Sequestration of Retinyl Esters Is Essential for Retinoid Signaling in the Zebrafish Embryo*

The biological importance of retinoids (vitamin A or all-trans retinol) is required in quantitative different amounts and spatiotemporal distribution for the production of retinoic acid, a nuclear hormone receptor ligand, and 11-cis retinal, the chromophore of visual pigments. We show here for zebrafish that embryonic retinoid homeostasis essentially depends on the activity of a lecithin:retinol acyltransferase (Lratb). During embryogenesis, lratb is expressed in mostly non-overlapping domains opposite to retinal dehydrogenase 2 (raldh2), the key enzyme for retinoic acid synthesis. Blocking retinyl ester formation by a targeted knock down of Lratb results in significantly increased retinoic acid levels, which lead to severe embryonic patterning defects. Thus, we provide evidence that a balanced competition between Lratb and Raldh2 for yolk vitamin A defines embryonic compartments either for retinyl ester or retinoic acid synthesis. This homeostatic mechanism dynamically adjusts embryonic retinoic acid levels for gene regulation, concomitantly sequestering excess yolk vitamin A in the form of retinyl esters for the establishment of larval vision later during development.

The biological importance of retinoids (vitamin A or all-trans retinol) and its derivatives for vertebrate development has long been known, because both deprivation and exposure to excess retinoids cause major embryonic abnormalities. The ROL derivative all-trans retinoic acid (RA) is a hormonal signaling molecule that acts in developing and adult tissues (1). RA regulates gene expression via heterodimeric nuclear receptors, the RA receptors and the retinoid X receptors (2, 3). Both RA regulates gene expression via heterodimeric nuclear receptors, the RA receptors and the retinoid X receptors (2, 3). Both are ligand-dependent transcription factors belonging to the superfamily of nuclear hormone receptors (4). RA is generated from maternal ROL via all-trans retinal (all-trans RAL) by a stepwise enzymatic oxidation. Hereby, the second oxidation step catalyzed by retinal dehydrogenases (Raldhs) is thought to be rate-limiting (5, 6). The first step in the turn-off mechanism of retinoid signaling is the catabolism of RA by a class of cytochrome P450 enzymes (Cyp26s) to more polar products, such as 4-hydroxy RA or 4-oxo RA (7).

The catabolic control of RA levels via Cyp26s is not sufficient to explain all aspects of retinoid homeostasis, considering for example the key role of vitamin A in vision. In photoreceptors, ROL is required for the synthesis of 11-cis retinal (11-cis RAL), the chromophore of visual pigments (8), in much higher amounts than for RA synthesis in gene regulation. This quantitatively different requirement for ROL calls for a mechanism that maintains the homeostasis between RA and 11-cis RAL synthesis. To elucidate the underlying control mechanism of retinoid homeostasis, we used the zebrafish as a model. In this oviparous vertebrate, yolk retinoids must be proportioned during embryonic development to adequately support both these processes. We found that sequestration of retinyl esters (RE) is essential for retinoid homeostasis during embryonic development. We molecularly and functionally characterized two different lecithin:retinol acyltransferases (Lrats), Lrata and Lratb that convert ROL to its ester form. Lrata is exclusively expressed in light-sensitive organs such as the pineal gland and the developing eyes, indicating that this Lrat contributes to retinoid metabolism in light perception (visual cycle). By contrast, lratb was already expressed during embryonic stages. Most interestingly, the expression domains of lratb were mutually exclusive to those of raldh2, suggesting that embryonic compartments for either RE or RA production exist. Targeted gene knock down of the Lrata function resulted in highly decreased embryonic RE but increased RA levels leading to severe patterning defects. Thus, a competition of Lrata and Raldh2 for the yolk ROL dynamically adjusts embryonic RA synthesis levels. This homeostatic control mechanism elegantly satisfies the different quantitative and temporal requirements for ROL in gene regulation and vision during zebrafish development.

EXPERIMENTAL PROCEDURES

Cloning of lrata and lratb—For cloning lrat orthologues from zebrafish, we searched the data base and found two full-length cDNA sequences (GenBank™ accession codes BC095753 and BC090301) encoding proteins with high overall sequence identity to the mouse and human Lrata, respectively. We here termed the corresponding genes lrata and lratb. For cloning, we per-
formed reverse transcription PCR with whole RNA preparations from 3-day post-fertilization larvae (irata) and liver of adult zebrafish (lrab) and the oligonucleotide primers irata-up ATGTGAGCTGCTGACTTTTC and irata-down ACCAGCACAAAGATGAGG for irata and lrab-up ATGTGAGCTGCTGACTTTTC and lrab-down ACCAGCACAAAGATGAGG for lrab. The corresponding irata and lrab cDNAs obtained were cloned into the vector pCRII-TOPO (Invitrogen) and verified by sequence analyses.

Construction of Drosophila S2 Schneider Cell Expression Vectors, Transient Transfection of S2 Cells, and Induction—For cloning of irata the primers forward, 5′-TTACTAGTAAAAATTAGTGCCAGCTGCTGACTTTTCT-3′, and reverse, 5′-TTCTCGAGGACCCGCCATCCAAAGATGACCTTTCT-3′, and lrab forward, 5′-TTACTAGTAAAAATTAGTGCCAGCTGCTGACTTTTCT-3′, and reverse, 5′-TTCTCGAGGACCCGCCATCCAAAGATGACCTTTCT-3′, were used. PCR products were cut with SpeI and XhoI and ligated into a likewise treated pMT-V5/HisA expression vector (Invitrogen). By this procedure both irata and lrab were linked to the V5/HisA epitope of the expression vector.

S2 cells were purchased from Invitrogen and grown in Schneider’s Drosophila medium (Invitrogen) containing 10% fetal bovine serum, 50 units of penicillin, and 50 µg of streptomycin/ml in 25-cm² cell culture flasks (Greiner Bio One). Cells were maintained at 28 °C.

On the day prior to transfection, cells were seeded in 30-mm (1.5 × 10⁶ cells/dish) culture dishes (Greiner). To ensure comparability, for each experiment a single pool of cells was used. Transient transfection with 2 µg of plasmid DNA was accomplished using Cellfectin reagent (Invitrogen) essentially according to the manufacturer’s protocol. Protein expression was induced 24 h post-transfection by addition of 1 µM copper sulfate. Assays were carried out 48 h post-transfection under dim red light (620 nm). For this purpose, the cell culture medium was replaced by 2 ml of fresh medium containing 5 mM L-lysine (Sigma-Aldrich)-coated coverslips. After 24 h of protein expression induction, coverslips were washed with PBS and removed from the dishes. Cells were fixed with ice-cold methanol for 5 min. Methanol was removed, and 4% paraformaldehyde was added for 10 min. Cells were washed with PBST (PBS containing 0.1% Triton X-100) and blocked with PBSTM (PBST containing 1% skim milk powder, w/v) for 30 min at room temperature. Coverslips were incubated in PBSTM containing anti-V5 antibody (1:10,000) (Invitrogen) overnight at 4 °C, washed three times with PBSTM, and incubated in anti-mouse-Cy3 antibody (1:400) (Jackson ImmunoResearch) for 1 h at room temperature. Following three washes with PBS, coverslips were mounted on microscope slides with Vectashield mounting medium containing 4′,6-diamidino-2-phenylindole (Vector Laboratories). Fluorescence signals were recorded on a Zeiss Axiohot epifluorescence microscope. Confocal laser scanning microscopy was carried out with a Leica TCS4D microscope.

Fish Maintenance and Strains—Zebrafish (strain AB/TL) were bred and maintained under standard conditions at 28.5 °C (9). Morphological features were used to determine the stage of the embryos in hours (hpf) or days post-fertilization (10). Embryos used for in situ hybridization experiments were raised in the presence of 200 µM 1-phenyl-2-thiourea (Sigma-Aldrich) to inhibit pigmentation.

Whole Mount in Situ Hybridization—Whole mount in situ hybridization (WISH) was performed as described (11). irata and lrab were cloned into the vector pCRII-TOPO (Invitrogen), and antisense RNA probes were synthesized with the T7 RNA polymerase. Additional RNA probes used for in situ hybridization experiments were raldh2 (12), cyp26a1 (13), shh (14), fgf8 (15), myod (16), krox-20 (17), otx2 (18), pax2.1 (19), and pax6.2 (20). The RNA probes were generated with the Dig RNA or the fluorescein labeling kit (Roche Applied Science) according to the manufacturer’s protocol.

Injections of Morpholino Oligonucleotide and mRNA—For targeted knock down of the Lrath protein an antisense morpholino oligonucleotide (MO) (GeneTools, LLC) was used covering +1 to +25 of the lrat mRNA, 5′-ACAGCAAGAGAGAGGAATCTAAACAT-3′. The MO was dissolved in 0.3× Danieau’s solution (1× Danieau is 5 mM Heps, pH 7.6, containing 58 mM NaCl, 0.7 mM KCl, 0.4 mM MgSO₄, 0.6 mM Ca(NO₃)₂) to obtain a stock concentration of 1 mM (8.57 mg/ml). The stock solution was diluted to 1.7 mg/ml. The injected volume was ~3 nl, corresponding to 5.1 ng of MO/egg. Rescue experiments were performed by co-injection of 5.1 ng of lrab MO and 12.7 ng of raldh2 MO (12). Additional rescue experiments were performed with lrab mRNA. For this purpose the lrab cDNA was cloned into the vector pCS2+ and mRNA was synthesized in vitro using the mMESSAGEMACHINE kit according to the manufacturer’s protocol (Ambion Europe Ltd. UK). Rescue by co-injection of 30 pg of the lrab mRNA demonstrated the specificity of lrab MO.

Retinoic Treatments of Embryos—Fish embryos (3–4 hpf) were treated with 10⁻³ M ROL, 10⁻² m RAL, 10⁻⁷ m RA, and 10⁻⁷ M 4-oxo RA in egg water. Retinoins were prepared from a stock of 10⁻³ M ROL, 10⁻³ m all-trans RAL, 10⁻⁴ M RA, and 10⁻⁴ M 4-oxo RA in dimethyl sulfoxide (Me₂SO) (Sigma-Aldrich). As controls, siblings were treated with equivalent amounts of Me₂SO alone. During treatment, embryos were kept under dim red light (620 nm). Retinoids were purchased from Sigma-Aldrich, and 4-oxo RA was synthesized as previously described (21).

HPLC Analysis of Retinoids of Zebrafish Embryo—For extraction of retinoids, staged zebrafish embryos were collected under dim red light (620 nm). For the determination of apolar retinoids, 200 µl of 2 M NH₂OH and 200 µl of 100% MeOH were added to the embryos (n = 50). After homogenization, retinoids were extracted and subjected to HPLC system 1 as previously described (22). For determination of RA levels, staged embryos (n = 100) were collected and homogenized in 350 µl of PBS buffer (137 mM NaCl, 2.7 mM KCl, 7.3 mM Na₂HPO₄, 1.47 KH₂PO₄, pH 7.2) and 150 µl of ethanol. Then 750 µl of ethylacetate/methylacetate (8:1, v/v) were added.
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results in the zebrafish embryo. Alignment of the deduced amino acid sequences of human (h-LRAT) and Lrata and Lratb from zebrafish. Conserved His and Cys residues (12) are marked by asterisks.

probes were mixed and subjected to centrifugation for 1 min at 5000 \( \times \) g at 24 °C. The supernatant was collected and transferred to a new reaction tube. Extraction of the aqueous phase was repeated. The collected supernatants were vacuum dried (Concentrator 5301, Eppendorf, Germany). The dried pellet was resolved in 100 \( \mu l \) of HPLC solvent. The HPLC solvent was \( n \)-hexane and ethylacetate (81:19, v,v) containing 12.5 \( \mu l \) of acetic acid/100 ml. HPLC system 2 consisted of a Hypersil 3- \( \mu m \) column (Knauer) and a Beckman System Gold (Beckman Instruments) equipped with a multidiode array model 166 (Beckman Instruments) with flow rate of 1 ml min \(^{-1}\). For quantification of the molar amounts of retinoids, peak integrals were scaled with defined amounts of reference substances from Sigma-Aldrich and were quantified using the 32 Karat software (Beckman Instruments). Student’s \( t \) test was used for statistical analysis.

Photography—Live embryos were photographed in egg water and stained whole mount embryos in 100% glycerol on a dissecting microscope (Leica MZ FLIII) with an Axiocam (Zeiss).

RESULTS

**RE and Lrat Exist in the Zebrafish Embryo**—In zebrafish, yolk ROL is mainly required for the establishment of functional visual pigments and is recovered in the eyes in the form of RE and 11-cis RAL already at early larval stages (23). Here we determined the metabolic fate of yolk ROL during embryogenesis. We found that RAL, the yolk storage form of ROL in this teleost (23), is largely converted to RE already during early embryogenesis. These compounds are the storage and transport form of ROL but are also intermediates for the synthesis of the visual chromophore 11-cis RAL (24). As development proceeds, the increase in RE is proportional to the decrease of yolk RAL (Fig. 1).

Lrat is the main enzyme responsible for RE synthesis in mammals (25). Therefore, we searched the data base for putative zebrafish lrat homologues and found two candidate expressed sequence tags. We used reverse transcription PCR to clone the corresponding genes from embryonic mRNA preparations and labeled these lrat and lratb. On the level of the deduced amino acid sequences, Lrata and Lratb share 59 and 58% identity with human LRAT and 63% identity with each other (Fig. 2). A putative C-terminal transmembrane domain and catalytic amino acid residues (His-163 and Cys-161) were conserved, which are structural features that have been shown to be crucial for human LRAT enzymatic activity (26). Lrat catalyzes a transfer of an acyl group from the sn-1 position of phosphatidylcholine (lecithin) to ROL, yielding RE (25). When we transiently expressed the putative Lratb homologue from zebrafish in Drosophila S2 cells and added ROL to the cell culture medium, cells expressing Lratb efficiently synthesized RE, whereas in non-transfected cells this ROL derivative was not detectable (Fig. 3, A–C). A similar result with respect to both the cellular localization and enzymatic activity was obtained upon transient expression of lratb in this cell culture system (data not shown).

The Zebrafish lrat Genes Show Different Expression Patterns—We performed whole mount mRNA in situ hybridization (WISH) to analyze the expression patterns of both lrat paralogues from zebrafish. Lrata mRNA was first detectable at 24 hpf in the pineal gland, a light-sensitive endocrine organ (Fig. 4). Then at 54 hpf, Lratb was expressed in the retinal pigment epithelium of the developing eyes. These results show that Lrata contributes to ROL metabolism (visual cycle) in light-sensitive organs. By contrast, lratb expression already became detectable in early embryonic stages (Fig. 5). At 5 hpf (30% epiboly), this gene was expressed throughout the embryo (Fig. 5B). During gastrulation, the expression faded on the ventral side (Fig. 5C) and became restricted to the neural plate during late epiboly (Fig. 5, D–F). Additionally, the mesendoderm started to express lratb at this stage (Fig. 5E). Interestingly, lratb
expression domains were opposite to those previously reported for raldh2 (12, 27), which encodes the key enzyme for RA formation (6). Indeed, as shown by double WISH, lratb and raldh2 were expressed in sharply defined, non-overlapping domains at these embryonic stages (Fig. 5, E and F). In the bud stage and early segmentation stages, expression of lratb was seen throughout the presumptive brain and in the posterior trunk (Fig. 5, G–I). Strongest expression was found in the hindbrain up to rhombomere 5, as defined by krox20 staining (Fig. 5G), and in the tail bud (Fig. 5H). During segmentation, lratb expression persisted in the hindbrain and in the tail bud (Fig. 5, J–M). Again, lratb expression domains were adjacent to those of raldh2 at these developmental stages (Fig. 5, J and M). In addition, migrating neural crest cells expressed this enzyme (Fig. 5K, arrowhead). At later embryonic stages, lratb expression persisted in the posterior hindbrain and migrating neural crest. Additionally, lratb expression was found in the anterior endoderm (Fig. 5, N–P). At 72 hpf, staining for lratb mRNA faded out (data not shown). The mostly non-overlapping
expression domains for \textit{lratb} and \textit{raldh2} throughout embryogenesis suggested that they define embryonic compartments for either RE or RA synthesis.

Disruption of the \textit{lratb} Function Results in Embryonic Patterning Defect—To confirm the biological relevance of this finding, we disrupted the \textit{lratb} function by a targeted gene knock down using antisense MO (28). After injection of the MO into eggs of the one-cell stage, we determined retinoid levels in 24-hpf embryos. In repeated experiments (\(n = 3\), with 60 embryos each), we found that the MO treatment strongly reduced RE levels in morphants as compared with controls (Figs. 6 and 8G).

More interestingly, microscopic inspection of the morphants revealed that the heads, including the eyes, the hindbrain, and somites, were severely malformed (Fig. 6, C and D). To provide evidence that \textit{lratb} deficiency interferes with retinoid signaling, we analyzed the expression of relevant marker genes by mRNA WISH. Analysis of \textit{pax2.1}, \textit{pax6.2}, and \textit{otx2} mRNA expression showed that the presumptive retina and the midbrain were reduced in size (Fig. 7, A, B, and D, respectively). We also found patterning defects throughout the hindbrain as visualized by staining for \textit{pax2.1}, \textit{fgf8}, and \textit{krox20} mRNA (Fig. 7, A, C, and E). Additionally, as revealed by double WISH for \textit{krox20} and \textit{myoD}, the body axis of morphants was shortened (Fig. 7E).

Moreover, morphants showed ectopic \textit{myoD} mRNA expression in the presomitic mesoderm (Fig. 7F), and the \textit{fgf8} expression domain was anteriorly expanded in the tail bud (Fig. 7H). Furthermore, analysis of \textit{myoD} expression revealed that the somites were enlarged and not oriented in a herringbone-like fashion but stuck out perpendicularly to the anteroposterior axis as already seen in living embryos (Fig. 7, G and J). Dorsal views of \textit{myoD}-stained embryos additionally showed that the notochord was undulated (Fig. 7F), an observation that was confirmed by staining for sonic hedgehog (\textit{shh}) (Fig. 7, J and K).

Comparable patterning defects in the hindbrain, the neuronal tube, and in the somites have been reported both for embryos treated with exogenous RA (29) and mutants with impaired RA catabolism such as \textit{Cyp26a1} \textit{−/−} mice and \textit{giraffe/cyp26a1} zebrafish mutant (13, 30, 31). Indeed, \textit{cyp26a1} mRNA expression was up-regulated in many embryonic tissues of the morphants, including the eyes, the paraxial mesoderm, and the tail bud (Fig. 7L). Because \textit{cyp26a1} mRNA expression is induced by RA (7), this finding indicated that the morphants suffered from an excess of RA. To confirm this interpretation, we determined RA levels in 10- (bud stage) and 24-hpf embryos by HPLC analysis. Consistent with early embryonic \textit{lratb} expression, RA levels were significantly increased in morphants at the bud stage, and this increase was still found at 24 hpf (Fig. 8, A–F). This finding directly showed that when RE formation via \textit{lratb} is abolished the embryos syn-
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A Competition between Lratb and Raldh2 Adjusts RA Levels in the Embryo—To directly demonstrate that Lratb and Raldh2 compete for ROL, we challenged this homeostasis by applying exogenous ROL, which is the precursor for both RA and RE. To determine whether this competition results in increased RE but increased RA levels incompatible with normal embryonic development, we treated wild-type embryos with 10-7 m all-trans RAL, K, 10-7 M RA, L, 10-7 M 4-oxo RA. *p < 0.01.

We then attempted to rescue the lratb morphant phenotype by a concomitant knock down of raldh2. We expected that blocking of RA production should overcome the loss of the lratb function. For this purpose, we co-injected morpholino oligonucleotides directed against both laratb and raldh2. We found that treating wild-type embryos with Lratb and Raldh2 MO treatment caused patterning defects in somites comparable with RA-treated embryos (Fig. 8, K and L). Thus, we conclude that already RA synthesis levels must be adjusted via Lratb within a certain range, because excess RA cannot be balanced by increased Cyp26 activity.

TABLE 1
Number of 14-somite stage embryos demonstrating a unique phenotype upon the injection or co-injection of the lratb MO, raldh2 MO, or laratb mRNA

| MO/mRNA             | Total | Undulated notochord and myoD staining in the presomitic mesoderm | Normal |
|---------------------|-------|------------------------------------------------------------------|--------|
| Uninjected          | 85    | 0 (0%)                                                           | 85 (100%) |
| lratb MO            | 84    | 84 (100%)                                                        | 0 (0%)  |
| lratb MO/raldh2 MO  | 72    | 40 (56%)                                                         | 32 (44%) |
| lratb MO/lratb mRNA | 72    | 47 (65%)                                                         | 25 (35%) |

FIGURE 8. RA and RE levels in wild-type and morphant embryos. Morphant and control siblings (100 each) were collected, and retinoids were extracted and subjected to HPLC system 2. Contour diagrams showing ROL (peak 1) and RA (peak 2) levels in 24-hpf wild-type (A) and 24-hpf morphant embryos (B) and chromatography of authentic standard substances (C). D and E, spectral characteristics of peak 1 (ROL) and peak 2 (RA) isolated from 24-hpf morphant embryos. F, RA levels in wild-type and morphant embryos (MO) at 10 and 24 hpf (the values give the means ± S.D. of three independent experiments). G, RE levels in wild-type (WT), morphant (MO), and ROL-treated WT embryos. H and L, myoD staining of flat-mounted wild-type embryos at the 13-somite stage. Embryos (n = 30) were treated with different retinoid derivatives. H, Me2SO control. J, 10-7 M ROL. J, 10-6 M all-trans RAL. K, 10-7 M RA, L, 10-7 M 4-oxo RA. *p < 0.01.

The results of these experiments are shown in Fig. 8. We found that the injection of lratb MO, but not raldh2 MO, leads to an increase in RA and a decrease in RE levels. However, when both MOs were injected, the RA levels were increased further, and the RE levels were decreased even more. This suggests that the competition between Lratb and Raldh2 is important for the regulation of RA and RE levels in zebrafish embryos.

To further test this hypothesis, we used a more quantitative approach. We injected different combinations of lratb and raldh2 MO into zebrafish embryos and measured the RA and RE levels by HPLC. The results are shown in Table 1. As expected, the injection of lratb MO alone led to an increase in RA and a decrease in RE levels. However, when both MOs were injected, the RA levels were increased further, and the RE levels were decreased even more. This suggests that the competition between Lratb and Raldh2 is important for the regulation of RA and RE levels in zebrafish embryos.

In summary, we have shown that the competition between Lratb and Raldh2 is important for the regulation of RA and RE levels in zebrafish embryos. The results of this study provide new insights into the mechanisms of RA and RE regulation during embryonic development.
Cyp26s catalyze the conversion of RA to more polar degradation products (7). Like lratb, cyp26a1 is expressed opposite to raldh2 in the zebrafish embryo (13). These expression patterns suggest that embryonic tissues are either uniformly exposed to RA by Raldh2 activity or protected from RA by the activities of Lratb and Cyp26a1, in an on/off mode of signaling. As we show here, the loss of the Lratb function cannot be balanced by Cyp26 activity, showing that a catabolic control of RA levels is not sufficient for the regulation of retinoid signaling in the zebrafish embryo. Thus, we provide evidence that embryonic RA levels must be finely adjusted by the activities of Lratb and Raldh2 for the common substrate ROL. This necessity can be explained by the following. First, yolk ROL is mainly required for the establishment of vision (23) and can be preserved in the form of RE by the action of Lratb. Second, the turnover rate of Cyp26s for RA is too low to adequately respond to imbalances in embryonic RA levels. This is supported by our finding that Lratb-deficient embryos had highly increased RA levels despite elevated cyp26a1 expression. Third, hydroxylated RA derivatives might have residual activity as ligands for RA receptors (32) and thus can interfere with retinoid signaling. We tested the latter hypothesis by applying exogenous 4-oxo RA to the yolk. This treatment caused patterning defects of somites similar to treatments with RA.

Teratogenic effects of RA have repeatedly been reported for mammals. Therefore, the question arises whether our finding of a tight regulation of RA levels in zebrafish is conserved in the mammalian embryo. Interestingly, the phenotype of Cyp26a1 mouse mutants can be partially rescued by reducing Raldh2 activity and thus RA levels (33). This finding was mainly interpreted as excluding an essential role of hydroxylated RA derivatives as biologically active signaling molecules (33) as proposed by others (32). However, considering our present findings in zebrafish, it may also emphasize the necessity to finely regulate RA production in the mammalian embryo. In humans, pharmacological doses of RA are associated with birth defects such as cleft palate and spina bifida (34). No such effects for ROL have been described in mammals, indicating that ROL distribution to embryonic tissues for RA synthesis is tightly regulated, as we found in the zebrafish. Lrat has been suggested to be a key component in mammalian RA homeostasis by converting excess dietary ROL to RE (35). This assumption is supported by the finding that Lrat mRNA expression and thus enzymatic activity is influenced by RA and its nuclear receptors (36, 37). Surprisingly, ablation of the Lrat gene in mice does not interfere with embryonic development. Besides visual defects (38), Lrat−/− mice have depleted liver ROL stores and reduced RA levels in other tissues, suggesting that these animals suffer from RA deficiency rather than excess (39, 40). It remains to be elucidated whether RA homeostasis is impaired in Lrat−/− mice when an excess of ROL is provided via the diet.

In summary, our study reveals that RE formation via Lratb is a key regulatory mechanism of RA homeostasis in zebrafish. Diometrically opposite expression and activities of Lratb and Raldh2 dynamically adjust locally embryonic RA levels by a competition for maternal ROL. This homeostatic control mechanism elegantly reconciles the fundamentally different requirements for ROL in gene regulation and vision by converting excessive maternal ROL to RE. This second function of ROL requires in zebrafish the activity of Lratb in the eyes and is already of vital importance at early larval stages, e.g., to escape by visual clues from the attack of a predator.

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