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Rbm19 is a nucleolar protein expressed in crypt/progenitor cells of the intestinal epithelium

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Abstract

Intestinal development and homeostasis rely on the coordination of proliferation and differentiation of the epithelium. To better understand this process, we are studying Rbm19, a gene expressed in the gut epithelium that is essential for intestinal morphogenesis and differentiation in the zebrafish (Development 130, 3917). Here we analyzed the expression of Rbm19 in several biological contexts that feature proliferation/differentiation cell fate decisions. In the undifferentiated embryonic gut tube, Rbm19 is expressed throughout the epithelium, but then becomes localized to the crypts of Lieberkühn of the adult intestine. Consistent with its expression in adult crypt/progenitor cells, expression is widespread in human colorectal carcinomas and dividing Caco-2 cells. Its expression in Caco-2 cells recapitulates the in vivo pattern, declining when the cells undergo confluence-induced arrest and differentiation. Rbm19 protein localizes to the nucleolus during interphase and to the perichromosomal sheath during mitosis, in accordance with the pattern described for other nucleolar proteins implicated in ribosome biogenesis. Interestingly, the loss of nucleolar rbm19, nucleolin/C23, and nucleophosmin/B23 in confluent Caco-2 cells did not signify loss of nucleoli as detected by electron microscopy. Taken together, these data point to the nucleolus as a possible locus for regulating the proliferation/differentiation cell fate decision in the intestinal epithelium.

Keywords: Intestine; Rbm19; Development; Nucleolus; Progenitor; Stem cell; Differentiation; Morphogenesis; Organogenesis; Caco-2 cells; Intestinal crypt; RNA-binding proteins; Nucleophosmin; Nucleolin; C23; B23; Preribosomal RNA processing; Ribosome biogenesis; Colorectal adenocarcinoma; Colon cancer; Mitosis

The vertebrate intestine offers a venue to study a broad range of basic biological processes: organ morphogenesis, growth, differentiation, homeostasis, and neoplasia. During development of the mammalian intestine, the epithelium changes from multilayer to single layer and the cells begin to express intestine-specific genes. A subset of cells remain undifferentiated and segregate to the basal portion of newly formed villi to form a progenitor compartment. Thereafter, progenitor cells continue to divide and differentiate as they migrate up the villus to replenish the epithelium on average every 3–5 days (Montgomery et al., 1999). The molecular control of the cell fate decisions that collectively establish and maintain the architecture of the intestine are being revealed through the manipulation of known candidate pathways (Sancho et al., 2004) and forward genetic screens (Amsterdam et al., 2004; Farber et al., 2001; Mayer and Fishman, 2003; Pack et al., 1996). Previously, we undertook a forward genetic approach to studying intestinal development in zebrafish and isolated the *nil per os* (*npo*) gene (Mayer and Fishman, 2003). The phenotype in the *npo* mutant is arrest of intestinal development at the primitive gut tube stage, with failure of subsequent growth and differentiation. Gene expression peaks in the gut

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immediately prior to villus morphogenesis, consistent with an essential function in this step. *npo* was found to encode a unique protein with six RNA recognition motif (RRM) domains (5 domains in fungi and plants) that is highly conserved throughout all eukaryotes (Bjork et al., 2002; Mayer and Fishman, 2003). Studies of the *npo* ortholog in yeast, in the dipteran *C. tentans*, and in *C. elegans*, all concluded that the gene product is essential for the production of the 18S ribosomal RNA during ribosome biogenesis (Bjork et al., 2002; Jin et al., 2002; Saijou et al., 2004). We will hereafter refer to this gene by its HUGO-approved name, Rbm19. In this work we sought to determine how Rbm19 might function in the intestine of higher vertebrates. As an initial step, we examined its expression during development, in adult intestine, in a colon carcinoma cell line and in neoplastic tissue. In each of these contexts, we found Rbm19 to be differentially expressed in epithelial cells that exhibit a 'crypt-progenitor' phenotype, suggesting that it may be part of a pathway regulating proliferation/differentiation cell fate decisions.

1. Results and discussion

1.1. Rbm19 expression in developing and adult intestinal epithelium

In the zebrafish, *rbm19* expression is highly dynamic (Mayer and Fishman, 2003). Prior to intestinal morphogenesis, the gene is expressed throughout the primitive gut tube. Then the gut undergoes an anterior–posterior wave of intestinal differentiation (Andre et al., 2000), and *rbm19* expression declines with the appearance of differentiated intestinal cells. A rare subset of cells continues to express *rbm19,* suggesting the possibility that these cells represent a remnant of undifferentiated endoderm fated to become epithelial progenitor cells. In mammals, epithelial morphogenesis of the intestine is well described, and the appearance of villi corresponds to the formation of a stem cell compartment in the intervillus epithelium (Klein, 1989). To detect Rbm19 expression in developing mouse intestine, we performed in situ hybridization (ISH), focusing on the stages during which the intestinal epithelium matures from the endoderm (Fig. 1). In whole mount specimens, we found the staining to be diffuse at E13.5, but increasingly restricted at E14.5. Expression then declines precipitously by E15.5. Rbm19 expression in E14.5 localizes to islands with a stereotypical spatial pattern, such that sharp bands of higher expression form in the rostral part of the island, and a gradient tapers off caudally (Fig. 1, panel B). These are highlighted in magnified views of the pylorus-duodenum junction (Fig. 1, panel B1) and the duodenal-jejunal border (Fig. 1, panel B2). At E15.5, the only expression detectable on whole-mount is in a segment of duodenum between the pylorus and the ampulla of Vater (Fig. 1D). Since, the temporal pattern in the mouse differed from that seen in

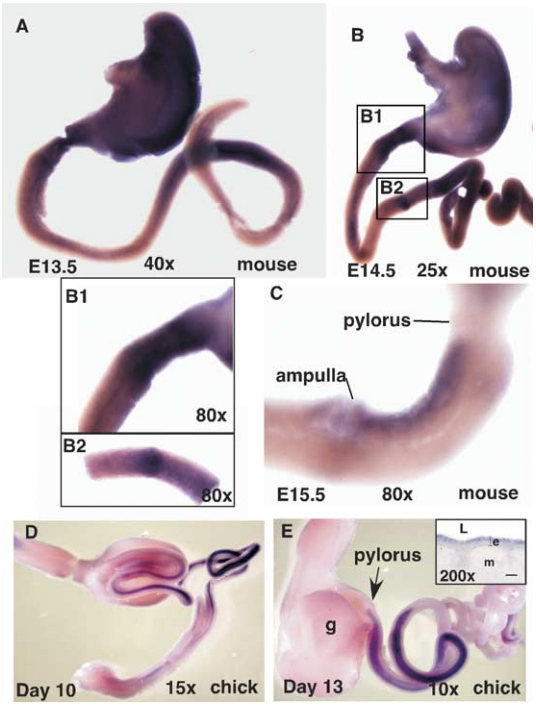

Fig. 1. Whole mount in situ hybridization for Rbm19 expression. Mouse (A–C) and chick (D–E). In the mouse Rbm19 is expressed in a dynamic, heterogeneous expression pattern, with more diffuse expression at E13.5, and progressive restriction of expression by E14.5. Magnified views of areas with increased expression show a distinct banding pattern (B1, B2). By E15.5 expression is undetectable except in the mesenteric aspect of the duodenum between the pylorus and ampulla of Vater (C). An overall similar pattern is seen in the developing chick intestine, with diffuse expression on day 10, then localization to the duodenum by day 13 (D and E). Histological section of chick intestine on E13 shows staining of the apical layer of the epithelium, in a pattern similar to the mouse intestine on E13.5 (Fig E, inset). Abbreviations: L, lumen, e, epithelium, m, mesenchyme.

zebrafish, we also performed in situ hybridization on chick intestine during the period of villus morphogenesis (Coulombre and Coulombre, 1958). In the chick, we found a similar developmental variation of Rbm19 compared with the mouse. At E10, Rbm19 expression is widely distributed across the intestine (Fig. 1D), and then at E13, expression is restricted to the duodenum (Fig. 1E). This pattern resembles the mouse intestine in the progression of diffuse to focal gene expression. We concluded that, while there may be interspecies differences in the exact developmental sequence, there is a general conservation of diffuse expression in the undifferentiated endoderm with progressive restriction of expression as the endoderm differentiates.

Microscopic examination of embryonic gut stained for Rbm19 mRNA demonstrates that expression is mostly in the endoderm (Fig. 2). In accordance with the pattern seen on whole mount, we found a dramatic reduction in the extent of Rbm19 expression with the onset of villus morphogenesis. At E12.5 expression is distributed in multiple foci throughout the endoderm, then at E13.5 the signal localizes