Journal of Cell Science 109, 3113-3119 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JCS9515

Expression and changing distribution of the human transferrin receptor in developing *Drosophila* oocytes and embryos

Mark S. Bretscher

Division of Cell Biology, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

SUMMARY

In order to understand better the membrane systems in a developing *Drosophila* oocyte, the human transferrin receptor has been expressed there. This was achieved using the *armadillo* promoter combined with *K10* or *oskar* trailer sequences; these enable the messenger RNA to be transcribed in nurse cells and then transported to, and translated in, oocytes. This is the first exogenous protein to be expressed in oocytes. At stage 8, the transferrin receptors are mainly concentrated towards the posterior pole of the oocyte and are associated with large cytoplasmic vesicles; when combined with the *shibire* mutation the transferrin receptors are transferred to the oolemma, demonstrating that they participate in an endocytic cycle. At stage 10, the transferrin receptors are localised either to the anterior margin of the oocyte or to the posterior pole, depending on where the mRNA is located. In newly laid eggs, all the transferrin receptors are found in large cytoplasmic vesicles. The results reveal remarkable sorting processes which occur as oocytes mature and show that ring canals, which separate the oolemma from nurse cell plasma membranes, act as barriers to prevent components in these two compartments from intermixing.

Key words: *Drosophila* oocyte, Endocytic cycle, *shibire*, Transferrin receptor

INTRODUCTION

The developing *Drosophila* oocyte is part of a multicellular complex in which the other cells assist in its growth and the establishment of its polar axes (reviewed by Spradling, 1993; St Johnston and Nüsslein-Volhard, 1992). The oocyte is linked to fifteen nurse cells, in which most of the biosynthetic processes occur, by ring canals that allow the transport of selected components to the oocyte. The follicle cells initially enclose the oocyte and associated nurse cells, but later several migrations occur so that by stage 10 they largely surround the oocyte. The cell surface interactions between oocyte and associated follicle cells are crucial in defining the axes of the future egg.

In order to begin to understand the relationships between these different cells at a cell biological level, I have transformed flies with a human transferrin receptor (TFR) construct which leads to the TFR being expressed in oocytes. The TFR was chosen because it is a useful membrane marker which could reveal the existence of any junctions which might separate membrane domains (see Rodriguez-Boulan and Powell, 1992). In addition, since it has a high affinity for coated pits and circulates rapidly in mammalian cells, it was hoped that the sequence recognized in it by mammalian adaptins (Pearse and Robinson, 1990) might also be recognized by the corresponding *Drosophila* adaptins. If this were the case, it might prove useful in looking at cell asymmetries which arise from the existence of polarised endocytic cycles (see Bretscher, 1984, 1996).

MATERIALS AND METHODS

Fly lines carrying the human TFR gene

Several DNA constructs derived successively from each other were assembled in Bluescript. The *armadillo-TFR-K10* construct contained: an *Eco*RI/*Kpn*I *armadillo* promoter (lifted out of AZE 16; Vincent et al., 1994); the human TFR region containing a *Kpn*I 5′ end, followed by the fly *Adh* ribosome binding site (GGTACCG-CAAAAAAGAAGTCACCA), followed by the complete human TFR coding region including 46 3′ noncoding residues, followed by *Sac*I and *Eco*RI sites and the *K10* 3′ trailer sequence from residues 3,411 to 5,401 bounded by *Eco*RI and *Sal*I sites. The entire region was lifted out of Bluescript and inserted into the Carnegie C20 vector. The *armadillo-TFR-olc21* construct contained the same components as above up to the *Sac*I site, followed by *olc21* bounded by *Sac*I and *Bam*HI sites; this was transferred to the Casper 4 vector for injection into flies. The *olc21* fragment was prepared by PCR from genomic DNA and included the 3′ sequence between residues 2,802 and 3,656 (Kim-Ha et al., 1991, 1993). Flies were transformed by standard procedures and were maintained at 23°C.

Several other constructs have been made with the *armadillo* and *K10* flanking regions. These include chimeric versions of thy-1 and the C terminus of the LDL receptor, which might circulate more rapidly than the TFR, and various C-terminal deletions of this construct. No expression of these could be detected in transformed flies.

Antibody and lectin staining

Standard procedures using 5% paraformaldehyde in PBS (plus heptane, for embryos) for 30 minutes were used for fixing samples; after fixation, ovaries were teased apart to provide better access for

reagents. For experiments in which whole ovaries were incubated prior to fixation, they were dissected in growth medium (lacking vitamins) (Robb, 1969) and rotated to assist aeration in this medium at 23°C or 32°C. They were then fixed in the same medium (but lacking amino acids and the salt increased to balance the ionic strength) with 5% formaldehyde for 5 minutes at the relevant temperature, followed by 25 minutes at room temperature in PBS/formaldehyde.

Samples were then stained by routine methods (Lawrence and Johnston, 1989) using either a rabbit antiserum to the TFR (for embryos) or mouse monoclonal antibodies (B 3/25 or D 65/30) for ovaries, or biotinylated lectins (Vector Lab kits BK 1000, 2000 and 3000). After lectin labelling only, samples were refixed in 5% formaldehyde/PBS for 15 minutes, washed and blocked in PBS containing 0.1% Tween-20 and 0.1 M glycylglycine for 30 minutes. Labelled samples were finally developed with Vector Elite horseradish peroxidase-AB complex, using diaminobenzidine. Selected oocytes and embryos were dehydrated, embedded in Durcupan and thick sections (about 5 μm) cut and mounted in Araldite. Samples were examined in a Zeiss Axiophot microscope; photographs were mainly taken in bright field.

For the localisation of *TFR* mRNAs, standard in situ procedures were used with RNA probes into which had been incorporated DIG-UTP. The probes were detected with the phosphatase substrate II from Vector Labs and processed as above.

Electron microscopy

I was unable to convert the stain produced by horseradish peroxidase and diaminobenzidine into an electron dense deposit visible in the electron microscope. I have therefore adapted a lead precipitation method (Lewis, 1977) based on alkaline phosphatase to localise antigenic determinants by electron microscopy. Ovaries were fixed and labelled with primary antibodies as above, followed by biotinylated anti-mouse antibodies. After washing, they were labelled with Vector alkaline phosphatase-AB complex, washed and developed with lead nitrate/cytidylic acid for 50 minutes; the samples were washed and then held overnight at 4°C in 1% glutaraldehyde in 0.1 M sodium phosphate, pH 7.2. To visualise the precipitated lead phosphate, samples were rinsed, then held at room temperature for 10 minutes in 0.05 M sodium sulphide in 0.1 M sodium phosphate, pH 7.2: the white lead phosphate is turned to the dark lead sulphide. Selected samples were then osmicated (2% osmium tetroxide in 0.1 M sodium phosphate, pH 7.2, for 30 minutes on ice), dehydrated and embedded in Araldite. Thin sections were examined without further staining. This cytological technique has its limitations, probably because of the slight solubilities of both lead phosphate and sulphide (about 0.2 and 3 μM, respectively; CRC Handbook of Physics and Chemistry, 1994). The solubilities of these lead deposits were partly suppressed by keeping labelled samples in 0.1 M phosphate buffer when possible. However, examination of labelled tissue strongly suggests that a part of the lead deposits is washed out at some stage, especially in samples in which there is little lead deposition, such as in control samples; elsewhere it sometimes spreads throughout structures where it clearly should not be (see Fig. 3B). In addition, follicle cell nuclei become labelled in a primary antibody-independent process.

RESULTS

Expression of the human TFR in oocytes and early embryos

In preliminary experiments, two DNA constructs were prepared containing the TFR coding region, followed by the 3′ fly *tubulin* trailer sequence and, as promoters, either the *hsp70* or *Adh* promoter. No significant labelling of either oocytes or early embryos from either set of transgenic flies could be detected. However, western blots of larvae showed that, after heat induction of the *hsp70* line, a new ~180 kDa band could be detected on non-reducing SDS gels, and a new ~90 kDa band on reducing gels (unpublished data): these are characteristic of the transferrin receptor in mammalian cells.

Transgenic flies were made using a related construct having the *armadillo* promoter, which is known to be highly active in nurse cells (Riggleman et al., 1989): staining ovaries of such flies for TFRs showed that the nurse cells had a low level of TFR expression, but undetectable levels in either oocytes or newly laid embryos. However, in later embryos (5 hour), the TFR was intensely expressed. I then replaced the 3′ *tubulin* trailer with that from the *K10* gene, as it had previously been shown that the *K10* RNA is transported from nurse cells to oocyte, and that this transport is effected by its 3′ trailer (Prost et al., 1988; Cheung et al., 1992). This had a dramatic effect: in transformed flies, the TFR, as detected by antibodies coupled to horseradish peroxidase (HRP), could now be readily seen in both oocytes and early embryos, but not in nurse cells (except in stage 7 and earlier where it was faintly visible).

The pattern of the expressed TFRs changes as the oocytes develop. Prior to stage 6, TFR-positive vesicles are present in nurse cells, and there is a light staining of oocyte membranes. By stage 7, the oocytes are much more strongly stained, this label being present in both the plasma membrane and intracellular vesicles. At stage 8, the TFRs are strongly localised towards the future posterior of the oocyte, as shown in the whole mount picture (Fig. 1A). By stage 10A, the pattern is reversed: most of the TFRs are found towards the future anterior of the oocyte (Fig. 1C). Rarely, an intermediate distribution is seen in which both posterior and anterior regions are labelled (Fig. 1B). In newly laid embryos (or eggs from virgin flies), the TFR is not detected on the cell surface, but is found inside the cell.

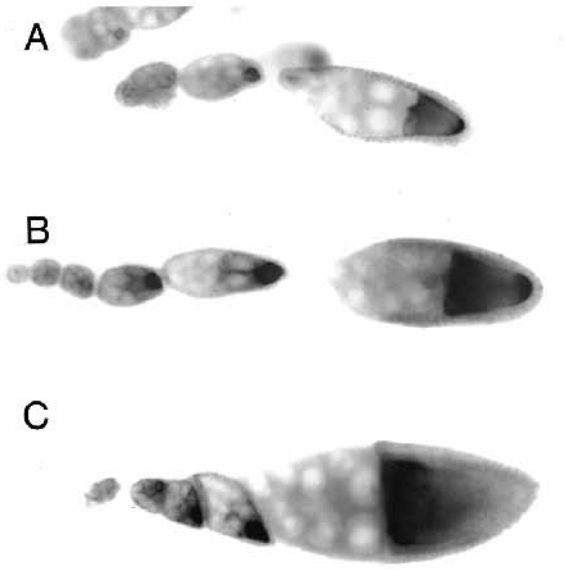

Fig. 1. TFR expression in follicles. Whole-mount views of: (A) a late stage 8 oocyte having a pronounced posterior accumulation of label; (B) a stage 9 oocyte, in which both anterior and posterior regions are labelled; and (C) a stage 10A oocyte having strong anterior labelling.

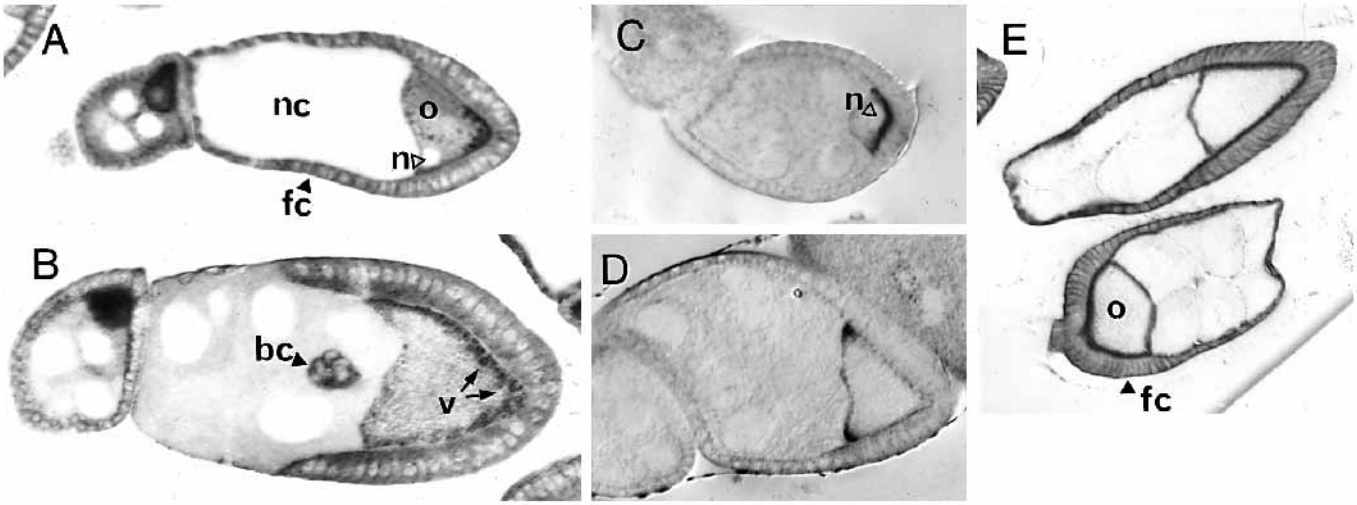

Fig. 2. TFR (A,B,E) and *TFR* mRNA (C,D) expression in stage 7-9 oocytes. Thick sections from the mid-planes of stage 7 (C), stage 8 (A,D) or stage 9 (B) oocytes. In E, stage 8 (lower) and stage 9 (upper) oocytes in a *shibire ts-1* background have been exposed to a 32°C treatment for 30 minutes before fixation. Note that the TFRs in A and B are mainly associated with large vesicles (v) in the posterior regions of oocytes (o) and that, after endocytosis has been blocked (in E), they are now found along the oocyte plasma membrane. A similar change occurs in the follicle cells (fc): compare A and B with E. A group of labelled border cells (bc) can be seen migrating through the nurse cells (nc) towards the anterior of the oocyte in B. In C, *TFR* mRNA is localised to the posterior of the oocyte, but by stage 8 this is now found at the anterior rim (D). Nuclei (n) are visible in A and C. In control experiments, the distributions of TFRs in *shibire ts-1* at 23°C and that of wild type at 32°C were found to be similar to those of the wild type at 23°C, as seen in A and B above.

Stage 8

In order to see more clearly where the TFR is, selected labelled oocytes were embedded in resin and thick sections cut. This revealed that most of the TFRs are localised in vesicles collected towards the cell's posterior, around the oocyte's edge (Fig. 2A). These vesicles vary greatly in size: some of the TFRs are also found along the oocyte plasma membrane. The TFR is also expressed in follicle cells and, in some central sections, in a cluster of cells amongst the nurse cells (Fig. 2B shows this in a stage 9 oocyte): these are presumably border cells migrating from the anterior end of the egg chamber to the anterior region of the oocyte.

The observed distribution of TFRs towards the posterior of these oocytes could possibly have arisen because its mRNA is localised there. In situ hybridisation with a TFR mRNA probe revealed that, in stage 7 oocytes, the mRNA is localised at the posterior pole of the oocyte (Fig. 2C), but by stage 8 it is now found at the anterior pole (Fig. 2D). This is not entirely surprising as it parallels the position in the oocyte of the *K10* mRNA, which itself is determined by its 3′ trailer (Serano and Cohen, 1995) and which is present in the TFR construct employed here. The observation that in stage 8 oocytes the mRNA is localised to the anterior, but the protein is localised towards the posterior, suggests that the one is not dependent on the other (but see also below).

To discover whether the TFRs are permanently localised to these different membrane compartments, or whether the images seen represent a snap-shot of TFRs circulating between different compartments, the *shibire ts-1* mutation was introduced into this *TFR* transgenic line. As the *shibire* mutation blocks endocytosis of circulating receptors by preventing formed coated pits from budding off into the cytoplasm (Kosaka and Ikeda, 1983a,b), its introduction might be expected to cause circulating TFRs to collect along the oocyte plasma membrane. Ovaries were incubated for 30 minutes at 32°C (the restrictive temperature) and, after staining and embedding, thick sections were examined. The most striking feature of this heat treatment is that the cytoplasm has largely become depleted of TFR-positive vesicles, and in some oocytes virtually no stained vesicles can be seen (Fig. 2E). By contrast, staining of the entire oolemma is greatly enhanced. This establishes that these receptors are indeed participating in a rapid endocytic cycle. A similar change is also visible in the surrounding follicle cells.

An attempt was made to examine the effect of the *shibire ts-1* mutation at the EM level. For this, the final detecting conjugate, streptavidin-HRP, was replaced with streptavidin-alkaline phosphatase (see Materials and Methods), and the TFRs localised by the precipitation of lead phosphate. Examination of thin sections in the electron microscope showed that the preservation of morphology is good, although individual membranes are not discernible. However, it is clear that much of the TFR in stage 8 oocytes at the permissive temperature is located in what would have been the membranes of small vesicles and of much larger (yolk?) granules (Fig. 3A,B). When oocytes from *shibire* females are incubated at the restrictive temperature, the TFRs become largely cleared from their vesicular structures and are now found along the plasma membrane and nearby (Fig. 3C,D), presumably in deep indentations which arise in *shibire* at 32°C (Kosaka and Ikeda, 1983a,b; Damke et al., 1994). These EM observations are in complete accord with the conclusions from thick sections. They further show that the intracellular TFRs at the posterior pole (as in Fig. 3A) are largely restricted to a subset of vesicles there; these are presumably those vesicles which were participating in the endocytic cycle.

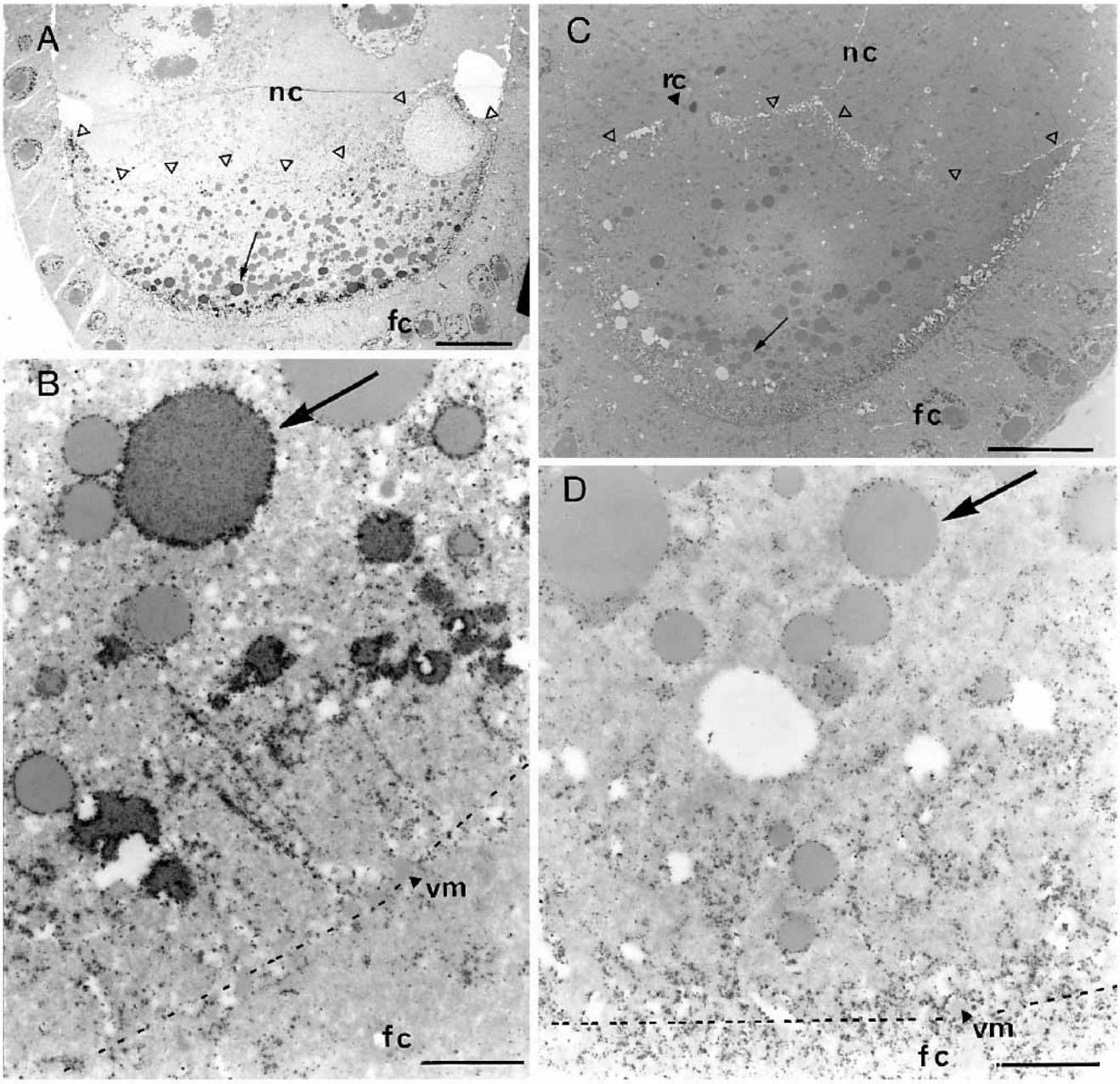

Fig. 3. TFR localisation in stage 8 oocytes. Thin sections of stage 8 oocytes in which the TFRs have been labelled by lead deposition in a wild-type background (A,B) or in a *shibire* background (C,D) after 20 minutes at 32°C. The higher magnifications shown in B,D are taken from A,C, and an arrow in each case highlights a prominent common vesicle. Note the high concentration of label associated with vesicles and presumed yolk granules near the oocyte plasma membrane in A and B, and how this label accumulates near the oocyte plasma membrane (adjacent to the follicle cell, fc) 20 minutes after endocytosis is blocked (C,D). The anterior regions of the oocytes are marked in A and C with open arrowheads; the borders between the oocytes and posterior follicle cells are marked by broken lines in B and D, where small parcels of vitelline membrane (vm) can be seen to be accumulating. In C, one of two ring canals (rc) is marked. The yolk granule marked with a large arrow in B has a light extensive precipitate of lead throughout it. This, and the nuclear labelling seen in the follicle cells (A,C), are non-specific (see Materials and Methods). nc, nurse cell. Bars: 10 μm (A,C) and 1 μm (B,D).

Stage 10

The posterior localisation of the TFR continues into early stage 9 oocytes; thereafter, there is a radical redistribution of the receptor. It is found along the anterior and anterior-lateral edges of the oocyte, tapering off towards the posterior (Fig. 4A). Both the follicle cells and anterior border cells are also labelled. Compared to the earlier distribution, the TFR appears to be more weakly expressed and absent from structures deep in the cytoplasm, where only a rare labelled vesicle is seen. When combined with *shibire ts-1*, and after an incubation of 30 minutes at 32°C, oocytes at this stage have an apparently unchanged pattern of staining, although the intensity of staining often seems diminished (Fig. 4B). This unchanged distribution is not necessarily surprising, as the endosomal

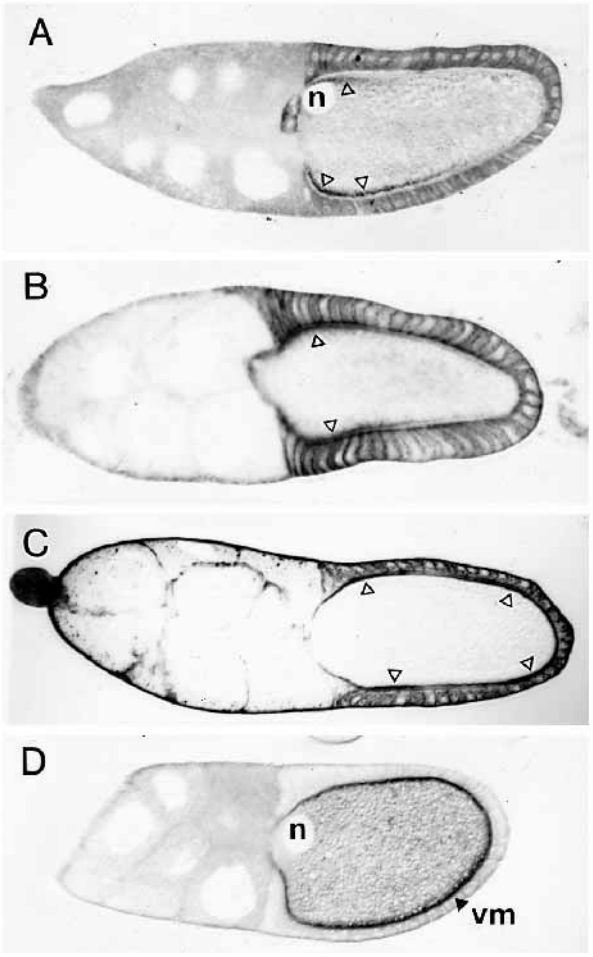

Fig. 4. TFR and lectin expression in stage 10 oocytes. Thick sections from the midplanes of stage 10 follicles in a wild-type background (A) or in the *shibire* background after incubation at 32°C for 30 minutes (B), stained for the TFR; a *shibire* follicle after incubation at 32°C for 30 minutes, stained for *Lycopersicon esculentum* lectin receptors (C), and a wild-type follicle stained for *Datura stramonium* lectin receptors (D). In A-C, the labelled oocyte plasma membranes are indicated by open arrowheads; oocyte nuclei (n) can be seen in A and D, as can the adjacent border cells (bc) in A; in D, the vitelline membrane (vm) is arrowed. Note the TFR label decreases along the oocyte plasma membrane from anterior to posterior in A,B, and that it becomes more intense in the follicle cell plasma membranes from *shibire* females after heat-treatment in B compared to A. Bar, 50 μm.

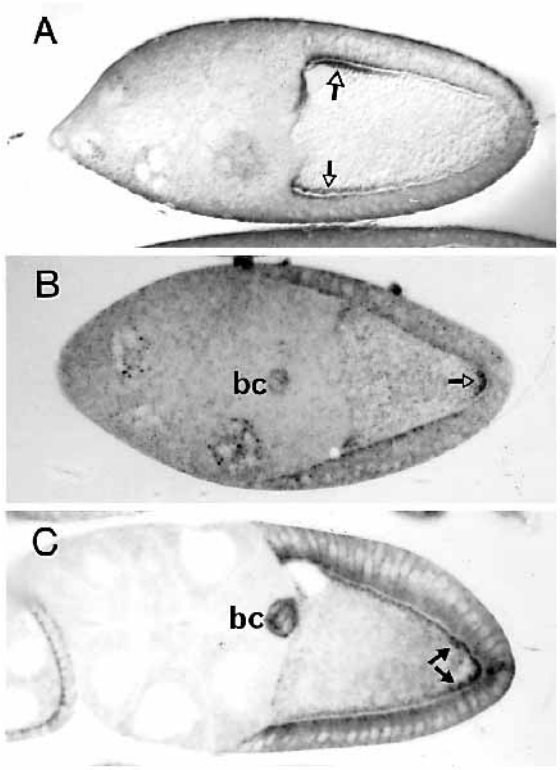

Fig. 5. TFR and *TFR* mRNA expression in stage late 9/10 oocytes. Thick sections through the midplanes of: (A) in situ *TFR* mRNA in *armadillo-TFR-K10* oocyte (stage 10); (B) in situ *TFR* mRNA in *armadillo-TFR-olc21* oocyte (stage 9); (C) TFRs in *armadillo-TFR-olc21* oocyte (stage 10). Open arrows, in situ *TFR* mRNAs; solid arrows, TFRs. Note that in the oocytes, there is a remarkably similar distribution of *TFR* mRNA in A compared with TFR protein in Fig. 4A, and that the *TFR* mRNA and protein are localised at the posteriors of the oocytes in B and C.

network in which most of the TFRs might reside lies just next to the plasma membrane in oocytes at this stage (Giorgi et al., 1993).

The distribution of TFRs seen in stage 10 oocytes towards the anterior region could be biased by a variety of factors, such as poor penetration of antibody reagents at the posterior, or a greater infolding of the plasma membrane at the anterior. To examine these possibilities I have screened a variety of lectins for their abilities to label oocytes. From this, one lectin (*Lycopersicon esculentum*, molecular mass ~71 kDa) was found which clearly labels the oocyte plasma membrane (Fig. 4C) but not, surprisingly, oocyte vesicles. For comparison, another lectin, that from *Datura stramonium*, selectively labels the vitelline membrane (Fig. 4D). These results show that the membrane at the posterior region is neither less accessible to reagents, nor less well-preserved, than elsewhere and hence that the sparseness of labelling of the TFR in these regions is caused by its sparseness there. This conclusion is supported by the ability of TFRs, which are localised at the posterior pole of the oocyte, to be labelled by the same procedures (see below).

In situ hybridisation revealed that the TFR mRNA is localised along the anterior margin of the oocyte in a distribution, as seen in thick sections, indistinguishable from that of the TFRs (compare the TFRs in Fig. 4A with the in situ in Fig. 5A). In order to determine whether this TFR distribution depends on that of its mRNA, a further construct was made. The 3′ UTR from *K10* was replaced with a segment from the 3′ UTR of *oskar*, *olc21* (Kim-Ha et al., 1993) which has been used to localise the *K10* RNA to the oocyte posterior and still allow its translation there (Serano and Cohen, 1995). In situ hybridisation using a *TFR* mRNA probe of oocytes from flies transformed with this construct (*armadillo-TFR-olc21*) showed that the *TFR* mRNA is barely detectable, even in thick sections, of stage 10 oocytes, although traces of it are occasionally visible at the posterior pole. Somewhat stronger expression is seen in stage 9 oocytes where this RNA is also expressed at the posterior pole (Fig. 5B). Antibody staining of

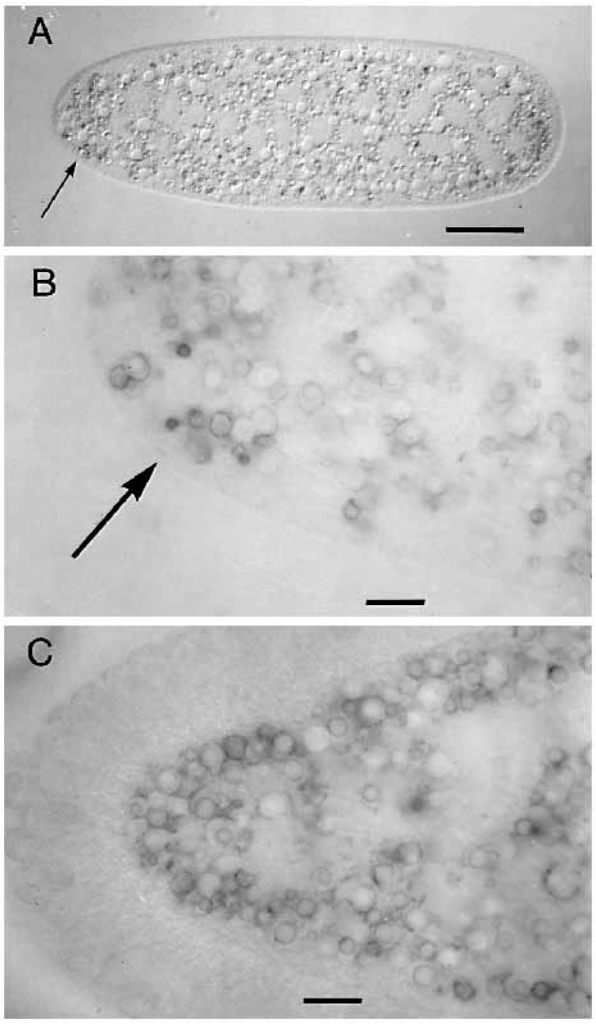

Fig. 6. TFR expression in early embryos. A newly laid embryo (A,B) and a blastoderm stage embryo (C) stained for TFRs. The region in A marked by an arrow is enlarged in B. Note the many variously sized vesicles which are labelled throughout the cytoplasm, and the absence of such label (in A,B) from the largest vesicles and from the plasma membrane, and (in C) from the cell layer of the blastoderm. Bars: 50 μm (A); 10 μm (B,C).

oocytes demonstrates that the follicle cells are well-stained. Sectioning a series of such oocytes reveals that the TFRs can occasionally be detected: in stages 8-10 they are now found concentrated towards the posterior pole and appear to be largely present in cytoplasmic vesicles (Fig. 5C shows this in a stage 10 oocyte). In other words, the pattern of TFR staining seen in stage 10 oocytes depends on where the mRNA is localised. Because of the difficulties in visualising these expressed TFRs, the effect of *shibire* on them has not been investigated.

Newly laid embryos

Thick sections of early embryos stained for the TFR show that all visible label has now been transferred to cytoplasmic vesicles: these vesicles vary in size from about 5 μm diameter downwards (Fig. 6A,B). The largest vesicles seen with Nomarski optics are devoid of label. The same distribution of TFRs is present in eggs laid by virgin flies; neither is noticeably affected, when combined with *shibire ts-1*, by incubation at 32°C for 15 minutes. As the embryos develop and the yolk granules are moved away from the embryo surface, so are the TFR-positive vesicles moved interiorly (Fig. 6C). The plasma membranes of the syncitial blastoderm remain unlabelled.

DISCUSSION

The transgenic *Drosophila* line described here is the first available which carries a readily detectable circulating receptor, the human TFR, in its oocytes. Expression of soluble proteins in nurse cells usually leads to their eventual deposition in the forming oocyte, but this appears not to be the case for the TFR. Expression of the TFR, under the control of the *armadillo* promoter and *tubulin* trailer sequences, led only to barely detectable labelling of the TFR in nurse cells: at no stage could any TFRs be detected in oocytes or eggs. Its expression in oocytes was achieved by the inclusion of the *K10* trailer sequence, which directs the transfer of mRNA from nurse cells to oocytes (Cheung et al., 1992). Now the TFR expression was essentially limited to the oocyte after stage 7. This can be clearly seen in Figs 1C and 2E, and shows that nurse cell and oocyte plasma membranes are not continuous structures with free diffusion across the ring canals which join them. Rather, it suggests that ring canals act as barriers to the free diffusion of proteins in what appears to be a continuous membrane. This further suggests that the plasma membrane proteins of oocytes may be distinct from those of nurse cells, and are likely to be synthesised in the oocyte.

In stage 8 oocytes, most of the TFRs are present at any moment in cytoplasmic vesicles localised towards the posterior pole of the cell. This distribution might reflect where they were synthesised: in stage 7 oocytes, the *TFR* mRNA bearing the *K10* trailer is localised to the posterior (Fig. 2C) and perdurance might leave the receptors there at stage 8 (by which time the mRNA is now anteriorly localised; Fig. 2D), provided that the receptors are comparatively immobile. This seems unlikely for two reasons. First, these TFRs are highly mobile: after an incubation at the restrictive temperature in a *shibire* background, the receptors are largely found along the entire plasma membrane (Fig. 2E). The time it takes for this relocalisation (30 minutes for that shown) is short compared to the time a follicle takes to proceed from stage 7 to stage 8 (about six hours, see Spradling, 1993), suggesting that much migration of receptors could occur in this interval (if, indeed, they survive that long). Second, in stage 7 oocytes in which the *K10* trailer is replaced by *olc 21*, the *TFR* mRNAs, which are only weakly expressed, are localised primarily at the anterior margin (not shown), as has been found for a different construct bearing the same *olc 21* trailer (Serano and Cohen, 1995). However, the receptors at stage 8 are still found at the posterior, further indicating that the site of stage 7 mRNA localisation does not determine where the TFRs are at stage 8. This all suggests that in stage 8 oocytes the TFRs are seen in these posterior vesicles because that is where they spend the majority of their time as they cycle.

The observation that the TFRs are transferred to the plasma membrane in *shibire* at high temperature shows that they are taking part in a coated pit driven endocytic cycle (Kosaka and

Ikeda, 1983a,b; Herskovits et al., 1993). This suggests that *Drosophila* coated pits recognise a signal in the human transferrin receptor for endocytosis, and therefore that such signals may be conserved between these species. In addition, the intracellular distribution of TFRs among the oocyte's vesicles is surprising: they seem mainly to access a limited population of vesicles and putative yolk particles which are near the oocyte plasma membrane and lie towards the posterior region of the cell (Figs 2A,B and 3A,B). An intriguing possibility is that this uneven distribution of TFRs is related to the requirement for gurken at the posterior pole of oocytes prior to stage 8 (González-Reyes et al., 1995); if so, polar exocytosis of both newly synthesised and circulating membranes at the oocyte posterior may occur. However, these oocytes are too small to allow this possibility to be tested (as has been done in large tissue culture cells; Bretscher, l983; see also Hopkins et al., 1994).

By stage 10 the distribution of TFRs in oocytes is quite different and depends on the localisation of its mRNA. When the RNA is localised to the anterior margin by the *K10* trailer, the TFRs have a distribution in the oocyte indistinguishable (at this level of resolution) from that of the mRNA. However, when its mRNA is localised to the posterior pole by the *oskar* trailer *olc21*, the TFRs are now found at the posterior pole of the stage 10 oocyte. These results show that the TFR distribution is now determined by that of its mRNA and therefore that no equilibrium distribution of it has been achieved. As such, the TFR distribution is uninformative as to whether a polarised endocytic cycle in oocytes exists at this stage. The lack of equilibration of TFRs presumably means that it has become too unstable: proteases must exist which degrade it.

Between stage 10 and a newly laid egg, a further astonishing reorganisation occurs. The TFRs are no longer found associated with the cell cortex; instead they are now present in large vesicles. They no longer circulate (although egg plasma membranes have an abundant supply of coated pits) and appear destined to be degraded with the remaining yolk in the embryonic gut. The sorting thus revealed presumably reflects a much wider membrane remodelling process between stage 10 of oogenesis and the newly laid egg.

These experiments would not have been possible without the generous help and advice of many colleagues, especially Peter Lawrence and the late Paul Johnston. I am also most grateful to Douglas Kershaw (for help with electron microscopy), to Gary Struhl for advice and vectors, to Ian Trowbridge for the TFR gene and monoclonal antibodies, to R. Cohen, A. Ephrussi, K. Hardwick, E. Mohier, J. Muller, S. Ner and J.-P.Vincent for help or advice and to M. Ashburner for the *shibire* strain.

REFERENCES

Bretscher, M. S. (1983). Distribution of receptors for transferrin and low density lipoprotein on the surface of giant HeLa cells. *Proc. Nat. Acad. Sci. USA* **80**, 454-458.

Bretscher, M. S. (1984). Endocytosis: Relation to capping and cell locomotion. *Science* **224**, 681-686.

Bretscher, M. S. (1996). Moving membrane up to the front of migrating cells. *Cell* **85**, 465-467.

CRC Handbook of Chemistry and Physics (1994). 75th edition. CRC Press, Boca Raton, FL.

Cheung, H.-K., Serano, T. L. and Cohen, R. S. (1992). Evidence for a highly selective RNA transport system and its role in establishing the dorsoventral axis of the *Drosophila* egg. *Development* **114**, 653-661.

Damke, H., Baba, T., Warnock, D. E. and Schmid, S. L. (1994). Induction of mutant dynamin specifically blocks endocytic coated vesicle formation. *J. Cell Biol.* **127**, 915-934.

Giorgi, F., Lucchesi, P., Morelli, A. and Bownes, M. (1993). Ultrastructural analysis of *Drosophila* ovarian follicles differing in yolk polypeptide (yps) composition. *Development* **117**, 319-328.

González-Reyes, A., Elliott, H. and St Johnston, D. (1995). Polarization of both major body axes in *Drosophila* by *gurken-torpedo* signalling. *Nature* **375**, 654-658.

Herskovits, J. S., Burgess, C. C., Obar, R. A. and Vallee, R. B. (1993). Effects of mutant rat dynamin on endocytosis. *J. Cell Biol.* **122**, 565-578.

Hopkins, C. R., Gibson, A., Shipman, M., Strickland, D. K. and Trowbridge, I. S. (1994). In migrating fibroblasts, recycling receptors are concentrated in narrow tubules in the pericentriolar area, and then routed to the plasma membrane of the leading lamella. *J. Cell Biol.* **125**, 1265-1274.

Kim-Ha, J., Smith, J. L. and Macdonald, P. M. (1991). *oskar* mRNA is localised to the posterior pole of the Drosophila oocyte. *Cell* **66**, 23-35.

Kim-Ha, J., Webster, P. J., Smith, J. L. and Macdonald, P. M. (1993). Multiple RNA regulatory elements mediate distinct steps in localisation of *oskar* mRNA. *Development* **119**, 169-178.

Kosaka, T. and Ikeda, K. (1983a). Possible temperature-dependent blockage of synaptic vesicle recycling induced by a single gene mutation in Drosophila. *J. Neurobiol.* **14**, 207-225.

Kosaka, T. and Ikeda, K. (1983b). Reversible blockage of membrane retrieval and endocytosis in the garland cell of the temperature-sensitive mutant of Drosophila melanogaster, *shibire ts1*. *J. Cell Biol.* **97**, 499-507.

Lawrence, P. A. and Johnston, P. (1989). Pattern formation in the *Drosophila* embryo: allocation of cells to parasegments by *even-skipped* and *fushi tarazu*. *Development* **105**, 761-767.

Lewis, P. R. (1977). Metal precipitation methods for hydrolytic enzymes. In *Staining Methods for Sectioned Material* (ed. P. R. Lewis and D. P. Knight), pp. 137-215. North Holland, Amsterdam.

Pearse, B. M. F. and Robinson, M. S. (1990). Clathrin, adaptors, and sorting. *Annu. Rev. Cell Biol.* **6**, 151-171.

Prost, E., Deryckere, F., Roos, C., Haenlin, M., Pantesco, V. and Mohier, E. (1988). Role of the oocyte nucleus in determination of the dorsoventral polarity of *Drosophila* as revealed by molecular analysis of the K10 gene. *Genes Dev.* **2**, 891-900.

Riggleman, B., Wieschaus, E. and Schedl, P. (1989). Molecular analysis of the *armadillo* locus: uniformly distributed transcripts and a protein with novel internal repeats are associated with a *Drosophila* segment polarity gene. *Genes Dev.* **3**, 96-113.

Robb, J. A. (1969). Maintenance of imaginal discs of *Drosophila melanogaster* in chemically defined media. *J. Cell Biol.* **41**, 876-885.

Rodriguez-Boulan, E. and Powell, S. K. (1992). Polarity of epithelial and neuronal cells. *Annu. Rev. Cell Biol.* **8**, 395-427.

Serano T. L. and Cohen, R. S. (1995). Gratuitous mRNA localisation in the Drosophila oocyte. *Development* **121**, 3013-3021.

Spradling, A. C. (1993). Developmental genetics of oogenesis. In *The Development of* Drosophila melanogaster (ed. M. Bate and A. Martinez Arias), pp. 1-90. Cold Spring Harbor Press, USA.

St Johnston, D. and Nüsslein-Volhard, C. (1992). The origin of pattern and polarity in the Drosophila embryo. *Cell* **68**, 201-219.

Vincent, J.-P., Girdham, C. H. and O'Farrell, P. H. (1994). A cell-autonomous, ubiquitous marker for the analysis of *Drosophila* genetic mosaics. *Dev. Biol.* **164**, 328-331.

(Received 25 June 1996 - Accepted 30 September 1996)