

# NUCLEO-CYTOPLASMIC RELATIONS IN SALIVARY-GLAND CELLS OF *DROSOPHILA*\*

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The importance of the nucleus in the control of cellular functions has been demonstrated by studies in many fields of experimental biology.<sup>1</sup> The results have indicated that sustained physiological activity of the cell and the expression of its genetic potentialities are dependent on the transfer of materials between nucleus and cytosome. Theoretical considerations of nucleo-cytoplasmic relations have engaged the attention of biologists since de Vries advanced the hypothesis of intracellular pangenesis in 1889, but the supporting cytological evidence has been extremely meager, being for the most part a series of observations of cytoplasmic particles that assumedly were of nuclear origin.<sup>2</sup> In some types of cells, such as microsporocytes of various plants,<sup>3</sup> these cytoplasmic particles were Feulgen-positive, indicating that they contained deoxyribonucleic acid; but it was not always possible to determine whether they had been ejected from the nucleus in the normal course of cellular activity or as a result of degenerative changes. In other types of cells, such as the oöcytes of amphibians and fishes,<sup>4</sup> the cytoplasmic particles were identified as nucleoli; and extrusion of nucleolar material through the nuclear membrane was reported to be induced by treatment of the living amphibian oöcyte with dilute acid.<sup>5</sup> Such findings indicated that particles of large size might pass through the nuclear membrane, although the mechanism responsible for their transport in the normal metabolizing cell was not determined. In the course of an electron microscope study of the salivary-gland cells of *Drosophila melanogaster*, structural configurations were observed that suggested a mechanism for exchange of materials between nucleus and cytosome.<sup>6</sup> The electron micrographs showed that outpocketings of the nuclear membrane were intimately associated with specific regions of the chromosomes. A survey of the experimental evidence is presented here.

*Materials and Methods.*—Salivary glands of well-fed third-instar larvae of *D. melanogaster* (Sw-b 6) were excised and fixed in ice-cold 1 per cent osmium tetroxide, isotonic with insect blood,<sup>7</sup> and buffered at pH 6.5.<sup>8</sup> The glands were imbedded in *n*-butyl methacrylate<sup>9</sup> and sectioned with a Porter-Blum ultramicrotome. Serial sections prepared by the method of Gay and Anderson<sup>10</sup> were examined in a Philips electron microscope without removal of the imbedding medium.

*Results.*—Electron micrographs of these ultrathin sections of salivary-gland cells showed well-preserved structural elements in the cytosome, including spherical secretion granules, mitochondria, and abundant endoplasmic reticulum in the form of narrow membranous sacs aligned parallel to each other and to the contours of the nucleus (Fig. 1). The nucleus contained fibrous chromosomes, whose outlines were readily discernible, although no limiting membranes were apparent. The nuclear membrane was composed of two layers, separated by a distance of about 150 Å; they were most clearly defined in cross-sections that were normal to the

nucleus (perpendicular to the tangent at the level of the section). The contour of the nucleus was undulous, and in some places the membrane protruded into the cytosome in the form of outpocketings or blebs (the arrows of Fig. 1).

It seemed necessary to establish with certainty that these outpocketings were not attributable to fixation distortion (even though the fine structural details characteristic of good fixation were apparent in cytoplasmic organelles<sup>11</sup>), and therefore a lengthy series of modifications of technical procedures was undertaken. They included variations in the salt concentration and pH of the fixative, in the time and temperature of fixation and subsequent treatment, and in the nature of the agents used in the postfixation processes. None of these modifications was effective in eliminating the uneven border of the nucleus and the outpocketings of the membrane. It should also be noted that undulatory nuclear contours have been reported for other types of secretory cells.<sup>12</sup> On the basis of these considerations, it seems reasonable to conclude that outpocketings of the nuclear membrane are characteristic structural features of the salivary-gland cells of third-instar larvae of *D. melanogaster*.

The outpocketings were not uncommon in this material, and single ultrathin sections sometimes showed as many as five or six. More significant than the number, however, was the fact that the chromosomal materials adjacent to the blebs appeared different from those in other parts of the nucleus. The strands were more highly electron-scattering and were often associated with a type of material that was not discernible in the body of the chromosome. The chromatin strands were in contact with the nuclear membrane at the region of blebbing and in some cases were observed to extend into the outpocketings (Figs. 2, 3, and 4).

Examination of serial sections in which an outpocketing could be traced through its entirety invariably revealed connections with the salivary-gland chromosomes. In some cases the connections were to single bands in intercalary regions and in others apparently to terminal chromatin. Occasionally the intercalary band was located in a "reverse repeat" (Figs. 6a-6c). No positive identification was possible in the ultrathin sections with respect to the specific subdivisions of chromosomes involved, but it should be kept in mind that some reverse repeats have been shown to contain intercalary heterochromatic regions.<sup>13</sup>

*Discussion.*—Observations on static systems such as are represented in sections of fixed material have limitations with respect to the interpretation of functional relationships, but the intimate association between chromosomal materials and membrane outpocketings revealed in these electron micrographs strongly suggests a mechanism for transfer of chromosomal products to the cytosome.

In evaluating this interpretation, the possibility must be considered that the outpocketings are merely transitory manifestations of a generalized surface activity at the nucleus-cytosome border. This possibility seems to be negated, however, by the observation that chromosomal material extends into the blebs, a fact which implies that the latter are formed at portions of the membrane that lie adjacent to specific regions of the chromosomes. It should also be kept in mind that the phenomenon reported here has been observed in only one type of cell at one stage of its development. This stage precedes by about 20 hours (at the temperature used) the time at which the larval salivary glands degenerate. The question may therefore be raised whether the nuclear blebs represent the first stages of nuclear degen-

eration. Several lines of evidence indicate that they do not. Peptidase activity is high in salivary glands at the stage of development represented in these studies and increases until about six hours after puparium formation.<sup>14</sup> Salivary glands of late larval and early pupal stages are active in producing a cement that serves to affix the puparium to the substrate.<sup>15</sup> No evidence of degeneration of nuclear or cytoplasmic structures was observed in any of the electron micrographs obtained in the present investigation. Finally, it may be noted that, if nuclear outpocketings and the associated materials are indicative of degenerative changes, the relation of the blebs to specific chromosomal bands indicates that this is not an indiscriminate phenomenon. Such a localized type of degeneration seems improbable. By all these criteria, the salivary glands of mid-third-instar larvae of *Drosophila* appear to be composed of functionally active, and healthy cells; and, by the same criteria, the outpocketings of the nuclear membrane appear to be a manifestation of normal cellular activity involving participation of the chromosomes.

If the structural features described above provide a mechanism for transfer of materials from nucleus to cytosome, it seems probable that the blebs become detached from the nucleus to lie in the cytosome. Several spherules whose walls showed the double-layered condition characteristic of the nuclear membrane were observed adjacent to the nuclei in the preparations studied, but conclusive proof of their origin was not possible by examination of the electron micrographs. Studies with the light-microscope of sections of salivary glands of *Drosophila* and *Chironomus* revealed that Feulgen-positive strands were oriented toward the nuclear membrane in several regions and that small droplets of faint pink-to-colorless material occurred on the cytoplasmic side of the membrane. These findings suggest that the shape and composition of the nuclear blebs and their contents may change rapidly upon detachment. The blebs, freed from their connection with the nucleus, could conceivably discharge their contents and become flattened to produce the saclike membranes of the endoplasmic reticulum. The similarity in structure of the nuclear membrane and the elements of the endoplasmic reticulum is indeed striking, as can be seen by inspecting Figures 4 and 5. As revealed in these transverse and tangential sections, both types of membrane are double-layered and are

#### PLATE I

FIGS. 1-6.—Electron micrographs of ultrathin sections of salivary-gland cells of third-instar larvae of *Drosophila melanogaster*. The marker in the upper right-hand corner of each figure indicates 1  $\mu$ . Abbreviations: *chr*, chromosome, *nu*, nucleus; *n.m.*, nuclear membrane; *cyt.*, cytosome; *s.g.*, secretion granule; *e.r.*, endoplasmic reticulum.

FIG. 1.—Section through nucleus and adjacent cytosome, showing nuclear and cytoplasmic structure. Arrows indicate regions of outpocketings of nuclear membrane.

FIG. 2.—Outpocketing of nuclear membrane, showing dense chromosomal material confluent with chromonemata.

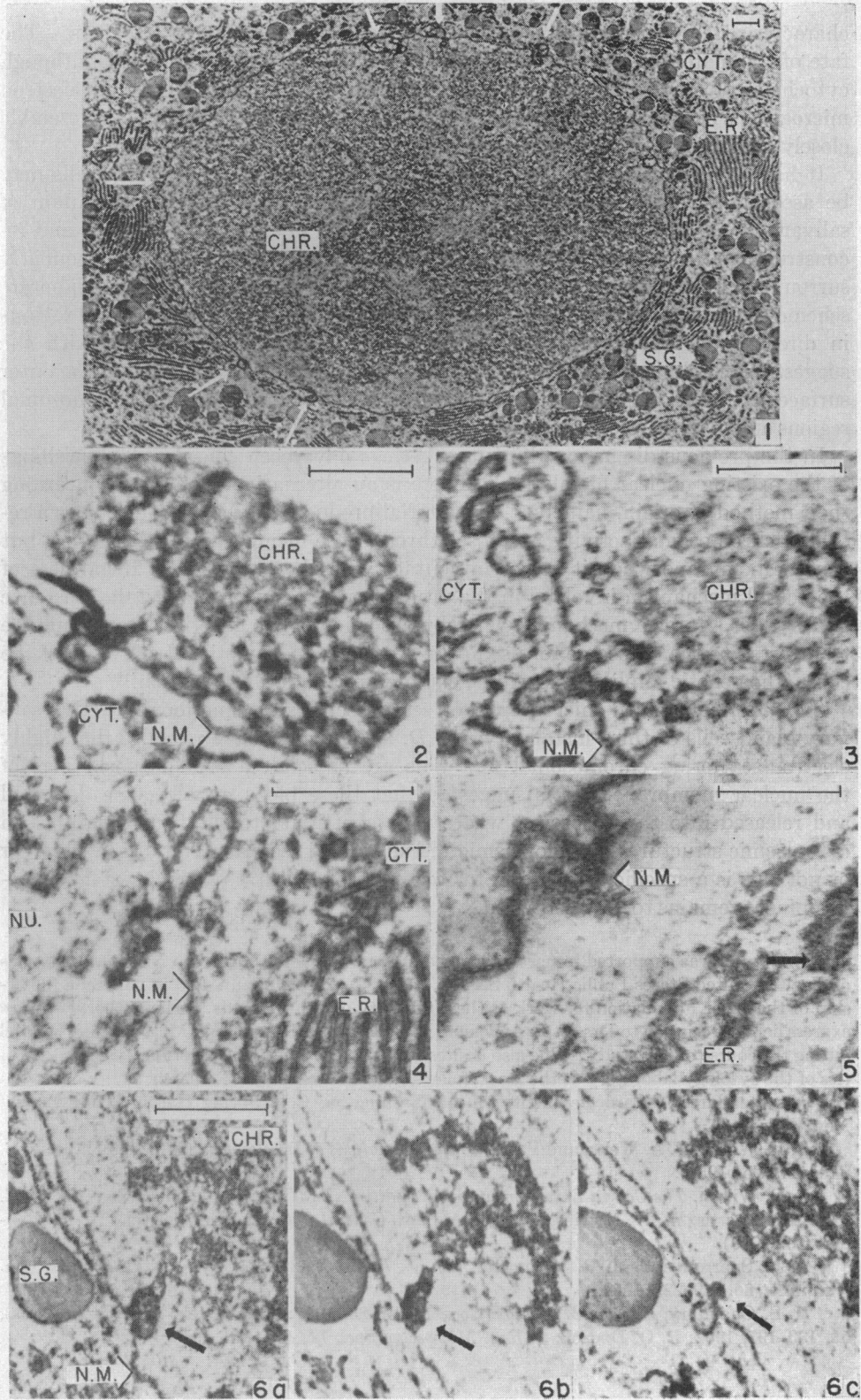
FIG. 3.—Projection from the nucleus, containing fibrous strands which connect with highly electron-scattering chromosomal material.

FIG. 4.—Outpocketing of nuclear membrane and adjacent cytoplasm. Chromatin near the bleb is electron-dense and contains spherical granules not seen in interior of nucleus. Similarity in structure of membranes of outpocketing and endoplasmic reticulum is apparent.

FIG. 5.—Tangential section through nuclear membrane and endoplasmic reticulum, showing characteristic reticulate pattern of the nuclear membrane and, at black arrow, a similar pattern of the endoplasmic reticulum.

FIGS. 6a-6c.—Three sections selected from a complete series of thirty, used to trace the connection between a single band in the chromosome and a small bleb in the nuclear membrane. Note dense connecting material at arrows.

PLATE I



characterized by periodically spaced "pores" and associated markings. The fate of the materials included within the blebs has not been determined (although cytochemical studies directed to this end have been initiated), but, in electron micrographs of some cells, cytoplasmic structures have been identified that resemble closely the chromosomal materials extending into the outpocketings.

It has been suggested that endoplasmic reticulum in mouse pancreas cells may be associated with the production of zymogen granules.<sup>16</sup> If the reticulum of salivary-gland cells is associated with the formation of secretion granules—and reconstructions from serial sections have shown that large granules are frequently surrounded by the membranous sheets of the endoplasmic reticulum—an elaborate scheme could be formulated with respect to the role of specific chromosomal regions in directing metabolic processes. Such a mechanism would conform with the suggestion of Schultz<sup>17</sup> that the nuclear membrane, having been formed at the outer surfaces of chromosomes at telophase, may be associated with specific chromosomal regions and may therefore control specific nucleocytoplasmic interactions.

In the previous discussion the outpocketings have been considered as swellings of the existing nuclear membrane. There is an alternative possibility concerning their method of origin, namely, that material produced within the nucleus as a result of chromosomal activity may pass through the membrane to form a new but similar membrane when it comes in contact with the cytoplasm. Regardless of the actual mechanism involved in the production of the membranes, the existence of the blebs affords a mechanism whereby a portion of the genetic material may be transferred to the cytosome for use in the synthetic and functional activities of the cell. The genetic implications will be explored in another publication.<sup>18</sup>

*Summary.*—Electron micrographs of serial sections of functionally active salivary-gland cells of third-instar larvae of *D. melanogaster* have revealed that highly electron-scattering chromosomal materials are associated with outpocketings of the nuclear membrane. It is suggested that these blebs may become detached and released into the cytosome, where they contribute to the formation of such cytoplasmic structures as endoplasmic reticulum and secretion granules. Whether or not this is a specific mechanism for one type of cell in a particular stage of development remains to be determined.

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## PHENOTYPIC VARIATION AND PSEUDO-ALLELISM AT THE FORKED LOCUS IN *DROSOPHILA MELANOGASTER*

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In previous reports<sup>1, 2</sup> it was demonstrated that, by use of a suppressor mutant, the otherwise phenotypically inseparable vermilion (*v*) eye-color mutants of *Drosophila melanogaster* could be separated into two classes—suppressed and unsuppressed. Moreover, pseudo-allelism is indicated, since recombination was demonstrated to occur between suppressed and unsuppressed *v* mutants. The description of a suppressor of the recessive, sex-linked forked bristle (*f*) mutants<sup>3</sup> plus the fact that *f* mutants recur frequently suggested that the observations made for the *v* mutants be extended to the *f* mutants.

Four independent *f* mutants, spontaneous in origin, have been used. These are *f*<sup>1</sup>, *f*<sup>3</sup>, *f*<sup>3n</sup>, and *f*<sup>5</sup>. All are characterized phenotypically by causing a gnarling or twisting of the bristles. The *f*<sup>5</sup> mutant differs from the others in that the microchaetes as well as the macrochaetes are forked. In the tests with the sex-linked suppressor of forked (*su-f*), the following mutants (with map locations) were used: *f* (56.7); Bar eye, *B* (57.0); carnation eye color, *car* (62.5); and *su-f* (64.0). Since *f*<sup>1</sup> is suppressed by *su-f*, a stock of the genotype *f*<sup>1</sup> *B su-f* was obtained. Tests for the suppression of any independent *f* mutant, designated *f*<sup>x</sup>, were made by first obtaining ♂♂ of the genotype *f*<sup>x</sup> *car*. These ♂♂ were crossed to ♀♀ *f*<sup>1</sup> *B su-f*, thereby producing ♀♀ of the genotype *f*<sup>1</sup> *B + su-f/f*<sup>x</sup> + *car* +. The ♂ progeny of these heterozygous ♀♀ were scored. Those ♂ progeny phenotypically *B*<sup>+</sup> *car*<sup>+</sup> must be genotypically *f*<sup>x</sup> *su-f*, and their bristle phenotype was compared with