A crucial role for the Anaplastic lymphoma kinase receptor tyrosine kinase in gut development in *Drosophila melanogaster*

Christina E. Lorén¹*, Camilla Englund¹*, Caroline Grabbe¹, Bengt Hallberg², Tony Hunter³ & Ruth H. Palmer^{1,4}

¹Umeå Center for Molecular Pathogenesis and ²Department of Pathology, Umeå University, Umeå, Sweden, and ³Molecular and Cell Biology Laboratory, The Salk Institute, La Jolla, California, USA

The *Drosophila melanogaster* gene *Anaplastic lymphoma kinase* (*Alk*) is homologous to mammalian *Alk*, which encodes a member of the Alk/Ltk family of receptor tyrosine kinases (RTKs). In humans, the t(2;5) translocation, which involves the *ALK* locus, produces an active form of ALK, which is the causative agent in non-Hodgkin's lymphoma. The physiological function of the Alk RTK, however, is unknown. In this paper, we describe loss-of-function mutants in the *Drosophila Alk* gene that cause a complete failure of the development of the gut. We propose that the main function of *Drosophila Alk* during early embryogenesis is in visceral mesoderm development.

EMBO reports 4, 781–786 (2003)

doi:10.1038/sj.embor.embor897

INTRODUCTION

Anaplastic lymphoma kinase (*Alk*) encodes a novel receptor tyrosine kinase (RTK), which was first described in non-Hodgkin's lymphoma in humans (Fujimoto *et al.*, 1996; Morris *et al.*, 1994). *ALK* has since been shown to be involved in many other translocations in anaplastic large-cell lymphoma (ALCL), as well as in other cancers, such as inflammatory myofibroblastic tumours (IMTs; Duyster *et al.*, 2001; Falini, 2001). Despite the identification of the mouse Alk RTK in 1997 (Iwahara *et al.*, 1997; Morris *et al.*, 1997), the function of Alk *in vivo* has remained elusive. This is also true of leukocyte tyrosine kinase (Ltk) RTK, the related Alk/Ltk family member in mice and humans. Recently, we identified an Alk relative in *Drosophila* (Loren *et al.*, 2001), which seems to encode just one member of the Alk/Ltk RTK family in its genome. The molecular similarity between *Drosophila Alk* and mammalian Alks suggests that mutants of *Alk* in flies may

affect similar processes. In this paper, we report that the *Drosophila Alk* RTK has a crucial function in gut development *in vivo*.

RESULTS AND DISCUSSION

We were initially interested in the physiological function of the Alk RTK. To investigate this, we set out to mutate the *Alk* locus in *Drosophila*, first by creation of a designer deletion over the *Alk53* locus (for details, see supplementary information online), which was subsequently used in an ethylmethane sulphonate (EMS) screen with the aim of identifying *Alk* mutant animals. Eleven independent *Alk* mutants were identified using this approach, and each showed similar phenotypes, as described below. The *Alk* mutations (*Alk*¹ to *Alk*¹⁰) and their associated molecular characteristics are shown in Fig. 1. Significantly, all the mutations identified were located in amino acids that are conserved between *Drosophila*, mouse and human Alk. The *Alk*¹¹ mutant line carried several amino-acid mutations and will not be discussed further.

The *Drosophila Alk* mutants are highly informative about Alk RTK function. The mutations can be divided into three groups: truncations (*Alk*¹ and *Alk*⁹), point mutations within the extracellular domain (*Alk*² to *Alk*⁷) and point mutations within the protein tyrosine-kinase (PTK) domain (*Alk*⁹ and *Alk*¹⁰). *Alk*¹ is a mutation of Gln 306 at the beginning of the first MAM (named after mephrins, A-5 protein and receptor protein tyrosine phosphatase mu) domain, which creates a stop codon and results in a truncated protein. This protein is estimated to have a molecular weight of ~33 kDa and, consistent with this, analyses of heterozygous *Alk*¹ mutant animals showed the presence of a truncated protein (see supplementary information online). The *Alk*¹ protein lacks any recognizable domain, and we consider this allele to be an Alk RTK functional null. The second mutation that causes a truncation, *Alk*⁹, arises from a splice-donor-site mutation and is predicted to generate a truncation just after the transmembrane domain. As no *Alk* mutant phenotypes are seen in *Alk*⁹ heterozygous animals, it seems that the mutant protein expressed does not act in the predicted dominant-negative manner, at least when expressed at endogenous levels. Interesting conclusions about the functional importance of the various Alk extracellular regions can be made from the *Alk* point mutations that lie within the extracellular domain.

¹Umeå Center for Molecular Pathogenesis, Building 6L, Umeå University, Umeå, S-901 87, Sweden

²Department of Pathology, Building 6M, Umeå University, Umeå, S-901 87, Sweden

³Molecular and Cell Biology Laboratory, The Salk Institute, 10010 N Torrey Pines Road, La Jolla, California 92037-1099, USA

*These authors contributed equally to this work

*Corresponding author. Tel: +46 90 785 6786; Fax: +46 90 778 007;

E-mail: ruth.palmer@ucmp.umu.se

Received 16 April 2003; revised 4 June 2003; accepted 11 June 2003

Published online 4 July 2003

Alk is the only RTK that contains a MAM domain in its extracellular region (Loren *et al.*, 2001). MAM domains are comprised of ~160 amino acids, and are present in transmembrane proteins such as the meprins and receptor protein-tyrosine phosphatases, in which they seem to function in cell-cell interactions (Beckmann & Bork, 1993; Jiang *et al.*, 1993). In *Drosophila* Alk, the second MAM domain seems to be important, as *Alk²* was identified as a mutation of Asp 681 in this domain. More surprisingly, the *Drosophila* Alk mutant screen underscores the importance of the glycine-rich region, a region that contains

stretches of up to six glycines in a row, which the Alk RTK shares with its relative, Ltk. In *Alk⁴*, *Alk⁵*, *Alk⁶* and *Alk⁷* mutants, a single glycine within the glycine-rich domain is mutated to an acidic amino acid. All of the glycines that are mutated in the *Drosophila* Alk mutants are conserved not only between the *Drosophila* and human Alks, but also in the Ltk RTK, thus suggesting an important role for this domain and highlighting the intolerance of an acidic residue in the stretches of glycine in this domain.

The third class of Alk mutants have point mutations in the intracellular domain. It is interesting to note that no mutations were found in the six potential phosphotyrosine motifs that lie outside the PTK domain, and although this may simply be due to chance, it may also indicate some plasticity in the signalling pathways downstream of the *Drosophila* Alk receptor. Both *Alk⁹* and *Alk¹⁰* have mutations that lie in the conserved PTK catalytic domain of the receptor, thus indicating that in the case of *Drosophila* Alk, the PTK activity of the receptor is indeed essential for its *in vivo* action. This is an important observation, as PTK activity is not essential for at least one RTK in *Drosophila* (Yoshikawa *et al.*, 2001). *Alk⁹* is a mutation in the conserved subdomain III of the kinase domain, in which the invariant glutamate (Glu 1244 in *Drosophila* Alk) in the C-helix, which is responsible for stabilizing the catalytic lysine and the α - and β -phosphates of Mg-ATP, is mutated to lysine. Last, *Alk¹⁰* has a mutation of the aspartic acid (Asp 1347 in *Drosophila* Alk) of the highly conserved triplet Asp-Phe-Gly (DFG), in subdomain VII, to asparagine. This aspartic acid is an invariant residue in protein kinases and is essential for activity, functioning to orientate the γ -phosphate of Mg-ATP for transfer to the substrate. Thus, from the ten Alk mutant alleles we have identified, we can infer the importance of the different domains in the *Drosophila* Alk RTK: functionally, the second MAM domain, the glycine-rich domain and the PTK domain are of crucial importance for *Drosophila* Alk function.

Fifty per cent of animals homozygous for Alk mutations died as embryos, and the rest died as first-instar larvae (Fig. 1C). In no case did an Alk mutant animal survive past the first-instar larval stage. Despite expression of Alk in the brain in mice and flies (Iwahara *et al.*, 1997; Loren *et al.*, 2001; Morris *et al.*, 1997), the central nervous system of mutant larvae seems to be generally normal, as visualized by staining with monoclonal antibody 22C10 (data not shown). Both human and *Drosophila* Alk have been reported to be expressed in the gut (Loren *et al.*, 2001; Morris *et al.*, 1994), with *Drosophila* Alk being highly expressed in the developing visceral mesoderm during embryogenesis (Loren *et al.*, 2001).

Visceral muscles are important components of many internal organ systems, particularly the gastrointestinal and urogenital tracts, respiratory tract and vascular system. In *Drosophila*, the visceral

Fig. 1 | Molecular organization of the *Drosophila* Anaplastic lymphoma kinase locus and characterization of mutant alleles. (A) Schematic representation of the genomic structure of the *Drosophila* Anaplastic lymphoma kinase (*Alk*) locus at 53C showing the intron-exon structure and open reading frame. Black and grey boxes represent coding and non-coding sequences, respectively. The locations of ethylmethane sulphonate (EMS) mutations in *Alk* (*Alk¹* to *Alk¹⁰*) are indicated by arrows. (B) Schematic representation of domain alterations in Alk alleles. The wild-type Alk domain structure is shown at the top, including the MAM (named after meprins, A-5 protein and receptor protein tyrosine phosphatase mu) domains (M), the glycine-rich domain (G), the transmembrane domain (TM) and the protein tyrosine kinase (PTK) domains. The locations of EMS mutations in Alk are indicated by arrows. (C) Effect of the *Alk¹* mutation on viability.

musculature is less diverse and primarily consists of the musculature of the digestive tract. Following the early subdivision of the mesoderm in the embryo, cells are specified to contribute to distinct tissues, which then perform coordinated migrations to form organs. The *Drosophila* visceral mesoderm is composed of two sets of muscles, an inner layer of circular muscles derived from cells along the trunk of the embryo, and an outer layer of longitudinal muscles derived from the posterior end of the embryo (Campos-Ortega & Hartenstein, 1997). The presumptive visceral mesoderm can first be identified as 12 metamereric clusters. The cells of these clusters migrate longitudinally to form two parallel bands along most of the length of the embryo, then ventrally and dorsally to form a closed tube, which is lined by endoderm (Nguyen & Xu, 1998). Longitudinal visceral-muscle precursors migrate over the circular muscle cells.

Using immunohistochemistry, we examined the expression of Alk protein in the developing visceral mesoderm in detail (Fig. 2A–F). Alk expression is first detected at germband extension (stage 10) as two Alk-positive cell groups per segment in the metamereric clusters that correspond to presumptive visceral mesoderm (Fig. 2A). During germband retraction (stage 11), the clusters on each side of the embryo fuse into a continuous band (Fig. 2B). At the end of germband retraction (stage 13), Alk is expressed in a broad band of visceral mesoderm on each side of the embryo (Fig. 2C,D). During stage 14, these two bilateral bands begin their expansion (Fig. 2E). By late embryogenesis, the cells of the visceral mesoderm have spread out dorsally and ventrally to encircle the entire gut (Fig. 2F). *Alk* mutant animals that survived to first-instar stages were analysed using a gut-coloration assay. Whereas heterozygous sibling animals were robust, with healthy appetites (Fig. 2G), *Alk* mutant animals did not seem to ingest food (Fig. 2, compare G and H). On fine dissection, these animals were found to lack discernable intestinal structures (data not shown). Further analysis of the developing gut in *Alk* mutant animals showed that the Alk-positive visceral mesoderm was severely disrupted and that no functional midgut was formed (Fig. 2, compare I and J).

The function of Alk in visceral mesoderm development was further analysed using the immunoglobulin domain adhesion molecule Fasciclin III (FasIII), which is a marker for differentiated visceral mesoderm in the *Drosophila* embryo. In wild-type embryos, Alk and FasIII expression patterns overlap perfectly in the visceral mesoderm as the midgut forms (Fig. 3A,B). Further analysis of *Alk* mutant embryos shows that there was a complete loss of Alk-positive and FasIII-positive cells, whereas FasIII staining in the epidermis was normal (Fig. 3, compare B and D). Similar results were obtained when antibodies to *Drosophila* Myocyte enhancer factor 2 (Mef2), which is produced in all muscle lineages of the *Drosophila* embryo (Lilly *et al.*, 1994; Nguyen *et al.*, 1994), were used (Fig. 3E–H). Furthermore, anti-myosin-heavy-chain (MHC) staining, which showed the thin layer of gut mesoderm in wild-type embryos (Fig. 3J), was absent from *Alk* mutant animals (Fig. 3L).

To test whether Alk is sufficient for FasIII activation, we expressed UAS-*Alk* (an *Alk* transgene under the control of the yeast Gal4 upstream activating sequence) using the mesodermal *twist-Gal4* driver. Indeed, we found that Alk induced ectopic expression of FasIII (Fig. 4, compare B and D), supporting the idea that Alk controls FasIII expression. This is an exciting possibility, as the forkhead-domain transcription factor, *Drosophila* FoxF/Biniou, has been reported to drive expression of visceral mesoderm markers, including FasIII (Perez Sanchez *et al.*, 2002; Zaffran *et al.*, 2001), and *Drosophila* FoxF/Biniou

Fig. 2 | A role for *Drosophila* Anaplastic lymphoma kinase during gut development. (A–F) In wild-type (WT) embryos Anaplastic lymphoma kinase (Alk) protein is expressed throughout visceral mesoderm development, as visualized by anti-Alk polyclonal antibodies. (A) Stage 10, lateral view. (B) Stage 11, lateral view. (C) Stage 12, lateral view. (D) Stage 13, lateral view. (E) Stage 14, ventral view. (F) Stage 15, ventral view. (G,H) *Alk* mutants are unable to eat. (G) Heterozygous sibling first-instar larva. (H) *Alk*¹ mutant first-instar larva. *Alk* mutants were identified by the absence of Kruppel–GFP (green fluorescent protein). (I,J) *Alk* mutants show severe visceral mesoderm defects. (I) Heterozygous sibling embryo, stage 15, ventral view. (J) *Alk*¹ mutant embryo, stage 15, ventral view. *Alk* mutant embryos were identified by the absence of a *lacZ* balancer. Embryos are orientated with the anterior to the left.

could potentially be activated by an Alk RTK signalling pathway, as Alk is a member of the Insulin receptor RTK superfamily (Loren *et al.*, 2001). As we saw induction of FasIII expression on Alk expression, we carefully re-examined *Alk* mutant embryos. Using confocal microscopy, we were able to locate scattered Alk-positive cells in *Alk*¹ mutant animals (Fig. 4H). This is possible as the anti-Alk antibodies are raised against the extreme amino-terminal end of Alk and therefore detect the Alk¹ truncated protein (see supplementary information online). On closer inspection, these cells are also seen to be weakly FasIII-positive (Fig. 4J). Thus, although FasIII expression seems to be significantly reduced in visceral mesoderm cells in *Alk* mutants, it is not absent. Nevertheless, full FasIII expression may still require Alk signalling through a FoxF/Biniou-mediated pathway, especially as it has been reported that there may be a positive-feedback pathway that reinforces FasIII expression (Zaffran *et al.*, 2001).

In previous work, we proposed that *Drosophila* Alk is the RTK responsible for the activation of extracellular-signal-related protein kinase (ERK) that is seen in the visceral mesoderm (Gabay *et al.*, 1997; Lorén *et al.*, 2001). The availability of *Alk* mutant embryos allowed us to formally test this. Analysis of *Alk* mutants showed that diphospho-ERK (dp-ERK) is absent from the visceral arches at stage 11 (Fig. 4, compare K and L). Thus, we conclude that the *Drosophila* Alk RTK is indeed crucial for ERK activation and for downstream ERK-mediated events in the visceral mesoderm.

Fig. 3 | *Drosophila* Anaplastic lymphoma kinase is crucial for visceral mesoderm development. Visceral mesoderm does not form in *Anaplastic lymphoma kinase* (*Alk*) mutant animals, as visualized by loss of several visceral mesoderm markers. Ventral views of stage 15 embryos are shown. (A) Wild-type (WT) embryo stained with anti-Alk. (B) WT embryo stained with anti-FasIII. (C) *Alk*¹ mutant embryo stained with anti-Alk. (D) *Alk*¹ mutant embryo stained with anti-FasIII. (E) WT embryo stained with anti-Alk. (F) WT embryo stained with anti-Mef2 (Myocyte enhancer factor 2). (G) *Alk*¹ mutant embryo stained with anti-Alk. (H) *Alk*¹ mutant embryo stained with anti-Mef2. (I) WT embryo stained with anti-Alk. (J) WT embryo stained with anti-MHC (myosin heavy chain). (K) *Alk*¹ mutant embryo stained with anti-Alk. (L) *Alk*¹ mutant embryo stained with anti-MHC. *Alk* mutant embryos were identified by the absence of a *lacZ* balancer.

We have shown here that the endogenous Alk RTK has a function in controlling gut development in *Drosophila*. The targets of Alk-mediated signalling remain to be discovered. The exciting possibility exists that Alk is the receptor for the newly discovered Jelly belly protein, which has been shown to be required for mesoderm migration and differentiation (Weiss *et al.*, 2001). Exploring these Alk pathways and targets is an important task for the future.

Fig. 4 | *Drosophila* Anaplastic lymphoma kinase regulation of Fasciclin III and extracellular-signal-related kinase in the visceral mesoderm. (A–D) Animals overexpressing Anaplastic lymphoma kinase (Alk) show induction of ectopic Fasciclin III (FasIII). Lateral views of stage 12 embryos are shown. (A) *Twist-Gal4* embryo stained with anti-Alk. (B) WT embryo stained with anti-FasIII. (C) *Twist-Gal4; UAS-Alk* embryo stained with anti-Alk (red). (D) *Twist-Gal4; UAS-Alk* embryo stained with anti-FasIII. (E–J) Alk-positive cells are scattered in *Alk*¹ mutant embryos. (E) WT embryo stained with anti-Alk. (F) WT embryo stained with anti-FasIII. (G) WT embryo stained with anti-FasIII. (H) Merge of (E) and (F). (I) *Alk*¹ mutant embryo stained with anti-Alk. (J) *Alk*¹ mutant embryo stained with anti-FasIII. (K) Merge of (H) and (J). (K–L) diphospho-extracellular-signal-related kinase (dp-ERK) staining of stage 11 embryos. (K) WT embryo. (L) *Alk*¹ mutant embryo. ERK activation was detected in the arches of the visceral mesoderm in WT embryos but not in the *Alk*¹ mutant embryos (indicated by arrows). Ventral views are shown. *Alk* mutant embryos were identified by the absence of a *lacZ* balancer. UAS, yeast Gal4 upstream activating sequence.

METHODS

Fly stocks. Standard *Drosophila* husbandry procedures were followed. All *Drosophila* strains were maintained on standard cornmeal-molasses medium. The *UAS-Alk* transgenic fly line has been described previously (Loren *et al.*, 2001). The following stocks were obtained from the Bloomington Stock Center: E10-2-40 (stock number 10815), *l(2)06503*, *P{neoFRT}42D* (stock number 2120), *Twist-Gal4* (stock number 2517), *w;TM3SbΔ23/Dr*, *CyO Kr-GFP* (stock number 5194) and *CyO Wg-LacZ*.

Isolation of *Alk* mutants. *Df(2R)AlkΔ21* was isolated using a designer-deletion approach (Cooley *et al.*, 1990). Briefly, two transposable elements flanking the *Alk* locus (10815 and *l(2)06503*) were recombined onto the same chromosome using standard genetic techniques. P-element mobilization followed by quantitative Southern blot analysis was used to identify deletion of the *Alk* locus, resulting in the creation of an *Alk* deficiency, *Df(2R)AlkΔ21*.

Subsequently, *Alk* mutants were isolated as follows: EMS mutagenesis of *P{neoFRT}42D* flies was carried out as described in Grigliatti (1998). Male flies were starved for 12 h before treatment with 25 mM EMS/1% sucrose for 12 h, and were mated to *ES/cy* virgin females. After 3–4 days, mutagenized males were discarded to ensure that only postmeiotic chromosomes were tested. Male *w¹/cy* progeny (each being potentially mutagenized within the deficiency region) were mated with virgin females of genotype *Df(2R)AlkΔ21*. In total, 4,760 *w¹/cy* males were tested. Sixty-eight fly strains were obtained that failed to complement *Df(2R)AlkΔ21*.

Complementation analysis was performed between the 68 fly strains obtained, leading to the identification of 8 independent complementation groups. *Alk* mutants were identified by sequence analysis. Genomic DNA from heterozygous adult flies was prepared, and sequence analysis was carried out. *Alk* mutants were identified from double peaks in the sequencing trace, and this was subsequently confirmed by cloning into the TOPO vector (Invitrogen) followed by further sequence analysis with vector-specific primers. These sequences showed single peaks in the sequencing trace, corresponding to either mutant or wild-type DNA.

Larval eating-assay. *Alk* mutant fly lines were balanced over *CyO Kr-GFP* (which carries a fusion of *Kruppel* (*Kr*) to the gene encoding green fluorescent protein (GFP)) to allow the identification of mutant first-instar larvae. Embryos were collected and raised on food that had been pre-coloured with food dye. *Alk* mutant larvae were identified by the absence of *Kr-GFP* expression.

Immunostaining. Embryos and larval brains were fixed and immunostained as described in Loren *et al.* (2001). The following primary antibodies were used: rabbit anti-β-galactosidase (diluted 1:1,500; Cappel), mouse anti-FasIII (monoclonal antibody 7G10; diluted 1:50) and monoclonal antibody 22C10 (diluted 1:20), the latter two from the Developmental Studies Hybridoma Bank, rabbit anti-Mef2 (diluted 1:500; kindly provided by B. Paterson), mouse anti-MHC (diluted 1:5) and mouse anti-dp-ERK (diluted 1:500; Sigma). The following secondary antibodies were used: biotin-conjugated goat anti-mouse IgG (diluted 1:1,000), Cy3-conjugated donkey anti-guinea-pig (diluted 1:200), Cy3-conjugated goat anti-mouse IgG (diluted 1:1,000), Cy2-conjugated goat anti-mouse IgG (diluted 1:1,000, all from Jackson Laboratories), and Cy2-conjugated goat anti-rabbit IgG (diluted

1:1,000; Amersham). Signals were developed using the Vectastain Elite ABC Kit (Vector Laboratories). Embryos were cleared in methyl salicylate (Sigma) before visualization. Embryo staging was carried out according to Campos-Ortega & Hartenstein (1997).

Anti-*Alk* antibodies. DNA encoding *Drosophila* *Alk* amino acids 30–316 was subcloned into pHIS8-3 to generate a 39-kDa extracellular HIS₆-*Alk* fusion protein. The HIS₆-*Alk* fusion protein was induced and purified from *Escherichia coli* (BL21(DE3)) bacterial lysates by standard protocols using Ni-NTA agarose (Qiagen). The resulting eluted HIS₆-*Alk* recombinant protein was used for rabbit and guinea-pig immunizations. Polyclonal antibodies against HIS₆-*Alk* were used at 1:1,000 for immunofluorescence analysis.

Other methods. SDS-polyacrylamide gel electrophoresis was carried out according to Laemmli (1970). Protein concentrations were determined by the method of Bradford (1976). Standard molecular techniques were used for DNA manipulation (Sambrook *et al.*, 1989).

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

R.H.P. is the recipient of grant 621-2001-1624 from the Swedish Research Council. This work was also supported by: the Swedish Society for Medical Research (SSMF), Åke Wibergs Fund, the Royal Swedish Academy of Sciences, Lars Hiertas Minne Fund and the Cancer Research Fund of Northern Sweden. T.H. is a Frank and Else Schilling American Cancer Society Research Professor.

REFERENCES

- Beckmann, G. & Bork, P. (1993) An adhesive domain detected in functionally diverse receptors. *Trends Biochem. Sci.*, **18**, 40–41.
- Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.
- Campos-Ortega, J.A. & Hartenstein, V. (1997) *The Embryonic Development of Drosophila melanogaster*. Springer, Berlin, Germany.
- Cooley, L., Thompson, D. & Spradling, A.C. (1990) Constructing deletions with defined endpoints in *Drosophila*. *Proc. Natl Acad. Sci. USA*, **87**, 3170–3173.
- Duyster, J., Bai, R.Y. & Morris, S.W. (2001) Translocations involving anaplastic lymphoma kinase (ALK). *Oncogene*, **20**, 5623–5637.
- Falini, B. (2001) Anaplastic large cell lymphoma: pathological, molecular and clinical features. *Br. J. Haematol.*, **114**, 741–760.
- Fujimoto, J., Shiota, M., Iwahara, T., Seki, N., Satoh, H., Mori, S. & Yamamoto, T. (1996) Characterization of the transforming activity of p80, a hyperphosphorylated protein in a Ki-1 lymphoma cell line with chromosomal translocation t(2;5). *Proc. Natl Acad. Sci. USA*, **93**, 4181–4186.
- Gabay, L., Seger, R. & Shilo, B.Z. (1997) MAP kinase *in situ* activation atlas during *Drosophila* embryogenesis. *Development*, **124**, 3535–3541.
- Grigliatti, T.A. (1998) in *Drosophila: A Practical Approach* (ed. Roberts, D.B.), 55–84. Oxford Univ. Press, Oxford, UK.
- Iwahara, T., Fujimoto, J., Wen, D., Cupples, R., Bucay, N., Arakawa, T., Mori, S., Ratzkin, B. & Yamamoto, T. (1997) Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. *Oncogene*, **14**, 439–449.
- Jiang, Y.P., Wang, H., D'Eustachio, P., Musacchio, J.M., Schlessinger, J. & Sap, J. (1993) Cloning and characterization of R-PTP-κ, a new member of the receptor protein tyrosine phosphatase family with a proteolytically cleaved cellular adhesion molecule-like extracellular region. *Mol. Cell. Biol.*, **13**, 2942–2951.
- Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.

- Lilly, B., Galewsky, S., Firulli, A.B., Schulz, R.A. & Olson, E.N. (1994) D-MEF2: a MADS box transcription factor expressed in differentiating mesoderm and muscle cell lineages during *Drosophila* embryogenesis. *Proc. Natl Acad. Sci. USA*, **91**, 5662–5666.
- Loren, C.E., Scully, A., Grabbe, C., Edeen, P.T., Thomas, J., McKeown, M., Hunter, T. & Palmer, R.H. (2001) Identification and characterization of DAlk: a novel *Drosophila* melanogaster RTK which drives ERK activation *in vivo*. *Genes Cells*, **6**, 531–544.
- Morris, S.W., Kirstein, M.N., Valentine, M.B., Dittmer, K.G., Shapiro, D.N., Saltman, D.L. & Look, A.T. (1994) Fusion of a kinase gene, *ALK*, to a nucleolar protein gene, *NPM*, in non-Hodgkin's lymphoma. *Science*, **263**, 1281–1284.
- Morris, S.W., Naeve, C., Mathew, P., James, P.L., Kirstein, M.N., Cui, X. & Witte, D.P. (1997) *ALK*, the chromosome 2 gene locus altered by the t(2;5) in non-Hodgkin's lymphoma, encodes a novel neural receptor tyrosine kinase that is highly related to leukocyte tyrosine kinase (LTK). *Oncogene*, **14**, 2175–2188.
- Nguyen, H.T. & Xu, X. (1998) *Drosophila* *mef2* expression during mesoderm development is controlled by a complex array of *cis*-acting regulatory modules. *Dev. Biol.*, **204**, 550–566.
- Nguyen, H.T., Bodmer, R., Abmayr, S.M., McDermott, J.C. & Spoerl, N.A. (1994) *D-mef2*: a *Drosophila* mesoderm-specific MADS box-containing gene with a biphasic expression profile during embryogenesis. *Proc. Natl Acad. Sci. USA*, **91**, 7520–7524.
- Perez Sanchez, C., Casas-Tinto, S., Sanchez, L., Rey-Campos, J. & Granadino, B. (2002) DmFoxF, a novel *Drosophila* fork head factor expressed in visceral mesoderm. *Mech. Dev.*, **111**, 163–166.
- Sambrook, J., Fritsch, E. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.
- Weiss, J.B., Suyama, K.L., Lee, H.H. & Scott, M.P. (2001) Jelly belly: a *Drosophila* LDL receptor repeat-containing signal required for mesoderm migration and differentiation. *Cell*, **107**, 387–398.
- Yoshikawa, S., Bonkowsky, J.L., Kokel, M., Shyn, S. & Thomas, J.B. (2001) The derailed guidance receptor does not require kinase activity *in vivo*. *J. Neurosci.*, **21**, RC119.
- Zaffran, S., Kuchler, A., Lee, H.H. & Frasch, M. (2001) Biniou (FoxF), a central component in a regulatory network controlling visceral mesoderm development and midgut morphogenesis in *Drosophila*. *Genes Dev.*, **15**, 2900–2915.