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Previous studies have indicated that the *pineapple eye* gene may encode a survival factor important for regulating cell number in the *Drosophila* eye. We have proposed to isolate the *pineapple eye* gene and characterize its role in survival. In the first year we have achieved our goals of identifying a mutation in the *pineapple eye* gene that is associated with a cytologically and molecularly detectable rearrangement. Genomic DNA encompassing this region has been isolated and mapped, and defines the minimal region wherein the *pienapple eye* gene must reside.

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CELL PROLIFERATION, CELL DEATH, AND SIZE REGULATION

INTRODUCTION

Cell proliferation and cell death come to attention through their roles in cancer and in degenerative diseases, but their normal function is in biological development. Cell death and division must be central features of biological size control, a fundamental property of organs and organisms about which almost nothing is known. In vivo, rates of cell division and of cell death must be linked to the current size of the organ and of the organism. The mechanisms by which this occurs have not been discovered.

A genetic approach might be the best way to investigate regulation of cell number in vivo. Drosophila could provide the ideal multicellular animal model. We have been investigating regulation of cell number in the developing Drosophila eye, and have isolated mutations in a gene that reduce cell number. These identify a candidate gene for size regulation in vivo.

Each Drosophila compound eye comprises a precise array of about 800 ommatidia ("unit eyes"). Each ommatidium is constructed by 22 cells. Individual cell types are determined after a nearly random proliferative phase with no detectable role of lineage (1)(2)(3). Each ommatidium arise through a sequence of post-mitotic single-cell inductions, each successive cell inducing the next(4)(5)(6). How can the undifferentiated retinal epithelium contain a multiple of 22 cells, prior to their specification by induction? A clue came from studies of mutants in which too few ommatidia form. In normal development, the first five cells of each ommatidium are specified during an arrest in G1 of the cell cycle. Remaining cells re enter the cell cycle, and are recruited to form the remaining ommatidial cells after another division (1)(4). In mutants where very few ommatidia initiate differentiation, all the remaining cells reenter the cell cycle, but almost all then arrest in G2. Exceptions are cells in contact with the few differentiating ommatidia, which proceed through mitosis. This established the existence of an inductive signal from the first five cells of each ommatidium, permitting nearby cells to pass the G2/M checkpoint (7). The supernumerary, G2 arrested cells subsequently undergo cell death. We further showed that such death occurs even if supernumerary cells were arrested in the prior G1 phase, and so was not dependent on the particular cell cycle stage (our unpublished results). This indicated that the first five cells of each ommatidium also provide a survival signal for nearby cells. We therefore concluded that the first five cells of each ommatidium control cell number by "counting" the cells in their neighborhood, through local control of cell division and cell survival (7).

We performed mutagenesis experiments to identify mutations affecting Drosophila eye development(8). A recessive mutation mapping at 43 centimorgans along the second chromosome has "rough" eyes and was named *pineapple eye (pie)*. In *pie* mutants the retina has too few cells, because of excess cell death during eye development. Therefore the *pie* gene is a candidate to encode a component of the hypothesized survival signal. We proposed to clone the *pie* gene and characterize the role of its product.

EXPERIMENTAL METHODS

Results obtained during the first year have led to some changes in the experiments performed, although the overall strategy remained similar.

The original scheme was to use the location of the pie gene at chromosome band 32A to isolate new mutant alleles associated with chromosome breakpoints. The scheme proposed involved using transgenic strains bearing genetically visible insertions in the vicinity of 32A to screen for chromosomal deletions and rearrangements in the 32A region as well as for new alleles of pie. These would then be used to locate the pie genomic region in DNA cloned by the Drosophila genome project. This is essentially what we have achieved, although the route taken has been different from that anticipated.

RESULTS

Early on we discovered that the initial mapping of the pie gene to chromosome band 32A was incorrect. This error arose because one of the deficiency strains used for mapping, *Df(2L)Mdh 30D-F;31F* in fact did not contain the indicated deficiency. We were able to reassign the pie gene to the nearby region 31E-F. This led us to use different transgenic lines as the substrate for X-ray mutagenesis of the 31E-F region, instead of region 32A.

66,500 flies derived from X-irradiated germ cells (4000 rads) were screened for rearrangements and for failure to complement the original $pie^{EB3}$ allele. Several individuals apparently carrying new pie mutations were recovered, but in no case did such individuals breed successfully, so lines carrying the putative new alleles could not be recovered.

In subsequent experiments we concluded that the $pie^{EB3}$*/Deficiency* genotype is in fact semilethal. That is, while some individuals of this genotype do survive and exhibit the characteristic pie phenotype, most die before emerging from the pupa. Those adults that do survive are extremely sickly, the females are invariable sterile and the males breed very poorly.

On the one hand, the properties of *pie/deficiency* flies rendered our proposed scheme for isolating new *pie* mutant alleles from adult flies problematic. On the other hand, it raised the possibility that new *pie* alleles could more readily be obtained through screening for a lethal phenotype. Fortunately, such a genetic screen for lethal mutations in the 31E-F region had already been reported by Grigliatti and colleagues (9). We obtained all their published lethal strains and determined that pie was allelic to a "lethal" complementation group, l(2)31Ek. Six more alleles of pie thus became available to us through this reassignment. One of these alleles, $l(2)31Ek^{G2-4}$, had been induced after X-ray mutagenesis. Thus, the original goals of our mutagenesis plan, to precisely define the pie locus and obtain X-ray induced mutations, were attained through a different mechanism than the anticipated one.

Genomic clones from the 31E region had been found amongst cosmid clones collected by the European component of the Drosophila genome project (10). We established a contig of three overlapping cosmid clones that extended from a Sequence-Tagged Site corresponding to the *daughterless* gene, which was determined to map distal to the pie locus,

to the end of the *Df(2L)J77* deficiency, determined to map proximal to the pie locus. Thus, *pie* genomic DNA must lie within this interval of ~ 120 kb.

We have restriction mapped this 120 kb region. Genomic Southern blotting with DNA from the *l(2)31Ek$^{G2-4}$* mutant identified a breakpoint at position +60 which is not present in the progenitor strain in which this mutation was induced. Further molecular and cytological analysis revealed that *l(2)31Ek$^{G2-4}$* was associated with a chromosomal inversion, which we characterized as *In(2L)31E-32F*.

DISCUSSION

Our original goals for the first 12 months of the project, as outlined in the statement of work, were: 1) to perform an X-ray screen for rearrangements and mutations in the *pie* region; 2) Perform cytological analysis of such chromosomes; 3) Establish an ordered set of DNA's from the region; 4) Begin locating DNA important for *pie* gene function. All these goals have been achieved, although the experiments took a different course from that anticipated.

Our X-ray screen and other genetic experiments determined that *pie* was located in chromosome region 31E-F, not 32A, and that the *pie/Df* phenotype was semilethal. Using this information we located novel mutant alleles of pie amongst the products of an unrelated genetic screen (9). One of these mutations is caused by a chromosomal inversion between the 31E region and 32F. An ordered set of DNA's has been established that spans the region defined genetically to include the *pie* locus, and the 31E inversion breakpoint mapped within this contig. This therefore maps the genomic DNA important for *pie* function.

Within the next 12 months we will proceed with the isolation of the *pie* gene exactly as envisaged in the original statement of work. Using Northern blotting and cDNA library screening, transcription units in the pie-critical region will be identified and mapped. Candidate pie transcription units will be sequenced. The transcription unit responsible for *pie* mutant function will be identified by the criteria that it should be altered in each of seven independent *pie* mutations now available to us, and/or by rescue of the *pie* mutant phenotype by transformed genomic DNA. Results from these experiments will leave us poised to investigate the mechanism of *pie* gene function and its role in cell survival during the third year of the grant.

CONCLUSIONS

A genomic region likely to contain the *pie* gene has been mapped.

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