SCIENCE CHINA
Life Sciences

• COVER ARTICLE •

November 2013 Vol.56 No.11: 975–984
doi: 10.1007/s11427-013-4563-4

Apolipoprotein C1 regulates epiboly during gastrulation in zebrafish

WANG Yang, ZHOU Li, LI Zhi, LI WenHua & GUI JianFang*

State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China

Received September 2, 2013; accepted October 9, 2013

Apolipoprotein C1 (Apoc1) is associated with lipoprotein metabolism, but its physiological role during embryogenesis is largely unknown. We reveal a new function of Apoc1b, a transcript isoform of Apoc1, in epiboly during zebrafish gastrulation. Apoc1b is expressed in yolk syncytial layers and in deep cells of the ventral and lateral region of the embryos. It displays a radial gradient with high levels in the interior layer and low levels in the superficial layer. Knockdown of Apoc1b by injecting antisense morpholino (MO) caused the epiboly arrest in deep cells. Moreover, we show that the radial intercalation and the radial gradient distribution of E-cadherin are disrupted both in Apoc1b knockdown and overexpressed embryos. Therefore, Apoc1b controls epiboly via E-cadherin-mediated radial intercalation in a gradient-dependent manner.

epiboly, apolipoprotein C1, E-cadherin, radial intercalation

Citation: Wang Y, Zhou L, Li Z, et al. Apolipoprotein C1 regulates epiboly during gastrulation in zebrafish. Sci China Life Sci, 2013, 56: 975−984, doi: 10.1007/s11427-013-4563-4

During vertebrate gastrulation, the evolutionarily conserved morphogenetic movements of epiboly, internalization, convergence and extension cooperate to generate the three germ layers and to sculpt the body plan [1−3]. Epiboly begins at the late blastula stage and ends at the bud stage, and constitutes the first active gastrula migration [4]. During epiboly, three cell layers, the yolk syncytial layer (YSL), the deep cells and the enveloping layer (EVL) expand towards the vegetal pole and thin the blastoderm over the large yolk cells.

The cellular and molecular processes underlying epiboly during gastrulation remain poorly understood [5]. An important recent development in gastrulation research is the demonstration that E-cadherin-mediated cell-cell adhesion is necessary for epiboly. Several zebrafish mutants (e.g., *half baked*, *avalanche*, *lawine*, and *weg*), in which epiboly is inhibited, have been reported to map to a single locus, *cdh1* gene encoding E-Cadherin [6−8]. Because of a decrease in cell-cell junctions, *cdh1* deficient embryos exhibit inhibited radial intercalation, during which the deep cells move superficially, thinning the deep cell layer [6].

Another major force generator is the cytoskeleton that plays many roles during epiboly. Disruption of microtubule networks in the yolk cell inhibits the epiboly of yolk syncytial nuclei and partially impairs the epiboly of the EVL and deep cells [9,10]. Microtubule polymerization is maintained by pregnenolone, a lipid product of Cyp11a1 enzyme in yolk cells, and is required for normal epiboly [11]. The leading edge of the EVL and YSL are connected by tight junctions. A punctate actin band overlaps with the process of a massive endocytosis that drives EVL epiboly [9,12,13]. It has also been hypothesized that two other actin rings at the margins of the EVL and deep cells provide the pursing force for epiboly [12,14]. These actomyosin rings drive the cell spread by a combination of cable-constriction and

*Corresponding author (email: jfgui@ihb.ac.cn)

flow-friction [15].

A number of other factors have been shown to affect cell motility during epiboly, such as Src-related kinases, *Fyn* and *Yes*, both known regulators of the cytoskeleton [16,17], T-box transcription factor Emoesodermin (*Emoes*) [18], POU domain transcription factor *Pou5f1/Oct4* [19–21], Dicer enzyme [22], prostaglandin E2 signaling via epinephrine receptor 4 [23], the Rac-GAP activity protein alpha2-Chimerin [24], an Src kinase encoded by angiomotin-like2 [25], an atypical pro-cadherin Flamingo [26], heterotrimeric G proteins of $G\alpha_{12}$ family ($G\alpha_{12}$ and $G\alpha_{13}$) [27] and αE-catenin [28], an actin-binding protein associated with E-cadherin-β-catenin-based adherences. However, the molecular basis of epiboly is not well understood.

Apolipoproteins, the major protein component of lipoproteins, play an important role in lipoprotein metabolism. They contribute to interorgan fuel (triglyceride) transport and to the balance of the cholesterol pool [29]. Abnormalities of apolipoproteins are associated with many human diseases, such as cardiocerebral vascular disease, obesity, insulin resistance, diabetes and Alzheimer's disease [30–34]. Recently, researchers have begun to identify links between apolipoproteins and some signaling molecules [35–37]. Some apolipoproteins are able to stimulate vascular endothelial cell growth and migration [38,39]. These findings suggest that much remains to be learned from this family.

Development of embryos depends on endogenous nutrition and energy supplied by the yolk repository in both fish and frogs. However, little is known about the regulatory mechanism by which the stock materials are transported and used in embryos. In prior studies, we have observed that gastrula embryos express a number of many apolipoproteins, including Apo-14 [40,41] and ApoC1 [42], suggesting they may play a significant role in early embryogenesis. Here, we report a new role for Apolipoprotein c1 during epiboly cell movement in zebrafish.

1 Materials and methods

1.1 Embryology

Wild-type zebrafish (AB strain) and embryos were maintained and raised at 28.5°C as described previously [43]. The stages were staged according to morphological criteria [44].

1.2 Morpholino oligonucleotides, constructs and mRNA injections

The ORF of Apoc1b was cloned into the pCS2+ vector for mRNA synthesis using Message Machine-Kit (Ambion, USA). Morpholino oligonucleotides of Apoc1b were purchased from Gene Tools and the microinjections were performed as described previously [45]. The following morpholinos blocking the translation were injected at the concentrations given in Table 1: *c1a*MO: 5′-AGCCAAGTACAATTTCATCTTGTCG-3′; *c1b*MO1: 5′-TCAGGTCCTCTGCAATCTCCTTCAT-3′; *c1b*MO2: 5′-CTGCAATCTCCTTCATCTGGGTCCC-3′; *5mm*-MO: 5′-TCACGTCGTCTGGAATCTGCTTGAT-3′.

1.3 Northern blot analysis

Total RNA (10 ng) was run on an 8% acrylamide denaturing (urea) gels, then transferred to a Zeta-Probe GT membrane (BioRad, USA). Biotin labeled oligonucleotide probes were hybridized to crosslinked membranes at 42°C in 7% SDS, 0.2 mol L^{-1} Na_2PO_4 (pH 7.0) overnight. Membranes were washed at 42°C, twice with 2× SSPE, 0.1% SDS and twice with 0.5× SSPE, 0.1% SDS, then detected with Chemiluminescence (Pierce, USA). The following probes were used during the Northern blot analysis: P1: 5′-TACAGCAAGTACAAGCATCAGCAC-3′-biotin; P2: 5′-GAAGGCGGTTTTGGTCTTATCTGCCAGGT-3′-biotin.

1.4 Whole-mount *in situ* hybridization and staining

In situ hybridization and antibody staining were carried out as described [46]. Anti-zE-cadherin polyclonal antiserum (1∶2500), DAPI (0.2 μg mL^{-1}, Sigma, USA) and phalloidin (66 nmol L^{-1}, Invitrogen, USA) were used for staining.

1.5 Antiserum preparation

Mice and rabbits were immunized with two different peptides of Apoc1 coupled to keyhole limpet hemocyanin (KLH), yielding two polyclonal antibodies against Apoc1a (C-EEPTLEQHFTKFGTQ-NH2) or against both Apoc1a and Apoc1b (C-YEKLKQKMTETFN-NH2), respectively (New East, China). The anti-sera were purified by using CNBr-activated Sepharose 4B (GE).

1.6 Cell division cycle analysis

Analysis of cell division cycles by flow cytometry was performed as previously described [47]. In brief, embryos were washed with 1/2 Ringer's solution then dissociated by trypsin digestion. After incubation with Brdu (10 μmol L^{-1}) for 30 min, the cells were washed and fixed, then stained with anti-BrdU. The cells were suspended in 50 μg mL^{-1} of PI (Sigma, USA) for flow cytometric analysis.

1.7 Microscopy and time-lapse imaging

Whole embryos were photographed using a Leica SP2 confocal microscope. For time-lapse records, dechorionated embryos were laid on a glass-bottom culture dish and were imaged at intervals. Afterward, the recordings were processed using ImageJ software. In Figure 5A, the images

from one plane of the time-lapse video recording were imported into Adobe Photoshop and pseudo-color was added to aid in presentation.

2 Results

2.1 Two isoforms of *apoc1* in zebrafish

To evaluate the developmental function of Apoc1, we searched the zebrafish genome database and obtained a homologous zebrafish *apoc1* located in the *apoe/apoc1/apoc2* gene cluster (Figure 1A). Interestingly, two *apoc1* cDNAs with different lengths were amplified by the 5′ RACE-PCR from zebrafish embryos (Figure 1B). Compared with the longer form (named *apoc1a*), the shorter form of *apoc1* (named *apoc1b*) has a truncation at the 5′-end, resulting in the absence of a signal peptide (Figure 1C and D). This suggests that *apoc1b* is an intracellular isoform.

2.2 The differential expression patterns of Apoc1s during embryogenesis

To confirm the existence of two different length *apoc1* molecules in zebrafish, we analyzed their expression during embryogenesis by Northern blot analysis using two 3′-biotin labeled oligonucleotide probes. Probe P1 targeting exon 2, only detected *apoc1a*, whereas probe P2 targeting the 3′ terminal of exon 3, detected both *apoc1a* and *apoc1b* (Figure 1C). *apoc1a* was first detected at the shield stage by both the P1 and P2 probes, while *apoc1b* was detected at the one-cell and 32-cell stage of embryos (Figure 2A), suggesting that *apoc1b*, but not *apoc1a*, was of maternal origin. The zygotic *apoc1b* was transcribed at the sphere stage and the level of expression was higher during embryogenesis than that of *apoc1a* (Figure 2A).

In addition, whole-mount *in situ* hybridization (WISH) was used to observe the spatial distribution of *apoc1a* and

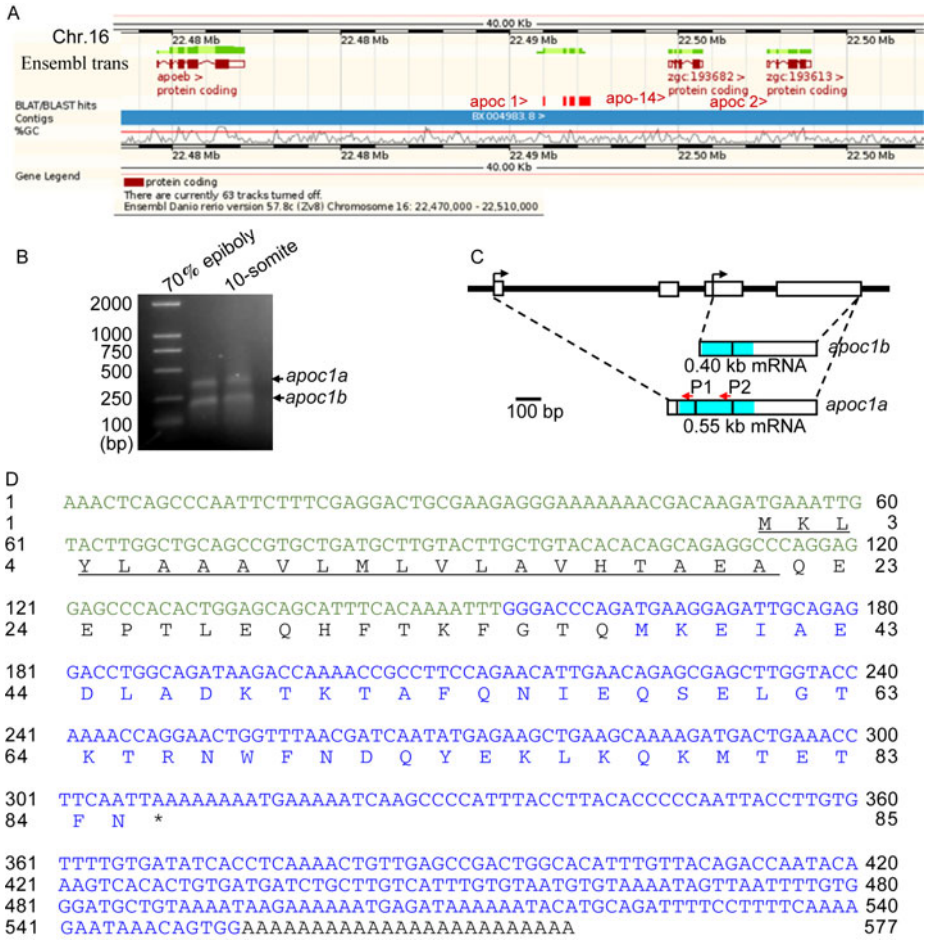

Figure 1 Identification and sequence characterization zebrafish *apoc1s*. A, Genomic mapping of *apoc1* locus (http://www.ensembl.org/). A conserved *apoe/apoc1/apoc2* gene cluster is found in linkage Group 16 (LG16) and *apoc1* consists of four exons and three introns. B, 5′ RACE-PCR in two SMART cDNA libraries from the gastrulae stage and 10-somite stage in zebrafish. C, Schematic representation of the *ApoC1* locus with the two different transcript structures. The cyan box indicates the open reading frame (ORF). D, Nucleotide and deduced amino acid sequences of *apoc1a* and *apoc1b* (blue color). *apoc1a* is 37 aa longer than *apoc1b* at the N terminus because of the inclusion of a signal peptide (underlined).

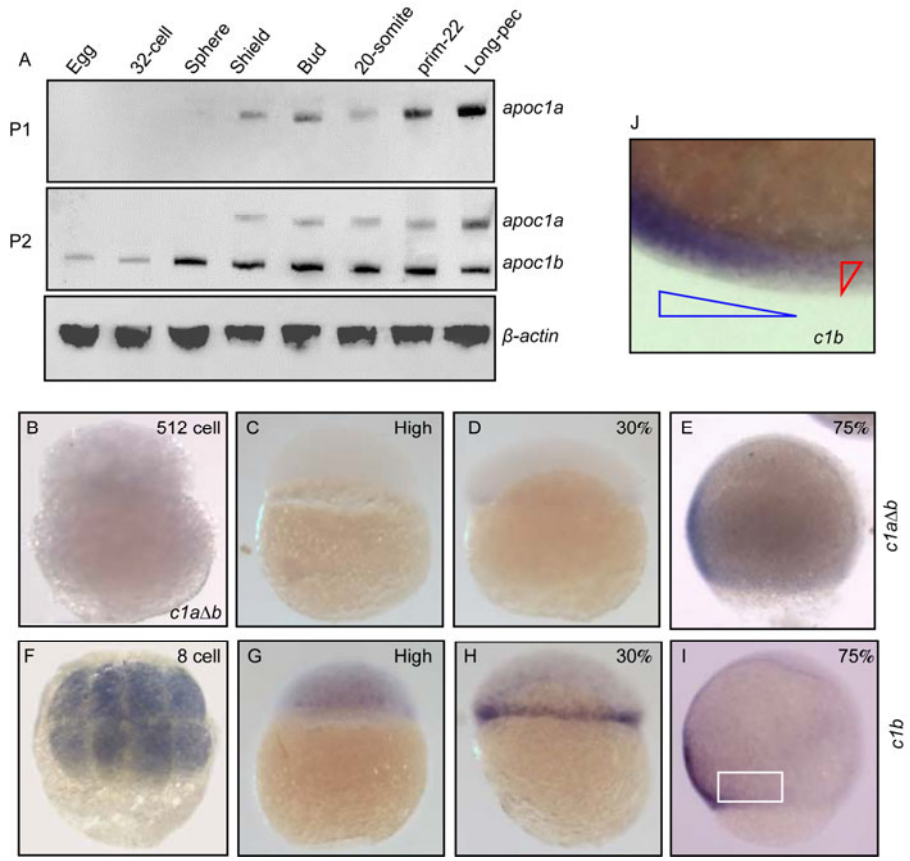

Figure 2 The expression patterns of *apoc1a* and *apoc1b* during embryogenesis in zebrafish. A, Northern blot analysis showing the size and time of *apoc1s* mRNA accumulation. As indicated in Figure 1C, probe P1 recognized only *apoc1a* (top) while P2 recognized both *apoc1a* and *apoc1b* (middle). β-actin acted as control (bottom). B–E, *In situ* hybridization analysis with antisense probe *c1aΔ1b* at 512-cell (B), high (C), 30%-epiboly (D) and 75%-epiboly (E) stage. Dorsal to the right and anterior to the top. F–J, *In situ* hybridization analysis with antisense probe *c1b* at 8-cell (F), high (G), 30%-epiboly (H) and 75%-epiboly (I) stage. Dorsal to the right and anterior to the top. Vegetal view of the box area in I is shown in higher magnification (J). Gradient WISH signaling is indicated by triangles.

apoc1b using two different antisense RNA probes *c1aΔb* (green color in Figure 1D) and *c1b* (blue color in Figure 1D). Consistent with the results of Northern blot analysis, *c1aΔb* (specific for *apoc1a*) did not yield a signal during the early stage (Figure 2B and C). However, applying another probe (specific for both *apoc1a* and *apoc1b*) yielded a positive signal that was ubiquitously and uniformly distributed in the cleavage cells (Figure 2F and G), suggesting that the probe was targeting *apoc1b* from maternal egg. Similarly, *apoc1a* was only weakly detected at 30% epiboly (Figure 2D), whereas *apoc1b* was expressed throughout the blastoderm and was strongly localized at the margin of the blastoderm (Figure 2H). From gastrulation, it was difficult to distinguish *apoc1a* and *apoc1b* using the two probes (Figure 2E compared with 2I). Significantly, during the onset of gastrulation, we observed a transcriptional gradient from ventral to dorsal, in which expression was highest on the ventral side and decreased progressively towards the dorsal side (Figure 2E and I and shown by the blue triangle in Figure 2J). Concurrently, we also observed another gradient characterized by high expression in deep cells and low in superficial cells in the ventral and lateral region of the embryos (shown by the red triangle in Figure 2J). Taken together, the results suggest that zebrafish *apoc1* would play some roles in a concentration-dependent manner during early embryogenesis.

2.3 Apoc1b is required for epiboly

To study the physiological function of Apoc1, endogenous Apoc1a and Apoc1b were knocked down by antisense morpholinos (MO), which specifically inhibited the expression of either Apoc1a or Apoc1b (Figure 3A). Injection with *apoc1a* MO did not yield a phenotypic response in the embryo (not shown), whereas injection of *apoc1b* morpholino (*c1b*MO1) disrupted gastrula morphogenesis (Figure 3). Thus, we concentrated further analysis on this small form. In addition, a control mismatched MO with five nucleotide changes (*c1b*Mis) did not affect gastrulation (Table 1). Another MO (*c1b*MO2), targeted to a different region, produced similar phenotypes when injected alone or mixed with *c1b*MO1 (Table 1). Therefore, disruption of the gas-

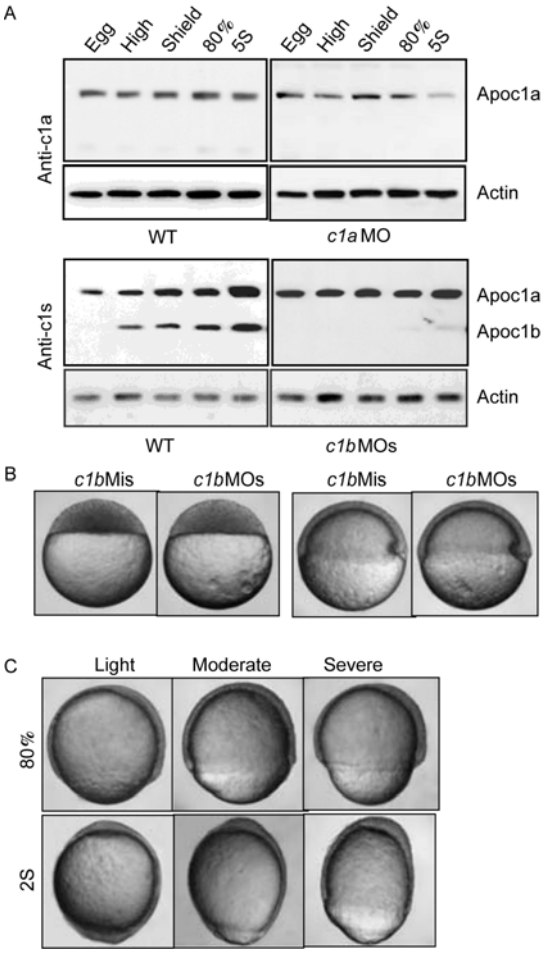

Figure 3 Apoc1b is required for gastrula morphogenesis in zebrafish. A, Western blot analysis of Apoc1a and Apoc1b protein in wild type and morpholino-injected eggs. Actin acted as control. B, Embryos injected with mismatched control and Apoc1b morpholinos at the dome and shield stage. C, Apoc1b morphants exhibit morphogenic defects at the late gastrulation and somite stage.

trula appears to be produced by specific knockdown of the Apoc1b protein with *apoc1b* MOs.

When compared with Mis or WT siblings, the early embryos appeared normal during the blastula and early gastrula stage (Figure 3B). However, at approximately 80% epiboly, Apoc1b morphants exhibited obvious morphogenetic defects in a dose-dependent manner (Figure 3C, Table 1). At the ideal dose (8 ng), 60.6% of morphants showed moderate defect, but still completed epiboly at the somite stage; 26.6% of embryos displayed a severe defect and never completed epiboly (Table 1). However, co-injection with an *apoc1b* mRNA lacking a targeted sequence (*rc*RNA) aggravated the morphogenetic defects (Table 1). These data suggest that the proper expression of Apoc1b is required for epiboly.

2.4 Knockdown of Apoc1b disrupts deep cell epiboly

To analyze the epiboly delay in more detail, we stained nuclei and the cytoskeleton to visualize the distribution of cells. This revealed the epiboly progression in deep cell, YSL and EVL compartments (Figure 4). The staining of fixed embryos revealed that the deep cells slowed and stopped their spread over the yolk cell in Apoc1b morphants (Figure 4B). The progression of epiboly in the EVL and YSL was very slightly delayed (Figure 4B). Both spread to the vegetal pole of the embryo by the end of gastrulation (data not shown). As epiboly progressed, the EVL margin (indicated by arrows) and YSL margin (indicated by blank triangles) moved far away from the deep cell margin (indicated by triangles), in contrast to the pattern in WT controls (Figure 4B compared with 4A). Apoc1b was expressed in a gradient characterized by high levels in deep cells and low levels in superficial cells, but was not expressed in the EVL. Knockdown of Apoc1b not only decreased the amount of protein, but also attenuated the gradient. Ubiquitous overexpression by coinjection with mRNA is unlikely to reestablish the gradient, but disrupts the gradient. Therefore, Apoc1b *rc*RNA did not rescue the epiboly defects caused by Apoc1b knockdown, but instead aggravated them (Figure 4C, Table 1).

Delay of deep cell epiboly can be caused by abnormal migratory or adhesive behavior, but also could result from a reduced number of deep cells [20]. To evaluate this latter possibility, we analyzed cell proliferation by examining cell division cycles in embryos at 3.3 hours post-fertilization and 60% epiboly stage using flow cytometry. Our data revealed that, at least until cell division cycle 13, the syn-

Table 1 Induction of gastrulation epiboly defeats at bud stage by microinjection

Experimental condition	Epiboly			*n* (times)
	95%–100%	80%–95%	<80%	
WT	100%	0	0	
Mis (8 ng)	100%	0	0	
*c1b*MO1 (4 ng)	69.5%	30.5%	0.0%	187 (3)
*c1b*MO1 (8 ng)	12.8%	60.6%	26.6%	475 (8)
*c1b*MO1 (4 ng)+*c1b*MO2 (4 ng): *c1b*MOs	13.5%	54.5%	32.1%	156 (2)
*c1b*MOs+*apoc1b rc*RNA (0.1 ng)	8.9%	37.9%	53.2%	235 (4)
*apoc1b rc*RNA (0.1 ng)	53.5%	42.5%	4.0%	226 (4)

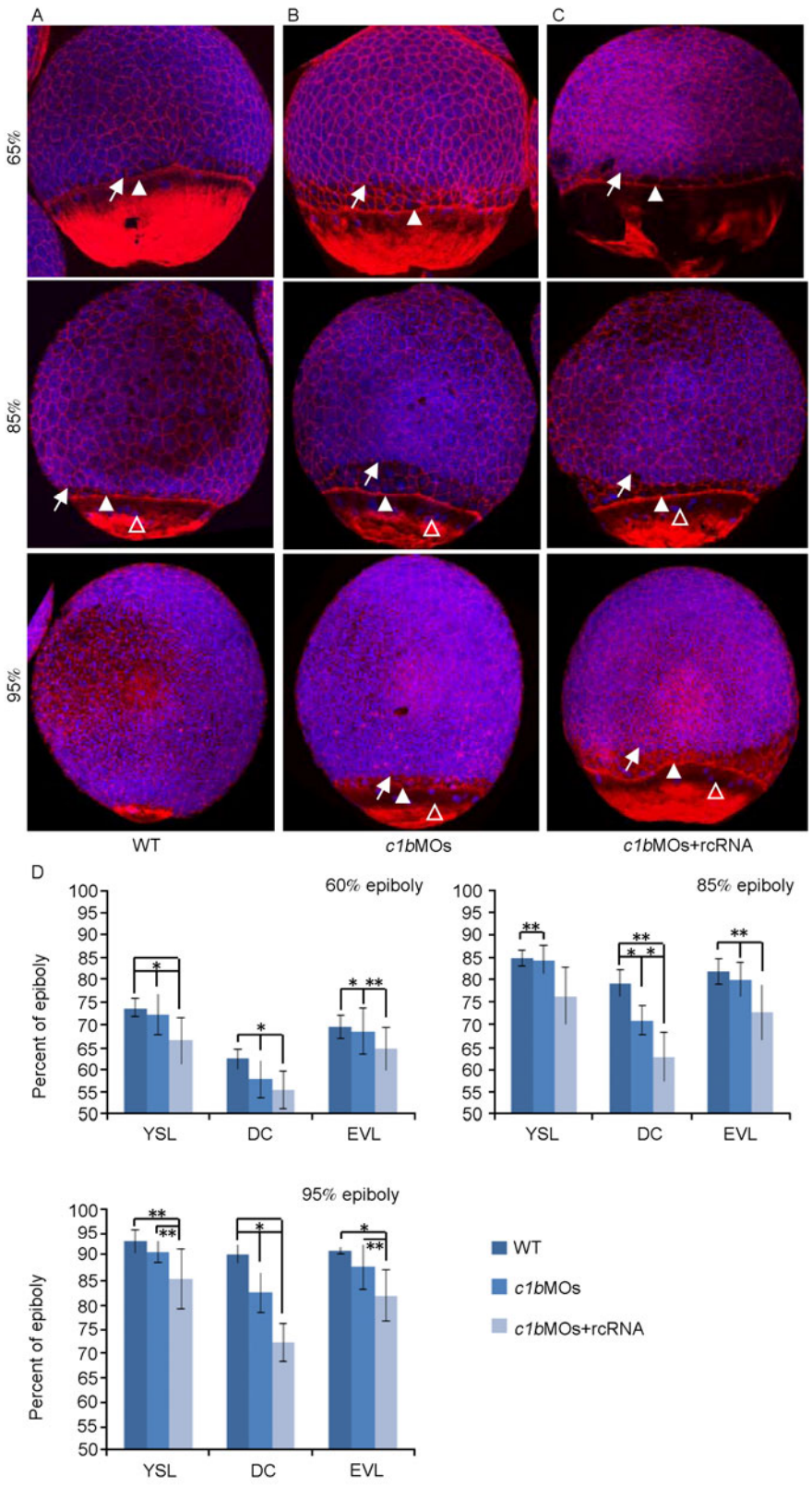

Figure 4 *Apoc1b* affects the epiboly of deep cells. A–C, Analysis of EVL deep cell and YSL epiboly in wild-type embryos (A), Apoc1b morphants (B) and Apoc1b morphant coinjected with *rc*RNA (C). Confocal Z-projections of co-stained embryos with DAPI (blue) and phalloidin (red) were shown. The margin of the EVL is indicated by white triangles. The margin of the deep cells is indicated by white arrows. The margin of the YSL is indicated by blank triangles. D, Graphs showing the percentage of epiboly in the three layers. n=10 embryos from three independent experiments for each sample. Error bars represent mean±SD; *, P<0.001; **, P<0.05, one way AVOVA using the Holm-Sidak method.

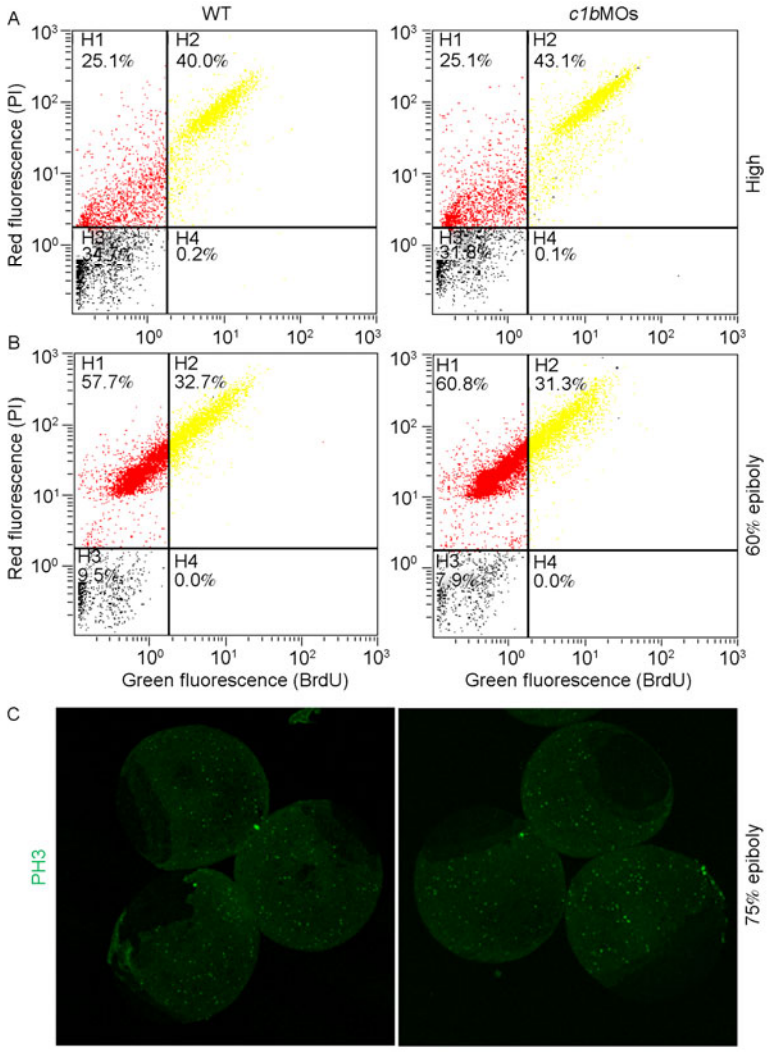

Figure 5 Cell proliferation is unaffected in Apoc1b morphants. A and B, Flow cytometric analysis of wild-type embryos and Apoc1b morphants at the high (A) and 60%-epiboly (B) stage. Dual parameter dot plots of PI (red) vs. BrdU (green) were generated by software within the Fac-Sort flow cytometer. Three independent experiments were performed for each sample. C, Z-projections of whole-mount immunofluorescence analyses of PH3 in wild-type and morphant embryos at 75% epiboly. n=23 from three independent experiments.

chronous pre-MBT and semisynchronous post-MBT cell division cycles proceeded at the same rate in wild-type and Apoc1b morphants (Figure 5, yellow dots). Fluorescence staining analysis using anti- phosphorylated histone H3 (PH3) revealed a similar pattern in the WT control and Apoc1b morphants (Figure 5C). Together, this indicates that the delay in epiboly is not caused by reduced deep cell number, but by an effect on cell movement.

2.5 Radial intercalation is disrupted by apoc1b knockdown

To directly observe the effects of Apoc1b on deep cell behavior during epiboly, we recorded the movements of ventral-lateral cells during late epiboly. In WT controls, the individual cells from the interior layer (via radial intercalation) entered the exterior layer, changed their shape, then became restricted to that layer (Movie S1 in Supporting Information). During this process, the combination of radial intercalation and cell shape changes drive the spreading of the blastoderm [6]. In Apoc1b morphants, cells forming the interior layers also intercalated into the exterior layer, but these cells never completely flattened out (Figure 6A; Movie S2 in Supporting Information). Furthermore, many of these cells 'de-intercalated' and moved back into the interior layer (blue cell in Figure 6A; Movie S2 in Supporting Information). The disordered cell movement in response to Apoc1b knockdown was reduced by co-injection with Apoc1b *rc*RNA, which even restricted the normal radial intercalation (Movie S3 in Supporting Information). Thus, co-injection with *rc*RNA did not rescue the epiboly defects in Apoc1b morphants, but induced a longer delay in epiboly. Therefore, deep cell epiboly requires both the presence of the Apoc1b protein and a gradient of its expression.

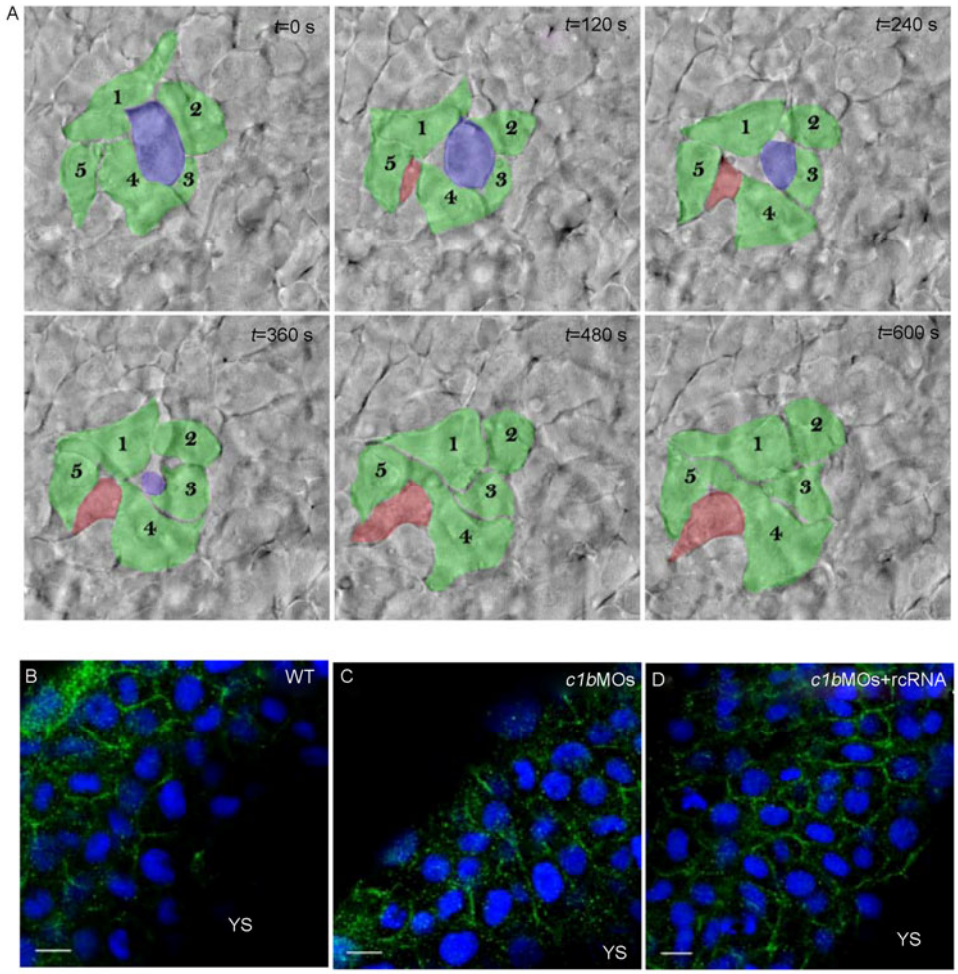

Figure 6 Apoc1b regulates the epiboly motility and disrupts the radial gradient of E-cadherin. A, Radial intercalation in Apoc1b morphant at ~80% epiboly (n=5). Cells from the interior layer of the epiblast (red) intercalate in among cohorts of cells of the exterior layer (green). Also cells frequently leave the exterior layer (blue) and migrate back into the interior layer. B–D, Ventral medial sagittal sections of 90% epiboly embryos stained with E-cadherin antibody (green) and DAPI (blue), lateral view. The radial gradient expression of E-cadherin (B) is inhibited both in Apoc1b knock down (C) and in rescued (D) embryos. YS, yolk sphere; scale bar, 10 μm.

2.6 The radial gradient of E-cadherin is disrupted by apoc1b knockdown

With low expression in deep cells and high in superficial cells, *e-cadherin* forms a gradient that is necessary for radial intercalation (Figure 6B). Therefore, we proposed that Apoc1b affects epiboly via the action of E-cadherin. To explore this, we examined the expression of E-cadherin in ventral cells. Knockdown of Apoc1b not only decreased the amount of E-cadherin in the plasma membrane (PM), but also impaired the gradient in the ventral region (Figure 6C). The dorsal gradient was unaffected in Apoc1b morphants (data not shown), in accordance with the ApoC1 ventral expression pattern (Figure 2E–G). Interestingly, the defect in subcellular distribution was compromised by co-injection with Apoc1b *rc*RNA, whereas disruption of the gradient was not (Figure 6D). This may explain why coinjection with Apoc1b *rc*RNA cannot reverse the delay in epiboly and the intercalation disorder. The ubiquitous expression of rescued Apoc1b mRNA cannot reestablish its gradient, but does rescue its intracellular effects on E-cadherin. Taken together, these data suggest that the proper pattern of Apoc1b expression is needed for correct E-cadherin distribution and for regulating the radial intercalation during epiboly during zebrafish gastrulation.

3 Discussion

Lipid homeostasis is critical for cells and organisms. Members of apolipoprotein family play fundamental roles in lipid transportation and lipid metabolism. In this paper, we have shown a new function for Apolipoprotein C1, independently to lipoprotein metabolism, in epiboly cell movement during zebrafish gastrulation.

We identified two different isoforms of Apolipoprotein C1 in zebrafish, designated as Apoc1a and Apoc1b. As in other species, Apoc1a is a secreted protein because of a

signal peptide situated at its N terminal. The yolk sphere, a unique extracellular structure consisting of large lipids, contains a maternal source of Apoc1a protein, which acts as a pool for early embryogenesis. Therefore, injection with Apoc1a morpholino only slightly reduced the amount of Apoc1a protein (Figure 3A). We speculate that this explains why Apoc1a knockdown does not affect embryogenesis. Its presence in the yolk sphere suggests that Apoc1a plays a role in lipid transport and metabolism, as in other species. In contrast, another transcript isoform (Apoc1b) is restricted in cells, owing to the lack of signal peptide (Figure 1C and D). The difference in distribution implies a differential function, in which Apoc1b regulates epiboly.

Apolipoproteins are key mediators of lipid homeostasis. As the epiboly progressed, the expression of Apoc1s increases in YSL (Figure 2E and F), where Apoc1s easily interact with yolk lipid. It is unlikely that only Apoc1a is expressed in YSL, since *apoc1b* is not a transcript splicing variant and shares the same genome with *apoc1a* (Figure 1). The cell membrane in YSL is largely absent [12], suggesting that the difference between secreted Apoc1a and non-secreted Apoc1b is small. Therefore, both of these Apoc1 proteins likely play similar roles in YSL during epiboly.

Epiboly is the initial morphogenetic process during fish gastrulation, and consists of the spreading movements of three layers. The interactions between the three layers occur throughout the whole epiboly process. The inner layer (yolk cell) likely acts as an epiboly motor, since the YSL moves more quickly when the blastoderm is removed in *Fundulus* [48]. The yolk cell produces a pulling force via its Claudin E-dependent tight junctions to the EVL margin [13]. The interaction between the EVL and deep cells is also important for epiboly. The basal surface of the EVL acts as a substrate for the epiboly movement of deep cells. Conversely, deep cells exert traction force on the EVL [49]. In our case, knockdown of Apoc1b significantly impaired the epiboly of deep cells. Apoc1b regulates the radial intercalation, through which deep cells move actively during epiboly. The epiboly of the EVL can be attenuated through the traction force produced by interacting with underlying deep cells, in which epiboly is impaired. The epiboly of YSL was very slightly weakened, although Apoc1b is expressed in this area. As with the EVL, this slight delay in YSL is likely due to the influence of deep cells.

During gastrulation, the transcription of apoc1s is tightly controlled and is characterized by two gradients. One is from ventral to dorsal, where transcription levels were highest in the ventral region and decreased progressively to the dorsal side (Figure 2E and F). The second gradient is from deep to superficial, with higher transcription levels in deeper cells. These suggest that not only Apoc1b itself, but also the gradient in its expression is required for gastrulation morphogenesis. Indeed, interfering with its expression by knockdown or overexpression of Apoc1b both influences gastrulation morphogenesis and inhibits epiboly (Figure 3). Both disrupt the Apoc1b gradient, although they have opposite effects on protein levels. Therefore, the underlying mechanisms differ in these two cases. In Apoc1b morphants, radial intercalation of deep cells is not impaired, but the cells in the outer layer move to the deeper region, concurrently (Figure 6A). This leads to defects in the blastoderm spreading while the cell movement is unlimited (Movie S2 in Supporting Information). In the case of overexpression, radial intercalation is reduced by injecting *rc*RNA and cells are likely confined to their current position (Movie S3 in Supporting Information). Together, these suggest that the mediation of epiboly by Apoc1b is correlated to cell-cell interactions.

E-cadherin is a critical cell-cell adhesion molecular at adherent junctions. It has been proven that E-cadherin plays pivotal roles both in epiboly and in C&E movement. The high level of E-cadherin expression in superficial cells and low levels in deep cells form a gradient during gastrulation in zebrafish. This means that the cell-cell adhesions in one cell differ in the inward and outward orientation. This results in a higher probability of cell motility in the exterior layer [50]. Interestingly, this gradient is reversed relative to the Apoc1b gradient. Coincidently, disruption of the Apoc1b gradient also impaired the E-cadherin gradient. Therefore, the Apoc1b gradient is required for corrected E-cadherin distribution, and thereby influences epiboly.

We thank J. A. Marrs for providing the zebrafish E-cadherin antibody. This work was supported by grants from the National Basic Research Program of China (2010CB126300), the National High Technology Research and Development Program of China (2012AA092201-3), Ministry of Agriculture of China within the special Fund for Agro-scientific Research in the Public Interest (200903045), and the National Natural Science Foundation of China (30971414).

1 Warga R M, Kimmel C B. Cell movements during epiboly and gastrulation in zebrafish. Development, 1990, 108: 569–580
2 Solnica-Krezel L. Conserved patterns of cell movements during vertebrate gastrulation. Curr Biol, 2005, 15: R213–R228
3 Niu X B, Shi H, Peng J R. The role of mesodermal signals during liver organogenesis in zebrafish. Sci China Life Sci, 2010, 53: 455–461
4 Arendt D, Nübler-Jung K. Rearranging gastrulation in the name of yolk: Evolution of gastrulation in yolk-rich amniote eggs. Mech Dev, 1999, 81: 3–22
5 Solnica-Krezel L. Gastrulation in zebrafish—all just about adhesion? Curr Opin Genet Dev, 2006, 16: 433–441
6 Kane D A, Mcfarland K N, Warga R M. Mutations in *half baked*/E-cadherin block cell behaviors that are necessary for teleost epiboly. Development, 2005, 132: 1105–1116
7 Kane D, Adams R. Life at the edge: Epiboly and involution in the zebrafish. Results Probl Cell Differ, 2002, 40: 117–135
8 Shimizu T, Yabe T, Muraoka O, et al. E-cadherin is required for gastrulation cell movements in zebrafish. Mech Dev, 2005, 122: 747–763
9 Solnica-Krezel L, Driever W. Microtubule arrays of the zebrafish yolk cell: organization and function during epiboly. Development, 1994, 120: 2443–2455
10 Wilkins S J, Yoong S, Verkade H, et al. Mtx2 directs zebrafish morphogenetic movements during epiboly by regulating microfilament formation. Dev Biol, 2008, 314: 12–22
11 Hsu H J, Liang M R, Chen C T, et al. Pregnenolone stabilizes micro-

tubules and promotes zebrafish embryonic cell movement. Nature, 2006, 439: 480–483
12 Cheng J C, Miller A L, Webb S E. Organization and function of microfilaments during late epiboly in zebrafish embryos. Dev Dyn, 2004, 231: 313–323
13 Siddiqui M, Sheikh H, Tran C, et al. The tight junction component Claudin E is required for zebrafish epiboly. Dev Dyn, 2010, 239: 715–722
14 Köppen M, Fernández B G, Carvalho L, et al. Coordinated cell-shape changes control epithelial movement in zebrafish and *Drosophila*. Development, 2006, 133: 2671–2681
15 Behrndt M, Salbreux G, Campinho P, et al. Forces driving epithelial spreading in zebrafish gastrulation. Science, 2012, 338: 257–260
16 Sharma D, Holets L, Zhang X, et al. Role of Fyn kinase in signaling associated with epiboly during zebrafish development. Dev Biol, 2005, 285: 462–476
17 Tsai W B, Zhang X, Sharma D, et al. Role of Yes kinase during early zebrafish development. Dev Biol, 2005, 277: 129–141
18 Bruce A E, Howley C, Dixon F M, et al. T-box gene eomesodermin and the homeobox-containing Mix/Bix gene mtx2 regulate epiboly movements in the zebrafish. Dev Dyn, 2005, 233: 105–114
19 Reim G, Mizoguchi T, Stainier D Y, et al. The POU domain protein Spg (Pou2/Oct4) is essential for endoderm formation in cooperation with the HMG domain protein Casanova. Dev Cell, 2004, 6: 91–101
20 Lachnit M, Kur E, Driever W. Alterations of the cytoskeleton in all three embryonic lineages contribute to the epiboly defect of Pou5f1/Oct4 deficient MZspg zebrafish embryos. Dev Biol, 2008, 315: 1–17
21 Lunde K, Belting H, Driever W. Zebrafish pou5f1/pou2, homolog of mammalian Oct4, functions in the endoderm specification cascade. Curr Biol, 2004, 14: 48–55
22 Giraldez A J, Cinalli R M, Glasner M E, et al. microRNAs regulate brain morphogenesis in zebrafish. Science, 2005, 308: 833–838
23 Cha Y I, Kim S H, Sepich D, et al. Cyclooxygenase-1-derived PGE2 promotes cell motility via the G-protein-coupled EP4 receptor during vertebrate gastrulation. Genes Dev, 2006, 20: 77–86
24 Leskow F C, Holloway B A, Wang H, et al. The zebrafish homologue of mammalian chimerin Rac-GAPs is implicated in epiboly progression during development. Proc Natl Acad Sci USA, 2006, 103: 5373–5378
25 Huang H, Lu F I, Jia S, et al. Amotl2 is essential for cell movements in zebrafish embryo and regulates c-Src translocation. Development, 2007, 134: 979–988
26 Carreira-Barbosa F, Kajita M, Morel V, et al. Flamingo regulates epiboly and convergence/extension movements through cell cohesive and signalling functions during zebrafish gastrulation. Development, 2009, 136: 383–392
27 Lin F, Chen S, Sepich D S, et al. Galpha12/13 regulate epiboly by inhibiting E-cadherin activity and modulating the actin cytoskeleton. J Cell Biol, 2009, 184: 909–921
28 Schepis A, Sepich D, Nelson W J. αE-catenin regulates cell-cell adhesion and membrane blebbing during zebrafish epiboly. Development, 2012, 139: 537–546
29 Dominiczak M H, Caslake M J. Apolipoproteins: Metabolic role and clinical biochemistry applications. Ann Clin Biochem, 2011, 48: 498–515
30 Bell R D, Winkler E A, Singh I, et al. Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. Nature, 2012, 485: 512–516
31 Bu G. Apolipoprotein E and its receptors in Alzheimer's disease: Pathways, pathogenesis and therapy. Nat Rev Neurosci, 2009, 10: 333–344
32 Hegele R A. Plasma lipoproteins: Genetic influences and clinical implications. Nat Rev Genet, 2009, 10: 109–121
33 Lusis A J, Pajukanta P. A treasure trove for lipoprotein biology. Nat Genet, 2008, 40: 129–130
34 Zou L, Huang Q, Li A. A genome-wide association study of Alzheimer's disease using random forests and enrichment analysis. Sci China Life Sci, 2012, 55: 618–625
35 Gafencu A V, Robciuc M R, Fuior E, et al. Inflammatory signaling pathways regulating ApoE gene expression in macrophages. J Biol Chem, 2007, 282: 21776–21785
36 Cho T, Jung Y, Koschinsky M L. Apolipoprotein(a), through its strong lysine-binding site in KIV_{10}, mediates increased endothelial cell contraction and permeability via a Rho/Rho kinase/ MYPT1-dependent pathway. J Biol Chem, 2008, 283: 30503–30512
37 Liu L, Craig A W, Meldrum H D, et al. Apolipoprotein(a) stimulates vascular endothelial cell growth and migration and signals through integrin αVβ3. Biochem J, 2009, 418: 325–336
38 Xia J H, Liu J X, Zhou L, et al. Apo-14 is required for digestive system organogenesis during fish embryogenesis and larval development. Int J Dev Biol, 2008, 52: 1089–1098
39 Zhou L, Wang Y, Yao B, et al. Molecular cloning and expression pattern of 14 kDa apolipoprotein in orange-spotted grouper, *Epinephelus coioides*. Comp Biochem Physiol B-Biochem Mol Biol, 2005, 142: 432–437
40 Wang Y, Zhou L, Li Z, et al. Molecular cloning and expression characterization of *ApoC-I* in the orange-spotted grouper. Fish Physiol Biochem, 2008, 34: 339–348
41 Westerfield M. The zebrafish book. A guide for the laboratory use of zebrafish (*Danio rerio*). 4th ed. Eugene: University of Oregon Press, 2000
42 Kimmel C B, Ballard W W, Kimmel S R, et al. Stages of embryonic development of the zebrafish. Dev Dyn, 1995, 203: 253–310
43 Mei J, Li Z, Gui J. Cooperation of Mtmr8 with PI3K regulates actin filament modeling and muscle development in zebrafish. PLoS ONE, 2009, 4: e4979
44 Liu J, Hu B, Wang Y, et al. Zebrafish eaf1 and eaf2/u19 mediate effective convergence and extension movements through the maintenance of wnt11 and wnt5 expression. J Biol Chem, 2009, 284: 16679–16692
45 Dolbeare F, Gratzner H, Pallavicini M G, et al. Flow cytometric measurement of total DNA content and incorporated bromodeoxyuridine. Proc Natl Acad Sci USA, 1983, 80: 5573–5577
46 Betchaku T, Trinkaus J P. Contact relations, surface activity, and cortical microfilaments of marginal cells of the enveloping layer and of the yolk syncytial and yolk cytoplasmic layers of *Fundulus* before and during epiboly. J Exp Zool, 1978, 206: 381–426
47 Lepage S E, Bruce A E. Zebrafish epiboly: Mechanics and mechanisms. Int J Dev Biol, 2010, 54: 1213–1228
48 Borghi N, Lowndes M, Maruthamuthu V, et al. Regulation of cell motile behavior by crosstalk between cadherin- and integrin-mediated adhesions. Proc Natl Acad Sci USA, 2010, 107: 13324–13329

Supporting Information

scls-2013-0107-movie S1–S3

The supporting information is available online at life.scichina.com and www.springerlink.com. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.