Casein kinase I epsilon somatic mutations found in breast cancer cause overgrowth in *Drosophila*

TOMAS DOLEZAL*,[1,2], KATERINA KUCEROVA[1], JANA NEUHOLD[1] and PETER J. BRYANT[3]

[1]*Department of Molecular Biology, Faculty of Science, University of South Bohemia in Ceske Budejovice,*
[2]*Institute of Entomology, Biology Centre of the Academy of Sciences of the Czech Republic, Czech Republic*
and [3]*Developmental Biology Center, University of California, Irvine, USA*

ABSTRACT We are using a candidate gene approach to identify genes contributing to cancer through somatic mutation. Somatic mutations were found in breast cancer samples in the human *casein kinase I epsilon (CKIε)* gene, a homolog of the *Drosophila* gene *dco* in which certain point mutations lead to imaginal disc overgrowth. We therefore created fly genotypes in which the *dco* gene carried point mutations homologous to those discovered in *CKIε*, and tested them *in vivo*. The results show that the most frequent mutation discovered in breast cancer, L39Q, causes a striking overgrowth phenotype in flies. Further experiments show that this mutation affects the newly recognized Fat/Warts signaling pathway, which controls organ size and shape in both flies and mammals. Another mutation, S101R, modifies the mutant phenotype so that the affected tissue disintegrates, mimicking more aggressive forms of breast cancer. Our results thus strongly support the conclusion that *CKIε* mutations play important roles in breast carcinogenesis.

KEY WORDS: *Drosophila, breast cancer, CKIε, Dco*

Introduction

Most of the genes known to be causally related to human cancer have been identified through their association with familial cancers, but this approach identifies only a very limited set of cancer-related genes. Strictly somatic mutations are probably extremely important in all stages of carcinogenesis but are not well characterized because of their probable heterogeneity and the lack of practically feasible methods to identify the affected genes. However, candidate genes that may be affected by somatic mutations can potentially be identified by their homology to genetically characterized cancer-related genes in model organisms, specifically mouse and *Drosophila*. Tests in model systems also provide the opportunity to distinguish functionally significant somatic mutations from bystander mutations that do not contribute to the malignant phenotype (Zender *et al.* 2006).

Fuja *et al.* (2004) screened breast cancer tissue for somatic mutations in candidate genes identified through their homology to tumor suppressor genes where mutations cause either hyperplastic or neoplastic overgrowth of the imaginal discs in larval *Drosophila*. They found a remarkably high rate of mutations in the human gene encoding Casein Kinase Iε (CKIε), homologous to the *Drosophila disc overgrown* (*dco*) gene in which an unusual allele *dco³* causes imaginal disc overgrowth (Jursnich *et al.* 1990; Zilian *et al.* 1999). All of the 11 identified human mutations were non-synonymous, ten of them altered residues conserved from *Drosophila* to human (Fig. 1B), and most of them were associated with loss of heterozygosity of closely linked markers, all indicating that these mutations were functionally significant and contributed to the cancer phenotype. One particular mutation, L39Q, was found in 5 out of 6 mutated samples, or 12% of 42 screened breast cancer patients. Although these are all indications that the identified mutations might be related to the process of carcinogenesis, a more functional study is required to test their real significance. In order to test for the predicted functional changes *in vivo*, we are recreating some of the identified human *CKIε* mutations in the highly conserved fly homolog *dco*.

---

*Address correspondence to: Tomas Dolezal. Department of Molecular Biology, Faculty of Science, University of South Bohemia, Branisovska 31, Ceske Budejovice 37005, Czech Republic. Fax: +42-038-777-2265. e-mail: tomas.dolezal@prf.jcu.cz - web: http://apendix.prf.jcu.cz/Dolezal/dolezal/

Results

Since overexpression or misexpression of an introduced gene construct could mask the effects of a mutation, we have introduced the mutations into a *dco* gene regulated by adjacent genomic regulatory sequences, using a large genomic clone containing the *dco* gene (Fig. 1A). This clone bears intact endogenous regulatory sequences surrounding the *dco* gene, demonstrated by the fact that when transgenically provided to flies it fully rescued the null *dco* mutants (Zilian *et al.* 1999). We have introduced selected human mutations into this clone by site-directed mutagenesis and injected the resulting clones into embryos to establish transgenic lines. We have introduced the mutant transgenes into a null *dco* background (achieved by combining the deletions *dco*[le88] and *Df(3R)A177der22*; Fig. 1A and Zilian *et al.*1999) so the only Dco protein present was the mutant version expressed from the natural promoter. For L39Q, we will refer to this genetic combination simply as the *dco*[L39Q] mutant.

The *dco*[L39Q] mutant causes an extended larval development (10 days compared to 5 days for wild-type larvae) associated with a striking overgrowth phenotype in larval imaginal discs (Fig. 2). Whereas the wild-type discs stop growing when they reach the appropriate size (as in Fig. 2A), the mutant discs continue growing during the extended larval period and before pupariation they reach about three times the normal final size. Although the mutant larvae eventually pupate they do not proceed through metamorphosis, and the pupae die without any signs of adult development.

The effect of *dco*[L39Q] on larval development and disc growth is similar to the phenotype of the originally isolated *dco*[3] mutant (compare discs in Fig. 2A), but the overall morphology of *dco*[L39Q] discs is much more abnormal, with more extensive rippling and loss of normal morphological features including the wing pouch. These features are recessive towards *dco*[3], since *dco*[L39Q]/*dco*[3] transheterozygotes appear similar to *dco*[3]. In spite of this overgrowth, confocal microscopy revealed that the *dco*[L39Q] mutant discs retain their monolayered epithelial structure (Fig. 3) showing that they exhibit a hyperplastic overgrowth phenotype as is typical for *dco*[3] and other mutants in the Fat/ Warts cascade (Bryant *et al.* 1988; Jursnich *et al.* 1990; Justice *et al.* 1995; Xu *et al.* 1995; Cho *et al.* 2006).

In breast cancer samples the *CKIε* L39Q mutation was usually observed in combination with additional mutations in the same gene (Fig. 1 and Fuja *et al.* 2004); for example, in two breast cancer patients L39Q was associated with another point mutation S101R. Therefore we tested this combination of mutations in flies, and found that *dco*[L39Q,S101R] mutants have extended larval development and a similar imaginal disc morphology to that seen in *dco*[L39Q] when they reach the size of wild-type discs (Fig. 2A). However, the surface of *dco*[L39Q,S101R] mutant discs is not smooth as in wild type or *dco*[L39Q], the discs do not grow larger than normal, and the tissue seems to disintegrate in places (Fig. 2A and in more detail in Fig. 2E). We conclude that the growth of *dco*[L39Q,S101R] discs is affected as in *dco*[L39Q] but that in addition the S101R mutation causes loss of tissue integrity. It is important to note that, although in both human cancer cases S101R was found in combination with L39Q, a heteroallelic combination could not be excluded. Therefore it will be interesting to test, using our *Drosophila* model, the effects of individual S101R mutation as well as other combinations of identified somatic mutations.

For both mutants, *dco*[L39Q] and *dco*[L39Q,S101R], two independent transgenic lines gave indistinguishable mutant phenotypes, making it unlikely that the phenotypes are caused by changed expression of *dco* due to a position effect on the transgenic insertion.

The *dco*[L39Q] mutant phenotype closely resembles that produced by mutants in the Fat/Warts signaling pathway (Cho *et al.* 2006 and Fig. 4A), namely overgrowth and rippled morphology of the imaginal discs (Justice *et al.* 1995; Bennett & Harvey, 2006). Furthermore, in two recent studies it was shown that the Dco kinase phosphorylates the Fat receptor (Feng *et al.* 2009; Sopko *et al.* 2009). Therefore, we are testing whether *dco*[L39Q] also affects Fat/Warts signaling. To do this we have taken advantage of the fact that the *dco*[L39Q] mutation expresses with low penetrance (less than 15%) a very mild dominant effect on a crossvein in the adult wing (Fig. 4C). As shown in Fig. 4C,
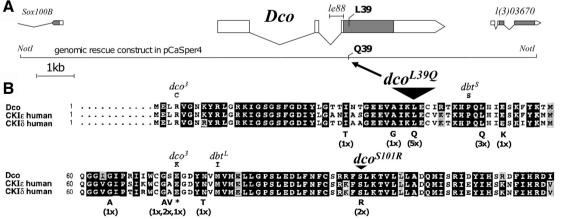

Fig. 1. Molecular design of mutagenesis and mutations in *dco* and *CKIε*. (A) Schematic map of the genomic region containing the dco *gene; exons are depicted by boxes with protein-coding region in grey. The position of leucine L39 which was mutated to glutamine Q39 is shown. The small 391-bp deletion* le88, *which removes the start codon of* dco, *was used with the* Df(3R)A177der22 *deficiency (covering a larger area than on the map) to make a null* dco *mutant background. The size and position of the genomic rescue construct (Zilian* et al. *1999) in which the mutations were introduced is also shown.* **(B)** *Sequence alignment of the first 129 amino acids of* Drosophila *Dco with human CKIε and CKIδ proteins. The previously identified* dco[3] *(Zilian et al. 1999) doubletime*[L/S] *(Kloss et al. 1998) and newly introduced* dco[L39Q,S101R] *mutations are shown above the alignment. Somatic mutations found in human CKIε in breast cancer are shown below the alignment with the number of their occurrences in parentheses.*
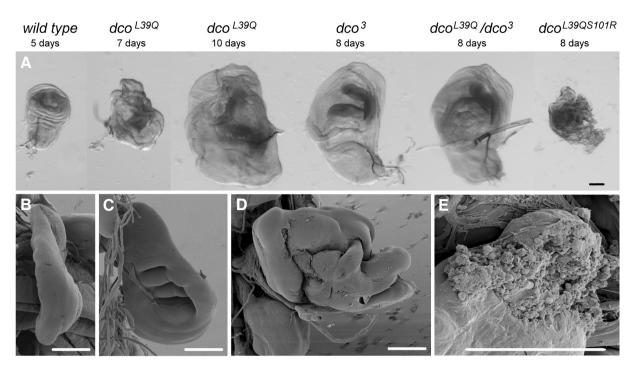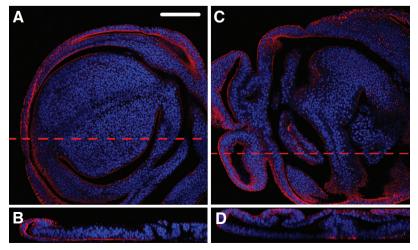
Fig. 2. Wing disc morphology of different *dco* mutants. (A) *Wing discs dissected from third-instar larvae of various genotypes indicated above each disc; the age of larvae in days after egg laying is also indicated.* **(B-E)** *Scanning electron microscopy of wing discs. Side view showing flat plane* **(B)** *and bottom view showing typical morphology of late third-instar wild-type wing disc* **(C)**. *Slant view of* dco^L39Q *mutant disc demonstrating overgrowth of tissue in all directions* **(D)**. *Detail of ruptured tissue of* dco^L39Q,S101R *mutant disc* **(E)**. *Scale bar on all pictures, 100 μm.*

while *warts^P2*/+ adult wings (Justice *et al.* 1995) do not show any abnormalities in vein pattern, *warts^P2* significantly enhances the mild dominant effect of *dco^L39Q*: the extra vein fragment is present in more than 90% of the *dco^L39Q* /*warts^P2* transheterozygous wings, the fragment is usually enlarged, and most of the wings show additional extra vein material not present in either heterozygous combination (Fig. 4C). More importantly, the heterozygous *dco^L39Q* mutation significantly enlarges adult wings compared to controls (Fig. 4B and Table 1). This is similar to the effect of mutation in *Warts* (Fig. 4B and Table 1), which plays a central role in the control of organ size by the Fat/Warts signaling cascade. The transheterozygous *dco^L39Q* /*warts^P2* combination therefore shows a significantly

enhancement over the effects of the individual mutations (Fig. 4B and Table 1).

Discussion

This work presents a functional study of the *CKIε* point mutations, which were found as somatic mutations in human breast cancer, using *Drosophila* as a model. The introduction of the *CKIε* point mutation L39Q into the highly conserved fly homolog *dco* causes a striking overgrowth phenotype in imaginal disc tissue. The newly discovered phosphorylation of the Fat receptor by Dco (Feng *et al.* 2009; Sopko *et al.* 2009), the similarity of the *dco^L39Q* mutant phenotype to that produced by mutants in Fat/Warts cascade, and the genetic interaction of *dco^L39Q* with *warts^P2* all support the idea that the *dco^L39Q* mutation affects the Fat/Warts signaling cascade (Fig 4A), possibly by causing abnormal phosphorylation of the Fat receptor.

The *fat*, *discs overgrown* and *warts* genes were discovered many years ago by their similar mutant phenotypes of hyperplastic imaginal disc overgrowth (Bryant *et al.* 1988; Jursnich *et al.* 1990; Justice *et al.*

Fig. 3. Confocal images of wing imaginal discs. (A,B) *Wing blade region of wild-type disc with optical cross section* **(B)** *marked by red intermittent line on* **(A)**. **(C,D)** *part of the* dco^L39Q *wing disc.* **(D)** *An optical section demonstrating that normal epithelial structure is retained despite extensive folding. Discs were stained by DAPI labeling nuclei (blue) and by anti-Discs large antibody (red). White scale bar common for both images represents 60 μm.*

1995; Xu *et al.* 1995), and until recently there was no reason to consider them functionally related at the molecular level. However, the products of these genes have now been linked into a common pathway which has been called the Fat/Warts signaling cascade (Cho *et al.* 2006). This pathway controls organ size and shape through interaction of the Fat transmembrane receptor with its ligand Dachsous, leading to inhibition of the growth-promoting transcription coactivator Yorkie (in flies; YAP in mammals) via phosphorylation by Warts (in flies; Lats in mammals; reviewed by Reddy & Irvine, 2008, see also Fig 4A). Rescue of the fly mutants by their mammalian counterparts has demonstrated the high evolutionary conservation of this pathway (Tao *et al.* 1999). Furthermore, components of the Fat/Warts pathway are now being linked to human cancer, including breast cancer. Frequent loss of heterozygosity has been re-
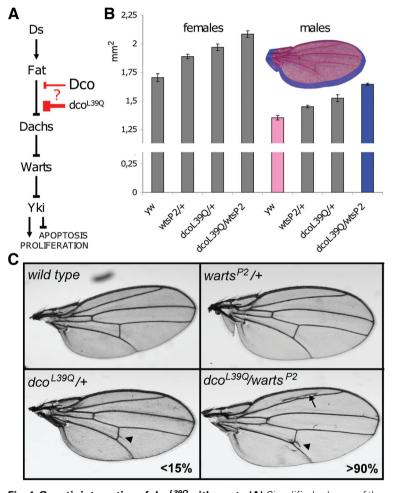
Compared genotypes	Females	Males
yw vs. *dco^{L39Q}*/+	P<1,01 x 10^{-08}	P<1,64 x 10^{-06}
yw vs. *wts^{P2}*/+	P<1,38 x 10^{-05}	P<2,94 x 10^{-06}
dco^{L39Q}/+ vs. *dco^{L39Q}*/*wts^{P2}*	P<4,01 x 10^{-06}	P<1,71 x 10^{-08}
wts^{P2}/+ vs. *dco^{L39Q}*/*wts^{P2}*	P<1,76 x 10^{-08}	P<1,45 x 10^{-18}

ported for regions containing *LATS1* and *LATS2*, the human homologs of *Drosophila Warts*, and promoter hypermethylation that silences these genes was detected in more than 50% of breast cancers (Takahashi *et al.* 2005). In a screen for gene copy-number changes in mouse mammary tumors, Overholtzer *et al.* (2006) identified an amplicon containing *YAP*, a mammalian homolog of *Drosophila Yorkie* and they further showed that in nontransformed mammary epithelial cells, overexpression of human *YAP* induces epithelial-to-mesenchymal transition. Finally, Qi *et al.* (2009) reported loss of mRNA expression of *Fat4*, a human homolog of *Drosophila Fat*, in 3 of 5 primary breast cancers and 3 of 6 breast-cancer cell lines. Thus alterations at all levels of the Fat/Warts signaling cascade, from receptor to transcriptional coactivator, are now being reported at high frequencies for breast cancer, suggesting that aberrations of this pathway might play a significant role in breast carcinogenesis. This is strongly supported by the results presented in this paper, in that the most frequent somatic mutation of *CKIε* found in breast cancer causes a phenotype associated with aberrant Fat/Warts signaling in flies.

Dco/CKIε mutations show pleiotropic effects, and different point mutations have distinct effects on Dco function. For example the Dco[3] mutant protein phosphorylates the circadian rhythm protein Period normally but shows altered phosphorylation of the Fat receptor, while Dco[Dbt-AR] shows altered Period phosphorylation (Feng and Irvine 2009). The normal function of Dco must be required for tissue growth, since the null mutant larvae are discless due to the reduction of apoptosis inhibitor DIAP1 (Guan *et al.* 2007). One possible mechanism could be that Dco fine-tunes the Fat/Warts inhibitory pathway via Fat phosphorylation (Fig. 4A). Null *dco* mutants, which lack this function, exhibit a reduction of DIAP1 (Guan *et al.* 2007), one of the Fat/Warts signaling targets. Furthermore, *dco^{L39Q}* like *dco[3]* could then lead to excessive inhibition of Fat/Warts signaling, via aberrant Fat phosphorylation, resulting in excessive tissue growth (Fig. 4A).

In conclusion, our work demonstrates that introduction of the *CKIε* point mutation L39Q, which was found as a somatic mutation in human breast cancer, into the highly conserved fly homolog *dco* causes a striking overgrowth phenotype in imaginal disc tissue. This first functional study of *CKIε* mutations *in vivo* therefore strongly suggests that they indeed play an important role in breast cancer. In addition, similar phenotypes and genetic interactions indicate that L39Q interacts with the recently

Fig. 4. Genetic interaction of *dco^{L39Q}* with *warts*. (A) *Simplified scheme of the Fat/Warts signaling cascade with a hypothetical role of Dco (see text for details).* **(B)** *Size of wings (in square millimeters; Mean +/- SEM) from females and males of the indicated genotypes. An image of overlapped wings of average size from* yw *control males (pink) and* dco^{L39Q} */*warts^{P2} *transheterozygous male (blue) is shown within the graph for comparison.* **(C)** *Adult wings from flies of the indicated genotypes. The wing vein pattern of* warts *heterozygotes is normal. In less than 15% of* dco^{L39Q} *heterozygotes, a small branch from the crossvein (arrowhead) is detected, and a similar feature is detected in more than 90% of* dco^{L39Q} */*warts^{P2} *transheterozygotes. The arrow points to extra vein material which is also detected in most of the wings of the transheterozygotes.*

recognized Fat/Warts signaling pathway, which is required for the cessation of cell proliferation when imaginal discs reach their genetically determined final size at the end of larval development. Thus our results support the idea that genes controlling this tissue-level mechanism of size control, in addition to genes controlling the basic mechanisms of cell proliferation and death, play an important role in cancer (Leevers & McNeill, 2005). Adding the S101R mutation worsens the mutant phenotype such that the disc tissue disintegrates, which is especially interesting in view of reports that an aggressive form of breast cancer, including metastases, is associated with aberrant LATS/Yap signaling (Takahashi *et al.* 2005; Overholtzer *et al.* 2006). Our work confirms that *Drosophila* can be used as an extremely useful model system to identify genes that might be important in cancer (Brumby & Richardson, 2005), and furthermore that it can provide important *in vivo* functional tests to characterize the importance of somatic mutations that have been discovered in human cancer.

Materials and Methods

Generation of mutant flies

Mutations L39Q and S101R were introduced into a genomic rescue construct "NotI of lambda88.9 in CasPer4#7" (described in Zilian *et al.* 1999) as follows: 6-kb fragment including *dco* coding sequence was cut out by *Xho*I and *Bam*HI and cloned into pBluescript II SK(+) (Stratagene). Mutations were introduced by QuickChange Multi Site-Directed Mutagenesis Kit (Stratagene) using following primers:
L39Q – gtggccatcaagcAggagtgcatccgc and
S101R – cttttgttcacgccgctttCGgttgaagacggttctgct (mismatches capitalized). Mutant sequences were cloned back to genomic rescue construct using *Swa*I and *Bsi*WI enzymes. The mutated constructs were injected into the *yw* embryos using a standard P-element transformation protocol. Two independent transgenic lines for each mutant construct on X chromosome were crossed with *yw; dco^{le88}/TM6B* and *yw; Df(3R)A177der22/TM6B* lines (provided by Markus Noll) to obtain *yw dco^{L39,/S101R}; dco^{le88}/Df(3R)A177der22* larvae with *dco^{L39Q,S101R}* in *dco* null background. *dco^{L39Q}* mutated sequence was verified by sequencing of PCR products amplified from genomic DNA of *yw dco^{L39Q,S101R}; dco^{le88}/Df(3R)A177der22* larvae – the only normal-length PCR product was from the *dco^{L39Q,S101R}* sequence.

Genetic interaction with warts

y w dco^{L39Q}; dco^{le88}/TM6B and *w; warts^{P2}/TM6B'*lines were crossed either to *y w* or to each other to obtain *y w dco^{L39Q}; dco^{le88}/+* or *y w; warts^{P2}/+* heterozygous flies (depicted as *dco^{L39Q}/+* or *warts^{P2}/+* in the text) and *y w dco^{L39Q}/w; dco^{le88} warts^+/dco^+ warts^{P2}* transheterozygous flies (depicted as *dco^{L39Q}/warts^{P2}* in the text). Adult wings were photographed on a stereomicroscope, with transmitted light for the wing veins pattern. A fixed zoom and camera resolution were used for wing area measurement, with the area of each wing being marked using the selection tool in the GNU Image Manipulation Program 2.6 and the number of pixels being determined using the histogram tool. The pixel number was then converted to square millimeters. A t-Test was used to compare the areas in different genotypes. Wings from more than 40 flies were analyzed for each genotype.

Imaginal disc preparation and analysis

Wing imaginal discs were dissected from third-instar larvae of the indicated genotype and age, and their size and morphology observed using a stereomicroscope using transmitted light. The epithelial architecture was analyzed using confocal microscopy; discs were fixed in 4% paraformaldehyde and stained using mouse anti-Disc large antibody diluted 1:400 (the mouse monoclonal antibody developed by Dr. Dan

Woods was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242) and by DAPI.

Scanning electron microscopy

Larval bodies were inverted in PBS on ice and the fat body, gut and salivary glands were dissected away. Imaginal discs attached to the integument were fixed in glutaraldehyde, dehydrated and dried, coated by gold and scanned using a JEOL JSM-7401F scanning electron microscope.

References

BENNETT FC, HARVEY KF (2006). Fat cadherin modulates organ size in *Drosophila* via the Salvador/Warts/Hippo signaling pathway. *Curr Biol* 16: 2101-2110.

BRUMBY AM, RICHARDSON HE (2005). Using *Drosophila Melanogaster* to map human cancer pathways. *Nat Rev Cancer* 5: 626-639.

BRYANT PJ, HUETTNER B, HELD LI JR, RYERSE J and SZIDONYA J (1988). Mutations at the fat locus interfere with cell proliferation control and epithelial morphogenesis in *Drosophila*. *Dev Biol* 129: 541-554.

CHO E, FENG Y, RAUSKOLB C, MAITRA S, FEHON R and IRVINE KD (2006). Delineation of a Fat tumor suppressor pathway. *Nat Genet* 38: 1142-1150.

FENG Y, IRVINE KD (2009). Processing and phosphorylation of the Fat receptor. *Proc Natl Acad Sci USA* 106: 11989-11994.

FUJA TJ, LIN F, OSANN KE and BRYANT PJ (2004). Somatic mutations and altered expression of the candidate tumor suppressors CSNK1 epsilon, DLG1, and EDD/hHYD in mammary ductal carcinoma. *Cancer Res* 64: 942-951.

GUAN J, LI H, ROGULJA A, AXELROD JD and CADIGAN KM (2007). The *Drosophila* casein kinase Iepsilon/delta Discs overgrown promotes cell survival via activation of DIAP1 expression. *Dev Biol* 303: 16–28.

JURSNICH VA, FRASER SE, HELD LI JR, RYERSE J and BRYANT PJ (1990). Defective gap-junctional communication associated with imaginal disc overgrowth and degeneration caused by mutations of the dco gene in *Drosophila*. *Dev Biol* 140: 413-429.

JUSTICE RW, ZILIAN O, WOODS DF, NOLL M and BRYANT PJ (1995). The *Drosophila* tumor suppressor gene warts encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. *Genes Dev* 9: 534-546.

KLOSS B, PRICE JL, SAEZ L, BLAU J, ROTHENFLUH A, WESLEY CS and YOUNG MW (1998). The *Drosophila* clock gene double-time encodes a protein closely related to human casein kinase Iepsilon. *Cell* 94: 97-107.

LEEVERS SJ, MCNEILL H (2005). Controlling the size of organs and organisms. *Curr Opin Cell Biol* 17: 604-609.

OVERHOLTZER M, ZHANG J, SMOLEN GA, MUIR B, LI W, SGROI DC, DENG CX, BRUGGE JS and HABER DA (2006). Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. *Proc Natl Acad Sci USA* 103: 12405-12410.

QI C, ZHU YT, HU L and ZHU YJ (2009). Identification of Fat4 as a candidate tumor suppressor gene in breast cancers. *Int J Cancer* 124: 793-798.

REDDY BV, IRVINE KD (2008). The Fat and Warts signaling pathways: new insights into their regulation, mechanism and conservation. *Development* 135: 2827-2838.

SOPKO R, SILVA E, CLAYTON L, GARDANO L, BARRIOS-RODILES M, WRANA J, VARELAS X, ARBOUZOVA NI, SHAW S, SABURI S, MATAKATSU H, BLAIR S and MCNEILL H (2009). Phosphorylation of the tumor suppressor fat is

regulated by its ligand Dachsous and the kinase discs overgrown. *Curr Biol* 19: 1112-1117.

TAKAHASHI Y, MIYOSHI Y, TAKAHATA C, IRAHARA N, TAGUCHI T, TAMAKI Y and NOGUCHI S (2005). Down-regulation of LATS1 and LATS2 mRNA expression by promoter hypermethylation and its association with biologically aggressive phenotype in human breast cancers. *Clin Cancer Res* 11: 1380-1385.

TAO W, ZHANG S, TURENCHALK GS, STEWART RA, ST JOHN MA, CHEN W and XU T (1999). Human homologue of the *Drosophila Melanogaster* lats tumour suppressor modulates CDC2 activity. *Nat Genet* 21: 177-181.

XU T, WANG W, ZHANG S, STEWART RA and YU W (1995). Identifying tumor

suppressors in genetic mosaics: the *Drosophila* lats gene encodes a putative protein kinase. *Development* 121: 1053-1063.

ZENDER L, SPECTOR MS, XUE W, FLEMMING P, CORDON-CARDO C, SILKE J, FAN ST, LUK JM, WIGLER M, HANNON GJ, MU D, LUCITO R, POWERS S and LOWE SW (2006). Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. *Cell* 125: 1253-1267.

ZILIAN O, FREI E, BURKE R, BRENTRUP D, GUTJAHR T, BRYANT PJ and NOLL M (1999). double-time is identical to discs overgrown, which is required for cell survival, proliferation and growth arrest in *Drosophila* imaginal discs. *Development* 126: 5409-5420.

Further Related Reading, published previously in the *Int. J. Dev. Biol.*

See our recent Special Issue *Placenta* edited by Joan S. Hunt and Kent L. Thornburg at:
http://www.ijdb.ehu.es/web/contents.php?vol=54&issue=2-3

The mob as tumor suppressor (mats1) gene is required for growth control in developing zebrafish embryos
Yuan Yuan, Shuo Lin, Zuoyan Zhu, Wenxia Zhang and Zhi-Chun Lai
Int. J. Dev. Biol. (2009) 53: 525-533

Using fruitflies to help understand the molecular mechanisms of human hereditary diffuse gastric cancer
Joana Caldeira, Paulo S. Pereira, Gianpaolo Suriano and Fernando Casares
Int. J. Dev. Biol. (2009) 53: 1557-1561

The cellular and genetic bases of organ size and shape in Drosophila
Antonio García-Bellido
Int. J. Dev. Biol. (2009) 53: 1291-1303

Tumor invasion and metastasis: getting more basic to come closer to the patient. An interview with Lance A. Liotta
Vincent Castronovo
Int. J. Dev. Biol. (2004) 48: 559-562

Tumor suppressor and overgrowth suppressor genes of Drosophila melanogaster: developmental aspects
E Gateff
Int. J. Dev. Biol. (1994) 38: 565-590

5 yr ISI Impact Factor (2008) = 3.271