

# Conserved signals and machinery for RNA transport in *Drosophila* oogenesis and embryogenesis

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**Localization of cytoplasmic messenger RNA transcripts is widely used to target proteins within cells. For many transcripts, localization depends on *cis*-acting elements within the transcripts and on microtubule-based motors; however, little is known about other components of the transport machinery or how these components recognize specific RNA cargoes. Here, we show that in *Drosophila* the same machinery and RNA signals drive specific accumulation of maternal RNAs in the early oocyte and apical transcript localization in blastoderm embryos. We demonstrate *in vivo* that Egalitarian (Egl) and Bicaudal D (BicD), maternal proteins required for oocyte determination, are selectively recruited by, and co-transported with, localizing transcripts in blastoderm embryos, and that interfering with the activities of Egl and BicD blocks apical localization. We propose that Egl and BicD are core components of a selective dynein motor complex that drives transcript localization in a variety of tissues.**

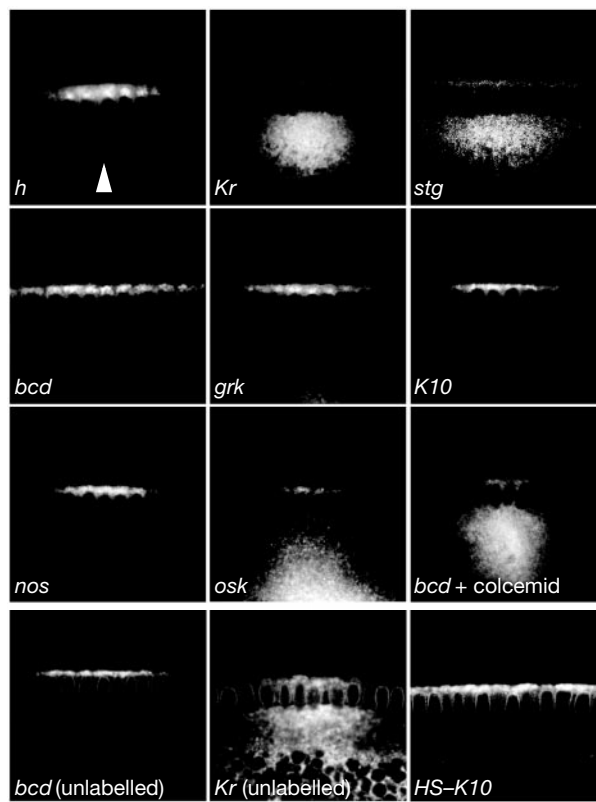
During *Drosophila* oogenesis, specification of the oocyte is associated with selective accumulation of RNA determinants supplied by the neighbouring, interconnecting ovarian nurse cells<sup>1,2</sup>. Subsequently, deposition of mRNA transcripts at selected sites within the oocyte leads to localized translation of the proteins that establish the prospective embryonic body axes. *gurken* (*grk*) transcripts reside first posteriorly and then anterodorsally, and sequentially establish the anteroposterior and dorsoventral axes<sup>3-5</sup>. *bicoid* (*bcd*) and *oskar* (*osk*) transcripts localize to the anterior and posterior of the oocyte, respectively, to pattern the anteroposterior body axis<sup>6-8</sup>.

Asymmetric RNA localization is also evident during zygotic development, especially in the unicellular syncytial blastoderm embryo. At this stage, several transcripts including those of the pair-rule and *wingless* (*wg*) segmentation genes lie exclusively apically of the layer of several thousand peripheral nuclei. Localization of these transcripts seems to be mediated by signals within their 3' untranslated regions (UTRs)<sup>9-11</sup>, and to be driven on microtubules<sup>10</sup> by the minus-end-directed molecular motor, dynein<sup>12</sup>. However, the linkers and other factors that provide the cargo specificity are unknown. Nor is it clear if transcript localization in blastoderm embryos relates to that in other types of cells.

Previously, we reported rapid apical localization of fluorescently labelled *fushi tarazu* (*ftz*) pair-rule transcripts injected into the basal cytoplasm of the cycle 14 blastoderm embryo<sup>10</sup>. Although these experiments indicated a requirement for nuclear proteins, more recently we, and others<sup>12</sup>, have found that fluorescein labelling compromises the structure of the transcripts, and that pair-rule (*even-skipped*, *hairy* (*h*), *ftz*, *paired* and *runt*) and *wg* transcripts labelled with several other fluorochromes localize apically within 5–8 min without the need for exogenous protein (Fig. 1 and Table 1; see also Methods). Indeed, injected unlabelled transcripts also localize apically (Fig. 1; see also Methods). The protein-free assay retains specificity for apical transport, as transcripts that are normally unlocalized (*Krüppel* (*Kr*), *huckebein*) or enriched in the basal cytoplasm (*string* (*stg*)) are not transported apically and instead diffuse away from the site of injection (Fig. 1 and Table 1).

## Shared localization signals and machinery

We used the injection assay to investigate whether any maternal transcripts that localize in the oocyte are recognized by the localiza-



**Figure 1** Localization of zygotic and maternal transcripts in blastoderm embryos. Confocal images of representative blastoderm embryos injected with various transcripts. Injected RNA is excluded from the layer of peripheral nuclei. Preinjection with colcemid inhibits localization of injected *bcd* transcripts (right panel, third row). The left and middle panels of the bottom row show apical localization of injected unlabelled *bcd* but not *Kr* transcripts, respectively. Apical localization of endogenously expressed *K10* transcripts (induced by heat shock; *HS-Kr* transcripts do not localize; not shown) is shown in the bottom row, right panel. Transcripts were visualized by fluorescence (top three rows) or by *in situ* hybridization and nuclear staining with Alexa 660-labelled WGA (bottom row). Arrowhead in the top left panel indicates approximate site of injection. Apical is to the top and basal to the bottom in this and all subsequent figures. Scale bar, 50  $\mu$ m.

**Table 1 Summary of transcript injections**

Transcript	Without colcemid	With colcemid	Recruitment of Egl and BicD
<i>even-skipped</i>	85 (40)	ND	ND
<i>ftz</i>	92 (122)	7* (15)	+
<i>h</i>	93 (83)	0 (18)	+
<i>paired</i>	92 (26)	ND	ND
<i>runt</i>	100 (25)	ND	ND
<i>wg</i>	97 (33)	ND	+
<i>huckebein</i>	0 (43)	N/A	ND
<i>Kr</i>	0 (36)	N/A	-
<i>stg</i>	0 (52)	N/A	-
<i>bcd</i>	100 (32)	18* (39)	+
<i>grk</i>	91 (77)	22* (23)	+
<i>K10</i>	100 (63)	20* (25)	+
<i>nos</i>	96 (26)	36* (22)	+
<i>osk</i>	77* (75)	14* (22)	+/-†
<i>bcd4496G → U</i>	22* (60)	ND	-
<i>Kstem5'</i>	5* (22)	N/A	-
<i>Kstem5'3'</i>	100* (28)	ND	ND
<i>Kr-K10TLS</i>	100 (21)	ND	+
<i>stg-K10TLS</i>	100 (39)	ND	+

Numbers indicate the percentage of embryos showing localization; numbers in parentheses indicate the number of embryos. N/A, not applicable; ND, not determined.

\*Weak localization (see text and *osk* transcript localization in Fig. 1).

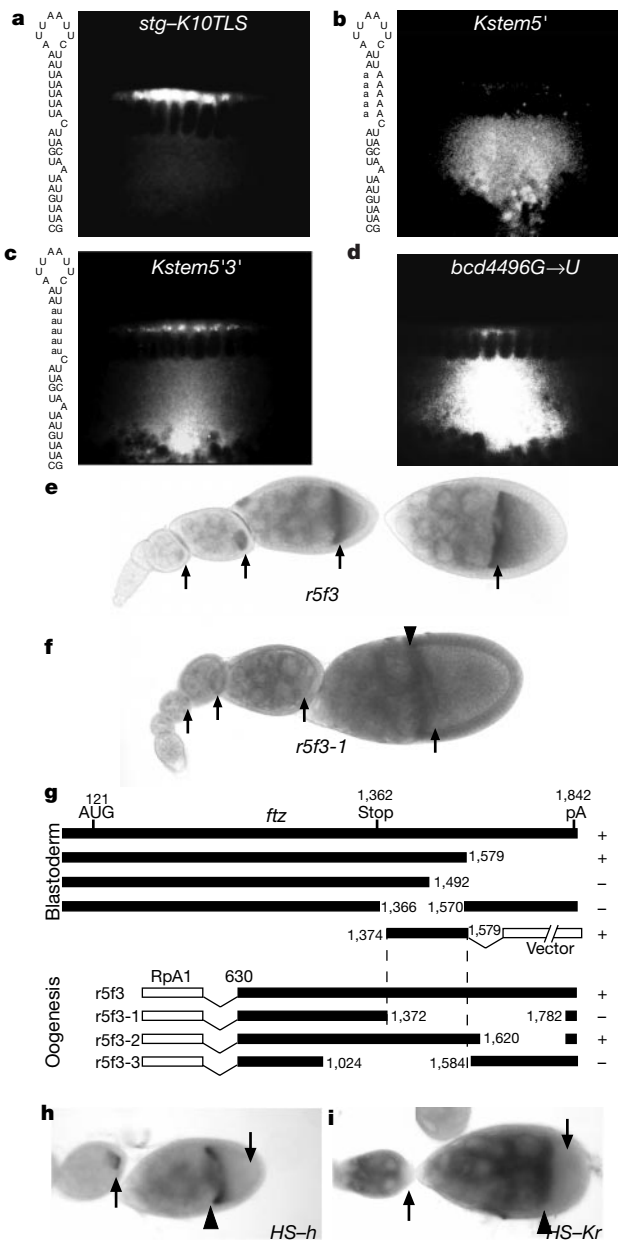
†Weak recruitment, only to localizing transcripts.

tion machinery of blastoderm embryos. We tested five such transcripts (*bcd*, *grk*, *nanos* (*nos*), *osk* and *female sterile (1) K10* (*K10*)), and found that all accumulate in the apical cytoplasm after injection (Fig. 1 and Table 1). With the exception of *osk* transcripts—only a small proportion of which localize apically—the efficiency of localization of these transcripts appears indistinguishable from that of pair-rule transcripts. Maternal transcripts also localize apically when zygotically expressed from endogenous transgenes (Fig. 1, *K10*; *bcd* 3' UTR<sup>9</sup>). Preinjection with colcemid severely inhibits apical localization of the injected maternal transcripts, indicating that their localization in blastoderm embryos, like that of the pair-rule transcripts<sup>10,12</sup>, is dependent on intact microtubules (Fig. 1 and Table 1).

Further experiments show that the same signals mediate transcript transport during oogenesis and apical localization in blastoderm embryos. We focused on transcripts of the *K10* gene, which localize through a 44-nucleotide region of the 3' UTR (transport/localization sequence; *K10TLS*)<sup>13</sup>—the shortest signal thus far shown to be active during oogenesis. This signal, which is predicted to form a stem-loop structure (Fig. 2a), mediates all aspects of *K10* transcript localization, that is, transport of transcripts from the nurse cells into the oocyte from stage 2 and localization at its anterior pole between stages 8–10 (ref. 13).

We found that the *K10TLS* is sufficient to drive apical localization in blastoderm embryos. Reporter *stg* and *Kr* transcripts into which we inserted the *K10TLS* (*stg-K10TLS* and *Kr-K10TLS*) localize apically in a way that is indistinguishable from pair-rule transcripts (Fig. 2a and Table 1). Moreover, the same regulatory signals are used for transcript localization during oogenesis and in the embryo. A 5-nucleotide transversion in the *K10* transcript that disrupts base pairing of the *K10TLS* stem-loop (Fig. 2b) abolishes all aspects of localization during oogenesis (*Kstem5'*; ref. 13), and prevents *K10* transcripts from localizing in our blastoderm injection assay (Fig. 2b and Table 1). *Kstem5'3'*, in which compensatory mutations restore base pairing in the stem (Fig. 2c), directs weak but significant localization in embryos (Fig. 2c and Table 1). The same signal also partially restores localization during oogenesis<sup>13</sup>. These results suggest that the same machinery is used in both cases.

The common aspect of maternal RNA localization measured in our experiments is unlikely to be transport within the oocyte, because the maternal transcripts tested are distinctly distributed in late stage oocytes by means of different motors and accessory factors<sup>2</sup>. However, all the transcripts—with the possible exception of *grk*<sup>14</sup>—are synthesized in adjacent nurse cells and reach the



**Figure 2** Conservation of RNA localization signals during oogenesis and embryogenesis. **a–c**, Predicted secondary structures (left) and correspondingly injected embryos (right) of the wild-type *K10* transport/localization sequence (*K10TLS*) in the context of a *stg* reporter transcript (**a**), *Kstem5'* in which the primary sequence is altered but the stem-loop is disrupted (**b**) and *Kstem5'3'* in which primary sequence is altered but the stem-loop is restored (**c**). **b** and **c** are in the context of the *K10* 3' UTR. Modified bases in secondary structures are indicated in lower-case letters. **d**, *bcd4496G→U* transcripts in which apical localization is abolished (compare with Fig. 1). Apical localization is efficient in **a** and weak but significant in **c**. **e**, Maternal *ftz* transcripts (*r5f3*) localize within and at the anterior of the oocyte. **f**, *r5f3-1* transcripts that lack the *ftz* 3' UTR do not accumulate specifically during oogenesis. Transcript accumulation anterior to the oldest oocyte is in somatic follicle cells (arrowhead), perhaps because of missing somatic transcript instability elements<sup>16</sup>. **g**, *ftz* constructs tested ( $n \approx 20$  embryos,  $\approx 100$  egg chambers) for transcript localization in blastoderm embryos or during oogenesis. Plus and minus signs indicate presence or absence of apical/oocyte-specific localization, respectively. The minimal signal in blastoderm embryos between positions 1,374 and 1,579 (S.L.B., unpublished observations) is also required for localization in ovaries. The vector tag RNA (1.5-kb fragment of pCR2.1TOPO, Invitrogen; see Methods) is not otherwise able to localize. **h**, **i**, Oocyte-specific localization of heat-shock-induced *h*, but not *Kr*, transcripts that are excluded from the oocyte (arrowhead indicates anterior border of oocyte). Egg chambers are positioned with anterior and earliest stages to the left; oocytes are labelled by arrows. Scale bar: **a–d**, 50  $\mu\text{m}$ ; **e**, **f**, **h**, **i**, 150  $\mu\text{m}$ .

oocyte by transport along microtubules<sup>2</sup>. To test whether this process is analogous to apical localization in blastoderm embryos, we injected a *bcd* transcript containing a single nucleotide change (4496G→U), which prevents early oocyte-specific transport (stages 4–6) without disrupting later (stage 6 onwards) import of transcripts into the oocyte or their subsequent accumulation at the anterior cortex<sup>15</sup>. This mutation inhibits apical *bcd* localization in blastoderm embryos (Fig. 2d and Table 1), suggesting that transcripts localize in our injection assay through the same machinery that transports transcripts into the early oocyte.

This proposal is supported by our finding that pair-rule transcripts accumulate in the early oocyte if synthesized ectopically during oogenesis. *r5f3* females express a hybrid transcript containing a portion of the *ftz* coding sequence and the entire 3' UTR under the control of the constitutively active *RpA1* promoter<sup>16</sup>. *r5f3* transcripts accumulate specifically in the oocyte from stage 3 (Fig. 2e), and concentrate at the anterior cortex of the oocyte between stages 8 and 10B, after which they become distributed throughout the oocyte. This pattern of localization is indistinguishable from that of *K10* transcripts and closely follows the distribution of the minus ends of microtubules<sup>17</sup>. Localization of *r5f3* is dependent on an intact microtubule cytoskeleton, as it is inhibited by prior treatment with colchicine<sup>18</sup> (not shown). A hybrid *ftz* transcript (*r5f3-1*) lacking the 3' UTR, and therefore the signal for apical localization in blastoderm embryos<sup>9</sup>, is retained in the nurse cells and not transported to the oocyte (Fig. 2f).

These results indicate that blastoderm localization signals can drive transcript transport during oogenesis. This view is supported by more detailed analysis of maternally expressed pair-rule transcripts. Our injection assay reveals a minimum region between positions 1,374 and 1,579 in *ftz* that is necessary and sufficient for localization in blastoderm embryos (Fig. 2g and S.L.B., unpublished results). A similar region of *ftz* seems to be required for localization of transcripts into the oocyte (Fig. 2g). Furthermore, *h* (Fig. 2h) and *runt* (data not shown) transcripts, driven maternally by the *Hsp70* promoter, also accumulate specifically in the oocyte and later reside at its anterior cortex, whereas *Kr* or truncated *h* transcripts lacking most of the 3' UTR (*HSH21*; ref. 19) fail to localize either in blastoderm embryos<sup>9</sup> or during oogenesis (Fig. 2i and data not shown).

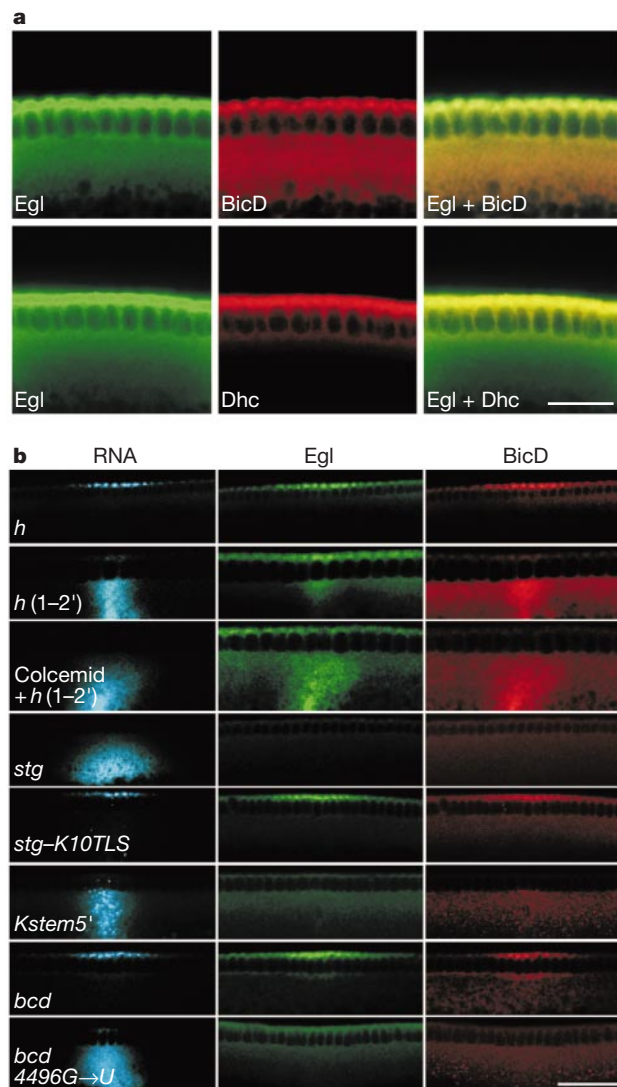
### Recruitment of Egl and BicD to localizing transcripts

The above data suggest that components of the blastoderm localization machinery are also likely to function in RNA transport into the early oocyte. Genetic screens for maternal mutations that affect formation of the embryonic axis have identified *egl* and *BicD* as genes required for oocyte differentiation and for specific RNA accumulation in the oocyte<sup>20–25</sup>. However, their exact activities are uncertain. *BicD* includes multiple heptad repeats<sup>26,27</sup>, which may mediate oligomerization and interactions with other proteins; *Egl* includes a domain shared with 3'–5' exonucleases<sup>28</sup>. During oogenesis, these two proteins form complexes together<sup>24</sup> and colocalize at the minus ends of microtubules<sup>24,29</sup>. The integrity of the microtubule cytoskeleton is defective in *egl* and *BicD* mutants, which has been proposed to explain subsequent defects in RNA localization<sup>23,29</sup>. Alternatively, *Egl* and *BicD* might act directly in RNA transport<sup>24,30</sup>. However, evidence that distinguishes between these two possibilities is lacking.

We first examined whether *Egl* and *BicD* are present in early embryos. Both proteins are supplied maternally to the embryo. They are noticeably enriched apically to the nuclei at blastoderm stages (Fig. 3a) where they colocalize with dynein heavy chain (*Dhc*; Fig. 3a)—a component of the motor associated with apical transcript transport<sup>12</sup>. Nevertheless, a large proportion of both of the proteins is present in the basal cytoplasm.

We next tested whether endogenous *Egl* and *BicD* can associate with injected localizing transcripts, as might be expected if they are

components of the RNA localization machinery. Injection of *h* transcripts leads to marked enrichment of *Egl* and *BicD* protein levels at the sites of RNA localization (Fig. 3b). Similar results are found on injection of the other tested maternal and zygotic localizing transcripts (*ftz*, *bcd*, *grk*, *K10*, *nos*, *osk* and *wg*) (Fig. 3b and Table 1). Both proteins accumulate basally at the site of injection within 1–2 min (Fig. 3b). Protein recruitment is not inhibited in embryos preincubated with colcemid, showing that it is not dependent on intact microtubules (Fig. 3b). Thus, the proteins are recruited locally before transport and are transported together apically with transcripts.



**Figure 3** Colocalization of *Egl*, *BicD* and *Dhc* proteins and recruitment of *Egl* and *BicD* to localizing transcripts. **a**, Immunostaining of cycle 14 blastoderm embryos for *Egl* and *BicD* (top panel, green and red, respectively) and for *Egl* and *Dhc* (bottom panel, green and red, respectively). All three proteins colocalize apically of the nuclei. Merged images are on the right. **b**, *Egl* (green) and *BicD* (red) protein distribution in embryos injected with localizing (*h*, *stg-K10TLS*, *bcd*) or non-localizing (*stg*, *Kstem5'*, *bcd4496G-U*) RNAs (blue), or preinjected with colcemid 10 min before RNA injection. There are increased levels of *Egl* and *BicD* at the sites of RNA localization. Embryos were fixed 5–8 min after RNA injection, except in the second and third rows when they were fixed 1–2 min after injection. Elevated concentrations of transcripts were used in these experiments to emphasize protein enrichment over injection site (Methods). *BicD* and *Egl* are also recruited to unlabelled localizing transcripts (not shown). Scale bar: **a**, 40 μm; **b**, 100 μm (except for the 2nd and 3rd rows where it represents 60 μm).

Interaction of injected transcripts with Egl/BicD is mediated by intact localization signals: we detected protein recruitment to *Kr-K10TLS* and *stg-K10TLS*, but not to *Kr*, *stg*, *Kstem5'* and *bcd4496G→U* (Fig. 3b and Table 1), or to a *h* transcript containing a 21-base-pair (bp) deletion within the localization signal that abolishes localization (S.L.B. and D.I.-H., unpublished data). When localization is weak, we only detected recruitment of Egl and BicD by transcripts that have localized apically (for example, *osk*; data not shown). The above results suggest that only transcripts that bind Egl/BicD can be transported apically.

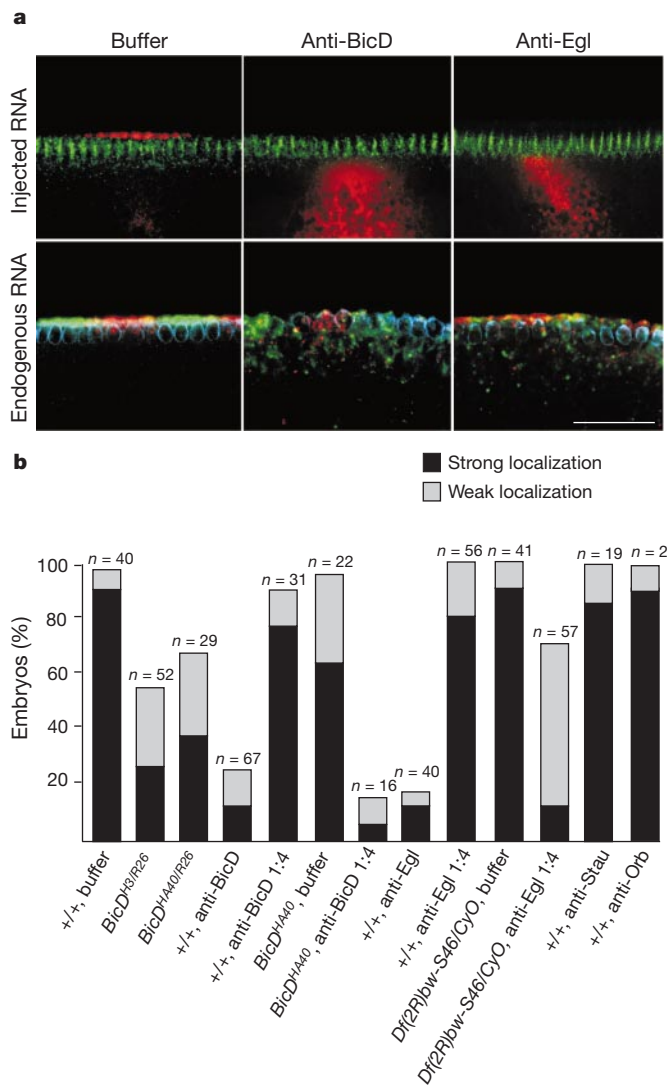
**Egl and BicD are required for apical RNA localization**

We next examined whether BicD and Egl are required for apical localization in blastoderm embryos. Strong *BicD* alleles block oogenesis early<sup>21,25–27</sup>, and weaker mutant mothers that lay fertilized eggs (*BicD<sup>HA40</sup>/BicD<sup>R26</sup>* and *BicD<sup>H3</sup>/BicD<sup>R26</sup>*; see Methods<sup>31</sup>) retain sufficient BicD activity for a normal apical distribution of endogenous pair-rule transcripts (data not shown). However, the reduced BicD activity in these embryos no longer supports efficient transport of injected transcripts: 62% of *BicD<sup>HA40</sup>/BicD<sup>R26</sup>* and 73% of *BicD<sup>H3</sup>/BicD<sup>R26</sup>* embryos show no or weak localization 5–8 min after injection, compared with 10% of wild-type embryos (Fig. 4b).

Moreover, we show that an antibody against BicD blocks RNA transport. Preinjection into the basal cytoplasm of anti-BicD antibody 4C2 (ref. 21) strongly inhibits the localization of injected *h* (Fig. 4a, b), *ftz*, *grk* and *stg-K10TLS* (data not shown) transcripts in 70–75% of embryos, whereas injection buffer alone has no effect on localization (Fig. 4a, b). Control anti-Staufen (Stau) and anti-Orb antibodies do not affect localization (Fig. 4b). The microtubule cytoskeleton is not obviously affected by the brief (≤20 min) antibody treatment (Fig. 4a), indicating that the effects on RNA transport are probably direct. Injection of anti-BicD antibody (but not injection buffer, anti-Orb or anti-Stau; Fig. 4a and not shown) prevents apical localization of endogenous pair-rule transcripts, also leading to anteroposterior smearing of their distribution (Fig. 4a). Thus, apical transcript localization seems to be important in restricting the range of activity of pair-rule genes, and allowing their combinatorial control of *Drosophila* segmentation.

To confirm that the antibody acts specifically on BicD, we assessed its blocking activity in embryos from mutant mothers (*BicD<sup>HA40</sup>*) that only express 6% of wild-type BicD protein levels<sup>31</sup> (Methods). This level of BicD production is sufficient for full maternal and zygotic function<sup>31</sup>, and permits normal apical localization of endogenous pair-rule transcripts in embryos (data not shown), although localization of injected transcripts is impaired in 32% of embryos (Fig. 4b). Notably, *BicD<sup>HA40</sup>* embryos are sensitive to dilutions of anti-BicD antibody that have little effect on wild-type embryos. For example, a 1:4 dilution of antibody inhibits the localization of injected transcripts in 94% of *BicD<sup>HA40</sup>* embryos compared with 23% of wild-type embryos (Fig. 4b). This demonstrates that the antibody inhibits localization by interacting with BicD, and confirms that BicD is required for transcript localization in blastoderm embryos.

Injecting blastoderm embryos with anti-Egl<sup>24</sup> also inhibits apical localization of both exogenous and endogenous pair-rule transcripts, without overtly disrupting the microtubule network (Fig. 4a, b). Moreover, its effect is more potent in embryos from mothers containing only a single copy of the *egl* gene (*Df(2R)bw-S46/CyO*; Fig. 4b), indicating that the antibody disrupts RNA localization by inhibiting the activity of Egl. Egl and BicD are probably also involved in transporting other cargoes. The arrangement of peripheral nuclei is disrupted after injection of antibodies to either of the two proteins, consistent with previous data showing a requirement for BicD in nuclear migration in eye imaginal disc cells<sup>32</sup>. Embryos injected with either antibody undergo abnormal morphogenesis (data not shown), which is also indicative of Egl and BicD transporting additional cargoes.



**Figure 4** Requirement for BicD and Egl for apical transcript localization in the blastoderm. **a**, Distribution of injected (*h* (red)) and two endogenous (*ftz* (red) and *h* (green)) transcripts after preinjection with injection buffer alone (buffer) or antibodies against BicD or Egl. Both antibodies block apical localization and lead to accumulation of injected and endogenous RNA in the basal cytoplasm. Top,  $\beta$ -tubulin (green) distribution (enriched between the nuclei) is not obviously affected by either antibody. Bottom, nuclear envelopes are stained with Alexa 660-conjugated WGA (blue). In control embryos, pair-rule transcripts lie in circumferential stripes 3–4 nuclei wide. Pair-rule transcripts have a broader distribution after antibody injection. Nuclear organization is disrupted after antibody injection. Scale bar, 50  $\mu$ m. **b**, Percentage of embryos displaying strong, weak or no localization of injected *h* transcripts (see Methods for classification). Localization is impaired in embryos from mothers with reduced BicD levels (*BicD<sup>H3</sup>/BicD<sup>R26</sup>*, *BicD<sup>HA40</sup>/BicD<sup>R26</sup>*) or after injection with anti-BicD or anti-Egl. Diluted antibodies block localization more effectively in embryos with reduced BicD (*BicD<sup>HA40</sup>*) or Egl (*Df(2R)bw-S46/CyO*) levels. Results are the total of two–three separate experiments. *n* is the number of embryos injected. Similar results were found with injected *ftz* transcripts (data not shown).

**Discussion**

Our results indicate that Egl and BicD are principal elements of a complex that transports RNA in blastoderm embryos. Egl and BicD appear to be present as pools of excess cytoplasmic protein that associate selectively with localizing transcripts and are transported together apically. Protein recruitment occurs before transport and does not require microtubule integrity; rather, transport depends on Egl and BicD activity. Egl and BicD probably act directly to mediate RNA transport associated with establishment and

maintenance of the oocyte. Thus, mutant transcripts that are defective in export from nurse cells into the oocyte fail to recruit Egl or BicD in blastoderm embryos. *grk* transcripts are also recognized by the Egl–BicD–microtubule transport pathway, which is consistent with the hypothesis that nurse cells are a source of these transcripts for the early oocyte<sup>33</sup> and that they do not derive exclusively from the oocyte nucleus<sup>14</sup>.

Egl/BicD is enriched at sites of RNA localization in both blastoderm embryos and oocytes<sup>21,24</sup>, presumably as the consequence of protein/RNA co-transport. The complex may have an additional role in anchoring transcripts at their destination. Alternatively, maintenance of localized transcripts might not depend on an independent anchorage step, but result from sustained minus-end-directed transport.

The structural basis of how the transport machinery and RNA signals recognize each other is unclear. The shortest signal defined to date, the *K10* transport/localization sequence<sup>13</sup>, probably relies on both primary and secondary structure. Thus, mutating bases in the stem (*Kstem5'*) inactivates the signal, and compensatory mutations that restore base pairing in the stem (*Kstem5'3'*) reactivates the signal. However, the *Kstem5'3'* signal is only partially active, indicating that primary sequence and possibly tertiary structure are also important. Nor were we able to identify shared structural features in several maternal and zygotic localization signals (S.L.B. and D.I.-H., unpublished data). This could be due to promiscuous or multiple adapter proteins, or because the motor protein complex allows alternative RNA contacts. Egl or BicD may contribute directly to determining RNA selectivity. Neither includes a well-characterised RNA-binding motif, but Egl includes a domain found in a variety of nucleic-acid-recognizing proteins (ref. 28 and J. Sgouros, personal communication). Other components of the complex may also contribute to selective RNA recognition in blastoderm embryos. However, none of the proteins currently implicated in localizing maternal transcripts are likely candidates for such adapters, being absent in blastoderm embryos (Orb<sup>34</sup>, Swallow (Swa)<sup>35</sup>, Exuperantia (Exu)<sup>36,37</sup>), not required for early transport of transcripts into the oocyte (Stau, Exu, Swa)<sup>38</sup>, or not recruited to localizing pair-rule transcripts (Stau; S. Lall, S.L.B. and D.I.-H., unpublished data).

Dhc, Egl and BicD have markedly similar distributions during oogenesis and in blastoderm embryos, and seem to function together in specifying oocyte identity<sup>32</sup>. We propose that an Egl/BicD complex links specific RNAs to dynein and the microtubules. The same machinery may operate elsewhere in *Drosophila*. For example, *inscuteable* transcripts, which localize asymmetrically in neuroblasts<sup>39</sup>, also localize apically when injected into blastoderm embryos (J. R. Hughes, S.L.B. and D.I.-H., unpublished data). Indeed, germline transcripts localize apically when expressed in follicle cells<sup>40</sup>. Egl and BicD homologues have been identified in *Caenorhabditis elegans* (GenBank accession numbers U58745 and U70848 for Egl and BicD, respectively) and mammals (BicD<sup>41</sup>), and might comprise part of an evolutionarily conserved cytoskeletal system for transporting transcripts and other cargoes. □

## Methods

### Fly culture

Wild-type flies are of the strain Oregon-R. The *r5f3*, *r5f3-1*, *r5f3-2* and *r5f3-3* transgenes include the *RpA1* promoter and 0.2 kb of its 5'-transcribed sequences<sup>16</sup>. Full genotypes of *BicD* mutant flies are: *BicD<sup>HA40</sup>*, (*P{BicD<sup>HA40</sup>}*); *BicD<sup>RS</sup>/Df(2L)TW119*); *BicD<sup>HA40</sup>/BicD<sup>R26</sup>*, (*P{BicD<sup>HA40</sup>}*); *BicD<sup>R26</sup>/Df(2L)TW119*); *BicD<sup>H3</sup>/BicD<sup>R26</sup>*, (*P{BicD<sup>H3</sup>}*); *BicD<sup>R26</sup>/Df(2L)TW119*). *BicD<sup>RS</sup>*, a protein null, is described in ref. 25. *BicD<sup>R26</sup>* (ref. 20), an antimorphic allele, *P{BicD<sup>H3</sup>}* and *P{BicD<sup>HA40</sup>}*, which expresses 12% as much BicD as the wild-type gene (6% of diploid production), are described in ref. 31. Transgenes containing the heat-inducible *Hsp70* promoter were used to drive ectopic expression of *h* (using *HSH33* and *HSH21* lines<sup>19</sup>), *Kr* (*HS-Kr*; G. Struhl, unpublished data) and *K10* (*HS-K10*; ref. 40).

For heat-shock-induction in egg chambers (nurse and follicle cells, but not oocytes),

adult females were incubated at 36.8 °C for 1 h and incubated for 6 h at 25 °C before fixation<sup>40</sup>. Cycle 13–14 blastoderm embryos were heat-shocked at 36.0 °C for 15 min and incubated at 25 °C for 15 min before fixation.

### Fluorescent RNA synthesis

Linearized plasmid template was transcribed in a solution containing 0.4 mM ATP, 0.4 mM CTP, 0.36 mM UTP, 0.04 mM Cy3-, Cy5- (NEN Life Sciences) or Alexa-488 (Molecular Probes) UTP, 0.12 mM GTP and 0.3 mM <sup>7</sup>mG(5')pppG cap analogue (Stratagene) using SP6, T3 or T7 polymerases (Roche) according to the manufacturer's instructions. UTP concentration was 0.4 mM for synthesis of unlabelled transcripts. RNA was treated with DNase I, extracted with phenol/chloroform, spun through a mini Quick Spin G50 column (Roche) to remove unincorporated nucleotides, and precipitated with NH<sub>4</sub>OAc/EtOH. Fluorescent RNAs typically contained 1 fluorochrome/250 nucleotides. In contrast to fluorescein-labelled transcripts<sup>10</sup>, Cy3- or Cy5- or Alexa 488-labelled RNAs localize apically without incubation with nuclear factors. Transcripts labelled with Alexa 488-UTP have a propensity to form large particles, which are nevertheless capable of localizing. Unlabelled *h*, *bcd* and *nos* transcripts (detected by *in situ* hybridization) also localize apically.

### Injections and histochemistry

Protein-free RNA was injected into embryos together with 1 μg μl<sup>-1</sup> cycloheximide to inhibit transcript degradation, as described<sup>10</sup>, although similar results were achieved when cycloheximide was omitted. In all of the experiments 200 ng μl<sup>-1</sup> RNA was injected, with the exception of experiments addressing Egl and BicD recruitment when RNA concentration was 1 μg μl<sup>-1</sup>. Typically, 15–30 blastoderm embryos were injected in a single experiment. In some experiments, injection buffer, colcemid (20 ng μl<sup>-1</sup>; final intracellular concentration is ~50 times less), anti-BicD antibody (mouse monoclonal 4C2 (ref. 21)), anti-Egl antibody (rabbit polyclonal<sup>24</sup>), anti-Orb antibody (mouse monoclonal 6H4 (ref. 42)) or anti-Stau antibody (rabbit polyclonal<sup>43</sup>) was injected into embryos 10 min before RNA injection. Unless stated otherwise, embryos were fixed 10 min after injection of the last embryo with RNA (~13 min after injection of the first embryo) in heptane saturated with 37% formaldehyde for 10 min, before hand-peeling. For detection of endogenous pair-rule transcripts, embryos were injected with injection buffer or antibody 25–30 min before fixation for 5 min in heptane saturated with 37% formaldehyde. This longer incubation increased the likelihood that we were not visualizing transcripts that were synthesized and transported before antibody injection.

For immunofluorescence, injected embryos were fixed for 5 min in heptane saturated with 37% formaldehyde, hand-peeled and washed in PBS/0.1% Triton X-100. Embryos were incubated overnight at 4 °C with mouse monoclonal anti-BicD antibody 1B11 (1:20; ref. 21), rabbit polyclonal anti-Egl antibody (1:2,000), mouse monoclonal anti-Dhc antibody (1:20; ref. 44) or mouse anti-β-tubulin antibody (1:100; Sigma). Secondary antibodies (Molecular Probes) were conjugated to Alexa 488 or Alexa 594. Mounted embryos were imaged with a Zeiss LSM 510 confocal microscope, and the proportion of injected RNA in the apical and basal cytoplasm was estimated using Zeiss LSM 510 software. Localization was designated as strong (most of the RNA in the apical cytoplasm, for example pair-rule transcripts), weak (most of the RNA remains basal but there are some apical caps of RNA, for example *osk* transcripts) or unlocalized (no apical concentration of RNA, for example *Kr* transcripts).

*In situ* detection of transcripts during oogenesis was performed essentially as described<sup>45</sup> except that we used labelled RNA probes. *In situ* hybridization of embryos was performed using fluorescein- and Cy3-tyramides (NEN) followed by Alexa 660-wheat germ agglutinin (WGA; Molecular Probes) labelling of nuclear pore complexes<sup>12</sup>.

### Plasmid construction

All transcripts were derived from full-length complementary DNAs, with the exception of *K10* and its derivatives, which was a *SalI*–*PstI* fragment corresponding to the entire 3' UTR and a 860-bp portion of the 3' genomic sequences. For production of *stg*–*K10TSL* and *Kr*–*K10TSL* constructs, a fragment corresponding to the entire 44-nucleotide *K10TSL* (residues 52–95; GenBank accession number U36587) flanked by artificial *BglII* and *BamHI* sites (gift of R. S. Cohen) was blunted and inserted into the *StuI* site of *stg* (position 1,727; M24909) or the *NruI* site of *Kr* (position 1,289; X03414), respectively. The *bcd4496G*→*U* mutation was introduced into full-length *bcd* cDNA using the Quickchange site-directed mutagenesis kit (Stratagene). Modified *ftz* constructs for mapping the blastoderm localization signal were generated by PCR or by Quickchange mutagenesis. The pCR2.1TOPO vector RNA is derived from a 1.5-kb *EcoRI*/*NcoI* fragment.

Received 10 July; accepted 12 September 2001.

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**Acknowledgements**

We are grateful to I. Davis, N. MacDougall, G. Wilkie, D. Baker, A. Flament and J. Hughes for discussions; R. Lehmann, R. Steward, D. Sharp, B. Suter, M. Jacobs-Lorena, D. St Johnston, G. Dollar, L. Gavis, B. Cohen and Developmental Studies Hybridoma Bank for providing reagents; and many colleagues for comments on the manuscript. This work was supported by the Imperial Cancer Research Fund and a fellowship from the Royal Commission for the Exhibition of 1851 (to S.L.B.).

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