Giant Polytene Chromosomes from the Ovaries of a *Drosophila* Mutant

Abstract. *The chromosomes of the ovarian nurse cells of* Drosophila melanogaster *fall apart during their cycles of endoreduplication. However, chromosomal synapsis occurs in the pseudonurse cells produced in certain mutant females. The resulting polytene chromosomes undergo developmental changes that are strikingly different from those recorded for the giant chromosomes of the larval salivary gland cells.*

The giant, polytene chromosomes of the salivary glands of larvae of *Drosophila melanogaster* have been studied extensively since they were first described by Painter 48 years ago (*1–3*). For comparative studies with this species, it would be useful to have a source of giant chromosomes from an adult tissue. In anopheline mosquitoes, large banded polytene chromosomes are present in both larval salivary gland cells and adult ovarian nurse cells (*4, 5*). Such is not the case for *Drosophila*, although the nurse cell nuclei undergo many rounds of DNA replication. At first, the homologous chromosomes in each nurse cell are conjoined, and all chromosomes adhere at their centromeric regions. However, as endomitosis proceeds the chromosomes uncoil, lengthen, and fall apart, so that by the time the maximum DNA content is reached, all 15 nuclei in each chamber contain jumbled masses of Feulgen-positive threads (*6*). Therefore, these chromosomes are unsuitable for cytological study.

Certain mutations of the ovarian tumor class (*7*) that result in female sterility generate pseudonurse cells (PNC) in which the replicating chromosomes remain in register. The first mutant of this type was isolated in 1925 by Calvin Bridges. He called the gene *fes* (female sterile) and located it near the left end of the second chromosome. King *et al.* (*8*) showed that *fes* females were sterile because their ovaries were filled with tumorous chambers, and they described PNC for the first time. However, the frequency of nuclei with polytene chromosomes in *fes* tumors was relatively low (*9*). In 1975 Gans *et al.* (*10*) reported the isolation of an X-linked ovarian tumor mutant, which they named *fs(1)231*. The ovaries of *fs231* females produce occasional PNC, and the frequency of PNC can be increased by rearing females at low temperatures (*11*).

We subsequently attempted to increase the frequency of PNC still further by combining *fs231* with other independently induced allelic mutations or with nonallelic mutations belonging to the ovarian tumor class. High frequencies of PNC were observed in the ovaries of females simultaneously homozygous for *fs231* and heterozygous for *fes*. We also found that PNC differentiation was stimulated by *fs116*. This mutation was isolated by Gans and shown to be an allele of *fs231*. The ovaries of 116 homozygotes contained typical tumors and chambers possessing PNC. Such chambers were usually free of tumor cells and sometimes contained an oocyte. There were far more PNC in ovaries of *231/116* females than in those from either *231* or *116* homozygotes, and more chromosomes in banded polytene stages were observed.

The diploid chromosomal complement for female *D. melanogaster* is two rod-shaped X chromosomes, two V-shaped second, two V-shaped third, and two dot-shaped fourth chromosomes. Since we generally observed five rod-shaped chromosomes in squash preparations, it is clear that the arms of chromosomes 2 and 3 were usually torn apart during our squashing procedure. However, in some preparations of PNC nuclei from young chambers, the pericentric heterochromatin appears as thin filaments connecting the arms of the two large autosomes. A microchromosome appears in some squashes, and it shows the quinacrine fluorescence typical of the fourth chromosome (*12*). Pseudonurse cell chromosomes can be grouped into three morphological classes: 1, condensed; 2, banded; and 3, diffuse. For each class, PNC chromosomes come in a variety of size groups. Class 1 chromosomes are 10 to 60 μm long and 4 to 6 μm wide. Class 2 chromosomes are 80 to 150 μm long and 5 to 10 μm wide, and they can be subdivided into nonpuffed (2A) and puffed (2B) classes. When comparing the banding patterns of chromosomes from PNC and salivary gland cells (SGC), only the largest chromosomes of class 2A are of use. Class 3 chromosomes are 30 to 70 μm long and 10 to 12 μm wide. The five chromosomes in each of classes 1 and 3 are quite similar, so it is difficult to decide whether a given chromosome represents the X or an arm of an autosome. It is not even clear which end bears the centromere and which the telo-

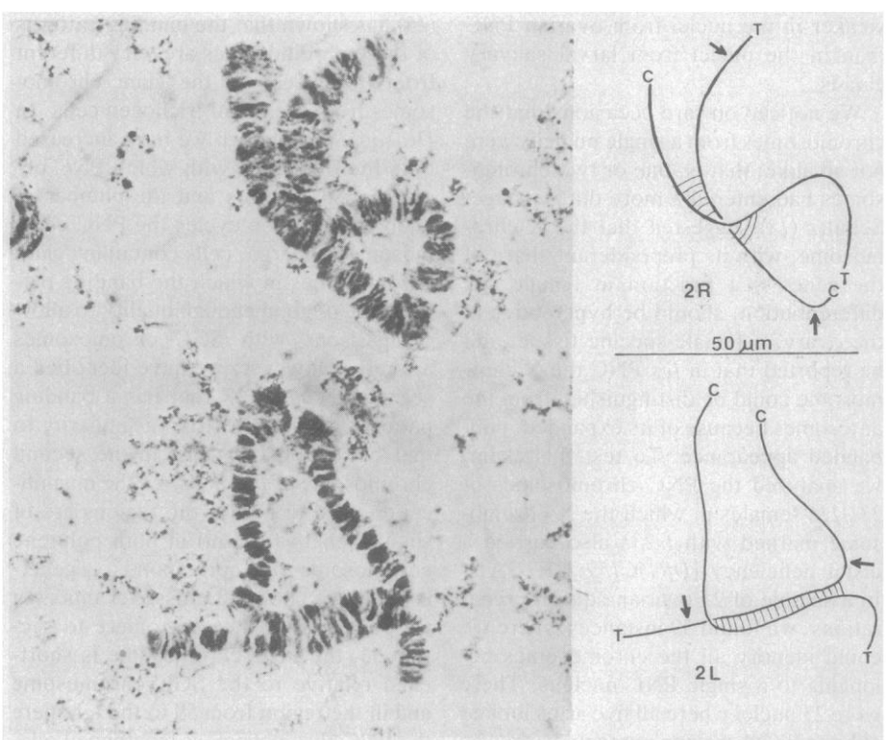

Fig. 1. Giant, banded, polytene chromosomes from an ovarian pseudonurse cell of an adult female *Drosophila*. Specific chromosomes (*2L* and *2R*) have been marked by paracentric inversions. The female had the genotype *fs231/fs116; fes/In(2L)CY + In(2R)Cy*. The accompanying drawing shows an interpretation of the pairing configurations of the homologs. Note that the centromeric (*C*) and telomeric (*T*) portions of the homologs are unsynapsed. Arrows bracket the inverted segments.

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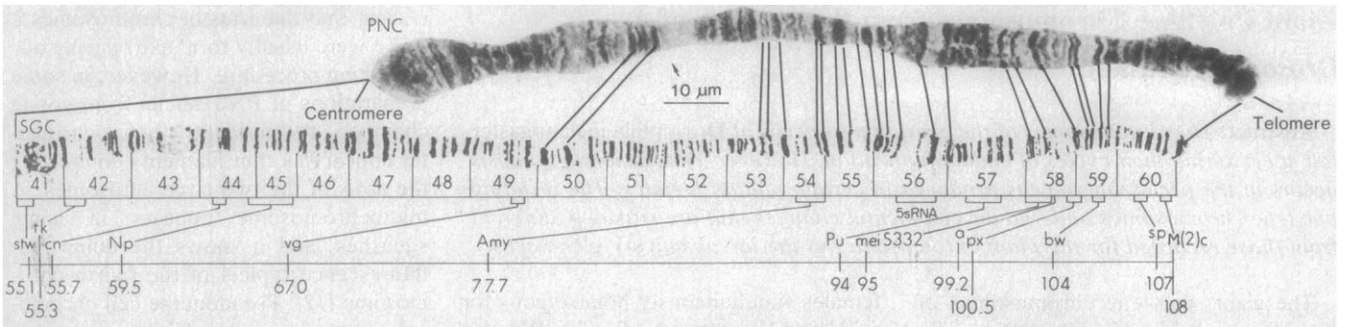

Fig. 2. Polytene chromosome 2R from a pseudonurse cell (*PNC*) and from a salivary gland cell (*SGC*) compared with the genetic map for the oocyte chromosome of *D. melanogaster*. The PNC chromosome photographed was 150 μm long. The genetic map contains the symbols for those genes that have been located on the SGC chromosome. Their loci (in crossover units) are shown below the line and their approximate locations on the SGC chromosome are bracketed. The photographic map of SGC 2R is from Lefevre (*2*) and the genetic map is adapted from King (*18*).

mere. Therefore, we introduced various chromosome aberrations into our *fs231* stocks to provide a cytological marker for each chromosome.

Figure 1 shows PNC chromosomes marked by specific paracentric inversions. The centromeric and telomeric portions of the homologs are unsynapsed. Such abnormal, "reverse" pairing configurations are seen in the majority of cases. In salivary glands stained and squashed under identical conditions, chromosomes containing the same inverted segments invariably paired throughout their lengths to produce typical inversion loops. Therefore the synaptic attraction of homologs is clearly weaker in the nuclei from ovarian PNC than in the nuclei from larval salivary glands.

We noticed on rare occasions that the chromosomes from a single nucleus were not all alike; that is, one or two chromosomes had entered a more diffuse stage. Schultz (*13*) suggested that the X chromosome, with its preponderant share of the genes that function in female sex differentiation, should be hyperactive in the ovary, a female-specific tissue, and he reported that in *fes* PNC the X chromosome could be distinguished from the autosomes because of its expanded, nonbanded appearance. To test this claim, we analyzed the PNC chromosomes of *231/116* females in which the X chromosome marked with *fs231* also carried a distal deficiency [*Df(1)C159*; 3E7-5A1]. In a sample of 25 ovarian squash preparations, we found 39 instances where we could identify all the chromosomes belonging to a single PNC nucleus. There were 25 nuclei where all five arms looked alike, nine where one autosome arm was diffuse, three where an X was diffuse, and two where two autosome arms were diffuse. So, when a chromosome enters a diffuse state in *fs231* PNC, it is not invariably the X. In those *fs231* nuclei that contained one diffuse chromosome that was not an X, it is clear that the left and right arms of an autosome were out of synchrony.

Beermann (*14*) emphasized the similarities between the banding patterns in the polytene chromosomes isolated from different somatic tissues of larvae of the midge, *Chironomus tentans*. Coluzzi *et al.* (*5*) compared polytene chromosomes from larval SGC and adult ovarian nurse cells of *Anopheles superpictus* and found marked differences in both banding and puffing patterns. In certain inbred strains of the blowfly *Calliphora erythrocephala*, banded polytene chromosomes occur in the ovarian nurse cells. Ribbert (*15*) has shown that the banding patterns of these chromosomes are very different from those seen in the giant chromosomes from the pupal trichogen cells. In *Drosophila*, although we have increased both the frequency with which PNC occur in *fs231* tumors and the number of endoreduplication cycles the PNC chromosomes undergo, cells containing giant chromosomes in which the banding patterns are of good enough quality to allow comparisons with SGC chromosomes are rare. However, we have identified a segment of PNC 2R that has a banding pattern that bears a striking similarity to that of sections 53 to 59 in the second chromosome of the SGC. At the magnification used in Fig. 2, the regions are of similar length (55 μm) in both polytene chromosomes and correspond to a genetic length of about 30 crossover units. In the region from the centromere to section 53, the PNC chromosome is shortened relative to the SGC chromosome and in the region from 59 to the telomere the PNC chromosome is lengthened relative to the SGC chromosome. It is generally impossible to homologize the banding patterns in regions such as 41 to 52 and 60, where the giant chromosomes from the two tissues are expanded to different degrees. The deficiency we used to mark the X chromosome divides it into left and right segments. In the salivary gland X chromosome the right segment (between the deficiency and the centromere) is six times longer than the left segment (between the deficiency and the telomere). In PNC X chromosomes in classes 1 and 2A the right segment is only two to three times longer than the left. It follows that the region of the X chromosome proximal to the centromere has shortened more than the distal region in PNC as opposed to SGC.

When one contrasts the relative positions of the same genes localized on the genetic and the salivary gland chromosome maps of *D. melanogaster*, broad regions proximal to the centromeres seem compressed and narrow regions adjacent to the telomere seem expanded in the oocyte chromosomes, as opposed to the salivary chromosomes (see Fig. 2). If the coiling behavior of PNC chromosomes at the banded polytene stage is more similar to that of oocyte chromosomes at pachynema than to salivary chromosomes, then PNC chromosomes should be relatively compressed in regions proximal to the centromeres when compared to salivary chromosomes. The above data show that this prediction holds true, at least for the X and 2R chromosomes.

By means of DNA-Feulgen cytophotometry (*16*) it has been estimated that the ovarian nurse cells of wild-type *D. melanogaster* undergo nine to ten cycles of DNA replication. In the case of *fs231* the largest banded polytene chromosomes measured from PNC have undergone 10 to 12 cycles of DNA replication (*17*). Therefore, the amount of DNA in the giant chromosomes of PNC often is equal to or greater than that in wild-type nurse cells or SGC.

Cytologists studying *Drosophila* giant chromosomes previously had to work

with the cells of the larval salivary gland. Therefore, any specific conclusions applied to one class of somatic cells having restricted synthetic activities during an immature developmental stage. Pseudonurse cells, in contrast, are derived from the germ line, are present in the reproductive system of the adult female, and contain chromosomes that undergo a greater variety of morphological changes during their growth than do salivary gland chromosomes. Since all chromosome arms can be cytologically marked, the synthetic activities of specific chromosome segments can be contrasted at various developmental stages. The differential replication of euchromatin and heterochromatin and the relation between the relative amounts of chromosomal proteins and the coiling behaviors of these giant chromosomes are also targets for future investigations.

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19. We are grateful to Dr. Madeleine Gans for supplying the initial cultures of *fs(1)231* and *116* and to Dr. George Lefevre for the *Df(1)C159* stock. Drs. Eun Ho Park, Michael R. Cummings, Ralph M. Sinibaldi, Ellen M. Rasch, and James B. Kitzmiller gave useful suggestions. This research was supported by National Science Foundation grant PCM7907597 and with funds from Lyun Joon Kim, former president of Hanyang University.
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Adenosine 3′,5′-Monophosphate Waves in *Dictyostelium discoideum*: A Demonstration by Isotope Dilution–Fluorography

Abstract. *The distribution of adenosine 3′,5′-monophosphate (cyclic AMP) in fields of aggregating amoebae of* Dictyostelium discoideum *was examined by a novel isotope dilution-fluorographic technique. Cellular cyclic AMP was visualized by its competition with exogenous ^{3}H-labeled cyclic AMP for high-affinity binding sites on protein kinase immobilized on a Millipore filter used to blot the monolayer. The cyclic AMP was distributed in spiral or concentric circular wave patterns which centered on the foci of the aggregations. These patterns were correlated with those of cell shape change that propagate through the monolayers: cells in regions of high concentrations of cyclic AMP were elongated (presumably moving up a cyclic AMP gradient), whereas those in regions of low cyclic AMP concentrations were randomly directed. The highest cyclic AMP concentrations were about 10^{-6}M. The widths of the regions of elevated cyclic AMP were about 0.3 to 1 millimeter which, assuming a wave velocity of 300 micrometers per minute, suggests that a cell signals for about 1 to 3 minutes. These observations support the hypothesis that the aggregation process in* Dictyostelium *is mediated by the periodic relay of cyclic AMP signals and suggest a simple scheme for the dynamics of the aggregation process.*

Although it is clear that cells must communicate during morphogenesis little is known about how they do so. The cellular slime mold *Dictyostelium discoideum* can be used to examine this question. During one phase of its life cycle, separated amoebae begin to communicate and aggregate to form a multicellular structure which later undergoes a series of shape changes (*1–5*). Monolayers of cells on agar become segregated into territories; cells within each territory are attracted to its center. Early in aggregation, cells move in steps, advancing for about 2 minutes and then stopping for about 5 minutes before moving again (*6, 7*). Moving cells are elongated, and in dark-field photographs appear lighter than stationary, rounded cells (*7*). Light bands (moving cells) form spiral or concentric circular patterns about a center (see Fig. 1). In time-lapse films, each light band can be seen spreading outwardly from a center as an enlarging ring as cells progressively farther from the center begin an inward movement step (*8*). It has been proposed that the chemotactic movement steps are coordinated by extracellular signals of adenosine 3′,5′-monophosphate (cyclic AMP) which are propagated outwardly from the center (*9*). In this scheme, a cell

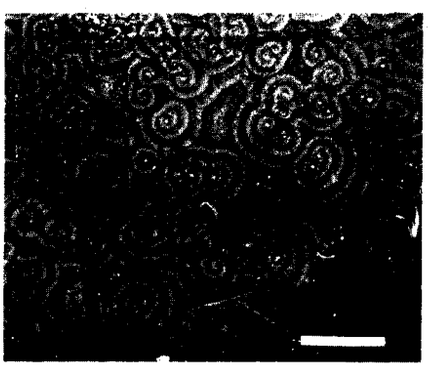

Fig. 1. Organized waves of cell movement during aggregation in *D. discoideum*. Techniques for growth and development of NC-4 amoebae closely followed those of Alcantara and Monk (*7*). "Starvation plates" were prepared just before use by pouring 9 ml of an autoclaved solution of 10 g of agar (Difco), 1.00 g of K_2HPO_4, 2.31 g of $KHPO_4$, and 0.5 g of $MGSO_4$ per liter into 100-mm petri dishes (Falcon 1001). Amoebae, grown in association with *Enterobacter aerogenes*, were freed of bacteria, and 1 ml of a cell suspension at a density of 8×10^7 cells per milliliter was added to each plate. The cell suspension was spread evenly by shaking the dish, and the cells were allowed to settle for 20 minutes. Excess fluid was removed and the lid was left off the dish for an additional 20 minutes or until a thin layer of surface fluid remained. The cultures were incubated at 7°C for 18 hours. Although the patterns shown were usually visible when the cultures were removed from the incubator, removal of the lid for about 15 to 20 minutes enhanced their appearance. Illumination was provided by a dark-field condensing lens system as described by Gross *et al.* (*8*). Calibration bar, 1 cm.

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