A New Marker for Mosaic Analysis in *Caenorhabditis elegans* Indicates a Fusion Between hyp6 and hyp7, Two Major Components of the Hypodermis

John Yochem,*,†,[1] Trent Gu*,[2] and Min Han*,‡

**Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309,*
†Umeå Center for Molecular Pathogenesis, Umeå University, S-901 87 Umeå, Sweden, and
‡Howard Hughes Medical Institute, University of Colorado, Boulder, Colorado 80309

Manuscript received January 26, 1998
Accepted for publication March 30, 1998

ABSTRACT

A fusion of the *sur-5* protein to the green fluorescent protein containing a nuclear localization signal is demonstrated as a marker for genetic mosaic analysis in the nematode *Caenorhabditis elegans*. Because of an extensive accumulation of bright fluorescence in many nuclei, normal growth plates, each containing hundreds of worms, can be rapidly screened with a dissecting microscope for rare mosaic individuals. As the marker can also be used to detect transgenic worms, the construction of strains for mosaic analyses can be minimized. In the course of examining rare mosaic animals, an unexpected pattern of fluorescence was noticed for hyp6, a syncytial component of the hypodermis, which indicated that the marker may serve as a means of assessing cellular fusions during development. Immunofluorescent staining of adherens junctions confirmed a postembryonic fusion of hyp6 with hyp7, the major syncytium of the hypodermis.

MUCH effort has been made to determine the pattern of expression of genes. Often more critical questions are when, and in what cells, genes must function, and whether this function is autonomous or nonautonomous with respect to cellular phenotype. These questions can be addressed genetically in certain experimental systems such as *Caenorhabditis elegans* by the examination of mosaic individuals, ones in which both mutant and wild-type genotypes are simultaneously present. Mosaic analysis is possible in this organism because of the behavior of extrachromosomal DNA during mitosis: free duplications of parts of the genome or extrachromosomal arrays that form following the injection of cloned DNA into the germline occasionally are not segregated to one of the daughter cells (Lackner *et al.* 1994; Hedgecock and Herman 1995). If the extrachromosomal DNA contains a functional copy of a gene whose two chromosomal copies have recessive mutations, further division of the daughter cell lacking the extrachromosomal DNA then generates a genotypically mutant clone within the otherwise wild-type individual. This type of analysis has proved to be particularly powerful for developmental studies of *C. elegans*, a nematode whose nearly invariant cell lineage has been determined (Sulston and Horvitz 1977; Sulston *et al.* 1983). For example, mosaic analysis of *lin-12* provided the first evidence that members of the *Notch* gene family encode receptors for extracellular signals (Seydoux and Greenwald 1989). The technique has also demonstrated a very limited requirement for the small G protein Ras during late embryonic and all postembryonic development (Yochem *et al.* 1997).

Mosaic analysis is more efficient in *C. elegans* if the extrachromosomal DNA is marked with an independent gene whose cell-autonomous activity can be detected in many cells; the presence or absence of the activity of the gene being tested can then be inferred. The most frequently used marker has been the *ncl-1(e1865)* mutation (Hedgecock and Herman 1995). It confers a recessive enlargement of the nucleoli of certain cells, a phenotype (Ncl) that can be detected at high magnification with Nomarski optics. Although this marker has worked well for many analyses, the approach can sometimes involve the mounting of several thousand individuals on slides for microscopic examination. This problem can sometimes be circumvented by exploiting genetic tricks such that only relevant parts of the cell lineage need be analyzed. However, this is not applicable to every case and, even when possible, may entail the construction of more complicated genotypes and the use of additional cloned DNA in the generation of extrachromosomal arrays.

A marker based on the green fluorescent protein (GFP) from *Aequorea victoria* (Chalfie *et al.* 1994) might circumvent these problems, provided that several features are present. The signal should be confined to nuclei, as they are the typical means of cellular identity for this organism (Sulston and Horvitz 1977). The signal must be broadly expressed with respect to the cell lineage and must be sufficiently bright to allow an

Corresponding author: Min Han, Howard Hughes Medical Institute, University of Colorado, Porter Biosciences Building, Boulder, CO 80309. E-mail: mhan@colorado.edu

[1] *Present address:* Umeå Center for Molecular Pathogenesis, Umeå University, S-901 87 Umeå, Sweden.

[2] *Present address:* Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711-5399.

assessment of inheritance before photodamage. Ideally, the signal should be bright enough to examine normal Petri plates rapidly for rare mosaic animals by means of a dissecting microscope. Finally, the fluorescence should not perdure such that genotypically mutant clones of cells cannot be detected. These conditions have been met for strains transgenic for high copies of a plasmid, pTG96, which encodes a fusion of SUR-5 and GFP. Moreover, mosaic analyses with this novel marker reveal an unexpected pattern of inheritance for hyp6, suggesting that it fuses with another hypodermal syncytium during postembryonic development. An investigation of this possibility has revealed a convenient means of assessing cell fusions in living animals.

MATERIALS AND METHODS

Construction of pTG96: pTG96 (Figure 1) was constructed for the purpose of assessing the pattern of expression of *sur-5*, a gene encoding a novel protein that was identified in a genetic screen for suppressors of a vulvaless phenotype associated with certain dominant-negative mutations in *let-60Ras* (Gu *et al.* 1998). The plasmid contains 7.3 kb of genomic DNA from the *sur-5* locus and a vector, pPD95.70 (A. Fire, J. Ahnn, G. Seydoux and S. Xu, personal communication), whose expression of GFP is dependent on the addition of heterologous DNA. The form of GFP encoded by the vector has a nuclear localization signal (A. Fire, personal communication) and an amino acid change (cysteine for serine at position 65) that increases the intensity of green fluorescence (Heim *et al.* 1995). Also, the DNA encoding GFP contains artificial introns for enhanced expression in transgenic *C. elegans* (A. Fire, personal communication).

Recessive mutations in *sur-5* can be complemented by a plasmid, pTG1-1, that contains 9.8 kb of genomic DNA from the *sur-5* locus (2.3 kb of which is upstream of the presumed initiation codon and 5 kb of which extends from the initiation codon to the termination codon; Gu *et al.* 1998). By means of the PCR technique (Saiki *et al.* 1985), pTG1-1 was modified such that the predicted stop codon and 3′ untranslated DNA were precisely replaced with a *Sma*I restriction site that had been incorporated into the downstream primer. Digestion of this DNA with *Sma*I therefore generates an end that has a CCC triplet in place of the stop codon. This permitted a fusion of the 3′-end of the open reading frame of *sur-5* with an exon encoding GFP in pPD95.70. This was achieved by ligating a 7.3-kb *Sph*I/*Sma*I fragment (obtained from an intermediate plasmid containing *sur-5* DNA) into the corresponding sites present in the multiple cloning region of pPD95.70.

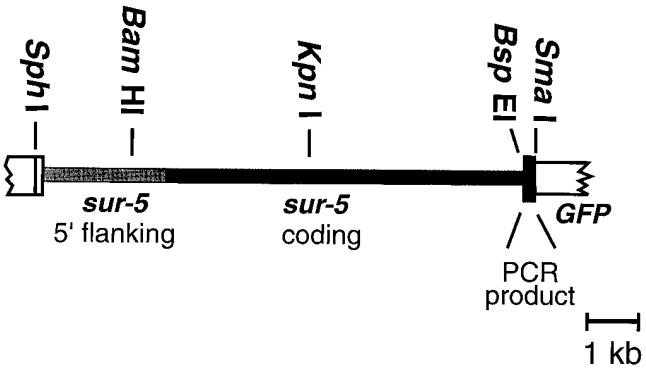

Figure 1.—The region of pTG96 that encodes a putative fusion protein, SUR-5GFP(NLS). Not shown are introns present in the *sur-5* coding region (Gu *et al.* 1998) and artificial introns in the region encoding GFP in the vector pPD95.70 (A. Fire, J. Ahnn, G. Seydoux and S. Xu, personal communication). The *Sph*I site is not present in *sur-5*; it and a small amount of flanking DNA derive from pUC19.

Construction of transgenic strains: The use of extrachromosomal arrays for mosaic analysis in *C. elegans* (Lackner *et al.* 1994) is based on the formation of these arrays *in vivo* following the microinjection of solutions of DNA into the gonadal syncytium (Mello *et al.* 1991). For mosaic analysis (and for the detection of transgenic lines), pTG96 was injected at concentrations between 80 and 100 μg/ml, and the most intensely fluorescing progeny were picked for the establishment of transgenic lines. In some cases it may be necessary to inject even higher concentrations in order to achieve the level of fluorescence evident in Figures 2 and 3. Animals that fluoresce less intensely than these examples should be avoided because the signal will most likely be too faint for screening with a dissecting microscope, and will be subjected to more rapid photobleaching at higher magnifications.

For the construction of MH1046 [complete genotype *unc-119(ed3)*; *him-5(e1490)*; *kuEx74(pTG96*; *unc-119*$^+$; *pBlueScript-SK*)], a strain homozygous for *unc-119(ed3)* was injected with a solution containing pTG96 at 100 μg/ml, pBlueScriptSK at 50 μg/ml, and pDP#MM016B at 30 μg/ml. Complementation of the uncoordinated phenotype conferred by *unc-119(ed3)* by pDP#MM016B (Maduro and Pilgrim 1995) was used to detect transgenic progeny. For MH1067 [complete genotype *unc-36(e251)*; *kuEx77(pTG96; unc-36*$^+$)], a strain homozygous for the *unc-36* mutation was injected with pTG96 at 100 μg/ml and R1p16 at 50 μg/ml. Complementation of the uncoordinated phenotype by R1p16 (Herman *et al.* 1995) was used to detect transgenic progeny. For MH1068 [complete genotype *ncl-1(e1865) unc-119(ed3)*; *kuEx78(pTG96; ncl-1*$^+$; unc-119$^+$)], a strain homozygous for both the *ncl-1* and the *unc-119* mutations was injected with pTG96 at 80 μg/ml, C33C3 at 75 μg/ml (for rescue of *ncl-1*; Miller *et al.* 1996), and pDP#MM016B at 30 μg/ml. Transgenic progeny were first identified as being fully coordinated and were then examined with a dissecting microscope for intense fluorescence and with Nomarski optics at a magnification of ×1000 for rescue of *ncl-1(e1865)*. For MH1066 [complete genotype *lrp-1(ku156)*; *him-8*; *kuEx76 (pTG96*; *lrp-1*$^+$)] worms having the genotype *lrp-1(ku156)/unc-13(e1091)*; *him-8* were injected with pTG96 at 100 μg/ml and two cosmids, F29D11 and C27G12 [for rescue of *lrp-1(ku156)*; J. Yochem and M. Han, unpublished results]. Second-generation progeny exhibiting fluorescence thoughout most of their bodies and especially in P_1, a progenitor of the germ line, were picked to individual plates that were examined later for a lack of segregation of the *unc-13* mutation, indicating homozygosity of the *lrp-1* mutation.

The rates of mitotic nondisjunction for the arrays above ranged from 1:200 per cell division for *kuEx78* to 1:500 for *kuEx77*. Each of the strains segregated a variety of mosaic animals, including those that appeared to have only one early or one late loss and those that had two or more independent losses. Segregants of each strain were also produced that failed to exhibit signs of mosaicism.

Microscopy of GFP: All growth plates examined directly with a stereoscopic dissecting microscope for fluorescent worms were standard NGM plates (Brenner 1974) containing either Sigma (A7002) or Difco Bacto agars and lawns of the *Escherichia coli* strain OP50. Most observations were made with a Leica microscope (model MZ12) capable of zoom magnification from ×8 to ×100. It possessed a 100-watt mercury arc lamp and a filter set (KSC 190–807; excitation 470–490 nm; emission ≥500 nm) permitting illumination of the surface of

the plates. Maximum working distance between the plate and objective for the manipulation of worms was achieved with a low-power objective (1.0; Leica number 445819). Observations have also been made with a dissecting microscope (Leica MZ6) having a maximum magnification of ×40. Although a dissecting microscope, especially one capable of zoom magnification to ×100, has obvious advantages for mosaic analysis, successful examinations and manipulations have been done with compound microscopes having a ×10 and, preferably, a ×5 objective. In these cases, plates are first taped to standard microscope slides.

Most observations of living worms (partially embedded in 5% Difco noble agar) at ×1000 were made with a Zeiss Axioskop equipped with a 100-watt mercury arc lamp and a number 9 filter set (excitation 450–490 nm; emission ≥515 nm). Observations have also been made with a Leica DMRB having an L4 filter set (excitation 450–490 nm; emission 515–560 nm). The signal is sufficiently strong so as to permit the scoring of worms in the presence of 10 mm sodium azide before detrimental photobleaching. Micrographs were generated with Adobe Photoshop from images derived from a Zeiss Axioplan (filter set 10; excitation 450–490 nm; emission 515–560 nm) having for a camera a charged-coupled device. They were printed to resemble as closely as possible the visualized image.

Mosaic analysis: Mosaicism was ultimately determined by examining living L3 and L4 larvae and young adults at a magnification of ×1000. The points of loss in the early cell lineage of extrachromosomal arrays containing pTG96 were inferred in part by scoring the following cells for the presence of nuclear fluorescence: from ABalap: CANL, RID, CANR, ALA, and RMED; from ABalpa: m3L and m3VL; from ABalpp: ASKL and ADLL; from ABarap: m3R and m3VR; from ABarpp: ALML, BDUL, ALMR, and BDUR; from ABplaa: ASIL; from ABplap: PLML, ALNL, HSNL, and the vulva (or its precursor); from ABplpa: excretory gland L, excretory cell, and rectal epithelium D; from ABplpp: hyp8/9, rectal epithelium VL, and anal depressor muscle; from ABpraa: ASKR, ADLR, and ASIR; from ABprap: PLMR, ALNR, HSNR, and the vulva (or its precursor); from ABprpa: excretory gland R; from ABprpp: hyp8/9, rectal epithelium VR, DVA, and body muscle; from MSaa: m7D, m8, and body muscles; from MSap: left coelomocytes, posterior distal tip cell, dorsal coelomocytes and those body muscles not from M; from MSpa: m6D and body muscles; from MSpp: right coelomocytes, anterior distal tip cell, and body muscles; from E: all intestinal cells; from Ca: DVC and body muscles; from Cp: hyp11 and body muscles; from D: body muscles. The origins and names of these cells and of other cells discussed in the text are based on Sulston *et al.* (1983).

Indirect immunofluorescence of adherens junctions: A population consisting of all developmental stages of wild-type *C. elegans* [N2 var. Bristol (Brenner 1974)] was harvested from normal NGM plates and fixed for immunofluorescence using conditions that permit greater than 95% of whole mount larvae and adults to be permeable to antibodies (Bettinger *et al.* 1996). The monoclonal antibody MH27 (Waterston 1988) was used at a dilution of 1:1000, and its binding was detected with a 1:1600 dilution of a cy3-conjugated secondary antibody (Jackson ImmunoResearch). Incubations (16 hr at 4°) and washes (room temperature over the course of several hours) were as described previously (Finney and Ruvkun 1990). DAPI was included in the penultimate wash at a concentration of 1 μg/ml so that nuclei could be visualized.

RESULTS

pTG96 directs the synthesis of bright fluorescence that remains localized to the nuclei of many cells: Microinjection of pTG96 at high concentrations (80–100 μg/ml) into the germline of hermaphrodites results in progeny transgenic for extrachromsomal arrays of DNA that confer bright nuclear fluorescence in many cells (Figure 2). Moreover, the fluorescence is unique in its nearly absolute localization to nuclei. This striking property is presumably the result of a gene product [henceforth referred to as SUR-5GFP(NLS), although its exact nature is unknown] that consists of a modified form of GFP fused to the carboxyl-terminus of SUR-5, a protein of unknown function. Fluorescence is first evident in embryos containing nearly 100 cells and is present without diminution during each of the four larval stages (L1-L4) of both sexes. It is also similarly present in young adults, but as they age, it decreases in some cell types, the intestine particularly so. For simplicity and because of a requirement that individuals be of a mini-

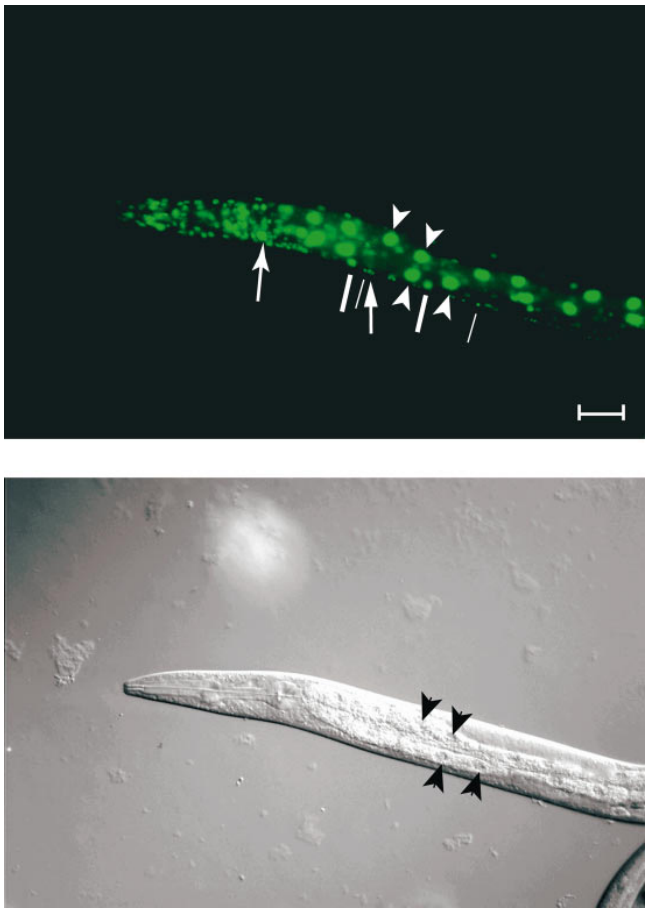

Figure 2.—pTG96 is expressed in the nuclei of many cells throughout the body. Shown are longitudinal views (median plane; anterior to the left and dorsal up) with fluorescence or Nomarski optics (magnification ×200) of the anterior half of an L4 hermaphrodite. The brightest nuclei are those of the intestine, four of which are indicated with arrowheads in both panels. Also very bright is the nucleus of the excretory cell (top, anterior-most arrow). The nuclei of two (slightly out-of-focus) body muscles (thick line), of two neurons in the ventral cord (thin lines), and of what was originally a Pn.p cell (posterior arrow) are also indicated. The bar in the top panel represents 40 μm.

mal size for convenient examination with a dissecting microscope, most of the results reported here are based on observations of hermaphrodites at the L3, L4, or young adult stages.

Although not all cells fluoresce (see below), those that do represent early branches of the somatic cell lineage (Sulston *et al.* 1983), including parts formed by the blastomeres ABa, ABp, MS, E, C, and D, suggesting a potential use of pTG96 as a marker for genetic mosaicism. For example, the fluorescence of most of the muscles of the body wall permits rapid scoring of cells derived from Ca, Cp, Da, and Dp. The anterior-most body muscles, the somatic gonad, and the terminal bulb of the pharynx are good for scoring MS, and an absence of fluorescing neurons in the head and ventral cord is consistent with the failure of AB (one of the daughters of the zygote) to inherit an extrachromosomal array containing pTG96. Some of the specific cells that can be scored are listed in materials and methods.

Based on analyses of four strains, each having an independently derived extrachromosomal array [*kuEx74(pTG96*; *unc-119*$^+$; *pBlueScriptSK*), *kuEx76(pTG96*; *lrp-1*$^+$), *kuEx77(pTG96; unc-36*$^+$), or *kuEx78(pTG96; ncl-1*$^+$; *unc-119*$^+$)], and on a strain homozygous for a genomic insertion of an array resembling *kuEx74*, it should be noted that the following cells usually do not express SUR-5GFP(NLS) or do so only very weakly: B, F, K′, K.a, U, Y, I5, hyp3, hyp12, the seam cells, the excretory duct, the amphid sheaths, and several others. (Individuals have occasionally been seen in which these nuclei do fluoresce, usually faintly. Some of these animals had a brighter than normal fluorescence of nearly all nuclei, suggesting that zygotes had inherited extra copies of an array or had inherited an array of increased size. In other cases, a clone of cells within an individual appeared to have inherited extra copies of an array because of nondisjunction at the time of formation of the clone's founder and its sister.) Also, the body muscles and the sex myoblasts derived from M usually cannot be scored, although the two coelomocytes can be. Never seen to fluoresce are the nuclei of the germ line. Mutant clones involving the germ line must therefore be inferred from preceding parts of the cell lineage, as is also the case when *ncl-1* is used (Hedgecock and Herman 1995).

SUR-5GFP(NLS) does not excessively perdure in genotypically mutant clones: Several observations indicate that perdurance of SUR-5GFP(NLS) does not preclude an analysis of mutant clones. First, segregants from MH1046, MH1066, MH1067, and MH1068 (see materials and methods for genotypes) were examined for mosaicism of their intestinal cells, as these all descend from one founder cell, E, and are therefore closely related to each other with respect to the cell lineage (Sulston *et al.* 1983). An examination of thousands of adults and L3 and L4 larvae at magnifications of 40- or 100-fold with a stereoscopic dissecting microscope capable of detecting fluorescence provided little evidence of perdurance in these cells. The more frequent case is to see large or small clones that completely lack fluorescence among positive clones that fluoresce brightly. The same trend is seen when individuals are randomly selected without prior knowledge of their states of fluorescence and then examined at higher magnification (×1000). However, a few cases have been noticed in which nuclei fluoresce faintly in clones that should be negative based on lineally related nuclei.

Another indication that perdurance is not excessive is based on observations of the strain MH1068, which contains an extrachromosomal array having both pTG96 and *ncl-1*$^+$ DNA. For one test, randomly selected L3 and L4 larvae were observed with Nomarski optics for the Ncl phenotype before any examination of fluorescence. In an examination of nine mosaic individuals for those nuclei that can be scored for both markers, the concurrence of nonfluorescence with a fully Ncl phenotype was 91% (204/223 nuclei) and concurrence of a normal level of fluorescence with a non-Ncl phenotype was 96% (324/339 nuclei; Table 1). Although there were a few exceptions, random segregants of this strain have also revealed an agreement between both markers for both early and late losses. For losses occurring at or before the 28-cell stage, both markers always implicated the same point of loss. This was also mostly the case for very late embryonic losses, including cells that were sisters of each other, although both markers sometimes exhibited inconsistencies with respect to each other. In these cases, the reliability of one marker versus the other could not be ascertained.

The excretory cell was also examined for perdurance of fluorescence because its nucleus is brightly fluorescent (Figure 2 and Figure 3, A and B) throughout postembryonic development, suggesting a consistently high level of SUR-5GFP(NLS). A lack of persistence of fluorescence relative to the Ncl marker is evident for this cell even in late losses (Figure 3, C and D). In the example here, the loss leading to the Ncl phenotype and lack of fluorescence did not occur too many generations earlier, as the nucleus of the dorsal rectal epithelial cell (ABplpappppp), a close relative of the excretory cell (ABplpappaap), was non-Ncl and brightly fluorescent (Figure 3, E and F). Thus, the great-great grandmother (ABplpapp) of these two cells must have inherited the array.

Certain mosaic types can be rapidly identified with a dissecting microscope: The bright fluorescence of SUR-5GFP(NLS) suggested that rare animals suffering losses early in development might be identified on normal growth plates by means of a dissecting microscope equipped with a mercury-arc lamp. This was first tested for the cell P_1, one of the daughters of the zygote, because of the nature by which the intestinal cells originate in the cell lineage (Sulston *et al.* 1983). The blastomere E is the immediate progenitor of all of these cells and

TABLE 1

Correlation of fluorescence and the Ncl phenotype

	Degree of fluorescence for 570 nuclei[a]			
Ncl phenotype	Normal	Weaker than normal	Very much weaker	Absent
Absent	324	15	0	0
Complete	2	9	8	204
Partial[b]	1	6	1	0

[a] Relative to that typically observed.
[b] Those nuclei in which the nucleolus appeared enlarged, but not to its maximum extent.

only of these cells; it is a daughter of EMS, which is itself a daughter of P_1 (Figure 4; E can also be designated as P_1ap). Thus, P_1 should be the point of loss in one out of three animals in which all of the intestinal nuclei are negative but in which other cells fluoresce. This was found to be the case for the strain MH1067 when 10 segregants completely negative for the intestine were examined at a magnification of ×1000: P_1 was the point of loss in 4 of them, EMS in 2, and E in 4. The detection of P_1 losses can be further enhanced by more detailed examination with the dissecting microscope, especially with those capable of magnifying ×100 (although it can still be accomplished with magnifications of 40 or ×50). If P_1 is the point of loss, the somatic gonad (entirely from MS), the terminal bulb of the pharynx (largely from MS), and all body muscles (except one) should not fluoresce, whereas many body muscles (those from C and D in addition to the one from AB) should fluoresce when EMS is the only point of loss.

It is also possible to detect with a dissecting microscope candidates that might have losses in AB or ABp. These will lack fluorescence in several nuclei that can often be scored for SUR-5GFP(NLS), including the nuclei of hyp8, hyp9, the excretory cell, the neurons in the ventral cord, many neurons anterior to the terminal bulb of the pharynx, and the left and right clusters of neurons in the tail. It is often easier to assess these candidates with the dissecting microscope if they also have an independent loss in E, because an absence of the extreme fluorescence of the intestinal nuclei can facilitate the scoring of fainter nuclei.

A unique feature of SUR-5GFP(NLS) is the ability to detect with a dissecting microscope very rare individuals that have had consecutive losses of an array such that only a restricted part of the cell lineage fluoresces. Not only does the restricted fluorescence provide a striking contrast to an otherwise dark body, but its intensity is almost always several fold greater than normal. (See Figure 5 for an example of an individual in which only the blastomere C appeared to have inherited pTG96 DNA; normally these nuclei do not fluoresce to this extent.)

Mosaic analysis of *unc-36* with SUR-5GFP(NLS) as a marker: As determined in previous work, animals in which ABp fails to inherit wild-type *unc-36* gene activity move in an uncoordinated fashion (Kenyon 1986). The *unc-36* gene was therefore chosen as a test of the feasibility of using SUR-5GFP(NLS) and a dissecting microscope for mosaic analyses. A strain, MH1067, with the genotype *unc-36(e251); kuEx77(pTG96; unc-36$^+$)*, was grown on normal growth plates, which were then examined by a dissecting microscope for fully coordinated segregants that were mosaic for green fluorescence. Consistent with the requirement in ABp, ~1 out of 500 had P_1 as the point of loss. (In every case, the true nature of the mosaicism was determined by examination at a magnification of ×1000 with a compound microscope.) In contrast, no coordinated animals with AB or ABp as the point of loss have been observed among a collection exceeding 8000, and none has been observed when coordinated individuals are randomly selected and viewed at high magnification.

The ability to examine with a dissecting microscope hundreds of worms on normal plates also facilitates a complementary analysis, the examination of mosaic animals that exhibit the phenotype under study. For example, several fully uncoordinated segregants that were also mosaic were noticed in the analysis of MH1067 described above. These ranged from ones in which only a few intestinal nuclei were fluorescent to four in which P_1 had inherited the array. In no cases, however, had ABp inherited the array.

SUR-5GFP(NLS) can be used as a marker for transgenic worms: Genetic strains containing a mutation in a gene essential for viability or reproduction must also have a functional copy of that gene. The necessity of "balancing" such mutations makes the addition of marker mutations such as *ncl-1(e1865)* for mosaic analysis more time-consuming. The use of SUR-5GFP(NLS) as a marker for both genetic mosaicism and for transformation can minimize the construction of strains. For example, the *lrp-1(ku156)* mutation must be balanced at all times (J. Yochem and M. Han, unpublished results). A strain with the simple genotype *lrp-1(ku156)/unc-13(e1091); him-8* was injected with pTG96 at 100 μg/ml and with DNA that complements the *lrp-1* mutation.

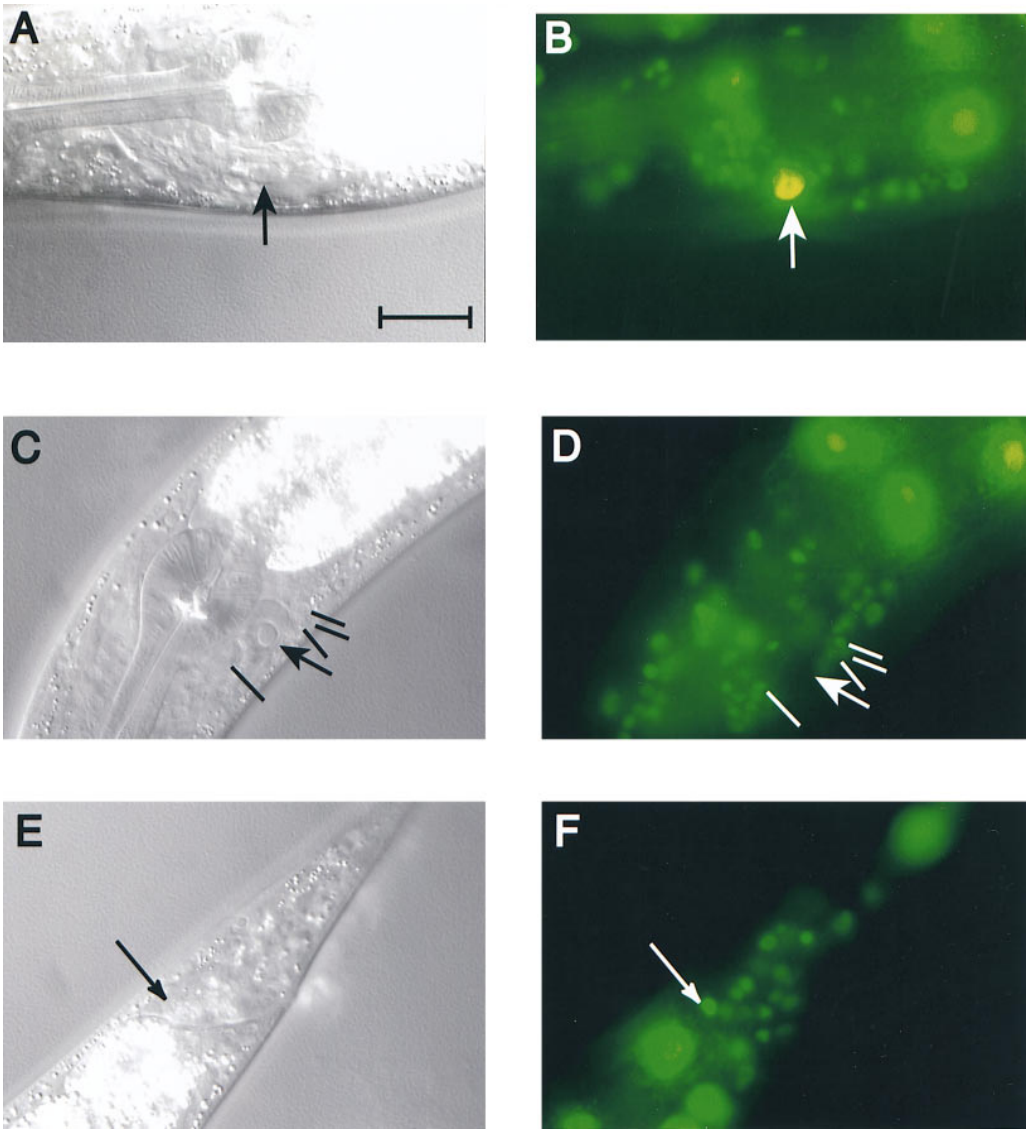

Figure 3.—Fluorescence does not excessively perdure. Shown are longitudinal views (median plane; ×1000 magnification) of two L4 larvae displayed with anterior to the left and dorsal up. (A) and (B) The nucleus of the excretory cell fluoresces brightly when non-Ncl. (A) Nomarski image of the nucleus (arrow) of a non-Ncl excretory cell. The smaller than normal nucleolus within the nucleus indicates inheritance of an extrachromosomal array containing many copies of *ncl-1*$^{+}$ DNA. (B) Intense fluorescence of the same nucleus (arrow) can be seen to saturate the color processing (yellow). (C) and (D) The nucleus of the excretory cell does not fluoresce when Ncl. (C) Nomarski image showing the enlarged nucleolus (the Ncl phenotype) present in the nucleus (arrow) of the excretory cell of another animal. (D) The same nucleus lacks fluorescence. The nuclei of several neurons (lines) serve as reference points. (E) and (F) A close relative of the excretory cell inherited pTG96. (E) Nomarski image of the posterior region of the same animal as in C and D. The position of the nucleus of the dorsal rectal epithelial cell is indicated. (F) An image of the same region indicating the typically bright fluorescence of this nucleus. All panels are to the same scale; the bar in A represents 20 μm.

An examination of fluorescence with a dissecting microscope 24 hr later revealed that injected worms had laid green eggs, and extrachromosomal arrays capable of continuous propagation from one generation to the next could be established with frequencies similar to those formed with pRF4, a commonly used transformation marker that confers a rolling behavior (Mello *et al.* 1991). After establishment of stably transgenic lines in which *lrp-1(ku156)* was complemented by extrachromosomal arrays, a line (MH1066) was directly used for mosaic analysis with unambiguous results (J. Yochem and M. Han, unpublished results).

Mosaic analyses with SUR-5GFP(NLS) indicate that hyp6 fuses with hyp7: Most of the hypodermis of *C. elegans* is a single syncytium, hyp7, that is composed of nuclei that descend from ABa, ABp, and C—the latter being a blastomere from the P_1 side of the cell lineage (Sulston *et al.* 1983). Immediately anterior to hyp7 is hyp6, a syncytium containing six nuclei, all of which descend from AB (Sulston *et al.* 1983). Thus, if one assumes an invariant lineage, these syncytia should exhibit a different pattern of inheritance in certain types of mosaic individuals. For example, all of the nuclei of hyp7 should fluoresce when either ABa, ABp, P_1, P_2, or C is the sole blastomere that inherits an extrachromosomal array containing pTG96 (Figure 4). That is, each nucleus—whether genotypically plus or minus for the array—should have equal access to the *sur*-5GFP(NLS)

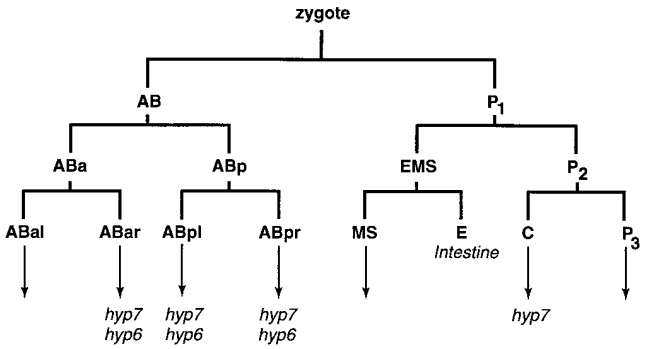

Figure 4.—Schematic representation of the first divisions of a zygote showing the early progenitors of the intestine and of hyp6 and hyp7. The lowercase a, p, l, and r represent anterior, postereior, left, or right daughters, respectively, following a cell division. The drawing is based on data determined by Suston *et al.* (1983).

protein synthesized in the common cytoplasm of the syncytium. In contrast, the nuclei of hyp6 should only fluoresce when either AB, ABa, or ABp has inherited the array; inheritance of pTG96 by P_1 should have no effect, as it should not contribute to this syncytium. However, two mosaic analyses did not support this prediction.

In the course of analyzing mosaic segregants from MH1066, the strain containing *lrp-1(ku156)*, only five individuals (either L4 larvae or adults) were identified in which AB apparently failed to inherit the extrachromosmal array *kuEx76*. It should be noted that this conclusion was well established, as it was based on scoring many nuclei from both ABa and ABp. Surprisingly, the six nuclei of hyp6 fluoresced in each case. The only other nuclei that descend from AB to fluoresce in these individuals were in hyp7, and an examination of nuclei that descend from P_1 revealed that C had inherited the array in every case.

As an independent analysis, adults and larvae at the L3 or L4 stages from MH1067, the strain containing *unc-36(e251)*, were also examined for the behavior of hyp6 when AB appeared not to have inherited pTG96. In this analysis, normal growth plates were examined with a dissecting microscope for uncoordinated segregants that exhibited green fluorescence. From a sample of nearly 8000 worms, only eight were found that had AB as the apparent point of loss of the array, based on scoring many nuclei at a magnification of ×1000. Again, the nuclei of hyp6 fluoresced (Figure 5) in every case, bringing the total number of cases to 13. As for the MH1066 analysis, the nuclei of hyp7 also fluoresced, and the blastomere C had clearly inherited the array in every case. An explanation for this deviation from the expected result in 13 out of 13 cases is that hyp6 is connected in some manner to hyp7, which itself has inherited pTG96 from those nuclei originating from C.

The observations above raise the possibility that SUR-5GFP(NLS) might serve as a convenient marker for assessing cellular fusions during development. Those descendants of the 8 of the 12 P cells that are known to fuse with hyp7 were therefore examined for fluorescence as a test of this idea. For the cells P1.p, P2.p, P9.p, P10.p, and P11.p, fusion occurs in the first larval stage; for the daughter nuclei of P3.p, P4.p, and P8.p, fusion occurs in the third larval stage soon after they and the cells P5.p, P6.p, and P7.p have divided (Sulston and Horvitz 1977). The descendants of P5.p, P6.p, and P7.p, however, do not fuse with hyp7 but form the vulva instead. In each of the 13 cases described above in which C but not AB had presumably inherited pTG96, the nuclei of the descendants of the P cells that should have fused with hyp7 always fluoresced, but the nuclei of the mature or incipient vulva did not (an L3 larva in which the vulval cells are still in the process of dividing is shown in Figure 5, E and F).

hyp6 fuses with hyp7 postembryonically: Mosaic analyses with SUR-5GFP(NLS) strongly indicated that hyp6 might fuse with hyp7 by the time of the third larval stage, the earliest stage examined. A fusion in wild-type worms was therefore investigated by an independent means, the immunological detection of adherens junctions between these syncytia with the monoclonal antibody MH27 (Podbilewicz and White 1994). Consistent with the presence of two distinct syncytia at hatching (Sulston *et al.* 1983; Podbilewicz and White 1994), 46 of 46 mid-L2 larvae had two continuous bands of strong staining adjoining and perpendicular to the left and right H0 seam cells in the location expected for the junction of hyp6 and hyp7 (Figure 6A). In contrast, these bands were much less intense and always discontinuous in 29 of 29 early- to mid-L3 larvae, and they could not be detected by the time of the L3 to L4 molt (6/6 cases). [Staining was also not evident in L4 larvae (20/20 cases) nor in adults (31/31 cases).] The stage at which a reduction of staining is first apparent was determined by examining the seam cells, which divide during the L2 to L3 molt (Sulston and Horvitz 1977), and the developmental state of the gonad (Kimble and Hirsh 1979). In late L2 larvae, a breach in the bands of staining occurs near their junctions with the H0 seam cells (4/4 cases), and the entire staining then becomes much reduced in intensity and in continuity during the subsequent molt (3/3 cases). By the time of division of the vulval precursor cells, only traces of staining can be detected (Figure 6, B and C). Thus, as predicted by the mosaic analyses, hyp6 appears to fuse with the major syncytium of the hypdermis. In contrast, the mosaic analyses and staining with MH27 have indicated that hyp5, the hypodermis immediately anterior to hyp6, never fuses during postembryonic development.

DISCUSSION

The use of SUR-5GFP(NLS) as a marker for transformations, mosaic analyses, and assessments of cell fusions in living animals is based on a striking property, a nearly

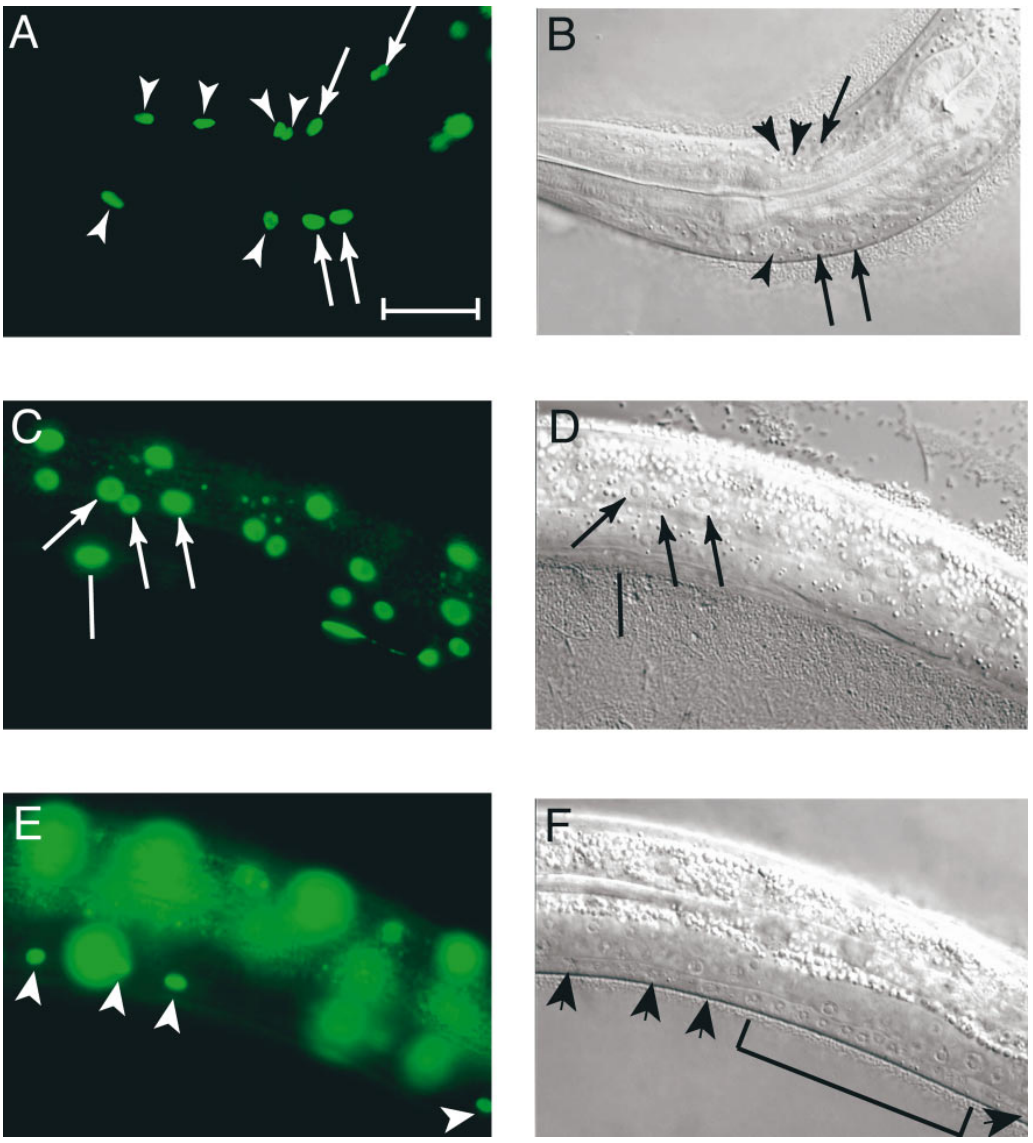

Figure 5.—hyp6 and hyp7 may be interconnected. Shown are longitudinal views (×1000 magnification; anterior to the left; dorsal up) of an L3 larva in which pTG96 appeared to be inherited only by the blastomere C. (A) Fluorescence of the six nuclei of hyp6 (arrowheads) and of the medial nuclei of hyp7 (arrows). The three anterior-most hyp6 nuclei are slightly out of the plane of focus. Even though this view encompasses most of the head, note the complete absence of fluorescence of other nuclei (compare with Figure 2 and Figure 3, A–D). (The fluorescence in the posterior region results from lateral hyp7 nuclei that are out of the plane of focus.) Note also the brightness of the signal. (B) A Nomarski image of the same region showing the typical morphology of hyp6 nuclei. The posterior-most ventral one provides the best example (ventral arrowhead); the anterior three nuclei are slightly out of the plane of focus. (C) Bright and equal fluorescence of left lateral hyp7 nuclei (three of which are indicated with arrows) present in the midbody. Consistent with inheritance of pTG96 by C and only C, several ventral and dorsal body muscles fluoresce slightly out of the plane of focus (a ventral one is indicated with a line). Autofluorescence of cytoplasmic granules present in the intestinal cells is also evident. (D) Nomarski view of the same region as C. (E) The median optical plane of the same region as in C and D. Four descendants of Pn.p cells that should have fused with hyp7 brightly fluoresce (arrowheads). (The blurry image near the middle one of the anterior set of three is the now extremely out-of-focus nucleus of the body muscle indicated in C.) (F) The nuclei of the 12 cells that will divide further to form the vulva are indicated with a bar (the posterior 4 appear to be either reforming after a round of cell division or initiating the next division). Note how they completely fail to fluoresce in E. Although the divisions of the vulval precursor cells were not monitored, the four fluorescent nuclei are probably descendants of P3.p (the anterior-most nucleus), P4.p (the two nuclei just anterior to the vulval nuclei), and P8.p (the posterior most nucleus). All panels are to the same scale; the bar in A represents 20 μm.

absolute localization of bright fluorescence to nuclei. (Worms are occasionally observed in which fluorescence is not completely localized to the nucleus of some cells, but these can often still be identified.) It must be the case that the fluorescent protein, besides having high affinity for nuclei, has few deleterious effects when present at high levels. (It is also possible that the intense signal seen in some cells results from an enhancement of fluorescence rather than from an increase in amount of the protein. However, some nuclei that fluoresce brightly have an increase in size, suggesting that they harbor a large amount of the protein.)

One question concerns how this degree of nuclear localization is achieved. Although the form of GFP used in the construction has an artificial nuclear localization signal (A. Fire, J. Ahnn, G. Seydoux and S. Xu, personal communication), we are not aware of other translational or transcriptional fusions to it that localize this well. *sur-5* itself serves as an example. The fluorescence resulting from a transcriptional fusion (present in plasmid pTG96-2) of *sur-5* to the same form of GFP does not localize well to nuclei (T. Gu and M. Han, unpublished results). [Recall that SUR-5GFP(NLS) should be a translational fusion, although its exact nature is unknown.]

Perhaps the striking localization of SUR-5GFP(NLS) to nuclei simply results from the presence of at least two signals, one being present in SUR-5 itself. Consistent with this idea, a translational fusion of SUR-5 to a form of GFP lacking an NLS also localizes to nuclei, but not nearly to the same degree as SUR-5GFP(NLS) (Gu *et al.* 1998). Whatever the reason for the strong localization to nuclei, this property may be of use for studies in addition to those described here. For example, it may help determine the effectiveness of laser microsurgery.

Advantages and disadvantages of using SUR-5GFP (NLS) as a marker for mosaic analysis: An advantage of SUR-5GFP(NLS) for mosaic analysis is the positive nature of its signal, a generally bright fluorescence of nuclei against a dark background. In contrast, the scoring of *ncl-1(e1865)* relies on an examination of the sizes of nucleoli with Nomarski microscopy, and this size varies to some degree among different cell types (Hedgecock and Herman 1995). Although the degree of fluorescence of SUR-5GFP(NLS) also varies among different cell types, less time and skill are required for scoring fluorescence against a dark background. This is also an advantage in scoring nuclei in adults, provided they are not too old.

One significant advantage of SUR-5GFP(NLS) is extremely bright expression in the nuclei of the intestinal cells, a tissue in which *ncl-1* usually cannot be scored. [It is sometimes possible to score the intestine for the Ncl phenotype based on extrachromosomal arrays that overly express the *ncl-1* gene product to such a degree that the nucleoli of positive cells are smaller than normal (Koga and Ohshima 1995).] The bright fluorescence and the nature of the origin of the intestinal cells together permit the convenient detection of worms mosaic for P_1, EMS, or E, especially when examined with a dissecting microscope. With practice, it is possible to examine a set of 12 plates, each containing about 200 late-stage worms (L3 and L4 larvae and adults), for these types of losses in less than 1 hr. Thus, a large part of the cell lineage can be examined in a short period of time.

Individuals mosaic for AB, ABa, or ABp are not as easily detected with a dissecting microscope, as this side of the lineage has fewer bright nuclei that are linearly related. For example, one of the brightest signals from the ABp side of the linage is the nucleus of the excretory cell, but this cell (ABplpappaap) cannot be soley relied on for this purpose because too many cell divisions precede its birth, unlike the case for the blastomere E (Figure 4) (Sulston *et al.* 1983). Nevertheless, it is still

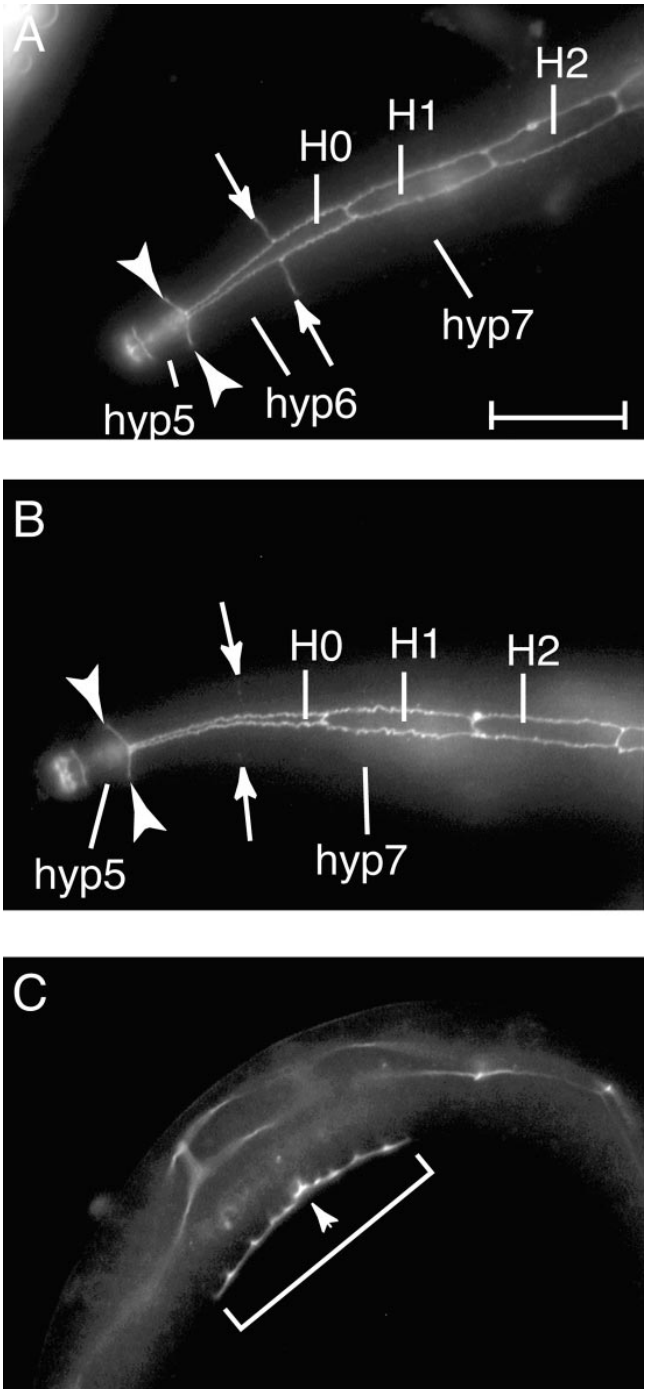

Figure 6.—Confirmation of the postembryonic fusion of hyp6 to hyp7 in whole mounts. (A) Left lateral view (anterior to the left) of MH27 staining indicating that hyp6 has not fused in a larva at the mid-L2 stage. Two lines (arrows) adjoining the left H0 seam cell form the boundary between hyp6 and hyp7. An examination of other focal planes revealed that each line was continuous from left to right seam cell. Also indicated are the left H1 and H2 seam cells and the boundary between hyp5 and hyp6 (arrowheads). These designations are based on Podbilewicz and White (1994). (B) Only faint traces of MH27 staining remain (arrows) between hyp7 and what was hyp6 in an L3 larva. (C) MH27 staining of 12 vulval precursor cells (bracket) of the same animal as in B, providing an estimate of its age. Invagination (arrowhead) of the central cells (also confirmed by bright-field microscopy) typically occurs more than midway through the third larval stage (Sulston and Horvitz 1977). The development of the vulval cells of this specimen is slightly more advanced than that of the L3 larva in Figure 5. The bar in panel A represents 20 μm.

possible to identify candidates that might have such losses.

The striking increase in brightness of fluorescence typically associated with consecutive losses of arrays containing pTG96 enhances the power of mosaic analysis: it is easy to identify with a dissecting microscope rare individuals in which an array was only inherited in a restricted part of the cell lineage. The most likely explanation for the increase in brightness is inheritance by one daughter cell of a doubled copy number of an array at each mitotic nondisjunction. Based on a visualization of DNA with a fluorescent dye and on the proportion of progeny inheriting a free duplication covering the *ncl-1* gene, Hedgecock and Herman (1995) have proposed that nondisjunction is the primary reason for mosaicism in *C. elegans*. pTG96 may therefore provide a stunning visualization of this process.

The ability to examine normal growth plates for SUR-5GFP(NLS) provides a final advantage, the complementary analysis of rare segregants that are both mutant and mosaic. Heretofore this analysis has been difficult or has required additional strain construction because of poor meiotic transmission of free duplications and extrachromosomal arrays (Mello *et al.* 1991; Herman 1995). For MH1067, there were no cases of fully uncoordinated segregants in which ABp had inherited the array, consistent with ABp being the focus for *unc-36* gene activity (Kenyon 1986). This activity, however, is presumably required in neuronal descendants of ABp. It may be possible to examine many thousands of uncoordinated segregants for those in which ABp had inherited *unc-36*$^{+}$ DNA. These might represent multiple, independent losses within the descendants of ABp and therefore might provide further insight into those cells that require the gene's activity. This type of analysis, however, could be compromised by aberrancies in the expression of arrays.

Although pTG96 has significant advantages over *ncl-1(e1865)* for mosaic analysis, it is not a perfect marker. It requires an expensive mercury vapor lamp, and analyses are best done with an expensive dissecting microscope capable of zoom magnification up to ×100. Also, the use of a dissecting microscope limits analyses to worms of sufficient size and renders less efficient the quantitation of the total number of worms analyzed.

Occasional discordance between nonfluorescence and the Ncl mutant phenotype was apparent in the analysis of MH1068 (Table 1). This may indicate, in some cases, perdurance of SUR-5GFP(NLS) relative to the Ncl phenotype, or the converse, in late losses. In some of the cases in Table 1, however, the nucleolus was not of maximum size, nor was the fluorescence of maximum brightness, indicating that both markers can sometimes persist, or are not properly expressed, to similar extents. Thus, neither marker may be perfect in these regards, but problems should not occur if early losses are emphasized. (The more critical question, of course, is the perdurability of the gene being tested by the mosaic analysis.) Also apparent in the analysis of MH1068 were inexplicable aberrancies. For example, the somatic gonad of one segregant appeared to be completely negative for the array based on both the Ncl phenotype and SUR-5GFP(NLS) except that one and only one cell (a distal tip cell) brightly fluoresced. In another animal P_1 appeared to be the point of loss except that the nucleus of one body muscle that should originate from the P_1 side of the cell lineage was faintly green and partly Ncl. These cases may represent aberrant expression of the genes composing the array or alterations that rendered the array unable to be replicated, resulting in unicellular inheritance. These aberrancies are easily visualized with SUR-5GFP(NLS) and help to emphasize that caution should be exercised when using arrays for mosaic analysis and other purposes (for discussions, see Herman 1995; Mello and Fire 1995; Miller *et al.* 1996).

A fusion of SUR-5 to a form of GFP lacking the signal for localization to nuclei is able to complement a mutation in *sur-5*. Thus, the possibility exists that the fusion protein discussed here has SUR-5 activity that might confuse analyses, especially those involving genes that might be involved in the Ras pathway. On the other hand, the brightness of the fluoresence and its resistance to photodamage suggest that nuclei appear quite tolerant of high amounts of the protein. Furthermore, the level of fluorescence can be several fold higher in certain types of mosaicism (see above) with no overt effect on animals.

As for *ncl-1(e1865)* (Hedgecock and Herman 1995), the limited expression of pTG96 can be a hindrance for assessing some types of mosaicism. For example, all of the cells (except for the two coelomocytes) that descend from the postembryonic blastomere M are difficult to score. As more of these can be scored for the Ncl-1 phenotype, it might be better to use this marker when interested in late losses in this part of the cell lineage. Given the advantage of SUR-5GFP(NLS) in assessing other types of losses, an alternative might be to incorporate both markers. In any case, a more efficient marker would be one that permits bright fluorescence in all nuclei. This might be achieved by means of a transcriptional fusion of a universally expressed gene to a form of GFP containing multiple signals for nuclear localization.

The use of SUR-5GFP(NLS) for the assessment of cellular fusions: Nomarski- and electron-microscopic examinations and stainings of fixed embryos with MH27, the antibody that recognizes adherens junctions, have suggested that hyp6 and hyp7 are separate syncytia (Podbilewicz and White 1994). Surprisingly, mosaic analyses of two strains containing arrays expressing SUR-5GFP(NLS) suggested that the nuclei of hyp6 have access to the cytoplasm of hyp7 by the time of the third larval stage. Another interpretation is that, of all of the

nuclei that descend from AB, only one or a few nuclei of hyp6 inherited the arrays. It is also possible that these exceptions resulted from variations in the cell lineage. Both of these possibilities, however, seem improbable, and the staining of whole mounts with the MH27 antibody is completely consistent with a fusion of hyp6 and hyp7 during larval development.

The significance, if any, of the fusion between hyp6 and hyp7 is unknown. Perhaps formation of the body plan during embryogenesis is best achieved with separate syncytia that can be differentially regulated. For instance, a separate syncytium like hyp6 may be necessary for forming a gradual tapering of the head (for a discussion, see White 1988). Once tapering is achieved during embryogenesis, hyp6 fuses with hyp7 either because their separation is no longer important or their fusion permits better regulation of postembryonic events such as molting.

The fusion of hyp6 and hyp7 emphasizes the diffusible nature of SUR-5GFP(NLS). In 11 of the 13 mosaic animals in which hyp7 but not hyp6 apparently inherited pTG96, the nuclei of hyp6 were as fluorescent as the nuclei of hyp7 (Figure 5A). (In the two exceptions, both L4 larvae, the nuclei of hyp6 uniformly fluoresced with respect to each other, but the fluorescence was slightly weaker than that of the nuclei of hyp7.) In almost all cases of mosaicism involving hyp7, SUR-5GFP(NLS) is equally distributed to all of the nuclei of this syncytium regardless of their inheritance of pTG96.

Although the significance of the fusion of hyp6 and hyp7 remains unknown, it has nevertheless revealed a marker for assessing certain cellular fusions: nuclei that have not inherited SUR-5GFP(NLS) should only fluoresce if they have been exposed to the cytoplasm of a cell that did inherit it. Although SUR-5GFP(NLS) may not be sufficient for precise timing of fusions because nuclei must internalize it to detectable levels, this approach has several advantages over electron microscopy and immunofluorescence, both of which require fixed specimens. Also, a disappearance of staining with the MH27 antibody may not always indicate fusion, as the epitope itself could disappear without a disappearance of adherens junctions. In contrast, SUR-5GFP(NLS) can be scored in living animals and is less restricted in its expression than the MH27 epitope. Although the approach described here relies on mosaic individuals, these can often be conveniently detected with the marker, and it is also possible that the need for mosaicism could be replaced by expressing SUR-5GFP(NLS) with cell-specific promoters. Thus, not only may SUR-5GFP(NLS) serve as a new marker for transformation and mosaic analysis, it may also permit a powerful means of assessing cell fusions during development of living animals.

We are grateful to Simon Tuck for comments on the manuscript and for providing space and support during the completion of this work. Many thanks are also due Camilla Englund for help with the photomicroscopy and Lars Nilsson and Simon Tuck for advice concerning antibodies. The following generously provided antibodies or DNA clones: Alan Coulson, Andy Fire, Mike Herman, Bob Herman, David Pilgrim, David Waring, and Bob Waterston. Support was from the U.S. Public Health Service (grant GM47869) and the American Cancer Society. T. Gu was a National Institutes of Health predoctoral trainee.

LITERATURE CITED

Bettinger, J. C., K. Lee and A. E. Rougvie, 1996 Stage-specific accumulation of the terminal differentiation factor LIN-29 during *Caenorhabditis elegans* development. Development **122:** 2517–2527.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans.* Genetics **77:** 71–94.

Chalfie, M., Y. Tu, G. Euskirchen, W. W. Ward and D. C. Prasher, 1994 Green fluorescent protein as a marker for gene expression. Science **263:** 802–805.

Finney, M., and G. Ruvkun, 1990 The *unc-86* gene product couples cell lineage and cell identity in *C. elegans.* Cell **63:** 895–905.

Gu, T., S. Orita and M. Han, 1998 *C. elegans* SUR-5, a novel but conserved protein, negatively regulates LET-60 Ras activity during vulval induction. Mol. Cell. Biol. (in press).

Hedgecock, E. M., and R. K. Herman, 1995 The *ncl-1* gene and genetic mosaics of *Caenorhabditis elegans.* Genetics **141:** 989–1006.

Heim, R., A. B. Cubitt and R. Y. Tsien, 1995 Improved green fluorescence. Nature **373:** 663–664.

Herman, R. K., 1995 Mosaic analysis, pp. 123–146 in *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, edited by H. F. Epstein and D. C. Shakes. Academic Press, San Diego.

Herman, M. A., L. L. Vassilieva, H. R. Horvitz, J. E. Shaw and R. K. Herman, 1995 The *C. elegans* gene *lin-44*, which controls the polarity of certain asymmetric cell divisions, encodes a Wnt protein and acts cell nonautonomously. Cell **83:** 101–110.

Kenyon, C., 1986 A gene involved in the development of the posterior body region of *C. elegans.* Cell **46:** 477–487.

Kimble, J., and D. Hirsh, 1979 The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans.* Dev. Biol. **70:** 396–417.

Koga, M., and Y. Ohshima, 1995 Mosaic analysis of the *let-23* gene function in vulval induction of *Caenorhabditis elegans.* Development **121:** 2655–2666.

Lackner, M. R., K. Kornfeld, L. M. Miller, H. R. Horvitz and S. K. Kim, 1994 A MAP kinase homolog, *mpk-1*, is involved in *ras*-mediated induction of vulval cell fates in *Caenorhabditis elegans.* Genes Dev. **8:** 160–173.

Maduro, M., and D. Pilgrim, 1995 Identification and cloning of *unc-119*, a gene expressed in the *Caenorhabditis elegans* nervous system. Genetics **141:** 977–988.

Mello, C., and A. Fire, 1995 DNA transformation, pp. 451–482 in *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, edited by H. F. Epstein and D. C. Shakes. Academic Press, San Diego.

Mello, C. C., J. M. Kramer, D. Stinchcomb and V. Ambros, 1991 Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J. **10:** 3959–3970.

Miller, L. M., D. A. Waring and S. K. Kim, 1996 Mosaic analysis using a *ncl-1(+)* extrachromosomal array reveals that *lin-31* acts in the Pn.p cells during *Caenorhabditis elegans* vulval development. Genetics **143:** 1181–1191.

Podbilewicz, B., and J. G. White, 1994 Cell fusions in the developing epithelia of *C. elegans.* Dev. Biol. **161:** 408–424.

Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn *et al.*, 1985 Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science **230:** 1350–1354.

Seydoux, G., and I. Greenwald, 1989 Cell autonomy of *lin-12* function in a cell fate decision in *C. elegans.* Cell **57:** 1237–1245.

Sulston, J. E., and H. R. Horvitz, 1977 Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans.* Dev. Biol. **56:** 110–156.

Sulston, J. E., E. Schierenberg, J. G. White and J. N. Thompson,

1983 The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. **100:** 64–119.

WATERSTON, R. H., 1988 Muscle, pp. 281–335 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

WHITE, J., 1988 The anatomy, pp. 81–122 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

YOCHEM, J., M. SUNDARAM and M. HAN, 1997 Ras is required for a limited number of cell fates and not for general proliferation in *Caenorhabditis elegans*. Mol. Cell. Biol. **17:** 2716–2722.

Communicating editor: I. GREENWALD