

CELLULAR DEGENERATION IN WING DEVELOPMENT OF THE MUTANT VESTIGIAL OF *DROSOPHILA MELANOGASTER*

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INTRODUCTION

The mutant vestigial (*vg*), in which only the basal part of the wing is present, is one of the most familiar and intensively studied mutants in *Drosophila melanogaster*. Goldschmidt (1935) and Waddington (1940) made observations on the development of the *vg* wing but arrived at different conclusions as to the way in which the *vg* phenotype was produced. Goldschmidt proposed that the reduction of the wing was a consequence of degradation of specific parts of the wing during development. Waddington, alternately, concluded that the missing regions of the wing simply failed to develop. Both of these studies, however, were done with the light microscope, and neither author detected any histological evidence of degeneration. An electron microscope study was undertaken to establish unequivocally whether degeneration occurs in the developing *vg* wing.

MATERIALS AND METHODS

Wing discs from wild type and *vg* late third instar larvae were dissected in *Drosophila* Ringer's and fixed for 1 hr at 4°C in a solution of 1% glutaraldehyde and 1% osmium tetroxide in 0.05 M sodium cacodylate buffer at pH 7.4. The discs were then dehydrated in ethanol and embedded in Epon. 1 μ sections for light microscopy were stained with methylene blue, and ultrathin sections for electron microscopy were stained with uranyl acetate and lead citrate.

RESULTS

A wing disc of a mature larva appears as a flattened, roughly circular structure composed of many small, undifferentiated cells. During metamorphosis these cells grow to form the wing and dorsal part of the thorax. Previous authors (e.g. Chen, 1929; Auerbach, 1936) observed that the part of the disc destined to form the wing blade is separated from the thoracic region by a pro-

nounced fold. In this study it was found that the presumptive wing blade, unlike the rest of the disc, contained numerous lipid inclusions.

The cells of the normal wing disc are typically embryonic with large numbers of free ribosomes, little endoplasmic reticulum, poorly developed Golgi apparatus, and small mitochondria with few cristae. In *vg* wing discs there are numerous bodies which have not been observed in normal discs. Many of these unique structures can be identified as degenerating cells (Fig. 1). They are concentrated in the lipid-containing, wing blade-forming region of the disc. They have also occasionally been observed in the presumptive thoracic area of wing discs and in leg discs of *vg* (at about 2% of the frequency in the wing anlagen).

The process of degeneration appears to involve a shrinkage and condensation of the entire cell followed by phagocytosis by a neighboring cell. After it has been engulfed, the degenerating cell is surrounded by two membranes, its own plasma membrane and that of the surrounding cell (Fig. 2). The cytoplasm and nucleus become further condensed, and the nucleolus forms a large homogeneous mass. Eventually, the plasma membrane of the degenerating cell breaks down, and the organelles begin to cytolize. The chromatin clumps, the nucleolus develops vacuolar spaces, and the outer nuclear membrane and its ribosomes are lost. The cytoplasmic ribosomes aggregate and then disappear. Other organelles become unrecognizable, and large myelin figures appear. Thus, at an advanced stage of degeneration, the cell appears as an intracellular inclusion bounded by a single membrane (the plasma membrane of the surrounding cell) and containing organelles in various stages of disintegration. Only the possession of a nucleus identifies it as a cell (Fig. 3). There are also a number of intracellular inclusions which do not contain any sign of nuclear remains. These are possibly autophagic vacuoles.

Several observations support the contention that the cellular degeneration in the developing wing ultimately results in the *vg* phenotype. It has long been known that the temperature during development influences the expression of *vg* (Roberts, 1918; Stanley, 1931); 18°–25°C produces the extreme *vg* wing, whereas 29°–31°C results in a relatively normal wing. In the present study, when *vg* flies were grown at 18° or 25°C, then, as mentioned above, a large number of degenerating cells

(20–30 per transverse section) was concentrated in the presumptive wing blade region of the third instar disc. Indeed, this part of the disc is already much smaller in size than normal, containing approximately one-third the number of cells in the corresponding region of a wild type disc of the same age. When *vg* flies were grown at 30°C, much smaller areas of the adult wing were missing and the wing disc was also more normal in appearance. Transverse sections through the presumptive wing blade contained only two to four degenerating cells per section. Hence, growth at 30°C results in both an increase in wing size and a decrease in the incidence of degenerating cells.

DISCUSSION

The occurrence of degenerating cells in vestigial wing discs, the concentration of these cells in the region of presumptive wing blade, and the correlation between the number of degenerating cells and the degree of expression of *vg* indicate that the vestigial phenotype results from a degeneration of specific regions of the developing wing. This is, in essence, what Goldschmidt proposed in 1935.

In other degenerating tissues, e.g. insect salivary glands during metamorphosis (Rasch and Gawlik, 1964; Schin and Clever, 1968) and rat prostate after castration (Swift and Hruban, 1964), the process of degeneration appears to be mediated by the activity of lysosomes, either by the release of their enzymes into the cytoplasm or by the formation of autophagic vacuoles. Although bodies resembling autophagic vacuoles have been observed in *vg* discs, it is not clear what part they play in the over-all process of involution. Earlier stages in development are now being studied, in the hope that they will provide information on the cellular processes leading to degeneration.

The detail with which the genetics and biology of *Drosophila* are known makes it a useful organism in which to study the molecular and cellular events that initiate degeneration. In addition to the mutant *vg*, there are other mutants in which part of the wing is missing (e.g. *cut*, *Beadex*, *apterous*) which may also involve degeneration. An electron microscope study of the developing wing discs of these mutants may be instructive.

SUMMARY

Wing discs from mature third instar larvae of wild type *Drosophila melanogaster* and the mutant strain

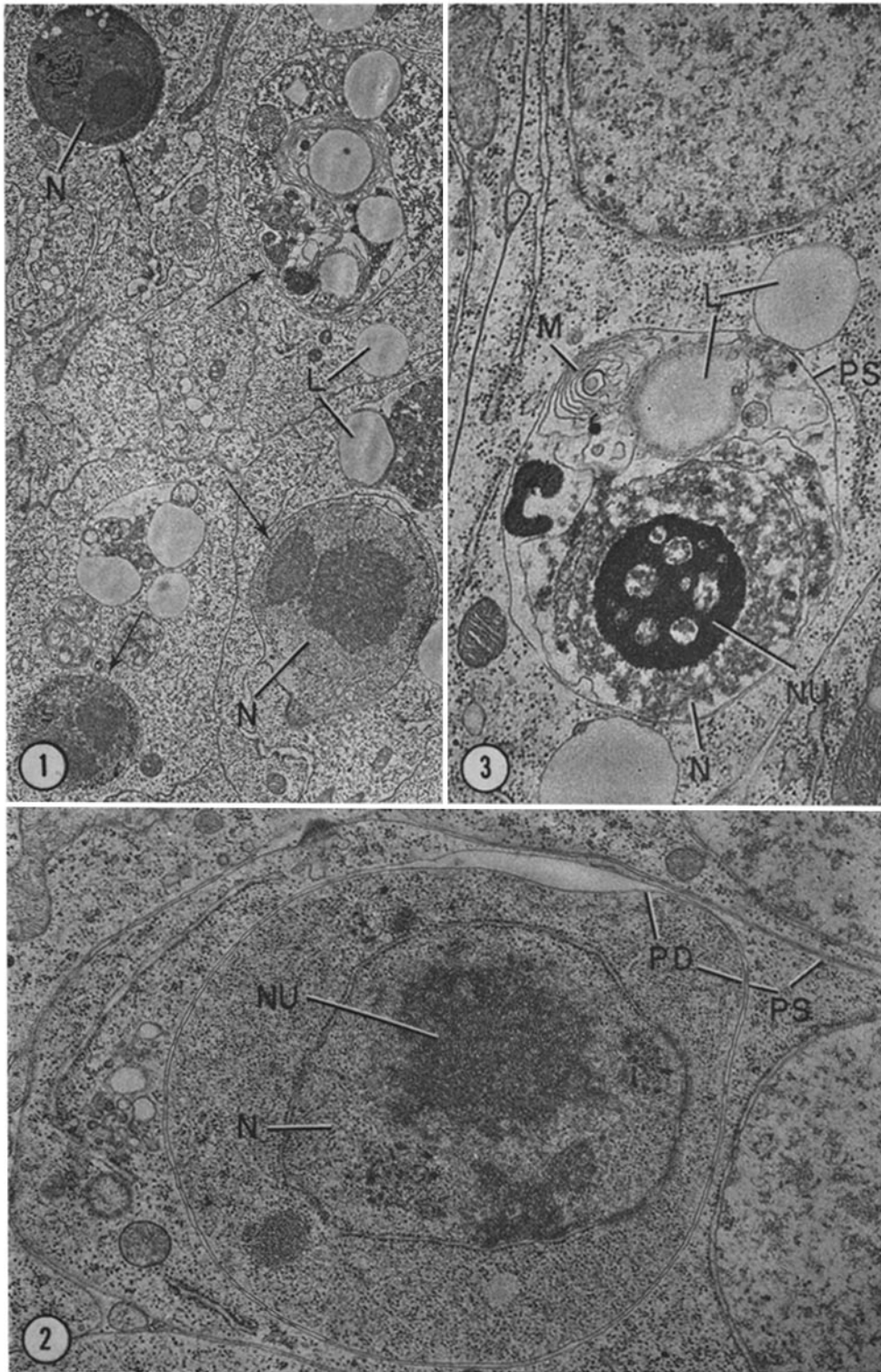


FIGURE 1 Degenerating cells (arrows) in the presumptive wing blade region of a vestigial wing disc. Some cells are sectioned through the highly condensed nucleus (*N*). *L*, lipid droplet. $\times 14,000$.

FIGURE 2 A cell in an early stage of degeneration engulfed by another cell. *N*, nucleus; *NU*, nucleolus; *PD*, plasma membrane of degenerating cell; *PS*, plasma membrane of surrounding cell. $\times 24,500$.

FIGURE 3 A cell in an advanced stage of degeneration. The limiting membrane is the plasma membrane of the surrounding cell (*PS*). Note the clumped chromatin (*N*) and the vacuolar areas in the nucleolus (*NU*). *L*, lipid droplet; *M*, myelin figure. $\times 28,500$.

vestigial were examined by light and electron microscopy. Numerous degenerating cells were observed in *vg* discs but not in those from wild type larvae. The degenerating cells were concentrated in the presumptive wing blade region of the disc. The process of involution involved condensation of cells and engulfment by neighboring cells. The number of degenerating cells was correlated with the degree of expression of *vg* obtained by rearing flies at different temperatures. These observations indicate that the *vg* phenotype results from cellular degeneration in a specific region of the wing disc.

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