RESEARCH ARTICLE

Zebrafish *foggy/spt5* Is Required for Migration of Facial Branchiomotor Neurons but Not for Their Survival

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Transcript elongation is a critical step in the production of mature messenger RNAs. Many factors have been identified that are required for transcript elongation, including Spt5. Studies in yeast determined that *spt5* is required for cell viability, and analyses in *Drosophila* indicate Spt5 is localized to sites of active transcription, suggesting it is required generally for transcription. However, the requirement for *spt5* for cell viability in a metazoan organism has not been addressed. We determined that zebrafish *foggy/spt5* is required cell-autonomously for the posterior migration of facial branchiomotor neurons from rhombomere 4 (r4) into r6 and r7 of the hindbrain. These genetic mosaics also give us the unique opportunity to determine whether *spt5* is required for mRNA transcription equivalently at all loci by addressing two processes within the same cell—neuronal migration and cell viability. In a wild-type host, *spt5* null facial branchiomotor neurons survive to at least 5 days postfertilization while failing to migrate posteriorly. This finding indicates that *spt5*-dependent transcript elongation is required cell-autonomously for a complex cell migration but not for the survival of these same cells. This work provides evidence that transcript elongation is not a global mechanism equivalently required by all loci and may actually be under more strict developmental regulation. *Developmental Dynamics 234:651–658, 2005.* © 2005 Wiley-Liss, Inc.

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INTRODUCTION

Embryonic development depends on proper transcription and translation of a myriad of genes required for cell viability, morphogenesis, and induction and realization of cell fates. Transcription is a multistep process that involves recruitment of RNA polymerase to a promoter by specific and general initiation factors, escape from the promoter, RNA chain elongation, and termination of the nascent transcript (Lee and Young, 2000). Transcript initiation at individual loci is regulated by factors that either promote or repress transcription of genes, and several genes identified in genetic screens for developmental defects regulate transcript initiation. Although most work has focused on regulation of transcript initiation, it has become clear recently that transcript elongation is another step at which gene expression can be developmentally regulated. Recent work in the zebrafish has shown that the transcript elongation factors *foggy/spt5* and *pandora/spt6* are required for embryo survival, gene expression, and acquisition of particular neuronal cell fates (Guo et al., 2000; Keegan et al., 2002).

Genetic evidence in yeast first indicated that *spt5* plays a role in transcript elongation, and biochemical analysis showed that Spt5 directly binds to the large subunit of RNA polymerase II (pol II) in vitro by means of four KOW homology domains (Hartzog et al., 1998). The regulation of transcript elongation by Spt5 can be either positive or negative, depending on the cellular context. For instance, Spt5 promotes transcript elongation in limiting nucleotide concentrations (Wada et al., 1998) or in response to heat shock stimulation (Andrulis et al., 2000; Keegan et al., 2002; Jennings et al.,

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2004). However, Spt5 is also a member of the DSIF (DRB sensitivity inducing factor) complex that is required for the elongation inhibitory activity of the ATP analogue 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB; Wada et al., 1998; Yamaguchi et al., 1999). Whereas these experimental conditions reveal positive and negative elongation functions for Spt5, the in vivo context of positive vs. negative control is still unknown.

Two in vivo studies in *Drosophila melanogaster* demonstrate that Spt5 colocalizes with actively transcribing phosphorylated RNA polymerase II (Pol II) to hundreds of sites on polytene chromosomes (Andrulis et al., 2000; Kaplan et al., 2000). This finding has been considered evidence that Spt5 has a general positive effect on virtually all active transcription; however, the negative elongation effect appears to be less general. The first allele of *foggy*/*spt5* to be described in the zebrafish, m806, a point mutant in the C terminus of the protein, abolishes the negative effect on elongation in vitro while leaving its positive function intact (Guo et al., 2000). *foggy*/*spt5*^{m806} mutant embryos have a specific reduction in dopamine-secreting neurons in the hypothalamus and related neurons in the eye and a corresponding increase in the number of serotonin-producing neurons in the hypothalamus. Recently, a point mutation in the *Drosophila spt5* that primarily affects the negative transcript elongation function revealed a specific maternal requirement for *spt5* to repress transcription of the pair-rule genes *even-skipped* and *runt* (Jennings et al., 2004).

The zebrafish *foggy*/*spt5* null mutant exhibits a combination of specific phenotypes, including heart defects and several neuronal defects (Keegan et al., 2002; this work). If *spt5* is required equivalently at all loci, then one would expect the null phenotype, which reflects loss of positive as well as negative transcript elongation, to have severe and potentially early embryonic defects. The null phenotype has led to the important question of how loss of a general transcript elongation factor can lead to specific phenotypes. One possibility is that *spt5* is required for elongation at many or all loci; the specific phenotypes in particular tissues of zygotic *spt5* mutants may be due to different rates of cell proliferation or to varying rates of *spt5* mRNA and/or protein turnover in different tissues. *spt5* mRNA is expressed maternally, and by this model the tissues most affected in *spt5*$^{-/-}$ embryos would be those that deplete this maternal expression the fastest. Cells lacking *spt5* function would be predicted to die from lack of transcription at loci required to maintain cellular homeostasis. A second possibility is that the requirement for the positive effect of *spt5* on elongation is not general but is preferentially required by specific loci and specific developmental contexts. By this second hypothesis, one might expect to identify cells that require *spt5* function for distinct aspects of their development but not for their survival.

We identified an allele of *foggy*/*spt5*, *fh20*, in a genetic screen to identify genes that control the posterior migration of facial branchiomotor neurons in the zebrafish hindbrain. In wild-type zebrafish embryos, facial branchiomotor neurons are born in rhombomere 4 (r4) of the developing hindbrain and subsequently migrate caudally to reside in r6 and r7. Several genes have been identified that are required for this migration to take place, some of which regulate transcription of unknown target genes (reviewed in Chandrasekhar, 2004). The identification of facial branchiomotor neuron migration defects in *foggy*/*spt5* mutant embryos allowed us to test the hypothesis that *spt5* is not required equivalently for production of all transcripts by addressing two processes within a single cell type. Using mosaic analysis, we show that the positive effect of *foggy*/*spt5* on elongation is required cell-autonomously for facial branchiomotor neurons to migrate posteriorly from r4 but is not required within these same cells for their survival. These data suggest that *foggy*/*spt5* is not required equivalently for production of all mRNA transcripts within a cell and provides further evidence that transcript elongation by means of *spt5* is a step at which control of gene expression may be developmentally regulated.

RESULTS

fh20 Is Required for Facial Branchiomotor Neuron Migration and Exhibits Pleiotropic Defects

We identified the *fh20* mutant in a genetic screen for defects in facial branchiomotor neuron migration in the isl1–green fluorescent protein (GFP) transgenic line that expresses GFP in a subset of cranial neurons (Higashijima et al., 2000). *fh20* mutant embryos display a pleiotropic morphological phenotype by 48 hours postfertilization (hpf), which includes developmental delay first evident at approximately 26 hpf, severely reduced pigmentation, and a poorly differentiated heart tube accompanied by pericardial edema (Fig. 1B). The somites of *fh20* mutant embryos initially form correctly, but subsequently degenerate between 36 and 48 hpf. By 48 hpf, TUNEL staining revealed widespread neural cell death in *fh20*$^{-/-}$ embryos compared with wild-type (Fig. 1C,D). *fh20*$^{-/-}$ embryos die by 4 days postfertilization (dpf), whereas heterozygous embryos have no detectable morphological phenotype (data not shown).

Facial branchiomotor neurons in wild-type isl1-GFP transgenic embryos migrate posteriorly from r4 to establish clusters in r6 and r7, leaving behind them axons that exit from lateral r4 to extend into the second branchial arch and innervate jaw support muscles (Higashijima et al., 2000; Fig. 1E, asterisk). In *fh20* mutant embryos, facial branchiomotor neurons fail to migrate from r4, instead forming a characteristic "footprint" of facial branchiomotor neuron cell bodies in r4 and anterior r5 (Fig. 1F, asterisk). The facial branchiomotor axons collect at lateral r4, where they exit the hindbrain correctly but fail to innervate their targets, presumably due to an absence of second arch-derived cartilages and muscles (data not shown).

In situ hybridization determined that expression of the genes *dlx2*, *eng3*, *hoxb1a*, *hoxb4*, *EphA4*, and *myoD* is indistinguishable between wild-type and *fh20* mutant embryos, suggesting that establishment of regional specification is unaffected. Ad-

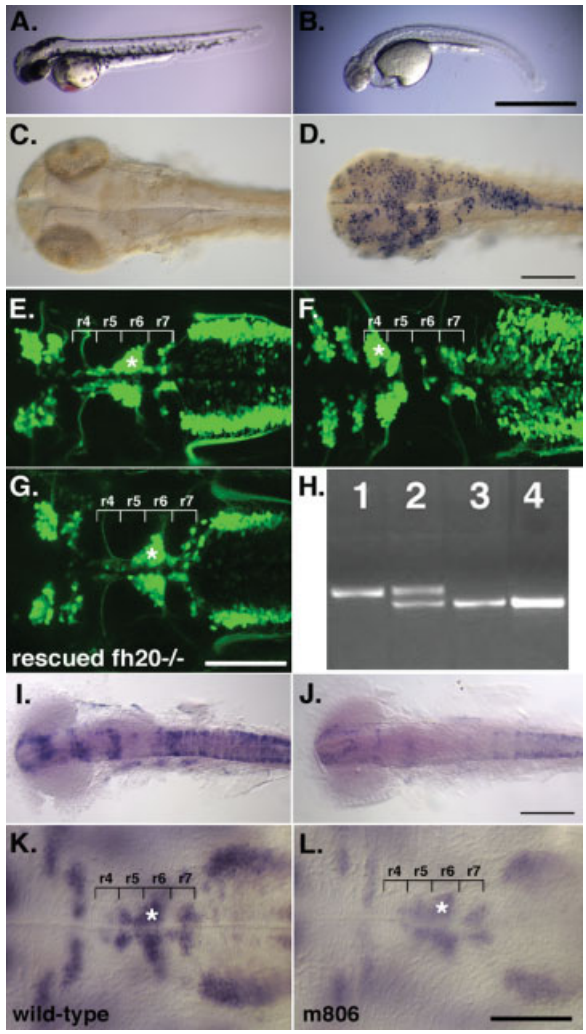

Fig. 1. **A:** *fh20*$^{-/-}$ mutant embryos exhibit multiple defects, including failure of facial branchiomotor neuron migration. Anterior is to the left in all panels. **B:** The morphological phenotype of *fh20*$^{-/-}$ embryos includes developmental delay and failure of tail elongation, absence of pigment formation, failure of the ventral retina to close, heart tube hypotrophy accompanied by pericardial edema, and degeneration of somites. **C,D:** The mutation is lethal at approximately 4 days postfertilization (dpf). Terminal deoxynucleotidyl transferase–mediated deoxyuridinetriphosphate nick end-labeling (TUNEL) labeling indicates that apoptotic cell death is increased in *fh20*$^{-/-}$ (D) compared with wild-type (C). **E,F:** Facial branchiomotor neurons (asterisks) fail to migrate posteriorly from r4 in isl1-GFP transgenic *fh20*$^{-/-}$ embryos (F) compared with wild-type (E). **G:** Full-length wild-type *spt5* mRNA injected into *fh20*$^{-/-}$ embryos rescues facial branchiomotor neuron migration. **H:** Polymerase chain reaction genotypes for an absolutely linked microsatellite marker (30020R9) in (1) homozygous wild-type, (2) *fh20*$^{+/-}$, (3) *fh20*$^{-/-}$, and (4) the rescued *fh20*$^{-/-}$ embryo shown in G. **I,J:** In situ hybridization for *ngn1* expression indicates a decrease in intensity that correlates with the *fh20* mutation: wild-type (I) and *fh20*$^{-/-}$ (J). **K,L:** Facial branchiomotor neurons, identified by *islet1* in situ hybridization, migrate normally in the m806 point mutant of *foggy/spt5* that abolishes the negative effect on transcript elongation. Scale bars = 900 μm in A,B, 100 μm in C–L.

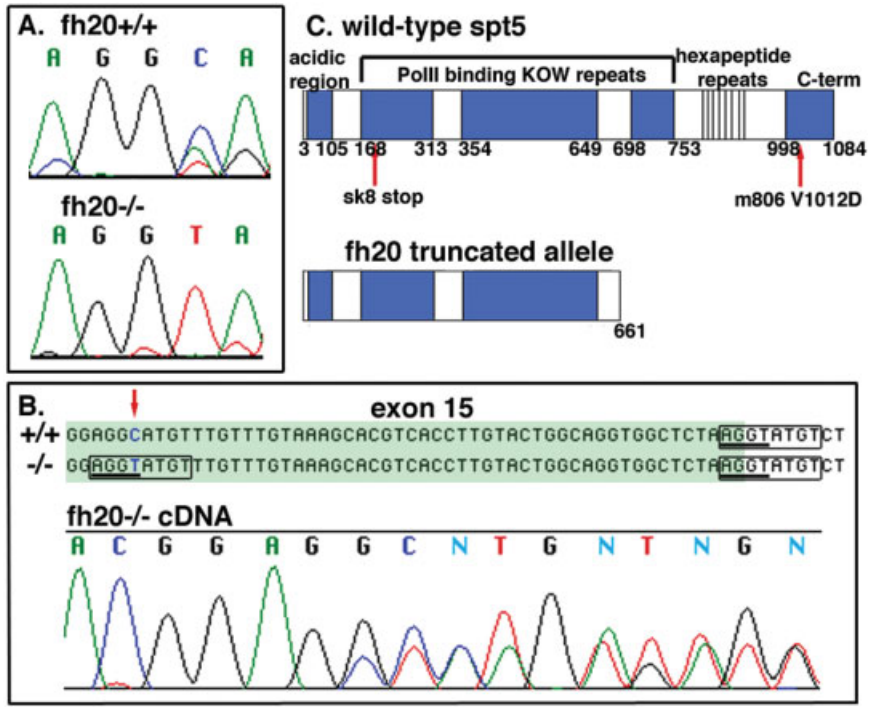

Fig. 2. *fh20* is a hypomorphic cryptic splice allele of *foggy/spt5*. **A:** Genomic sequence analysis indicates the *fh20* allele is a C-T transition corresponding to position 1904 of the *spt5* mRNA. **B:** This base change (red arrow) results in the creation of an AGGT consensus splice donor sequence (underlined), and the eight base region including the change (box in *fh20*) matches eight bases around the endogenous splice donor (boxes in wild-type and *fh20*) for exon 15 (shaded green). Sequence analysis of *fh20*$^{-/-}$ cDNA shows a double trace, indicating that correctly and incorrectly spliced mRNA transcripts are present in the same *fh20* mutant. Thus, the *fh20* allele is a hypomorph. **C:** The mis-spliced mRNA results in a frameshift, and the protein terminates at amino acid (~676), before the fourth KOW RNA pol II binding motif.

ditionally, expression of the neurogenic *delta* genes, *deltaA*, *deltaB*, and *deltaD*, is comparable in mutant and wild-type embryos. However, reduction of *ngn1* mRNA expression in approximately 27% of embryos from an $fh20^{+/-}$ incross (24 of 86) was detected as early as the 8-somite stage, and this difference was maintained as late as the 18-somite stage (Fig. 1I,J). When these embryos were genotyped for the tightly linked marker 30020R9, we found that 19 of 24 faintly expressing embryos were genotypically $fh20^{-/-}$, whereas only 4 of 62 darkly expressing embryos were $fh20^{-/-}$, indicating a strong correlation between *ngn1* expression intensity and genotype ($P < 0.001$). However, no neurogenic phenotype was apparent in $fh20^{-/-}$ embryos.

fh20 Is a Hypomorphic Splice Allele of Zebrafish *foggy/spt5*

We placed *fh20* on linkage group 15 between microsatellites z20993 and z7871, an interval that includes the transcript elongation factor *foggy/spt5*. We tested whether *fh20* is an allele of *foggy/spt5* by complementation to the *sk8* null allele of *spt5* (Keegan et al., 2002) and rescue with wild-type *spt5* mRNA. Crosses of $fh20^{+/-}$ to $sk8^{+/-}$ fish yielded 23 phenotypic mutants of 97 embryos (23.7%), showing the two mutants fail to complement, and *fh20* is likely an allele of *foggy/spt5* (data not shown). We generated full-length *spt5* mRNA for microinjection into one-cell stage embryos from an $fh20^{+/-}$;isl1-GFP intercross to address whether wild-type *spt5* mRNA can rescue *fh20* mutant embryos. Seventeen percent (8 of 47) of phenotypically wild-type embryos (Fig. 1G) were genotypically $fh20^{-/-}$ as determined by polymerase chain reaction (PCR) amplification of a tightly linked microsatellite marker (Fig. 1H), indicating wild-type *spt5* mRNA rescues the *fh20* phenotype.

The protein structure of *spt5* consists of an amino terminal acidic region, four conserved KOW Pol II binding repeats, and a carboxy-terminal hexapeptide repeat region (Ivanov et al., 2000). Genomic sequence from wild-type and *fh20* mutant products identified a single base transition from C to T (Fig. 2A) that creates a conserved AGGT splice donor sequence and makes the eight nucleotide region around the change identical to the endogenous exon 15 splice donor, which appears 50 bp downstream. Homozygotes express both correctly and incorrectly spliced products, as evidenced by a double trace from directly sequenced cDNA, indicating that the *fh20* allele is a hypomorphic splice mutation that causes premature splicing from the middle of exon 15 to the splice acceptor of exon 16 (Fig. 2B). Thus, the close proximity of the two splice donor sites leads to a hypomorphic mix of correctly and incorrectly spliced mRNA and a hypomorphic phenotype. The single base transition in a correctly spliced mRNA is a silent mutation, but the incorrectly spliced mRNA results in a frameshift that subsequently truncates the protein at an in-frame stop codon after amino acid 661, N-terminal to the fourth RNA Pol II binding repeat (Fig. 2C).

The *fh20* allele of *foggy/spt5* is expected to affect both the positive and negative aspects of transcript elongation, because it is truncated in the region required for RNA Pol II binding, and the phenotype closely resembles that of the null allele. To determine whether the facial branchiomotor neuron migration defects observed in *fh20* mutants are primarily due to loss of positive or negative regulation, we examined facial branchiomotor neurons in the hypomorphic $foggy/spt5^{m806}$ allele that is defective in the negative aspect of transcript elongation (Guo et al., 2000). Facial branchiomotor neurons in $foggy/spt5^{m806}$ mutant embryos, detected by in situ hybridization for *islet1* mRNA, migrate normally into r6 and r7 (Fig. 1K,L), indicating that facial branchiomotor neuron migration is primarily dependent on the positive transcript elongation function of *spt5*.

foggy/spt5 Is Required Cell-Autonomously for Facial Branchiomotor Neuron Migration but Not for Neuron Survival

foggy/spt5 is expressed ubiquitously in the developing embryo and is presumably required for the elongation of several mRNA transcripts. To address whether *spt5* is required cell-autonomously within facial branchiomotor neurons or in the environment of these cells as they migrate posteriorly through the hindbrain, we made genetic mosaics at gastrula stage (Fig. 3A) and analyzed the transplant hosts by confocal microscopy at 48 hpf. In these mosaics, donor-derived cells are red, and donor-derived motor neurons, which also express GFP, are yellow. Wild-type facial branchiomotor neurons placed in a wild-type host migrate normally through the hindbrain to take up residence in r6 and r7 (Fig. 3B; n = 14 of 14). Facial branchiomotor neurons from a wild-type donor placed in a $foggy/spt5^{fh20}$ mutant host migrate further posteriorly than do $foggy/spt5^{fh20}$ cells (Fig. 3C; n = 14 of 20), although less completely than in wild-type to wild-type control mosaics. This partial non–cell-autonomous effect is likely due in part to the community effect we described previously where facial branchiomotor neurons migrate more completely in larger numbers than they do as individuals (Cooper et al., 2003). In contrast, $foggy/spt5^{fh20}$ mutant facial branchiomotor neurons placed in a wild-type host fail to leave r4 but project axons into the periphery and look otherwise normal (Fig. 3D; n = 11 of 11). These data suggest that *spt5* is required primarily cell-autonomously for facial branchiomotor neuron migration.

Because $foggy/spt5^{fh20}$ mutant embryos die by 4 dpf and exhibit widespread neuronal cell death by 48 hpf (Fig. 1D), it seemed possible that the cell-autonomous migration defect might reflect a role for *spt5* in facial branchiomotor neuron survival, which would be expected to affect the neurons' ability to migrate. We analyzed hindbrains of wild-type hosts containing $foggy/spt5^{fh20}$ mutant donor-derived facial branchiomotor neurons at 5 dpf and observed that not only did $foggy/spt5^{fh20}$ motor neurons survive in wild-type hosts but they also remain in r4 4 days after their wild-type counterparts have migrated posteriorly (Fig. 4B; n = 12 of 13). Thus, the cell-autonomous effect on facial branchiomotor neuron migration revealed by the hypomorphic $foggy/spt5^{fh20}$ al-

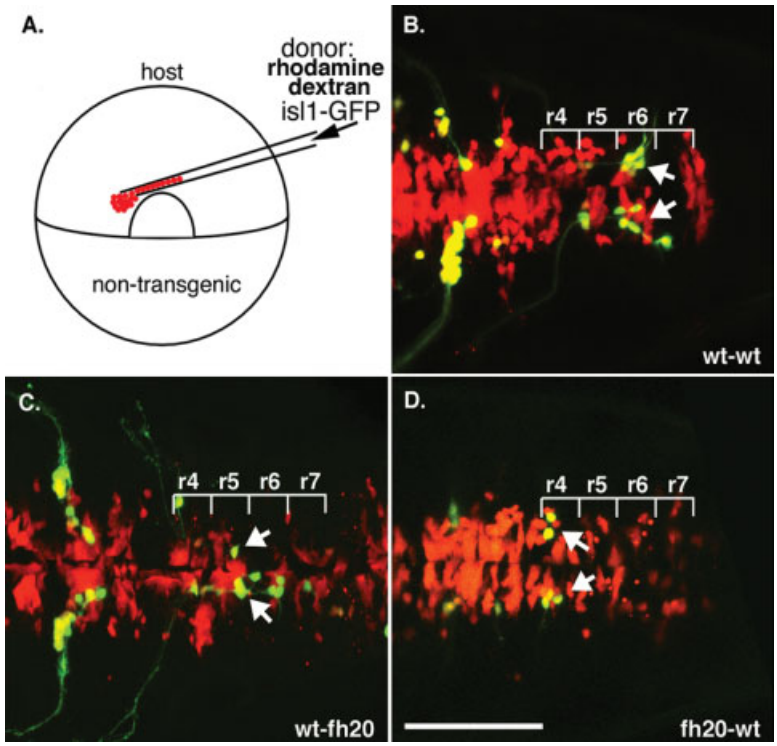

Fig. 3. *foggy/spt5* is required cell-autonomously for facial branchiomotor neuron migration. **A:** Genetic mosaics were generated by transplanting cells from rhodamine dextran-labeled isl1–green fluorescent protein (GFP) transgenic donor cells to unlabeled hosts at gastrula stage (6 hours postfertilization [hpf]), and confocal images were collected at 36 hpf. Donor-derived cells are red, and donor derived motor neurons, which express GFP, are yellow. **B:** Wild-type facial motor neurons (arrow) migrate posteriorly in a wild-type host; n = 14 of 14. **C:** Wild-type facial motor neurons (arrows) also migrate posteriorly from rhombomere 4 (r4) in a *foggy/spt5*^{fh20} mutant host, although to a lesser extent than in wild-type to wild-type control mosaics; n = 14 of 20. **D:** *foggy/spt5*^{fh20} facial motor neurons (arrow) fail to migrate from r4 in a wild-type host; n = 11 of 11. Scale bar = 100 μm.

lele does not reflect a delay to migration or an effect on cell survival.

Because the *fh20* allele is hypomorphic, it remained possible that *spt5* is in fact required for cell survival but that genes required for survival are less sensitive to loss of *spt5*-dependent transcription than are genes required for facial branchiomotor neurons to migrate out of r4. To distinguish these hypotheses, we used the null *sk8* allele of *foggy/spt5* (Keegan et al., 2002) to perform genetic mosaic experiments similar to those performed using the *fh20* allele. Facial branchiomotor neurons in 48 hpf *foggy/spt5*^{sk8} mutant embryos fail to migrate posteriorly from r4 (Fig. 4C) and appear indistinguishable from the hypomorphic *fh20* facial branchiomotor neuron phenotype (Fig. 1F). *foggy/spt5*^{sk8} null cells were placed in wild-type hosts, and the host embryos were raised to 5 dpf. Confocal imaging of dissected fixed host brains revealed that *foggy/*

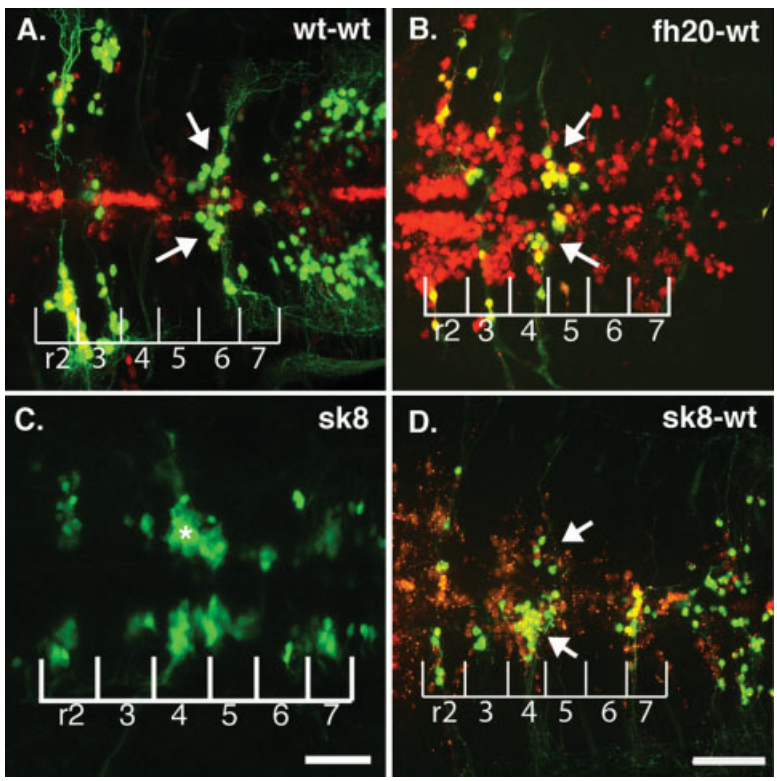

Fig. 4. *spt5* is not required for facial branchiomotor neuron survival to at least 5 days postfertilization (dpf). Wild-type transplant hosts containing wild-type donor cells (red) and donor-derived motor neurons (yellow) at 5 dpf. **A:** Wild-type facial motor neurons (arrow) in a wild-type host migrate posteriorly, differentiate, and extend normal axonal processes from rhombomere 4 (r4). **B:** Hypomorphic *foggy/spt5*^{fh20} facial motor neurons (arrow) placed in a wild-type host fail to migrate but are still detectable by green fluorescent protein (GFP) expression and have normal morphology with axons extending from r4 at 5 dpf; n = 12 of 13. **C:** The isl1-GFP motor neuron phenotype of the *foggy/spt5*^{sk8} null allele at 48 hours postfertilization (hpf) demonstrates that facial motor neurons fail to migrate posteriorly from r4 (asterisk). **D:** *foggy/spt5*^{sk8} null facial motor neurons (arrow) placed in a wild-type host fail to migrate normally but have axons extending from r4 at 5 dpf; n = 8 of 8. Scale bars = 40 μm in D (applies to A,B,D).

spt5^{sk8} null facial branchiomotor neurons persist in the hindbrain and have axons that extend into the periphery (Fig. 4D; n = 8 of 8). The presence of *foggy*/*spt5* null facial branchiomotor neurons with normal morphology in r4 suggests that *spt5* is required for neuronal migration but not for survival of these same cells at least until 5 dpf.

DISCUSSION

Previous work has suggested that *spt5* is required broadly for transcript elongation in yeast and *Drosophila*. Coimmunofluorescence in *Drosophila* showed that Spt5 colocalizes at sites of elongating phosphorylated Pol II on polytene chromosomes and that Spt5 is recruited to heat shock genes after heat shock (Andrulis et al., 2000; Kaplan et al., 2000). While this evidence has been used to suggest that Spt5 is required generally for transcript elongation, no loss of function study in *Drosophila*, or any other metazoan has provided evidence that *spt5* is indeed required equivalently for all mRNA production. Zebrafish *foggy*/*spt5* mutant embryos have multiple defects but survive until 4 dpf. One explanation for the pleiotropy of the *foggy*/*spt5* mutant phenotype is maternal perdurance and the nonequivalent loss of maternal *spt5* in different tissues. Maternal *spt5* mRNA persists until 10 hpf (Keegan et al., 2002), and the stability of *spt5* protein has not been determined. Whereas this is a plausible explanation for certain aspects of the *foggy*/*spt5* phenotype, it fails to explain the effects that we observe within individual cells—that *spt5* is required for a complex migration behavior evident at 2 dpf but is not required for the survival of these same cells until at least 5 dpf. The cell-autonomous failure of migration suggests that there is a deficit in the production of *spt5*-dependent transcripts, but the viability of these cells suggests that transcripts required cell-autonomously for facial branchiomotor neuron cell viability are *spt5*-independent until at least 5 dpf.

It remains plausible that, rather than affecting specific transcripts required for neuronal migration, *spt5* increases the overall efficiency of transcript elongation and that neuronal migration is more sensitive to this effect than neuronal survival. In addition to *spt5*, the genes *spt4* and *spt6* also code for transcript elongation factors (Hartzog et al., 1998). The zebrafish mutant or morpholino phenotype of *spt4* is unknown, but the *spt6*$^{-/-}$ morphological phenotype closely resembles that of *spt5* mutants (Keegan et al., 2002). The observation that loss of function of *spt5* and *spt6* together results in morphological and heat shock induction defects more severe than loss of either single gene suggests neither alone is a complete loss of transcript elongation. This finding is not likely due to a simple functional redundancy, because *spt5* mRNA does not rescue the *spt6* mutant phenotype, nor does *spt6* mRNA rescue loss of *spt5* (Keegan et al., 2002). It is also important to note that *spt6* has additional chromatin remodeling characteristics distinct from the function of *spt5* that might account for its enhancement of the *spt5*$^{-/-}$ phenotype (Bortvin and Winston, 1996).

Recent work suggested that the negative elongation effect of *spt5* is required at distinct loci (Guo et al., 2000; Jennings et al., 2004), possibly in a manner specific to *cis*-acting regulatory features rather than transcript characteristics such as length or GC content (Jennings et al., 2004). Although specific targets of Spt5 in zebrafish have not been identified, our genetic mosaic results looking at migration vs. survival of facial branchiomotor neurons suggest that the positive effect on transcript elongation is also more specific than previously thought. Several genes have been identified that are required cell-autonomously for facial branchiomotor neuron migration in vertebrates and might be good candidates for Spt5 targets. These genes include the transcription factors *hoxb1* (Studer et al., 1996; Goddard et al., 1996; McClintock et al., 2002; Cooper et al., 2003; Arenkiel et al., 2004), *pbx4* (Cooper et al., 2003), *nkx6.1* (Muller et al., 2003), and *ebf1* (Garel et al., 2000). Of these, only the functions of *hoxb1a* and *pbx4* have been shown to be required for facial branchiomotor neuron migration in zebrafish (Cooper et al., 2003). In situ hybridization to investigate *hoxb1a* expression showed no difference between wild-type and *foggy*/*spt5* mutants (data not shown), although the broad expression of *hoxb1a* throughout r4 precludes the ability to investigate expression specifically within facial branchiomotor neurons.

Holstege et al. (1998) showed that not all transcriptional machinery components are required equivalently for the production of mRNAs. A comparison of microarray expression data between yeast mutants for various transcription initiation components revealed that most are general in that they are required for the production of an equivalent set of transcripts that also require RNA polymerase II. Surprisingly, however, several transcriptional components were required for the expression of only a relatively small subset of these genes. For instance, several pheromone-responsive genes depend on the mediator component Srb5, and the cyclin-dependent kinase Srb10 appears to repress genes regulating nutrient starvation response. Our results are consistent with a model in which transcript elongation components function with some degree of specificity on downstream genes. A similar microarray comparison in either *Drosophila* or zebrafish might uncover a surprisingly specific requirement for the positive and negative effects of *spt5* on distinct subsets of developmentally regulated genes. Since Spt5 has been seen to localize globally to sites of active transcription (Andrulis et al., 2000; Kaplan et al., 2000), this specificity could be conferred by biochemical modifications to the hexapeptide repeat region or physical interactions with Spt5 that regulate its activity rather than subcellular localization. Further biochemical and cell biological analyses of *spt5* will likely reveal the mechanisms by which transcriptional components can exert specific regulation over gene expression.

EXPERIMENTAL PROCEDURES

Fish Husbandry

Zebrafish and embryos were raised at 28.5°C and staged as previously described (Kimmel et al., 1995).

Genetic Screen, Linkage Analysis, and Cloning

The *fh20* allele was identified in a forward genetic screen of the isl1-GFP transgenic zebrafish line (Higashijima et al., 2000) by using the early pressure (EP) method (Johnson et al., 1995). To map the *fh20* mutation, heterozygous females were crossed to wild-type males from the polymorphic Wik strain, and embryos were raised to adulthood. Linkage mapping was initiated in pools of 24 wild-type and *fh20*$^{-/-}$ gynogenetic diploid embryos generated by EP; this strategy placed *fh20* on chromosome 15. Further fine mapping using diploid mutant embryos localized *fh20* to a 0.4 cM region between z20993 (4 recombinants/2334 meioses) and z7871 (2/682) containing the *spt5* candidate gene.

Two overlapping fragments of *spt5* cDNA (1.7-kb 5′ and 2.2-kb 3′) were amplified from wild-type and *fh20* mutant embryos by reverse transcriptase-PCR. Direct sequencing of gel-purified PCR products was performed in both directions.

Embryo Rescue and Complementation Analysis

Full-length *spt5* mRNA for injections was generated using the SP6 mMessage mMachine kit (Keegan et al., 2002) following manufacturers' instructions (Ambion). Injections were performed into dechorionated one- to two-cell stage embryos of an *fh20*$^{+/-}$; isl1-GFP intercross. Embryos were raised to 48 hpf and sorted based on the extent of facial branchiomotor neuron migration. These embryos were subsequently lysed, and DNA was used to genotype individual embryos by PCR amplification of a microsatellite marker that is tightly linked to the *fh20* locus (0/3677). The microsatellite was identified from sequencing data produced by the Zebrafish Sequencing Group at the Sanger Institute and annotated at the Ensembl Zebrafish Genome Browser (version 16.2.1; 30020R9For 5′-TTTTGTTAGTAGTTGTTTTTGGTC-3′; 30020R9Rev 5′-ATTCTGTCCACACAAATTCTTTAT-3′). We also performed complementation analysis by crossing an *fh20*$^{+/-}$ male to an *sk8*$^{+/-}$ female.

Genetic Mosaics

Embryos from either an *fh20*$^{+/-}$;isl1-GFP$^{tg/+}$ or an *sk8*$^{+/-}$;isl1-GFP$^{tg/+}$ intercross were used for donors and hosts to generate genetic mosaics. Donor embryos were injected with 2.5% lysinated rhodamine dextran (10-kDa Fluor-Ruby, Molecular Probes) in 0.2 M KCl. Transplants were performed at shield stage (6 hpf) as in Moens and Fritz (1999). All confocal analysis was performed as in Cooper et al. (2003). The image of the *sk8* to wild-type transplant host in Figure 4D had faint GFP expression. Enhancement of the GFP channel caused bleed-through into the rhodamine channel resulting in yellow–red donor cells.

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