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Zebrafish transgenic lines co-expressing a hybrid Gal4 activator and eGFP in tissue-restricted patterns

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ABSTRACT

We have used a Tol2-derived trapping vector, carrying a hybrid Gal4 gene and a UAS:eGFP reporter cassette, to identify 16 transgenic zebrafish lines expressing the fluorescent marker eGFP in tissue-restricted patterns during development. Most lines show co-expression of eGFP and a hybrid Gal4 transcription activator containing a truncated VP16 domain that facilitate induction of other UAS-transgenes (*UAS:RFP*). Notably, many of the transgenic lines are expressed in particular areas of the central nervous system, such as the retina. We mapped the genomic positions of most of the activated insertions, and for three retina-specific lines we also demonstrate that eGFP reports the expression of particular endogenous genes. One of the identified zebrafish genes shows expression in ventral retina, and encodes a protein containing a repulsive guidance molecule (RGM) domain, suggesting a role in axonal guidance during optic nerve formation. Among the lines labeling other tissues, three show early co-expression of eGFP and Gal4-VP16 in blood vessels, erythrocytes and other hematopoietic cells. Interestingly, the activated insertion in the erythrocyte line was mapped to a site near the globin cluster on chromosome 3. All the reported lines co-expressing eGFP and the hybrid Gal4 activator may have potential as genetic tools to study developmental processes.

1. Results and discussion

In vivo labeling and genetic manipulation of cells have been established as important tools to study developmental processes. In particular, these techniques have become essential for investigations of neural development and function in the zebrafish model. Following the first demonstration of gene- and enhancer-trapping in zebrafish using transposon or retroviral vectors (Balciunas et al., 2004; Ellingsen et al., 2005; Kawakami et al., 2004; Parinov et al., 2004), more advanced methods have been established by combining the Gal4/UAS system with the use of target genes encoding various fluorescent markers and/or other proteins, such as toxins (Curado et al., 2007; Davison et al., 2007; Distel et al., 2009; Scott, 2009; Scott and Baier, 2009). The Gal4/UAS system is based on the yeast Gal4 transcriptional activator and its ability to bind upstream activator sequences (UAS), and several modifications have made this system more efficient and useful in zebrafish. By fusing the Gal4 DNA binding domain to the transcriptional activator domain of the herpes simplex virus (HSV) VP16, the transactivation potential was strongly improved (Koster and Fraser, 2001). However, partial truncations of the highly potent VP16 activation domain are required to avoid deleterious effects on embryonic development (Asakawa and Kawakami, 2008; Asakawa et al., 2008; Ogura et al., 2009; Scott et al., 2007).

Several vector constructs derived from the medaka fish Tol2 transposon have been successfully used to generate Gal4 gene- and enhancer trap lines (Asakawa and Kawakami, 2008, 2009; Davison et al., 2007; Scott et al., 2007). The majority of these constructs generate Gal4 expressing driver lines that require a second UAS effector line to visualize the expression patterns (Asakawa and Kawakami, 2009; Asakawa et al., 2008; Scott et al., 2007). One important exception is the self-reporting SAGVG vector, which

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contains a modified Gal4-VP16 sequence followed by a UAS:eGFP reporter cassette (Davison et al., 2007). The partial VP16 truncation in the Gal4-VP16 fusion protein generated from this vector is not harmful, and when activated by endogenous enhancers co-expression of eGFP is observed (Davison et al., 2007; Ogura et al., 2009).

We have previously identified and characterized several SAGVG transgenic lines that display cell-type and region specific expression during retina development (Zhao et al., 2009a,b). In this report, we describe 16 SAGVG lines that mainly show co-expression of eGFP and modified Gal4-VP16 in specific neural tissues. The genomic positions of most of the activated insertions were mapped, and for three of the retina-specific lines, we have identified the endogenous genes that are regulated by the same enhancers. Our identification of three transgenic lines co-expressing Gal4-VP16 and eGFP in specific blood tissues may also provide genetic tools to investigate hematopoietic processes.

1.1. General features of transgenic lines generated with the SAGVG construct

We have previously described four retina-specific enhancer trap lines obtained from a screen by using the Tol2-derived SAGVG construct (Zhao et al., 2009a,b), which was originally designed for gene-trapping, but apparently works more efficiently as an enhancer trap vector (Davison et al., 2007). In this report, we describe 16 additional transgenic lines that were identified and characterized from the same screen. When crossed to non-transgenic wild-type fish, the activated insertions were inherited in a Mendelian fashion, and for all lines we observed reproducible expression patterns for more than three generations (data not shown). Hence, the study presented in this report is based on analysis of F3 or later progeny from these crosses. At one day post-fertilization (dpf), the 16 stable transgenic lines show tissue-restricted patterns of eGFP (enhanced green fluorescent protein) expression as presented in the overview panel in Fig. 1 (Figs. S1–S16 show additional detailed images of each line). We selected these 16 lines from a larger group of ~100 tissue-restricted lines, mainly on the basis of their expression in neural tissues. Hence, 12 of the lines display expression in the retina and/or other parts of the central nervous system (CNS) at 1 dpf and later developmental stages (Figs. 1–4). Most of these 'neural' lines also express eGFP in other tissues, such as muscle, notochord, pronephros, pharyngeal arches and pectoral fin buds (Figs. 1, 2 and 4). In addition, we describe four lines showing highly specific expression patterns in muscle, blood and cardiovascular tissues (Figs. 1, 2 and 4).

We mapped the vector insertions associated with the eGFP expression of 13 of the transgenic lines (Table 1; Section 2). Similar to the retina-specific lines described previously (Zhao et al., 2009a,b), the insertion sites of four of the lines were mapped to intergenic regions (Table 1). Hence, we found intronic insertions in nine of the lines. Two of these insertions (*ub14*, *ub17*) were oriented in opposite direction relative to transcription of the genomic host genes, and for two of the other lines (*ub65*, *ub79*) expression patterns were shown to correspond to genes located further away from the insertions (Table 1; Fig. 5). For the five remaining lines with intronic insertions, we cannot exclude the possibility that one or more of these may be genuine gene trap lines. Among the relatively few previously characterized lines with SAGVG insertions, only one line has been shown to result from such gene trapping (Davison et al., 2007).

In the SAGVG construct, the Gal4 DNA-binding domain is fused to an activation domain sequence of HSV VP16 (Koster and Fraser, 2001; Ogura et al., 2009; Scott et al., 2007). The highly potent transactivation domain of VP16, which interacts with several components of the transcriptional machinery, may inhibit transcription and elicit abnormal gene regulation, causing deleterious effects on embryonic development (Gilbert et al., 1993; Ogura et al., 2009). However, the Gal4-VP16 fusion used in the SAGVG construct (Davison et al., 2007) included only a truncated VP16 activation domain (aa 413–470), which is approximately 10-fold less active and not toxic to embryonic development (Barlev et al., 1995; Ogura et al., 2009). Consistent with this, we did not detect any abnormalities in transgenic lines generated with the SAGVG construct.

At 1 dpf, when the zebrafish embryos have already completed early stages of organogenesis and show clear signs of cell differentiation in many tissues, all the lines express eGFP strongly in one or more organ systems (Fig. 1). One example is the *ub44* line, which shows eGFP labeling in the floor plate and pronephric duct at this stage (Fig. 1). Fluorescent cells are visible along the entire lengths of both tissues, and this eGFP expression occurs a few hours after their initial differentiation (Ertzer et al., 2007; Schafer et al., 2007; Wingert and Davidson, 2008). A similar pattern of eGFP expression was observed in *ub44* at later larval stages (Figs. 2 and S7). Notably, the intensity of eGFP labeling of individual cells within the floor plate and pronephric duct is quite variable, and in some cases undetectable (Figs. 1 and 2 and S7). Similar mosaic patterns of eGFP labeling were observed in several of the other transgenic lines, and we have previously reported the same type of variegated expression from SAGVG vector insertions in two retina-specific enhancer trap lines (Zhao et al., 2009a). The same

Table 1
Characterization of stable transgenic SAGVG lines with respect to expression, transactivation and vector insertion sites in the zebrafish genome. The position and orientation of each activated insert relative to the nearest gene are indicated. Abbreviations: CNS, central nervous system; FP, floor plate; NC, notochord; OP, olfactory placode; PF, pectoral fin; PNS, peripheral nervous system; SC, spinal cord.

Line ID	Expression pattern	Transactivation	Genomic position	Relative position and orientation
ub14	Midbrain, muscle	Yes	1:9689438	Intron 5 of trpc5; reverse
ub15	Blood cells, SC neurons	Yes	17:20424318	34.5 kb 3′ of zgc:162183; same
ub17	Blood cells	Yes	3:55922123	Intron 5 of nprl3; reverse
ub28	SC, mesoderm, gills, forebrain	Yes	3:43355477	74.6 kb 3′ of CR762486.1; same
ub38	Eye lens, retina, CNS, gills	Yes	4:21863109	Intron 2 of magi2; same
ub39	Retina, forebrain	No	5:36679168	43.8 kb 3' of tmem174; reverse
ub44	FP, pronephros, SC neurons	No	n.d.	1.6 kb 5′ of zgc:110289; reverse
ub45	retina, CNS, myotomes	Yes	22:39416165	Intron 1 of si:ch211-138h3.1; same
ub61	Retina, rhombomeres, FP, OP, myotomes, PF	Yes	18:25687033	Intron 1 of zgc:154079; same
ub65	Retina, eye lens, midbrain, PF, SC	Yes	11:28912757	Intron 1 of si:dkey-267l16.1; same
ub67	CNS	No	9:38764366	Intron 3 of hsd11b3a; same
ub79	Retina, forebrain, SC, muscle, NC	No	22:19882087	Intron 1 of klf12b; same
ub80	Midbrain, hindbrain, FP, NC, PNS	Yes	9:31767771	Intron 1 of zgc:162298; same
ub95	Blood cells, blood vessels, heart	Yes	14:27810330	
ub121	Rhombomeres, myotomes	Yes	n.d.	
ub148	Myotomes	Yes	n.d.	