Cell Clones and Pattern Formation: Studies on sevenless, a Mutant of Drosophila melanogaster

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Summary. sev^{LY3}, the only existing allele at the sev locus (1-33,2 ± 0,2), behaves as strongly hypomorph or even as amorph. Ommatidia in a sev compound eye have only seven receptor cells, the position of the R7 pattern element being vacant. Various criteria showing that the missing cell is R7 have been verified. These include (i) anatomical characteristics of sev ommatidia; (ii) behaviour of central R cells in sev rdgB double mutants; (iii) medulary projection of central R cell axons; and (iv) mitotic pattern of sev imaginal discs. The analysis of morphogenetic sev-sev^{+} mosaics has shown that sev is expressed autonomously by R7 cells, indicating that the sev phenotype is not due to a sev genotype of ommatidial pattern elements other than R7. The study of third instar sev imaginal discs has not brought any direct evidence for death of clustered presumptive R7 cells; however, clonal analysis of the developing sev compound eye has given evidence of developmental parameters comparable to those of sev^{+}, therefore favouring the hypothesis that R7 cells die in sev mutants. On the other hand, sev^{+} seems to be required for the determination of the R7 cells, since the sev phenotype cannot be uncovered during the last mitoses of heterozygous mutant cells.

Key words: Compound eye – Development – Determination of R7 cells – sevenless mutant analysis – Drosophila.

Introduction

One of the central problems in developmental biology concerns the mechanism generating spatial patterns which are expressed by the regular arrangements of different cell types. The compound eye of Drosophila melanogaster provides a particularly suitable model system for the analysis of pattern formation since it is essentially a repetitive array of an elementary pattern, the ommatidium, which consists of only a few cell types representing some 20 singly identifiable
pattern elements. In the mutant sevenless (sev), isolated by Benzer and co-workers (see Harris et al., 1976), the site of one particular photoreceptor (or retinula, R) cell, R7, is empty while the remainder of the ommatidium appears normal. The sev ommatidia, therefore, contain seven instead of the normal complement of eight receptor cells. Provided that the sev phenotype can be attributed to the absence of identical cells, and this lack does not result from failures in the process of cytodifferentiation, the developmental analysis of the sev mutant might yield information about the origin of a particular pattern element of the compound eye, and hence of cellular diversity in the ommatidium.

We have studied in detail the phenotype of the sev mutant and the development of the sev compound eye to assess the identity of the missing receptor cells. We also report experiments on the means by which the sev pattern originates during development.

Material and Methods

Strains. The following strains of Drosophila melanogaster have been used for the present study. Wild-type: OregonR. Mutants: w (1-1.5), sevL73 (1-33.2 ± 0.2), rdgBKS222 and rdgBE170 (1-42.7 ± 0.6), M(1)oSy (1-56.6), Df(1)vL3, Df(1)vL2, Df(1)RA37 and Df(1)N71. sev, rdgBKS222 and rdgBE170 were kindly provided by S. Benzer, M(1)oSy by A. Garcia-Bellido and D(1)vL3, Df(1)RA37 and Df(1)N71 by J. Hall. w sev, w rdgBKS222, w rdgBE170, sev rdgBKS222, sev rdgBE170 and w sev rdgBE170 chromosomes were made by recombination.

rdgBKS222 and rdgBE170 are different alleles at the rdgB locus, isolated by Hotta and Benzer (1970; Benzer, personal communication). Both alleles are recessive and produce in hemi- or homozygous condition degeneration of photoreceptor cells. In rdgBKS222 R1 to R6 cells completely degenerate within a period of 7-10 days, being phagocytosed by secondary pigment cells, whereas the remaining cells of the ommatidium are normal. In rdgBE170 degeneration also affects about 70% of all R7 as well as a few R8 cells. The degenerative process is induced in both mutants by light of a given wavelength and requires about 7-10 days to be accomplished. Room temperature of 25-30°C accelerates the process (Harris and Stark, 1977).

Two different techniques, reduced corneal pseudopupil and optical neutralisation of lenses (Franceschini and Kirschfeld, 1971; Franceschini, 1972), enable a rapid observation of the rhabdomeric pattern to be made. The pseudopupil of living flies was observed using a Wild M-8 binocular stereomicroscope provided with dark field illumination. Optical neutralisation was achieved by means of immersion oil on eyes of half-head preparations. Subsequent observation of the pattern of rhabdomeres was performed with a Leitz-Orthoplan light microscope at 250× magnification. Both techniques were very useful during the synthesis of the chromosomes mentioned above.

Genetic labelling was induced with different doses of X-rays (see Table 1) (100 Kv, 15 mA, 20 cm distance, 0.7 mm Al filter) in the larvae indicated in Table 1. In order to allow complete degeneration of homozygous rdgB cells, rdgBKS222 and rdgBE170 homo- and heterozygotes irradiated as larvae were kept for 14 to 20 days in a room at 28°C, 65% humidity and constant light covering the range between 360 to 590 nm. A variable number of compound eyes from each one of the crosses (see Table 1) was processed for histology.

3H-thymidine autoradiography. Two different kinds of 3H-thymidine labelling have been used. In the first, brain and eye-antennal imaginal discs of OregonR and sev mid-third instar larvae were dissected out and incubated "in vitro" in a bath containing 0.5 ml Schneider's medium and 1 μl 3H-thymidine (spec. act. 21 Ci/mM) for 30 min. After incubation the preparations were washed in Drosophila-Ringer and fixed for histology. In the second, mid-third instar OregonR and sev larvae were injected with a micropipette with about 0.1 μl of 3H-thymidine (conc. 1 Ci/ml). The injected larvae were allowed to complete development. After eclosion the compound eye of the flies was fixed, embedded in Durcupan (Fluka) and serial sections of the eye were processed for autoradiography as described in Campos-Ortega and Gateff (1976).