Manuscript submitted to: Volume 2, Issue 1, 25-53.

AIMS Genetics DOI: 10.3934/genet.2015.1.25

Received date 18 November 2014, Accepted date 27 January 2015, Published date 1 February 2015

Review

Drosophila models of metastasis

Michael J. Murray *

School of BioSciences, Faculty of Science, University of Melbourne, Victoria 3010, Melbourne, Australia

*** Correspondence:** Email: murraym@unimelb.edu.au; Tel: (61) 3 8344 7139.

Abstract: An important goal in the fight against cancer is to understand how tumors become invasive and metastatic. A crucial early step in metastasis is thought to be the epithelial mesenchymal transition (EMT), the process in which epithelial cells transition into a more migratory and invasive, mesenchymal state. Since the genetic regulatory networks driving EMT in tumors derive from those used in development, analysis of EMTs in genetic model organisms such as the vinegar fly, *Drosophila melanogaster*, can provide great insight into cancer. In this review I highlight the many ways in which studies in the fly are shedding light on cancer metastasis. The review covers both normal developmental events in which epithelial cells become migratory, as well as induced events, whereby normal epithelial cells become metastatic due to genetic manipulations. The ability to make such precise genetic perturbations in the context of a normal, in vivo environment, complete with a working innate immune system, is making the fly increasingly important in understanding metastasis.

Keywords: *Drosophila*; metastasis; epithelial mesenchymal transition

1. Metastasis—reuse of conserved developmental programs

One of the most important goals in the battle against cancer is to prevent metastasis. Metastatic tumor cells employ a complex repertoire of behaviors that allow them to break free of the primary tumor and disseminate throughout the body to establish secondary tumors. Since it is these metastases that are the primary cause of cancer related deaths, understanding the genetic regulation of metastasis is crucial. In this quest, genetic model organisms such as the vinegar fly, *Drosophila melanogaster*, can play a key role. For years, studies in the fly have contributed greatly towards our understanding of many basic cellular processes such as the cell-cycle, apoptosis, polarity, and motility, and have led to the identification and elucidation of signaling pathways that play a central role in cancer (e.g. Hippo, Wingless (Wg)/Wnt, Notch, Ras, Hedgehog etc.). In this review I

highlight the many ways in which the fly is also helping us understand metastasis.

Although metastasis can affect tumors of both epithelial and non-epithelial origin, the focus of this review will be the metastasis of epithelial tumors, which account for the majority of cancers. To metastasize, epithelial tumor cells must break free of the epithelial constraints and become migratory and invasive, allowing them to break through the basement membrane, and migrate into the adjacent tissue. To progress from local invasion to metastasis to distant sites, cells must then enter blood vessels or lymphatic vessels (i.e. intravasate), and subsequently leave the vessels (extravasate) to reinitiate proliferation and establish the secondary tumor mass.

In recent years a popular model for this process has been that metastasizing cells lose their epithelial characteristics, such as apico-basal polarity and the zonula adherens (ZA) (the circumferential belt of E-Cadherin-based cell-cell adhesive junctions) and become dissociated, migratory, mesenchymal cells. This characteristic sequence of events is known as an epithelial mesenchymal transition (EMT), and is also crucial during animal development, in events such as gastrulation and vertebrate neural crest formation [1]. Since secondary tumors exhibit epithelial characteristics, however, this model also includes the idea that cancer cells can undergo the reverse transition of mesenchymal epithelial transition (MET) at the secondary site, and this is thought to be important for the growth of the secondary tumor [2,3]. More recently, however, this EMT model for metastasis is being revised, since in many cases it is clear that metastatic cells do not undergo a "full EMT" but instead continue to express epithelial markers and migrate as collectives [4]. Similarly, during development there exist many examples where epithelial cells undergo only a partial EMT resulting in an intermediate phenotype, with both epithelial and mesenchymal characteristics. It has also been suggested that metastasis might involve cooperation between "EMT cells", which provide tumors with the necessary invasive properties, and "non-EMT cells", which are responsible for the formation of secondary tumors [5,6,7]. Alternatively, there exist tumors that are highly invasive but still express full epithelial features, such as those expressing podoplanin [8,9]. Similar events occur in development where epithelial sheets migrate due to leading edge cell motility. These considerations are leading to a more widely applicable concept of epithelial plasticity, in which cells can move backwards and forwards along an axis of epithelial-mesenchymal traits [10].

To achieve the complex sequence of behaviors required for metastasis, cancer cells take advantage of existing genetic regulatory networks used during development. For example in flies, the transcription factor Twist sits at the apex of a hierarchy of regulatory events that together lead mesodermal cells to fold inwards, undergo an EMT, and become migratory. Thus, when a cancer cell upregulates a gene like Twist1, it can put into play a coordinated and complex genetic program that together promotes metastasis. Consequently, significant insight into metastasis can be gained by studying analogous processes during development.

In this review the focus will be on the first stage of metastasis, when cells lose their epithelial properties and become migratory, i.e. the EMT. The subsequent process of migration is obviously also crucial, and readers are referred to other reviews on cell migration in *Drosophila* [11-16]. I begin by covering several normal developmental events in the fly that have provided key insights into the fundamental mechanisms of EMT and metastasis. The second part of the review covers situations in which researchers induce metastatic behavior via specific genetic perturbations. Since the events of metastasis depend greatly on the surrounding cellular microenvironment, the ability to make such perturbations in the context of a normal, in vivo, cellular environment is one of the great strengths of *Drosophila* as a system for modelling for metastasis. Conversely, it must be recognized

that there are inherent limitations in how completely one can model metastasis in flies. A major difference is that flies have an open circulatory system and therefore do not possess blood or lymphatic vessels, which are key factors in metastasis.

2. Developmental models of metastasis

In this section I review four normal developmental events that involve loss of epithelial structure/integrity and acquisition of cell motility. These are mesoderm formation, endoderm formation, wing disc eversion, and border cell delamination (Figure 1).

The question arises as to whether these events should be classified as EMT or partial-EMT. A widely used definition of full EMT is that it involves complete loss of E-Cadherin (E-Cad) expression due to transcriptional repression by an EMT-inducing transcription factor such as Snail [17]. According to this definition, none of these events would be classified as EMTs. Although mesoderm formation does involve Snail repression of *shotgun (shg),* the *Drosophila* orthologue of E-Cad (see Table 1 for list of *Drosophila* genes and human orthologues), and epithelial structure and apico-basal polarity are completely lost, levels of E-Cad protein remain high and may even be important for the migration. In the endodermal EMT, cells do lose epithelial structure and polarity, but there is no transcriptional repression of *shg* and E-Cad is again maintained at significant levels. In disc eversion, epithelial cells in some regions undergo dismantling of ZAs and loss of apico-basal polarity, but away from this area the epithelium persists, and it is not known whether Snail-family transcription factors are involved. Finally, in the case of the border cells, a cluster of cells breaks free of the follicular epithelium, but their subsequent migration is as a structured, and tightly adhered collective, which is dependent upon E-Cad.

For the sake of this review, a broader definition of EMT will be adopted, in which EMT entails only the loss of epithelial structure (i.e. apico-basal polarity and ZAs) and gain of motility. Thus the mesoderm and endoderm will be considered EMTs, while the eversion and border cell delamination will be considered partial EMTs. Regardless of the terminology, these four events represent a valuable and diverse set of EMT paradigms involving four, distinct genetic regulatory hierarchies. In addition to these EMTs, there are several other developmental events in which epithelial cells exhibit plasticity but still maintain ZA connections with their original neighbors, such as the branching morphogenesis of the trachea, and the epithelial sheet migrations of dorsal and thorax closure. While these involve cellular behaviors that are relevant to metastasis, they will not be covered here and the reader is referred to recent reviews [18,19].

2.1. Mesodermal EMT

The first EMT during *Drosophila* embryogenesis, and indeed the first for any animal, occurs at gastrulation. In *Drosophila*, a ventral band of cells constrict their apical surface creating a furrow which folds inwards. Once internalized, mesodermal cells undergo a wave of cell division as they lose their epithelial structure (Figure 1A), and then they migrate laterally upon the inner surface of the ectoderm. All of these behaviors are under the control of two key transcription factors that are specifically expressed in presumptive mesodermal cells, Twist and Snail. These genes, which were identified over 30 years ago [20], have since become two of the most important cancer genes in humans [21,22]. The fact that over 1000 cancer papers have now been published on Twist and Snail

family proteins, half of these within the last 3 years, highlights the importance of the mesodermal EMT, as a model system for cancer studies.

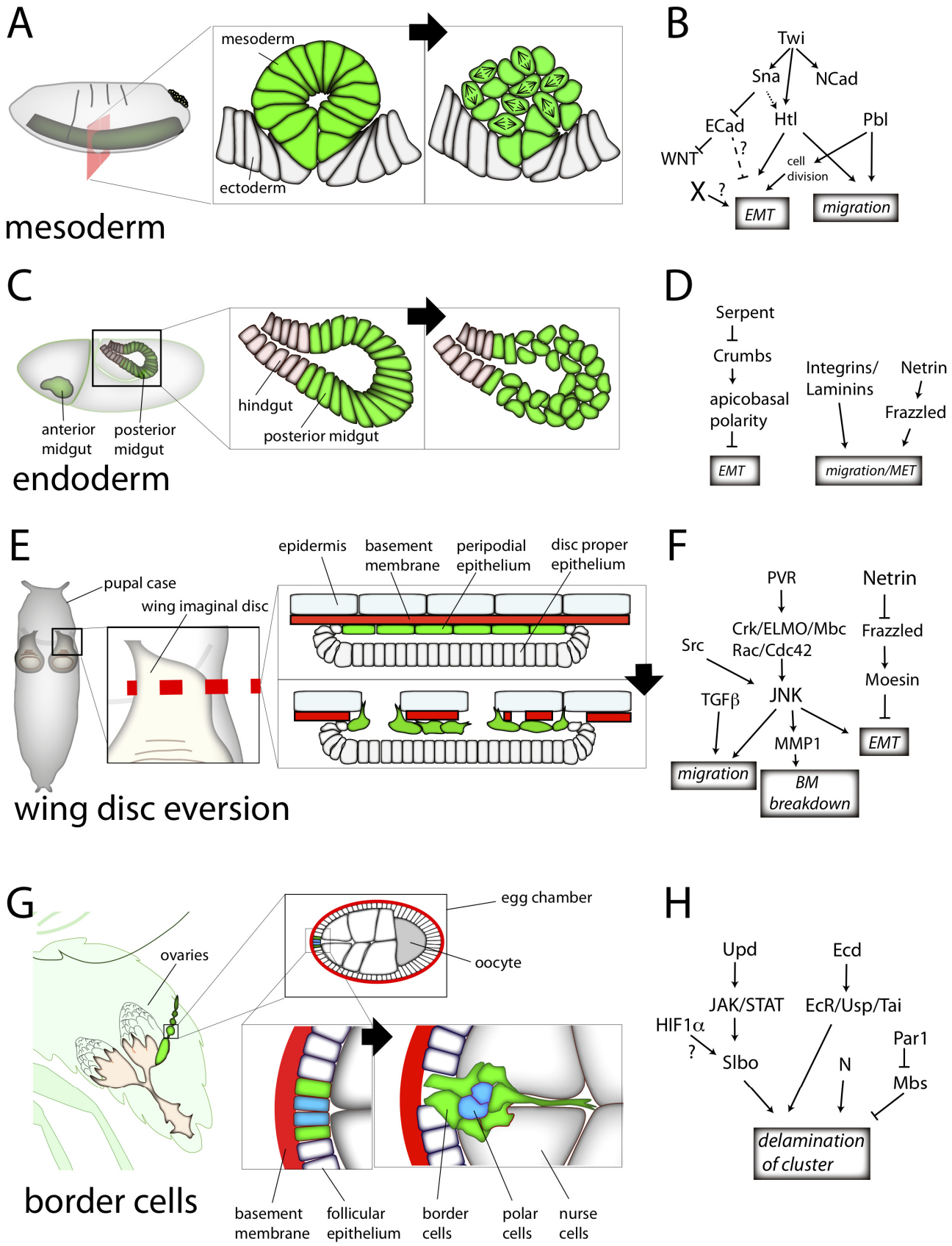

Figure 1. Normal developmental EMT and partial-EMT events in *Drosophila*. (A) Mesoderm formation. The mesoderm forms from a ventral band of cells that furrow inwards to form an epithelial tube (green). The cells then undergo a combined EMT and round of cell division, and subsequently migrate laterally over the inner surface of the ectoderm (grey). (B) Genetic regulation of the mesodermal EMT. The transcription factor Twist activates expression of a number of genes including the transciption factor Snail, the FGF receptor Heartless (Htl) and Neural-Cadherin (N-Cad). Snail represses transcription of E-Cad, but activates transcription of other genes like *htl* in conjunction with Twist. Repression of E-Cad helps activate WNT signaling but is not necessary for the EMT. The EMT is promoted by Htl, cell division, and perhaps rounding of cells due to Pebble (Pbl). Both Htl and Pbl are necessary for the subsequent migration. (C) Endoderm formation. The endoderm forms from two primordia at the anterior and posterior poles of the embryo. The posterior midgut (green) is initially continuous with the hindgut epithelium (grey). The posterior midgut cells then undergo an EMT and begin migrating along the visceral mesoderm (not shown). (D) Genetic regulation of the endodermal EMT. The GATA factor Serpent represses transcription of the apical polarity determinant Crumbs, which leads to loss of apico-basal polarity and epithelial structure. The subsequent migration and MET events require both integrin and Frazzled receptors and their respective ligands, the Laminins and Netrins. (E) Wing disc eversion. The wing imaginal discs are epithelial sacs attached to the inner surface of the larval epidermis. Around 3.5 hours after puparium formation the wing disc comes into apposition with the epidermis. The basement membrane (red) is degraded, and the cells of the peripodial epithelium (green) lose polarity and cell-cell junctions, become motile and invade through the overlying epidermis. Holes form in the epidermis allowing the disc to move up and over the epidermis (not shown). (F) Genetic regulation of eversion. The Jun kinase pathway (JNK) is activated by the PVR receptor and Src family kinases, leading to expression of MMP1, and, together with the TGFβ pathway, acquisition of cell motility. JNK activation also promotes loss of apico-basal polarity and epithelial cell-cell junctions. The Frazzled receptor opposes the EMT via Moesin, but undergoes endocytosis and Netrin-dependent degradation to promote the EMT. (G) Border cell delamination. Within the ovary, each ovariole (green) consists of a chain of developing egg chambers. Each egg chamber consists of an oocyte at the posterior end connected to 15 supporting nurse cells. These germ cells are surrounded by a somatic follicular epithelium and a basement membrane (red). (H) Genetic regulation of border cell delamination. The cytokine Unpaired (Upd) is expressed by the two polar cells (blue) activating the JAK/STAT pathway in adjacent border cells (green) leading to upregulation of Slow Border Cells (Slbo). HIF1α may contribute to Slbo expression. The timing of delamination is controlled by the steroid hormone Ecdysone which activates the Ecd pathway via its heterodimeric receptor EcR/Ultraspiracle (Usp), and their co-activator Taiman (Tai).

Table 1. *Drosophila* gene function and human orthologues.

Gene symbol	Gene name	Molecular function	Human orthologues
ab	*abrupt*	BTB-Zinc Finger Transcription Factor	
Abl	*Abl tyrosine kinase*	Cytoplasmic tyrosine kinase	ABL1, ABL2
arm	*armadillo*	Catenin (adherens junction component)	CTNNB1
ban	*bantam*	microRNA	
ben	*bendless*	Part of Ben/dUev1a E2 ubiquitin-conjugating enzyme complex	UBE2Z
bsk	*basket*	JUN kinase	MAPK8, MAPK9, MAPK10
N-Cad	*N-Cadherin*	N-Cadherin	CDH2 *
Cdc42	*Cdc42*	Rho GTPase	CDC42
Ced-12	*Ced-12*	PH-domain protein, complexes with DOCK180	ELMO1, ELMO2, ELMO3
cher	*cheerio*	Filamin protein	FLNA, FLNB, FLNC
chic	*chickadee*	Profilin protein	PFN1, PFN2, PFN3, PFN4 *
conu	*conundrum*	Rho GAP	ARHGAP40
crb	*crumbs*	transmembrane apical polarity complex protein	CRB1,CRB2
Crk	*Crk*	SH2/SH3-containing adaptor protein	CRKL
Csk	*C-terminal Src kinase*	cytoplasmic tyrosine kinase, negative regulator of Src	CSK
cta	*concertina*	$G\alpha_{12/13}$ subunit	GNA12, GNA13
dlg1	*discs large 1*	MAGUK scaffolding protein, part of Scrib/Dlg/Lgl complex	DLG4
dome	*domeless*	gp130-like JAK/STAT Receptor	
dpp	*decapentaplegic*	TGF ligand	BMP2, BMP4
EcR	*Ecdysone receptor*	part of the EcR/Usp ecdysone receptor heterodimer	NR1H4
egr	*eiger*	Tumor Necrosis Factor	
ena	*enabled*	actin-associated protein	VASP, ENL
fog	*folded gastrulation*	secreted protein	
fra	*frazzled*	Netrin receptor	DCC, NEO1
hep	*hemipterous*	Jun kinase kinase	MAP2K7
HIF-1α	*Hypoxia Induced Factor 1α*	Hypoxia Induced Factor	HIF-1α *
htl	*heartless*	FGF Receptor	FGFR1, FGFR2, FGFR3, FGFR4
Jra (Jun)	*Jun-related antigen*	part of Jun/Fos (AP-1) transcription factor	JUN, JUNB, JUND

Kay(Fos)	*kayak*	part of JUN/FOS (AP-1) transcription factor	FOS, FOSL1, FOSL2, FOSB
l(2)gl	*lethal (2) giant larvae*	Scaffolding protein, part of Scrib/Dlg/Lgl complex	LLGL1, LLGL2
mbc	*myoblast city*	DOCK-family GTP Exchange Factor (GEF)	DOCK1, DOCK2, DOCK5
mist	*mesoderm-invaginati on signal transducer*	G-protein-coupled Receptor	
Mmp1	*Matrix metalloproteinase 1*	Matrix Metalloproteinase	MMP19, MMP28 *
Mmp2	*Matrix metalloproteinase 2*	Matrix Metalloproteinase	MMP1, MMP3, MMP7, MMP8, MMP10, MMP11, MMP13, MMP14, MMP15, MMP17,MMP20, MMP24, MMP25, MMP27, AP000789.1
Moe	*Moesin*	ERM-family protein, F-Actin/trans-membrane linker	EZR, MSN, RDX
N	*Notch*	Notch receptor	NOTCH1, NOTCH2, NOTCH3, NOTCH4, SNED1
NetA	*Netrin-A*	Netrin, secreted chemotactic factor	NTN1, NTN3, NTN4, NTN5, NTNG1, NTNG2, LAMB3
NetB	*Netrin-B*	Netrin, secreted chemotactic factor	NTN1, NTN3, NTN4, NTN5, NTNG1, NTNG2, LAMB3
p120ctn	*Adherens junction protein p120*	p120 Catenin (adherens junction component)	CTNND1, CTNND2, PKP1, PKP2, PKP3, PKP4, ARVCF
par-1	*par-1*	serine/threonine kinase	MARK1, MARK2, MARK3, MARK4
pbl	*pebble*	Rho GTP Exchange Factor (RhoGEF)	ECT2
ph-d	*polyhomeotic distal*	Polycomb Group repressor complex subunit	PHC1, PHC2, PHC3
Pvr	*PDGF- and VEGF-receptor related*	PDGF/VEGF-Receptor Tyrosine Kinase	PDGFRA, PDGFRB, FLT1, FLT3, FLT4, CSF1R, KDR, KIT
pyr (FGF8-lik e2)	*pyramus*	FGF ligand	
Rac1	*Rac1*	Rho GTPase	RAC1, RAC2, RAC3
Ras85D	*Ras oncogene at 85D*	Ras GTPase	ERAS, KRAS, HRAS, HRAS

Rho1	*Rho1*	Rho GTPase	RHOA, RHOB, RHOC
RhoGEF2	*Rho guanine nucleotide exchange factor 2*	Rho GTP Exchange Factor (RhoGEF)	ARHGEF1, ARHGEF11, ARHGEF12
scb	*scab*	Integrin alpha-subunit	ITGA1, ITGA11, ITG4, ITGA2, ITGAD, ITGAL, ITGAE, ITGA9, ITGA10, ITGAX, ITGAM
scrib	*scribbled*	LRR/PDZ scaffolding protein, part of Scrib/Dlg/Lgl complex	SCRIB, LRRC1
sdt	*stardust*	MAGUK family of scaffolding protein, part of Crumbs apical complex	MPP5
shg (E-Cad)	*shotgun*	Epithelial Cadherin (adherens junction component)	CDH1 *
Sin3A	*Sin3A*	Sin3-family Histone Deacetylase	SIN3A, SIN3B
slbo	*slow border cells*	C/EBP transcription factor	
slik	*Sterile20-like kinase*	Sterile20 kinase	SLK, SLK10
slpr	*slipper*	Jun kinase kinase kinase	MAP3K9, MAP3K10, MAP3K11, RP5-862P8.2
sna	*snail*	Snail family transcription factor	SNAI1, SNAI2, SNAI3
Socs36E	*Suppressor of cytokine signaling at 36E*	cytoplasmic suppressors of JAK/STAT signaling	SOCS4, SOCS5
srp	*serpent*	GATA-factor	hGATA4, hGATA5, hGATA6 *
T48	*Transcript 48*	membrane protein	
tai	*taiman*	Steroid receptor co-activator	AIB1 *
Tak1	*TGF-beta activated kinase 1*	Jun kinase kinase kinase	MAP3K7
ths (FGF8-like1)	*thisbe*	FGF ligand	
Traf6	*TNF-receptor-associated factor 6*	TNF Receptor-associated factor	
tsr	*twinstar*	Cofilin/Actin Depolymerising Factor	CFL1, CFL2, ADF *
twi	*twist*	Twist transcription factor	TWIST1, TWIST2
Uev1A	*Ubiquitin-conjugating enzyme variant 1A*	Part of Ben/dUev1a E2 ubiquitin-conjugating enzyme complex	UBE2V1, UBE2V2
Upd	*Unpaired*	Interleukin-like JAK/STAT ligand	IL-6 *
upd2	*unpaired 2*	Interleukin-like JAK/STAT	IL-6 *

		ligand	
upd3	*unpaired 3*	Interleukin-like JAK/STAT ligand	IL-6 *
usp	*ultraspiracle*	part of the EcR/Usp ecdysone receptor heterodimer	RXRA, RXRB, RXRG
wg	*wingless*	WNT/Wingless secreted ligand	WNT1
wgn	*wengen*	TNF Receptor	
zip	*zipper*	non-muscle myosin II	

* These human orthologs were curated from *Drosophila* literature. All others were extracted from Ensembl Biomart.

As a master regulator of mesodermal cell fate, Twist activates hundreds of downstream targets, including *snail* [23]. For some of these, the link to cell behaviors has been determined. The initial apical constriction that drives invagination begins with the Twist target *folded-in-gastrulation (fog)*. Fog is a secreted protein that activates the G-protein Coupled Receptor, *mesoderm invagination signal transducer (mist)*, which is itself a Snail target. This is thought to lead to activation of the Gα subunit Concertina, and subsequent activation and recruitment to the membrane of RhoGEF2, facilitated by another Twist target the transmembrane protein T48 [24,25]. Shortly after internalization, the epithelial tube undergoes a collective EMT, in which the mesodermal cells lose their epithelial structure en masse, and subsequently spread out over the underlying ectoderm. This migration is dependent upon activation of the FGF Receptor Heartless (Htl), in response to two FGF8-like ligands, Pyramus and Thisbe, and upon the RhoGEF Pebble (Pbl) [reviewed in 16]. Interestingly, although Pbl plays a positive role in migration, too much Pbl at the earlier, furrowing stage causes phenotypes. *pbl* is actually one of the genes that is repressed by Snail in the presumptive mesoderm, and when this repression is blocked, or when Pbl is overexpressed, internalization is disrupted [26].

The mesodermal EMT exemplifies one of the classic hallmarks of EMT in both development and cancer: cadherin switching [27]. This is the process in which E-Cad is repressed, while N-Cadherin (N-Cad), a form associated with non-epithelial cells such as neurons and mesodermal cells, is upregulated. Loss or mutation of E-Cad, and upregulation of N-Cad [28,29], are strongly associated with metastasis and poor prognosis. In the case of *Drosophila* mesodermal cells, Snail represses *shg/E-Cad* while Twist upregulates *N-Cad* [30]. A natural assumption of the mechanism of mesodermal EMT, therefore, would be that Snail-dependent repression of *E-Cad* adhesion drives the EMT, and the switch to N-Cad is important for the subsequent migration. However, two recent publications are challenging our notions about this canonical EMT event.

Firstly, the functional importance of the cadherin switch has recently been put to the test [31]. Surprisingly, neither expression of N-Cad nor loss of E-Cad is necessary for the EMT or for the subsequent segregation of mesoderm and ectodermal cells. In fact, the EMT can proceed normally even when E-Cad is overexpressed, pointing to powerful, but as yet unidentified, factors driving the dismantling of the ZA. Since E-Cad is still expressed in wild type mesodermal cells, and even appears to play a positive role in the migration [32], the mesodermal EMT has been referred to as a partial EMT. However, although mesodermal cells do move together, and might be thought of as a collective migration event, single cell labelling experiments have provided evidence that mesodermal cells also have the capacity to migrate independently from one another [33]. Given that transcriptional repression of *E-Cad* does not seem important, an outstanding question is: what drives

the ZA breakdown. Two factors that are known to contribute at least partly to this process are Htl, and the round of cell division that the cells undergo. Previous studies using mutants for *string (stg)*, the *Drosophila cdc25* ortholog, have shown that the EMT can proceed in the absence of cell division, and it also occurs normally in *htl* mutants. However, in *stg;htl* double mutants the EMT is significantly delayed [32]. How cell division contributes is not clear, but one possibility is that the cell's cortical tension increases during mitosis due to the RhoGEF Pbl. During cytokinesis, Pbl activates Rho1 at the cytokinetic furrow driving the actin-myosin contraction. In *pbl* mutants, in addition to cytokinesis failure, mesodermal cells are less rounded/dissociated, suggesting that Pbl may also contribute to Rho1-dependent actin-myosin contractility throughout the cortex [34].

Rounding of cells undergoing EMT is also a feature of gastrulating cells in the mouse primitive streak [35] and in the zebrafish neural crest EMT, where a Rho-kinase/myosin II-dependent blebbing behavior has been described [36]. This type of blebbing/ameoboid behavior is also a mode of migration employed by cancer cells and is indicative of high levels of Rho1 contractility [4]. Finally, Rho1-dependent constriction is also a mechanism by which epithelial cells can extrude other cells due to aberrant gene expression, or overcrowding ([37] and see below). Thus activation of Rho contractility pathways may be an important conserved feature of EMT.

Another finding from the cadherin-switching study was that higher levels of E-Cad inhibited activation of the WNT pathway. E-Cad and WNT signaling are intimately linked due to the dual roles of βCatenin (βCat) as a component of the adherens junction (AJ), and as a transcription co-factor for the WNT pathway. Excess E-Cad, therefore, can potentially inhibit WNT pathway activation by acting as a sink for βCat. Thus, part of the function of downregulation of E-Cad may be to potentiate the WNT pathway. The links between the WNT pathway and cancer are extensive [38], and changes in βCat localisation from the cortex to the nucleus, are a common feature of the metastatic tumor-host interface [28].

The second recent insight from this system, which has important implications for metastasis, is that Snail, traditionally thought of as a transcriptional repressor, can also directly bind, and help activate, mesodermal genes in cooperation with Twist, such as *htl* and *N-Cad* [39]. Snail and Twist binding sites were often closely associated, and approximately half of the genes bound by Snail were downregulated in *snail* mutants. In vitro tests showed that although Snail could not activate transcription itself, it potentiated activation by Twist. Intriguingly the authors also identified a binding motif associated with these positive enhancer elements - but the identity of the transcription factor involved is not yet known. In vertebrates, similar examples are emerging in which Snail/Slug factors positively regulate factors that promote EMT. For example Snail can activate transcription of the EMT-inducing transcription factor ZNF281 [40], and WNT target genes independently of its repressor functionality [41], while Slug can activate expression of the EMT factor ZEB1 [42]. In mice, Snai1 activates MMP15, an important factor in invasiveness [43].

Taken together the results suggest that our "canonical" views of the role Snail-family transcription factors in EMT, and the loss E-Cad, may need refinement. In the *Drosophila* mesoderm, levels of E-Cad are not crucial, and Snail clearly plays a broader role. Given the key role that Twist, Snail, and the FGFR and Rho signaling pathways are known to play in cancer, the *Drosophila* mesoderm will remain an important model system providing insight into fundamental mechanisms of metastasis, and no doubt will also continue to challenge established models.

2.2. Endodermal EMT and MET

The second EMT occurs ~ 1.5 hrs later with the formation of the endoderm. The *Drosophila* endoderm (or midgut) forms from two primordia at opposite poles of the embryo, which undergo an EMT, and then migrate towards each other along a cellular substrate, the visceral mesoderm (Figure 1C), and eventually undergo an MET to form the midgut epithelium.

Recently, the molecular basis for the EMT of the posterior midgut rudiment has been described. In this case the EMT does not involve repression of E-Cad, but rather the loss of apico-basal polarity. The driver of the process is the GATA-factor, Serpent, which represses the key apical polarity determinant *crumbs* [44]. This leads to loss of apico-basal polarity and delocalization of E-Cad around the cell membranes of the midgut cells. This mechanism represents a new paradigm for EMT that is likely to be conserved in vertebrates. Loss of apico-basal polarity has been strongly linked to cancer [45,46,47]. In addition, two human orthologs of Serpent, hGATA-4 and hGATA-6, are expressed in gut epithelial tissue [48,49], and, as in *Drosophila*, hGATA-6 transcriptionally represses Crumbs2, but not Epithelial Cadherin. Overexpression of hGATA-6 is also found in cancer cells [50,51,52], and can induce EMT in MDCK cells in vitro [44].

The subsequent migration event is dependent upon integrins, which are expressed by both the migrating midgut cells and the visceral mesodermal cells that they use as a substrate, and upon the integrin ligand Laminin, which lies at the interface between these two tissues [53-56]. Recent results show migration also requires the axon-guidance receptor Frazzled, which is expressed in midgut cells and responds to Netrins emanating from the visceral mesoderm [57]. As in the mesoderm, a feature of the midgut system is the continued expression of E-Cad during migration, with cell-cell spot adherens junctions existing between midgut cells [58]. The expression of E-Cad may be particularly important in the midgut since migration is followed by a MET. Our understanding of the molecular basis for this MET is rudimentary, but the formation of a columnar monolayer is disrupted by loss of either E-Cad or Laminin [59,60]. The Netrin/Frazzled pathway is also important. In embryos lacking netrins, midgut cells fail to form a columnar monolayer and apico-basal markers such as Filamin-1 and E-Cad, and the basally polarised receptor Frazzled, are all mislocalised [57]. Understanding how the Netrin and Integrin molecular pathways interact during both the migration and MET events are important future goals. Since the midgut system represents one of the few well characterized, developmental events in which an EMT is followed by a migration and MET event, it is likely to grow in importance as a model system for metastasis.

2.3. Disc eversion partial-EMT and invasion

The third well-characterized EMT event, which occurs at the onset of metamorphosis, was discovered relatively recently. In 2004, Pastor-Pareja et al. [61] showed that an EMT-like event takes place during the eversion of the wing imaginal disc. This is an important model for metastasis since, unlike the mesoderm and endoderm system, it involves basement membrane (BM) degradation and the invasion of another tissue.

Imaginal discs are epithelial sacs that proliferate and are patterned during larval development, and finally, during metamorphosis, evert, migrate, and fuse with other discs to eventually generate a continuous adult epidermis. During eversion the peripodial epithelial cells (Figure 1E) undergo a partial EMT in which their AJs are disrupted, and the cells become motile and invasive. They break

through the BM lying between them and the epidermis, invade the epidermal layer, and then undergo an epithelial sheet migration over the epidermis. The molecular control of this differs again from the mesodermal and endodermal EMTs. It is mediated by two pathways that appear to act in parallel: the Jun-kinase (JNK) pathway and a novel EMT pathway involving the axonal chemo-attractant Netrin.

The JNK pathway has long been associated with the processes of eversion and thorax closure and affects all aspects of the process [61,62]. The JNK pathway is activated downstream of Src family kinases [63] and the PVR receptor which appears to act through Crk, Mbc, ELMO, Rac and Cdc42 [64]. JNK activation leads to delocalization of cell-cell junction proteins, activation of cell motility and expression of the matrix metalloproteinases, MMP1 and MMP2, which degrade the BM [61,65]. Following the epithelial dissociation and BM breakdown, peripodial cells must invade the epidermis and undergo an epithelial sheet migration event. This process involves the JNK and TGFβ pathways [62] and is likely to also involve Integrins since integrins are known to be required for the closely related process of embryonic dorsal closure [66], and RNAi knockdown of the αPS3 subunit *scab* generates thoracic clefts (R. Manhire-Heath and M. J. Murray, unpublished observations). In flies, activation of the JNK pathway appears to be a central feature of epithelial cells becoming invasive, both during normal events like wing eversion, and in situations where genetic changes induce loss of epithelial integrity (see below). There are also extensive links between JNK activation and metastasis in humans [67].

In a recently discovered pathway, which appears to act in parallel, expression of the axonal chemo-attractant NetA promotes ZA breakdown in the peripodial epithelium, via degradation of its receptor Frazzled (Fra) [68]. Fra plays an opposing role in this process. Overexpression of *fra* phenocopies eversion defects associated with loss of *netA,* while *fra*-RNAi can rescue *netA*-RNAi eversion failure, and can accelerate epithelial dissociation in vitro. Exactly how Fra opposes the dissociation is not clear but it appears to act through the ERM family protein Moesin (Moe). Moe becomes phosphorylated in response to increased Fra levels and is required for *netA*-RNAi eversion failure. *fra* and *moe* have both previously been shown to be required for epithelial integrity (see below) [69,70].

This finding that Netrins can regulate an EMT-like event is of interest since vertebrate Netrins and their receptors are already strongly associated with metastasis. In vertebrates there are two orthologs to Fra, Deleted in Colorectal Carcinoma (DCC) and Neogenin1. DCC can suppress metastasis by acting as a "dependence receptor" for its trophic ligand Netrin-1 [71]. Similarly, overexpression of Netrin-1, which can protect cells from apoptosis, has been observed in metastatic breast cancers [72] and ovarian cancers [73], and can promote the formation of adenocarcinomas in the mouse gut [74]. Netrin-1 and DCC can also regulate cell migration and epithelial plasticity in development [75], and Netrin-1 can increase the invasiveness of cancer cells in vivo and in vitro [76,77,78], and has been implicated in an EMT-like event in mice [79]. Conversely, DCC can inhibit an induced EMT in vitro apparently by promoting cell adhesion via ERM-M family proteins [80], and can also inhibit the invasiveness of cancer cells [78]. Finally, given the role of Fra in inhibiting epithelial dissociation and promoting the midgut MET it is worth noting that Neogenin is required to maintain cell polarity and epithelial structure in the neural tube [81].

An important question for the future is how the JNK and Netrin pathways converge on bringing about the EMT.

2.4. Border cell delamination

The fourth developmental event occurs in the developing egg-chamber. Here, a small cluster of cells delaminate from the follicular epithelium and undergo a unique, stereotyped, collective migration towards the oocyte (Figure 1G). This system, with its accessibility to live imaging, and ability to precisely manipulate gene expression within the cluster, has provided a wealth of information concerning both the delamination and collective migration. Since these events have recently been comprehensively reviewed [12] the description here will be brief, and focused on the delamination event.

The process begins when two follicle cells at the anterior pole of the egg chamber become specified as "polar cells" and secrete the cytokine Unpaired (Upd), an ortholog to vertebrate Interleukin-6. Neighboring follicle cells receive this signal through the Domeless receptor (a gp130-like cytokine receptor) leading to activation of a JAK/STAT pathway and expression of several target genes. Of these, one of the most important is Slow Border Cells (Slbo), an ortholog of vertebrate C/EBP. Slbo is essential for the delamination and subsequent migration of the border cell cluster.

At the time of delamination the border cell cluster extend a long protrusion in the direction of future movement, called the "long cellular extension" (LCE), which is dependent upon the receipt of the graded directional cue of the growth factors, and upon the Cadherin-based adhesion between the LCE and the nurse cells through which the cluster migrates [82]. The delamination of the cluster is an active process that requires actin-myosin contractility, with Par-1 promoting myosin II contractility in the rear of the border cell cluster by phosphorylating and inhibiting Myosin Phosphatase [82,83].

The delamination process also involves the Notch pathway. Notch activation is refined to the border cells at the time of delamination, and is required for their migration [84], but also for the ability of border cells to detach from the follicular epithelium [85]. The precise timing of delamination is controlled by the activation of the Ecdysone steroid hormone pathway via EcR and Ultraspiracle, together with their co-activator Taiman. An initial imbalance in Ecd signaling in border cells becomes amplified by feedback between the JAK/STAT and EcR pathways through the BTB protein Abrupt, eventually leading to the detachment of the cluster. See reference [12] for details.

Each of these molecular factors controlling border cell delamination is implicated in human metastasis. Firstly, the closest ortholog of Upd, Interleukin-6, is strongly associated with metastasis, as is the JAK/STAT pathway [86,87]. The vertebrate orthologue of Slbo, C/EBP, is elevated in carcinoma, and promotes VEGF3 and VEGFR3 expression, which increase lymphangiogenesis and pulmonary metastases [88]. Interestingly, in this system there is also an intimate link between C/EBP-d and hypoxia, which may be partly conserved in flies. Hypoxia induces C/EBP-d expression and C/EBP-d regulates HIF-1a expression. Blocking HIF-1α activity blocked CEBP-d-induced VEGF-C. In flies, the migration of the border cells is responsive to both the levels of oxygen, and levels of the *Drosophila* hypoxia-inducible factor HIF1α/Sim. Moreover, cells mutant for HIF1α have reduced levels of Slbo [89]. The Tai orthologue in humans AIB1 (Amplified In Breast cancer 1) is also amplified in several cancers [90]. Finally, although the mechanism by which Notch promotes border cell delamination is not known, in vertebrates Notch signaling can repress matrix adhesion genes [91].

Thus, the border cell delamination event is again complementary to the other three scenarios in

that it involves a different set of genetic regulators, all of which are important in understanding human metastasis.

3. Genetically induced models of metastasis

While normal developmental EMTs provide great insight into metastatic processes, one can also induce metastatic behavior via genetic manipulations. The ability to generate cells with complex genotypes in a normal, in vivo environment, and track those cells with live markers like GFP is an extremely powerful tool for uncovering basic principles underlying metastasis. The fact that the experiments are done in vivo is very important since the response of such cells is dependent upon the microenvironment that surrounds them, and can be modulated by system wide responses such as the innate immune system.

3.1. Cell extrusion and the JNK pathway

Although epithelia are sometimes thought of as stationary cells in a relatively inert 2D sheet, live imaging shows us that epithelial cells are quite dynamic and undergo rapid remodeling of their connections with neighboring cells. Epithelial cells must maintain homeostasis, so mechanisms exist by which aberrant cells can be removed when necessary. In terms of metastasis, one of the most common ways that an epithelial cell can lose its place in an epithelium is by loss of polarity. However, there are many other examples in *Drosophila*, where cells that are genetically different from their neighbors are expelled from the main epithelium. For example, expression of oncogenic Ras^{V12} [92], loss of the Polycomb Group gene *polyhomeotic* [93], and loss of Dpp signaling [94] all lead to groups of epithelial cells detaching from the primary epithelium to form cysts (Figure 2B). In fact, mechanisms exist to extrude even wild type cells if overcrowded [95].

In epithelial cells, polarity is actively maintained by a number of complexes that define membrane compartments in the apical-basal axis via mutually antagonistic interactions [96]. An important complex in the discussions to follow is the basolateral Lgl/Dlgl/Scrib, complex which is essential for epithelial integrity [97]. Mutant clones for genes like *scrib* lose their columnar, mono-layered arrangement and become multilayered and rounded [98]. Crucially, they also activate the JNK pathway which leads to apoptosis and removal from the tissue. Elimination of cells also involves activation of a non-apoptotic JNK response in neighboring cells involving the Pvr/ELMO-Mbc pathway, which leads to phagocytic engulfment [99]. JNK activation leads not only to apoptosis, however, but to invasive behavior through expression of target genes such as *mmp1* [65,100], which mediates BM degradation, and the actin binding proteins, *cheerio* (i.e. Filamin-1) [101] and *chickadee* (Profilin) [102], which promote motility.

In another example where loss of polarity led to JNK activation and apoptosis, Dekanty et al. [103] looked at the effects of inducing chromosomal instability (CIN) through manipulation of several cell cycle related genes. As with *scrib* mutant cells, CIN cells lost apico-basal polarity, delaminated from the epithelium and activated the JNK pathway leading to apoptosis and upregulation of MMP1 and Wg. How CIN resulted in loss of polarity was not explored but it was speculated that general stress associated with aneuploidy-induced protein imbalances might drive this process.

Similar results have been shown for three other genes: *moesin (moe)*, the sole *Drosophila* ERM (ezrin/radixin/moesin) gene [69], *slik*, the Sterile-20 kinase [104], and *fra* [70]. Loss of *moe* disrupts

epithelial integrity leading to mutant cells delaminating from the wing disc epithelium and becoming invasive (Figure 2C) [69]. This process is strongly inhibited by reduction of Rho1, and can be phenocopied by activated Rho1, suggesting that Moe's primary role in epithelia is to inhibit Rho1. A likely mechanism for this has recently been reported in which Moesin recruits the RhoGAP Conundrum to the plasma membrane [105]. The Sterile-20 kinase Slik appears to act upstream of Moesin in maintaining epithelial integrity [104]. *slik* mutant cells exhibit the same types of phenotypes as *moe* mutants such as loss of polarity, delamination, apoptosis and invasive behavior. Slik was shown to phosphorylate Moesin, and *slik* phenotypes could be greatly rescued by either expression of an active form of Moesin, or by loss of Rho1, consistent with the results for *moe*. Similarly, *fra* mutant clones become invasive and migratory, activate ERK and JNK pathways and MMP1 expression, and invasion can be suppressed by dominant negative Rho^{N19} [70].

How then might Rho1 activity lead cells to leave the epithelium? As previously mentioned several developmental EMT events involve cells rounding up in a Rho1 dependent manner, but another possibility is via the JNK pathway. It has been known for some time that several Rho-family GTPases can activate the JNK pathway, including Rac1 and Cdc42, and Rho1 [106,107]. In the case of Rho1, two potential mechanisms have been reported. Firstly, it has been shown that Rho1 can directly interact with the JNK Kinase Kinase Slipper, and, consistent with this, increases in cortical Rho1 associated with loss of *moesin*, can recruit Slipper to the membrane [108]. An alternative pathway involving a RhoGEF2/Rho1/Rok/myosin II pathway has also been reported, though the mechanism by which myosin II leads to JNK activation remains to be established [109].

Another pathway to JNK activation and invasion is through the Src family kinases (SFKs). For example when Csk, a negative regulator of Src, is knocked down by expression of *Csk-IR* (i.e. an inverted repeat construct) delamination occurs (Figure 2E). *Csk-IR* cells delaminate and undergo JNK-dependent migratory behavior, BM breakdown and apoptosis [110]. As with other systems the JNK activation, invasion and migration is dependent upon Rho1. Invasion is also dependent upon MMP1, and p120ctn, a well-known SFK target. Surprisingly, invasion is inhibited by reduction in E-Cad, suggesting that delamination is an active process that involves AJ adhesion and remodelling. Rudrapatna et al. [111] have also found that the Src activation of JNK is via Rho1 and, is dependent upon the Actin Regulatory Proteins Cofilin and Enabled. Thus, cytoskeletal regulators appear not only to act downstream of JNK during invasion but, like myosin II, are able to activate the JNK pathway as well, though again the mechanism is not clear.

SFKs may not always act through Rho1 however. Expression of the oncogene Abelson kinase, in the wing stripe assay causes a similar phenotype to *Csk*-RNAi, with cell delamination, activation of JNK and expression of MMP1 [112]. Abl appears to act downstream of SFKs, since the effects of *Csk*-RNAi are inhibited by *Abl*-RNAi. Abl could also activate SFKs indicating the potential for a positive feedback loop. However, in contrast to Rudrapatna et al. [111] the downstream effects of Abl, including JNK activation, were dependent, not upon Rho1, but on the three Rac GTPases, suggesting multiple pathways from SFKs to JNK activation exist.

A potential upstream regulator of *Csk* has also been reported. Knockdown of the Histone Deacetylase, Sin3a causes excessive delamination, upregulation of N-Cad, MMP1, and degradation of the BM, particularly when combined with overexpression of the oncogene Ret^{MEN2} [113]. Using Histone Deacetylation marks, CHIP-seq and RTPCR the authors found Sin3A, positively regulates *Csk* (and interestingly, *fra*) while negatively regulating *RhoGEF2* and *Rho1*, consistent with their respective roles in regulating epithelial stability.

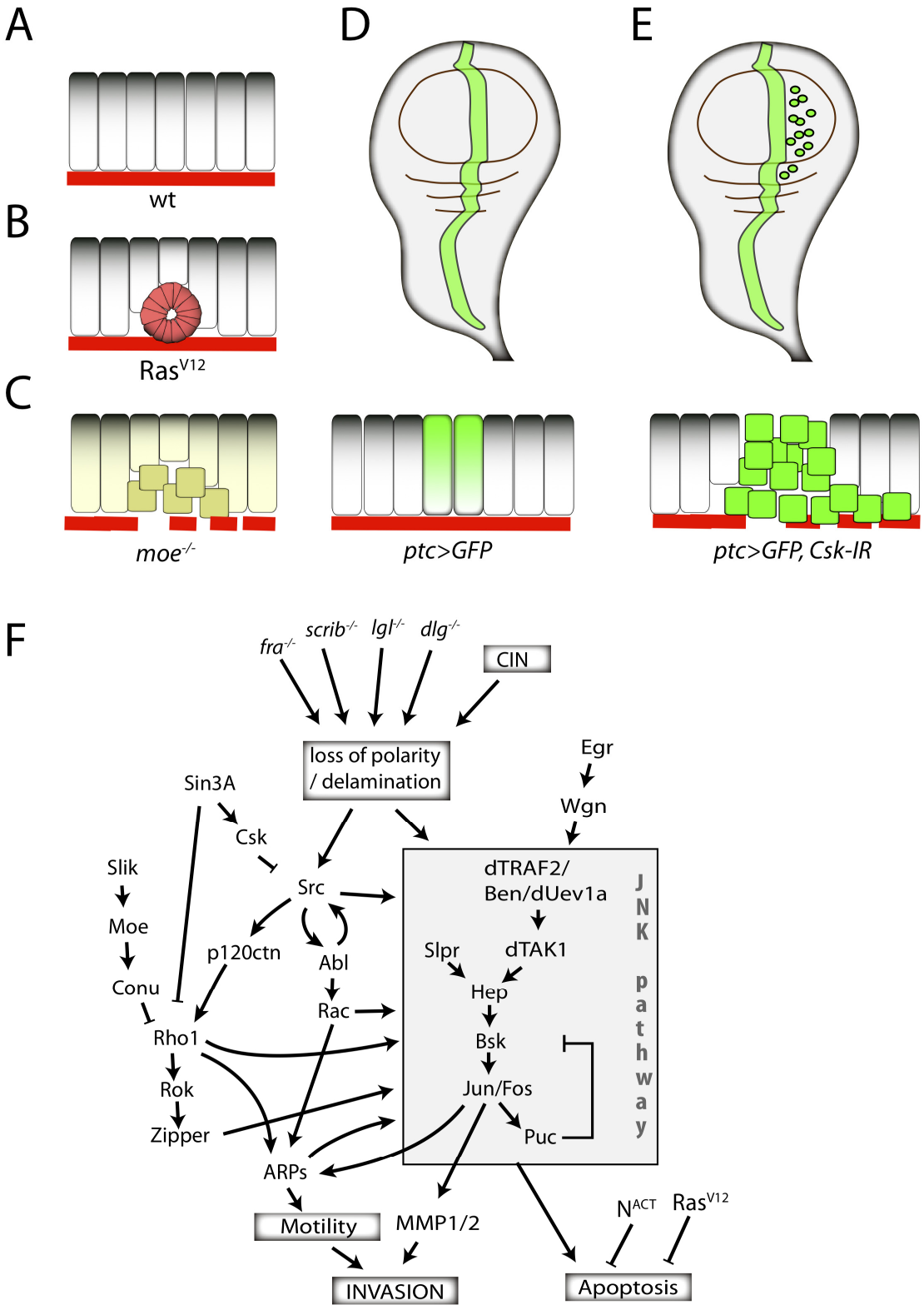

Figure 2. Genetic perturbations leading to epithelial delamination and invasion. (A) Normal wild type epithelium. (B) Clones of cells that are genetically different from surrounding normal cells can be extruded to form epithelial cysts (red). (C) In certain genetic backgrounds such as *moesin*$^{-/-}$ some cells delaminate, lose polarity

and are extruded basally, leading to basement membrane breakdown and lateral movement. (D) The stripe assay whereby a GAL4 driver such as patched (ptc), which has a sharply demarcated edge is used to express a marker such as GFP and other transgenes. (E) Expression of the inverted repeat RNAi construct Csk-IR causes cells to lose polarity and delaminate basally, leading to basement membrane breakdown and migration into the posterior compartment. (F) Hypothetical composite of the many genetic regulatory pathways known to regulate aberrant loss of epithelial stability and invasion. Activation of JNK occurs downstream of many of the molecular perturbations in this review such as loss of polarity regulators like scrib, Src activation, RhoGTPases Rac and Rho1 and Actin Regulatory Proteins (ARPs). Some of the arrows leading to the JNK pathway may be redundant. For example it may be the JNK activation in response to loss of *scrib* is always via Src kinases. Similarly the mechanism by which the RhoGTPase Rac activates JNK may involve ARPs, given the well-known role of Rac1 in regulating of F-Actin.

3.2. Cooperativity of loss of polarity with oncogenes and cell survival

Thus the response of cells to various stresses and loss of polarity is the activation of the JNK pathway, which promotes both apoptosis and invasive behaviors. Consequently, when apoptosis is prevented by expression of oncogenes the result is highly metastatic tumors.

For example, Brumby et al. [114] found that while $scrib^{-/-}$ clones underwent JNK-dependent apoptosis (Figure 3D), co-expression of the activated oncogenes, $Notch^{ACT}$ or Ras^{V12}, resulted in massive overgrowth and fusion of the eye-antennal discs with each other and with neighboring tissue, suggesting invasive behavior (Figure 3F). Further screening for genetic interactors in this process determined that the activation of JNK was likely to be via a RhoGEF pathway [98].

Similarly, Pagliarini et al. [115] induced Ras^{V12} tumors in the eye-antennal disc, and then screened for mutations that could mediate metastatic transformation. Loss of *scrib* created highly metastatic tumors that induced BM breakdown and migrated down the nerve cord, exhibiting protrusive leading edges (Figure 3B). Similar results could be obtained with other polarity genes such as *lgl*, *dlg*, *sdt*, *baz*, and *cdc42*, and in each case the invasive behavior was due to JNK activation [116].

Further screening identified *Src42A* as an essential mediator acting downstream of the $Ras^{V12}/lgl^{-/-}$ tumor cascade. Src42A-RNAi could block invasion and JNK activation of $Ras^{V12}/lgl^{-/-}$ tumors, and the posterior delamination/migration of *ptc* > *lgl-IR* cells in the wing disc [117]. In this case, Src42A has been shown to act through the Bendless/dUEV1a ubiquitin-conjugating E2 enzyme complex and the E3 ligase dTRAF2, in activating JNK. How the Rho GTPases and ARP pathways interact with these upstream components of the JNK pathway is an important question for future studies to address.

Similar results are obtained when apoptosis is inhibited by other methods. In the previous example of CIN cells [103], expression of the caspase-inhibitor p35, led to tumor cells that when injected into host abdomens could metastasize to ovaries (Figure 3C). Similarly, Rudrapatna et al. [118] showed that by precisely controlling the level of caspase activity one could prevent JNK-dependent apoptosis, while still allowing the JNK invasive pathway. This result may explain why human cancer progression is often associated with increased levels of effector caspases [119].

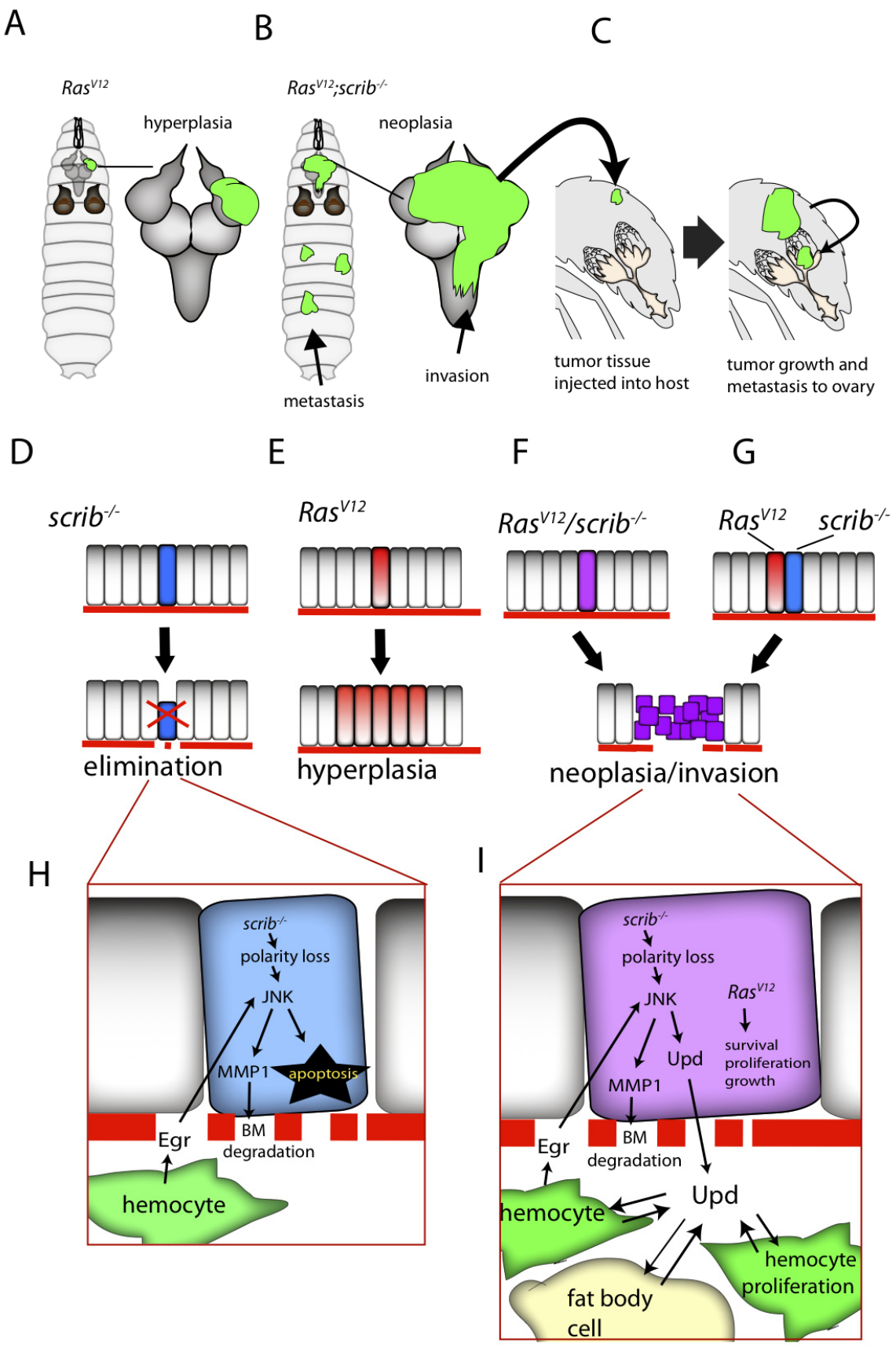

Figure 3. Mechanisms of metastasis in *Drosophila*. (A) Expression of the oncogene

Ras^{V12} in clones (which are also marked with GFP) causes massive proliferation without loss of epithelial structure. i.e. hyperplasia (B) Combining Ras^{V12} expression with loss of a polarity regulator, such as *gl* or *scrib*, leads to massive overgrowth and loss of epithelial structure (i.e. neoplasia). In addition, tumor cells can invade and migrate down the nerve cord and also metastasise to other positions within the larval body. (C) The allograft technique involves injecting fragments of tumorous tissue into the abdomen of an adult female host. Tumor cells continue to proliferate and can metastasise to the ovaries. This is an invasive event since ovarioles are ensheathed in a layer of muscles covered on both sides by basement membrane. (D) $scrib^{-/-}$ cells lose epithelial integrity and activate the JNK pathway leading to basement membrane degradation, but are then eliminated via apoptosis and engulfment (not shown). (E) Ras^{V12} expressing clones overproliferate but maintain epithelial structure. (F) Combined expression of Ras^{V12} and loss of *scrib* creates neoplastic tumors that massively overgrow and become metastatic. (G) Clonal cooperation. Activation of the JNK pathway in $scrib^{-/-}$ cells can propagate to the neighbouring cells expressing Ras^{V12}, again causing neoplasia. (H) Signaling associated with elimination of $scrib^{-/-}$ cells. Basement membrane degradation attracts hemocytes, which express the TNF ortholog, Eiger (Egr). This activates the JNK pathway leading to apoptosis. (I) When Ras^{V12} is also expressed cells avoid apoptosis and massively overgrow. JNK pathway activation leads to expression of the cytokine Unpaired (Upd). This causes proliferation of hemocytes, which, together with fat body cells, express Upd, creating a systemic increase in Upd that facilitates tumor growth and metastasis.

3.3. Non-cell autonomous effects on metastatic behavior

Strikingly, it has also been shown that loss of *scrib* does not actually need to be in the same cells that are overexpressing the oncogene Ras^{V12}. When clones of $scrib^{-/-}$ cells are adjacent to Ras^{V12} expressing cells the same invasive tumors result (Figure 3G) [120]. The mechanism involves JNK pathway activation, which occurs in $scrib^{-/-}$ cells, propagating to the Ras^{V12} cells, via some as yet unknown mechanism. This study also highlighted the importance of the cytokine Upd. JNK activation in both the $scrib^{-/-}$ cells and the Ras^{V12} cells increased expression of *Upd* and this was necessary for invasion. This study also placed the JAK/STAT pathway downstream of JNK, in that Bsk^{DN} was able to prevent $Ras^{V12}/scrib^{-/-}$ tumors from invading, but not Ras^{V12}/Upd tumors.

A separate study has confirmed the synergistic interaction between Ras and JAK/STAT pathways. Herranz et al. [121] found that when the EGFR receptor, which acts upstream of Ras, is overexpressed, coexpression of the miRNA bantam produced massive neoplastic tumors with loss of apico-basal polarity, and upregulation of mmp1 and snail. Further study determined that bantam was repressing the JAK/STAT repressor Socs36E, and knocking down *socs36E* in the EGFR overexpression background also produced neoplastic, metastatic tumors.

3.4. Metastasis and the immune system

Drosophila is also helping us understand the complex relationship between tumor cells and

inflammation. Tumor-associated inflammation has long been known to both facilitate and inhibit tumor growth, invasion and metastasis [122,123,124]. In *Drosophila,* the cellular part of the innate immune system consists blood cells called hemocytes, which circulate in the hemolymph that bathes the larval organs. Recent work shows that hemocytes are attracted to sites of tissue damage, and to the $scrib^{-/-}$ and $Ras^{V12}/scrib^{-/-}$ tumors due to the BM breakdown on their basal surface, and can inhibit the growth of $scrib^{-/-}$ tumors (Figure 3H) [125]. Furthermore, $Ras^{V12}/scrib^{-/-}$ tumors were shown to upregulate the cytokines Upd, Upd2 and Upd3, which in turn led to increased JAK/STAT signaling and Upd3 expression in hemocytes and the fat body cells, creating a system wide increase in cytokines, and in the number of hemocytes (Figure 3I) [125]. Cordero et al. [126] have since shown that Tumor-Associated Hemocytes (TAHs) express Eiger, the sole *Drosophila* ortholog of Tumor Necrosis Factor (TNF), and this is necessary for the removal of $lgl^{-/-}$ tumors. In the case of the $Ras^{V12}/scrib^{-/-}$ tumors, however, the activation of JNK led to invasiveness—providing a clear example in *Drosophila* of the immune system promoting metastatic behavior.

3.5. Invasive behavior through expression of other oncogenes

The effects of several other oncogenes that are often upregulated in cancers have also been tested by overexpression in imaginal disc tissue. For example, overexpression of the transcription factors TBX2 and TBX3 is a common feature of several human cancers. Shen et al., [127] found that overexpression of the *Drosophila* ortholog, *optomotor-blind (omb),* or indeed the human gene TBX2, caused downregulation of E-Cad, induction of cell motility, which allowed cells to disperse through the wing disc epithelium—without disruption of the BM. The centrosomal kinase, Nek2 is also highly expressed in cancer and given its role in mitosis, had been thought to contribute to late stage cancers through promoting CIN. Studies of the fly Nek2, however, suggest an earlier role [128]. Nek2 cooperates with the oncogenic RTK Ret^{MEN2B} in promoting dissemination in the wing stripe assay**,** and increases dissemination of $Ras^{V12}/Csk^{-/-}$ tumor cells in the eye-antennal discs. Also, when mutant tissue is injected into the notum of an adult fly, distant metastatic tumors are seen in other parts of the animal in the case of $Nek2/Ras^{V12}/Csk^{-/-}$ cells but not for *Nek2* or $Ras^{V12}/Csk^{-/-}$ cells [128].

Finally activation of the Notch pathway, in conjunction with other factors, such as loss of *scrib*, expression of the epigenetic regulators Lola and Pipsqueak, Atonal, Mef2 and others, can be a potent trigger for metastasis. For a recent and extensive review on Notch pathways to metastasis in flies see [129].

4. Future directions

What might the future hold for *Drosophila* research into metastasis? Analysis of normal developmental EMT/MET events will no doubt continue since there clearly remains much to be discovered. For example, although the mesodermal EMT was first described over 25 years ago, the molecular factors that drive the loss of epithelial structure are still not clear. In the case of the endoderm, the molecular mechanisms acting downstream of Integrins and Frazzled, to drive the MET, remain unexplored.

In the case of induced metastatic behavior, future research avenues are really limited only by the imagination, and will undoubtedly grow in complexity and sophistication. For example, the ability to generate sister cells with different genotypes, as was done for Ras^{V12} and $scrib^{-/-}$ [120], should allow further exploration of models in which a mix of cell types (e.g. "EMT" and "non-EMT" cells)

promote metastasis [7]. Methods for manipulating and assaying gene expression will continue to grow, as each year brings powerful new tools such as CrispR [130], genome-wide libraries of *GAL4* drivers [131], and methods for in vivo transcriptional profiling [132].

One aspect of metastasis that has yet to receive much attention is the way disseminated cells can grow into a secondary tumor. Given the importance of MET to metastasis, of particular interest will be those that exhibit some epithelial characteristics. While tumors caused by loss of polarity factors like *scrib* would not be expected to show any epithelial structure [97] others might. For example, ectopic expression of Delta in the eye generates secondary growths that appear structured and contain differentiated cell types [133]. Since such tumors can apparently attract tracheal branches [129], they may also provide a useful model for tumor neovascularisation. Indeed, the mechanisms of morphogenesis in the *Drosophila* tracheal system are already providing key insights into tumor angiogenesis [18]. Fundamental principles, such as Notch-based selection of leader cells, and partial EMT in response to FGF ligands, closely parallel the mechanisms of branching of blood vessels.

Another likely growth area will be testing the effect of therapeutic drugs on metastasis. The combination of drug treatments and genetic analysis in a whole-animal context is particularly powerful. For example, Willoughby et al. [134] used the $Ras^{V12}/scrib^{-/-}$ model to test the bioavailability, efficacy and toxicity of drugs such as MEK inhibitors to inhibit metastasis and tumor growth. In a screen of 2000 compounds the glutamine analogue, acivicin, was shown to reduce tumor overgrowth, and subsequent genetic analysis revealed CTP synthase to be its likely target. Similarly, in the MEN2 model in which the activated Ret receptor is expressed, researchers showed that the drug Vandetanib had high efficacy but low toxicity [135]. In subsequent experiments the efficacy and toxicity of various Ret-inhibitors was compared, and the importance of coordinated inhibition of other signaling pathways such as Src and Raf was demonstrated [136]. Flies may even become a standard workhorse in personalized medicine. The ability to firstly model a primary cancer-causing oncogene, and then analyse the efficacy of drugs and the consequences of secondary mutations may provide a way of identifying functionally important genomic changes identified by genomic sequencing of tumors [137].

5. Conclusion

For those that do not work with flies it may not be obvious what they have to tell us about human processes such as metastasis. However, as the examples in this review demonstrate, there exists an extraordinary level of conservation, both of the molecular pathways, and of the cellular processes that they control. This, together with the extensive array of genetic tools, means that studies in the fly can elucidate important underlying principles that are directly relevant to human cancer. While one will always be able to find differences between flies and humans, recognizing and exploiting the many similarities, can bring substantial rewards.

Acknowledgements

I apologize to all the colleagues whose studies could not be covered due to space limitations and thank S. Golenkina and H. Weerasinghe for critical reading of the manuscript, and the reviewers for helpful comments and suggestions.

Conflict of Interest

Author declares no conflicts of interest in this paper.

References

1. Thiery JP, Acloque H, Huang RY, et al. (2009) Epithelial-mesenchymal transitions in development and disease. *Cell* 139: 871-890.
2. Chaffer CL, Thompson EW, Williams ED (2007) Mesenchymal to Epithelial Transition in Development and Disease. *Cells Tissues Organs* 185: 7-19.
3. Yao D, Dai C, Peng S (2011) Mechanism of the Mesenchymal-Epithelial Transition and Its Relationship with Metastatic Tumor Formation. *Mol Cancer Res* 9: 1608-1620.
4. Friedl P, Wolf K (2003) Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat Rev Cancer* 3: 362-374.
5. Calbo J, van Montfort E, Proost N, et al. (2011) A functional role for tumor cell heterogeneity in a mouse model of small cell lung cancer. *Cancer Cell* 19: 244-256.
6. Mateo F, Meca-Cortes O, Celia-Terrassa T, et al. (2014) SPARC mediates metastatic cooperation between CSC and non-CSC prostate cancer cell subpopulations. *Mol Cancer* 13: 237.
7. Tsuji T, Ibaragi S, Hu GF (2009) Epithelial-mesenchymal transition and cell cooperativity in metastasis. *Cancer Res* 69: 7135-7139.
8. Nakashima Y, Yoshinaga K, Kitao H, et al. (2013) Podoplanin is expressed at the invasive front of esophageal squamous cell carcinomas and is involved in collective cell invasion. *Cancer Sci* 104: 1718-1725.
9. Wicki A, Lehembre F, Wick N, et al. (2006) Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. *Cancer Cell* 9: 261-272.
10. Nieto MA (2013) Epithelial plasticity: a common theme in embryonic and cancer cells. *Science* 342: 1234850.
11. Montell DJ (2006) The social lives of migrating cells in Drosophila. *Curr Opin Genet Dev* 16: 374-383.
12. Montell DJ, Yoon WH, Starz-Gaiano M (2012) Group choreography: mechanisms orchestrating the collective movement of border cells. *Nat Rev Mol Cell Biol* 13: 631-645.
13. Pocha SM, Montell DJ (2014) Cellular and Molecular Mechanisms of Single and Collective Cell Migrations in Drosophila: Themes and Variations. *Annu Rev Genet* 48: 295-318.
14. Ribeiro C, Petit V, Affolter M (2003) Signaling systems, guided cell migration, and organogenesis: insights from genetic studies in Drosophila. *Dev Biol* 260: 1-8.
15. Wilson R, Leptin M (2000) Fibroblast growth factor receptor-dependent morphogenesis of the Drosophila mesoderm. *Philos Trans R Soc Lond B Biol Sci* 355: 891-895.
16. Winklbauer R, Muller HA (2011) Mesoderm layer formation in Xenopus and Drosophila gastrulation. *Phys Biol* 8: 045001.
17. Nieto MA (2011) The ins and outs of the epithelial to mesenchymal transition in health and disease. *Annu Rev Cell Dev Biol* 27: 347-376.

18. Affolter M, Caussinus E (2008) Tracheal branching morphogenesis in Drosophila: new insights into cell behaviour and organ architecture. *Development* 135: 2055-2064.
19. Harris TJ, Sawyer JK, Peifer M (2009) How the cytoskeleton helps build the embryonic body plan: models of morphogenesis from Drosophila. *Curr Top Dev Biol* 89: 55-85.
20. Simpson P (1983) Maternal-Zygotic Gene Interactions during Formation of the Dorsoventral Pattern in Drosophila Embryos. *Genetics* 105: 615-632.
21. Khan MA, Chen HC, Zhang D, et al. (2013) Twist: a molecular target in cancer therapeutics. *Tumour Biol* 34: 2497-2506.
22. Wang Y, Shi J, Chai K, et al. (2013) The Role of Snail in EMT and Tumorigenesis. *Curr Cancer Drug Targets* 13: 963-972.
23. Sandmann T, Girardot C, Brehme M, et al. (2007) A core transcriptional network for early mesoderm development in Drosophila melanogaster. *Genes Dev* 21: 436-449.
24. Leptin M (2005) Gastrulation movements: the logic and the nuts and bolts. *Dev Cell* 8: 305-320.
25. Manning AJ, Rogers SL (2014) The Fog signaling pathway: insights into signaling in morphogenesis. *Dev Biol* 394: 6-14.
26. Murray MJ, Southall TD, Liu W, et al. (2012) Snail-dependent repression of the RhoGEF pebble is required for gastrulation consistency in Drosophila melanogaster. *Dev Genes Evol* 222: 361-368.
27. Wheelock MJ, Shintani Y, Maeda M, et al. (2008) Cadherin switching. *J Cell Sci* 121: 727-735.
28. Jeanes A, Gottardi CJ, Yap AS (2008) Cadherins and cancer: how does cadherin dysfunction promote tumor progression? *Oncogene* 27: 6920-6929.
29. Mariotti A, Perotti A, Sessa C, et al. (2007) N-cadherin as a therapeutic target in cancer. *Expert Opin Investig Drugs* 16: 451-465.
30. Oda H, Tsukita S, Takeichi M (1998) Dynamic behavior of the cadherin-based cell-cell adhesion system during Drosophila gastrulation. *Dev Biol* 203: 435-450.
31. Schafer G, Narasimha M, Vogelsang E, et al. (2014) Cadherin switching during the formation and differentiation of the Drosophila mesoderm - implications for epithelial-to-mesenchymal transitions. *J Cell Sci* 127: 1511-1522.
32. Clark IB, Muha V, Klingseisen A, et al. (2011) Fibroblast growth factor signalling controls successive cell behaviours during mesoderm layer formation in Drosophila. *Development* 138: 2705-2715.
33. Murray MJ, Saint R (2007) Photoactivatable GFP resolves Drosophila mesoderm migration behaviour. *Development* 134: 3975-3983.
34. Smallhorn M, Murray MJ, Saint R (2004) The epithelial-mesenchymal transition of the Drosophila mesoderm requires the Rho GTP exchange factor Pebble. *Development* 131: 2641-2651.
35. Williams M, Burdsal C, Periasamy A, et al. (2012) Mouse primitive streak forms in situ by initiation of epithelial to mesenchymal transition without migration of a cell population. *Dev Dyn* 241: 270-283.
36. Berndt JD, Clay MR, Langenberg T, et al. (2008) Rho-kinase and myosin II affect dynamic neural crest cell behaviors during epithelial to mesenchymal transition in vivo. *Dev Biol* 324: 236-244.
37. Eisenhoffer GT, Rosenblatt J (2013) Bringing balance by force: live cell extrusion controls epithelial cell numbers. *Trends Cell Biol* 23: 185-192.

38. Polakis P (2012) Wnt signaling in cancer. *Cold Spring Harb Perspect Biol* 4.
39. Rembold M, Ciglar L, Yanez-Cuna JO, et al. (2014) A conserved role for Snail as a potentiator of active transcription. *Genes Dev* 28: 167-181.
40. Hahn S, Jackstadt R, Siemens H, et al. (2013) SNAIL and miR-34a feed-forward regulation of ZNF281/ZBP99 promotes epithelial-mesenchymal transition. *EMBO J* 32: 3079-3095.
41. Stemmer V, de Craene B, Berx G, et al. (2008) Snail promotes Wnt target gene expression and interacts with beta-catenin. *Oncogene* 27: 5075-5080.
42. Wels C, Joshi S, Koefinger P, et al. (2011) Transcriptional activation of ZEB1 by Slug leads to cooperative regulation of the epithelial-mesenchymal transition-like phenotype in melanoma. *J Invest Dermatol* 131: 1877-1885.
43. Tao G, Levay AK, Gridley T, et al. (2011) Mmp15 is a direct target of Snai1 during endothelial to mesenchymal transformation and endocardial cushion development. *Dev Biol* 359: 209-221.
44. Campbell K, Whissell G, Franch-Marro X, et al. (2011) Specific GATA factors act as conserved inducers of an endodermal-EMT. *Dev Cell* 21: 1051-1061.
45. Godde NJ, Pearson HB, Smith LK, et al. (2014) Dissecting the role of polarity regulators in cancer through the use of mouse models. *Exp Cell Res* 328: 249-257.
46. Humbert PO, Grzeschik NA, Brumby AM, et al. (2008) Control of tumourigenesis by the Scribble/Dlg/Lgl polarity module. *Oncogene* 27: 6888-6907.
47. Khursheed M, Bashyam MD (2014) Apico-basal polarity complex and cancer. *J Biosci* 39: 145-155.
48. Gao X, Sedgwick T, Shi YB, et al. (1998) Distinct functions are implicated for the GATA-4, -5, and -6 transcription factors in the regulation of intestine epithelial cell differentiation. *Mol Cell Biol* 18: 2901-2911.
49. Molkentin JD, Antos C, Mercer B, et al. (2000) Direct activation of a GATA6 cardiac enhancer by Nkx2.5: evidence for a reinforcing regulatory network of Nkx2.5 and GATA transcription factors in the developing heart. *Dev Biol* 217: 301-309.
50. Fu B, Luo M, Lakkur S, et al. (2008) Frequent genomic copy number gain and overexpression of GATA-6 in pancreatic carcinoma. *Cancer Biol Ther* 7: 1593-1601.
51. Kwei KA, Bashyam MD, Kao J, et al. (2008) Genomic profiling identifies GATA6 as a candidate oncogene amplified in pancreatobiliary cancer. *PLoS Genet* 4: e1000081.
52. Shureiqi I, Zuo X, Broaddus R, et al. (2007) The transcription factor GATA-6 is overexpressed in vivo and contributes to silencing 15-LOX-1 in vitro in human colon cancer. *FASEB J* 21: 743-753.
53. Bokel C, Brown NH (2002) Integrins in development: moving on, responding to, and sticking to the extracellular matrix. *Dev Cell* 3: 311-321.
54. Devenport D, Brown NH (2004) Morphogenesis in the absence of integrins: mutation of both Drosophila beta subunits prevents midgut migration. *Development* 131: 5405-5415.
55. Martin-Bermudo MD, Alvarez-Garcia I, Brown NH (1999) Migration of the Drosophila primordial midgut cells requires coordination of diverse PS integrin functions. *Development* 126: 5161-5169.
56. Urbano JM, Dominguez-Gimenez P, Estrada B, et al. (2011) PS integrins and laminins: key regulators of cell migration during Drosophila embryogenesis. *PLoS One* 6: e23893.

57. Pert M, Gan M, Saint R, et al. (2015) Netrins and Frazzled/DCC promote the migration and mesenchymal to epithelial transition of *Drosophila* midgut cells. *Biology Open*. doi:10.1242/bio.201410827 [Epub ahead of print]
58. Tepass U, Hartenstein V (1994) The development of cellular junctions in the Drosophila embryo. *Dev Biol* 161: 563-596.
59. Tepass U, Hartenstein V (1994) Epithelium formation in the Drosophila midgut depends on the interaction of endoderm and mesoderm. *Development* 120: 579-590.
60. Yarnitzky T, Volk T (1995) Laminin is required for heart, somatic muscles, and gut development in the Drosophila embryo. *Dev Biol* 169: 609-618.
61. Pastor-Pareja JC, Grawe F, Martin-Blanco E, et al. (2004) Invasive cell behavior during Drosophila imaginal disc eversion is mediated by the JNK signaling cascade. *Dev Cell* 7: 387-399.
62. Martin-Blanco E, Pastor-Pareja JC, Garcia-Bellido A (2000) JNK and decapentaplegic signaling control adhesiveness and cytoskeleton dynamics during thorax closure in Drosophila. *Proc Natl Acad Sci U S A* 97: 7888-7893.
63. Tateno M, Nishida Y, Adachi-Yamada T (2000) Regulation of JNK by Src during Drosophila development. *Science* 287: 324-327.
64. Ishimaru S, Ueda R, Hinohara Y, et al. (2004) PVR plays a critical role via JNK activation in thorax closure during Drosophila metamorphosis. *EMBO J* 23: 3984-3994.
65. Srivastava A, Pastor-Pareja JC, Igaki T, et al. (2007) Basement membrane remodeling is essential for Drosophila disc eversion and tumor invasion. *Proc Natl Acad Sci U S A* 104: 2721-2726.
66. Homsy JG, Jasper H, Peralta XG, et al. (2006) JNK signaling coordinates integrin and actin functions during Drosophila embryogenesis. *Dev Dyn* 235: 427-434.
67. Bubici C, Papa S (2014) JNK signalling in cancer: in need of new, smarter therapeutic targets. *Br J Pharmacol* 171: 24-37.
68. Manhire-Heath R, Golenkina S, Saint R, et al. (2013) Netrin-dependent downregulation of Frazzled/DCC is required for the dissociation of the peripodial epithelium in Drosophila. *Nat Commun* 4: 2790.
69. Speck O, Hughes SC, Noren NK, et al. (2003) Moesin functions antagonistically to the Rho pathway to maintain epithelial integrity. *Nature* 421: 83-87.
70. VanZomeren-Dohm A, Sarro J, Flannery E, et al. (2011) The Drosophila Netrin receptor frazzled/DCC functions as an invasive tumor suppressor. *BMC Dev Biol* 11: 41.
71. Castets M, Broutier L, Molin Y, et al. (2012) DCC constrains tumour progression via its dependence receptor activity. *Nature* 482: 534-537.
72. Fitamant J, Guenebeaud C, Coissieux MM, et al. (2008) Netrin-1 expression confers a selective advantage for tumor cell survival in metastatic breast cancer. *Proc Natl Acad Sci U S A* 105: 4850-4855.
73. Papanastasiou AD, Pampalakis G, Katsaros D, et al. (2011) Netrin-1 overexpression is predictive of ovarian malignancies. *Oncotarget* 2: 363-367.
74. Mazelin L, Bernet A, Bonod-Bidaud C, et al. (2004) Netrin-1 controls colorectal tumorigenesis by regulating apoptosis. *Nature* 431: 80-84.
75. Lai Wing Sun K, Correia JP, Kennedy TE (2011) Netrins: versatile extracellular cues with diverse functions. *Development* 138: 2153-2169.

76. Dumartin L, Quemener C, Laklai H, et al. (2010) Netrin-1 mediates early events in pancreatic adenocarcinoma progression, acting on tumor and endothelial cells. *Gastroenterology* 138: 1595-1606, 1606 e1591-1598.
77. Kaufmann S, Kuphal S, Schubert T, et al. (2009) Functional implication of Netrin expression in malignant melanoma. *Cell Oncol* 31: 415-422.
78. Rodrigues S, De Wever O, Bruyneel E, et al. (2007) Opposing roles of netrin-1 and the dependence receptor DCC in cancer cell invasion, tumor growth and metastasis. *Oncogene* 26: 5615-5625.
79. Salminen M, Meyer BI, Bober E, et al. (2000) Netrin 1 is required for semicircular canal formation in the mouse inner ear. *Development* 127: 13-22.
80. Martin M, Simon-Assmann P, Kedinger M, et al. (2006) DCC regulates cell adhesion in human colon cancer derived HT-29 cells and associates with ezrin. *Eur J Cell Biol* 85: 769-783.
81. Kee N, Wilson N, De Vries M, et al. (2008) Neogenin and RGMa control neural tube closure and neuroepithelial morphology by regulating cell polarity. *J Neurosci* 28: 12643-12653.
82. Fulga TA, Rorth P (2002) Invasive cell migration is initiated by guided growth of long cellular extensions. *Nat Cell Biol* 4: 715-719.
83. Majumder P, Aranjuez G, Amick J, et al. (2012) Par-1 controls myosin-II activity through myosin phosphatase to regulate border cell migration. *Curr Biol* 22: 363-372.
84. Wang X, Adam JC, Montell D (2007) Spatially localized Kuzbanian required for specific activation of Notch during border cell migration. *Dev Biol* 301: 532-540.
85. Prasad M, Montell DJ (2007) Cellular and molecular mechanisms of border cell migration analyzed using time-lapse live-cell imaging. *Dev Cell* 12: 997-1005.
86. Ara T, Declerck YA (2010) Interleukin-6 in bone metastasis and cancer progression. *Eur J Cancer* 46: 1223-1231.
87. Sullivan NJ, Sasser AK, Axel AE, et al. (2009) Interleukin-6 induces an epithelial-mesenchymal transition phenotype in human breast cancer cells. *Oncogene* 28: 2940-2947.
88. Min Y, Ghose S, Boelte K, et al. (2011) C/EBP-delta regulates VEGF-C autocrine signaling in lymphangiogenesis and metastasis of lung cancer through HIF-1alpha. *Oncogene* 30: 4901-4909.
89. Doronkin S, Djagaeva I, Nagle ME, et al. (2010) Dose-dependent modulation of HIF-1alpha/sima controls the rate of cell migration and invasion in Drosophila ovary border cells. *Oncogene* 29: 1123-1134.
90. Anzick SL, Kononen J, Walker RL, et al. (1997) AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science* 277: 965-968.
91. Mazzone M, Selfors LM, Albeck J, et al. (2010) Dose-dependent induction of distinct phenotypic responses to Notch pathway activation in mammary epithelial cells. *Proc Natl Acad Sci U S A* 107: 5012-5017.
92. Bell GP, Thompson BJ (2014) Colorectal cancer progression: lessons from Drosophila? *Semin Cell Dev Biol* 28: 70-77.
93. Gandille P, Narbonne-Reveau K, Boissonneau E, et al. (2010) Mutations in the polycomb group gene polyhomeotic lead to epithelial instability in both the ovary and wing imaginal disc in Drosophila. *PLoS One* 5: e13946.
94. Widmann TJ, Dahmann C (2009) Dpp signaling promotes the cuboidal-to-columnar shape transition of Drosophila wing disc epithelia by regulating Rho1. *J Cell Sci* 122: 1362-1373.

95. Marinari E, Mehonic A, Curran S, et al. (2012) Live-cell delamination counterbalances epithelial growth to limit tissue overcrowding. *Nature* 484: 542-545.
96. Tepass U (2012) The apical polarity protein network in Drosophila epithelial cells: regulation of polarity, junctions, morphogenesis, cell growth, and survival. *Annu Rev Cell Dev Biol* 28: 655-685.
97. Bilder D, Li M, Perrimon N (2000) Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. *Science* 289: 113-116.
98. Brumby AM, Goulding KR, Schlosser T, et al. (2011) Identification of novel Ras-cooperating oncogenes in Drosophila melanogaster: a RhoGEF/Rho-family/JNK pathway is a central driver of tumorigenesis. *Genetics* 188: 105-125.
99. Ohsawa S, Sugimura K, Takino K, et al. (2011) Elimination of oncogenic neighbors by JNK-mediated engulfment in Drosophila. *Dev Cell* 20: 315-328.
100. Uhlirova M, Bohmann D (2006) JNK- and Fos-regulated Mmp1 expression cooperates with Ras to induce invasive tumors in Drosophila. *EMBO J* 25: 5294-5304.
101. Kulshammer E, Uhlirova M (2013) The actin cross-linker Filamin/Cheerio mediates tumor malignancy downstream of JNK signaling. *J Cell Sci* 126: 927-938.
102. Brock AR, Wang Y, Berger S, et al. (2012) Transcriptional regulation of Profilin during wound closure in Drosophila larvae. *J Cell Sci* 125: 5667-5676.
103. Dekanty A, Barrio L, Muzzopappa M, et al. (2012) Aneuploidy-induced delaminating cells drive tumorigenesis in Drosophila epithelia. *Proc Natl Acad Sci U S A* 109: 20549-20554.
104. Hipfner DR, Keller N, Cohen SM (2004) Slik Sterile-20 kinase regulates Moesin activity to promote epithelial integrity during tissue growth. *Genes Dev* 18: 2243-2248.
105. Neisch AL, Formstecher E, Fehon RG (2013) Conundrum, an ARHGAP18 orthologue, regulates RhoA and proliferation through interactions with Moesin. *Mol Biol Cell* 24: 1420-1433.
106. Coso OA, Chiariello M, Yu JC, et al. (1995) The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell* 81: 1137-1146.
107. Teramoto H, Crespo P, Coso OA, et al. (1996) The small GTP-binding protein rho activates c-Jun N-terminal kinases/stress-activated protein kinases in human kidney 293T cells. Evidence for a Pak-independent signaling pathway. *J Biol Chem* 271: 25731-25734.
108. Neisch AL, Speck O, Stronach B, et al. (2010) Rho1 regulates apoptosis via activation of the JNK signaling pathway at the plasma membrane. *J Cell Biol* 189: 311-323.
109. Khoo P, Allan K, Willoughby L, et al. (2013) In Drosophila, RhoGEF2 cooperates with activated Ras in tumorigenesis through a pathway involving Rho1-Rok-Myosin-II and JNK signalling. *Dis Model Mech* 6: 661-678.
110. Vidal M, Larson DE, Cagan RL (2006) Csk-deficient boundary cells are eliminated from normal Drosophila epithelia by exclusion, migration, and apoptosis. *Dev Cell* 10: 33-44.
111. Rudrapatna VA, Bangi E, Cagan RL (2014) A Jnk-Rho-Actin remodeling positive feedback network directs Src-driven invasion. *Oncogene* 33: 2801-2806.
112. Singh J, Aaronson SA, Mlodzik M (2010) Drosophila Abelson kinase mediates cell invasion and proliferation through two distinct MAPK pathways. *Oncogene* 29: 4033-4045.
113. Das TK, Sangodkar J, Negre N, et al. (2013) Sin3a acts through a multi-gene module to regulate invasion in Drosophila and human tumors. *Oncogene* 32: 3184-3197.

114. Brumby AM, Richardson HE (2003) scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. *EMBO J* 22: 5769-5779.
115. Pagliarini RA, Xu T (2003) A genetic screen in Drosophila for metastatic behavior. *Science* 302: 1227-1231.
116. Igaki T, Pagliarini RA, Xu T (2006) Loss of cell polarity drives tumor growth and invasion through JNK activation in Drosophila. *Curr Biol* 16: 1139-1146.
117. Ma X, Shao Y, Zheng H, et al. (2013) Src42A modulates tumor invasion and cell death via Ben/dUev1a-mediated JNK activation in Drosophila. *Cell Death Dis* 4: e864.
118. Rudrapatna VA, Bangi E, Cagan RL (2013) Caspase signalling in the absence of apoptosis drives Jnk-dependent invasion. *EMBO Rep* 14: 172-177.
119. Yang L, Cao Z, Yan H, et al. (2003) Coexistence of high levels of apoptotic signaling and inhibitor of apoptosis proteins in human tumor cells: implication for cancer specific therapy. *Cancer Res* 63: 6815-6824.
120. Wu M, Pastor-Pareja JC, Xu T (2010) Interaction between Ras(V12) and scribbled clones induces tumour growth and invasion. *Nature* 463: 545-548.
121. Herranz H, Hong X, Hung NT, et al. (2012) Oncogenic cooperation between SOCS family proteins and EGFR identified using a Drosophila epithelial transformation model. *Genes Dev* 26: 1602-1611.
122. de Visser KE, Eichten A, Coussens LM (2006) Paradoxical roles of the immune system during cancer development. *Nat Rev Cancer* 6: 24-37.
123. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144: 646-674.
124. Landskron G, De la Fuente M, Thuwajit P, et al. (2014) Chronic inflammation and cytokines in the tumor microenvironment. *J Immunol Res* 2014: 149185.
125. Pastor-Pareja JC, Wu M, Xu T (2008) An innate immune response of blood cells to tumors and tissue damage in Drosophila. *Dis Model Mech* 1: 144-154; discussion 153.
126. Cordero JB, Macagno JP, Stefanatos RK, et al. (2010) Oncogenic Ras diverts a host TNF tumor suppressor activity into tumor promoter. *Dev Cell* 18: 999-1011.
127. Shen J, Lu J, Sui L, et al. (2014) The orthologous Tbx transcription factors Omb and TBX2 induce epithelial cell migration and extrusion in vivo without involvement of matrix metalloproteinases. *Oncotarget* 5: 11998-12015.
128. Das TK, Dana D, Paroly SS, et al. (2013) Centrosomal kinase Nek2 cooperates with oncogenic pathways to promote metastasis. *Oncogenesis* 2: e69.
129. Dominguez M (2014) Oncogenic programmes and Notch activity: an 'organized crime'? *Semin Cell Dev Biol* 28: 78-85.
130. Gratz SJ, Wildonger J, Harrison MM, et al. (2013) CRISPR/Cas9-mediated genome engineering and the promise of designer flies on demand. *Fly (Austin)* 7: 249-255.
131. Kvon EZ, Kazmar T, Stampfel G, et al. (2014) Genome-scale functional characterization of Drosophila developmental enhancers in vivo. *Nature* 512: 91-95.
132. Southall TD, Gold KS, Egger B, et al. (2013) Cell-type-specific profiling of gene expression and chromatin binding without cell isolation: assaying RNA Pol II occupancy in neural stem cells. *Dev Cell* 26: 101-112.
133. Ferres-Marco D, Gutierrez-Garcia I, Vallejo DM, et al. (2006) Epigenetic silencers and Notch collaborate to promote malignant tumours by Rb silencing. *Nature* 439: 430-436.

134. Willoughby LF, Schlosser T, Manning SA, et al. (2013) An in vivo large-scale chemical screening platform using Drosophila for anti-cancer drug discovery. *Dis Model Mech* 6: 521-529.
135. Vidal M, Wells S, Ryan A, et al. (2005) ZD6474 suppresses oncogenic RET isoforms in a Drosophila model for type 2 multiple endocrine neoplasia syndromes and papillary thyroid carcinoma. *Cancer Res* 65: 3538-3541.
136. Dar AC, Das TK, Shokat KM, et al. (2012) Chemical genetic discovery of targets and anti-targets for cancer polypharmacology. *Nature* 486: 80-84.
137. Kasai Y, Cagan R (2010) Drosophila as a tool for personalized medicine: a primer. *Per Med* 7: 621-632.