin B sensitive (pbs2)* knockout in yeast (Suzanne *et al*., 1999). PBS2 is the MKK for HOG1, the yeast p38 homolog which participates in the high osmolarity response (Herskowitz, 1995). Similar approaches were taken to isolate *Drosophila* p38 genes, for which there are two, *D-p38a* and *D-p38b*. *D-p38a* was isolated by degenerate PCR screening of cDNA libraries (Han *et al*., 1998a,b) and *D-p38b* was isolated by two groups independently, both for its homology to *D-p38a* (Han *et al*., 1998b) and for its ability to functionally compensate for HOG1 (Adachi-Yamada *et al*., 1999).

To examine whether DMKK3/lic is required for *Drosophila* development, mutations in the gene were sought. Since *DMMK3/lic* is directly adjacent to *MKK7/hep* on the X chromosome, a *P* element insertion in *hep* was used to generate excision deletions of the two loci (Suzanne *et al*., 1999). Then, by providing Hep function from a transgene, defects specific to loss of *DMKK3/lic* could be analysed in the background of a deficiency for both genes. Given that *DMKK3/lic* transcripts are expressed highly in early precellular blastoderm embryos, and indicate a large maternal contribution, germline clonal analysis was performed to address the function of *DMKK3/lic* in early development. This analysis demonstrated a role of DMKK3/lic in several processes, including organization of the posterior pole plasm and abdominal segments in embryos, and establishment of both anterior/posterior and dorsal/ventral axes in the developing oocyte (Suzanne *et al*., 1999). Eggs laid by mothers mutant in their germline for *DMKK3/lic* were short and round, usually unfertilized, and moderately to severely ventralized, exhibiting a shift in the position of the pair of dorsal breathing appendages of the eggshell toward the dorsal side often to the point of fusion into a single appendage. Although the eggshell is secreted by somatic cells surrounding the germline, which would be heterozygous and functionally wildtype for *DMKK3/lic*, the nonautonomy of the eggshell phenotype suggests that *lic* may affect a secreted signal between the germline and soma. Indeed, gurken (grk), a TGF-alpha ligand, establishes the dorsal/ventral axis as a localized signal emanating from the oocyte (Gonzales-Reyes *et al*., 1995). Grk signal is transmitted to the follicle cells via the EGF receptor (DER) coupled to the MAPK pathway (Gonzales-Reyes *et al*., 1995). The dorsal/ventral defect observed in *DMKK3/lic* germline clones occurs as a result of a posttranscriptional effect on *grk* mRNA (Suzanne *et al*., 1999). *grk* mRNA is properly localized but the protein signal is reduced or diffuses away, resulting in a reduction in DER signaling and inability to specify the full range of dorsal fates. The nature of the *lic* effect on Grk translation, anchoring, or secretion is still unknown however. Examination of the role of p38 signaling during oogenesis has uncovered a striking connection where one MAPK pathway is used in a specific cell type (germline) to provide a ligand for activation of a different MAPK pathway in an adjacent cell type (somatic follicle cells). It will be very interesting to see if this sort of cellular linkage is working in other developmental phenomena.

Additional phenotypes associated with the small, round shape of the eggshell are characteristic of anterior/posterior polarity defects and failure of the supporting germline nurse cells to dump their contents to the oocyte during the latter stages of oogenesis. These multiple defects raise the question whether the p38 kinase, DMKK3/lic, may perform several 'independent' functions during oogenesis, to establish both coordinate axes, or may regulate the activity of downstream effectors that contribute to the overall spatial organization of the developing oocyte and egg chamber, which when compromised, leads to defects in overall axial polarity. It is not clear from these analyses if p38 activation regulates transcription in oogenesis, since the oocyte nucleus is thought to be transcriptionally silent. Nurse cell nuclei are highly transcriptionally active, though, and p38 may control transcription factors that participate in regulation of gene expression whose products are subsequently required in the oocyte. It is formally possible that the defects associated with *DMKK3/lic* loss-of-function result from failure to regulate effectors in the oocyte in a manner independent of the transcriptional machinery, but tissue culture and *in vitro* assays have confirmed the ability of *Drosophila* p38 kinases to phosphorylate and activate mammalian ATF2 (Adachi-Yamada *et al*., 1999; Han *et al*., 1998b) suggesting that transcription factors are likely to be physiological targets of p38 activity *in vivo* in fly tissues.

Although mutations in either *D-p38* gene have not yet been reported, transgenic analysis of dominant negative versions and antisense *D-p38b* constructs revealed a role for this protein in wing morphogenesis (Adachi-Yamada *et al*., 1999). Reduction in *D-p38b* activity appears to disrupt *dpp* signaling specifically. Expression of DN-D-p38b can give rise to a wing phenotype resembling *dpp* loss-of-function in the wing, can enhance the phenotype of a weak *dpp* mutant wing, and can suppress the defects associated with hyperactivity of a constitutive form of the Dpp receptor, Tkv. Consistent with these genetic interactions, various epistasis tests place D-p38b downstream of Tkv. In larvae expressing activated Tkv, only a slight increase in the level of phospho-D-p38b is detected. But levels of phospho-D-p38b are dramatically increased in cultured fly cells or whole adult flies subjected to heatshock, suggesting that even though p38 activation may be regulated during normal development, activation may also be inducible after stress treatment like heat shock (Adachi-Yamada *et al*., 1999; Han *et al*., 1998a). Moreover, both D-p38a and D-p38b phosphorylation and catalytic activity are upregulated in *Drosophila* S2 cells subjected to UV irradiation (Han *et al*., 1998b). Continuing investigations into the role of p38 and DMKK3/lic in oogenesis, embryogenesis, postembryonic development, and homeostasis will surely an exciting field to follow.

Stress signaling in immunity

The widespread use of stress signaling pathways in the vertebrate immune system is well documented (Ip and Davis, 1998; Kyriakis and Avruch, 1996). Both JNK and p38/ERK signaling pathways are responsive to inflammatory cytokines and result in transcriptional

regulation of genes required for the inflammatory response. While research on insect immunity intensifies, it is intriguing to look for parallels in the use of stress signaling in the *Drosophila* immune response. To date, ascertaining whether certain components of stress signaling are utilized *in vivo* during an immune response in the fly has been hampered by the fact that many of the genes cause embryonic lethality precluding analysis of mutant larvae or adults. Although several systems for generating mutant clones of tissue in an otherwise wildtype background have been used with success in various tissues, tools for generating and investigating clones of mutant immune cells have lagged. Nonetheless, initial evidence substantiating a role for JNK and p38 pathway components in *Drosophila* immunity has come from insect cell culture systems. Treatment of a *Drosophila* mbn-2 cell line, derived from macrophage-like hemocytes, with bacterial LPS (endotoxin), which is capable of eliciting a physiological response *in vivo*, leads to rapid stimulation of Bsk (JNK) activity monitored by an *in vitro* kinase assay with its substrate DJun (Sluss *et al*., 1996). Similarly, LPS can stimulate phosphorylation and activity of D-p38a and D-p38b in transfected cells (Han *et al*., 1998a,b.) Thus, both stress pathways appear to be induced by inflammatory stimuli in *Drosophila* cultured cells. Interestingly, an anti-inflammatory drug, SB203580, that has been shown to specifically target and disable human p38 kinases, is capable of inhibiting the catalytic activity of the *Drosophila* p38 isoforms *in vitro* (Han *et al*., 1998b). Furthermore, addition of this drug to LPS-treated p38-transfected S2 cells significantly prolongs the LPS response, measured by antimicrobial peptide target gene expression, suggesting that inhibition of p38 function may potentiate the immune response in the fly, contradicting the presumed role of this drug for anti-inflammation. To test the hypothesis that p38s may play a role in downregulation of the immune response *in vivo*, a transgenic *D-p38a* construct was expressed under an inducible heat shock promotor in larvae that were subsequently subjected to bacterial challenge. *D-p38a* overexpression resulted in supression of an immune response, again measured by the levels of target gene expression (Han *et al*., 1998b). Taken together, these data strongly suggest that p38s can be induced by an immune challenge but then serve to downregulate or attenuate the response, so as not to prolong inflammation. Carefully designed mutant screens to identify molecules functioning in the immune system have recently been conducted and may uncover new (viable) alleles of stress signaling components deficient in immune response (Wu and Anderson, 1998). This sort of genetic analysis will certainly prove valuable to confirm the role of JNK and p38 signal transduction pathways in insect immunity and to shed light on relevant tissue-specific signals and responses.

Concluding remarks

Drosophila is no exception when it comes to the presence and use of multiple MAPK signal transduction cascades during development and homeostasis. We have discussed how signaling cascades in the fly functionally related to the stress induced signaling pathways in vertebrates are utilized repeatedly in different cellular and developmental contexts, with distinct inputs and outcomes. Major roles for the JNK pathway in *Drosophila* include the regulation of complex morphogenetic movements during embryogenesis and generation of tissue polarity in the adult. The recently identified p38 pathway also appears to participate in morphogenetic processes and the generation of polarity in the egg chamber. Preliminary evidence also sets the stage for further characterization of JNK and p38 cascades in insect immunity.

One could imagine that morphogenesis, or the generation of form and shape in the context of multicellular organisms, such as that described in *Drosophila* DC, is an extension of the use of the same conserved pathways operating in unicellular yeast to control membrane and cytoskeletal organization and thus cell shape (osmotic response, mating protrusion), polarity (bud site selection), cell cycle, growth and differentiation. Furthermore, not only are the sequence and order of molecules in signaling cassettes like the JNK and Dpp pathway conserved, but the use of these two pathways sequentially to drive major morphogenetic movements has been documented from fly to man. The diverse processes that utilize MAPK and the stress signaling pathways, in particular, clearly underscore the exquisite versatility and specificity provided by the use of multiprotein phosphorylation cassettes throughout evolution. As in most scientific endeavors, new answers lead to new questions. Investigating the role of stress signaling genetically in *Drosophila* has stimulated a whole field of inquiry into regulation of tissue morphogenesis and polarity during development.

References

Adachi-Yamada T, Nakamura M, Irie K, Tomoyasu Y, Sano Y, Mori E, Goto S, Ueno N, Nishida Y and Matsumoto K. (1999). *Mol. Cell. Biol.*, **19,** 2322–2329.

Affolter M, Nellen D, Nussbaumer U and Basler K. (1994). *Development*, **120,** 3105–3117.

Arch RH, Gedrich RW and Thompson CB. (1998). *Genes Dev.*, **12,** 2821–2830.

Arora K, Dai H, Kazuko SG, Jamal J, O'Connor MB, Letsou A and Warrior R. (1995). *Cell*, **81,** 781–790.

Artavanis-Tsakonas S, Rand MD and Lake RJ. (1999). *Science*, **284,** 770–776.

Blake KJ, Myette G and Jack J. (1998). *Dev. Biol.*, **203,** 177–188.

Borchiellini C, Coulon J and Le Parco Y. (1996). *Roux's Arch. Dev. Biol.*, **205,** 468–475.

Boutros M, Paricio N, Strutt DI and Mlodzik M. (1998). *Cell*, **94,** 109–118.

Brown NH. (1994). *Development*, **120,** 1221–1231.

Brummel TJ, Twombly V, Marques G, Wrana JL, Newfeld SJ, Attisano L, Massague J, O'Connor MB and Gelbart WM. (1994). *Cell*, **78,** 251–261.

Campos-Ortega JA and Hartenstein V. (1985) *The embryonic development of Drosophila melanogaster*. Springer-Verlag, Berlin.

Chou T and Perrimon N. (1996). *Genetics*, **144,** 1673–1679.

Coso OA, Chiariello M, Yu JC, Teramoto H, Crespo P, Xu N, Miki T and Gutkind JS. (1995). *Cell*, **81,** 1137–1146.

Das P, Maduzia LL, Wang H, Finelle AL, Cho S-H, Smith MM and Padgett RW. (1998). *Development*, **125,** 1519–1528.

Erickson MR, Galletta BJ and Abmayr SM. (1997). *J. Cell Biol.*, **138,** 589 – 603.
Fehon RG, Dawson IA and Artavanis-Tsakonas S. (1994). *Development*, **120,** 545 – 557.
Garrington TP and Johnson GL. (1999). *Curr. Opin. Cell Biol.*, **11,** 211 – 218.
Glise B, Bourbon H and Noselli S. (1995). *Cell*, **83,** 451 – 461.
Glise B and Noselli S. (1997). *Genes Dev.*, **11,** 1738 – 1747.
Gonzales-Reyes A, Elliott H and St Johnston D. (1995). *Nature*, **375,** 654 – 658.
Grieder NC, Nellen D, Burke R, Basler K and Affolter M. (1995). *Cell*, **81,** 791 – 800.
Gustin MC, Albertyn J, Alexander M and Davenport K. (1998). *Microbiol. Mol. Biol. Rev.*, **62,** 1264 – 1300.
Hafen E, Dickson B, Brunner T and Raabe T. (1993). *Philos. Trans. Soc. Lond. Biol. Sci.*, **340,** 273 – 278.
Hamada K, Takuwa N, Yokoyama K and Takuwa Y. (1998). *J. Biol. Chem.*, **273,** 6334 – 6340.
Han S-J, Choi K-Y, Brey PT and Lee W-J. (1998a). *J. Biol. Chem.*, **273,** 369 – 374.
Han ZS, Enslen H, Hu X, Meng X, Wu I-H, Barrett T, Davis RJ and Ip YT. (1998b). *Mol. Cell. Biol.*, **18,** 3527 – 3539.
Harden N, Lee J, Loh HY, Ong YM, Tan I, Leung T, Manser E and Lim L. (1996). *Mol. Cell Biol.*, **16,** 1896 – 1908.
Harden N, Loh HY, Chia W and Lim L. (1995). *Development*, **121,** 903 – 914.
Harden N, Ricos M, Ong YM, Chia W and Lim L. (1999). *J. Cell Sci.*, **112,** 273 – 284.
Hasegawa H, Kiyokawa E, Tanaka S, Nagashima K, Gotoh N, Shibuya M, Kurata T and Matsuda M. (1996). *Mol. Cell. Biol.*, **16,** 1770 – 1776.
Herskowitz I. (1995). *Cell*, **80,** 187 – 197.
Holland PM, Suzanne M, Campbell JS, Noselli S and Cooper JA. (1997). *J. Biol. Chem.*, **272,** 24994 – 24998.
Hou XS, Goldstein ES and Perrimon N. (1997). *Genes Dev.*, **11,** 1728 – 1737.
Hudson JB, Podos SD, Keith K, Simpson SL and Ferguson EL. (1998). *Development*, **125,** 1407 – 1420.
Ip YT and Davis RJ. (1998). *Curr. Biol.*, **10,** 205 – 219.
Itoh M, Nagafuchi A, Yonemuta S, Kitani-Yasuda T, Tsukita S and Tsukita S. (1993). *J. Cell Biol.*, **121,** 491 – 502.
Jurgens G, Wieschaus E, Nusslein-Volhard C and Kluding H. (1984). *Roux's Arch.*, **193.**
Kim SK. (1995). *Curr. Opin. Cell Biol.*, **7,** 641 – 649.
Kockel L, Zeitlinger J, Staszewski LM, Mlodzik M and Bohmann D. (1997). *Genes Dev.*, **11,** 1748 – 1758.
Komuro I, Kudo S, Yamazaki T, Zou Y, Shiojima I and Yazaki Y. (1996). *FASEB J.*, **10,** 631 – 636.
Kyriakis JM. (1999). *Biochem. Soc. Symp.*, **64,** 29 – 48.
Kyriakis JM and Avruch J. (1996). *Bioessays*, **18,** 567 – 577.
Lai Z-C and Rubin G. (1992). *Cell*, **70,** 609 – 620.
Letsou A, Arora K, Wrana JL, Simin K, Twombly V, Jamal J, Staehling-Hampton K, Hoffman FM, Gelbart WM, Massague J and O'Connor MB. (1995). *Cell*, **80,** 899 – 908.
Liu H, Su Y-C, Becker E, Treisman J and Skolnik EY. (1999). *Curr. Biol.*, **9,** 101 – 104.
Lu Y and Settleman J. (1999). *Genes Dev.*, **13,** 1168 – 1180.
Luo L, Yiao YJ, Jan LY and Jan YN. (1994). *Genes Dev.*, **8,** 1781 – 1802.
MacKenna DA, Dolfi F, Vuori K and Ruoslahti E. (1998). *J. Clin. Invest.*, **101,** 301 – 310.
Madhani HD and Fink GR. (1998). *TIG*, **14,** 151 – 155.
Martin-Blanco E, Gampel A, Ring J, Virdee K, Kirov N, Tolkovsky AM and Martinez-Arias A. (1998). *Genes Dev.*, **12,** 557 – 570.
Minden A, Lin A, Claret FX, Abo A and Karin M. (1995). *Cell*, **81,** 1147 – 1157.
Miyamoto H, Nihonmatsu I, Kondo S, Ueda R, Togashi S, Hirata K, Ikegami Y and Yamamoto D. (1995). *Genes Dev.*, **9,** 612 – 625.
Morrison DK and Cutler RE. (1997). *Curr. Opin. Cell Biol.*, **9,** 174 – 179.
Nellen D, Affolter M and Basler K. (1994). *Cell*, **78,** 225 – 237.
Nolan KM, Barrett Lu, Y Hu-Q, Vincent S and Settleman J. (1998). *Genes Dev.*, **12,** 3337 – 3342.
Nusslein-Volhard C, Wieschaus E and Kluding, K. (1984). *Roux's Arch. Dev. Biol.*, **193,** 267 – 282.
Padgett RW, St Johnston RD and Gelbart WM. (1987). *Nature*, **325,** 81 – 84.
Paul A, Wilson S, Belham CM, Robison CJ, Scott PH, Gould GW and Plevin R. (1997). *Cell Signal*, **9,** 403 – 410.
Penton A, Chen Y, Staehling-Hampton K, Wrana JL, Attisano L, Szidonya J, Cassill JA, Massague J and Hoffman FM. (1994). *Cell*, **78,** 239 – 250.
Perrimon N. (1988). *Dev. Biol.*, **127,** 392 – 407.
Perrimon N, Engstrom L and Mahowald AP. (1989). *Genetics*, **121,** 333 – 352.
Ponting CP. (1995). *Trends Biochem. Sci.*, **20,** 265 – 266.
Ricos MG, Harden N, Sem KP, Lim L and Chia W. (1999). *J. Cell Sci.*, **112,** 1225 – 1235.
Riesgo-Escovar JR and Hafen E. (1997). *Genes Dev.*, **11,** 1717 – 1727.
Riesgo-Escovar JR, Jenni M, Fritz A and Hafen, E. (1996). *Genes Dev.*, **10,** 2759 – 2768.
Ring JM and Martinez-Arias A. (1993). *Development (Suppl.)*, **121,** 251 – 259.
Ruberte E, Marty T, Nellen D, Affolter M and Basler K. (1995). *Cell*, **80,** 889 – 897.
Rushton E, Drysdale R, Abmayr SM, Michelson A and Bate M. (1995). *Development*, **121,** 1979 – 1988.
Sluss HK, Han Z, Barret T, Davis RJ and Ip YT. (1996). *Genes Dev.*, **10,** 2745 – 2758.
St Johnston RD and Gelbart WM. (1987). *EMBO J.*, **6,** 2785 – 2791.
Staehling-Hampton K, Laughon AS and Hoffmann FM. (1995). *Development*, **121,** 3393 – 3403.
Stark KA, Yee GH, Roote CE, Williams EL, Zusman S and Hynes RO. (1997). *Development*, **124,** 4583 – 4594.
Stevenson BR, Siliciano JD, Mooseker MS and Goodenough DA. (1986). *J. Cell Biol.*, **103,** 755 – 766.
Strutt DI, Weber U and Mlodzik M. (1997). *Nature*, **387,** 292 – 295.
Su Y-C, Treisman JE and Skolnik EY. (1998). *Genes Dev.*, **12,** 2371 – 2380.
Suzanne M, Irie K, Glise B, Agnes F, Mori E, Matsumoto K and Noselli S. (1999). *Genes Dev.*, **13,** 1464 – 1474.
Takahashi K, Matsuo T, Katsube T, Ueda R and Yamamoto D. (1998). *MOD*, **78,** 97 – 111.
Toone WM and Jones N. (1998). *Genes Cells*, **1998,** 8.
Treisman JE, Ito N and Rubin GM. (1997). *Gene*, **186,** 119 – 125.
Vincent S and Settleman J. (1997). *Mol. Cell. Biol.*, **17,** 2247 – 2256.
Wassarman DA, Therrien M and Rubin GM. (1995). *Curr. Opin. Genet. Dev.*, **5,** 44 – 50.
Widmann C, Gibson S, Jarpe MB and Johnson GL. (1999). *Physiol. Rev.*, **79,** 143 – 180.
Wisotzkey RG, Mehra A, Sutherland DJ, Dobens LL, Liu X, Dohrmann C, Attisano L and Raftery LA. (1998). *Development*, **125,** 1433 – 1445.
Wu LP and Anderson KV. (1998). *Nature*, **392,** 93 – 97.
Young PE, Richman AM, Ketchum AS and Kiehart DP. (1993). *Genes Dev.*, **7,** 29 – 41.
Zecchini V, Brennan K and Martinez-Arias A. (1999). *Curr. Biol.*, **9,** 460 – 469.
Zeitlinger J, Kockel L, Peverali FA, Jackson DB, Mlodzik M and Bohmann D. (1997). *EMBO J.*, **16,** 7393 – 7401.