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genesis 34:51–57 (2002)

Analysis of Twenty-Four Gal4 Lines in *Drosophila melanogaster*

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Received 5 July 2002; Accepted 9 July 2002

The Gal4/UAS system is a powerful technique that allows the expression of a particular target gene in a tissue-specific manner (Brand and Perrimon, 1993). We isolated twenty-four Gal4 insertions with interesting expression patterns and characterized their chromosome location and whether the insertions were associated with zygotic lethality (Table 1).

The Gal4 enhancer trap insertion that we used is described in Brand and Perrimon (1993). We characterized a number of lines originally generated by G. Technau and by Manseau *et al.* (1997), which are labeled T and C insertions, respectively. We used two stocks: *w; Sco/CyO* and *w; Ly/TM3, Sb*, to establish balanced stocks and determine whether the chromosome that carries the Gal4 insertion was homozygous viable. We characterized the expression patterns in ovaries, testis, embryos, larvae, and imaginal tissues.

To determine the expression pattern of Gal4, we chose to express a membrane tethered green fluorescent protein (GFP) fusion molecule that allows rapid in vivo imaging as well as the analysis of fixed tissue. We crossed each of the Gal4 lines to *UAS-mCD8-GFP* (Lee and Luo, 1999) and analyzed the GFP expression in the progeny using either a Leica TCS-NT confocal microscope or a Zeiss Axiophot 2 fluorescent microscope equipped with a Zeiss Axiocam CCD camera.

Embryos were collected overnight, dechorionated in 50% bleach, and fixed with 10% formaldehyde in PBS for 20 min. A variety of specific expression patterns were found and are summarized in Table 2 (Fig. 1). All Gal4 lines show expression in salivary glands, presumably due to a salivary gland-specific enhancer in the original construct (Brand and Perrimon, 1993).

To analyze the general larval expression pattern, whole late 3rd instar larvae were dissected in Ringer's solution and visualized live using fluorescence microscopy (Fig. 2). For a more detailed analysis of specific larval tissue, we dissected the discs in Ringer's solution and examined them live (Fig. 3). The Gal4 expression patterns in larval brains, larval wing discs, eye discs, leg discs, and whole larvae are summarized in Table 3.

We also determined the Gal4 expression pattern in the male and female reproductive systems. Ovaries from 5-day-old, well-fed female progeny were dissected in PBS and fixed with 4% formaldehyde in PBS-0.1% Triton X-100 for 20 min. All stages of oogenesis were examined for particular Gal4 expression patterns (Fig. 4, Table 2). To analyze the male reproductive system, the reproductive tract was dissected in PBS and visualized live without prior fixation (Fig. 5). GFP expression was noted for any pattern in the male germ line cells and associated somatic lineages within the testis, for the muscle and pigment cells that ensheath the testis, and for the somatic cells of the seminal vesicles, ejaculatory duct, and accessory glands of the reproductive tract (Table 2). In

Table 1
Chromosome Location and Viability for Each Individual Gal4 Line

GAL4 line	Chromosome location	Viability
C135	3	viable
C147	2	viable
C253	2	viable
C289b11	3	viable
C355	X	viable
C564	2	viable
C729	3	viable
C754	X	viable
C784	2	viable
C805	2	lethal
C825	2	viable
C833	3	viable
C855	2	lethal
C855a	3	viable
MJ12a	2	viable
MJ33a	3	viable
MJ49	2	viable
T32	2	lethal
T76	2	lethal
T80	2	ND
T98	2	viable
T100	2	viable
T110	2	viable
T155	3	viable

ND = not determined.

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DOI: 10.1002/gene.10125

Table 2
GFP Expression in Adult Ovaries, Male Reproductive System, and Embryos

GAL4 line	Ovaries	Male reproductive tract	Embryos
C135	squamous FC and PTC, but not in mainbody CC	AC(s), SV strong, ED strong, CC, GC(sc)	ring-shaped subset of proventriculus
C147	NE	AC(s), CC weak	NE
C253	NE	ND	subset of EC st. 12 onwards, some uncharacterized cells st. 16
C289b11	weak CC, but not PTC at st. 10, strong ventral, CT	ND	subset of A cells and subset of PNS from st. 13 onwards
C355	strong ubiquitous expression	NE	NE
C564	NE	AC(s), SV, ED, PC patchy, CC(late) weak, GC(sc)	small number of uncharacterized cells at late stages
C729	stripes at stage 8, CC but not posterior PTC at st. 10	AC(s), PC, CC	NE
C754	NE	NE	NE
C784	some egg chambers at st. 9 and dead egg chambers	CC weak, GC weak	subset of PNS from st. 13, CNS subset from st. 16 onwards
C805	NE	AC(s), PC, CC	NE
C825	strong ubiquitous expression at the beginning of st. 9	AC(s), SV	A at st. 14/15
C833	NE	AC(s), CC, H	subset of PNS from st. 13, CNS subset from st. 16 onwards
C855	NE	AC(s) weak, CC weak, GC(sc) weak	NE
C855a	PTC at st. 9, PTC and dorsal anterior CC st. 10	SV, M	NE
MJ12a	NE	PC	posterior spiracles
MJ33a	NE	PC weak, CC	subset of A at st. 14
MJ49	NE	NE	NE
T32	NE	ND	subset of A from st. 12 onwards
T76	NE	SV strong, ED strong, CC(late) weak, GC(16)	strong ubi., A and EC starting at st 9/10
T80	early stalk cells, after st. 6 in all FC	AC(m) weak, AC(s) strong, ED, PC, CC(late) weak, GC(sc)	weak, ubi. from st. 11 onwards
T98	NE	GC(16) strong, CC(late) weak	PNS subset from st. 13, CNS subset from st. 16 onwards
T100	NE	AC(s), GC(sc) weak	subset of PNS from st. 13, CNS subset from st. 16 onwards
T110	NE	AC, ED, GC(16)	subset of PNS from st. 13, CNS subset from st. 16 onwards
T155	ubiquitous in FC	AC(s), CC(late) strong	NE

Ovaries: FC = follicle cells, PTC = posterior terminal cells, CC = columnar cells, CT = centripetal cells, NE = no expression observed.
Male reproductive tract: AC = accessory glands, (m) = main secretory cells, (s) = secondary secretory cell; SV = seminal vesicle, connects testis to duct; ED = ejaculatory duct; PC = pigment cells, ensheath testis; M = muscle cells, ensheath testis; CC = cyst cells, somatic cells in the testis, (early) = cyst cells in mitotic zone, (late) = cyst cells past mitotic zone; H = hub cells, somatic cells at tip of testis; GC = germ cells, (mz) = mitotic zone, (16) = 16-spermatogonia, (sc) = spermatocytes, NE = no expression observed, ND = not determined.
Embryos: NE = no expression observed, A = amnioserosa, PNS = peripheral nervous system, CNS = central nervous system, EC = epidermal cells, ubi = ubiquitous.

the male germ line, localization of GFP to the cell membrane made it difficult to distinguish GFP contribution from germ cells versus cyst cells.

The detailed characterization of these twenty-four Gal4 lines identified a variety of interesting and specific expression patterns. These lines might be of great use in investigating a variety of relevant developmental processes.

ACKNOWLEDGMENTS

We thank G. Technau, L. Manseau, L. Luo for sending stocks. This work was supported by J. Collins Childs (A. Kiger), NIH (C. Micchelli), HFSP (F. Schöck), Boehringer Ingelheim Fonds (M. Schober) and the Howard Hughes Medical Institute.

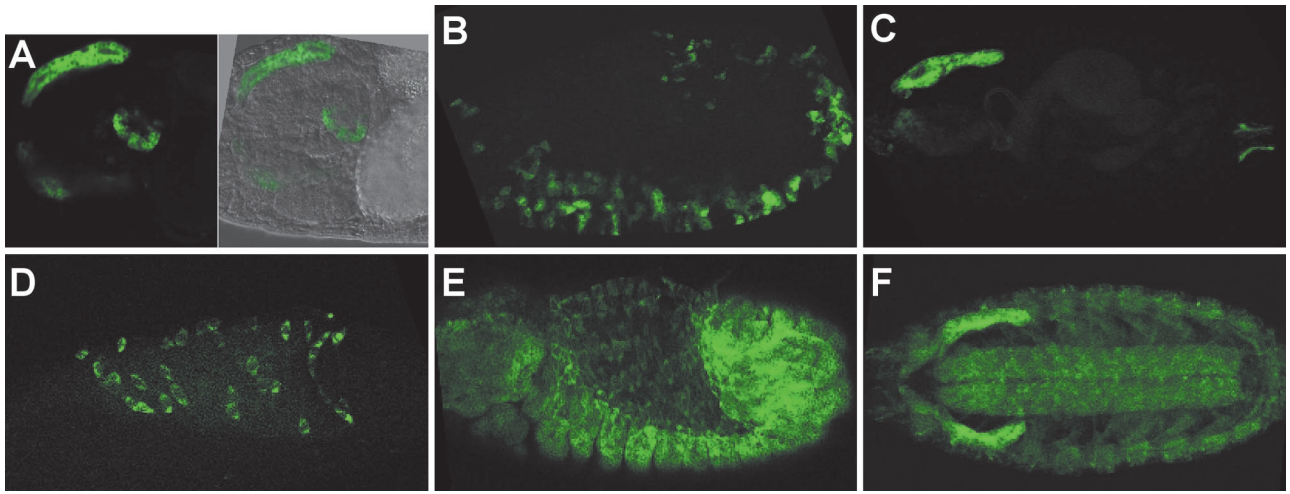

FIG. 1. Embryonic expression of Gal4 lines visualized with *UAS-mCD8-GFP*. **A:** GFP and GFP/DIC overlay of *c135-Gal4* showing expression in a ring-shaped subset of proventriculus cells in a stage 16 embryo. **B:** *c253-Gal4* expression in a subset of epidermal cells in a stage 12 embryo. **C:** *MJ12a-Gal4* expression in the posterior spiracles of a stage 17 embryo. **D:** *T32-Gal4* expression in a subset of amnioserosa cells at embryonic stage 12. **E:** *T76b-Gal4* exhibiting ubiquitous and strong expression in amnioserosa and germ band at embryonic stage 12. **F:** *T80-Gal4* expression in muscles and central nervous system at embryonic stage 16.

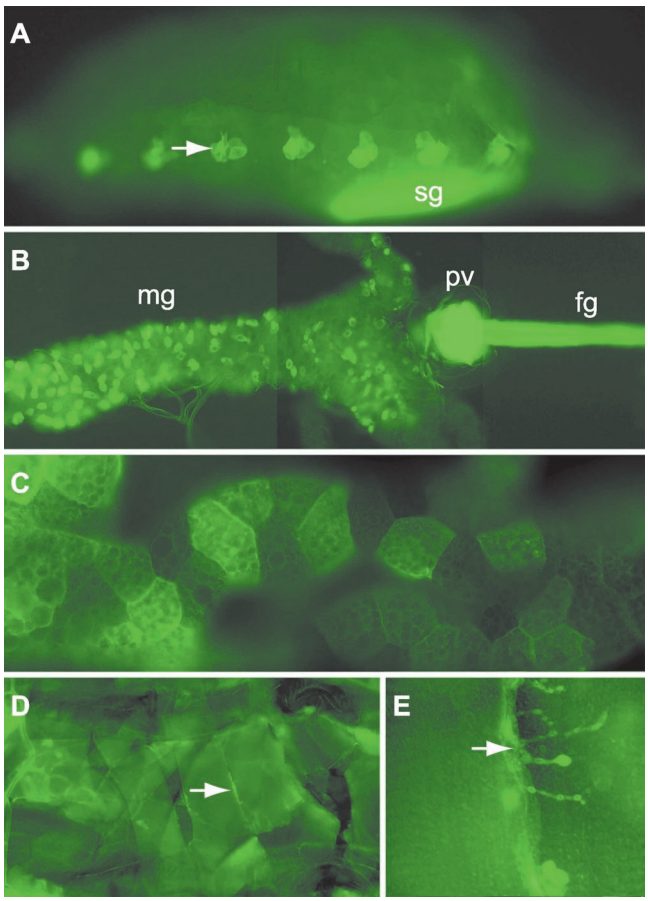

FIG. 2. Expression pattern of Gal4 insertions in unfixed late 3rd instar larval tissues. **A:** *c805* expression can be detected in clusters of cells along the anterior–posterior axis that are closely associated with the larval body wall (arrow). Signal can also be detected in the salivary glands (SG). Anterior is left, dorsal is up. *MJ12a* and *C754* display a pattern similar to *c805* (not shown). **B:** *c805* expression can be detected in the larval gut, including the foregut (FG), proventriculus (PV), and individual cells along the length of the midgut (MG). Anterior is left. **C:** *c564* is expressed ubiquitously in the larval fat body. Fat body cells express the transgene at varying levels. **D:** *T80* is expressed in the somatic muscle. *T80* is expressed at high levels in puncta associated with the muscle (arrow). **E:** High magnification of the preparation seen in **D**. Note the high levels of expression in structures that may be synaptic boutons at the neuromuscular junction (arrow).

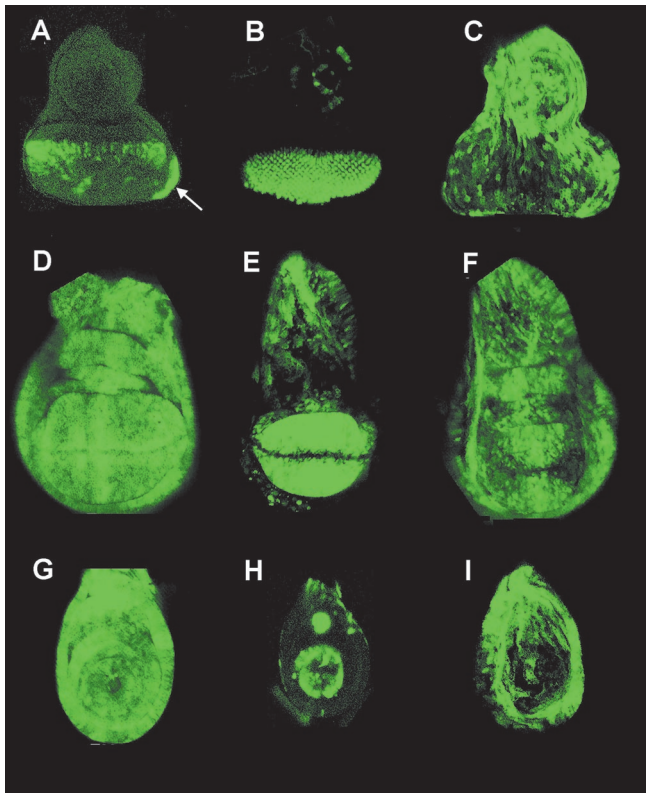

FIG. 3. Imaginal disc expression of Gal4 insertions of the genotypes indicated. **A–C:** Eye-antennal imaginal discs. **A:** *c289b11* drives transgene expression in the morphogenetic furrow and restricted domain of the peripodial epithelium (arrow). **B:** *T76* is expressed posterior to the morphogenetic furrow. **C:** *c855a* is expressed in the peripodial epithelium. **D–F:** Wing imaginal discs. **D:** *T80* drives ubiquitous transgene expression, with heightened levels along the A/P and D/V axes. **E:** *c355* expression is high throughout the wing pouch but not expressed along the presumptive wing margin. **F:** *c855a* is expressed in a central domain of the wing pouch as well as in the medial edge cells which mediate metamorphic interdisc fusion. **G–I:** Leg imaginal discs. **G:** *T80* expression is ubiquitous in the leg disc. **H:** *T76* is expressed in a distal ring and a more dorsal group of cells. **I:** *c855a* is predominantly expressed in peripodial cells in the leg disc.

Table 3
GFP Expression in Larval Brains, Wing Discs, Eye Discs, Leg Discs, and Whole Larvae

GAL4 line	Larval brain	Larval wing disc	Larval eye disc	Larval leg disc	Whole larvae
C135	P	NE	glia?	NE	G, FB
C147	P	NE	NE	NE	SG
C253	P, optic lobe	P in hinge region	NE	NE	ND
C289b11	P	NE	morphogenetic furrow	NE	ND
C355	SP	P, higher in blade	P	ubiquitous	M, FB
C564	P	NE	NE	P	FB, G, SG
C729	NE	NE	NE	NE	SG
C754	P	NE	NE	NE	FB, H?
C784	NE	diffuse P, elevated in pouch	weak, ubiquitous	weak P, ubiquitous	SG
C805	P, ring gland	NE	NE	NE	HB, G, MT, RG, S
C825	NE	NE	NE	NE	NE
C833	SP	diffuse, elevated in pouch	diffuse P throughout disc	diffuse P throughout disc	FB
C855	NE	NE	NE	NE	ND
C855a	P, optic lobe	SP centered on A/P	peripodial membrane	mostly peripodial membrane	ND
MJ12a	P	NE	NE	NE	G, SG, H?
MJ33a	NE	weak P centered on A/P	anterior to furrow	ubiquitous	SG, G
MJ49	SP	NE	Bolwig's nerve, not in disc	NE	SG
T32	SP	elevated in blade/hinge	NE	NE	ND
T76	SP	NE	behind furrow/ photoreceptors	proximal ring, P	T, FB
T80	P	ubiquitous, elevated vein/intervein	ubiquitous, weak P	ND	SG, M, G
T98	P	random cells throughout pouch	some peripodial cells, behind furrow	NE	SG
T100	P	wing pouch	ND	ND	SG
T110	weak	random cells throughout pouch	NE	NE	SG
T155	SP	diffuse P throughout disc	diffuse P throughout disc	diffuse P throughout disc	SG, G

Larval brain, larval wing, eye, and leg disc, whole larvae: P = pattern, SP = strong pattern, NE = no expression observed, A/P = anterior/posterior boundary, ND = not determined, SG = salivary glands, M = muscle, G = gut, T = trachae, H = histoblast, PS = posterior spiracles, CNS = central nervous system, PNS = peripheral nervous system, FB = fat body, RG = ring gland, MT = malpigian tubules.

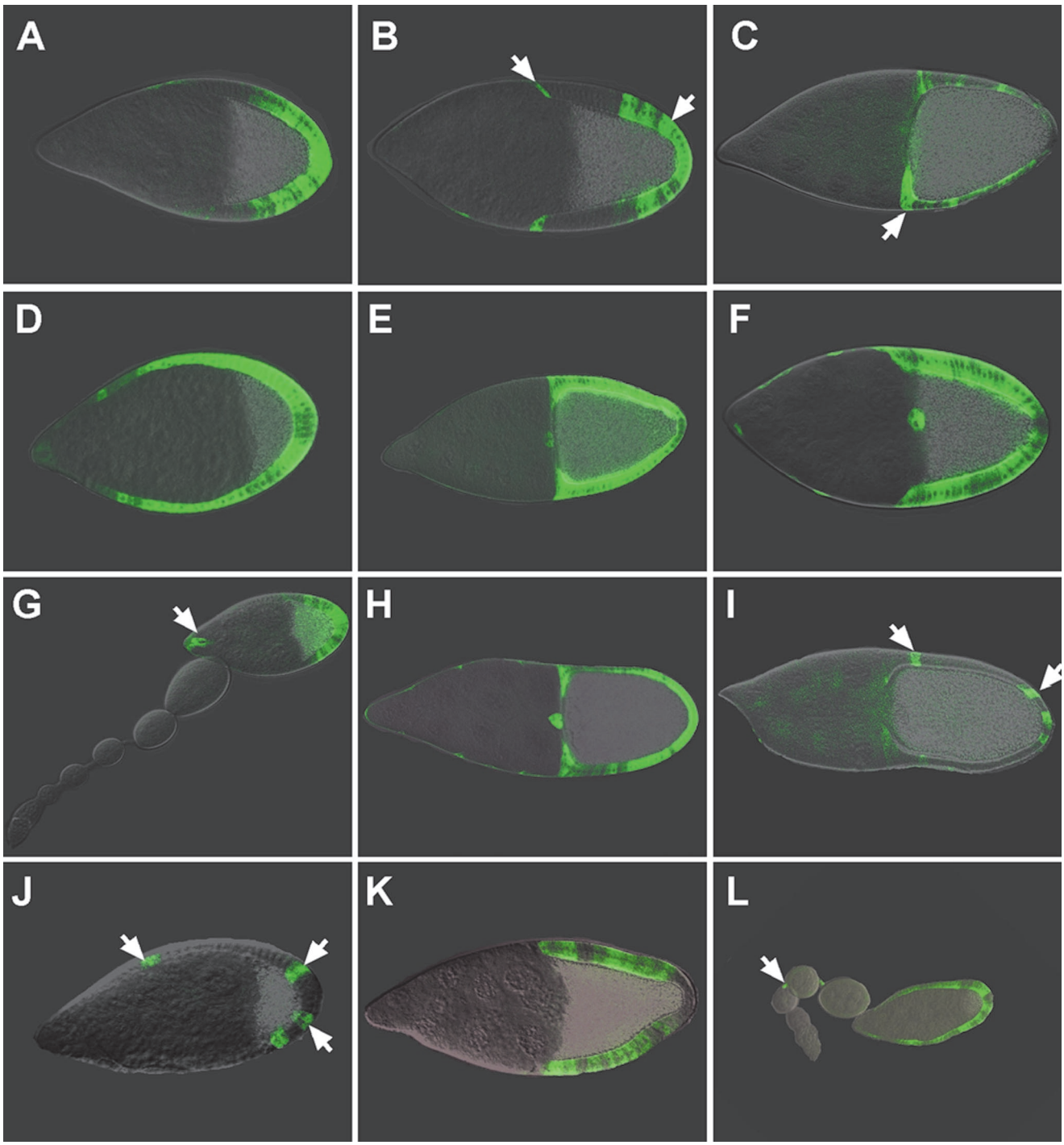

FIG. 4. Ovarian follicle cell expression of the Gal4 lines indicated. **A,B:** *c135* is expressed in posterior follicle cells at stage 9 (**A**) and in squamous cells, centripetal cells (arrow), and the posterior follicle cells at stage 10 (**B**). **C:** *c289b11* is expressed in the columnar follicle cells and preferentially upregulated in ventral centripetal cells. **D,E:** *c355* is strongly expressed in border cells and columnar follicle cells, but not in squamous cells. **F:** *T80* is ubiquitously expressed at stage 10. **G,H:** *c825* is expressed in border cells and posterior cells, beginning at stage 9 (**G**) and ubiquitous afterwards (**H**). **I:** *c855a* is expressed at the posterior pole and in the anterior, columnar follicle cells overlaying the germinal vesicle at late stage 10. **J,K:** *c729* is expressed in stripes in the follicle cell epithelium at early stages (**J**). At stage 10 (**K**) expression is specific to the columnar follicle cells except the cells at the posterior pole. **L:** *T80* is detectable specifically in interfollicular stalk cells (arrow) before its expression becomes ubiquitous at stage 8.

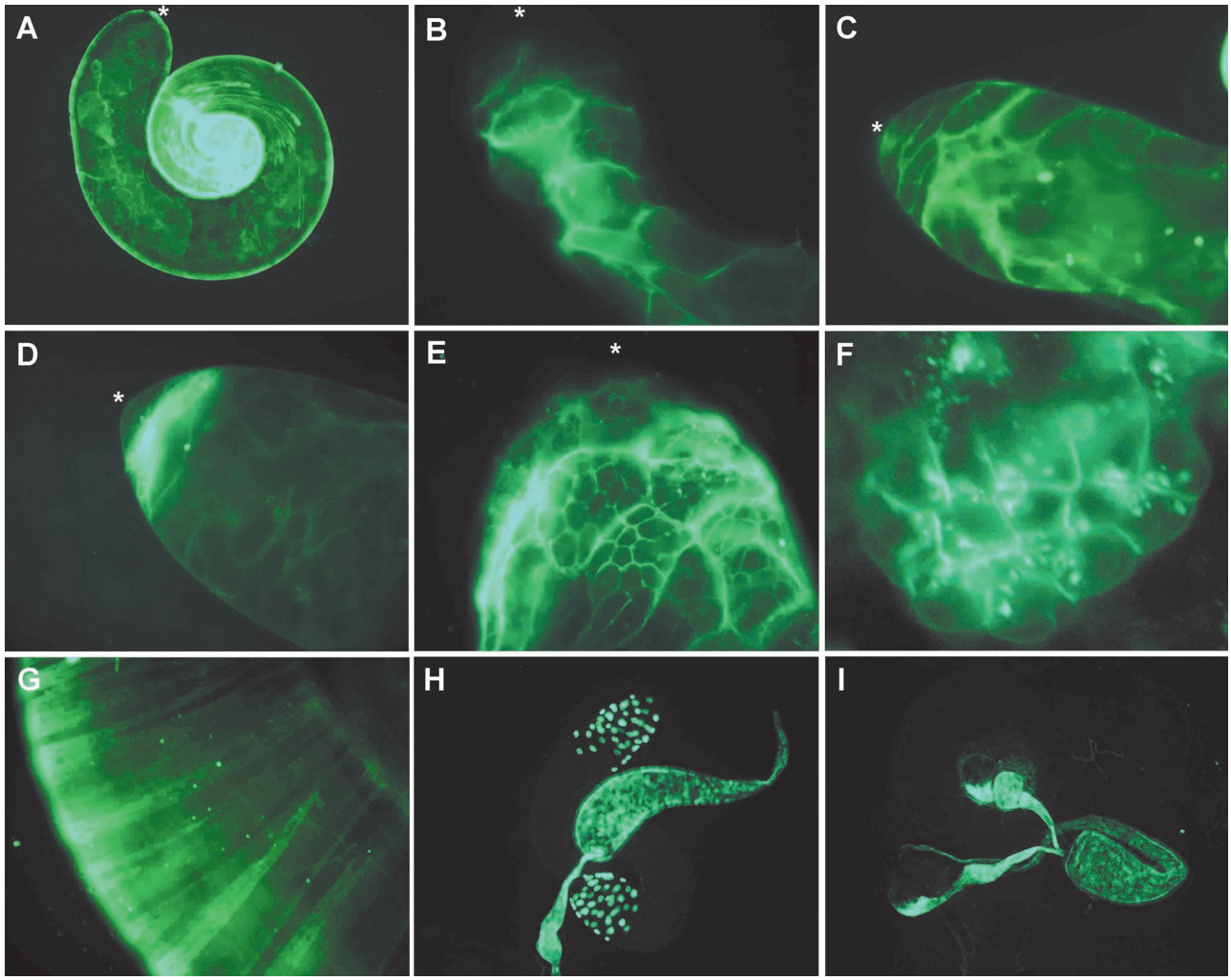

FIG. 5. Gal4 expression of *UAS-mCD8-GFP* in testes and the male reproductive tract. Apical tip of testis (*). **A:** *MJ12a* expressed in pigment cells ensheathing testis, entire coiled testis shown. **B–E:** Only apical tip of testis shown. **B:** *T155* expression detected in late somatic cyst cells enclosing 16-cell spermatocyte cysts. **C:** *C833* expression in somatic cyst cells and possibly apical hub cells. **D:** *T98* strongly expressed in the mitotic proliferation zone and weakly expressed in late somatic cyst cells. **E:** *C135* expressed in somatic cyst cells and weakly in the encysted germ cells. **F:** enlarged view of *T76* germ cell expression detected in 16-cell spermatocyte cysts. **G:** Enlarged view of *C855a* expression in muscle cells surrounding the testis. **H:** *T80* ubiquitously detected in male reproductive tissues. Strong expression in seminal vesicles, ejaculatory duct, and secondary secretory cells of the accessory glands; weak expression in the main secretory cells of the accessory gland not detected in this exposure. **I:** *T76* strongly expressed in seminal vesicles and ejaculatory duct.

LITERATURE CITED

Brand AH, Perrimon N. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118:401–415.

Lee T, Luo L. 1999. Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22:451–461.

Manseau L, Baradaran A, Brower D, Budhu A, Elefant F, Phan H, Philp AV, Yang M, Glover D, Kaiser K, Palter K, Selleck S. 1997. GAL4 enhancer traps expressed in the embryo, larval brain, imaginal discs, and ovary of *Drosophila*. Dev Dyn 209:310–322.