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hnf1b Genes in Zebrafish Hindbrain Development

Seong-Kyu Choe,* Nicolas Hirsch,*,** Xiaolan Zhang, and Charles G. Sagerström

Abstract

The Hnf1b transcription factor acts during formation of rhombomeres (r) 5 and 6 in the hindbrain. To determine if *hnf1b* is absolutely required in r5/r6, we examined the *hnf1b*^{hi2169} and *hnf1b*^{hi1843} retroviral insertion alleles. *Hnf1b*^{hi2169} shows highly variable residual expression of several genes in r5/r6, but this is not due to full-length *hnf1b* transcripts persisting in *hnf1b*^{hi2169} embryos, nor to *hnf1bl*, a novel *hnf1* family member expressed in r5 that we identified. Instead, we find evidence for a virus-*hnf1b* fusion transcript in *hnf1b*^{hi2169} embryos and demonstrate that morpholino-mediated knockdown of this transcript leads to near-undetectable r5 gene expression. The *hnf1b*^{hi1843} allele has a more severe phenotype with near-undetectable expression of r5/r6 genes. We next examined if *hoxb1b*, which functions upstream of *hnf1b* in r5/r6 formation, can induce expression of r5/r6 genes in *hnf1b* mutants. We find that microinjected *hoxb1b* mRNA induces ectopic gene expression anterior to the hindbrain in *hnf1b*^{hi2169} and *hnf1b*^{hi1843} embryos, but cannot restore gene expression in r5/r6 of the mutants. We conclude that *hnf1b*^{hi2169} is hypomorphic to *hnf1b*^{hi1843} and that, while *hnf1b* is required for r5/r6 gene expression in the hindbrain, r5/r6 gene expression can be experimentally induced independently of *hnf1b* anterior to the hindbrain.

Introduction

The vertebrate hindbrain is transiently subdivided into rhombomeres during embryonic development. The segmented nature of the embryonic hindbrain ensures proper spatial positioning of differentiating neurons (e.g., reticulospinal interneurons and motornuclei of the cranial nerves) and also patterns neural crest cells that migrate from the hindbrain and contribute to numerous structures (e.g., peripheral nervous system and craniofacial structures). Ongoing efforts to understand hindbrain patterning have identified a number of genes required for rhombomere formation.[1] In particular, several genes have been implicated in the formation of rhombomere (r) 5 and/or r6, including the homeodomain transcription factor *variable hepatocyte nuclear factor 1/transcription factor 2/hnf1 homeobox b* (*vhnf1/tcf2/hnf1b*), the bZip transcription factor *mafB/kreisler/valentino* (*mafB/Kr/val*), the zinc finger transcription factor *krüppel box 20/early growth response 2* (*krox20/egr2*), and homeodomain transcription factors from *hox* paralog group 3 (PG3 *hox*).

Previous work has suggested a pathway wherein *hnf1b* is required for activation of *val*, which in turn is required for activation of *krox20* and *PG3 hox* genes. Accordingly, *hnf1b* expression precedes *val* expression, *val* expression is disrupted in r5-r6 of *hnf1b* mutants[2–4] and the *val* promoter contains functional *hnf1b* binding sites.[5] Further, essential *val* (*mafB*) binding sites are present in the *hoxa3* and *hoxb3* promoter region,[6–8] and expression of *hoxa3* and *hoxb3* is absent from r5-r6 in *kreisler* mutant mice and *valentino* mutant zebrafish.[9–12] Lastly, *krox20* expression is disrupted in r5 of both

Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts.
*These authors equally contributed to this work.
**Current address: Department of Biology, Hiram College, Hiram, Ohio.

hnf1b and *val* mutant zebrafish,[2–4,13] as well as in *kreisler* mutant mice.[9] However, more recent work has brought this proposed simple linear regulatory cascade into question. For instance, it appears that *hnf1b* may directly activate *krox20* expression without requiring *val*.[14] In addition, *mafB* proteins may feed back to maintain *hnf1b* expression,[15] suggesting that these genes may constitute a regulatory network. Further, several aspects of the published *hnf1b* mutant phenotype, including *val* expression in r5/r6, appear to be variable,[2] suggesting that *hnf1b* may not be absolutely required for *val* expression or r5/r6 formation.

Here we characterize the phenotype of the *hnf1b*^{hi2169} retroviral insertion allele in greater detail. We find highly variable residual expression of several r5/r6 genes in *hnf1b*^{hi2169} embryos. This residual gene expression is not the result of full-length *hnf1b* transcripts persisting in *hnf1b*^{hi2169} embryos, nor it is due to *hnf1bl*, a novel *hnf1* family member expressed in r5 whose activity is indistinguishable from that of *hnf1b*. Instead, we find evidence for a virus-*hnf1b* fusion transcript. Although we lack antibodies to directly detect the presence of a protein produced from this transcript, we demonstrate that morpholino-mediated knockdown of this transcript leads to a stronger phenotype with near-undetectable gene expression in r5. Turning next to the *hnf1b*^{hi1843} allele, we find that it has a more severe phenotype with near-undetectable expression of several r5/r6 genes. We conclude that *hnf1b*^{hi2169} is hypomorphic to *hnf1b*^{hi1843} and that *hnf1b* is required for gene expression in r5/r6. Lastly, we find that *hoxb1b*, which functions upstream of *hnf1b* in the regulation of r5/r6 formation,[16] can induce ectopic expression of r5/r6 genes anterior to the hindbrain in *hnf1b*^{hi2169} and *hnf1b*^{hi1843} embryos, but cannot restore r5/r6 gene expression to the hindbrain of these mutants, indicating that *hnf1b* is required for induction of r5/r6 gene expression in the hindbrain, but not further anteriorly in the embryo.

Results

Variable gene expression in the caudal hindbrain of hnf1b^{hi2169} *embryos*

We have noted significant variations in the expression of several genes in r5 and r6 of *hnf1b*^{hi2169} mutant embryos. The phenotype ranges from near-complete loss of *krox20* gene expression in r5 (Fig. 1E) to relatively robust expression (Fig. 1B). Similarly, *hoxa3* expression, which is normally observed in r5 and r6, appears completely lost in the hindbrain of some *hnf1b*^{hi2169} embryos (Fig. 1J), but is readily detectable in other *hnf1b*^{hi2169} embryos (Fig. 1G, H). In a representative experiment we find that only 5% (6/120) of embryos from an *hnf1b*^{hi2169} incross lack r5/r6 gene expression. This is significantly less than 25%, indicating that most homozygous *hnf1b*^{hi2169} mutant embryos retain r5/r6 gene expression.

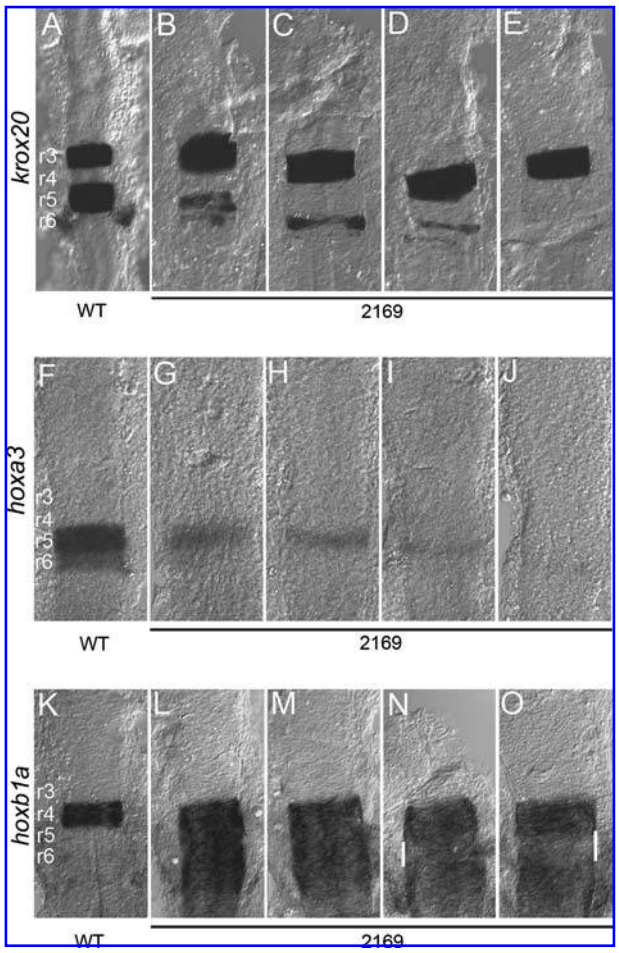


FIG. 1. Variable gene expression in the hindbrain of *hnf1b*^{hi2169} mutant embryos. Wild-type (**A, F, K**) and *hnf1b*^{hi2169} (**B–E, G–J, L–O**) embryos were assayed for expression of *krox20* (**A–E**), *hoxa3* (**F–J**), and *hoxb1a* (**K–O**) by *in situ* hybridization. All panels are dorsal views of flat-mounted hindbrains with anterior to the top. Rhombomere numbering is indicated in panels (**A**), (**F**), and (**K**). White bars in panels (**N**) and (**O**) indicate regions of reduced *hoxb1a* expression.

It has been reported that *hoxb1a* expression expands from r4 into the caudal hindbrain of *hnf1b*^{hi2169} embryos.[4] We observe variations also in *hoxb1a* expression among *hnf1b*^{hi2169} embryos. In particular, while most *hnf1b*^{hi2169} embryos show uniform *hoxb1a* expression throughout the caudal hindbrain (Fig. 1L, M), some embryos display reduced *hoxb1a* expression in the r5/r6 region (Fig. 1N, O). We hypothesize that embryos with reduced *hoxb1a* expression in r5/r6 correspond to the ones with higher expression of r5/r6 genes, since several r5/r6 genes are reported to repress *hoxb1* expression.[17–19]

We conclude that *hnf1b*^{hi2169} embryos retain variable r5/r6 gene expression. These findings do not distinguish whether residual r5/r6 gene expression in *hnf1b*^{hi2169} embryos is due to this allele retaining some *hnf1b* activity, or whether *hnf1b* is not absolutely required for r5/r6 gene expression.

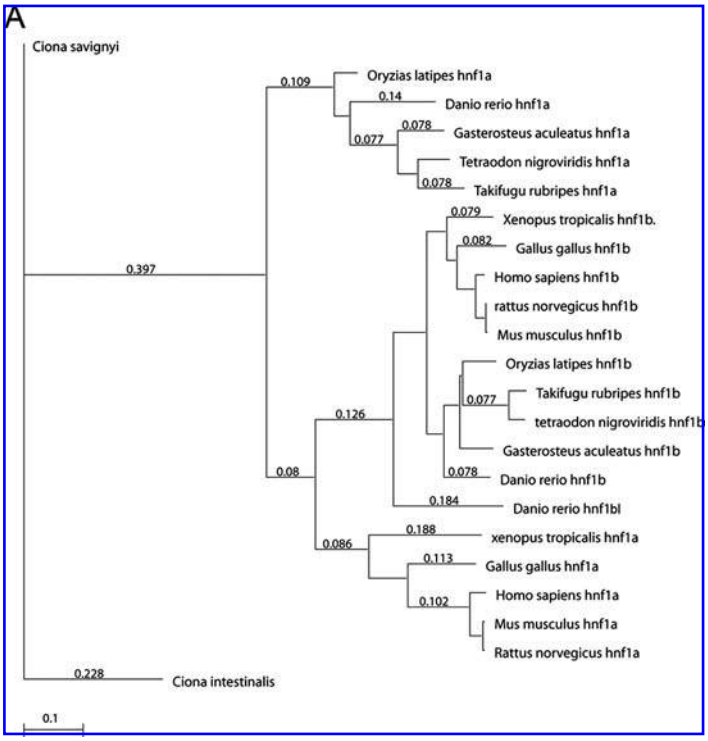
Hnf1bl *expression in r5 does not compensate for* hnf1b *activity in* hnf1b^{hi2169} *mutants*

The *hnf1* family contains two members (*hnf1a* and *hnf1b*) with only *hnf1b* being expressed in r5/r6. However, in a microarray screen for *hoxb1b*-regulated genes (manuscript in preparation), we identified a third *hnf1* gene identical to NCBI entry AF250352. While this gene has been previously named *hnf1γ*, we think it is unlikely to represent a third *hnf1* family member since other vertebrates do not appear to have a third *hnf1* gene. Instead, this gene may represent a duplicated copy of *hnf1b*. Indeed, sequence alignment reveals it to be more similar to *hnf1b* than to *hnf1a*, and it clusters with *hnf1b* in a phylogenetic tree (Fig. 2A). Further, this gene is on zebrafish LG21 and *hnf1b* is on LG15. We find that at least three adjacent genes (*aldoc*, *sfrs1*, and *dnl2*) are duplicated between LG21 and LG15 in zebrafish. In contrast, *hnf1b*, *aldoc*, and *sfrs1* map to a single chromosome in human (Hs17) and in mouse (Mm11). Lastly, the expression pattern of the novel gene is similar to that of *hnf1b* (Fig. 2B–F). We conclude that this *hnf1* gene likely resulted from the genome duplication reported to have occurred in the teleost lineage[20] and have named it *hnf1b-like* (*hnf1bl*).

We do not detect *hnf1bl* expression until 10 hpf, at which point it is expressed in the intermediate mesoderm (Fig. 2B). This is in contrast to *hnf1b*, which is expressed in both intermediate mesoderm and the caudal hindbrain (including r5/r6) at this stage (Fig. 2C; Sun and Hopkins[4]). *Hnf1bl* expression appears in r5 by 14 hpf (Fig. 2D, E), a stage when *hnf1b* expression has already regressed caudally (Fig. 2F), suggesting that *hnf1bl* might take over the role of *hnf1b* in r5/r6 after *hnf1b* is lost in this region. *Hnf1bl* expression then diminishes by 18 hpf.

Misexpression of *hnf1bl* by mRNA injection produced a phenotype where *hoxb1a* expression in r4 is reduced or lost and gene expression from r3 and r5 expands into r4 (Fig. 2L–N). This phenotype is indistinguishable from that observed upon injection of *hnf1b* mRNA,[2–4,16] demonstrating that *hnf1bl* has similar activity to *hnf1b* and raising the possibility that *hnf1bl* may compensate for the loss of *hnf1b* activity in *hnf1b*^{hi2169} embryos.

To test whether *hnf1bl* might act redundantly with *hnf1b*, we designed antisense morpholino oligonucleotides (MOs) to *hnf1bl* (see Materials and Methods). *Hnf1bl* MOs efficiently blocked expression of an Hnf1bl-GFP fusion protein *in vivo* (Supplemental Fig. 1, available online at www.liebertpub.com/fpd), demonstrating the efficacy of these MOs. We do not observe any effect on r5 gene expression upon injecting *hnf1bl* MOs into wild-type embryos (at concentrations up to 1 mM; not shown), indicating that *hnf1bl* is either not required for r5 gene expression or functions redundantly with *hnf1b*. To test the latter possibility, we examined the effect of injecting *hnf1bl* MOs into *hnf1b*^{hi2169} embryos. We find that injecting *hnf1bl* MOs results in 26% (13/50) of *hnf1b*^{hi2169} homozygous embryos lacking r5 gene expression. This is only slightly higher than the 19% (6/32) of uninjected *hnf1b*^{hi2169} homozygous embryos that lack r5 gene expression. Further, coinjecting *hnf1bl* MOs with *hnf1b* MOs into wild-type embryos did not produce a more severe effect than injecting *hnf1b* MOs alone (47% of embryos with little or no r5 staining after coinjection of *hnf1bl* and *hnf1b* MOs vs. 57% after injection of *hnf1b* MOs alone in a representative experiment). Lastly, we examined *hnf1bl* expression in *hnf1b*^{hi2169} and *val*^{b337} mutant embryos. We find that *hnf1bl* is lost in both mutants (Fig. 2G, H), demonstrating that *hnf1bl* expression



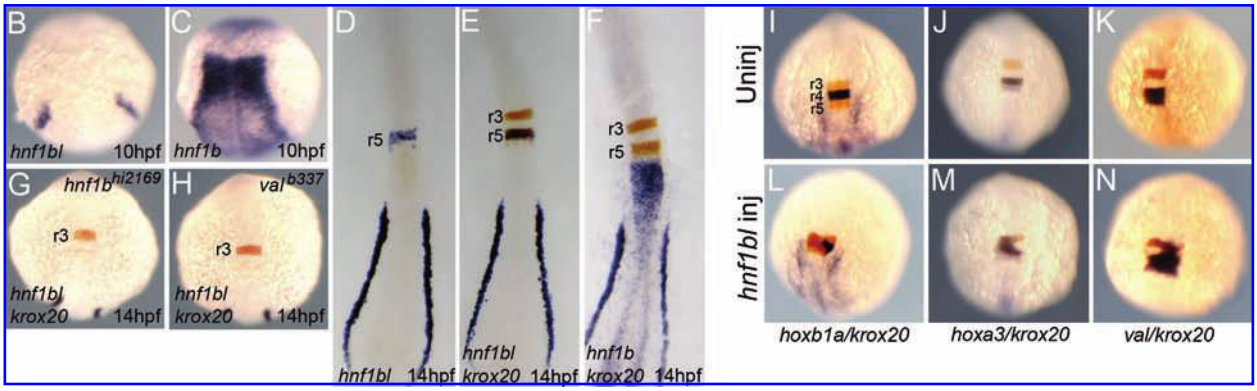


FIG. 2. A novel *hnf1bl* gene expressed in r5. (**A**) Phylogenetic tree demonstrating that *hnf1bl* clusters with *hnf1b* genes rather than *hnf1a* genes. (**B–H**) Wild-type (**B–F**), *hnf1b*^{hi2169} (**G**), and *val*^{b337} (**H**) embryos were assayed for expression of *hnf1bl* (**B, D**), *hnf1b* (**C**), *hnf1bl* + *krox20* (**E, G, H**), or *hnf1b* + *krox20* (**F**) by *in situ* hybridization. Note that *hnf1b* and *hnf1bl* are detected in blue, while *krox20* is detected in red. (**B**), (**C**), (**G**), and (**H**) are whole mounts while (**D**), (**E**), and (**F**) are flat mounts. Anterior is to the top in all panels. (**I–N**) Uninjected (**I–K**) and *hnf1bl*-injected (**L–N**) wild-type embryos were assayed for expression of *hoxb1a* + *krox20* (**I, L**), *hoxa3* + *krox20* (**J, M**), or *val* + *krox20* (**K, N**). Note that *hoxb1a*, *hoxa3*, and *val* are detected in blue, while *krox20* is detected in red. All embryos are whole mounts in dorsal view with anterior to the top.

requires both *hnf1b* and *val*. We conclude that *hnf1bl* acts downstream of *hnf1b* and *val*, and that *hnf1bl* activity cannot account for the residual r5/r6 gene expression in *hnf1b*^{hi2169} embryos.

The hnf1b^{hi1843} *allele displays a more severe phenotype than the* hnf1b^{hi2169} *allele*

Our analysis of the *hnf1b*^{hi2169} allele suggests that either *hnf1b* is not absolutely required for r5/r6 formation, or residual *hnf1b* activity persists in *hnf1b*^{2169} embryos. To address this question, we turned to additional *hnf1b* alleles. Of the three *hnf1b* alleles identified in the original insertion screen, *hnf1b*^{hi548} was reported as the weakest, while *hnf1b*^{hi2169} and *hnf1b*^{hi1843} gave similar phenotypes that were more severe than the *hnf1b*^{hi548} phenotype.[4] We therefore focused on the *hnf1b*^{hi1843} allele and find that it has a more severe hindbrain phenotype than *hnf1b*^{hi2169}. In particular, *hnf1b*^{hi1843} embryos appear to completely lack *krox20* expression in r5 (Fig. 3B–E) and *hoxa3* expression in r5/r6 (Fig. 3G–J) compared to *hnf1b*^{hi2169} embryos that showed variable residual expression (Fig. 1). However, we have also noted that some outcrosses of *hnf1b*^{hi1843} fish occasionally reveal detectable r5/r6 gene expression, suggesting that the *hnf1b*^{hi1843} phenotype may be modified on some genetic backgrounds (e.g., Tupfel longfin; not shown). *Hnf1b*^{hi1843} embryos also

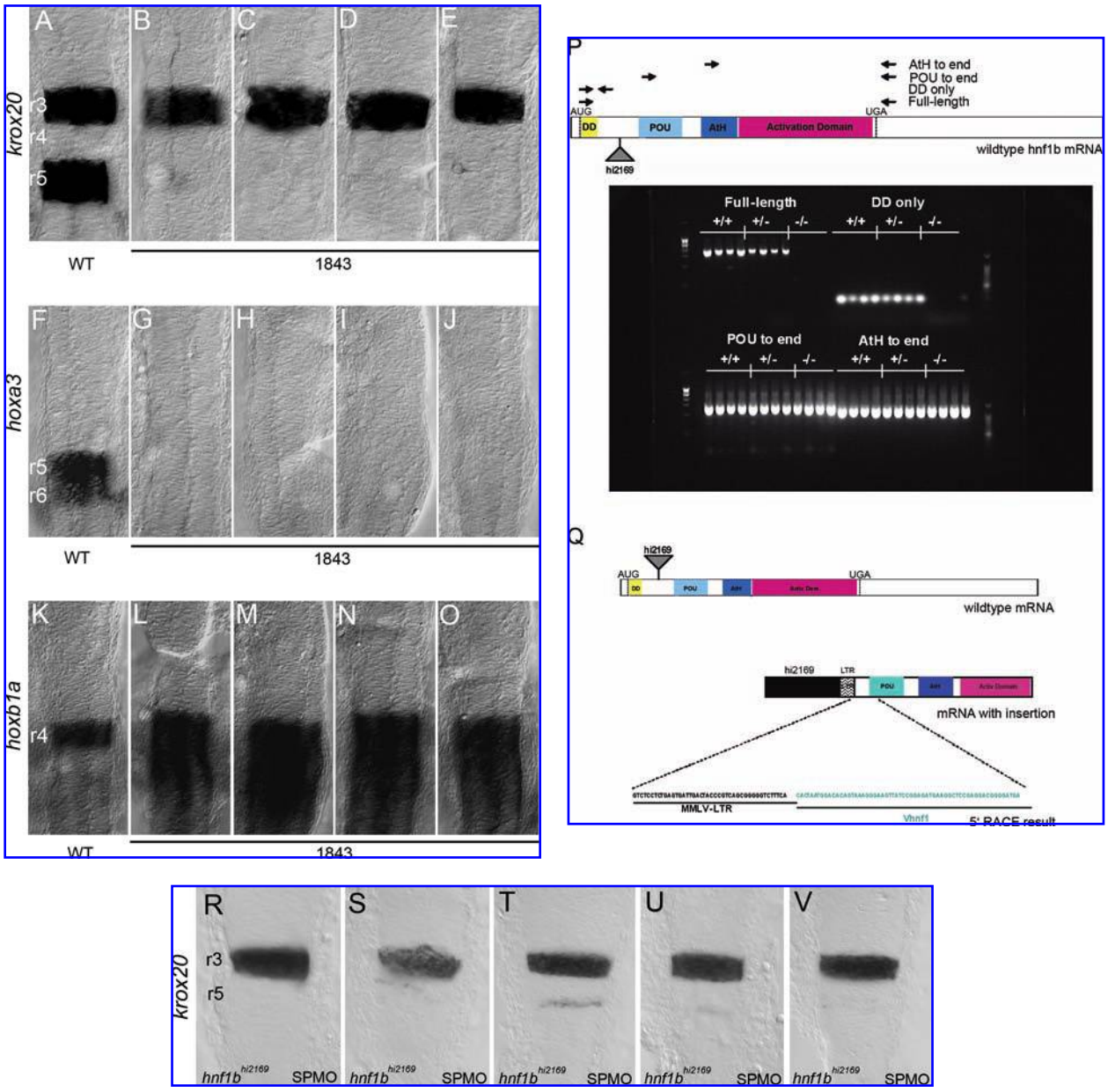


FIG. 3. (**A–O**) Wild-type (**A, F, K**) and *hnf1b*^{1843} (**B–E, G–J, L–O**) embryos were assayed for expression of *krox20* (**A–E**), *hoxa3* (**F–J**), and *hoxb1a* (**K–O**) by *in situ* hybridization. All panels are dorsal views of flat-mounted hindbrains with anterior to the top. Rhombomere numbering is indicated in panels (**A**), (**F**), and (**K**). (**P**) Wild-type (+/+), heterozygous (+/−), and homozygous *hnf1b*^{hi2169} mutant (−/−) embryos were assayed for presence of *hnf1b* transcripts by RT-PCR. Virus integration site and locations of primers used for RT-PCR analysis are indicated in diagram at top. RT-PCR products were resolved by gel electrophoresis, and the resulting gel is shown at the bottom. (**Q**) Primer extension analysis of virus-*hnf1b* transcript. Top panel shows structure of wild-type *hnf1b* mRNA and indicates retroviral insertion site (triangle) in the *hnf1b*^{hi2169} allele. Bottom panel shows putative viral-mRNA fusion transcript and indicates sequence obtained from primer extension analysis. (**R–V**) *hnf1b*^{hi2169} embryos injected with an *hnf1b* splice-blocking morpholino (SPMO) show near-complete loss of *krox20* expression in r5 compared to Figure 1B–E.

display robust uniform expression of *hoxb1a* throughout the caudal hindbrain (Fig. 3L–O), in contrast to *hnf1b*^{hi2169} embryos that sometimes showed reduced expression of *hoxb1a* in the r5/r6 region (Fig. 1). Our results indicate that *hnf1b*^{hi2169} is hypomorphic to *hnf1b*^{hi1843} and suggest that residual *hnf1b* activity persists in the *hnf1b*^{hi2169} allele. Notably, the differences between these alleles also rule out the possibility that the residual r5/r6 gene expression

in *hnf1b*^{hi2169} embryos is due to maternal *hnf1b* mRNA.

To explore the basis of the weaker phenotype of the *hnf1b*^{hi2169} allele, we next examined *hnf1b* transcripts in this mutant. PCR amplification with primers spanning the entire *hnf1b* open reading frame (ORF) did not detect a full-length *hnf1b* transcript in homozygous *hnf1b*^{hi2169} embryos (Fig. 3P), in agreement with previous reports.[4] Primers amplifying the 5′ end of the ORF (upstream of the viral integration site including the dimerization domain; "DD only" in Fig. 3P) also did not yield a product in homozygous *hnf1b*^{hi2169} embryos, suggesting that transcripts originating at the endogenous *hnf1b* promoter may not be stable in *hnf1b*^{hi2169} mutants. In contrast, a series of primer pairs amplifying regions of the *hnf1b* transcript downstream of the integration site yielded fragments encoding the POU domain, the homeodomain, and the activation domain ("POU to end" and "AtH to end" in Fig. 3P). Hence, a partial *hnf1b* transcript is present in *hnf1b*^{hi2169} embryos. Using primer-extension analysis we found that this partial transcript extends at least 43 bp into the viral long terminal repeat (LTR) sequence (Fig. 3Q). Since retroviral LTRs contain promoter activity, it is possible that the transcript originates in the 3′ LTR of the integrated retrovirus. Although we cannot rule out the possibility that the transcript originates at the endogenous *hnf1b* promoter and uses a splice site within the virus to create a fusion transcript, this appears unlikely since the transcript appears to lack the 5′ end of *hnf1b* (Fig. 3P). Since we lack antibodies to zebrafish Hnf1b, we cannot test directly whether this fusion transcript produces a protein. Instead, we generated a splice MO (sMO) that targets the intron 1/exon 2 junction, which is located downstream of the virus integration site in exon 1. The intron 1/exon 2 boundary corresponds to the N-terminal end of the POU domain, and the sMO is therefore expected to interfere with splicing of the fusion transcript and to disrupt translation of any protein produced from the fusion transcript. Accordingly, the sMO reduces the level of the properly spliced transcript by approximately six- to sevenfold (Supplemental Fig. 2, available online at www.liebertpub.com/fpd). We find that the *hnf1b* sMO reduces residual *krox20* expression in r5 of *hnf1b*^{hi2169} embryos (Fig. 3R–V, compare to Fig. 1B–E). We conclude that a fusion transcript in *hnf1b*^{hi2169} embryos is responsible for residual gene expression in r5.

Hoxb1b *induces ectopic r5-r6 gene expression in* hnf1b^{hi2169} *and* hnf1b^{hi1843}, *but not in* val^{b337} *embryos*

We have reported that *hoxb1b* (acting together with its *meis* and *pbx* cofactors) is required for r5/r6 gene expression[16,21,22] and can induce ectopic expression of r5/r6 genes in zebrafish embryos.[23] To determine if *hnf1b* is absolutely required for induction of r5/r6 gene expression, we tested whether *hoxb1b* can induce r5/r6 gene expression in *hnf1b*^{hi2169} or *hnf1b*^{hi1843} embryos. As expected, coinjection of *hoxb1b* and *meis3* mRNA (*pbx* mRNA need not be injected as *pbx2* and *pbx4* are ubiquitously expressed up to 24 hpf[24–26]) induces ectopic expression of r5/r6 genes anterior to the hindbrain in wild-type embryos (Fig. 4A, E). *Hoxb1b/meis3* injection also induces ectopic *hoxa3* expression in homozygous *hnf1b*^{hi2169} (19/19) and homozygous *hnf1b*^{hi1843} (8/9) mutant embryos (Fig. 4B, C), but not in homozygous *val*^{b337} mutant embryos (0/7; Fig. 4D). Similarly, *hoxb1b/meis3* induces ectopic expression of both *val* (Fig. 4E–G) and *krox20* (not shown) in both *hnf1b*^{hi2169} (13/13 for *val* and 10/10 for *krox20*) and *hnf1b*^{hi1843} (11/12 for *val*) mutant embryos. Notably, while *hoxb1b/meis3* can induce ectopic r5/r6 gene expression, it cannot restore normal gene expression to r5/r6 of *hnf1b* mutant embryos. This result indicates that *hnf1b* is required for induction of r5/r6 gene expression in the hindbrain, but not further anteriorly in the embryo. In contrast, *val* is required for induction of r5/r6 gene expression throughout the embryo.

Discussion

Previous work led to the suggestion that *hnf1b* is required for activation of r5/r6 gene expression in the zebrafish hindbrain. However, the *hnf1b*^{hi2169} retroviral insertion allele used in most studies shows residual r5/r6 gene expression (Fig. 1), indicating that this may not represent a null allele, or alternatively, that

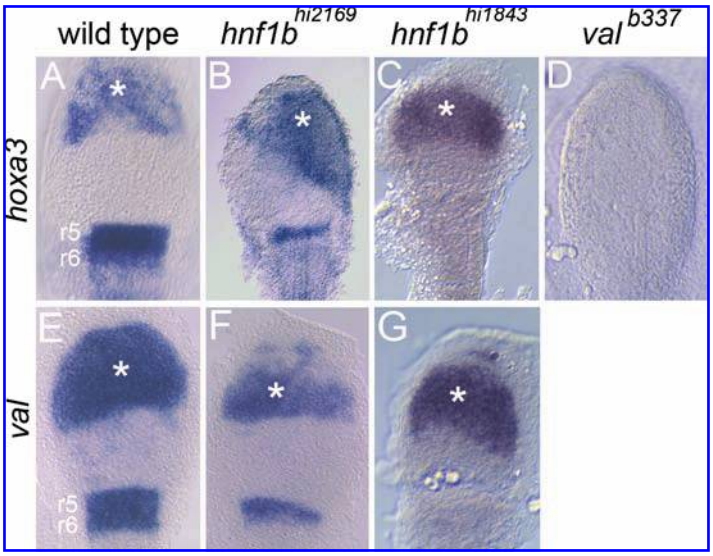


FIG. 4. *hoxb1b* activates r5/r6 gene expression independently of *hnf1b* in the anterior embryo, but not in the hindbrain. Wild-type (**A, E**), $hnf1b^{hi2169}$ (**B, F**), $hnf1b^{hi1843}$ (**C, G**), and val^{b337} (**D**) embryos were injected with *hoxb1b* and *meis3* mRNA and assayed for expression of *hoxa3* (**A–D**) and *val* (**E–G**). Ectopic gene expression anterior to the hindbrain is indicated by asterisks. All panels are flat-mounted dorsal views of the anterior embryo with anterior to the top.

hnf1b is not absolutely required for r5/r6 gene expression. We detect a virus-*hnf1b* fusion transcript in $hnf1b^{hi2169}$ embryos and demonstrate that MO-mediated knockdown of this transcript produces a more severe phenotype with almost complete loss of r5/r6 gene expression (Fig. 3). While we have not detected a protein produced from this transcript, we note that translation would likely be initiated at one of two methionines (positions 120 and 131) at the N-terminal end of the POU domain. In addition, a second retroviral insertion allele ($hnf1b^{hi1843}$) also displays almost complete loss of r5/r6 gene expression (Fig. 3). We conclude that $hnf1b^{2169}$ is not a null allele and that residual r5/r6 gene expression in $hnf1b^{hi2169}$ embryos is likely mediated by a virus-*hnf1b* fusion transcript. Hence, our results are consistent with *hnf1b* being required for r5/r6 gene expression. Surprisingly, we detect a virus-*hnf1b* fusion transcript also in $hnf1b^{1843}$ embryos (not shown), but this transcript may not be fully active since the retrovirus integrated into exon 2 (which encodes the POU homeodomain) in the $hnf1b^{hi1843}$ allele.

Using *hoxb1b*-mediated induction of r5/r6 gene expression as an assay, we also demonstrate that *hnf1b* is not required for ectopic expression of r5/r6 genes in the anterior embryo (Fig. 4). In contrast, *hnf1b* appears to be required for restoration r5/r6 gene expression in the hindbrain (Fig. 4). This result indicates that *hnf1b* plays a unique role in the hindbrain. While it is not completely clear what this role might be, it has been demonstrated that *hnf1b* is required to repress *hoxb1a* expression in the caudal hindbrain.[2–4,16] Since *hoxb1a* is not expressed in the anterior central nervous system (CNS), such a repressive *hnf1b* activity might not be needed outside the hindbrain. An interesting corollary to this hypothesis is that the primary role for *hnf1b* in r5/r6 may not be to activate r5/r6 genes, but to repress expression of genes such as *hoxb1a* that promote competing rhombomere fates. Indeed, there are reports of *hnf1b* proteins acting as repressors.[27]

Val is thought to act downstream of *hnf1b* in the induction of r5/r6 gene expression, and we therefore used the same assay to examine the requirement for *val* in *hoxb1b*-mediated induction of r5/r6 gene expression. In contrast to the situation for *hnf1b*, *val* function is absolutely required for induction of ectopic r5/r6 gene expression (Fig. 4). This finding is consistent with a role for *val* in activation of r5/r6 gene expression and underscores the fact that *vhnf1* and *val* may act by distinct mechanisms to promote r5/r6 gene expression.

Materials and Methods

Zebrafish lines

$Hnf1b^{hi2169}$ and $hnf1b^{hi1843}$ fish were obtained from N. Hopkins and the Zebrafish Resource Center. Val^{b337} fish were obtained from C. Moens.

Plasmid morpholinos and oligonucleotide primers

A full-length *hnf1bl* cDNA clone was purchased from Open Biosystems (Huntsville, AL). For mRNA synthesis, cDNAs were cloned into the pCS2MT vector and mRNA prepared using the mMessage mMachine kit (Ambion, Austin, TX). Translation start site MOs to *hnf1b* (5′-CTAGAGAGGGAAATGCGGTATTGTG-3′) and *hnf1bl* (5′-CTTGGACACCATGTCAGTA AA-3′) as well as an sMO to *hnf1b* (5′-TCCTC CCTGAAAAGATCGGAAACAT-3′) were purchased from Genetools (Philomath, OR)/Open Biosystems. PCR primers for the amplification of *hnf1b* cDNA sequences were as follows: Full length forward 5′-CACAATACCGCATTTCCC TCTCTAG-3′; Full length reverse 5′-GTCACT AAATTGGGCGCCATGTTGATCA-3′; DD reverse 5′-CTTCATCTCCGGATAACTTCCCTTT AC-3′; POU forward 5′-CATGATCAAAGGC TACATGCAGCAGCAC-3′; AtH forward 5′-G CGCCACCATGTTAGACAAAGGAAATCAG-3′. The 5′ RACE was carried out using the SMART RACE kit (Clonetech, Mountain View, CA), primed with oligo 5′-CATCATCACCTGG CTGGACCATACCTT-3′, and amplified with nested primer 5′-GATCTCCCGCTGTTTCCTC ACATACCA-3′. *Hnf1b*^{hi2169} and *hnf1b*^{hi1843} were genotyped by detection of the retroviral insertion in the *hnf1b* gene using primers 5′-CA-CAATACCGCATTTCCCTCTCTAG-3′, 5′-TCC GGATAACTTCCCTTTACTGTG-3′, and 5′-CTGTTCCATCTGTTCCTGAC-3′ for *hnf1b*^{hi2169} and primers 5′-TTCCTATGTAATTGTGTCCG ATGATAG-3′, 5′-CAAGCAGGCTCAATGGCA GC-3′, and 5′-GCTAGCTTGCCAAACCTACA GGT-3′ for *hnf1b*^{hi1843}. *Val*^{b337} fish were genotyped using primers 5′-CCCGCAGACGTTAAG CCTCAC-3′ and 5′-GATCGCGCCGTACTGG TGTT-3′ for PCR amplification followed by digestion with Pvu II. The efficacy of the *hnf1b* sMO was tested with primers PCR1 5′-AC-CGCCAATTCTCAAAGAGCTC-3′, PCR2 5′-GC GACAGGTGCGACTGATTCAG-3′, and PCR3 5′-CAATTGACCGCACTTGCAAAAT-3′.

Microinjections and in situ *hybridizations*

mRNA injections were carried out as reported[16] using 140 ng/μL *hnf1bl*, 166 ng/μL *hoxb1b*, 166 ng/μL *meis3*, 0.1–1 mM *hnf1bl* translation MO, 1 mM *hnf1b* translation MO, and 2 mM *hnf1b* sMO. *In situ* hybridizations were carried out as reported previously.[22]

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Disclosure Statement

No competing financial interests exist.

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Address reprint requests to:
Charles G. Sagerström, Ph.D.
Department of Biochemistry
and Molecular Pharmacology
University of Massachusetts Medical School
364 Plantation St./LRB822
Worcester, MA 01605-2324

E-mail: charles.sagerstrom@umassmed.edu