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An ABC transporter controls export of a *Drosophila* germ cell attractant

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Directed cell migration, which is critical for embryonic development, leukocyte trafficking and cell metastasis, depends on chemoattraction. HMG-CoA reductase regulates the production of an attractant for *Drosophila* germ cells that may itself be geranylated. Chemoattractants are commonly secreted through a classical, signal peptide-dependent pathway, but a geranyl-modified attractant would require an alternative pathway. In budding yeast, pheromones produced by α -cells are farnesylated and secreted in a signal peptide-independent manner, requiring the ABC transporter Ste6p. Here, we show that germ cell migration uses a similar pathway, demonstrating that germ cells, like yeast, are attracted to lipid-modified peptides. Components of this unconventional export pathway are highly conserved, suggesting that this pathway may control the production of similarly modified chemoattractants from yeast to man.

In most organisms germ cells migrate from their site of origin to the somatic part of the gonad where they develop into egg and sperm. In *Drosophila*, germ cells migrate as single cells in a stereotyped manner and are guided by repellent and attractive cues toward the somatic gonad in the mesoderm (1). HMG-CoA reductase (3-hydroxyl-3-methyl-glutaryl-CoA reductase or Hmgcr) activity controls germ cell attraction to the mesoderm and recruitment of germ cells to the somatic gonad. In *hmgcr* mutant embryos germ cells fail to reach the somatic gonad; moreover, ectopic Hmgcr expression is sufficient to attract germ cells to a new location (2). Embryos mutant for several enzymes in the *hmgcr* pathway, including the β subunit of type I geranylgeranyl transferase, β GGTI, are similarly defective in germ cell migration, suggesting that geranylation is critical in attracting germ cells to the mesoderm (3).

Because *hmgcr* or *β ggtI* mutant embryos show a rather specific germ cell migration defect, we favored the idea that the Hmgcr pathway was required to geranylate a critical germ cell attractant rather than regulating a pathway that controlled synthesis or secretion of the attractant (3). This idea posits secretion of a geranyl modified germ cell attractant, which would preclude secretion by a classic signal-peptide dependent secretory pathway and require an alternative export mechanism. Such a mechanism has been described in yeast, where ABC transporters export farnesylated pheromones required for cell mating (4,5). We therefore asked whether a similar export mechanism exists in *Drosophila* and is required for germ cell attraction.

ABC transporters are conserved from bacteria to human and shuttle hydrophobic, lipophilic compounds in an ATP dependent manner (6,7). In *S. cerevisiae* and *S. pombe*, the ABCB family members Ste6 and Mam1 play essential roles in the export of farnesyl-modified α -type

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Sentence summarizing most important point of paper: A HMGCoA reductase-based, secreted germ cell chemoattractant in *Drosophila* uses a non-conventional ABCB transporter system for export; conservation of the processing and export machinery from yeast to man suggests widespread use of such chemoattractants.

and M-type mating factors, respectively. The human ABCB family member *mdr1* (*multi-drug-resistant*) gene is amplified in multi-drug resistant cells and its homolog *mdr3* is a functional homolog of *STE6* (8,9), suggesting a close relationship between the ability of this class of transporters to export drugs and lipid modified signaling molecules. To determine whether ABCB transporters have a role in exporting the putative *Drosophila* germ cell attractant, we analyzed expression patterns and germ cell migration in embryos mutant for ABCB transporters (7) (Table S1). Among these, only *mdr49* showed an expression pattern and mutant phenotype consistent with a role in germ cell migration (Fig 1A, Fig S1, Table S2). We generated a strong loss of function allele, *mdr49*^{δ3.16}, (Fig 1B, Fig S1A, Table S2), and embryos mutant for *mdr49*^{δ3.16} showed defects in germ cell migration, like mutants in the Hmgcr pathway: germ cells migrated through the posterior midgut but then failed to associate with the somatic gonad (Fig 1C), which was properly specified (Fig S1E). This migration phenotype was only observed in *mdr49* mutants and not in mutants for other ABCB transporters, such as *Mdr50*, *Mdr65* and *CG7955* (Table S1). To determine whether *mdr49* function is required in the mesoderm we restored *mdr49* expression selectively in the mesoderm (Fig. S1F) and found that it fully rescued the *mdr49* mutant phenotype (Fig. 1D). To test whether *Mdr49* acts as an ABCB transporter we asked whether *Ste6p* rescued the germ cell migration phenotype observed in *mdr49* embryos. Expressing *STE6* in the mesoderm rescued the migration defect (Figs. 1D, S1F), suggesting that *Mdr49* is functionally equivalent to *Ste6p* and acts in mesodermal cells to attract germ cells.

The germ cell migration phenotypes caused by mutations in *Mdr49* and in components of the Hmgcr pathway, such as Geranylgeranyl-Diphosphate Synthase and βGGTI, are strikingly similar (2,3). Thus, to determine whether *Mdr49* acts as a transporter for a germ cell attractant that is geranyl-geranyl modified by the Hmgcr pathway, we tested the genetic epistasis between *hmgcr* and *mdr49*. Previous experiments showed that over-expression of *hmgcr* in the central nervous system (CNS) is sufficient to attract germ cells to this tissue (2). We therefore reasoned that *Mdr49* function should be necessary for the export of the ectopically produced attractant and that mutations in *mdr49* should suppress the *hmgcr* misexpression phenotype. Indeed, germ cell migration to the CNS was suppressed by reducing *mdr49* copy number using either the *mdr49* deficiency or P-element mutation. (Fig 2 and Table S3). Mutations in other ABCB transporters, such as *mdr50*, *mdr65* and *CG7955* did not significantly suppress the *hmgcr* overexpression phenotype (Table S1), consistent with a specific role for *Mdr49* as an exporter for an Hmgcr-dependent geranyl-modified germ cell attractant.

Prenylated proteins require additional modifications to be fully functional: cleavage of the AAX residues, prenyl proteolysis, and carboxymethylation (10) (Fig 3A). We therefore asked whether the enzymes that catalyze these reactions are found in *Drosophila* and are required for germ cell migration. Prenyl proteolysis is catalyzed by *Ste24p* (prenyl protease type I) and *Rce1p* (prenyl protease type II), with the former being essential for a-factor modification (11,12). Carboxymethylation is achieved exclusively through the activity of *Ste14p* (13). Orthologues of these enzymes are also found in mammals where they have similar substrates (Table S4). A genomic search in *Drosophila* revealed predicted orthologs for: (1) prenyl protease type I as a cluster of genes *CG9000/CG9001/CG9002*, which we term *Dmel/Ste24*, (2) prenyl protease type II termed *Sras*, and (3) the isoprenylcysteine carboxylmethyltransferase as *CG11268*, termed *Dmel/Ste14* (Table S4, Fig S2).

We next assessed the role of each enzyme in germ cell migration. Since specific mutations were not available for these enzymes we analyzed deficiencies that deleted each gene (as well as other genes (14)). Embryos homozygous mutant for *Df(2R)Dmel/ste24*, which deletes the three *Drosophila ste24* genes, showed a specific germ cell migration phenotype similar to that of embryos mutant for *mdr49*. On average, six germ cells did not associate with the somatic gonad (Fig 3B and Table S2). Next, we asked whether the single *Drosophila* isoprenylcysteine

carboxylmethyltransferase, Dmel\ste14, is required for germ cell migration. We were unable to analyze the germ cell migration phenotype of embryos homozygous for the deletion *Df(3L)ED4486 (Df(3L)Dmel\ste14)* due to embryonic patterning defects, which resembled those observed in mutants for Drosophila Rho GTPases and gave additional evidence that Dmel\ste14 encodes a general post-prenylation processing enzyme that modifies all prenylated proteins (15–17) (Fig S4). Instead of analyzing homozygous mutants we asked whether reducing Dmel\ste14 gene dosage was able to suppress germ cell mis-migration induced by ectopic expression of *hmgcr* in the CNS. As observed for the ABC transporter *mdr49*, reducing Dmel\ste14 gene dosage significantly suppressed Hmgcr-dependent ectopic germ cell migration, suggesting a requirement of this enzyme in attractant modification (Fig 3C, Table S5).

Our genetic analyses strongly suggest that a geranylgeranylated germ cell attractant is produced and modified in the somatic gonad and exported via an ABCB transporter. In order to more directly test for the production of a molecule that can act as a diffusible attractant, we devised an *in vitro* germ cell migration assay. We used FACS-sorted germ cells from 2–10 hour old embryos that expressed Moesin-GFP specifically in germ cells (18). Germ cells were placed in the upper well of a transwell chemotaxis chamber and scored for their migration toward Kc cell-conditioned medium (19), in the lower well (Fig. 4A). Germ cells migrated to the bottom well containing conditioned medium from cells overexpressing *hmgcr* and *mdr49* (Fig. 4B). Fewer cells moved toward unconditioned control medium or medium conditioned from parental Kc cells, which express low levels of *hmgcr* and *mdr49* (19) (Fig S5A). To determine whether migration toward parental Kc cells is dependent upon *hmgcr* and *mdr49* we used RNA interference (RNAi) to reduce the expression of Hmgcr pathway members in Kc cells. We found that reducing *hmgcr*, geranylgeranyl transferase 1 (*βGGT1*) and ABC transporter (*mdr49*) expression fully blocked germ cell migration toward Kc cell-conditioned medium (Fig 4C, Fig S5B). These results are consistent with our genetic data and support the notion that the prenylated Drosophila germ cell attractant is active in a secreted form.

To test whether the classic, signal-peptide dependent pathway is also required for secretion of the germ cell attractant, we used RNAi to reduce the levels of *syntaxin 5*, which encodes a SNARE essential for constitutive secretion (20). Germ cells were similarly attracted to conditioned medium from parental cells and *syntaxin 5* deficient KC cells, indicating that production of the attractant does not depend upon constitutive secretion (Figs. 4C, S5A). Our results demonstrate that the Drosophila germ cell attractant is geranylgeranylated and secreted by mesodermal cells in a signal peptide-independent manner through an ABCB transporter. The modifications, processing and export pathway used to generate and secrete the germ cell attractant strikingly resemble that of a-factor in mating yeast.

ABC transporters have been studied mostly in the context of cancer drug resistance or their role in toxin protection. Our findings reveal a new function for this conserved, non-conventional secretory pathway in a multicellular organism and suggest that this pathway may be used to export signals required for cell-cell communication in organisms other than Drosophila and yeast (21–23). Yeast lacks many of the secreted cell signaling molecules such as Hh, Wnt, and BMP used by multicellular organisms to communicate between cells. The use of a prenylated signal may thus be an ancient mechanism of cell communication. It is striking that this pathway is used in yeast and flies to facilitate the migration and adhesion of germ cells, the essential cells for reproduction.

Supplementary Material

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References and Notes

1. Santos AC, Lehmann R. *Curr Biol* Jul 27;2004 14:R578. [PubMed: 15268881]
2. Van Doren M, Broihier HT, Moore LA, Lehmann R. *Nature* Dec 3;1998 396:466. [PubMed: 9853754]
3. Santos AC, Lehmann R. *Dev Cell* Feb;2004 6:283. [PubMed: 14960281]
4. Christensen PU, Davey J, Nielsen O. *Mol Gen Genet* Jun;1997 255:226. [PubMed: 9236781]
5. McGrath JP, Varshavsky A. *Nature* Aug 3;1989 340:400. [PubMed: 2569166]
6. Higgins CF. *Annu Rev Cell Biol* 1992;8:67. [PubMed: 1282354]
7. Dean M, Hamon Y, Chimini G. *J Lipid Res* Jul;2001 42:1007. [PubMed: 11441126]
8. Raymond M, Gros P, Whiteway M, Thomas DY. *Science* Apr 10;1992 256:232. [PubMed: 1348873]
9. Roninson IB, et al. *Proc Natl Acad Sci U S A* Jun;1986 83:4538. [PubMed: 3459187]
10. Winter-Vann AM, Casey PJ. *Nat Rev Cancer* May;2005 5:405. [PubMed: 15864282]
11. Tam A, et al. *J Cell Biol* Aug 10;1998 142:635. [PubMed: 9700155]
12. Boyartchuk VL, Ashby MN, Rine J. *Science* Mar 21;1997 275:1796. [PubMed: 9065405]
13. Hrycyna CA, Sapperstein SK, Clarke S, Michaelis S. *Embo J* Jul;1991 10:1699. [PubMed: 2050108]
14. Materials and Methods.
15. Johndrow JE, Magie CR, Parkhurst SM. *Biochem Cell Biol* Dec;2004 82:643. [PubMed: 15674432]
16. Settleman J. *Dev Cell* Sep;2001 1:321. [PubMed: 11702944]
17. Magie CR, Meyer MR, Gorsuch MS, Parkhurst SM. *Development* Dec;1999 126:5353. [PubMed: 10556060]
18. Sano H, Renault AD, Lehmann R. *J Cell Biol* Nov 21;2005 171:675. [PubMed: 16301333]
19. Echaliier G, Ohanessian A. *C R Acad Sci Hebd Seances Acad Sci D* Mar 31;1969 268:1771. [PubMed: 4976834]
20. Bard F, et al. *Nature* Feb 2;2006 439:604. [PubMed: 16452979]
21. Schinkel AH. *Semin Cancer Biol* Jun;1997 8:161. [PubMed: 9441946]
22. Smit JJ, et al. *Cell* Nov 5;1993 75:451. [PubMed: 8106172]
23. Leung GK, et al. *J Biol Chem* Aug 3;2001 276:29051. [PubMed: 11399759]

Figure 1.

The ABC transporter *mdr49* is required for germ cell migration to the mesoderm. (A) in situ hybridization to wild-type embryos shows *mdr49* mRNA expression (purple) specifically in mesodermal tissues (B) RT-PCR of *mdr49*^{Δ3.16} allele (3), compared to a revertant line (2) or wild-type embryos (1) (rp49 loading control). Embryos in C and D are stage 14, germ cells marked by Vasa (brown). (C) *mdr49* phenotype. *mdr49*^{Δ3.16} embryo (bottom) shows lost germ cells (top). Quantification of different *mdr49* mutant lines (blue: 0–3 germ cells; red: 4–6 germ cells; yellow: 7–9 germ cells; green: 10–12 germ cells; black: >12 germ cells). Genotypes are homozygous or as indicated. (D) Rescue of *mdr49*^{Δ3.16} phenotype by expression of the yeast gene *ste6* or Drosophila *mdr49* in the mesoderm [1- *twi*gal4, *mdr49*^{Δ3.16}/CyO (n=77); 2-

twigal4, *mdr49* ^{$\delta^{3.16}$} (n=194); 3- twigal4, *mdr49* ^{$\delta^{3.16}$} ; UAS*mdr49*/+ (n=123); 4- twigal4, *mdr49* ^{$\delta^{3.16}$} ; UAS*STE6*/+ (n=92)] (** p<0.0001, * p<0.0005).

Figure 2.

Hmgcr dependent germ cell migration to the CNS is suppressed by reducing *mdr49* activity. All embryos are stage 13. (a) In more than 50% of embryos overexpressing *hmgcr* in the CNS >5 germ cells (brown) migrate to CNS (between lines). Ectopic migration is suppressed when *mdr49* dosage is reduced either using the deficiency (b) or a P-element mutation (c). Quantification is shown above (germ cells in CNS: blue: 0–3 germ cells, light blue: 4–5 germ cells; magenta: > 5 germ cells).

Figure 3.

Prenyl proteases Type I and isoprenylcysteine carboxymethyltransferases are required for germ cell migration to the mesoderm.

(A) A schematic of the prenyl processing pathway analyzed. CAAX represents the prenylation motif to which a farnesyl (F) or geranylgeranyl (GG) group is added.

(B) Embryos carrying a deficiency of *Dmel\ste24* show germ cells (brown) lost in the posterior mesoderm. Sibling control (top) and mutant (bottom) embryos are shown at left and quantification is shown on the right (blue: 0–3 germ cells; red: 4–6 germ cells; yellow: 7–9 germ cells; green: 10–12 germ cells; black: >12 germ cells).

(C) *Df(3L)Dmel\ste14* suppresses *hmgcr* dependent ectopic germ cell migration. Sibling control (top) and suppressed (bottom) embryos at left, quantification on the right (germ cells at ectopic sites, blue: 0–5 germ cells; light blue: 6–10 germ cells; magenta: > 10 germ cells). All embryos are at stage 14/15.

Figure 4.

Germ cells migrate towards a diffusible attractant. (A) Scheme of transwell migration assay procedure. (a) The box represents the germ cell population collected (b, c) FACS sorted germ cell population is homogenous and expresses germ cell markers: germ cell-specific *Egfp-moe* from *P_{nos}::egfp-moe::nos* 3'UTR transgene is expressed at the membrane (green b, c) and the *Vasa* germ cell marker in cytoplasm (c, red). (B) Quantification of migration towards serum-free media (control), Kc cells and Kc cells overexpressing *hmgcr* and *mdr49*. * $p < 0.001$, ** $p < 0.0001$ (C) Quantification of migration towards attractant in control and RNAi knock-down experiments, *** $p < 0.001$, ** $p < 0.005$, * $p < 0.01$, Kc+ds *syx5*, $p = 0.010$. All experiments repeated at least 3 times, in triplicate.