Abnormal organization of ovarian follicles in the mutant *l(1)su(f)$^{mad\text{-}ts}$* of *Drosophila melanogaster*

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Summary. The morphology of ovarian follicles in the temperature-sensitive female-sterile mutant *l(1)su(f)$^{mad\text{-}ts}$* has been studied by means of light and electron microscopy. As the follicle cells gradually degenerate at the restrictive temperature (29 °C) the follicles become increasingly disorganized with respect to shape and polarity.

The anterior-posterior polarity in the eggs of *Drosophila* must already be determined at the time of egg deposition since all known mutants affecting the polarity of the egg are maternal effect mutants[2-4]. Unfortunately, very little is known about the sequence of events leading to the axial determination of the ovarian follicle. The follicle cells are of particular interest in this context since they envelop the follicle containing the oocyte and 15 nurse cells. Several mutations have been previously reported which result in extensive follicle cell death[5-7].

We have studied the morphology of follicles carrying the mutation *l(1)su(f)$^{mad\text{-}ts}$* (henceforth referred to as mad-ts). After the 3rd day following a shift to 29 °C (d_3, indicates the number of days mad-ts flies were cultured at the restrictive temperature [29 °C]) mad-ts follicles do not develop beyond stage 9 (Jürgens[7], for stages see King[8]). Figure 1 shows an ovariole that contains a stage 9 follicle with apparently normal morphology, but posterior to the follicle where normally a late vitellogenic follicle is to be expected, the morphology is grossly abnormal. These abnormalities, however, usually become evident prior to stage 9. The most interesting aspect of the mutant syndrome is the gradual disappearance of follicle cells concomitant with the breakdown of the intrafollicular polarity. Young follicles appear more normal in morphology than older follicles in a more posterior position in the ovariole. The part of the ovariole shown in figure 2 is a case in point; a small number of follicle cells still surrounds the anterior follicles (arrowheads) and their round shape, characteristic for this early vitellogenic stage, is retained. The posterior follicle, however, lacks follicle cells and the ooplasm assumes an abnormal position, so that yolk accumulates in a central area of the follicle (fig. 2, open arrow). Frequently cytoplasmic extrusions can be observed which fill the space between 2 follicles and the epithelial sheath surrounding the ovariole. The cytoplasmic extrusions either consist of ooplasm, and are, therefore, filled with yolk granules (not shown), or of nurse cell cytoplasm (fig. 3a). Finally, follicles devoid of follicle cells fuse to form an elongated tube. There is no apparent anterior-posterior polarity, and nurse cell cytoplasm and ooplasm alternate in an irregular fashion.

The development of the mutant syndrome is illustrated in a drawing that shows part of 2 neighbouring follicles (fig. 3a). Some follicle cells are still found around the anterior chamber but these cells are not viable as indicated by the reduced size, irregular shape (fig. 3a) and various cytolyso-

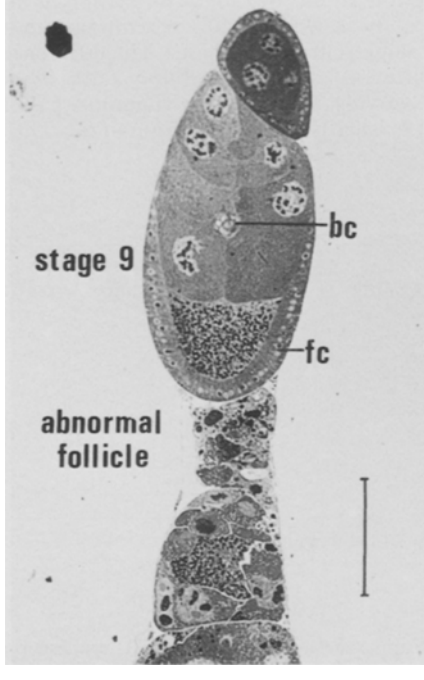

Figure 1. Semithin section through a d_5 ovariole showing 2 follicles with apparently normal morphology. Posterior to the stage 9 follicle the chambers become grossly abnormal. bc, Border cells (specialized follicle cells), fc, columnar follicle cells surrounding the posterior pole of the stage 9 follicle; the anterior pole is covered by very large but extremely thin follicle cells which cannot be seen at this magnification. Bar: 100 µm.

Figure 2. Section through a d_7 ovariole. Only few follicle cells are still present in 2 follicles (arrowheads). The older vitellogenic follicle in a more posterior position in the ovariole is devoid of follicle cells and accumulates yolk in a central area of the follicle (open arrow) indicating the breakdown of polarity. eps, Epithelial sheath covering the entire ovariole. Bar: 30 µm.

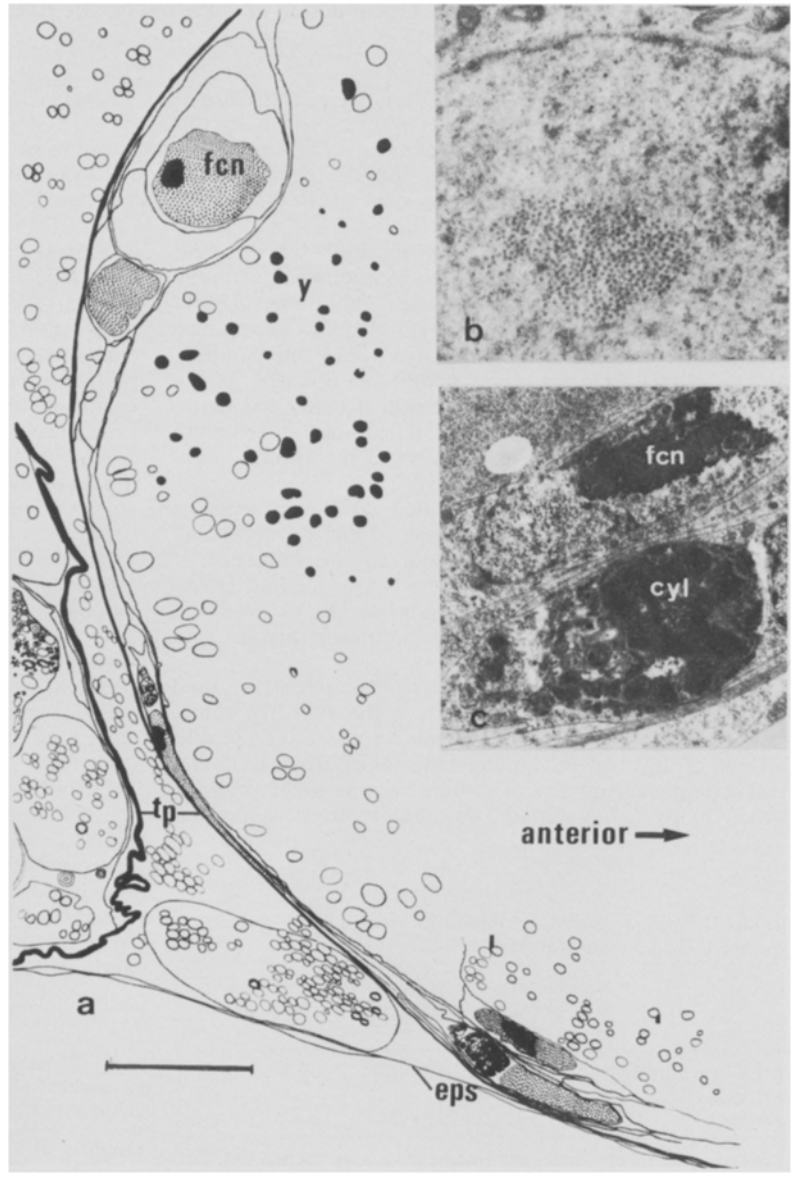

Figure 3. *a* Drawing based on electron micrographs showing a cytoplasmic extrusion between 2 d_7 follicles. The posterior follicle lacks follicle cells completely while the anterior follicle is surrounded by follicle cells in various stages of degeneration. tp, Tunica propria; eps, epithelial sheath; y, irregularly shaped yolk 'spheres'; fcn, follicle cell nucleus; open circles, lipid droplets. Bar: 10 µm. *b* Virus-like particles in the nucleus of a degenerating follicle cell. × 15,900. *c* Cytolysosome-like bodies (cyl) in a degenerating follicle cell. fcn: follicle cell nucleus of a different cell. × 8640.

some-like bodies (fig. 3c) which are typical for degenerating cells. The follicle cells of the interfollicular stalk are missing. The cytoplasmic extrusion is continuous with the cytoplasm of the posteriorly located follicle (fig. 3a), whose tunica propria is thrown into several folds, while the tunica of the anterior follicle is rather straight and normal-looking. This observation is taken as a further indication that the follicle cells play an essential role in the control of the follicular shape. Frequently cytoplasmic islands lined by membranes and filled with abnormally structured cytoplasm can be detected (fig. 3a, posterior follicle and cytoplasmic extrusion). These islands may contain, for example, condensed material or very lipid-rich cytoplasm. Other ultrastructural features include clusters of mitochondria and multilamellar bodies which occasionally aggregate to form very large membranous complexes. Some small and irregularly shaped yolk platelets were seen occasionally (anterior follicle in fig. 3a). Several nuclei of degenerating follicle cells contained large numbers of virus-like particles (fig. 3b); such particles have previously been found in a number of mutant and wild-type tissues (for review see Gateff[9]).

The morphology of the 16 cystocytes and the mesodermal profollicle cells in the germarium of d_7 ovarioles appears to be normal (not shown). Since the presumptive nurse cells and oocyte differentiate very early in the germarium[8] the mad-ts mutation appears to affect the maintenance of polarity rather than its establishment.

1 Acknowledgments. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 46). We wish to thank Mrs R. Koppa for excellent technical assistance and Mrs G. Mahlke for photographic work. We are also indebted to Dr D. Zissler, Dr E. Gateff and Prof. K. Sander for many helpful suggestions.
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