The Zebrafish Retinol Dehydrogenase, rdh1l, Is Essential for Intestinal Development and Is Regulated by the Tumor Suppressor Adenomatous Polyposis Coli*

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Lincoln D. Nadauld‡§, Dawne N. Shelton‡§, Stephanie Chidester§, H. Joseph Yost‡§, and David A. Jones‡§¶

From the Departments of Oncological Sciences and Medicinal Chemistry and the Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah 84112

Retinoic acid (RA) is a potent signaling molecule that plays important roles in multiple and diverse developmental processes. The contribution of retinoic acid to promoting the development and differentiation of the vertebrate intestine and the factors that regulate RA production in the gut remain poorly defined. Herein, we report that the novel retinol dehydrogenase, rdh1l, is required for proper gut development and differentiation. rdh1l is expressed ubiquitously during early development but becomes restricted to the gut by 3 days postfertilization. Knockdown of rdh1l results in a robust RA-deficient phenotype including lack of intestinal differentiation, which can be rescued by the addition of exogenous retinoic acid. We report that adenomatous polyposis coli (APC) mutant zebrafish harbor an RA-deficient phenotype including aberrant intestinal differentiation and that these mutants can be rescued by treatment with retinoic acid or injection of rdh1l mRNA. Further, we have found that although APC mutants are deficient in rdh1l expression, they harbor increased expression of raldh2 suggesting the control of RA production by APC is via retinol dehydrogenase activity. These results provide genetic evidence that retinoic acid is required for vertebrate gut development and that the tumor suppressor APC controls the production of RA in the gut by regulating the expression of the retinol dehydrogenase, rdh1l.

Dietary vitamin A, retinol, is a major source of retinoids in vertebrates. Conversion of retinol into its active form, retinoic acid (RA),1 is achieved via a two-step enzymatic process (1). The first step, conversion of retinol into retinal, is catalyzed by retinol dehydrogenases (RDH), members of the short chain dehydrogenase-reductase family of enzymes. The second step, conversion of retinal to retinoic acid, is performed by retinaldehydes (RALDH). The RA signaling pathway is engaged when plasma retinol is absorbed by cells or liberated from the plasma membrane and converted via RDHs and RALDHs into RA. RA in turn binds to a subset of nuclear hormone receptors, RARs and RXRs, to effect gene expression changes (2).

Several studies indicate an important role for retinoids in maintenance of the normal gut epithelium and in tumor biology. For example, vitamin A-deficient animals display various colonic defects highlighted by decreased mucus production, expansion of crypt proliferative zones, and disrupted ion exchange (3–9). Further, induction of colon carcinomas in rats by 5-azoxymethane is blocked by addition of retinoid analogs (3, 10–13). A role for RA in the intestine is further supported by reports that treatment of colon cancer cell lines with retinoids induces markers of differentiation, inhibits cell growth, and reduces invasiveness (14–17). Additionally, vitamin A-deficient rats are incapable of proper intestinal adaptation and response following small bowel injury (18).

Despite the data implicating RA in intestinal cell functions, a number of key issues remain unresolved. First, vitamin A deficiency in rodents does not appear to cause a complete loss of intestinal epithelial differentiation (3–5,9), thus challenging the notion that vitamin A is essential for differentiation of the intestinal epithelium. Second, the importance of RDHs in RA biosynthesis in vivo remains in question due, in part, to the relatively mild phenotypes of various alcohol dehydrogenase (ADH) mutants and double mutants compared with raldh2 null mice (19). In addition, reports of RDH-independent RA biosynthetic pathways suggest RDHs to be nonessential.

An important recent finding links the regulation of RA biosynthesis to the adenomatous polyposis coli (APC) tumor suppressor gene, which is mutated in as many as 85% of human colon carcinomas (20, 21). Specifically, retinol dehydrogenases were found to be reduced in human colon adenomas and adenocarcinomas carrying mutated APC. Introduction of wild type APC into colon tumor cells rescued the expression of the retinol dehydrogenase, RDHL, and increased cellular RA production.

Our recent work (22) shows that knockdown of retinol dehydrogenase activity in vivo is sufficient to produce a robust phenotype characteristic of vitamin A deficiency in zebrafish. Further, the zebrafish intestine appears to depend upon RDHs and RA signaling for appropriate patterning and development. Specifically, the adult zebrafish intestine expresses the retinol dehydrogenase, rdh1l (formerly referred to as zRDHB), in a graded fashion anterior to posterior with the highest expression appearing in the adult anterior gut (22). Consistent with this finding, abrogation of rdh1l in embryos appeared to affect only the anterior intestine, whereas the posterior intestine...
developed normally. This suggested two possibilities, (i) the posterior intestine does not require retinoic acid or (ii) a separate, posterior RDH is responsible for producing RA thus allowing proper development and differentiation of that intestinal segment in the absence of rdh1.

We have now investigated the role of a second zebrafish retinol dehydrogenase, rdh1l (formerly referred to as zRDHA), in zebrafish gut development and patterning. We found that morpholino-mediated knockdown of rdh1l resulted in loss of differentiation in both the anterior and posterior intestine. The expression of gut markers trypsin and i-FABP were rescued in morphant embryos by application of RA, confirming rdh1l as an in vivo RDH and underscoring the role of retinoic acid in intestinal cell differentiation. Further, we have utilized APC genetic mutant zebrafish to ascertain whether rdh1l is controlled by APC in vivo. We found that APC mutants were deficient in rdh1l expression throughout the gut and that these mutants also lacked trypsin and i-FABP. Interestingly, APC mutant embryos displayed increased expression of raldh2, suggesting a specific role for APC in positively regulating RDHs.

To characterize the role of rdh1l in the developing zebrafish, we first sought to analyze its expression pattern at various time points. To this end, we performed whole mount in situ hybridization on 6, 14, 24, 36, and 96 hpf zebrafish embryos using antisense probe against rdh1l (Fig. 1). We found rdh1l transcripts to be present throughout the embryo at 6 hpf with increased intensity in the migrating mesendoderm. During early segmentation (14 hpf) rdh1l maintained a ubiquitous expression pattern with particularly strong expression in the developing somites. At 24 and 36 hpf rdh1l expression remained ubiquitously increased in the developing eye, hindbrain, pectoral fins, branchial arches, and presumptive macrophages (Fig. 1). By 96 hpf rdh1l expression was localized to the gut tube of developing larvae, and positive staining was also noted in the liver (Fig. 1). A control probe for neomycin showed no staining in any structures at any of these time points (data not shown).

rdh1l Is Required for Jaw, Fin, and Gut Development—We previously determined that cells transfected with rdh1l and treated with retinol were capable of producing 3-fold more retinoic acid than control cells thereby verifying rdh1l as a bona fide retinol dehydrogenase in vitro (22). To determine whether rdh1l acts as a retinol dehydrogenase in vivo, we sought to abrogate rdh1l function in developing zebrafish embryos using antisense morpholino oligonucleotides. We designed a splicing-blocking morpholino (rdh1l MO) against the exonic splice-donor site of rdh1l to block splicing and effectively abrogate rdh1l activity. RT-PCR using primers targeted to exonic and intronic rdh1l sequences revealed that rdh1l MO labeled riboprobes for rdh1l, insulin, trypsin, and i-FABP were generated using the Roche Light Cycler instrument and software, version 2.0. Control and experimental embryos were allowed to develop at 28.5 °C. Control and experimental embryos were raised in 0.003% phenylthiourea to inhibit pigment formation (22).

Whole Mount in Situ Hybrdizations—Zebrafish embryos were fixed in sucrose-buffered 4% paraformaldehyde, rinsed in phosphate-buffered saline, dehydrated in methanol, and stored at −20 °C. Digoxigenin-labeled riboprobes for rdh1l, insulin, trypsin, and i-FABP were generated and described previously (22). The raldh2 riboprobe was generated by linearization of pCRIV (Invitrogen) containing a raldh2 cDNA followed by in vitro transcription with T7 RNA polymerase (Roche Applied Science). Whole mount in situ hybridizations were carried out as described previously (22). Embryos were cleared in 70% glycerol in phosphate-buffered saline and photographed using an Olympus DP12 digital camera.

RT-PCR—Single-stranded cDNA was synthesized from 1 μg of total RNA using SuperScript III (Invitrogen). PCR primers used were as follows: rdh1l exonic primers, forward, 5′-GCTGGATCATGAGGTGATCAGCTG-3′; reverse, 5′-ACTCGACCCTTGGCTTTCTT-3′; rdh1l intronic primers, forward, 5′-CCGTCGACTTCATTTATCCTA-3′; reverse, 5′-TGAGGGTCAGCGGATAGA-3′. β-Actin primers were as described previously (22).

Quantitative RT-PCR—Single-stranded cDNA was synthesized from 1 μg of total RNA using SuperScript III (Invitrogen). PCR was performed using the Roche Light Cycler instrument and software, version 3.5 (Roche Diagnostics). Primers were as follows: trypsin, forward, 5′-ATGAAGGCTTTCATTCTTCTG-3′; reverse, 5′-TCTGTCAGTGACTCACCCTTCTGTC-3′; raldh2, forward, 5′-ATCAAGAAGAGACGCAGGAAA-3′; reverse, 5′-GAGGTCGCTGTGTCATGGT-3′. PCR conditions were as follows: 35 cycles of amplification with a 10-s denaturation at 95 °C, 5 s of annealing at 57 °C, and a 10-s extension at 72 °C. A template-free negative control was included in each experiment.

Morpholino and RNA Injections—Antisense morpholino oligonucleotides were obtained from Gene Tools LLC. The rdh1l MO splicing blocking morpholino (5′-TCCTGGTGTCATGACTCCTCTGGT-3′), raldh2 Master SYBR Green I kit (Roche Diagnostics). PCR conditions were as follows: 35 cycles of amplification with a 10-s denaturation at 95 °C, 5 s of annealing at 57 °C, and a 10-s extension at 72 °C. A template-free negative control was included in each experiment.

For RNA injection experiments, full-length rdh1l RNA was synthesized from a linearized pCRII/rdh1l construct using mMessage mMachine (Ambion) according to the manufacturer’s protocol. Full-length rdh1l or GFP RNAs were injected into embryos at the one-cell stage.

RA Rescue Experiments—To rescue rdh1l morphants and APC mutants by application of retinoic acid, embryos were incubated in 900 nM all-trans-retinoic acid in Me2SO at 75% epiboly for 1 h. Embryos were then washed in embryo water. At 30 and 54 hpf embryos were treated with 20 nM all-trans-retinoic acid for 1 h then washed in embryo water. Control embryos were treated over these periods with an equal volume of Me2SO.

Histological Analyses—Embryos were fixed in 10% neutral buffered formalin, rinsed in phosphate-buffered saline, and embedded in paraffin. Six-micron sections were cut using a Leica microtome and stained in hematoxylin and eosin. Sections were analyzed using an Olympus compound microscope, and pictures were taken using a Zeiss AxioCam.

RESULTS

Developmental Expression Pattern of rdh1l—To characterize the role of rdh1l in the developing zebrafish, we first sought to analyze its expression pattern at various time points. To this end, we performed whole mount in situ hybridization on 6, 14, 24, 36, and 96 hpf zebrafish embryos using an antisense probe against rdh1l (Fig. 1). We found rdh1l transcripts to be present throughout the embryo at 6 hpf with increased intensity in the migrating mesendoderm. During early segmentation (14 hpf) rdh1l maintained a ubiquitous expression pattern with particularly strong expression in the developing somites. At 24 and 36 hpf rdh1l expression remained ubiquitous with increased intensity in the developing eye, hindbrain, pectoral fins, branchial arches, and presumptive macrophages (Fig. 1). By 96 hpf rdh1l expression was localized to the gut tube of developing larvae, and positive staining was also noted in the liver (Fig. 1). A control probe for neomycin showed no staining in any structures at any of these time points (data not shown).

rdh1l Controls RA Biosynthesis in the Intestine

FIG. 1. Expression analysis of rdh1l in developing zebrafish. Whole mount in situ hybridization was performed on wild type zebrafish embryos at 6, 14, 24, 36, and 96 hpf (upper panels) using digoxigenin-labeled antisense RNA probes against rdh1l. In all cases the embryos are positioned with anterior to the top. Lower panels, at 14 hpf the white arrow designates rdh1l expression in developing somites. At 24 hpf the black arrowhead identifies presumptive macrophages. Yellow arrows at 24 hpf indicate presumptive fin buds. The black arrow at 24 hpf identifies presumptive macrophages in the tail. At 36 hpf rdh1l is present throughout the head and trunk.
enzyme ruled out the possibility of genomic DNA contamination (Fig. 2A).

Injection of the rdh1l morpholino resulted in embryos characterized by small eyes, absence of pectoral fins, and failed jaw formation (Fig. 2, B and C). Similar phenotypes were seen upon injection of a translation-blocking morpholino targeted to the ATG start of the rdh1l mRNA (data not shown). To verify rdh1l as a gene involved in retinoic acid production, we sought to rescue fin formation in rdh1l morphants by the addition of exogenous retinoic acid. To accomplish this, we injected one-cell stage embryos with a control morpholino and with exogenous retinoic acid. To quantify the decrease in trypsin expression we performed qRT-PCR using total RNA isolated from rdh1l morphant and control embryos at 80 hpf. This analysis revealed trypsin expression in rdh1l morphants to be ~9% of that found in control embryos (Fig. 3A). The addition of RA to rdh1l morphants restored trypsin expression to nearly double the expression of trypsin. Similarly, by whole mount in situ hybridization, we noted that i-FABP expression was present in only 10% (n = 70) of rdh1l morphants (Fig. 3, A and C). Further, treatment with RA restored i-FABP expression in 42% (n = 43) of rdh1l morphants.

rdh1l Morphant Intestines Phenocopy Neckless and APC Mutant Intestines—We reported previously that knockdown of another retinol dehydrogenase, rdh1, prevented epithelial differentiation only in the anterior intestine of developing zebrafish, whereas knockdown of APC impaired differentiation throughout the intestine. Because rdh1 expression was confined to the anterior gut and because rdh1 is expressed throughout the gut, we reasoned that rdh1l may provide the retinoic acid for proper development of the posterior gut. To determine which portion, if any, of the intestine was affected in rdh1l morphants compared with APC mutants, we harvested 96 hpf rdh1l morphants and APC mutants for sectioning and hematoxylin and eosin staining. We found that rdh1l morphants and APC mutants had formed a gut tube, which was lined by a single layer of cells (Fig. 4A). In addition, both types of embryos expressed GATA-6 and hepatocyte nuclear factor-4α, markers of patterned, immature endoderm (26–28) (Fig. 4D and data not shown). However, compared with control-injected embryos, rdh1l morphant and APC mutant intestines lacked wild type morphological characteristics, including developing villi and columnar epithelial cells (Fig. 4A). These defects were present along the entire anteroposterior gut axis (Fig. 4A) in rdh1l morphants and APC mutants.

The rdh1l morphant and APC mutant intestine also revealed an apparent reduction in cell number comprising the intestine...
**rdh11 Controls RA Biosynthesis in the Intestine**

Control and experimental larvae were fixed, sectioned, and stained with hematoxylin and eosin at 80 hpf to analyze gut development. A, sections from the foregut (left panels), midgut (middle panels), and hindgut (right panels) were compared from control injected morphants (top panels), rdh11 morphants (middle panels), or APC mutants (bottom panels). B, the number of cells comprising the intestinal bulb of the 96-hpf larval intestine was determined by counting the number of nuclei present in the single-cell layer of a 20-cell rdh11 morphant, APC mutant, and wild type zebrafish. C, APC mutant (mt) larvae (right panels) or wild type (wt) APC siblings (left panels) were harvested at 80 hpf and in situ hybridized with antisense probes for i-FABP (top panels) or trypsin (bottom panels). D, whole mount in situ hybridization with an antisense probe for GATA-6 was performed on wild type APC siblings (left panels), APC mutants (middle panels), and rdh11 morphants (right panels) at 80 hpf.

The morphant gut remained at 20 cells at the same anteroposterior position in the gut tube compared with control injected 96-hpf larvae (data not shown).

**APC Mutants Display Decreased rdh1l and Increased raldh2 Expression**—The striking similarities in the gross and molecular phenotypes between APC mutants and RA-deficient morphants led us to ask whether the APC mutants lacked expression of the RA biosynthetic enzymes. Therefore, we performed in situ hybridization with antisense probes for rdh1l and raldh2 in APC mutant larvae. At 72 hpf, rdh1l expression is strong in the gut and liver of wild type APC siblings. However, rdh1l expression in APC mutants was not detectable (Fig. 5A). In contrast, raldh2 expression was robust in wild type APC siblings and appeared to intensify in APC mutants (Fig. 5A). To verify and quantitate these apparent changes in expression levels, we performed qRT-PCR using total RNA from 72-hpf APC mutants. By comparison, raldh2 expression increased 4.2-fold in APC mutants.

**APC Mutants Are Rescued by RA or rdh1l—**In light of the observation that APC mutants display a retinoic-deficient phenotype and maintain decreased rdh1l expression, we hypothesized that they might be rescued by application of retinoic acid or rdh1l overexpression. To test this we treated APC mutants with all-trans-retinoic acid for 1 h each day for 3 days. We then harvested the larvae and performed whole mount in situ hybridization with antisense probes for i-FABP and trypsin. Staining for i-FABP revealed that ~32% (n = 71) of APC mutants stained positively for this marker of intestinal differentiation following treatment with RA (Fig. 6A). In contrast, 3% of vehicle-treated mutant embryos stained positively for i-FABP (n = 37). Trypsin expression was restored in 60% of mutants treated with RA (n = 43), whereas 10% of vehicle-treated mutants (n = 34) were trypsin-positive (Fig. 6A). The expression of both i-FABP and trypsin was present in 100% of wild type APC siblings.

We also sought to rescue APC mutants by overexpression of rdh1l. To accomplish this we injected APC mutants with rdh1l or GFP mRNA at the one-cell stage. At 3 days postfertilization we harvested the injected larvae and analyzed them by whole mount in situ hybridization for expression of i-FABP and trypsin. Staining for i-FABP revealed that 4% of APC mutants injected with GTF mRNA stained positively for i-FABP (n = 45). In contrast, following injection of rdh1l mRNA, 22% of embryos (n = 36) were i-FABP positive by in situ hybridization (Fig. 6B). Trypsin expression was present in 14% of GFP-injected APC mutants (n = 35), whereas 46% of APC mutants (n = 37) injected with rdh1l were trypsin-positive (Fig. 6B).

**DISCUSSION**

Studies in mouse and chicken have suggested that RALDHs, rather than RDHs, represent the primary rate-limiting step in retinoic acid production in vivo (19). Herein, we establish that rdh1l is essential for normal development in zebrafish. Specifically, knockdown of rdh1l function resulted in several, well known RA-deficient phenotypes including loss of pectoral fin formation, lack of jaw development, small eyes, absence of differentiated exocrine pancreas, and aberrant intestinal development. These phenotypes are also present in raldh2 (neckless) mutants and are consistent with vitamin A and RA signaling deficiencies (24, 25). Importantly, treatment of rdh1l

**FIG. 4.** rdh1l knockdown affects intestinal development and differentiation. A, sections from the foregut (left panels), midgut (middle panels), and hindgut (right panels) were compared from control injected morphants (top panels), rdh11 morphants (middle panels), or APC mutants (bottom panels). B, the number of cells comprising the intestinal bulb of the 96-hpf larval intestine was determined by counting the number of nuclei present in the single-cell layer of a 20-cell rdh11 morphant, APC mutant, and wild type zebrafish. C, APC mutant (mt) larvae (right panels) or wild type (wt) APC siblings (left panels) were harvested at 80 hpf and in situ hybridized with antisense probes for i-FABP (top panels) or trypsin (bottom panels). D, whole mount in situ hybridization with an antisense probe for GATA-6 was performed on wild type APC siblings (left panels), APC mutants (middle panels), and rdh11 morphants (right panels) at 80 hpf.

**FIG. 5.** APC mutants display decreased rdh1l expression and increased raldh2 expression. A, in situ hybridization was performed on APC mutants (mt) (right panels) and wild type (wt) siblings (left panels) at 80 hpf with digoxigenin-labeled antisense probes for rdh1l (top panels) and raldh2 (bottom panels). B, qRT-PCR was performed using total RNA harvested from 96-hpf APC mutants and wild type siblings to measure levels of rdh1l and raldh2 expression. The bar graph indicates the fold-change in expression levels of indicated genes (calculated as APC mutant gene expression levels/wild type sibling gene expression levels).
morphant embryos with retinoic acid rescued the above defects. Our data, therefore, suggest tissue-specific, rate-limiting roles for retinol dehydrogenases in zebrafish.

This raises the question of whether a contradiction exists between knock-out of rdh1l in zebrafish and current models regarding tissue-specific production of retinoic acid in other vertebrates. For example, mice carrying genetic knock-outs of tissue-restricted ADH1 or ADH4 showed surprisingly few phenotypic defects except under vitamin A-deficient conditions (30, 31). This is in contrast to the knock-out of ubiquitously expressed ADH3, which produced mice showing reduced survival and growth defects (30). These observations support a current model wherein ADH3 provides redundancy in the absence of ADH1 and ADH4 and places RALDH expression as the point of tissue-specific control (19).

Thus far, we have examined knockdown of two zebrafish RDHs (rdh1 and rdh1l) each of which resulted in robust, tissue-specific defects. We proposed two explanations for the apparent contradiction. First, the direct ortholog of rdh1l has yet to be tested in rodent models. Second, it is possible that fish lack the ubiquitous equivalent of ADH3 and, thus, lack redundancy within tissues as seen in mice. An identification and manipulation of additional retinoid biosynthetic genes in both fish and mice may help clarify these issues.

Additional lines of evidence support RDHs as critical control points for RA production. First, RDHs show tissue-specific expression, thus indicating a tissue-specific requirement for conversion of retinol into retinaldehyde. As an example, rdh1l is ubiquitously expressed throughout somitogenesis and early organogenesis but becomes highly restricted to the intestine and liver at 96 hpf and beyond. Similarly, previous studies have shown that zebrafish rdh1 is highly gut-specific but shows the highest levels in anterior gut (22). This is comparable with the unique tissue distribution of hRDH-TBE/3α-HSD/RDHL in humans (21). Second, RDH expression levels can be regulated by key signaling pathways. In this respect, Li et al. (32) have demonstrated dynamic regulation of estRDH levels in rat uterus during the estrous cycle, and Jette et al. (21) have described transcriptional regulation of human hRDH-TBE/3α-HSD/RDHL by APC and cdx2 (21, 32–34). Consistent with this, we have found that rdh1l expression levels decreased more than 3-fold in zebrafish carrying mutations in the APC tumor suppressor gene as compared with wild type animals. In contrast, rdh2l expression increased 4-fold in the APC mutants, thus supporting the regulation and loss of RDH activity as a primary explanation for lack of RA signaling in APC mutants. Lastly, mutations in RDH12 have been identified as the causative incident in Leber congenital amaurosis, a condition associated with severe rod-cone dystrophy and macular atrophy, thus demonstrating that RDH mutation is sufficient to cause human disease (35, 36).

Consistent with the absence of rdh1l in APC mutant embryos and APC control of RA production, many of the phenotypes present in rdh1l morphant embryos, including lack of jaw, arrested pectoral fins, small eyes, and abnormal gut development, are strikingly similar to those present in APC mutant and morphant zebrafish (22, 37). In addition, APC mutant embryos were rescued, in part, by rdh1l overexpression. These observations lend support to our previously proposed model wherein APC controls retinoic acid biosynthesis by controlling RDH levels. Loss of APC, therefore, results in retinoic acid signaling deficiencies (21, 22).

The defects in intestinal development along the entire anteroposterior axis of rdh1l morphants recapitulate abnormalities in the APC mutant and mutant gut (22). This stands in contrast to knockdown of a previously reported gut-specific RDH, rdh1, which affects the differentiation of only the anterior gut. The phenotypic discrepancy between these two RDHs is supported by RT-PCR expression data in adult zebrafish where rdh1l is expressed equally along the entire anteroposterior axis, whereas rdh1 is expressed in a graded fashion anterior to posterior with highest expression lying in the anterior gut (22). The differences in expression profiles and knockdown phenotypes point to a model wherein a gradient of retinoic acid production is required to appropriately pattern and differentiate the developing intestine. This would be consistent with RA gradients that appear to be important in establishing and patterning several structures. For example, anteroposterior patterning of the mouse limb is dependent on the proper formation of an RA signaling gradient (40).

The finding that fewer cells exist in the rdh1l morphant and APC mutant intestinal tubes compared with wild type suggests that perturbation of APC or rdh1l affects the intestinal cell population number. This is consistent with a previously described role for retinoic acid in recruiting and specifying the correct cell number in such diverse tissues as heart, blood, brain, and uterus (32, 41–44). However, the presence of GATA-6 and hepatocyte nuclear factor-4α staining in APC mutants and rdh1l morphants suggests that patterning of the early endodermal tube is not altered in APC mutants and rdh1l.
morphants and that the defect in these guts is because of a later event in development. These findings are consistent with a dual role for APC in retinoic acid in specifying cell number and cellular differentiation within the intestine.

Our finding that loss of the tumor suppressor APC can be compensated by exogenous retinoic acid has important implications for colon cancer biology. These data ascribe to APC a dual role for APC and retinoic acid in specifying cell number and this study.

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