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Germ Cell Migration in Zebrafish Is Dependent on HMGCoA Reductase Activity and Prenylation

Short Article

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Summary

Hydroxymethylglutaryl coenzyme A reductase (HMG CoAR) is required for isoprenoid and cholesterol biosynthesis. In *Drosophila*, reduced HMGCoAR activity results in germ cell migration defects. We show that pharmacological HMGCoAR inhibition alters zebrafish development and germ cell migration. Embryos treated with atorvastatin (Lipitor) exhibited germ cell migration defects and mild morphologic abnormalities. The effects induced by atorvastatin were completely rescued by prior injection of mevalonate, the product of HMGCoAR activity, or the prenylation precursors farnesol and geranylgeraniol. In contrast, squalene, a cholesterol intermediate further down the pathway, failed to rescue statin-induced defects. Moreover, pharmacologic inhibition of geranylgeranyl transferase 1 (GGT1) protein prenylation activity also resulted in abnormal germ cell migration. Thus, our pharmacological inhibition-and-rescue approach provided detailed information about the elements of isoprenoid biosynthesis that contribute to germ cell migration. Together with data from *Drosophila* (Santos and Lehmann, 2004, this issue), our results highlight a conserved role for protein geranylgeranylation in this context.

Introduction

A key regulatory step in the synthesis of cholesterol is the reduction of hydroxymethylglutaryl-Coenzyme A to mevalonate by the enzyme Hydroxymethylglutaryl coenzyme A reductase (HMGCoAR) (Figure 1) (Sever et al., 2003). Evidence from studies in *Drosophila* indicates that there are cholesterol-independent developmental defects associated with genes in the mevalonate pathway. In flies lacking HMGCoAR, primordial germ cells (PGCs) fail to reach their correct target in the mesoderm (Van Doren et al., 1998). Importantly, cholesterol or its derivatives do not participate in producing the signal required for germ cell migration, because *Drosophila* lacks a number of enzymes necessary for de novo cholesterol synthesis (Santos and Lehmann, 2004).

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PGC migration in zebrafish can be divided into several steps that appear to rely on the integrity of specific somatic structures. During somitogenesis, bilateral clusters of PGCs migrate posteriorly until they reach the anterior of the yolk extension, the position where the gonad develops (Weidinger et al., 2002). Recently, the chemokine SDF-1a (stromal cell derived factor 1a) (Doitsidou et al., 2002) and its receptor CXCR4b (Doitsidou et al., 2002; Knaut et al., 2003) were identified as molecules essential for proper migration of zebrafish PGCs. When expression of *cxcr4b* or its ligand is inhibited, the migration of germ cells is disrupted, indicating that SDF-1a acts as a potent chemoattractant for zebrafish PGCs.

In the present study, we used statins, a class of potent competitive HMGCoAR inhibitors (Evans and Rees, 2002), to show that HMGCoAR activity is also required for vertebrate PGC migration. We found that the effect of statins could be overcome by injection of mevalonate but not squalene, indicating that cholesterol and its immediate precursors are not required for PGC migration. Further analysis of downstream components of the mevalonate pathway including the geranylgeranyl transferase 1 (GGT1), an enzyme responsible for the prenylation of proteins, suggests that geranylgeranylation is essential for correct PGC migration.

Results

Cloning of Zebrafish HMGCoAR Orthologs

To examine a possible role of HMCCoAR in zebrafish PGC migration, we identified partial cDNAs of two HMGCoAR orthologs (*hmgcr1* and *hmgcr2*) (Supplemental Figure S1 [http://www.developmentalcell.com/cgi/content/full/6/2/295/DC1]). Maternal *hmgcr2* transcripts were evident in 4-cell stage embryos and uniform expression was observed during gastrulation, suggesting that the translated protein is present where PGCs migrate. *hmgcr1* expression was observed in the liver and intestine of day 4 embryos and not at earlier stages.

HMGCoAR Is Required for Proper PGC Migration

To determine the effects of HMGCoAR during vertebrate development and PGC migration, we incubated early stage zebrafish embryos (2–4 hr post fertilization [hpf]) in media containing statins. Embryos treated overnight with either mevinolin (Lovastatin) or simvastatin (Zocor) exhibited developmental arrest, improper axis elongation, and compressed somites (Supplemental Figure S2). The developmental arrest and axis elongation phenotypes along with the EC_{50}s of these phenotypes were identical for both drugs. After mevinolin or simvastatin treatment, PGCs were widely dispersed, whereas untreated animals had a compact cluster of PGCs in the anterior yolk extension (data not shown). To demonstrate that the PGC migration phenotype represented a primary defect in this process and was not due to defects in somatic patterning (Supplemental Figure S2), embryos were treated with atorvastatin, the only statin that lacked a somatic phenotype in our assay (Figure 2).

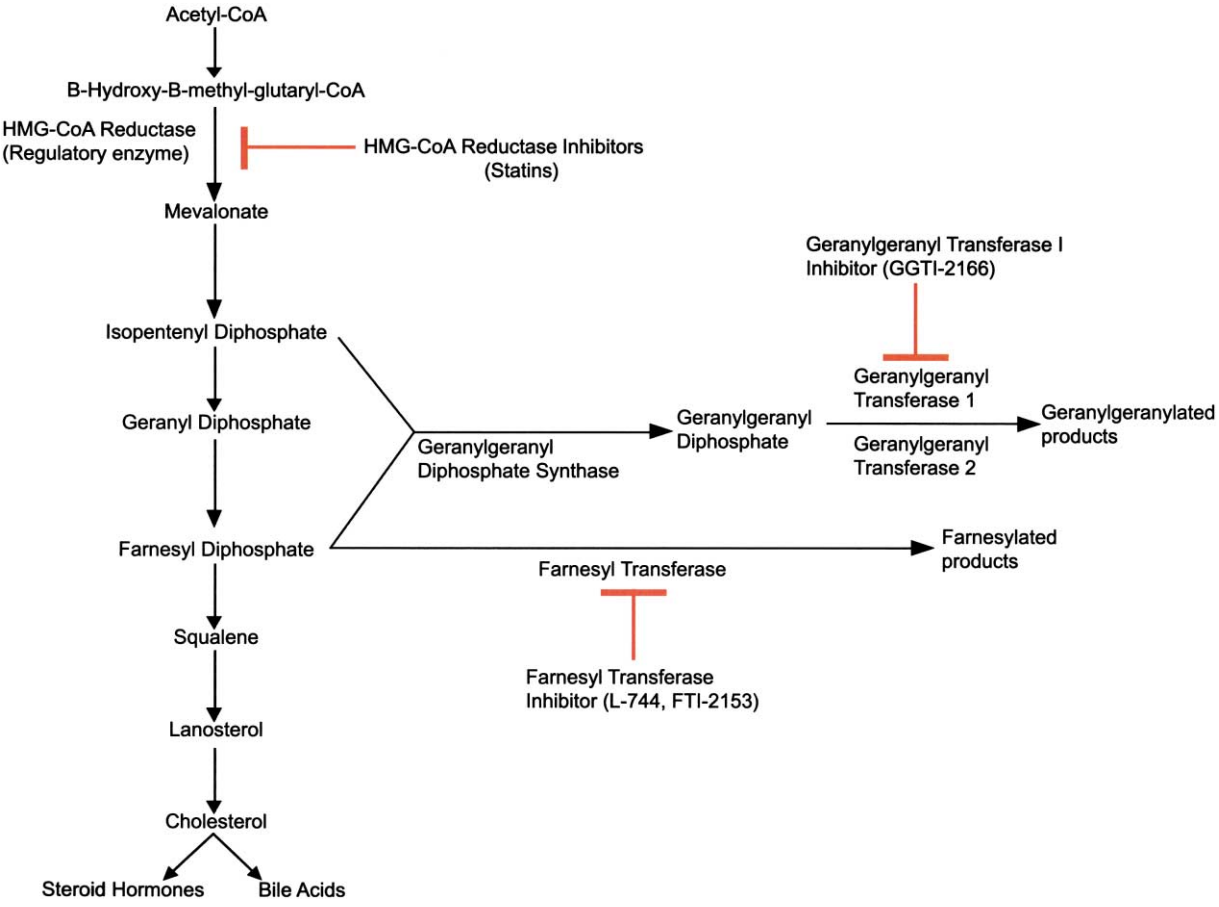

Figure 1. Cholesterol and Isoprenoid Biosynthesis Pathway

Atorvastatin treatment resulted in impaired PGC migration (Figures 2A–2C) without the severe morphological defects observed with the other statins (Figure 2D). Embryos treated with atorvastatin showed a PGC migration phenotype at the end of gastrulation, resulting in PGCs that failed to align along the anterior and lateral borders of the trunk mesoderm. By the 3-somite stage, over 90% of the embryos had ectopic germ cells compared to less than 20% in the wild-type (Figure 2E). Furthermore, the effect of atorvastatin on PGC migration was dose dependent (EC_{50} 4 μM) (Figure 2F). As development proceeded, some of the ectopic PGCs eventually migrated to the correct location, but still, by 24 hr, more than 50% of the treated embryos had ectopic PGCs (compared to less than 10% in the wild-type) (Figure 2E). Time-lapse studies of atorvastatin-treated embryos indicate that GFP-labeled PGCs were motile (Figures 2G and 2H and Supplemental Movie S1). This suggests that inhibition of HMGCoAr activity leads to PGC migration defects either by affecting the speed of migration or by impairing directional migration.

Rescue of Statin-Induced Defects by Mevalonate

To examine the specificity of the statin-induced effects and provide evidence that the observed phenotype resulted from the inhibition of HMGCoAR, we injected embryos with mevalonate (the immediate product of HMGCoAR activity; see Figure 1) prior to statin treatment. Mevalonate injections resulted in a complete rescue of the PGC migration phenotype caused by all three statins (Figure 3C). As a control, embryos were injected with sodium citrate, a salt with a similar molecular weight as mevalonate, prior to statin treatment, and this failed to reverse the PGC phenotype (data not shown). Mevalonate not only reversed the PGC migration defects caused by statin treatment, but also fully rescued the somatic phenotype caused by mevinolin and simvastatin (Figure 3D). Thus, both PGC migration as well as embryonic patterning require components of the HMGCoAR pathway. Moreover, these data suggest that HMGCoAR activity does not provide directional information to PGCs since its product, mevalonate, can allow proper PGC migration even when it is uniformly applied.

Injections with Farnesol and Geranylgeraniol Rescue Atorvastatin Effects

Mevalonate is utilized to synthesize a variety of cellular compounds that include ubiquinones, carotenoids, isoprenoids, and cholesterol (Santos and Lehmann, 2004). To determine directly whether cholesterol or its derivatives played a role in zebrafish PGC migration, we tested squalene, an intermediate in the pathway specifically required for de novo cholesterol synthesis (Figure 1). Squalene injection failed to rescue the effects of statin

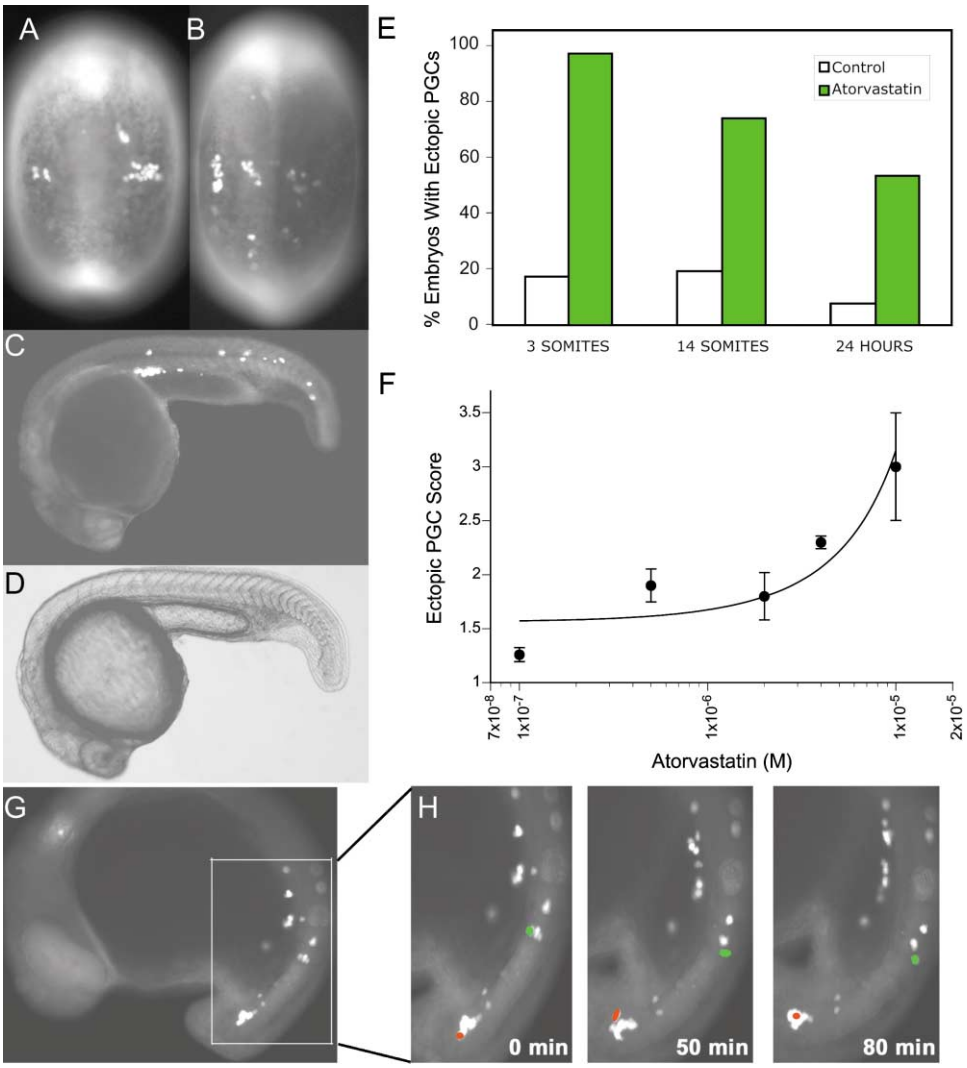

Figure 2. Migration of Primordial Germ Cells Is Altered by Atorvastatin Treatment

(A) A single fluorescent image from a time lapse (1-somite stage, first capture) of the movement of PGCs in a wild-type embryo injected at the 1-cell stage with *gfp-nanos* mRNA (1–2 nl, 60 ng/μl).

(B) Embryos (1-somite stage) soaked in atorvastatin (10 μM) and injected with *gfp-nanos* mRNA exhibit ectopic PGCs that fail to cluster.

(C) Later stage embryos (22-somite stage) exhibit many ectopic germ cells following atorvastatin (10 μM) treatment.

(D) Brightfield image of an embryo (24 hpf) injected with *gfp-nanos* mRNA and treated with atorvastatin (10 μM) that exhibits only a mild tail defect.

(E) Analysis of the effect of atorvastatin treatment on PGC migration at different developmental stages. The number of embryos at a given stage with more than two ectopic PGCs was determined and expressed as a percent of the total number of embryos examined (n = 64–84 for each stage).

(F) The effect of atorvastatin on numbers of ectopic PGCs is dose dependent. Embryos were soaked in atorvastatin (10 μM). At 24 hpf, embryos were each scored on the degree of ectopic PGCs (a level 1 embryo had a wild-type single gonadal cluster and a level 4 embryo had no discernable cluster). Data represent the mean ± SEM from three to four experiments/dose.

(G) Initial image from a time-lapse movie of an atorvastatin-treated (10 μM) embryo, taken at the 14-somite stage.

(H) Three captured images from the time-lapse movie as in (G) taken at 1, 50, and 80 min after the 14-somite stage.

treatment (data not shown), suggesting that cholesterol-independent compounds of the HMGCoAR pathway control PGC migration.

To test whether the isoprenoid synthesis branch of the pathway (via geranylgeranyl transferases and farnesyl transferases) mediates the effect of statins on PGC migration, we injected embryos with compounds that would increase the cellular levels of the substrates of these enzymes before immersion in atovastatin (10 μM). Embryos injected with geranylgeraniol, an alcohol that is readily converted to geranylgeranyl diphosphate (Crick et al., 1994) prior to statin treatment exhibited normal PGC migration and were as equally protected as embryos injected with mevalonate (Figure 3E). Farnesol is an alcohol that can elevate cellular farnesyl diphosphate, a precursor required both by geranylgeranyl transferase and farnesyl transferase (Figure 1). Injection of farnesol also rescued the PGC migration defect of embryos from statin treatment (Figure 3E). Our results support the idea that the PGC migration defect induced

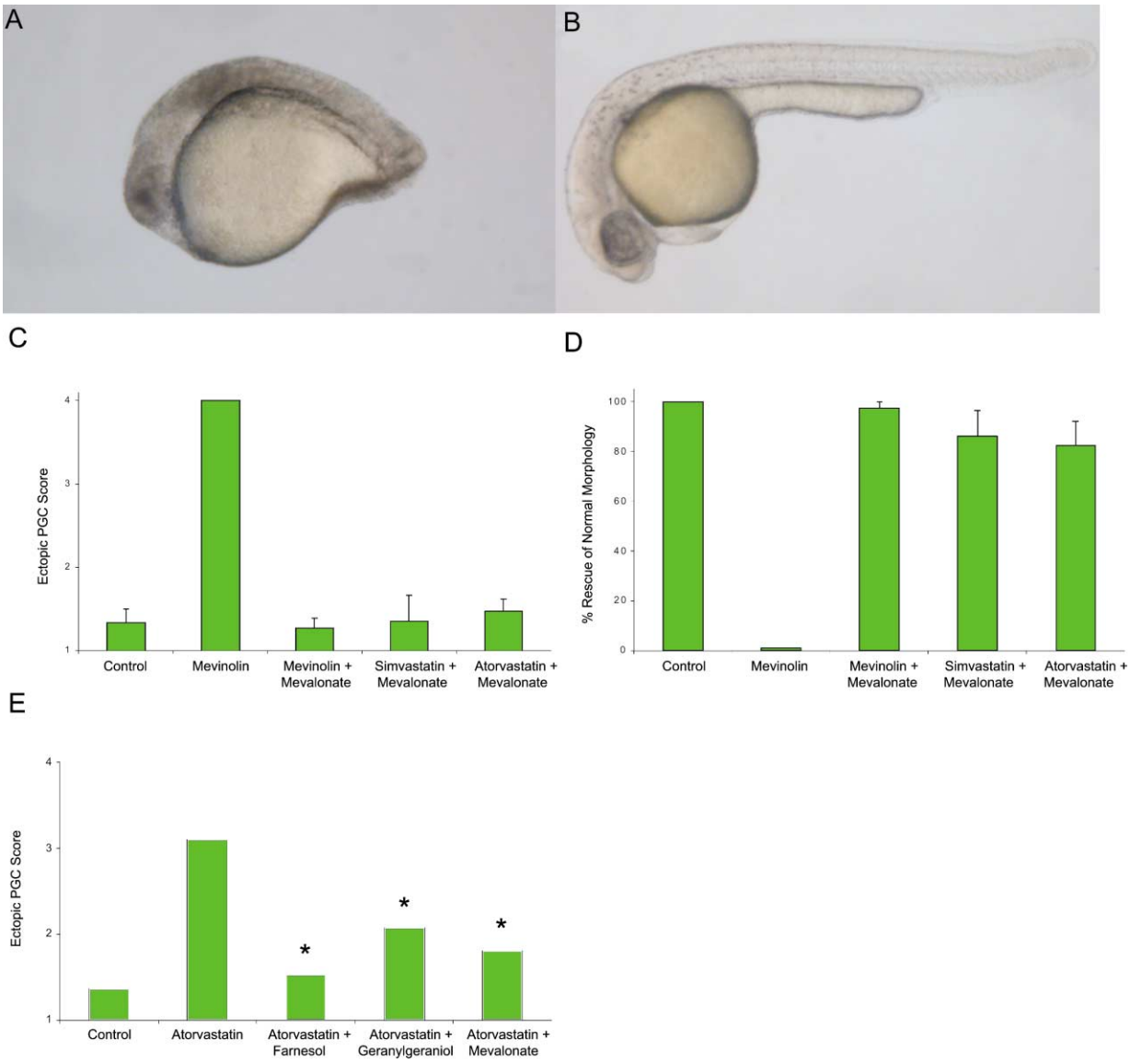

Figure 3. Injection of Isoprenoid Intermediates Abrogates the Effects of Statins

(A) Uninjected embryos show severe developmental defects after 24 hr of mevinolin (1.2 μM) treatment.

(B) Embryos injected at early cell stages (1–16 cell stage) with mevalonate (1–2 nl, 0.5 M) and then soaked in mevinolin (1.2 μM, 24 hr) exhibit normal morphology.

(C) The appearance of ectopic PGCs following statin treatment is prevented by mevalonate injections. Embryos at early cell stages were injected with *gfp-nanos* mRNA and mevalonate as in (B) and soaked overnight in statin drugs (mevinolin [1.2 μM], simvastatin [2.0 μM], and atorvastatin [10 μM]). At 24 hpf, the PGC score was determined. Data represent the mean ± SEM from three to four experiments.

(D) Mevalonate injection rescues the somatic defects observed in embryos treated with statins. Embryos at early cell stages were injected with mevalonate as in (B) and soaked overnight in statin drugs: mevinolin (1.2 μM), simvastatin (2.0 μM), and atorvastatin (10 μM). At 24 hpf, developmental defects were scored. Data represent the mean ± SEM from three to four experiments.

(E) Embryos injected at the 1–16 cell stage with farnesol (1–2 nl, 1 M), geranylgeraniol (0.5–1 nl, 1 M) or mevalonate (1–2 nl, 0.5 M) and then soaked overnight with atorvastatin (10 μM) show normal PGC migration. Statistics were performed using ANOVA with a post hoc test that utilizes a Bonferroni correction. Data represent mean ± SEM, * $p < 0.01$ difference from atorvastatin alone.

by statins is the result of impaired geranylgeranylation of target proteins caused by reduction in cellular geranylgeranyl diphosphate (Figure 1). Furthermore, these results are consistent with genetic studies in *Drosophila* where mutations in geranylgeranyl diphosphate synthase and farnesyl diphosphate synthase, the enzymes that produce farnesyl diphosphate and geranylgeranyl diphosphate, respectively, result in germ cell migration defects (Santos and Lehmann, 2004).

Geranylgeranyl Transferase Activity Is Required for Correct PGC Migration

To examine whether geranylgeranyl transferase (GGT1) or farnesyl transferase activities influence PGC migration, embryos were treated with different doses of specific inhibitors and the position of PGCs was determined after 1 day of development. High doses of the selective farnesyl transferase inhibitor FTI-2153 (FTase IC_{50}, 1.4 nM over GGT I IC_{50}, 1700 nM) (Crespo et al., 2001; Sun

et al., 1999) had no effect on PGC migration (data not shown). A second farnesyl transferase inhibitor (L-744) (Kohl et al., 1995) also had no effect on PGC migration (data not shown). In contrast, injection of a selective inhibitor of GGT1 (GGTI-2166, 100-fold more selective at inhibiting Rap1A [IC_{50}, 300 nM] over farnesyl transferase as measured by H-Ras processing) (Sun et al., 1999) caused a strong PGC migration phenotype (Figures 4A–4D) and only mild morphological defects, which were manifested as a slight kink in the notochord (Figure 4D, arrowhead).

The effect of GGTI-2166 on PGC migration was dose dependent (approximate EC_{50} 30 μM) (Figure 4E). To determine the cellular basis for the GGTI-2166-induced abnormal cell migration, the behavior of GFP-labeled PGCs was analyzed by time-lapse microscopy (Supplemental Movies S2 and S3). The formation of cellular protrusions was normal in PGCs treated with GGTI-2166. However, a significant reduction in the migration speed in treated PGCs was observed (from 2.170 $\pm$ 0.155 to 1.515 $\pm$ 0.095 μm/min in GGT1-treated embryos $p < 0.001$). These data suggest that protein prenylation, specifically by GGT1, is required for correct PGC migration and that HMGCoAR activity is needed to provide GGT1 substrates.

To assess the potential role of GGT1 activity on PGC migration, we identified the zebrafish ortholog of this gene. Zebrafish GGT1 is approximately 70% identical to the human ortholog at the amino acid level (Supplemental Figure S3). Similar to *hmgcr*2 expression, maternal *ggt1* transcripts were evident in 4-cell stage embryos and uniform expression was observed during gastrulation (Supplemental Figures S1 and S3). These data suggest that GGT1 is present in positions where the PGCs migrate.

Do SDF-1 and HMGCoAR Act in the Same Pathway to Regulate PGC Migration?

Previously, it was shown that the G protein-coupled receptor CXCR4b and its ligand SDF-1a provide directional guidance cues to migrating PGCs. One possibility to explain the effect of statin treatment on PGC migration could be that HMGCoAR activity is required for the expression or activity of the SDF-1 ligand. We therefore analyzed the expression of *sdf-1a* in statin-treated embryos and found that atorvastatin treatment did not alter *sdf-1a* expression (Figures 4F and 4G). To further test if statins and the SDF-1a receptor CXCR4b function together or independently, we impaired both pathways by injecting atorvastatin and morpholino antisense oligonucleotides directed against *cxcr4b* (Heasman et al., 2000; Nasevicius and Ekker, 2000). Doses were selected to induce mild migration defects. The combined effect of atorvastatin and *cxcr4b* morpholino was not greater than the sum of each independent treatment (Figure 4H), indicating that HMGCoAR and SDF-1a function in distinct pathways.

Discussion

The results of this study indicate that pharmacologic inhibition of protein prenylation, either through the inhibition of GGT1 directly or by limiting the supply of precursors for the reaction it catalyzes, impairs the migration of PGCs. The observation that correct PGC migration in zebrafish depends on HMGCoAR activity and isoprenylation is consistent with studies in *Drosophila* (Santos and Lehmann, 2004; Van Doren et al., 1998) and suggests that this pathway is evolutionarily conserved.

Understanding the mechanisms that regulate PGC migration is likely to help formulate a general model for long-range cell migration during development and disease. This is illustrated by the observation that the chemokine, SDF-1, and its receptor, CXCR4, provide guidance cues to direct PGCs to the developing gonad of mice and fish (Ara et al., 2003; Doitsidou et al., 2002; Molyneaux et al., 2003) and that a receptor related to CXCR4 is required for PGC migration in *Drosophila* (Kunwar et al., 2003). Further, a number of reports indicate that SDF-1 plays a critical role in determining the destination of metastatic tumor cells (Muller et al., 2001) and the migration of other cell types during development (Peled et al., 1999).

The parallels between proteins that influence PGC migration and those that regulate cancer cell metastasis are further substantiated by our observation that inhibition of GGT1 results in altered PGC migration. Several studies find that statins or specific prenylation inhibitors impair the chemotactic migration of cancer cells (Morgan et al., 2003), the migration of human monocytes (Wong et al., 2001), and the migration of lymphocytes (Walters et al., 2002).

The present study utilizes highly specific enzyme inhibitors to identify likely regulators of PGC migration. This approach has a number of advantages in that multiple isoforms and not individual proteins can be targeted. Additionally, when genetic studies impair zygotic gene expression, residual maternally derived protein, which may be experimentally difficult if not impossible to eliminate, can mask the corresponding phenotype. Conversely, it can be difficult to establish that the phenotype resulting from soaking whole embryos in a pharmacologic reagent results from the specific inhibition of a protein or protein family. We addressed this issue by injecting embryos with mevalonate prior to their exposure to statins. This procedure rescued all phenotypes associated with statin treatment, supporting the notion that the observed effect is specific to the mevalonate pathway (Figure 3).

Although mevalonate rescued the effect of all the statins utilized in this study, the phenotypes induced by the different statins were not identical. Simvastatin and mevinolin produced more severe somatic phenotypes than atorvastatin, one of the most hydrophobic statins. One possibility is that atorvastatin partitions into the yolk, which may limit both the availability and concentration of the drug within the embryo.

Our observation that atorvastatin-induced PGC migration defects were rescued by both geranylgeraniol and farnesol points to a potential role for geranylgeranyl transferase or farnesyl transferase activities in this process. However, two farnesyl transferase inhibitors failed to show any effect on PGC migration, while a GGT1-specific inhibitor (Sun et al., 1999) demonstrated a role for geranylgeranylated rather than farnesylated proteins in the process. The fact that farnesol also rescued atorvastatin-induced migration defects could be due to the presence of enough cellular isopentenyl diphosphate

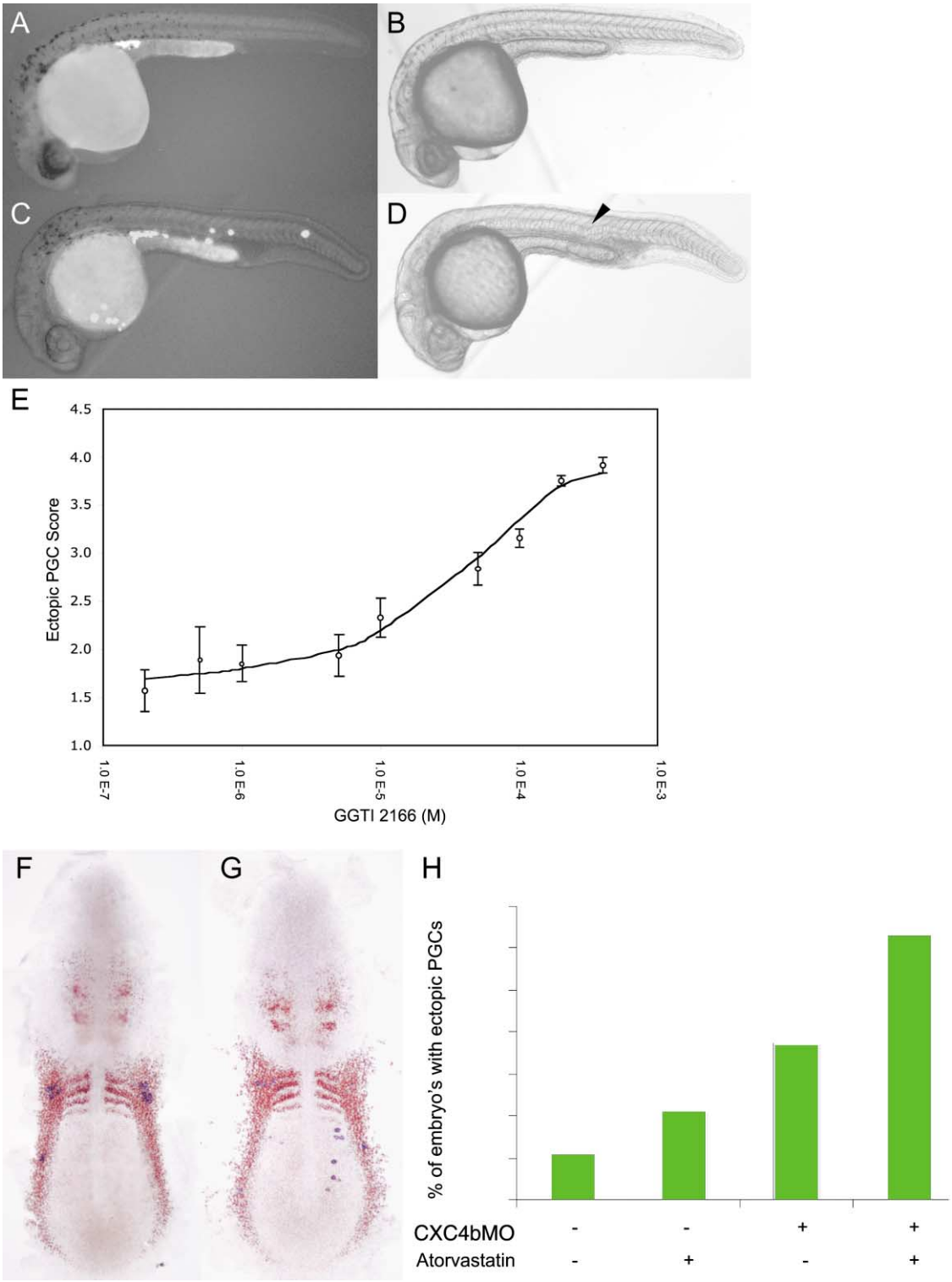

Figure 4. GGT1 Activity Is Required for PGC Migration

(A) A fluorescent image of a wild-type embryo (24 hpf) injected with *gfp-nanos* mRNA at the 1-cell stage reveals a cluster of PGCs.

(B) A brightfield image of the embryo in (A).

(C) A fluorescent image of an embryo (24 hpf) that was injected with *gfp-nanos* mRNA at the 1-cell stage and immediately soaked in the geranylgeranyl transferase inhibitor (GGTI-2166; 40 μM), resulting in ectopic migration of many PGCs.

(D) A brightfield image of the embryo in (G) showing a mild notochord defect (arrowhead).

(E) Dose response of GGTI-2166 on PGC migration defect. Data represent the mean ± SEM from three to six experiments for each dose.

sdf-1a expression is not altered in atorvastatin-treated embryos. A two-color whole mount in situ hybridization experiment was performed using *sdf-1a* (brown) and *nanos*-1 (blue, marks germ cells) antisense riboprobes on wild-type (F) and atorvastatin-treated embryos (G).

(H) Embryos injected with a *cxcr4b* morpholino to inhibit translation of *cxcr4b* exhibited a PGC phenotype that is additive with atorvastatin treatment. All embryos were injected with *gfp-nanos* mRNA and a control morpholino (1–2 nl, 0.02 mM) or *cxcr4b* morpholino (1–2 nl, 0.02 mM) at the 1-cell stage. Half of the injected embryos from each group were immediately treated with atorvastatin (4.0 μM) or embryo medium.

(even after HMGCoA reductase inhibition) such that farnesyl diphospate can be converted to geranylgeranyl diphosphate by the action of geranylgeranyl diphosphate synthase (see Figure 1).

Despite the striking conservation for the role of HMGCoAR in *Drosophila* and zebrafish PGC migration, an important difference between the two systems should be noted. In *Drosophila*, the expression pattern of HMGCoAR can determine the direction of PGC migration. In zebrafish, however, the spatial distribution of HMGCoAR activity fails to provide the PGCs with directional cues since zebrafish HMGCoAR genes are not specifically expressed in regions toward which the PGC migrate (Supplemental Figure S1) and uniform application of mevalonate simply rescued rather than interfered with PGC migration. One possibility is that HMGCoAr activity merely provides a factor necessary for PGC migration, while directional cues are under the sole control of SDF-1a (Doitsidou et al., 2002). Alternatively, while isoprenylation itself may not be spatially controlled, the target of its function, i.e., the substrate for isoprenylation, may be expressed along the PGC migratory path and provide directional cues for PGCs independent of SDF-1.

Our data suggest that a mevalonate-dependent prenylation reaction mediated by GGT1 is important for correct PGC migration. It has been proposed that protein prenylation mediates more than just the association of a protein to the membrane, but can act as a specific mediator of protein-protein interactions (Sinensky, 2000). Our results, together with those of Santos and Lehmann (2004), further suggest that there are yet-to-be-discovered proteins whose prenylation is essential for correct PGC migration as part of a highly conserved signaling pathway.

Experimental Procedures

Zebrafish

Methods for breeding and raising zebrafish were followed as described (Westerfield, 1995). Embryos were obtained from natural matings of wild-type (Oregon, AB) fish and staged according to criteria previously outlined (Kimmel et al., 1995) and by hours postfertilization (hpf).

Statin Pharmacology

To inhibit HMGCoAR activity, embryos were treated with statins, competitive inhibitors of HMGCoAR. Embryos were incubated overnight at 28°C in petri dishes (35 × 10 mm) containing mevinolin (Sigma Chemical), simvastatin (LKT Laboratories), and atorvastatin (LKT Laboratories) in embryo medium (EM) (Westerfield, 1995).

Statistical Analysis of Atorvastatin Phenotype

Embryos were treated with atorvastatin (10 μM) and then fixed at a variety of stages and subject to two-color in situ hybridization using *sdf-1a* and *nanos* riboprobes (Doitsidou et al., 2002; Koprunner et al., 2001). The ratio of the number of embryos with more than two ectopic PGCs to the total number of assayed embryos was determined.

Microinjections

Embryos were injected with glass microelectrodes fitted to a gas pressure injector (PLI-100, Harvard Apparatus, Cambridge, MA). Electrodes were pulled (P-97, Flamining/Brown) and filled with a stock solution of mevalonate (500 mM, Mevalonolactone, Sigma Chemical), sodium citrate (500 mM, FischerBiotech), farnesol (1 M, Sigma Chemical), geraynlgeraniol (1 M, Sigma Chemical) and/or *gfp-nanos* mRNA. Phenol red solution (0.2% final concentration) was added to all injection solutions to visualize injected embryos. Typically, embryos were initially injected with *gfp-nanos* mRNA and then sorted into groups that were subject to a second injection of mevalonate or buffer.

Farnesyl and Geranylgeranyl Transferase Inhibitors

Ras farnesyltransferase inhibitor (L744; Sigma) was made up to a final concentration of 4 mM. 1-cell stage embryos were injected with 1–2 nl of farnesyltransferase inhibitor and 1–2 nl of *gfp-nanos* mRNA. Some embryos were also soaked in farnesyltransferase inhibitor overnight. GGT1 inhibitor GGTI-2166 (Mol. Wt. 434.53) and farnsyl transferase inhibitor FTI-2153 (Mol. Wt. 539) were resuspended in DMSO (50 mM).

Microscopy

Embryos were anesthetized (Tricaine, 170 μg/ml) and placed on depression slides. Images were taken on a Zeiss Axio Vision 3.0 Camera mounted on a Leica MZFL-III stereomicroscope. Some images were captured using a Zeiss Axio Vision 3.0 camera mounted on a Zeiss Axioplan II microscope.

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References

Ara, T., Nakamura, Y., Egawa, T., Sugiyama, T., Abe, K., Kishimoto, T., Matsui, Y., and Nagasawa, T. (2003). Impaired colonization of the gonads by primordial germ cells in mice lacking a chemokine, stromal cell-derived factor-1 (SDF-1). Proc. Natl. Acad. Sci. USA *100*, 5319–5323.

Crespo, N.C., Ohkanda, J., Yen, T.J., Hamilton, A.D., and Sebti, S.M. (2001). The farnesyltransferase inhibitor, FTI-2153, blocks bipolar spindle formation and chromosome alignment and causes prometaphase accumulation during mitosis of human lung cancer cells. J. Biol. Chem. *276*, 16161–16167.

Crick, D.C., Waechter, C.J., and Andres, D.A. (1994). Utilization of geranylgeraniol for protein isoprenylation in C6 glial cells. Biochem. Biophys. Res. Commun. *205*, 955–961.

Doitsidou, M., Reichman-Fried, M., Stebler, J., Koprunner, M., Dorries, J., Meyer, D., Esguerra, C.V., Leung, T., and Raz, E. (2002). Guidance of primordial germ cell migration by the chemokine SDF-1. Cell *111*, 647–659.

Evans, M., and Rees, A. (2002). The myotoxicity of statins. Curr. Opin. Lipidol. *13*, 415–420.

Heasman, J., Kofron, M., and Wylie, C. (2000). Beta-catenin signaling activity dissected in the early Xenopus embryo: a novel antisense approach. Dev. Biol. *222*, 124–134.

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., and Schilling, T.F. (1995). Stages of embryonic development of the zebrafish. Dev. Dyn. *203*, 253–310.

Knaut, H., Werz, C., Geisler, R., and Nusslein-Volhard, C. (2003). A zebrafish homologue of the chemokine receptor Cxcr4 is a germ-cell guidance receptor. Nature *421*, 279–282.

Kohl, N.E., Omer, C.A., Conner, M.W., Anthony, N.J., Davide, J.P., deSolms, S.J., Giuliani, E.A., Gomez, R.P., Graham, S.L., Hamilton, K., et al. (1995). Inhibition of farnesyltransferase induces regression

of mammary and salivary carcinomas in ras transgenic mice. Nat. Med. *1*, 792–797.

Koprunner, M., Thisse, C., Thisse, B., and Raz, E. (2001). A zebrafish nanos-related gene is essential for the development of primordial germ cells. Genes Dev. *15*, 2877–2885.

Kunwar, P.S., Starz-Gaiano, M., Bainton, R.J., Heberlein, U., and Lehmann, R. (2003). Tre1, a G protein-coupled receptor, directs transepithelial migration of *Drosophila* germ cells. PLoS Biol. *1*, E80.

Molyneaux, K.A., Zinszner, H., Kunwar, P.S., Schaible, K., Stebler, J., Sunshine, M.J., O'Brien, W., Raz, E., Littman, D., Wylie, C., and Lehmann, R. (2003). The chemokine SDF1/CXCL12 and its receptor CXCR4 regulate mouse germ cell migration and survival. Development *130*, 4279–4286.

Morgan, M.A., Wegner, J., Aydilek, E., Ganser, A., and Reuter, C.W. (2003). Synergistic cytotoxic effects in myeloid leukemia cells upon cotreatment with farnesyltransferase and geranylgeranyl transferase-I inhibitors. Leukemia *17*, 1508–1520.

Muller, A., Homey, B., Soto, H., Ge, N., Catron, D., Buchanan, M.E., McClanahan, T., Murphy, E., Yuan, W., Wagner, S.N., et al. (2001). Involvement of chemokine receptors in breast cancer metastasis. Nature *410*, 50–56.

Nasevicius, A., and Ekker, S.C. (2000). Effective targeted gene 'knockdown' in zebrafish. Nat. Genet. *26*, 216–220.

Peled, A., Petit, I., Kollet, O., Magid, M., Ponomaryov, T., Byk, T., Nagler, A., Ben-Hur, H., Many, A., Shultz, L., et al. (1999). Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science *283*, 845–848.

Santos, A.C., and Lehmann, R. (2004). Isoprenoids control germ cell migration downstream of HMGCoA reductase. Dev. Cell *6*, this issue, 283–293.

Sever, N., Yang, T., Brown, M.S., Goldstein, J.L., and DeBose-Boyd, R.A. (2003). Accelerated degradation of HMG CoA reductase mediated by binding of insig-1 to its sterol-sensing domain. Mol. Cell *11*, 25–33.

Sinensky, M. (2000). Functional aspects of polyisoprenoid protein substituents: roles in protein-protein interaction and trafficking. Biochim. Biophys. Acta *1529*, 203–209.

Sun, J., Blaskovich, M.A., Knowles, D., Qian, Y., Ohkanda, J., Bailey, R.D., Hamilton, A.D., and Sebti, S.M. (1999). Antitumor efficacy of a novel class of non-thiol-containing peptidomimetic inhibitors of farnesyltransferase and geranylgeranyltransferase I: combination therapy with the cytotoxic agents cisplatin, Taxol, and gemcitabine. Cancer Res. *59*, 4919–4926.

Van Doren, M., Broihier, H.T., Moore, L.A., and Lehmann, R. (1998). HMG-CoA reductase guides migrating primordial germ cells. Nature *396*, 466–469.

Walters, C.E., Pryce, G., Hankey, D.J., Sebti, S.M., Hamilton, A.D., Baker, D., Greenwood, J., and Adamson, P. (2002). Inhibition of Rho GTPases with protein prenyltransferase inhibitors prevents leukocyte recruitment to the central nervous system and attenuates clinical signs of disease in an animal model of multiple sclerosis. J. Immunol. *168*, 4087–4094.

Weidinger, G., Wolke, U., Koprunner, M., Thisse, C., Thisse, B., and Raz, E. (2002). Regulation of zebrafish primordial germ cell migration by attraction towards an intermediate target. Development *129*, 25–36.

Westerfield, M. (1995). The Zebrafish Book, 3rd edn. (Eugene, OR: University of Oregon).

Wong, B., Lumma, W.C., Smith, A.M., Sisko, J.T., Wright, S.D., and Cai, T.Q. (2001). Statins suppress THP-1 cell migration and secretion of matrix metalloproteinase 9 by inhibiting geranylgeranylation. J. Leukoc. Biol. *69*, 959–962.