Characterization of a Temperature-sensitive Female Sterile Mutant \((l(l)1074^{ts})\) in \textit{Drosophila melanogaster}

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Abstract

A new X-linked temperature-sensitive female sterile mutant \((l(l)1074^{ts})\) is described. The non-permissive temperature for this mutant is 29°C. There are two temperature-sensitive periods during development—one between the 6th and 12th hours of embryogenesis and a second commencing during the first larval instar and terminating at mid pupation. Embryological abnormalities first become apparent during gastrulation and eventually these result in the breakdown of organogenesis and the complete absence of normal muscular contractions. Preconditioning mutant females at the non-permissive temperature for up to 48 h enhances the abnormal embryological effects produced by the mutant.

When developing gastrulae of the mutant are fragmented and cultured \textit{in vitro} at 29°C, many cell clumps fail to differentiate. This does not appear to be a consequence of cell death. Fragmented gastrulae of wild-type embryos always show cell differentiation following \textit{in vitro} culture.

On the basis of the studies reported in this paper, it is suggested that the \(l(l)1074^{+}\) gene product is deposited within the ovum during oogenesis and is vital for a number of early embryonic developmental processes rather than being critical for any specific developmental event such as the differentiation of a particular cell type.

Introduction

Large numbers of female sterile mutants have been isolated (for review see King and Mohler 1975). Many of these have now been characterized and have been found to affect specific steps in oogenesis. However, in a number of cases oogenesis is apparently normal, but the fertilized oocyte is incapable of supporting normal development. In these cases visible developmental abnormalities usually become apparent at some time up to and including gastrulation although developmental arrest may not actually occur until the end of embryogenesis or even during early larval life (Tarasoff and Suzuki 1970; Bakken 1973; Fullilove and Woodruff 1974; Gans et al. 1975; Rice and Garen 1975; Zalokar et al. 1975). The isolation of temperature-sensitive female sterile mutants has been of considerable importance in that it not only allows a more accurate estimate of the stage of embryogenesis when the maternal product is required, but it also enables investigators to determine whether there are other stages of development which require the presence of a particular maternal gene product or alternatively the functioning of that particular gene within the zygote. For example, flies developing from eggs laid by females homozygous for the \textit{amx} female sterile mutation can survive under certain environmental conditions and thus escape the embryonic lethal effect of the mutation, but they often hatch as adults which show characteristic phenotypic abnormalities (Shannon 1972, 1973).
possibly indicates a second period of gene activity during imaginal disc growth and differentiation. In addition, Tarasoff and Suzuki (1970), Fullilove and Woodruff (1974) and Gans et al. (1975) have each described mutants which appear to be biphasic in that they have two temperature-sensitive periods—one during embryogenesis and a second occurring later in development.

The primary developmental lesion produced by female sterile mutants is little understood. However, it has been suggested that the cytoplasm of the zygote, which comes almost exclusively from the maternal gamete, probably has an important role in the initiation of developmental commitments (Schneiderman and Bryant 1971; Gehring 1973; Postlethwait and Schneiderman 1973; Gans et al. 1975; Garcia-Bellido 1975), and also in the maintenance and compartmentalization of development which Morata and Lawrence (1977) have proposed as a theory to account for the progressive restriction of the developmental potential of groups of cells. Thus some important factors that may be affected by female sterile mutations include: (1) the distribution of cytoplasm within the egg, (2) the normal 'housekeeping functions' of the cell, (3) cell determination or differentiation, or both, (4) critical genetic regulatory events early in development, and (5) cell recognition factors that may be important for normal gastrulation and cell differentiation.

Two recent studies have attempted to investigate the nature of the primary developmental lesion in female sterile mutants which are not rescuable by the male genome. Kuroda (1974) reported that in vitro cell differentiation in embryonic cultures established from the mutant deep orange was abnormal in that there were no muscle cells whilst other cell types were sometimes aberrant. Zalokar et al. (1975) observed that there was a general disturbance to metabolic functions in a number of female sterile mutations which they recently analysed. However, in no case did they find that particular morphogenetic events were specifically affected. A number of mutants died very early in cleavage and in some cases developing eggs were found to be haploid, which may suggest that oocyte organization was affected.

In this paper the genetic and developmental effects of a new temperature-sensitive female sterile mutation (l(l)1074d) are described. In addition, the affect of this mutation on in vitro cell differentiation has been investigated in order to establish whether it is affecting specific developmental events associated with the differentiation of particular cell types, similar to that reported by Kuroda (1974) for deep orange, or whether it is producing some more generalized metabolic disturbance, such as that suggested by Zalokar et al. (1975) for the mutants that they investigated.

Materials and Methods

The mutant l(l)1074d was isolated from a routine sex-linked lethal test in which Oregon-R males were treated with the mutagen ethyl methane-sulphonate and then mated to Muller-5 females (Lindsley and Grell 1967). The permissive temperature for the mutant was found to be 22°C, whilst the non-permissive temperature was 29°C (Kneebone 1972). Stocks of the mutant are routinely maintained on treacle–semolina–yeast–agar medium at room temperature (22°C). Other stocks used in the experiments reported in this paper are described by Lindsley and Grell (1967).

Tests for Female Sterility

Progeny tests of two types of crosses have been carried out.

1. l(l)1074d males were mated to females from an attached-X (y=+/w11) stock and the progeny examined for the presence of both males and females. If only female progeny were obtained, then this would indicate that the mutant was a post-fertilization lethal, but if male progeny were also present then this may indicate that l(l)1074d is a female sterile mutant.
(2) Wild-type Oregon-R males were crossed to homozygous \( I(1)1074^a \) females. Progeny were scored for the presence of both males and females. No progeny would indicate that \( I(1)1074^a \) was a female sterile mutant, whilst the presence of only female progeny would suggest that the mutant was either a recessive lethal or alternatively a female sterile mutant which was rescuable by the paternal genome.

The possible outcomes from these two crosses are illustrated in Fig. 1.

\[
(1) \quad \hat{X}X/Y^{\varphi} \times I(1)1074/Y^{\sigma'}
\]

\[
\begin{align*}
\hat{X}X/I(1)1074^{\varphi}, & \quad Y/Y^{\sigma'}, \quad I(1)1074/Y^{\sigma'}, \quad \hat{X}X/Y^{\varphi} \\
22^\circ C & \quad \text{Dies} & \quad 29^\circ C
\end{align*}
\]

\[
\begin{align*}
I(1)1074/Y^{\sigma'} \quad \text{and} & \quad \hat{X}X/Y^{\varphi} \text{ survive but either} \\
\hat{X}X/Y^{\varphi} \text{ survive} & \quad (i) \quad I(1)1074/Y^{\sigma'} \text{ die,} \\
& \quad \text{therefore } I(1)1074 \text{ is a temperature-sensitive} \\
& \quad \text{post-fertilization lethal, or} \\
& \quad (ii) \quad I(1)1074/Y^{\sigma'} \text{ survive,} \\
& \quad \text{therefore } I(1)1074 \text{ is a temperature-sensitive} \\
& \quad \text{female sterile mutant}
\end{align*}
\]

\[
(2) \quad I(1)1074/I(1)1074/Y^{\varphi} \times +/-Y^{\sigma'}
\]

\[
\begin{align*}
I(1)1074/+ & \quad \text{Either} \\
I(1)1074/Y^{\sigma'} \text{ survive} & \quad (i) \quad \text{all progeny die,} \\
& \quad \text{therefore } I(1)1074 \text{ is a female sterile mutant, or} \\
& \quad (ii) \quad I(1)1074/+ \text{ survive and } I(1)1074/Y^{\sigma'} \text{ die,} \\
& \quad \text{therefore } I(1)1074 \text{ is a post-fertilization lethal or a} \\
& \quad \text{female sterile mutant rescued by the paternal genome}
\end{align*}
\]

Fig. 1. Two test crosses made to determine whether \( I(1)1074 \) is a temperature-sensitive female sterile mutant or a post-fertilization lethal.

\textbf{Tests for Fecundity, Egg Length, Frequency of Egg Hatch and Ovary Length}

Oregon-R or \( I(1)1074^a \) females were collected as virgins and maintained on standard medium fortified with yeast paste for 5 days before being placed individually in egg-laying chambers with two males of the same strain. Females were permitted to lay eggs for 24 h on agar egg plates blackened with charcoal and coated with yeast paste. After 24 h flies were transferred to fresh egg plates for a further egg-laying period, whilst the old plates were incubated for 24–30 h to allow the completion of embryonic development. At the end of this time the egg hatch frequency was determined. These egg-laying experiments were carried out at either 22 or 29°C. Some of the eggs were dechorionated in a saturated solution of calcium hypochlorite and their length measured.

A similarly treated group of females had their ovaries removed and fixed in a solution of formalin, alcohol and acetic acid. The length of each ovary was measured and the average of the two ovaries
was taken as the mean length for any particular female. In some experiments females were collected as virgins, kept at 22°C on medium fortified with yeast paste for 5 days and then transferred to 29°C for 36 h preconditioning before the egg-laying tests and ovary dissections were made.

**Temperature Shift Experiments**

The temperature-sensitive periods for *I(1)1074ts* were investigated by 'shift up' and 'shift down' experiments. The experimental design is shown in Figs 2a and 2b.

(a)  

![Diagram of temperature shifts](image)

(b) Egg Larval instars Pupation

![Diagram of temperature shifts](image)

**Fig. 2.** Experimental procedure used to determine temperature-sensitive periods. (a) *I(1)1074*/Y*L(1)1074ts/Y*. ‘Shift up’ (solid line) and ‘shift down’ (dotted line) every 3 h during embryogenesis to determine embryonic temperature-sensitive period. (b) *y*: = /w*/Y*X/l(1)1074*/Y. ‘Shift up’ (solid line) and ‘shift down’ (dotted line) every day after egg laying to determine later periods of temperature sensitivity.

To investigate the embryonic temperature-sensitive period, stocks of the mutant were maintained in temperature-controlled cages and allowed to deposit eggs on charcoal-blackened plates coated with fortified yeast paste. Groups of plates were removed from the cage every 3 h to ensure that the stage of embryonic development was relatively homogeneous. To determine the temperature-sensitive period, groups of eggs were shifted between the two temperatures every 3 h (Fig. 2a) and the egg
hatch frequency scored. Flies were conditioned at the high temperature for periods of 12, 24, 36 or 48 h prior to egg laying and the carrying out of the shift experiments.

To investigate the possibility of a second temperature-sensitive period later in development, \(I(1)1074^{a}\) males were mated to attached-X females and the developing offspring transferred between the two temperatures at daily intervals until the time of eclosion (Fig. 2b). The numbers of male and female progeny emerging from the culture vials were scored.

**Genetic Mapping**

Virgin \(I(1)1074^{a}\) females were crossed to \(y \ w^* \ cv \ ct \ v \ f(Y)(Xpl)\) males at room temperature. The \(F_1\) was inbred at 29°C and the \(F_2\) male progeny scored for genotype.

**Developmental Studies on Live Embryos**

Eggs were collected over a 3-h period from 5–8-day-old flies maintained in temperature-controlled cages (29°C) for 12 h. They were washed, dechorionated in a saturated solution of calcium hypochlorite for 10 min, and embryos at the syncitial blastoderm stage selected and mounted in a column drop of water in Sykes–Moore Chambers where development proceeded until the time of hatching. During this time embryos were observed under the microscope with bright field illumination. The temperature was maintained at 29°C using a Zeiss Airstream incubator (Model AS-I 400). Photographs were taken at regular intervals until the time of hatching.

**Establishment of Cell Cultures**

Eggs were collected from \(Oregon-R\) or \(I(1)1074^{a}\) stocks over a 3-h period. After collection, the eggs were washed with glass-distilled water and dechorionated in a saturated solution of calcium hypochlorite. After 10 min the hypochlorite was removed and the eggs washed three times with sterile distilled water and then three times with culture medium. Embryos in which the posterior midgut invagination had progressed halfway along the dorsal surface of the egg (about 15 min after the onset of gastrulation) were selected and placed in a small drop of culture medium on a cover glass. The embryo was torn apart into small fragments using a pair of fine tungsten needles. The cover glass containing the embryo fragments suspended in a drop of medium was then inverted over a cavity slide (similar to that described by Shields et al. 1975) to form a column culture. The slides were kept inverted for 1 h to enable sufficient time for the tissue fragments to adhere to the cover glass, before they were placed at 29°C for a further 3 days of incubation. Cultures of mutant and wild-type embryos were made at the same time using the same batch of medium.

**Culture Medium**

The culture medium used in this investigation was that described by Shields et al. (1975) with the following modifications (Shields, personal communication). KCl and NaCl were deleted and monopotassium glutamate (788 mg), bis-tris (105 mg) and cis-oxaloacetic acid (25 mg) added. In addition the amount of monosodium glutamate was increased to 653 mg. Medium was made up in batches of 100 ml.

**Examination of Cultures**

Cultures were viewed with phase contrast microscopy and selected areas photographed at intervals so that differentiation of groups of cells could be followed over a 72-h period. In some experiments cultures were stained with the vital stains neutral red and trypan blue after 24 h. The staining procedure described by DeRenzis and Schechterman (1973) was modified in that both stains were suspended in the culture medium at a final concentration of 0.25%. The stain was placed on the cover glass for 15 min before being removed and replaced with fresh culture medium prior to the re-establishment of the culture column. Live cells containing lipid or fat droplets (e.g. fat body cells) stained red whilst dead cells stained blue. Cultures were incubated for a further 2 days following staining.

**Results**

**Establishment of the Female Sterile Phenotype**

To determine whether \(I(1)1074^{a}\) has a female sterile phenotype the crosses illustrated in Fig. 1 were carried out. The results are shown in Table 1.
The cross between \( I(1)1074^{ts} \) males and attached-X females shows that no male progeny were obtained and furthermore many dead larvae and pupae were present in the culture vials (Table 1a). This could indicate that \( I(1)1074^{ts} \) is a post-fertilization lethal. On the other hand, the cross between wild-type males and \( I(1)1074^{ts} \) females failed to produce any offspring at all except for some live females and many dead larvae from the first egg laying (see Table 1b). This would indicate that the mutant is female sterile, with the maternal substance being inadequate to support any development of eggs laid during the second and third 24-h periods. Consequently there were no dead larvae in these vials, only unhatched eggs. A possible explanation for the discrepant results of the first cross is given in the discussion.

**Table 1.** Number of adult males and females observed when (a) \( I(1)1074^{ts}/Y \) males were crossed to attached-X (XX) females, and (b) \( I(1)1074^{ts} \) females were crossed to Oregon-R males at 29 and 22°C

At 29°C parents were transferred to new vials after 2 and 5 days

<table>
<thead>
<tr>
<th>No. of progeny</th>
<th>1st lay</th>
<th>29°C</th>
<th>2nd lay</th>
<th>3rd lay</th>
<th>22°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) ( I(1)1074^{ts}/Y ) males × XX females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>(b) ( I(1)1074^{ts} ) females × Oregon-R males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
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</tbody>
</table>

\( ^{a} \) Dead larvae were present.

**Table 2.** Ovary length, egg length, number of eggs laid and percentage hatch in strains \( I(1)1074^{ts} \) and Oregon-R at 22 and 29°C

<table>
<thead>
<tr>
<th>Genetic strain</th>
<th>Egg laying temperature (°C)</th>
<th>Time of preconditioning (h)</th>
<th>No. of eggs laid</th>
<th>% of eggs hatched</th>
<th>Ovary length (µm)</th>
<th>Egg length (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( I(1)1074^{ts} )</td>
<td>22</td>
<td>—</td>
<td>3031</td>
<td>75</td>
<td>1242 ± 32.5</td>
<td>586 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>—</td>
<td>1302</td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>—</td>
<td>1285</td>
<td>81</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>—</td>
<td>1665</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>36</td>
<td>490</td>
<td>5</td>
<td>1024 ± 14.4</td>
<td>547 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>36</td>
<td>546</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oregon-R</td>
<td>22</td>
<td>—</td>
<td>2640</td>
<td>95</td>
<td>1368 ± 41.4</td>
<td>606 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>—</td>
<td>2387</td>
<td>97</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>22</td>
<td>—</td>
<td>3677</td>
<td>98</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>—</td>
<td>2647</td>
<td>96</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>—</td>
<td>2038</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>36</td>
<td>6715</td>
<td>86</td>
<td>1458 ± 25.7</td>
<td>591 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>36</td>
<td>6269</td>
<td>89</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>36</td>
<td>4725</td>
<td>87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fecundity, Fertility and Ovarian Development**

The results of these experiments are shown in Table 2. At 29°C fewer eggs were laid and of these fewer hatched, particularly after 36 h of preconditioning of the adult females. This is correlated with a significant reduction in the ovary length \((t = 14.02; \ P < 0.001)\) as well as the egg length \((t = 5.26; \ P < 0.001)\).
The Temperature-sensitive Periods

(i) The embryonic temperature-sensitive period

The results for the 'shift down' experiment are shown in Fig. 3. There appears to be a sharp decrease in embryonic survival when transfers to the permissive temperature are not made within the first 9 h. Superimposed on this pattern is a general decrease in the percentage of eggs hatching with increased preconditioning times. Thus 48 h of preconditioning reduces the frequency of eggs hatching to below 5% even when developing eggs were shifted to 22°C within the first 6 h, whereas 12 h of preconditioning still enables about 40–60% of eggs to hatch as long as the 'shift down' is made within the first 6 h after the initiation of development. It is also evident that shorter preconditioning times result in more of the hatched eggs proceeding right through development to hatch as adults following a 'shift down' within the first 6 h.

![Histograms showing the frequency of eggs hatching (open bars) and frequency of adults eclosing (black bars) following the 'shift down' of developing eggs from 29 to 22°C at various times after being laid. Flies were preconditioned for (a) 12, (b) 24 (c) 36 or (d) 48 h prior to egg laying. The numbers shown above the white bars represent the number of eggs laid.](image)

Fig. 3. Histograms showing the frequency of eggs hatching (open bars) and frequency of adults eclosing (black bars) following the 'shift down' of developing eggs from 29 to 22°C at various times after being laid. Flies were preconditioned for (a) 12, (b) 24 (c) 36 or (d) 48 h prior to egg laying. The numbers shown above the white bars represent the number of eggs laid.

'Shift up' experiments, on the other hand, show that only after 21 h of development at 22°C does embryonic survival increase substantially following transfer to the non-permissive temperature, possibly signifying an end to the temperature-sensitive period (see Fig. 4).

(ii) Temperature-sensitive periods later in development

The possibility of a temperature-sensitive period occurring later in development was investigated by mating mutant males to attached-X females in order to eliminate the maternal effect of 1(l)1074'. The presence or absence of male progeny would indicate whether there was a second temperature-sensitive period. The results are shown in Fig. 5.
Fig. 4. Histogram showing the frequency of eggs hatching following the 'shift up' of developing embryos from 22 to 29°C at various times after the eggs were laid. Numbers shown on top of the bars represent the number of eggs laid.

Fig. 5. Graph showing the frequency of male offspring obtained when I(1)1074ts males were crossed with attached-X females. Developing offspring were kept at either 29°C (●) or 22°C (▲) for varying periods of time before being shifted to the permissive or non-permissive temperature.
There was a marked decrease in the frequency of male offspring between the 2nd and 4th day (corresponding to second and early third larval instars) in the ‘shift down’ experiment, and a correspondingly steep increase in the frequency of male offspring between the 10th and 12th day (corresponding to mid pupal development) in the ‘shift up’ experiment. In addition, many of the males emerging on these days exhibited an abnormal wing phenotype (see Fig. 6).

Length of Time between the Two Temperature-sensitive Periods

To determine whether there is a temperature-insensitive period between the two temperature-sensitive periods, an experiment was carried out in which eggs laid by mutant mothers were shifted from 22 to 29°C every 3 h up until 27 h. After 27 h the hatched and unhatched eggs in each shift were divided into four approximately equal sized groups and either (1) left at 29°C for an additional 10 days, (2) shifted back to 22°C after 4 days at 29°C, (3) shifted back to 22°C after 2 days at 29°C, or (4) shifted back to 22°C immediately and maintained at this temperature for 14 days. In each case, the presence of live adult progeny and/or the presence of dead larvae was scored. The results are shown in Table 3.

Generally, only those developing embryos shifted back to the permissive temperature at 27 h appeared to continue normal development and hatch as viable adults. A few adult progeny were obtained when developing larvae were shifted to the permissive temperature within 48 h of the initial shift, but the large numbers of dead larvae present suggest that the temperature-insensitive period is probably quite short.

Genetic Mapping Experiments

\(I(1)1074^a\) is a sex-linked mutation and its location on the X-chromosome has been determined by crossing the mutant to a strain carrying several visible sex-linked
recessive markers and scoring the class and frequency of recombinant progeny in the 
F₂ (see Table 4). The mutant appears to be located at about 16·3 map units on the 
X chromosome.

Table 3. Location of the two temperature-sensitive periods for I(I)1074ts

One period occurs during early embryogenesis, and the second during larval development. —, No 
larvae or adults; *, dead larvae; +, < 5 adults present; ++, 5–20 adults; ++++, 20–150 adults; 
+++++, 150–400 adults

<table>
<thead>
<tr>
<th>Time (h) of shift after egg laying:</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>18</th>
<th>21</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approx. No. of eggs:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kept at 29°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>→ 22°C after 96 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>→ 22°C after 48 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td>**</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>→ 22°C after 27 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td>**</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Embryological Studies

A comparison of the morphological changes occurring during embryonic develop-
ment in Oregon-R and I(I)1074ts strains is shown in Fig. 7. Differences first become 
evident at gastrulation. Thus in the mutant there is often no cephalic furrow or 
anterior midgut rudiment and furthermore there appear to be a number of irregular folds and furrows on the dorsal surface of the embryo. The yolk mass at the anterior 
end of the embryo is irregularly shaped. As development in the mutant proceeds,

Table 4. Number of various male genotypes obtained when I(I)1074ts/y wΔ cv ct v f 
females were crossed to I(I)1074ts/Y males

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of offspring</th>
<th>Genotype</th>
<th>No. of offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-crossover</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>y wΔ cv ct v f</td>
<td></td>
<td>196</td>
<td></td>
</tr>
<tr>
<td>Single crossover</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>y wΔ cv ct v +</td>
<td></td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>y wΔ cv ct + +</td>
<td></td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>y wΔ cv + + +</td>
<td></td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>y + + + + +</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>+ wΔ cv ct v f</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>+ + cv ct v f</td>
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<tr>
<td>+ ++ + v f</td>
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<tr>
<td>+ + + + + f</td>
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<tr>
<td>Double crossover</td>
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<tr>
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<tr>
<td>+ + cv ct v +</td>
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Genetic map of X chromosome indicating likely position of I(I)1074ts

segmentation is abnormal or absent, there is no organ formation and the embryo 
contains irregular masses of undigested yolk. These abnormalities result in the 
cessation of development at various stages (see Table 5).
In vitro Cell Culture of Fragmented Gastrulae

(i) Characteristics of wild-type embryonic fragments in culture

The disaggregation of wild-type gastrulae 15 min after the commencement of gastrulation produced cells which differentiated over the following 24–72 h into most of the cell types already described by Shields and Sang (1970) and Shields et al. (1975). Nerve cells differentiated within 18–24 h and were always present. They generally occurred in clusters of 10–20 cells some of which developed long and usually branched axons (40–70 μm long). Each cell in the cluster had a diameter of 5 μm. Clusters of nerve cells appeared to arise from angular cells with dense granular cytoplasm which migrated out from the periphery of many cell aggregates following dissociation of the embryo. A sequence of nerve cell differentiation is shown in Fig. 8a–d. The nerve cell clusters presumably arose from these angular cells by division as already suggested by Seecof et al. (1973). Generally, the number of nerve cells within the aggregates is consistent with them having arisen by a series of eight budding divisions followed by an equational division as described by Seecof et al. (1973). However, in some of the cultures established during the present series of experiments, groups of two or four cells were observed. These cells also tended to be larger (7 μm), pear-shaped and with a fibre extending from only one pole. It would appear then that there may be more than one type of nerve cell differentiation.

Table 5. Stages during embryogenesis at which developmental arrest occurs in strain I(I)1074* at 29 and 22°C

<table>
<thead>
<tr>
<th>No. of embryos examined</th>
<th>Temp. (°C)</th>
<th>Developmental arrest at:</th>
<th>Organ diff’n</th>
<th>No. of hatched larvae</th>
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<tr>
<td></td>
<td></td>
<td>Cleavage</td>
<td>Blastoderm</td>
<td>Gastrulation</td>
</tr>
<tr>
<td>26</td>
<td>29</td>
<td>12</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>22</td>
<td>3</td>
<td>—</td>
<td>0</td>
</tr>
</tbody>
</table>

Muscle cells may be present as either long spindle-shaped bipolar cells or alternatively as multinucleate myotubes. They begin to differentiate after about 24 h (Figs 9a and 9b). The number of muscle cells observed in any culture was between 10 and 20. Generally they were clustered and occasionally they were observed to pulsate.

Fat body cells, similar to those described by Shields et al. (1975), were always found in culture after about 48 h of incubation. They generally developed from flat angular epithelial-like cells which migrated out from undifferentiated embryonic cell masses after 24 h (Figs 9a and 9b). These cells eventually withdrew their processes, rounded up and accumulated fat or oil droplets. They often clump together in large aggregates and appear to be identical at the morphological level to those present in larval fat body tissue. The final diameter of these cells may be between 25 and 30 μm, particularly if cultures were kept for up to 5 days.

Other types of cell which were regularly found in cultures were chitin secreting cells and imaginal disc cells which were clustered in large spherical masses. Both these types of cell are as described by Shields et al. (1975), and in the present investigation they generally appeared between the 2nd and 3rd day of culture.
Fig. 7. Series of photographs showing stages of embryonic development in *Oregon-R* strain at 29°C. (a) Blastoderm stage. (b) Onset of gastrulation. (c) 30 min after the onset of gastrulation. (d) Formation of stomodaeum. (e) Segmentation. (f) After c. 20 h of development. Scale is 50 µm.
Fig. 8. Series of photographs showing stages of embryonic development in $I(1)1074^a$ strain at 29°C. The stages are comparable with those shown for Oregon-R strain in Fig. 7. Scale is 50 μm.
(ii) Characteristics of l(1)1074<sup>ns</sup> embryonic fragments in culture

Fragmentation of mutant gastrulae and subsequent culturing of the cells produced all those cell types already described for wild-type embryos (Fig. 10a and Table 6). The only two features which possibly suggest that there may be some difference in the in vitro cell differentiation patterns of the two strains were as follows.

![Series of photographs showing nerve cell differentiation in dissociated Oregon-R gastrulae: (a) 35 min after establishment of culture, showing undifferentiated clump of cells; (b) 8 h, showing angular epithelial-like cells (AE) migrating out of cell clump; (c) 18 h, showing groups of differentiated nerve cells (NC) some of which have developed branching axons, and (d) 54 h, showing nerve cell (NC) differentiation complete. Axons well developed. Scale is 50 μm.](image)

1. In about 25% of mutant cultures observed, almost all clumps of cells from dissociated embryos failed to differentiate (Fig. 10b). This did not appear to be correlated with any distinctly visible abnormality associated with gastrulation (e.g. absence of the cephalic furrow or irregular furrowing), nor was it associated with any marked degree of cell death since very few cells absorbed trypan blue. It thus appears that the cells lacked the competence to differentiate although they still remained viable.

2. There were no differentiated muscle cells in some mutant cultures. However, the apparently normal differentiation of myocytes in other cultures suggests that the lack of such cells in these cultures may be due to a slight lag in muscle cell determination in the mutant.
Fig. 10. *In vitro* muscle cell and fat body cell differentiation in fragmented *Oregon-R* gastrulae: (a) 18 h after culture establishment, showing elongating myocytes (M) and migrating flat angular epithelial-like cells (AE); and (b) 54 h, showing complete muscle cell differentiation (MC) and epithelial like cells accumulating fat and differentiating into fat body cells (FB). Scale is 50 μm.
Discussion

The results show that the temperature-sensitive sex-linked lethal mutant $I(1)1074\text{a}$ has the following properties.

(1) It is a female sterile mutant and the maternal factors necessary for normal development are probably acquired by the developing oocyte during the last stages of its growth.

(2) It appears to have at least two temperature-sensitive periods during development, indicating a requirement for the normal gene product at both these times. The first period of temperature sensitivity, which depends on a functional maternally inherited gene product or initiating factor being present in the cytoplasm of the egg, begins during gastrulation (or possibly earlier) and extends into the period of organ differentiation and the onset of muscular contractions. The second temperature-sensitive period begins during early larval development and extends into mid pupation. Normal development at this time requires the presence of a functional gene product which is presumably made by transcription and translation of nuclear genes before or during this period of development. This is indicated by the progeny types obtained when attached-X females are crossed to $I(1)1074\text{a}$ males. As expected there appears to be a close correlation between the genetic and embryological observations during these two periods (see Table 7).

(3) It does not appear to affect the determination or differentiation of particular cell types following \textit{in vitro} culture of fragmented embryos, but it does seem to totally prevent determination–differentiation of cell clumps in a proportion of fragmented gastrulae.

Gene mapping indicates that $I(1)1074\text{a}$ is in fact a single mutation. Examination of the salivary gland chromosomes confirms that there are no deletions or other structural alterations associated with the region of the X chromosome to which the mutant maps. Nevertheless, there is a remote possibility that $I(1)1074\text{a}$ could be two different genetic loci which are very closely linked and inseparable by recombination in the studies so far undertaken. The fact that $I(1)1074\text{a}$ appears to be a biphasic lethal mutant is not unusual as such mutants have been frequently described and indeed three such other mutants have been reported within the same vicinity of the X.
Mutant $l(I)1074^{st}$ in *Drosophila melanogaster*

chromosome. Tarasoff and Suzuki (1970) have isolated a maternal effect mutant ($l(I)E25^{st}$) which maps at 15·5 map units and has a temperature-sensitive period during the 12th to 15th hours of embryonic development and again during the second larval instar. Female sterile mutant $fs(1)1456$ isolated by Gans *et al.* (1975) is a biphasic

![In vitro cell differentiation in fragmented gastrulae of strain $l(I)1074^{st}$](image)

Fig. 11. *In vitro* cell differentiation in fragmented gastrulae of strain $l(I)1074^{st}$. (a) Normal differentiation after 54 h. NC, Nerve cells; MC, muscle cells; FB, fat body cells. (b) Undifferentiated clump after 54 h. Scale is 50 μm.

<table>
<thead>
<tr>
<th>Genetic observations</th>
<th>Morphological observations</th>
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<td>$l(I)1074$ shows a maternal effect inheritance pattern.</td>
<td>There is a reduction in fecundity and fertility at 29°C and this is correlated with a reduction in ovarian size and oocyte length.</td>
</tr>
<tr>
<td>The first temperature-sensitive period of development occurs between 6 and 12 h.</td>
<td>Gastrulation is abnormal and there is irregular yolk distribution and digestion. Organogenesis almost non-existent.</td>
</tr>
<tr>
<td>Second temperature-sensitive period of development occurs between late 1st instar and mid pupation.</td>
<td>Abnormal wing differentiation amongst survivors in temperature shift experiments.</td>
</tr>
</tbody>
</table>

maternal effect mutant located at 17 map units and with lethal phases during embryogenesis and again during the pupal stage. This mutant is temperature sensitive and females emerging at an intermediate temperature have abnormal wings and legs. Fullilove and Woodruff (1974) have isolated a temperature-sensitive maternal effect mutant located at 18 map units and which has temperature-sensitive periods during embryogenesis and again during late larval–pupal development. This clustering of
mutants with similar developmental effects is of interest and warrants further experimental investigation.

Non-permissive temperatures applied during the last 24–48 h of oogenesis in the mutant deprive the developing oocyte of some essential metabolite–developmental factor which is critical for normal embryonic development and developmental arrest usually occurs prior to blastulation. According to King (1970) this stage of egg maturation is noted for macromolecular synthesis in nurse cells and the transport of these macromolecules to the developing oocyte. Also during this time, follicle cells are laying down the vitelline membrane. There is some evidence from the present study that embryos ceasing development before or during blastulation have defective vitelline membranes. However, if females were not preconditioned prior to egg laying, developing oocytes will contain enough maternal gene product to support early development before it is depleted and developmental arrest occurs. There could be some delay in early developmental functions if the amount of maternal product is insufficient to support completely normal development.

The nature of the l(1)1074+ gene product as well as its primary effect on development remain unknown. The failure of cell clumps to differentiate when some mutant gastrulae are fragmented and cultured could be the result of some basic metabolic disturbance preventing groups of cells from becoming determined and/or differentiated. This same metabolic disturbance could explain the abnormal cell movements evident at gastrulation. According to the compartment hypothesis of Drosophila development (Morata and Lawrence 1977), groups or clones of cells become progressively restricted in their developmental potential as development proceeds. This restriction apparently depends on both maternally derived factors as well as differential nuclear gene activity. Perhaps the abnormal movement of cells at gastrulation in l(1)1074+ results from absent or dysfunctional genetic information located within the cytoplasm of the mutant oocyte. This abnormal movement of cells may then prevent them from becoming potentiated to undergo further development and differentiation. This hypothesis is currently being investigated.

Acknowledgments

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References


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