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Induction of mutations in the zebrafish with ultraviolet light

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Summary

Recessive lethal germline and specific locus somatic mutations were induced efficiently in the zebrafish by exposure of mature sperm to UV light. Mutagenesis of sperm yielded mosaic individuals: clones bearing novel mutations represented approximately 12–25% of the haploid germ cells and 25–50% of the somatic tissue. Simple methods are described for the reliable identification and propagation of newly arising developmental mutations in zebrafish.

1. Introduction

The study of mutant organisms has significantly enhanced our understanding of the regulation of development. Mutational analyses have identified sequential intermediates in the development of *Dictyostelium discoideum* (Godfrey & Sussman, 1982; Kaiser, 1986), necessary cell interactions in haemopoiesis in the mouse (Altus *et al.* 1971; Copeland *et al.* 1990), and spatial frameworks within which developmental processes operate in *Drosophila* (Lewis, 1978; Nusslein-Volhard & Wieschaus, 1980). Progress in understanding the role of developmentally active genes has rested on the isolation of multiple allelic forms and has served to re-emphasize the importance of obtaining non-null alleles (Stadler, 1954; Beckwith & Rossow, 1974). For example, particular revealing phenotypes associated with the *lin-12* locus of *C. elegans* or the *fused* locus of the mouse result only from the action of neomorphic or hypermorphic alleles (Greenwald *et al.* 1983; Greenspan & O'Brien, 1986).

We have begun a mutational analysis of the development of the zebrafish (*Brachydanio rerio*), a vertebrate generously amenable to genetic analyses (Streisinger *et al.* 1981, 1986) and attractive for developmental studies (Kimmel & Warga, 1986, 1988; Streisinger *et al.* 1989; Eisen *et al.* 1986). Previously we have described the induction of mutations in zebrafish by gamma irradiation (Chakrabarti *et al.* 1983; Walker & Streisinger, 1983). Comparison of the frequencies of recessive lethal and specific locus mutations led to the conclusion that most γ-ray-induced mutations were deficiencies or structural rearrangements affecting large portions of the genome. Genetic evidence that some of these mutations suppress meiotic recombination (Streisinger *et al.* 1986) in the region of the affected locus supports the interpretation that mutations generated in this manner are deletions or chromosomal rearrangements.

The purpose of the studies reported here and in the following paper was to develop methods for effective mutagenesis with agents known to induce predominantly point mutations. Ultraviolet (UV) light has been shown to induce point mutations, frameshift mutations, but rarely large deletions or mutations attributable to double-strand breaks in bacteriophage T4 (Drake, 1969). It has served as an effective mutagen in a variety of cell types including bacteria, yeast, and mammalian cells (deSerres, 1980; Maher & McCormick, 1980). Based on the experience of one of us in the field of bacteriophage mutagenesis (Streisinger *et al.* 1966) and the resemblance between phage and sperm both in structure and as obligatory parasites, we proposed that sperm might prove sensitive to the mutagenic effects of UV light, while providing little target for non-genetic cytotoxic effects. In this report we demonstrate that exposure of mature sperm to UV light is a simple and effective means to introduce germ line recessive lethal mutations in zebrafish.

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2. Materials and methods

(i) *Strains and mutations*

The origins of the standard strain and of the two clonal strains, C29 and C32, have been described (Chakrabarti *et al.* 1983). The *gol-1(b1)* and *alb-1(b4)* mutations, both of which are recessive and affect pigmentation, have also been described (Streisinger *et al.* 1986). Since they are the only mutant alleles at these loci used in the present study, they are referred to simply as *gol-1* and *alb-1*. Fish homozygous for these alleles were fully viable and expressed a defect in pigmentation easily observed at 2–3 days postfertilization in the pigmented retina of the embryo (Streisinger *et al.* 1989). The *alb-1* and *gol-1* loci are unlinked.

(ii) In vitro *fertilization*

Techniques have been described for the collection of gametes, standard fertilization of eggs *in vitro*, preparation of genetically impotent 'UV-treated' sperm used for activating eggs, and production of homozygous offspring from eggs (Streisinger *et al.* 1981; Chakrabarti *et al.* 1983). Briefly, to produce completely homozygous embryos: eggs were collected from individual females, activated with sperm rendered genetically impotent following exposure to a high dose of UV-irradiation, and incubated at 40 °C from 13 to 15 min postfertilization. This procedure, called heat-shock treatment (HS), yields diploid homozygous progeny by activating the replication of the maternal chromosomes and suppressing the first mitotic division.

Embryos were raised at 28·5 °C in embryo-rearing water [ER water = glass-distilled water containing 0·06 g l^{-1} sea salts (Instant Ocean, Aquarium Systems, Inc.)]. Developmental times in the text refer to hours or days postfertilization at 28·5 °C. In the present mutagenesis studies, immediately following ($\leqslant$ 15 s) fertilization of eggs with irradiated sperm, the eggs were placed in the dark, to avoid possible photoreaction effects (Novick & Szilard, 1949), where they remained for at least 3 h.

(iii) *Irradiation of sperm*

Sperm were expelled from anesthetized males and collected in cold Hank's saline (Streisinger *et al.* 1981; Chakrabarti *et al.* 1983) at a final concentration of approximately 5×10^7 ml^{-1}. The sperm solution ($< 0{\cdot}6$ ml) was transferred to an 8 cm watch glass supported by a bed of ice and was exposed to UV light from a 43 cm germicidal (Sylvania) tube. The dose rate experienced by the sperm was 65 ergs mm^{-2} s^{-1} as determined with a YSI-Kettering model 65 radiometer. Sperm were exposed for up to 10 s of continuous irradiation. Irradiated sperm were maintained on ice and used for fertilizations within 90 min following treatment.

3. Results

(i) *Dominant effects of irradiated sperm*

To optimize use of UV light as a potential mutagen in zebrafish, we investigated the effect of UV light dose applied to sperm on the ability of the sperm to activate wild-type eggs and support their normal development. Many mutagenic agents have been shown to cause dominant lethality in gametes (Alexander, 1954; Generoso, 1969). The viability of developing zebrafish embryos was depressed, in a dose dependent manner, when sperm were exposed to UV light prior to fertilization. Survival was measured in 7-day larvae by the development of the swimbladder, whose appearance was previously shown to be correlated highly with prospective survival (Streisinger *et al.* 1981). As Fig. 1 illustrates, no decrease in the viability of embryos was observed when up to 130 ergs mm^{-2} UV light were applied to mature sperm. Greater exposure to UV light resulted in a significant decrease in the normal development and survival of fertilized eggs. The data indicate that dominant toxicity is induced in sperm by UV light with greater than single hit kinetics. In contrast to its effect on embryonic development, the ability of irradiated sperm to activate eggs to initiate cleavage was not diminished even at the greatest dose tested (data not shown).

The genetic constitution of the egg appeared to influence the expression of potential dominant lesions in sperm. Preliminary experiments had indicated that exposure of sperm to approximately 300 ergs mm^{-2} UV light would provide a sensitive measure of induced dominant lethality (Fig. 1). When eggs from standard strain (non-inbred) fish were fertilized with sperm that had been treated in this sensitive range, we observed wide variation in the survival of eggs from different females. In addition, eggs of different genetic backgrounds (Fig. 1, opposing triangles) were fertilized with samples of the same irradiated sperm, collected from clonal strain males. The survival of fertilized eggs differed significantly between the two groups, eggs from C29 females being the least sensitive to the lethal effects of irradiated sperm. The genetic differences observed in these experiments have not been further investigated; in particular it is not known whether these differences would influence the frequency with which recessive lethal mutations would be induced.

(ii) *Somatic test for specific locus mutations*

The frequency with which mutations are induced at a specific locus may be estimated in a single generation somatic or 'spot' test. Measurements similar to the ones described here have been performed in *Drosophila*

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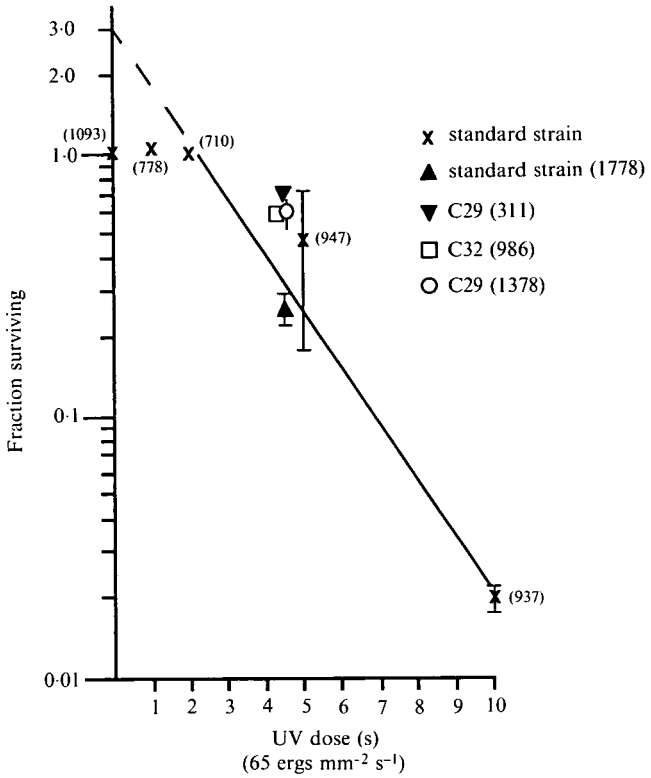

Fig. 1. Dominant lethality is induced in sperm by UV-irradiation. Wild-type sperm were exposed to UV light at a dose rate of 65 ergs mm^{-2} s^{-1} for varied periods of time prior to fertilization of eggs. The number of eggs that initiated cleavage (fertile eggs) in each experiment is presented in parentheses. Survival was calculated as the fraction of fertile eggs that developed swimbladders at 8 days, normalized with respect to the survival following 0 dose UV light. Each point represents mean survival of progeny of several females. Error bars (indicated where larger than the symbol itself) represent the standard error of the mean survival of the progeny of different females. Different symbols indicate independently performed experiments, with the exception that opposing triangles indicate the survival of eggs of differing origin that had been fertilized with a single population of mutagenized sperm.

(Alexander, 1954), mouse (Russell, 1951, 1977; Searle, 1974) and zebrafish (Chakrabarti *et al.* 1983). In these experiments mature gametes are mutagenized and examined for the inability to complement a defined recessive mutation or set of such mutations. In general the recessive alleles affect easily detectable morphological phenotypes, such as the extent or pattern of pigmentation. Two mutations that define unlinked loci in the zebrafish, *gol-1(b1)* and *alb-1(b4)*, are well suited for this purpose (Streisinger *et al.* 1986). Embryos homozygous for either of these mutations fail to produce normal pigment. This characteristic is most easily recognized in the pigmented epithelium of the retina, a sheet of cells that is normally black in the 2-day embryo and in which even single non-pigmented cells may be distinguished (Streisinger *et al.* 1989).

Zebrafish sperm were exposed to UV light and then used to fertilize eggs bearing recessive mutations at the *gol-1* and/or *alb-1* loci. The pigmented retinae of developing embryos were examined at 2 days for pigmentless tissue. We anticipated that this treatment would induce patches of pigmentless tissue in the retina (mosaicism). Since UV irradiation is known to induce lesions primarily affecting only a single strand of the chromosome (Drake, 1969), and since the pre-mutational DNA lesions induced by UV light may not be fixed immediately as frank mutations (Krieg, 1963), it seemed likely that only a fraction of the descendants of a sperm chromosome would harbour a new mutation.

The fraction of mosaic individuals present among developing embryos increased in a dose-dependent manner (Fig. 2). Sperm exposed to 130 ergs mm^{-2} UV light gave rise to a measurable fraction (0·5%) of mosaic embryos even though this sperm bore no detectable dominant toxicity (Fig. 1). At the highest dose tested, 650 ergs mm^{-2}, 9·6% of the embryos that developed to 2 days displayed patches of pigmentless tissue. Despite the increase in the proportion of mosaic individuals among embryos that survived to 2 days, the mutational yield (Haynes & Eckhardt, 1980), expressed as mutants recovered/mutagenized sperm tested by the fertilization of eggs, was highest following exposure to about 300 ergs mm^{-2} and decreased substantially at the highest dose examined. As illustrated in Fig. 2, the relation between dose and the induction of mosaicism appears to be biphasic, although further experiments are needed to clarify this issue. Omitting the data from the highest dose, the only dose at which a majority of embryos failed to survive until 2 days, the curve extrapolates to 1·05 at 0 dose, indicating first order kinetics for the induction of mosaicism at moderate doses.

UV irradiation of sperm led to mosaicism for the *gol-1* or *alb-1* locus with similar incidence. Eggs from females homozygous for *alb-1* and harboring either 1 or 2 copies of the mutant *gol-1* allele were fertilized with sperm that had been exposed to 292 ergs mm^{-2}. The incidence of mosaicism measured as mosaic embryos scored per mutant allele per egg was the same (2·1%) regardless of whether the *gol-1* mutation was segregating among the eggs (Fig. 2). A similar incidence of mosaicism per locus tested (2·4%) was obtained when eggs from *gol-1(b1)*/+ heterozygous females were fertilized with sperm that had received a slightly greater dose of irradiation. Thus, the presence of mutant *alb-1* or *gol-1* alleles in the eggs had a simple additive effect on the net yield of mosaic embryos, indicating that UV light affects both loci with equal probability. Similarly, sperm from clonal or standard strain males were equally sensitive to the effects of UV-irradiation as measured in the somatic test.

It is likely that the molecular events that lead to mosaicism originate on one strand of the DNA duplex of a chromosome in the sperm. UV light-induced lesions that would inactivate entire chromosomes or genes in the sperm would result in entirely golden

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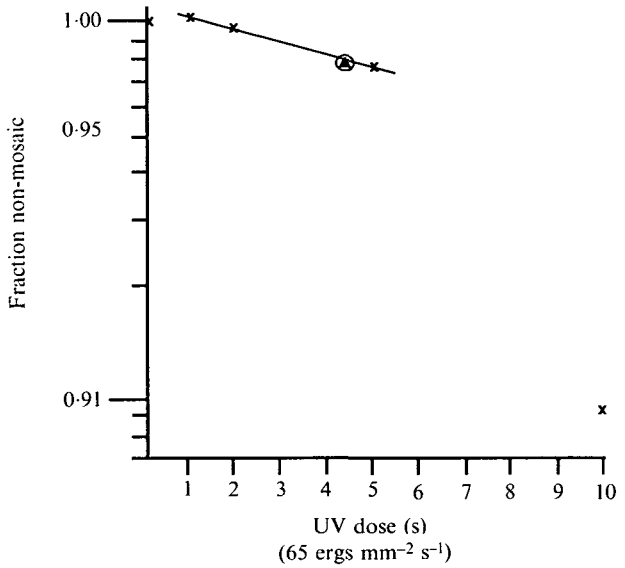

Fig. 2. Induction of somatic mutations by UV-irradiation. Eggs bearing recessive pigmentation alleles were fertilized by sperm that had been exposed for varied periods of time to UV light at a dose rate of 65 ergs mm^{-2} s^{-1}. At 2 and 3 days, embryos were examined for the presence of pigmentless cells in the pigmented retina. The fraction of non-mosaic embryos was calculated as: 1 − [fraction of mosaic embryos/average number of recessive pigmentation mutations present per egg]. ×, eggs were derived from *gol-1(b1)*/+females. The number of embryos scored: 0 dose, 936; 1 s, 739,; 2 s, 876; 5 s, 839; 10 s, 67. ○, Eggs from *alb-1(b4)/alb-1(b4)*; *gol-1(b1)*/+females: 4·5 s, 1056 embryos scored. △, Eggs from *alb-1(b4)/alb-1(b4)*; *gol-1(b1)/gol-1(b1)* females: 4·5 s, 1847 embryos.

offspring. Embryos that were entirely golden or albino appeared rarely following mutagenesis, representing less than 2% of the observed mosaics, and usually displayed morphological characteristics observed in haploid embryos.

The extent of mosaicism in the pigmented retina may be taken as representative of the mosaicism throughout the embryo since the pigmented retina of each eye is formed from a moderately large pool of approximately 40 precursor cells (Streisinger *et al.* 1989). The distribution of the fraction of pigmentless tissue in 65 mosaic embryos following sperm mutagenesis is presented in Fig. 3*a*. In the majority of the mosaic embryos ⩾ 25% of the pigmented retina consisted of mutant cells, and in greater than one-third of the embryos at least 50% of the cells in this tissue were pigmentless. Therefore, following mutagenesis of sperm, *gol-1* or *alb-1* cells were established at the 2-cell or 4-cell stage, i.e. following replication of the paternal chromosomes. These results are consistent with our understanding, derived from experiments with microorganisms, of UV light-induced mutagenesis and indicate that newly arising mutations induced by UV irradiation of sperm will be present among less than or equal to 50% of the paternally derived chromosomes.

(iii) *Induction of lethal mutations*

The ability of UV light treatment of sperm to induce germ-line lethal mutations was examined by two methods. To generate 'mutagenized G0' zebrafish, sperm from a homozygous clone of males (and therefore free of lethal mutations) were exposed to UV light and used to fertilize eggs from a related homozygous clone of females. The induction of germ-line lethal mutations was measured by comparing the viability of homozygous progeny of mutagenized G0 zebrafish with the viability of the homozygous progeny of control individuals. This test yielded an estimate of the average number of lethal mutations per egg of a mutagenized female. Second, the homozygous progeny of mutagenized females were examined for the presence of clusters of individuals that displayed a common aberrant morphological syndrome. The fraction of homozygous offspring of an individual female that exhibited a mutant phenotype represented the fraction of the germ line harbouring a particular

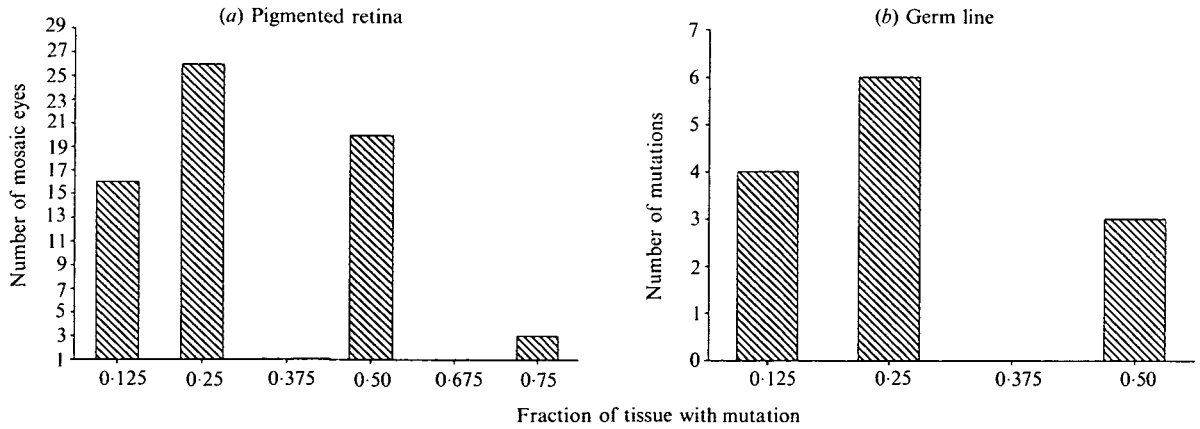

Fig. 3. Fraction of tissue harbouring a novel mutation following UV-irradiation of sperm. The fraction of the pigmented retina with pigmentless cells was determined by direct microscopic observation of mosaic eyes. The fraction of haploid germ cells with a novel mutation was determined as the fraction of homozygous progeny from individual G0 mutagenized females that exhibited a common developmentally aberrant phenotype.

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UV-induced mutation and therefore is informative regarding the ease with which UV-induced mutations may be recovered and propagated. In selected cases females that gave rise to clones of variant progeny were mated to wild-type males and the heritability of the syndrome was tested. Each of these methods has limitations for providing a measurement of the frequency of induced germ-line mutations, and both are likely to yield underestimates as discussed later.

In the first test, sperm from C29 males were exposed to 292 ergs mm^{-2} UV light prior to fertilization of eggs from C32 females. This dose of irradiation was chosen because it conferred only moderate dominant lethality to the sperm (Fig. 1) and it induced mosaicism efficiently in the somatic spot test. Embryos resulting from fertilization with treated sperm or with control sperm of the same origin were permitted to develop into mature adults. Eggs were collected from individual mutagenized or control G0 females, activated with genetically impotent sperm, and rendered homozygous and diploid by the HS treatment [see Section 2(ii)]. The fraction of eggs that initiated cleavage normally (fertility), the fraction of fertile eggs that developed into embryos with normal morphological appearance at 28 h, and the fraction of fertile eggs that yielded free-swimming larvae with swimbladders at 8 days is presented in Table 1.

Homozygous progeny of mutagenized females survived poorly overall as compared with offspring of control females. As reported previously (Streisinger *et al.* 1981) the HS treatment itself is damaging to the development of eggs: only 22% of eggs from control females, which lacked lethal mutations, survived. Although the viability of the homozygous progeny of mutagenized females varied between individual mothers, on average these progeny survived 56% as well as their control counterparts. This diminution of viability was not evident in the ability of eggs from mutagenized females to initiate cleavage (76% treated *vs.* 74% controls) nor in the apparent early development of the homozygous embryos as scored at 28 h (36% treated *vs.* 36% control 'normal appearing embryos'). In addition crosses between mutagenized females and wild type males yielded progeny of normal viability (data not shown). Thus mutagenized females were apparently of normal health, their eggs were of normal fertility and did not harbor a detectable number of dominant lethal mutations, and their homozygous offspring died due to recessive genetic factors affecting late embryonic and larval development.

An estimate of the average number of fully penetrant recessive lethal mutations (lethal 'hits') per egg of a mutagenized female zebrafish may be derived from the Poisson equation if we (1) note that surviving homozygous progeny do not have lethal mutations; (2) compare the fraction of eggs from mutagenized females that developed and survived with the survival of control eggs known not to harbor lethal mutations; and (3) assume that UV light-induced mutations are distributed normally among sperm. Hence the fraction of eggs from mutagenized females that had no lethal mutations was $0{\cdot}124/0{\cdot}223 = 0{\cdot}56$, and the average number, m, of recessive lethal mutations per haploid genome is 0·57 ($0{\cdot}56 = e^{-m}$). As indicated by the somatic test, UV light-induced events do not generally alter the genetic information on both DNA strands of a locus. Since the portion of the germ line occupied by a particular mutation is not known, the average number of mutations per germ line does not permit us to determine the total number of UV light-induced mutations; it is only a lower limit for the true number.

In a second series of experiments, sperm from clonal males were irradiated with UV light (292 ergs mm^{-2}) and used to fertilize eggs from lethal mutation-free clonal females. The resulting embryos were permitted to develop to adulthood. The germ lines of individual mutagenized females were then screened for distinctive recessive lethal mutations. Eggs from mutagenized and control females were subjected to the HS treatment and homozygous embryos were examined daily with the aid of a dissecting microscope for the presence of clusters of offspring that exhibited morphologically identifiable embryonic syndromes.

Among the homozygous progeny of 29 mutagenized females (representing a distinct group from that presented in Table 1), 13 clones of mutants with aberrant embryonic phenotypes were identified, whereas no mutant clones were observed among the progeny of 16 control females. Clones of mutants were recognized when 4 or more siblings presented a common phenotype and were further examined as live embryos using Nomarski optics. Six of the 13 mutant phenotypes were identified by the presence of large numbers of degenerating cells usually centered in the nervous system. In three of these clones cell death was largely restricted to the brain. Three additional clones had no evidence of cell death but displayed characteristic spinal abnormalities such as twisted trunks or a tail that formed improperly leading to the migration or leakage of groups of cells out of the rear of the tail. One of the clones developed small, poorly formed eyes, and another lacked eyes altogether. One of the clones was identified because it failed to exhibit spontaneous or responsive movements even though it resembled wild-type embryos superficially. A final class of mutants had well-developed axial structures with normal-appearing somites, muscle, notochord, spinal column, and circulatory system, but had a shortened tail and no head nor any discernible brain structures.

Since the homozygous diploid progeny resulting from the HS treatment directly reflected the genotype of the maternal germ line, the fraction of the germ line that harboured a particular mutation was the same as the fraction of offspring that displayed a characteristic mutant syndrome. As presented in Fig. 3*b*, six of the mutant clones represented approximately $\frac{1}{4}$ of the offspring, four clones represented approximately $\frac{1}{8}$ of

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Table 1. *Survival of homozygous progeny of control and UV light-mutagenized zebrafish*

	Fertility[a] (no. of fertile eggs)	Normal morphology[b] at 28 h	Survival[c] at day 8
Controls			
1	0·68 (151)	0·44	0·24
2	0·52 (98)	0·40	0·26
3	0·83 (203)	0·61	0·45
4	0·49 (134)	0·60	0·32
5	0·83 (178)	0·43	0·21
6	0·82 (116)	0·18	0·12
7	0·72 (161)	0·13	0·07
8	0·84 (86)	0·05	0·02
9	0·82 (256)	0·30	0·20
10	0·80 (78)	0·22	0·12
11	0·85 (173)	0·57	0·39
12	0·86 (150)	0·49	0·33
13	0·77 (148)	0·29	0·17
14	0·63 (179)	0·51	0·35
15	0·66 (185)	0·25	0·10
Total[d] ($n = 15$)	0·74 ± 0·03	0·36 ± 0·04	0·223 ± 0·033
UV-treated			
1	0·83 (236)	0·53	0·13
2	0·78 (175)	0·57	0·09
3	0·70 (155)	0·48	0·18
4	0·49 (53)	0·11	0·02
5	0·70 (192)	0·33	0·19
6	0·56 (85)	0·32	0·13
7	0·77 (206)	0·36	0·14
8	0·82 (230)	0·40	0·19
9	0·85 (271)	0·37	0·16
10	0·90 (328)	0·20	0·09
11	0·68 (144)	0·20	0·03
12	0·82 (164)	0·32	0·08
13	0·80 (61)	0·39	0·26
14	0·83 (208)	0·38	0·03
15	0·86 (125)	0·36	0·08
16	0·74 (68)	0·40	0·19
Total[d] ($n = 16$)	0·76 ± 0·03	0·36 ± 0·03	0·124 ± 0·017

[a] Measured at 2–4 h postfertilization as the fraction of eggs that initiated cleavage divisions. The total number of fertile eggs is indicated in parentheses.
[b] Fraction of fertile eggs that appeared as morphologically normal embryos at 28 h postfertilization.
[c] Fraction of fertile eggs that developed into free-swimming larvae, measured by the appearance of the swimbladder at day 8 postfertilization.
[d] Mean survival of progeny of different females ± S.E.M. Only survival at day 8 was significantly different between controls and UV-treated ($P < 0·01$, Mann–Whitney U test).

the offspring, and 3 clones each comprised $\frac{1}{2}$ of the homozygous progeny. These numbers have practical value for predicting the ease of recovery of novel mutations following mutagenesis of sperm with UV light. However, they may not fully depict the total number of induced mutations nor their true distribution in the germ line or soma for several reasons. In the screen described above, mutations present in a small fraction of the eggs would be more likely to escape detection than would mutations that occupied a large portion of the germ line. Similarly, mutations that did not result in grossly altered morphology would have been missed. In addition, the recovery of mutations and their distribution in the germ line would be influenced by the number and genotype of pregonial cells that were selected to form the germ line, a number that is often very small (Walker & Streisinger, 1983). Nevertheless these findings indicate that in general, $\frac{1}{4}$ of the chromosome sets in the germ line harboured a particular mutation. Thus most of the lesions induced in the DNA of sperm by irradiation with UV light result in mutations harboured by about $\frac{1}{2}$ of the paternally derived chromosomes of a mutagenized individual.

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The heritability of the mutant phenotypes was tested by further breeding of 4 females that had given rise to homozygous progeny among which 5 mutant clones had been observed. Each female was mated with clonal males, and their G1 progeny were raised to adulthood. The inheritance of the mutation was examined by progeny testing of the G1 females. Eggs from G1 females were made diploid and homozygous by the HS treatment, and embryos were examined for the presence of mutant clones representing approximately 50% of the embryos. Each of the original mutagenized females had G1 daughters that inherited the original mutation, and 4 of the 5 characteristic syndromes were observed among the homozygous G2 progeny. It is possible that further progeny testing would have revealed inheritance of the final mutation, which was expected in only 12% of the G1 daughters, since only 2 potential carriers were screened for this mutation. The morphological screen is thus sufficiently conservative to be a reliable method for the identification of novel mutations that may be propagated and recovered in further generations.

4. Discussion

(i) *Induction of recessive lethal mutations*

The results presented here demonstrate that UV irradiation is an efficient mutagen in mature sperm of zebrafish. Comparison of the viability of the homozygous offspring of mutagenized females with that of the homozygous progeny of appropriate control females permitted us to calculate that each gamete of an adult zebrafish resulting from the union of wild-type eggs with sperm that had been exposed to 292 ergs mm^{-2} UV light harboured, on average, 0·57 recessive lethal mutations. Examination of the distribution of mutant cells in both somatic and germ tissue indicated that UV light-induced mutations were expressed in less than or equal to $\frac{1}{2}$ of the chromosomes that were descendants of the paternal genome, or $\frac{1}{4}$ of the germ-line chromosome sets. Thus, consideration of the portion of the germ line occupied by a particular recessive lethal mutation leads to the conclusion that zebrafish mutagenized in this manner harbour 0·57 (lethal hits per gamete) × 4 (mutations per lethal hit per gamete) = 2·3 lethal mutations in their germ line. The estimate represents a lower limit and would be augmented if a considerable fraction of the induced mutations were represented by less than $\frac{1}{2}$ of the paternally derived chromosome sets.

Four of 5 mutations that were first identified by the presence of clones of morphologically aberrant embryos among the homozygous progeny of 4 mutagenized females were found to be heritable and displayed segregation patterns among G0 progeny consistent with a single locus origin for each. Failure to recover the fifth mutation is probably due to the fact that only two G1 potential carriers were screened for this mutation. Alternatively, this mutation might have been a dominant lethal, an unexpected result since the majority of UV light-induced mutations would be fixed by the 2- or 4-cell stage of the original mutagenized parent and hence are likely to express their lethal effects in the G0 generation. The recognition of phenotypically aberrant offspring among the homozygous progeny of UV light-mutagenized females is thus a valuable method for identifying recoverable mutations in zebrafish.

We observed 13 mutant clones among the homozygous progeny of 29 mutagenized fish (0·45 mutations recovered per G0 mutagenized individual), representing five-fold fewer mutations than would have been expected from the viability experiments. A likely reason for this difference is that recognition of mutant clones with anatomically distinctive features is a conservative method for the identification of newly arising lethal mutations. Since even the lethal mutation-free embryos survived poorly following HS treatment (Table 1), it would have been difficult to detect lethal phenotypes that were not spectacular. In addition, mutations that were present as a small fraction of the germ line would be preferentially missed in this screen.

Although the studies described in this report represent but a preliminary set of UV light-induced mutations in zebrafish, some features of the mutants are noteworthy. In the experiments designed to investigate the relative viability of homozygous offspring of mutagenized females, we examined the ability of eggs to proceed through blastogenesis and gastrulation and to form normal-appearing 28 h embryos, a superficial test for the normal development of axial structures, somites, head, and brain morphology. In general, homozygous embryos from mutagenized females could not be distinguished from their wild-type counterparts at these early stages of development (Table 1). Rather, lethal effects were exhibited during late embryonic and larval development. Our results are consistent with the interpretation that only a small fraction of zygotically active genes contribute to establishing the body plan of the embryo. In *Drosophila* only a few per cent of the genes that are active post-zygotically are essential for establishment of pattern formation in the early embryo (Nusslein-Volhard & Wieschaus, 1980). This has been interpreted as indicating a substantial maternal contribution to early embryogenesis. Although little is known about the extent to which the maternal genotype contributes to early vertebrate development, it is clear that some maternally supplied factors profoundly influence developmental decisions such as specification of the embryonic axis (Gerhart *et al.* 1989).

Among the 13 mutant clones that were observed, approximately $\frac{1}{2}$ displayed characteristic patterns of tissue degeneration usually restricted to portions of the central nervous system. We have observed similar phenotypes in a large fraction of γ-ray-induced and

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ethyl nitrosourea-induced mutants (Grunwald *et al.* 1988; Walker, Kimmel, Grunwald and Streisinger, unpublished observations). This phenotype may simply reflect the active destruction of malformed structures. However it may also indicate that in vertebrates the *maintenance* as well as the differentiation of neurons may require the action of a substantial number of genes. Maintenance might be dependent on the presence of several other cell types and factors (Berg, 1984).

(ii) *Induction of specific locus somatic mutations*

Eggs bearing mutant alleles at the *gol-1* or *alb-1* locus that were fertilized with UV-irradiated sperm developed into mosaic embryos comprised of both pigmented and pigmentless tissue. In principle, pigmentless cells may arise from at least three distinct origins: (1) somatic recombination between homologous chromosomes resulting in the daughter cells homozygous for pre-existing mutant alleles; (2) nondisjunction or loss of part of a paternal chromosome in some cells leading to aneuploid descendants that are hemizygous at either *alb-1* or *gol-1*; or (3) newly arising mutations at the *alb-1* or *gol-1* locus. Although we have not investigated the genetic origin of the mutant cells, our results indicate that symmetrical exchange between homologous is not the primary source of mosaicism. Such recombinational events would be expected to affect different loci on the basis of their positions with respect to a centromere. Since *alb-1* is genetically close to its centromere and *gol-1* is genetically distant from its respective centromere (Streisinger *et al.* 1986), the observation that UV light-induced alterations leading to the formation of pigmentless cells occur with equal probability at the *gol-1* and *alb-1* loci excludes an origin influenced strongly by gene–centromere distances. Furthermore, in the accompanying report, we show that mutagenesis of sperm with ethyl nitrosourea (ENU) also yields mosaic embryos in a similar somatic spot test (Grunwald & Streisinger, 1991). In those studies, we demonstrated that wild-type chromosomes needed to be exposed to the mutagen in order to obtain somatic mosaics at the observed incidence, a result indicating that somatic mosaics did not arise from symmetrical recombination events.

(iii) *Comparison of specific locus and recessive lethal mutation frequencies*

Newly induced germ-line mutations at *gol-1* or *alb-1* are likely to be expressed also in the pigmented retina since it is a valid representative of the genetic constitution of the embryo: most, if not all, of the early blastomeres contribute descendants to the tissue (Streisinger *et al.* 1989). In contrast the germ line is formed from only a few precursor cells (Walker & Streisinger, 1983) and many mutations that are expressed in somatic tissue may not participate in the germ line. We have shown that approximately 10% of the ENU-induced mosaics that develop to adulthood bear in their germ line a newly induced allele at the *gol-1* locus (Grunwald & Streisinger, 1991). If we assume that the fraction of surviving mosaic individuals with novel germ-line mutations at *alb-1* or *gol-1* is similar after mutagenesis with ENU or UV light, then we may estimate the fraction of mutagenized adults that harbour germ-line mutations at a specific locus.

Following UV light mutagenesis, the fraction of mosaic embryos that survived to the swimbladder stage was only 0·40 that of their pigmented siblings. Thus exposure of sperm to 292 ergs mm^{-2} UV light (Fig. 2) would be expected to yield surviving progeny of which 0·84% (2·1% × 0·4) would be mosaic. Approximately 10% of these survivors, or 8 out of every 10000 G0 adults, mutagenized by UV treatment of sperm, would be expected to harbour a new germ-like mutation at *alb-1* or *gol-1*. We can compare the incidence of recessive lethal mutations induced per mutagenized adult with our estimate of the incidence of specific locus mutations per mutagenized adult following exposure of sperm to 292 ergs mm^{-2} UV light. For these purposes we shall estimate the number of embryonic lethal mutations per mutagenized adult as 2·3. The ratio of these frequencies, which is an estimate of the number of essential genes that contribute to embryogenesis in zebrafish, is $2{\cdot}3/8 \times 10^{-4} = 3000$. This estimate is further discussed in Grunwald & Streisinger (1992).

(iv) *Effect of the genotype of eggs*

Differences in egg genotype were shown to influence the expression of dominant lethal effects induced in sperm by UV irradiation. Repair pathways have been shown to operate in eggs of mice (Generoso *et al.* 1979), and the degree of repair efficiency following damage of DNA with UV light is known to vary according to genotype in cells from lower as well as higher eukaryotes (deSerres, 1980; Maher & McCormick, 1980). Since the molecular basis of the dominant lethal effects observed in these studies is not known, we do not know whether variations in egg genotype may also influence the frequency with which germ line recessive lethal mutations would be induced following exposure of sperm to UV light.

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