The dynamics of *Drosophila melanogaster* spermatogenesis in *in vitro* cultures

By DAVID P. CROSS¹ AND DAVID L. SHELLENBARGE R²

From the Department of Zoology, the University of British Columbia, Vancouver, B.C., Canada

SUMMARY

A photographic description of meiosis and spermatogenesis in single sperm cysts of *Drosophila melanogaster* cultured *in vitro* is presented. In addition to the utility of this description for the study of spermatogenesis in whole live testes, the culture procedure provides a means for studying the dynamic development of individual sperm cysts from flies carrying male-sterile mutations, as illustrated with *X/O* males. Several deviations of spermatogenesis *in vitro* from that *in vivo* suggest that sperm morphogenesis is not rigidly coordinated.

INTRODUCTION

Spermatogenesis in *Drosophila melanogaster* is a well-defined sequence of dramatic morphological changes which have been especially well described in fixed material at both the light and electron microscope levels (for references, see Lifschytz & Hareven, 1977; Shellenbarger & Cross, 1979a). This report presents the results of the culturing *in vitro* of single cysts from wild-type and *X/O* testes. These studies were undertaken for three main reasons: (1) to develop a system which would aid in defining the developmental potential of mutants causing blocks in spermatogenesis; (2) to provide a more detailed light microscopic description of the timing and ordering of the various stages of the process than had previously been available from fixed material (Kiefer, 1966; Lindsley & Lifschytz, 1972; Tokuyasu, 1975); and (3) to determine whether the description of *in vitro* spermiogenesis in *Drosophila hydei* (Fowler & Johannisson, 1976) would hold for *Drosophila melanogaster*, where male-sterile mutations are readily available for analysis. The photographic description of the dynamics of spermatogenesis presented here is the most complete available and is extremely helpful for the identification of stage-specific defects in male-sterile mutants (Shellenbarger & Cross, 1979a, b).

¹ Author’s address: Department of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8S 4K1.
² Author’s address: Department of Biochemistry, University of Washington, Seattle, Washington, U.S.A. 98195.
MATERIALS AND METHODS

Flies were cultured on a standard yeast, cornmeal, sugar and agar medium at room temperature (22 °C). Wild-type testes were dissected from pupae (aged 24–50 h after puparium formation) which had been obtained from cultures that generated only males (C(l)DX,y f/ Y females × In(l)w^m4/Y^hb- males). Y-deficient (X/O) testes were obtained from pupae resulting from the mating C(l)RM/O females × +/Y males. Details of the special chromosomes used may be found in Lindsley & Grell (1968). Pupae were surface sterilized in 3% sodium hypochlorite and washed in sterile distilled water. Testes were removed in a drop of culture medium and washed in several fresh drops of medium. An individual testis was disrupted with tungsten needles in a 0.02 ml drop of medium on a siliconized cavity slide. A plain coverslip was sealed into position with petroleum jelly such that the culture formed a column drop. Slides were kept in an inverted position so that testis cysts could adhere to the coverslip. The slides were incubated in the dark and viewed with an inverted microscope employing phase-contrast optics.

The culture medium, designated M3(BF), was that of Shields & Sang (1977), as modified by Cross & Sang (1978), and included 10% foetal calf serum.

FIGURE 1
Phase-contrast micrographs illustrating the sequence of meiosis and spermiogenesis in isolated developing cysts. (A) 16-cell spermatocyte cyst (4 h prior to meiosis). (B) A different 16-cell cyst in the process of the first meiotic division; note the elongate nuclear morphology (0 h). (C) The 32-cell stage (1.1 h). (D) The second meiotic division; the nuclear morphologies at first and second divisions are very similar (1.6 h). (E) Towards the end of the second division (2.1 h). (F) The 64-cell stage immediately after meiosis (2.3 h). (G) Nebenkerns (nk), paired with nuclei (n), become apparent (2.8 h). (H) Spermatids prior to nebenkern elongation and division (4.6 h). (I) Nebenkerns become less distinct as they prepare for elongation (6.6 h). (J) A different cyst. Nebenkerns elongate and are indistinct (approx. 11 h). (K) Nuclear migration and nebenkern elongation establishes a clear morphological polarity within the cyst (approx. 12.5 h). (L) Nuclear migration continues and elongation of the cyst commences (approx. 14.5 h). (M), (N) and (O) A different cyst. The cyst grows considerably in length as axoneme elongation occurs (approx. 18, 20 and 24.5 h, respectively). (P) A different cyst. A fully elongated cyst; sperm individualization takes place in the region of the cystic bulge (cb) which moves from right (head end) to left in this cyst. (Q) A different cyst. A fully elongated cyst showing the start of coiling at the curved head end. The cystic bulge has not progressed as far as that of the previous cyst. (R) A different cyst. The cyst shortens as coiling progresses. The cystic bulge has almost terminalized. (S) Coiling is nearing completion as the tails are drawn into the coiled mass, and the cystic bulge has terminalized. (T) Coiling completed. Each bar = 50 µm; (A) to (O) are to the same scale. Times are given as elapsed hours after the first meiotic division at 22 °C.
Table 1. *Approximate duration of meiosis and stages of spermiogenesis in vitro*

<table>
<thead>
<tr>
<th>Stage</th>
<th>Photograph (Fig. 1)</th>
<th>Duration* (h)</th>
<th>Number of cysts observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meiosis I</td>
<td>B</td>
<td>0.5</td>
<td>12</td>
</tr>
<tr>
<td>32-cell</td>
<td>C</td>
<td>0.8</td>
<td>13</td>
</tr>
<tr>
<td>Meiosis II</td>
<td>D, E</td>
<td>0.8</td>
<td>15</td>
</tr>
<tr>
<td>‘Onion’ nebenkern</td>
<td>G, H</td>
<td>6.5</td>
<td>15</td>
</tr>
<tr>
<td>Initiation of nebenkern elongation</td>
<td>J</td>
<td>2.0</td>
<td>18</td>
</tr>
<tr>
<td>Nuclear migration</td>
<td>K</td>
<td>3.0</td>
<td>19</td>
</tr>
<tr>
<td>Axoneme elongation 1 (nuclei visible)</td>
<td>L, M</td>
<td>4.0</td>
<td>18</td>
</tr>
<tr>
<td>Axoneme elongation 2</td>
<td>O, P</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Individualization</td>
<td>Q, T</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

* Our estimates for each stage are based on three types of observations: (1) two observations within a stage (minimum durations), (2) observations in each flanking stage (maximum durations), and (3) observations spanning multiple stages (sums of sequential durations).

RESULTS AND DISCUSSION

A photographic record of the typical sequences of meiosis and spermiogenesis observed in vitro is presented in Fig. 1. Some individual cysts were followed in culture from meiosis through elongation and individualization to a completely coiled state, but sperm motility was never observed. Figure 1 is a composite of several cysts since repeated exposure to light caused cysts to develop abnormal morphologies and to burst. This photographic sequence is basically consistent with that inferred from fixed material and has the advantage that the novice can use it to readily identify stages in live testes preparations. The sequence described parallels that for *Drosophila hydei* (Fowler & Johansson, 1976). Several dynamic features, which can be visualized especially well in cultured cysts, are the morphological changes in nuclei during meiosis (Fig. 1A–F); the development of nuclei and mitochondrial nebenkerns and their relationship in early spermiogenesis (elongation, Fig. 1G–L); the movement of the cystic bulge away from the sperm heads during individualization (Fig. 1P–R); and the coiling process (Fig. 1Q–T).

Dynamic observations on 32 individual cysts provide an estimate of the timing of meiosis and spermiogenesis in culture (Table 1). Our estimate of the duration of the various stages is based on cysts which entered and passed through a given stage while in culture; meiosis and the coiled state served as reference points for the early and later parts of the process, respectively. The mean overall time from meiosis to the completion of coiling was estimated to be about 45 h; this is close to the actual time observed for a single meiotic cyst to reach a coiled state (41.8 h).
The process of spermatogenesis was very labile in culture. In one series of cultures, 56 out of 134 spermatocyte cysts entered meiosis during the period of observation (48–72 h). Of these 56, only six reached a fully coiled state while the remainder were arrested primarily during elongation. The spermatocyte stage lasts about 3 days in vivo (Lindsley & Lifschytz, 1972) and a longer period of observation in vitro might have revealed a higher proportion entering meiosis. On the other hand, young spermatocyte cysts may not be competent to develop in culture. A slightly higher proportion of cysts reached a completely coiled state in culture when they were introduced into the culture medium during the axoneme elongation state (13 of 79).

Spermatocyte cysts from X/O males achieved a development in vitro (Fig. 2) which closely paralleled that observed in vivo (Kiefer, 1966). This suggests that the system will be useful for dynamic observations on cysts isolated from a variety of male-sterile mutants (Shellenbarger & Cross, 1919b).

Several observations on developing cysts in culture are atypical of events in vivo and are either not detected or not discussed by Fowler & Johannisson (1976). These include: (1) the maximum length achieved by elongating cysts was roughly 500 μm, or less than one-third of normal; (2) individualization, which normally occurs only in fully elongated cysts in vivo (Tokuyasu, Peacock & Hardy, 1972a), nevertheless took place in the short cysts which developed in vitro; (3) an apparently complete coiling took place in culture, even though this process is normally associated with the embedding of sperm heads in the...
terminal epithelium of the testis (Tokuyasu, Peacock & Hardy, 1972b); (4) though sperm coiling is thought to follow the completion of individualization in vivo (Tokuyasu et al. 1972b), this was not necessarily true in culture; indeed, the initiation of coiling could occur at variable stages of the individualization process (Fig. 1P and Q); and (5) the duration of in vitro development from meiosis to a coiled state (44.6 h) was less than one-half the estimated in vivo time of 96 h (Lindsley & Lifschytz, 1972; Tokuyasu, 1975). A specific difference of opinion with Fowler & Johannisson (1976) concerns the in vitro development time, which Fowler and Johannisson considered normal and which we feel is drastically foreshortened from that in vivo.

Some of the above observations suggest that cyst development in vitro is abnormal; nevertheless, the deviations from development in vivo may be highly informative concerning the control and co-ordination of the complex morphological changes in sperm development. The overall process of spermiogenesis was shortened, possibly due to an early termination of elongation, yet the processes of individualization and coiling were brought forward in time arguing against a rigid 'clock' mechanism for their initiation. It is also clear that in the elongation-individualization-coiling sequence, the proper completion of one process is not a prerequisite for the initiation of the next; thus, sperm morphogenesis may not be subject to a highly rigid sequential control. Furthermore, the fact that extensive cyst development occurred outside the testis (e.g. the embedding of sperm heads in the terminal epithelium was not necessary for coiling) argues against a strict structural control.

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REFERENCES


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