Transvection Arising from Transgene Interactions in Zebrafish

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Abstract

There has been a rapid expansion in use of transgenic technologies in zebrafish. We report a novel example of transinteractions of genetic elements, or transvection. This interaction led to a novel expression pattern and illustrates a precautionary example regarding use of transgenes in zebrafish.

Keywords: transvection, zebrafish, transgenic, UAS, Gal4

RAPID ADVANCES IN THE TECHNOLOGIES for genetic manipulation in zebrafish have led to an extensive array of transgenic lines. Recently we encountered a novel phenomenon that demonstrates a precautionary principle regarding use of these approaches.

We generated a Tol2-based transgenic line expressing the human inwardly rectifying potassium channel gene *kir2.1* (*KCNJ2*), Tg(*UAS:hkir2.1-mCherry*). *kir2.1* has been used in prior studies including in zebrafish to hyperpolarize and silence neurons. Interestingly, when we crossed this line to a double transgenic line we have used previously, the triple-transgenic Tg(*foxP2.A.2:Gal4*), Tg(*UAS:GFP*), and Tg(*UAS:kir2.1-mCherry*) embryos had GFP expression throughout the brain and not just in the expected subset of *foxP2* neurons (Fig. 1A–D) (“green brain” phenotype). There was no expansion of endogenous *foxP2* expression (*in situ* data, not shown). Intriguingly, *UAS:GFP* and *UAS:kir2.1-mCherry* expression did not match (Fig. 1B–D); neither transgene’s expression matched the normal expression pattern of *foxP2.A.2*.¹ This was unexpected, given that both *UAS:GFP* and *UAS:kir2.1-mCherry* transgenes share the same *UAS* enhancer and minimal core promoter (MCP) sequences. Using a *GCaMP5* reporter, inhibition of electrical activity matched the altered expression pattern of *UAS:hkir2.1-mCherry* labeling. For experiments we used the widely used *Gal4-VPI6*_{413–470} and *10x UAS* constructs. Although we did not formally test whether any of the transgenes had more than one insertion, we have used these lines over multiple generations and not noted any variegation of expression, suggesting that they are either single copy; or if multiple copies, are closely linked.

We were unable to recapitulate the green brain using chemical or genetic mechanisms to disrupt neuronal activity,

including glutamate receptor antagonists (AP5, CNQX), a sodium channel inhibitor (MS-222), increased extracellular K⁺, or crossing to tetanus toxin Tg(*UAS:TeTx-CFP*) fish. The potassium channel blocker chloroquine that specifically inhibits *kir2.1* did not suppress the green brain phenotype. We did not observe a similar expansion in GFP labeling using other Gal4 driver lines. However, a Gal4 morpholino prevented the green brain phenotype (Fig. 1F, G). The expansion of GFP segregated with only the joint presence of the *foxP2.A.2:Gal4* and *UAS:kir2.1-mCherry* transgenes, and we could create a “red brain” phenotype in *foxP2.A.2:Gal4*, *UAS:RFP*, and *UAS:kir2.1-mCherry* embryos (Fig. 1E). Outcrosses demonstrated that the transgenes were not at the same or a nearby genomic locus. Thus, the green brain is *Gal4/UAS* dependent, is dependent on two specific transgenes (*foxP2.A.2:Gal4* and *UAS:hkir2.1-mCherry*), but is distinct from the normal expression pattern of *foxP2.A.2*.

This phenomenon is consistent with transvection, the *trans* interaction of genetic elements.² Transvection can involve *cis* and/or *trans* interactions of genetic elements, often between paired (allelic) insertions (Fig. 1H).³ Transvection can occur in *trans* when an enhancer drives expression by binding and activating expression at the paired allele. For example, in *Drosophila*, inserting two transgenes with different enhancers but a shared MCP into the same genomic location, including for *UAS*-driven transgenes, is sufficient to elicit transvection.⁴

Many transgenes in the zebrafish community use the same MCP containing the viral E1b TATA box fused to the carp β -actin 5' UTR. We hypothesize that the green brain phenotype is because of transvection between the *UAS:hKir2.1-mCherry* and *foxP2.A.2:Gal4* transgenes, containing the same MCP, which by chance have inserted into interacting positions

FIG. 1. (A–E) Maximum intensity confocal z-stack projections of whole-mount embryo immunofluorescence; (ob, olfactory bulb, tel, telencephalon, ey, eye, ot, optic tectum). (A) Neurons labeled by *foxP2A.2:Gal4; UAS:GFP* (green) show the expected expression pattern for *foxP2A.2* enhancer. (B–D) *Trans* interaction between *foxP2A.2:Gal4* and *UAS:hkir2.1-mCherry* expands *UAS:GFP* labeling to produce the “green brain” phenotype. Although both *UAS:hkir2.1-mCherry* (red) and *UAS:GFP* (green) transgenes have the same UAS and MCP, they have different expression patterns. (E) A “red brain” phenotype occurs in *foxP2A.2:Gal4; UAS:kir2.1-mCherry* embryos when crossed to *UAS:RFP*. (F, G) Live whole embryos at 24 hpf. *foxP2A.2:Gal4; UAS:hkir2.1-mCherry, UAS:GFP* embryos show green brain phenotype (closed arrowheads) in uninjected sibling controls. In *Gal4* morpholino-injected triple transgenic animals, the green brain phenotype is absent (open arrowheads). (H) Somatic pairing of transgenes causes transvection, where the regulatory region of *gene A* influences transcription in *trans* of *gene B*. The ability of an enhancer to bind a transcriptional activator (MCP) is sufficient to drive transcription in *trans*. Many zebrafish transgenes contain the same MCP sequence. (I) In our model, the *trans* interaction of the UAS enhancer from *UAS:hkir2.1-mCherry* transgene, with the MCP of the *foxP2A.2:Gal4* transgene, expands the expected *Gal4* expression pattern because of genomic pairing of these transgenes. We hypothesize that endogenous *cis*-acting elements near the transgene insertion for *UAS:hkir2.1-mCherry* constrains the UAS enhancer, leading to an unexpected expression pattern for *UAS:hkir2.1-mCherry*. MCP, minimal core promoter.

in the genome. The *UAS:hKir2.1:mCherry* may have inserted into a genomic region where an endogenous *cis*-regulatory element constrains its UAS expression, but leads to *trans*-driven expression of *foxP2A.2:Gal4*, and the subsequent expansion of GFP (or any other UAS-dependent gene) (Fig. 1I). Our findings are an important reminder to include controls for expected transgene expression patterns because of the potential for transvection interactions when using multiple transgenes in zebrafish.

Disclosure Statement

No competing financial interests exist.

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