THE DEVELOPMENTAL HISTORY OF GERMARIA IN PARTHENOGENETIC FEMALE APHIDS[1]

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INTRODUCTION

That germaria play a significant role in determination of differential features between gamic female and parthenogenetic female ovarioles of the aphid *Macrosiphum solanifolii* was suggested by Lawson (1939b). The ovarioles of both types of females consist of a tubular vitellarium on the end of which is attached a spherical germarium containing nurse cells and germ cells. The germ cells in both types of ovarioles descend from the germarium into the vitellarium where development occurs, but the development of germ cells in gamic female ovarioles differs from the development of germ cells in parthenogenetic female ovarioles. In the vitellarium of a gamic female ovariole the eggs grow large by the accumulation of much yolk, presumably undergo meiosis and require fertilization and deposition before embryonic development can occur. In the vitellarium of the parthenogenetic female ovariole the germ cells are stimulated to develop parthenogenetically into embryos which are born alive upon reaching the proper stage of development. The fundamental difference between the two types of ovarioles is the difference between the two types of germ cells that develop within the ovarioles. These germ cells are produced by the germaria and, according to the theory proposed by Lawson, each germarium determines the subsequent development that its germ cells shall undergo. Other differences between parthenogenetic female and gamic female ovarioles are supposedly secondary to the germ cell difference and are determined also by the original differentiation between germaria.

If the germarial theory is correct it also should explain the production of (1) structures other than ovarioles which differentiate gamic and parthenogenetic females, (2) aphids intermediate between gamic and parthenogenetic females, (3) winged

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and wingless parthenogenetic females, and (4) aphids intermediate between winged and wingless parthenogenetic females. These forms are interrelated in such a way that variations in the same mechanism must be responsible for the production of all.

The first step in a study of the relation of germaria to determination of various aphid types is an understanding of the developmental history of the germaria in the parthenogenetic female aphid. This paper is an attempt to lay such a foundation.

FIRST APPEARANCE OF GERMARIAL CELLS

There is a stage in the development of a parthenogenetic female aphid embryo shortly after the germ band is formed when one end of the embryo opens to allow "symbionts" or "mycetocytes" to enter. The symbionts fill the entire central cavity of the blastula shaped embryo with the exception of a small space immediately dorsad of the germ band. Lying in this space between the germ band and the symbionts is a group of cells whose descendants make up the germaria (fig. 1). Each of these cells has a large nucleus with a very thin layer of cytoplasm around it. The nucleus contains a prominent nucleolus and either a number of chromosomes that appear split longitudinally in preparation for mitotic division or a thick, heavily staining late prophase strand. The cells are larger than any other cells in the embryo except those cells that become hosts for the symbionts. These host cells can be distinguished from the germarial cells by virtue of their containing chromatin in a very thin lightly staining spireme.

The group of cells that have been designated "germarial cells" could also be called "germ cells" in contrast to the somatic cells which make up the rest of the embryo. However, as all of the original group of cells do not become reproductive cells it is thought best to use "germarial cells" for the cells in early stages of development and to reserve "germ cells" for the actual reproductive cells that differentiate later.

IDENTIFICATION OF GERMARIAL CELLS IN EARLY EMBRYONIC STAGES

During the early stages of development which precede revolution of the embryo the germarial cells lie in a compact group at the posterior end of the embryo, surrounded by a thin epithelial membrane. The cells can be recognized easily by their size, which is usually greater than adjacent somatic cells, and by the nature of their nuclei which usually contain chromatin in various prophase stages. A further mark of recognition is the position of the germarial cells in relation to the symbionts. These symbiotic cells collect at the posterior end of the embryo during early developmental stages. Lying next to them and sometimes partially surrounded by them are the germarial cells.

EARLY DEVELOPMENT OF THE GERMARIAL CELLS

Though the germarial cells are isolated somewhat from the somatic cells of the embryo, they go through a series of developmental stages which parallels the development of the soma. A series of mitotic divisions occurs in the germarial cells between the germ band stage and the revolution of the embryo. Metaphase stages are occasionally observed while prophase stages are common. During this period of mitotic division the germarial cells are evidently segregated into groups for whenever metaphase stages are observed they occur in groups of adjacent cells only and not in the entire group of cells. The cells not in metaphase exhibit the more common prophase stages.

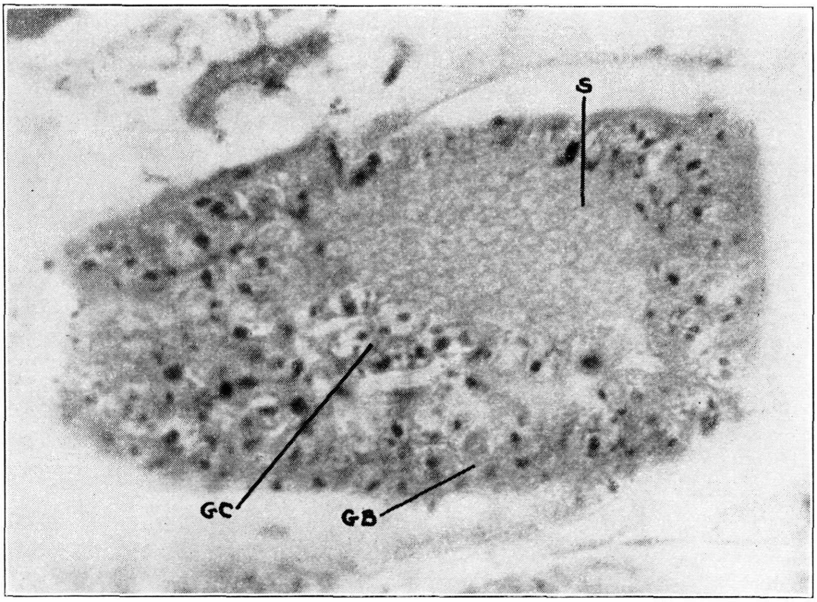

Fig. 1. Longitudinal section through an aphid embryo at stage of development of germ band. GB—germ band, GC—germarial cells, S—symbionts.

FORMATION OF GERMARIA

A change in the germarial cells that is evident at least as early as the formation of appendage buds in the soma is an increase in the cytoplasm of each cell. This cytoplasm does not form evenly around the nucleus but concentrates at one side forming a blunt, cone-shaped tail. At about the same time that the cytoplasmic cone is formed on each cell, the cells are arranged more or less loosely into a number of spherical groups. In each sphere of cells the nucleus of each cell lies near the periphery and the cytoplasmic cone lies between the nucleus and the center of the sphere. In a cross section of such a group the nuclei form a ring in the center of which is a circular area of cytoplasm made up of several cytoplasmic cones lying side by side. Metaphase stages of cell division have been observed in these spheres of germarial cells. Either shortly before or during the revolution of the embryo each group of germarial cells becomes surrounded by a thin epithelial membrane and henceforth can be called a germarium.

POSITION OF THE GERMARIA

The position of the germaria relative to the rest of the embryo is changed during the revolution of the embryo. Prior to this event the germaria, or in early stages the germarial cells, are found at the posterior

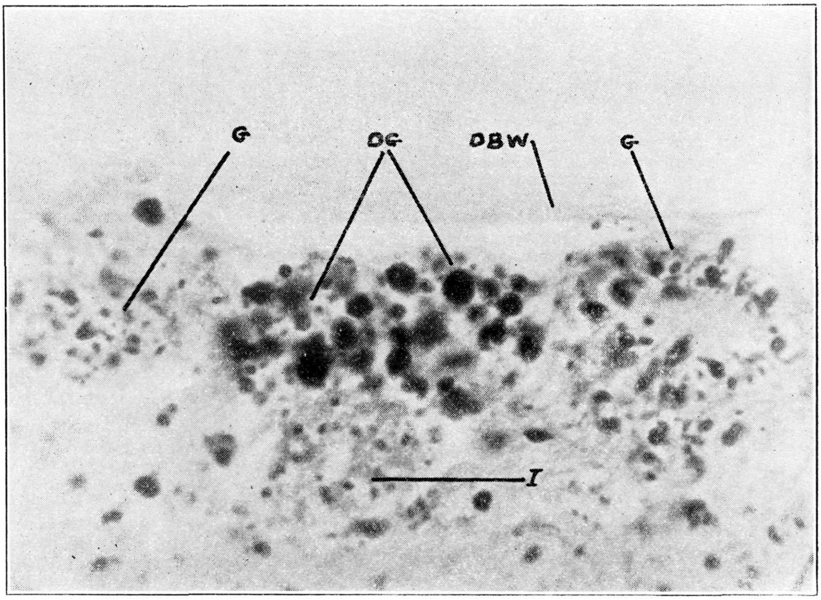

Fig. 2. Cross section through dorsal half of aphid embryo at a stage before dorsal body wall is fully developed. DBW—dorsal body wall, DG—degenerating germaria, G—germarium, I—intestine.

end of the embryo in close contact with the symbionts and in a sense outside of the body of the embryo. After the revolution of the embryo the germaria and the symbionts are located within the newly formed abdominal cavity. At this time the embryo has developed a head, thorax, appendages and a body wall. The body wall, however, is incomplete on the dorsal side of the abdomen, so that here the abdominal cavity opens directly to the outside. The germaria lie just within this opening forming a plate-like group of cells which extends from one lateral side of the abdomen to the other and from near the anterior end of the abdomen to near the posterior end. Immediately ventrad of the germaria are the symbionts which fill the greater part of the abdominal cavity.

DEGENERATION OF MID-DORSAL GERMARIA

Several groups of germaria which lie in the mid-dorsal region of the abdominal cavity degenerate after revolution of the embryo and before the dorsal body wall closes (fig. 2). During this degeneration the cells round up, become smaller than other germarial cells and stain an intense black with iron-hematoxylin. These cells are completely gone by the time the dorsal body wall closes.

After the degeneration of the mid-dorsal germaria two sets of germaria remain, one on each lateral side of the abdominal cavity. One group usually contains five germaria and the other contains four or occasionally five. This is the number and arrangement of germaria in older more fully developed embryos.

DIFFERENTIATION OF NURSE CELLS AND GERM CELLS

The germarial cells differentiate into two types of cells, nurse cells and germ cells, sometime during embryonic development between the germ band stage and closure of the abdominal wall. The exact time that this differentiation takes place is difficult to ascertain because of the similarity between the two types of cells. A distinction can be made after the germaria are fully formed, for the nurse cells then are grouped in a sphere, while the germ cells lie at the posterior end of the germarium between the ball of nurse cells and the epithelial covering of the germarium. Prior to the formation of the germaria the nuclei of the cells occasionally exhibit different prophase configurations, but this does not differentiate the two types of cells. The cytoplasm of the germarial cells becomes cone shaped before germaria are fully formed, and it was thought that the presence of such cones on certain cells would identify them as nurse cells, while the absence of such cones on other cells would identify them as germ cells. No such distinction could be made with certainty, though it may exist. Differing sizes of cells as a criterion is equally uncertain, for nurse cells sometimes vary in size within the same germarium and, also, in different germaria.

DEVELOPMENT OF THE OVARIOLES

After the formation of the germaria the epithelial covering of each germarium grows back as a small cellular tube in a posteriad-ventrad direction toward the region where the vagina later develops. These backward growing cellular tubes are the ovarioles or more precisely, the vitellarial region of the ovarioles. There are two groups of ovarioles, one for each lateral group of germaria. The ovarioles in each group unite a short distance behind the germaria to continue toward the vaginal region as a single tube which becomes the oviduct of the adult. As there are two groups of germaria, and two groups of ovarioles there are likewise two oviducts. In adult anatomy the ovariole includes the epithelial covering of the germarium and the tube that leads from the germarium to the oviduct, while the vitellarium refers only to that part of the ovariole that leads from the germarium to the oviduct.

PARTHENOGENETIC DEVELOPMENT OF GERM CELLS

Following the establishment of the ovarioles, the germ cells begin parthenogenetic development. This development begins at about the same time that the first faint indication of pigment appears in the eyes of the embryo. Usually one germ cell in each germarium begins to grow through an increase in the amount of cytoplasmic material (fig. 3). The probable source of this additional material is the ball of nurse cells, for with the beginning of germ cell growth, a stream of substance is evident extending from the center of the ball of nurse cells into the growing germ cells. This stream maintains contact with the oöcyte

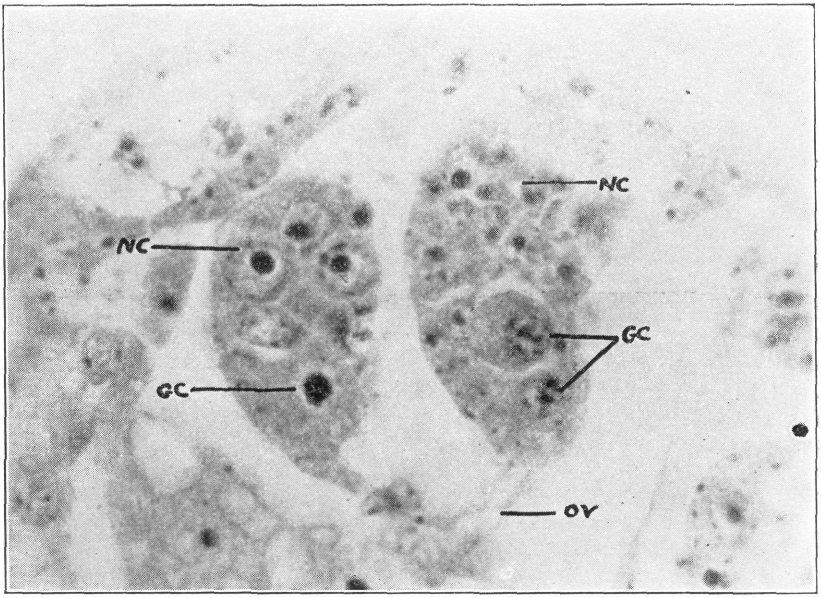

Fig. 3. Cross section through dorsal half of aphid embryo showing a longitudinal section through two germaria. GC—germ cell, NC—nurse cell, OV—ovariole (vitellarium).

until cleavage is well under way. The exact nature of this substance is uncertain, though a nutrient function is indicated. In a previous publication (Lawson 1939b) the secreted substance was called "yolk" and the stream or thread extending from the nurse cells to the growing germ cell was called a "yolk-stream." This choice of terms may be unfortunate for there is no certainty that the substance is yolk. Growth of the germ cell continues within the germarium until the germ cell has about tripled its size when it moves downward into the vitellarium. This germ cell or oöcyte, as it may now be called, continues to increase in size, throws off a polar body, and begins cleavage. After cleavage begins another germ cell grows, slips into the vitellarium and goes through the same developmental stages as the first cell. A third cell follows the second into the vitellarium before the embryo is born, so that in the oldest embryos each vitellarium commonly contains one growing oöcyte, one egg in early cleavage and one in late cleavage.

POSSIBLE SIGNIFICANCE OF GERMARIAL ACTIVITY

The determination of the winged or wingless condition in the adult aphid occurs before birth (Shull 1928). Hence, if the germarial theory is to have any application to wing determination there must be some event in the embryonic development of germaria that directly affects wing development. The initial stimulation of germ cells to parthenogenetic development may be the visible consequence of such an event.

Shull (1938) has shown that wings begin to develop sometime after pigmentation begins in the eyes of the embryo, but before one-fourth of the ommatidia are pigmented. The first indications of germ cell growth appéars at the same time that the first faint traces of eye pigment can be seen. Thus, it may be concluded that germ cells are stimulated before wing differentiation begins. Shull (1938) has estimated that wing determination occurs 10 or 12 hours before wing differentiation. That germ cell stimulation likewise occurs 10 or 12 hours before wing differentiation cannot be said, but that it occurs very near this time is possible.

A variation occurs in the time of initial stimulation of germ cells in the various germaria in the same embryo. Numerous cases have been found where two or three vitellaria contain oöcytes while the other vitellaria in the same embryo have none, and in some cases where all of the ovarioles show germ cell activity some of the oöcytes are more advanced in development than others. This variation supports the theory that germaria are determined, develop and function independently of one another, and, furthermore, it suggests a possible mechanism for the germarial control of wing determination.

To correlate germarial activity with wing determination it is necessary to know, at the time of initial germ cell stimulation, whether or not a given embryo is to be winged or wingless. This could not be determined in the embryos used in this study, so the problem must wait until such information concerning the embryos can be obtained. The use of the constant and intermittent light technique described by Shull (1938) should enable us to get this information.

SUMMARY

The developmental history of the germaria in parthenogenetic female aphids is described beginning with the appearance of the germarial cells in the blastula. The germarial cells are arranged in groups, each of which becomes a germarium. All of the germaria that are formed do not persist, for those lying in the mid-dorsal abdominal region degenerate. The remaining germaria form two groups, one on each dorso-lateral side of the abdominal cavity. Within each germarium the cells

differentiate into nurse cells and germ cells. An ovariole grows out from each germarium and connects to the oviduct, and into the vitellarium of each ovariole the germ cells descend, grow and undergo cleavage. The growth of the germ cells is aided by a substance secreted by the nurse cells. The time when germ cells are first stimulated to parthenogenetic development approximates the time when wing determination takes place and the suggestion is made that the determination of the winged or wingless condition in the adult aphid may be controlled by the germaria.

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