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The G Protein-Coupled Receptor Agtrl1b Regulates Early Development of Myocardial Progenitors

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SUMMARY

While many factors that modulate the morphogenesis and patterning of the embryonic heart have been identified, relatively little is known about the molecular events that regulate the differentiation of progenitor cells fated to form the myocardium. Here, we show that zebrafish *grinch* (*grn*) mutants form a reduced number of myocardial progenitor cells, which results in a profound deficit in cardiomyocyte numbers in the most severe cases. We show that *grn* encodes the G protein-coupled receptor (GPCR) Agtrl1b, a known regulator of adult cardiovascular physiology. Ectopic expression of Apelin, an Agtrl1b ligand, results in the complete absence of cardiomyocytes. Data from transplantation and transgenic approaches indicate that Agtrl1 signaling plays a cell-autonomous role in myocardial specification, with activity being required coincident with the onset of gastrulation movements. These results support a model in which *agtrl1b* regulates the migration of cells fated to form myocardial progenitors.

INTRODUCTION

Organogenesis begins with the development of progenitor cells that later differentiate to form the specialized tissues required for organ function. In vertebrates, myocardial progenitors can first be distinguished by *nkx2.5* expression in two bilateral stripes of cells in the anterior lateral plate mesoderm (ALPM) (Harvey, 1996). Prior to the onset of *nkx2.5* expression, fate-mapping studies in several vertebrates have shown that myocardial progenitor cells ingress early during gastrulation (Stainier et al., 1993; Parameswaran and Tam, 1995; Schoenwolf and Garcia-Martinez, 1995; Keegan et al., 2004). Multiple signaling pathways, acting in both stimulatory and inhibitory fashions, act to restrict cardiomyogenesis to a defined domain in the ALPM. These signals include TGF-βs, Fgfs, Shh, and Wnts/Wnt inhibitors (Schultheiss et al., 1997; Reifers et al., 2000; Schneider and Mercola, 2001; Zhang et al., 2001).

Relatively little is known regarding mechanisms that specifically affect myocardial progenitor specification and migration to the ALPM prior to the onset of *nkx2.5* expression. Explant studies in chick have suggested that signals are required for cardiomyogenesis prior to gastrulation (Antin et al., 1994). In gastrula-stage zebrafish embryos, retinoic acid (RA) signaling acts to restrict the number of myocardial progenitor cells formed (Keegan et al., 2005). Members of the *Mesp* transcription factor gene family are broadly expressed in the murine primitive streak, and they are required for the ingression of cardiovascular progenitors during gastrulation (Saga et al., 1999). Heart development is severely perturbed in *Mesp1/2* double mutants (Kitajima et al., 2000), as well as in ascidian embryos depleted of *mesp* function by injection of antisense oligonucleotides (Satou et al., 2004).

In this study, we used a forward genetic approach in zebrafish to identify possible novel regulators of early cardiac development. We describe a mutation, *grinch*, that greatly inhibits the development of the myocardium. Activity of the gene affected by this mutation, *agtrl1b*, is shown to be required early in myocardial progenitors, coincident with the onset of gastrulation. To our knowledge, these results therefore identify a novel signaling pathway required for myocardial development.

RESULTS

grinch Mutants Have a Specific and Early Defect in Cardiomyogenesis

In order to identify additional regulators of cardiac development, we carried out a forward genetic screen and assayed heart morphology at 52 and 96 hours postfertilization (hpf) (Beis et al., 2005). A single allele of a novel recessive mutation, *grinch*^{s608} (*grn*), was identified and caused a marked reduction in the size of the heart (Figures 1A and 1B). While the expressivity of the *grn* phenotype is