

P_{GALT} promoter on p426GAL1 vector (22) was induced with 0.1 μ M β -estradiol with a Gal4.ER.VP16 chimeric protein (23). Ten-milliliter cell cultures were harvested after 10 hours of induction (absorbance at 600 nm = 0.5 to 1) and labeled for 30 s with 35 S-EXPRESS (0.7 mCi/ml; New England Nuclear). Cycloheximide and NEM (final concentrations of 0.5 mg/ml and 0.1 M, respectively) were then added, cell extracts were prepared from 0.2 ml of the resulting sample, and immunoprecipitations were carried out as described (17), with antibodies to ha (12CA5 Boehringer), β gal (Promega), and/or FLAG (M2; Eastman Kodak), as appropriate. Total handling time to the beginning of cell lysis was 45 s. In the eukaryotic cell types examined, the rate of translation is 2 to 10 residues per second (24). Assuming a rate of five residues per second in *S. cerevisiae*, this pulse is seven times shorter than the duration of translation of the fusion protein. Immunoprecipitates were fractionated by 13% SDS-polyacrylamide gel electrophoresis, and band intensities were quantitated by PhosphorImager (Molecular Dynamics).

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14. The procedure was as in (7), except that cells were labeled for 30 min with 0.11 mCi of 35 S-EXPRESS, then pelleted, resuspended in 0.8 ml of ice-cold lysis buffer, and processed for immunoprecipitation (17).
15. For example, with Re κ - β gal, where C/A was 0.52 in wild-type cells and 0.86 in *ubr1 Δ* , the level of the Ubr1p-dependent cotranslational degradation was $100\% \times (1 - 0.52/0.86) = 40\%$.
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25. We thank F. L  vy and N. Johnsson for their contributions to early stages of this work and R. Deshaies, T. Iverson, T.-M. Yi, and members of the Varshavsky laboratory for helpful discussions and comments on the manuscript. This study was supported by a grant to A.V. from NIH. G.T. was supported in part by Amgen.

15 March 2000; accepted 31 July 2000

A Function for Kinesin I in the Posterior Transport of *oskar* mRNA and Stauf  n Protein

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The asymmetric localization of messenger RNA (mRNA) and protein determinants plays an important role in the establishment of complex body plans. In *Drosophila* oocytes, the anterior localization of *bicoid* mRNA and the posterior localization of *oskar* mRNA are key events in establishing the anterior-posterior axis. Although the mechanisms that drive *bicoid* and *oskar* localization have been elusive, oocyte microtubules are known to be essential. Here we report that the plus end-directed microtubule motor kinesin I is required for the posterior localization of *oskar* mRNA and an associated protein, Stauf  n, but not for the anterior-posterior localization of other asymmetric factors. Thus, a complex containing *oskar* mRNA and Stauf  n may be transported along microtubules to the posterior pole by kinesin I.

The *Drosophila* oocyte provides an excellent model system for studies of how the localization of mRNAs helps to establish complex body plans (1, 2). During the late stages of oogenesis, *bicoid* (*bcd*) and *oskar* (*osk*) mRNAs are localized to the anterior and posterior poles of the oocyte, respectively. Subsequent localized expression of Bicoid protein is necessary for anterior patterning, whereas localized expression of Oskar is necessary for posterior patterning and germ cell development. Genes required for the localization of *bcd* mRNA (e.g., *exuperantia* and *swallow*) and *osk* mRNA (e.g., *staufer* and *orb*) have been identified (1, 2), but the mechanisms of localization are not well understood.

Before stage 7 of oogenesis, the oocyte nucleus and a microtubule organizing center

(MTOC) are positioned at the posterior pole, generating a high concentration of microtubule minus ends near the posterior cortex (3–6). During stages 7 and 8, MTOC components switch to the anterior end, resulting in the reorganization of microtubules to place plus ends toward the posterior cortex (3–6). The oocyte nucleus then migrates from the posterior to the anterior end in a microtubule-dependent manner (1, 2, 7).

The localization of *bcd* and *osk* mRNAs between stages 8 and 12 is sensitive to microtubule-depolymerizing drugs, consistent with active transport of the mRNAs along microtubules by motor proteins (5, 8, 9). According to this model, *bcd* is moved to the anterior cortex by minus end-directed motors, such as dyneins, and *osk* is moved to the posterior cortex by plus end-directed motors, such as kinesins. Indeed, a dynein light chain can bind directly to Swallow, a putative RNA-binding protein necessary for anterior *bcd* localization (10).

If *osk* is transported to the posterior pole along microtubules, one or more of the more than 20 *Drosophila* kinesin superfamily members (11) might drive the movement. We focused our attention on *Kinesin heavy chain* (*Khc*) and its encoded protein KHC. KHC is the force-producing subunit of the tetrameric adenosine triphosphatase kinesin I (conventional kinesin), which in *Drosophila* and other metazoan animals serves as a motor for plus end-directed fast axonal transport of membranous organelles (12).

To determine if kinesin I is involved in oocyte patterning, we used mitotic recombination to generate mosaic female flies that contained clones of homozygous *Khc* null germ line stem cells (13–16). Western blots confirmed the depletion of KHC in embryos produced from such germ line clones (Fig. 1) (17). The production of eggs and embryos by the mosaic females suggests that germ line stem cells can proliferate and proceed through oogenesis without kinesin I. However, embryogenesis failed, despite fertilization by wild-type males. Most embryos arrested before blastoderm formation, but a few proceeded into early gastrulation stages. This maternal lethal effect was completely rescued by a wild-type *Khc* transgene (14, 18). Thus, germ line expression of KHC was required for normal embryogenesis, contrary to previous results from studies of a hypomorphic temperature-sensitive *Khc* allele (13).

Examination of embryos that reached the blastoderm stage revealed an absence of pole cells, the germ line precursors. To assay for earlier defects, we examined the distributions of *osk* and *bcd* mRNAs in *Khc* null oocytes (19). The localization of *bcd* mRNA was normal (Fig. 2, A and B), concentrated at the anterior during stages 8 to 10 (193 observed). In contrast, the localization of *osk* mRNA was defective (Fig. 2, C to F). It normally accumulates transiently at the anterior pole early in stage 8 and then moves to the poste-

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rior pole (20, 21). In *Khc* null stage 8 to 10 egg chambers, *osk* mRNA accumulated excessively at the anterior pole and was never concentrated at the posterior pole (72 observed). This localization defect was completely rescued by a wild-type *Khc* transgene. Thus, although KHC was not required for anterior localization of either *bcd* or *osk*, it was required for the posterior localization of *osk*. Perhaps kinesin I transports *osk* mRNA along microtubules toward their plus ends and the posterior pole.

Fig. 1. Depletion of KHC in embryos produced from germ line stem cell clones. Embryos from wild-type female flies (left lane) or females that carried *Khc*²⁷/*Khc*²⁷ germ line clones (right lane) were homogenized and tested for the presence of KHC by Western blotting with anti-KHC (76). Anti-actin staining (Act) showed that the two lanes had equivalent protein loadings.

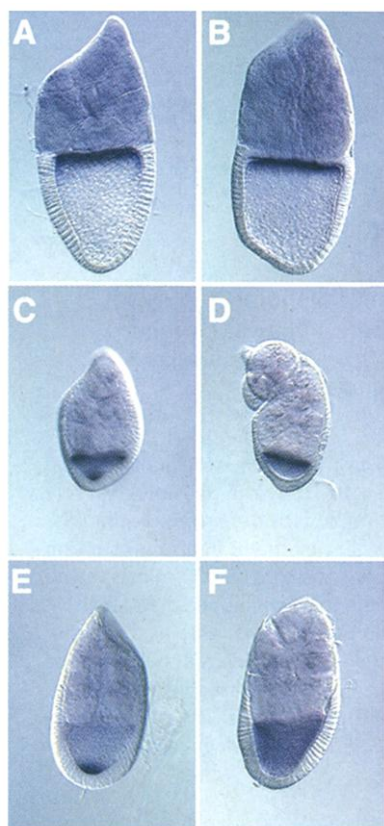
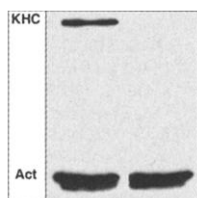


Fig. 2. Localization of anterior-posterior mRNA determinants in *Khc* null oocytes. In situ hybridization was used to test the distribution of *bcd* and *osk* mRNAs. *bcd* is shown in (A) stage 10A wild-type and (B) stage 10B *Khc*²⁷/*Khc*²⁷ egg chambers. *osk* is shown in (C) stage 8 wild-type, (D) stage 8 *Khc*²⁷/*Khc*²⁷, (E) stage 9 wild-type, and (F) stage 9 *Khc*²⁷/*Khc*²⁷ egg chambers.

Given that microtubule-disrupting drugs prevent the posterior localization of *osk* mRNA during oogenesis (5, 8), we considered the possibility that the absence of KHC blocks *osk* localization indirectly by disturbing oocyte microtubules. The shift of the oocyte nucleus from posterior to anterior poles during stage 6, which is microtubule-dependent (7), appeared normal in *Khc* null oocytes. Furthermore, the anterior localization of the MTOC component Centrosomin was normal (Fig. 3, A and B). As described above, the anterior localization of *bcd* mRNA, which is microtubule-dependent (9), was not affected by the loss of KHC.

We tested microtubule organization further by localizing a hybrid protein composed of the motor domain of KHC fused to a reporter enzyme, β -galactosidase (β -Gal). KHC:: β -Gal is thought to localize in regions of cells with high concentrations of microtubule plus ends (22). It is important to note that this chimeric protein did not rescue patterning defects in *Khc* null oocytes. In wild-type stage 9 to 10a oocytes, KHC:: β -Gal concentrated at the posterior pole in 79 cases (Fig. 3C) and elsewhere in 2 cases. In *Khc* null oocytes, KHC:: β -Gal concentrated at the posterior pole in 11 instances (Fig. 3C) and elsewhere in 4 instances. This suggests that in 73% of the stage 9 to 10a null oocytes with detectable amounts of KHC:: β -Gal, microtubule plus ends were concentrated at the posterior pole. This, and the indications that microtubules in the anterior end are normal, suggests that *osk* mRNA mislocalization in *Khc* null oocytes is not due to a disruption of

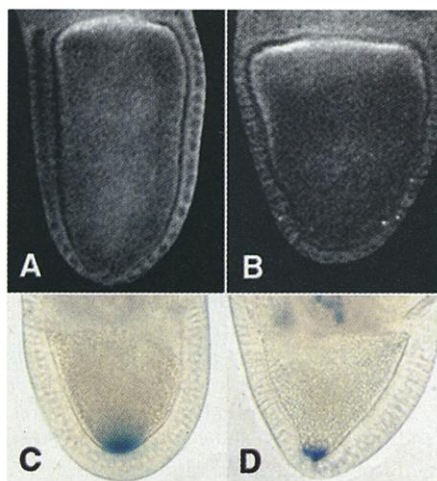


Fig. 3. Polarity of the oocyte microtubule cytoskeleton in *Khc* null oocytes. The localization of MTOC material was tested with antibodies to Centrosomin. (A) Wild-type and (B) *Khc*²⁷/*Khc*²⁷ stage 10A oocytes. The orientation of microtubule plus ends was tested by localizing a KHC:: β -Gal fusion protein. (C) Wild-type and (D) *Khc*²⁷/*Khc*²⁷ late stage 9 egg chambers. The wild-type genotype included two copies of the fusion gene, whereas the mutant included one copy.

microtubule organization. In *Khc* null oocytes, KHC:: β -Gal staining was often not detected in oocytes, although it was visible in nurse cells. Perhaps efficient transport of the hybrid protein from nurse cells to oocyte requires the presence of native KHC.

Orb protein is concentrated along the posterior and lateral oocyte cortex during stages 7 to 9 (23). It is thought to hold *osk* mRNA at the posterior pole and help regulate its translation (24, 25). To determine if the *osk* mislocalization in *Khc* null oocytes was secondary to a mislocalization of Orb, we stained with antibodies to Orb (anti-Orb) (22). The distribution of Orb in *Khc* null oocytes was normal (Fig. 4). Thus, the failure of *osk* localization may be due to a defect in *osk* transport rather than in posterior retention.

Posterior transport of *osk* mRNA is thought to depend on Stauf protein (26). Stauf is transiently localized at the anterior end of the oocyte during stage 8 where it may form a complex with *osk* mRNA (27). If kinesin I transports such *osk*-Stauf complexes along microtubules to the posterior pole, then Stauf protein should be mislocalized in *Khc* null oocytes. Immunostaining with anti-Stauf (28) confirmed this prediction (Fig. 5). In wild-type stage 8 to 10 oocytes, Stauf concentrated at the anterior end early, appeared in granules along the cortex, and then concentrated at the posterior end. Granular Stauf distribution

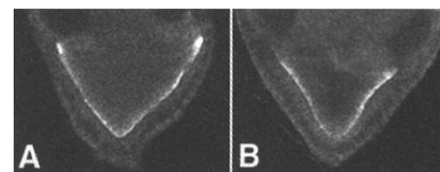


Fig. 4. Distribution of Orb, an *osk* mRNA binding protein that may anchor *osk* to the posterior cortex. Anti-Orb was used to stain stage 9 egg chambers. (A) Wild-type; (B) *Khc*²⁷/*Khc*²⁷.

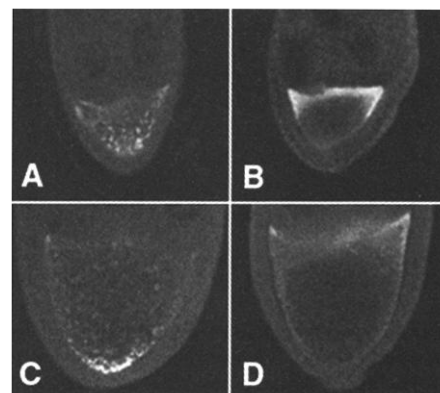


Fig. 5. Distribution of Stauf, an *osk* mRNA binding protein thought to be required for posterior *osk* transport. Anti-Stauf was used to stain egg chambers. (A) Stage 8 wild type, (B) stage 8 *Khc*²⁷/*Khc*²⁷, (C) stage 9 wild type, and (D) stage 9 *Khc*²⁷/*Khc*²⁷.

was detected in 61 of the 69 oocytes observed. This is consistent with the hypothesis that Staufen and *osk* mRNA form complexes at the anterior cortex that are transported to the posterior pole. In *Khc* mutant oocytes, Staufen protein overaccumulated in the anterior end during stage 8, was not detected in granules, and did not concentrate at the posterior pole (>90 oocytes observed). Normal Staufen distribution patterns were restored in *Khc* null oocytes by the addition of a wild-type *Khc* transgene (18).

Thus, KHC, the force-generating component of the plus end-directed microtubule motor kinesin I, is required for the posterior localization of both *osk* mRNA and Staufen protein. The participation of kinesin I in this mRNA motility process could be direct. It might attach specifically to *osk*-Staufen complexes at the anterior pole and transport them toward the posterior pole. However, our initial tests for coimmunoprecipitation of KHC and Staufen from *Drosophila* ovary cytosol have not revealed any robust association, so perhaps the linkage is less direct. It is generally accepted that kinesin I transports membranous organelles toward microtubule plus ends (12). Thus, *osk* and Staufen could localize to the posterior pole by virtue of association with mitochondria or other organelles carried by kinesin I. An alternative to these models is derived from the effect of a loss of KHC on the particulate staining pattern of Staufen (Fig. 5). Before stages 7 to 8, while microtubules are still oriented with their plus ends toward the anterior, kinesin I might deliver to the cortex materials necessary for the assembly of transport-competent *osk*-Staufen complexes. Thus, the lack of visible Staufen particles in *Khc* null oocytes may indicate that their assembly or persistence depends on kinesin I activity. New studies, using green fluorescent protein tags to follow the localization dynamics of *osk* mRNA, Staufen, and organelles, may distinguish between these models and provide further insight into the mechanisms that drive the movements of maternal determinants for early developmental patterning.

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18. All defects detected in *Khc* null oocytes and embryos were rescued by a transgenic copy of the wild-type *Khc* gene (13). This was determined by generating germ line clones in females carrying the genotype *y w P{hs-FLP}/ w; P{w⁺, FRT}42B, P{Ovo^{D1}}55D / P{w⁺, FRT}42B c Khc²⁷; P{w⁺, Khc⁺}/ +*.
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22. *Khc* null clones were induced in females as described (15) with the addition of a third chromosome carrying *P{Khc::LacZ}* (5). Localization of KHC::β-Gal activity in oocytes was performed according to Ghiglione et al. [*Cell* **96**, 847 (1999)]. Centrosomin was localized as described by Roth et al. [S. Roth, F. S. Neuman-Silberberg, G. Barcelo, T. Schupbach, *Cell* **81**, 967 (1995)] using anti-CNN at 1:800 dilution (6). Orb was localized with undiluted mouse anti-Orb as described (23, 25).
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28. Ovarioles were fixed in 200 μl of 2% paraformaldehyde, 0.5% NP-40 in phosphate-buffered saline (PBS) mixed with 600 μl of heptane (20 min), rinsed in PBS–0.2% Tween-20, and incubated in rabbit anti-Staufen (25) at 1:1600 dilution (overnight at 4°C). After extensive rinsing, ovarioles were stained with fluorescein-conjugated goat anti-rabbit immunoglobulin G (Jackson Laboratory, Bar Harbor, ME) at 1:500. All fluorescence images were generated with a Bio-Rad MRC600 scanning confocal fluorescence microscope.
29. Supported by NIH grant R01GM46295 (W.M.S.).

25 May 2000; accepted 25 August 2000

Role of Brain Insulin Receptor in Control of Body Weight and Reproduction

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Insulin receptors (IRs) and insulin signaling proteins are widely distributed throughout the central nervous system (CNS). To study the physiological role of insulin signaling in the brain, we created mice with a neuron-specific disruption of the IR gene (NIRKO mice). Inactivation of the IR had no impact on brain development or neuronal survival. However, female NIRKO mice showed increased food intake, and both male and female mice developed diet-sensitive obesity with increases in body fat and plasma leptin levels, mild insulin resistance, elevated plasma insulin levels, and hypertriglyceridemia. NIRKO mice also exhibited impaired spermatogenesis and ovarian follicle maturation because of hypothalamic dysregulation of luteinizing hormone. Thus, IR signaling in the CNS plays an important role in regulation of energy disposal, fuel metabolism, and reproduction.

Insulin receptors (IR) are expressed in most tissues of the body, including classic insulin-sensitive tissues (liver, muscle, and fat), as well as “insulin-insensitive” tissue, such as red blood cells and the neuronal tissue of the CNS. In the CNS, the IR displays distinct patterns of expression in the olfactory bulb, the hypothalamus, and the pituitary (1–3), although its function in these regions remains

largely unknown. Previous experiments have suggested a role for insulin signaling in the regulation of food intake (4, 5) and neuronal growth and differentiation (6, 7). Moreover, insulin has been shown to regulate neurotransmitter release and synaptic plasticity (8, 9), and dysregulation of insulin signaling in the CNS has been linked to the pathogenesis of neurodegenerative dis-