EVIDENCE FOR A POLARIZED MOVEMENT OF
THE LATERAL LOOPS OF NEWT LAMPBRUSH
CHROMOSOMES DURING OOGENESIS

M. H. L. SNOW AND H. G. CALLAN
Department of Zoology, The University, St. Andrews, Fife, Scotland

SUMMARY
Actinomycin D inhibits RNA synthesis on the lateral loops of newt lampbrush chromosomes. Partial inhibition does not provoke marked morphological alteration of ordinary lateral loops, most of which recover to the full their capacity for RNA synthesis within 2 days of treatment. However, occasional ordinary loops do not recover completely within the first few days after treatment, and in such loops RNA-synthesizing capacity is restricted to a region adjoining the thinner insertion in the parent chromomere. A greater degree of inhibition of RNA synthesis is accompanied by loss of matrix from ordinary lateral loops, and in the extreme case the loop axes retract to their parent chromomeres and neighbouring chromomeres coalesce; for the ordinary loops, full recovery from this stripped condition is nevertheless possible. Some 20 μ per loop extends during the first day following exposure to actinomycin, and normal morphology and RNA-synthesizing capacity are regained within 2–4 days. The giant granular loop of *Triturus cristatus cristatus* chromosome XII responds to extreme actinomycin D poisoning in different fashion. Matrix does not at once slough off its loop axis, but the loop present at the time of treatment is progressively replaced by a new granular loop which develops between the parent chromomere and the original loop’s dense tip. These observations support the theory that the DNA-containing axes of all lateral loops of lampbrush chromosomes continually extend from their parent chromomeres, engage in RNA synthesis while extended, and carry the associated RNP matrix along as they move towards the return insertions in the parent chromomeres, where loop axis retraction occurs.

INTRODUCTION
The great majority of the lateral loops of the lampbrush chromosomes of urodele Amphibia are asymmetrical structures, each with a thinner and a thicker insertion in a ‘parent’ chromomere (Fig. 4). Whenever, as a result of transverse breakage of a chromomere, a pair of loops forms a double bridge spanning the break, both members of the pair show asymmetry having the same polarity. Moreover, relative to the chromosome as a whole, the polarity evident in double bridges formed by loops which can be identified is constant. Thus, for example, whenever the giant granular loops of *Triturus cristatus cristatus* chromosome XII respond to extreme actinomycin D poisoning in different fashion. Matrix does not at once slough off its loop axis, but the loop present at the time of treatment is progressively replaced by a new granular loop which develops between the parent chromomere and the original loop’s dense tip. These observations support the theory that the DNA-containing axes of all lateral loops of lampbrush chromosomes continually extend from their parent chromomeres, engage in RNA synthesis while extended, and carry the associated RNP matrix along as they move towards the return insertions in the parent chromomeres, where loop axis retraction occurs.
at the base of the loop, as the thinner insertion; after it has been engaged for some
time in the synthesis of ribonucleoprotein (RNP) loop matrix, which accumulates
around the loop axis, this part of the axis will have moved round towards the thicker
insertion, will there shed its matrix and thereafter retract into the ‘right-hand’
portion of the parent chromomere (Fig. 1). In other words, the asymmetrical distribu-
tion of loop matrix was envisaged as resulting from the differing lengths of time for
which different portions of the loop axis had been involved in RNP synthesis, the
thinner insertion being the ‘youngest’ and the thicker insertion the ‘oldest’ portion.

Fig. 1. Diagram illustrating the theory of polarized lateral loop extension and retraction.
The chromomere is drawn quadripartite, indicating the planes in which it is known
to be capable of cleavage.

Gall & Callan (1962) set out to test this proposal by following the pattern of
incorporation of \(^3\text{H}\)uridine in lateral loop RNA. By good fortune \(T. c. \text{ cristatus}\) was
chosen for this study, and evidence for the envisaged movement was obtained for one
pair of loops, the aforementioned giant granular loops on chromosome XII (Figs. 2A;
6). Oocytes sampled a few hours after the provision of \(^3\text{H}\)uridine show labelled RNA
confined to the dense thinner tips of the giant granular loops (Fig. 7). Oocytes sampled
on subsequent days show progressively greater and greater lengths of the giant
granular loops labelled from the thinner towards the thicker insertion (Fig. 8 shows the
situation at 6 days after \(^3\text{H}\)uridine was administered) and under normal circumstances,
from the evidence of these experiments, 10 days must elapse before the giant granular
loops are labelled throughout their length.

How are these observations to be interpreted? The possibility that spasmodic waves
of RNA synthesis pass over the giant granular loops can be ruled out, for many different
oocytes sampled at a given time after the provision of \(^3\text{H}\)uridine all show their giant
granular loops labelled in the same region and to the same extent, these being depen-
dent on the time interval between \([^{3}H]\)uridine administration and sampling. However, there remain two alternative possibilities. Either loop axis and matrix move together from the thin towards the thick insertion, or the matrix alone moves over a stationary axis.

The first of these interpretations appears the likelier alternative. Loop axes undoubtedly do extend from their parent chromomeres in young oocytes, when the diplotene chromosomes first take on their lampbrush form; and it is equally apparent that they retract into the parent chromomeres just before oocytes mature. This could well be a continuous process, by means of which all the chromomeric DNA sooner or later extends and engages in RNA synthesis, rather than an initial extension, followed by a long stationary phase (during which most of the DNA remains compact and inert in the chromomere), followed by an ultimate retraction. However, the second interpretation, matrix movement over a stationary loop axis, is not excluded. The experiments to be described in this paper were carried out in an attempt to decide which of the two interpretations is correct.

In the American urodele *Triturus viridescens*, Gall (1963) has found a pair of lateral loops which label with \([^{3}H]\)uridine in similar fashion to the giant granular loops of *T. c. cristatus*. However, it needs to be emphasized that this sequential pattern of labelling is exceptional. The overwhelming majority of the other lateral loops of both these species, and certainly all of the ‘normal’ loops whose axes are coated with a fine fibrous RNP matrix, are labelled uniformly throughout their lengths shortly after \([^{3}H]\)uridine becomes available for incorporation into RNA (Fig. 5). Different normal loops acquire label at different rates, but they are alike in showing uniform degrees of labelling per unit of loop length at any given time after the provision of \([^{3}H]\)uridine, and this condition holds until the supply of labelled precursor has been exhausted.

Despite their uniform labelling with \([^{3}H]\)uridine, asymmetrical distribution of loop matrix is as much a feature of normal loops (Fig. 4) as it is of the giant granular loops. If one assumes that movement of axis plus matrix is universally responsible for producing loop asymmetry, the giant granular loop would be exceptional only in that its axis disengages from RNA synthesis before returning to the parent chromomere, yet retains for a while association with the RNA it has already synthesized. If one assumes on the contrary that movement of matrix relative to a stationary axis is responsible for loop asymmetry, then the giant granular loop differs from normal loops in that only a portion of its axis ever engages in RNA synthesis.

The antibiotic actinomycin D is a specific inhibitor of DNA-primed RNA synthesis (Reich & Goldberg, 1964). Our original aim was to suppress RNA synthesis on the lampbrush loops of *T. c. cristatus* by exposing oocytes to actinomycin D, then to remove the inhibitor, and investigate the recovery of RNA-synthesizing capacity by autoradiography. A tacit assumption was that actinomycin D would probably bind more readily to DNA exposed in the loop axes than to chromomeric DNA (see, however, Ebstein, 1967); and that after removal of unbound actinomycin, if the axes of normal loops continue to extend from their parent chromomeres, normal loops should show, during the first few days of their recovery, patterns of \([^{3}H]\)uridine labelling reminiscent of the giant granular loops; that is, with label confined to
regions neighbouring their thin insertions. As will be explained in this paper, two unexpected complications partly confounded the approach outlined above, and rendered the observations equivocal. Thereafter we turned our attention to morphological changes shown by the giant granular loops during recovery from actinomycin D poisoning, and this approach had a fruitful outcome.

MATERIALS AND METHODS

Materials

Females of *Triturus cristatus cristatus* were purchased from L. Haig and Son of Newdigate, Surrey, England. Most of the experiments were carried out during winter and spring months on newts which had been captured during the previous breeding season. The ovaries of such newts generally contain an abundance of small (0.7–0.9 mm diameter) white oocytes in which yolk is starting to accumulate. In oocytes within this size range the lampbrush chromosomes have well-developed lateral loops and the oocyte nuclei are large enough to permit isolation of the chromosomes without undue difficulty.

Samples of actinomycin D were kindly supplied by Dr H. J. Robinson, of the Merck Institute for Therapeutic Research. [3H]Uridine, at specific activities ranging from 760 to 1100 mc/mM, was purchased from the Radiochemical Centre, Amersham.

Methods

Lampbrush chromosomes were isolated in the manner described by Callan & Lloyd (1960). The nuclear sap of normal oocytes of *T. c. cristatus* in the size range 0.7–0.9 mm diameter does not fully disperse in the saline which we used for isolating oocyte nuclei, 0.1 M KCl and NaCl mixed in the proportions 5:1, but it does disperse if this saline contains a trace of CaCl2. We used CaCl2 at a concentration of 0.5 x 10^-3 M in the 0.1 M K/NaCl mixture.

For preparations to be examined in a fresh state the contents of oocyte nuclei were dispersed in saline in observation chambers designed by Dr J. G. Gall and illustrated by Callan & Lloyd (1960). After transfer of an oocyte nucleus to saline in such a chamber, and immediately following removal of the nuclear membrane, the preparation was exposed to the vapour of neutralized formalin for 2 min; thereafter, to prevent evaporation from the saline, a coverslip was placed over the top of the chamber, and its edges sealed to the slide with petroleum jelly.

Freshly isolated preparations of lampbrush chromosomes were examined by means of a Zeiss inverted Plankton microscope fitted with phase-contrast optics, and were photographed using a Zeiss Ukatron flash unit.

For autoradiographic observations each chamber was made up by sealing an ordinary 3 in. x 1 in. glass slide, instead of a coverslip, to the bored slide. Each freshly made preparation was treated briefly with formalin vapour but no top coverslip was applied thereafter. Instead the preparation was placed in a Petri dish containing a filter-paper circle wetted with saline, the dish covered and left undisturbed for 1½–2 h. At the end of this time 1 ml of acidified formalin (1 % acetic acid in formalin) was pipetted on to the filter-paper circle in the Petri dish and 15 min later the preparation was removed and placed in 5 % formalin in a Coplin jar.

This procedure generally ensured that the lampbrush chromosomes were firmly attached to the slide forming the base of the chamber, and not seriously contaminated with coagulated nuclear sap. An hour or more after being placed in formalin, a razor blade was used to separate the bored slide from the slide carrying the chromosomes, and such preparations were thereafter stored in 5 % formalin until further processed for autoradiography.

When sufficient preparations had accumulated for further processing, they were first washed in running tap water to remove the formalin, then placed for 5 min in ice-cold, freshly prepared 5 % trichloroacetic acid for extraction of unincorporated [3H]uridine, then again washed in running tap water. Thereafter they were rinsed in distilled water, taken up through an alcohol series followed by 2 baths in xylene to remove the paraffin wax, 2 baths in acetone to remove the xylene and air-dried. The slides were now subbed in chrome-alum gelatine (around but not over the chromosome preparations), dried while protected from dust and filmed in a dark room with
Movement of oocyte chromosomes' lateral loops

Kodak NTB-2 dipping emulsion diluted with distilled water to half its original strength. After drying in a stream of air the preparations were placed in light-tight boxes sealed with adhesive tape, and the boxes stored in a refrigerator during exposure.

The autoradiographs were developed for 2.5 min in Kodak D 19b at 20 °C, washed in distilled water, fixed in Kodak Metafix for 5 min, rinsed briefly in running tap water, hardened in 2% formalin for 5 min, washed in running tap water for 10 min, rinsed in distilled water and air-dried while protected from dust. When an autoradiograph was to be studied, a drop of distilled water was placed on the preparation and a coverslip applied. In such wet preparations (see Gall & Callan, 1962) the chromosomes can be observed by phase contrast. The Zeiss x 40 apochromatic oil-immersion phase-contrast objective fitted with an iris diaphragm is a particularly convenient lens for studying these wet autoradiographs, for the bright haloes around silver grains, which sometimes seriously interfere with images of the underlying chromosomes, can be reduced by shutting down the objective iris without an intolerable loss of resolution.

The wet preparations should not be allowed to dry out with the coverslip in place. Once the preparation has been studied the coverslip must be removed, otherwise it will seal tightly to the autoradiographic emulsion, and cannot thereafter be removed without damage to the preparation.

In a few experiments [3H]uridine was administered to newts by injection, but the more usual procedure was to test oocytes' capacity to incorporate [3H]uridine in vitro. For such a test, a dozen or so oocytes in the size range 0.7-0.9 mm diameter, together with adherent smaller oocytes, were excised from a newt ovary bathed in frog Ringer solution, pH 7. These oocytes were then transferred to a solid watch glass in which 25 μc of [3H]uridine in distilled water had previously been allowed to evaporate to dryness. The oocytes were drained of saline before transfer, but sufficient saline remained associated with them to form a pool in which the labelled uridine could dissolve. The oocytes were moved to and fro a few times to ensure distribution of the labelled uridine through the sample, the watch glass was then covered with a glass square, sealed with petroleum jelly to prevent further evaporation of water and set aside at room temperature (about 18 °C). Four hours later preparations of the lampbrush chromosomes were made from the selected oocytes and further processed as already described.

RESULTS

Experiments with actinomycin D in vitro

A female T. c. cristatus (Act D) was anaesthetized in a 0.1% solution of MS 222 (Sandoz). Its ovaries were then excised and placed in frog Ringer solution (lacking glucose) with pH adjusted to 7. A sample of oocytes of the standard size was removed, and a few preparations of the lampbrush chromosomes were made to establish that the sample was morphologically normal. This done, one ovary was transferred to frog Ringer solution containing 20 μg/ml actinomycin D, with pH again adjusted to 7, the other left in Ringer without actinomycin. One hour later part of the actinomycin-treated ovary was removed, thoroughly washed in Ringer, a dozen oocytes of standard size excised and placed in contact with 25 μc [3H]uridine for 4 h. A control sample of oocytes from the untreated ovary was similarly provided with labelled uridine. After a further hour other samples of treated and control oocytes were handled likewise.

Chromosome preparations from treated and control oocytes were made for autoradiography. It had been intended to make further preparations from oocytes which had been treated for 4 h with actinomycin, but this was deemed unnecessary. An effect of actinomycin on nuclear sap consistency was already evident during the making of preparations from oocytes which had been treated for 1 h; the sap was unusually fluid, allowing the chromosomes to disperse very rapidly. This was still more noticeable in preparations from oocytes treated for 2 h.

After fixation of these preparations the chambers were split in the usual way and the
preparations examined by phase contrast at low magnification. The control preparations were normal in all respects, but the actinomycin-treated preparations showed that the majority of the lateral loops had lost all or most of their RNP matrix, their axes being retracted to the chromosome axes. Matrix had been retained on a few loops: the 'lumpy' loops of chromosome II, the giant fusing and giant granular loops of chromosome XII and occasional other unidentified loops. This stripping of loop matrix by actinomycin D has already been recorded by Izawa, Allfrey & Mirsky (1963); we have found it to be of regular occurrence in oocytes treated as described above.

The preparations from actinomycin-treated oocytes were also peculiar in that large numbers of small granules (1–4 μ diameter) were scattered over the slides around the stripped chromosomes. These granules are probably aggregates of matrix stripped from the lateral loops. Such free granules are not absent from normal preparations, but they are far less numerous.

Autoradiographs of these preparations were exposed for 12 days. The control preparations were as expected, with abundant silver grains over most of the loops and with the giant granular loops labelled only at their dense tips (Fig. 9). Ribonuclease-treated control preparations (2 h in RNase, 0.1 mg/ml 0.01 M phosphate buffer, pH 6, 40 °C) were not entirely lacking silver grains over the loops, but the over-all grain count per 100 μ of loop length, averaged from representative samples, was only some 15% of that of undigested controls.

In the actinomycin-treated preparations some sparsely distributed silver grains overlaid the chromosome axes (to which the stripped or partially stripped loops had retracted). There were scarcely any grains over the few unstripped loops remaining extended, nor over the numerous free granules scattered amongst the chromosomes (Fig. 10).

We may therefore conclude that 1 or 2 h of treatment of excised T. c. cristatus oocytes with 20 μg actinomycin D per ml Ringer effectively inhibits RNA synthesis on the lampbrush chromosomes and causes stripping of RNP matrix from most of the lateral loops. Control excised oocytes kept for the same length of time in Ringer do not lose their capacity to synthesize RNA, and the normal morphology of their lampbrush chromosomes is maintained.

It is appropriate to mention here that Izawa et al. (1963) observed extensive stripping of loop matrix when lampbrush chromosomes were isolated from untreated oocytes and then immersed in a medium containing actinomycin D at 2 μg/ml. We have not been able to confirm this observation. If the contents of oocyte nuclei are allowed to disperse in 0.1 M saline, lightly buffered with phosphate to pH 6.8–7.0, and containing actinomycin D at concentrations of up to 20 μg/ml, the resulting preparations of lampbrush chromosomes cannot be distinguished from controls. When making observations of this kind it is of crucial importance to control the pH of the dispersing medium. The contracted and abnormally refractile lampbrush chromosomes illustrated in fig. 3A in the 1963 paper by Izawa et al., which the authors claim demonstrates the direct action of actinomycin, are almost certainly attributable instead to low pH.
Experiments with actinomycin D in vivo

General observations. As was mentioned in the Introduction, our original intention had been to inhibit RNA synthesis on the lampbrush chromosomes with actinomycin D, then remove the inhibitor and study by autoradiography the pattern of recovery of RNA-synthesizing capacity in the general run of lateral loops. The observation that actinomycin D strips normal loops of their matrix rendered this approach less promising and less critical, for in order to distinguish between supposedly ‘old’ loop in which inhibition has occurred and supposedly ‘new’ loop extending after removal of the inhibition, the old loop must remain in place, unstripped.

In order to follow recovery extending over several days, actinomycin treatment had to be given to ovaries not excised from newts; that is, ovaries retaining their connexion with the circulatory system. A newt (Act A) was anaesthetized, a slit made in its body wall and the right ovary dragged out through the slit. A control sample of standard oocytes gave normal lampbrush chromosome preparations. The entire animal was placed in actinomycin D in frog Ringer at 20 µg/ml; the saline contained sufficient MS 222 to maintain anaesthesia and pH was adjusted to 7. One hour later the newt was thoroughly washed in frog Ringer, a sample of oocytes taken, the rest of the ovary pushed back into the newt's body cavity and the body wall stitched up. The oocytes sampled after 1 h exposure to actinomycin D at 20 µg/ml gave lampbrush chromosome preparations indistinguishable from the controls, with no sign of matrix having been stripped off the loops.

Three days later the newt was anaesthetized again, and the remainder of its right ovary removed and placed in frog Ringer. A sample of oocytes was excised from this ovary and put in contact with [3H]uridine according to the standardized procedure. Later these gave lampbrush chromosome preparations which were processed as autoradiographs.

In the meantime the newt's left ovary was dragged out through the slit in the body wall, and the entire animal placed in actinomycin D in frog Ringer at 50 µg/ml, and containing MS 222. The ovary was sliced in a few places to ensure penetration of actinomycin into the ovarian cavity. An hour later the newt was again thoroughly washed in frog Ringer, a sample of oocytes taken from the left ovary, the rest of the left ovary pushed back into the body cavity and the body wall stitched. The oocytes sampled after 1 h exposure to actinomycin D at 50 µg/ml gave lampbrush chromosome preparations showing some sign of matrix stripping and increased numbers of granular matrix aggregates in the nuclear sap.

Three days later the newt was anaesthetized again, and the remainder of its left ovary removed. A sample of oocytes taken from this ovary gave lampbrush chromosome preparations which showed considerable stripping of matrix, more so than was evident immediately after exposure to actinomycin D. At first sight it seemed reasonable to conclude from this experiment that 1 h exposure to actinomycin D at 50 µg/ml was too severe a treatment, that oocytes do not recover thereafter (at least not within 3 days of treatment), and that if evidence for loop movement was to be sought in this manner exposure to a lower concentration of actinomycin would be necessary. It will
be shown later that neither of these conclusions was valid, and that the consequences 
of damage to the ovarian blood circulation had been overlooked. Autoradiographs 
were not prepared from the final sample of oocytes because of the extensive matrix 
stripping observed in the freshly made lampbrush chromosome preparations.

Autoradiographs from the oocytes sampled 3 days after treatment with actinomycin 
D at 20 μg/ml were exposed for 12 days. The general run of ordinary loops proved to 
be labelled throughout their lengths, but occasional ordinary loops (Fig. 11) were 
only partially labelled; in all such examples the label was confined to a region adjacent 
to the thinner loop insertion. Such a labelling pattern is reminiscent of the giant 
granular loop in normal circumstances.

Another female newt (Act C) was anaesthetized, its body wall slit on both sides and 
both ovaries dragged out. A piece of one ovary was excised and placed temporarily in 
frog Ringer, and the newt with exposed ovaries placed in frog Ringer containing
anaesthetic and actinomycin D at 20 μg/ml, pH adjusted to 7, and so treated for 4 h.
Autoradiographs of lampbrush chromosomes from the excised oocytes served as 
controls, of which 4 preparations were digested for 2 h with RNase prior to filming.

At the end of treatment with actinomycin D a sample of oocytes was assembled and 
similarly processed for autoradiography; the chromosomes from these oocytes showed 
no sign of matrix having stripped when the preparations were cursorily examined after 
fixation.

After thorough washing in frog Ringer the ovaries were re-inserted into the body 
cavity of this newt and the slits in its body wall sewn together. Further oocyte samples 
were taken at 1, 2, 4 and 8 days after the actinomycin treatment, and all were processed 
for autoradiography. None of the preparations showed sign of matrix having stripped.

The autoradiographs were exposed for 12 days. Silver grains were counted over 
several well-extended but otherwise randomly chosen lateral loops in 4 preparations 
from each of the oocyte samples. The counts were expressed per 100 μ of loop length, 
and averaged within a sample; they are given in Table 1.

Of the grain counts in controls it will be seen that again some 15% come from 
material which withstands RNase digestion. The resistant fraction may perhaps 
represent RNA base-paired with loop axis DNA, but this question has not been 
investigated further. Immediately after actinomycin D treatment the RNA-synthesizing 
capacity had fallen to 75% of that of controls, a day later to 32%; but given 2 days of 
recovery the oocytes were back to 80% of normality, and given 8 days of recovery, 
likewise. In the preparations from oocytes sampled 2 and 4 days after actinomycin D 
treatment there were again occasional partly labelled loops, with grains confined to the 
region adjacent to the thin insertion (Figs. 14, 15), but most of the loops appeared to be 
labelled uniformly throughout their lengths. No partly labelled loops were observed in 
the preparations from oocytes taken 8 days after actinomycin D treatment.

Similar experiments were carried out on 2 more newts. One (Act B) was treated for 
2 h with actinomycin D at 20 μg/ml. Matrix stripping did not occur, and occasional 
partially labelled loops were observed in preparations from oocytes allowed 2 days of 
recovery (Fig. 13). Another newt (Act E) was treated for 2 h with actinomycin D at 
50 μg/ml. Matrix stripping was observed in some but not all of the preparations from
Movement of oocyte chromosomes' lateral loops

Oocytes sampled immediately after actinomycin D treatment. Among oocytes sampled after one day of recovery, some gave preparations with loops much shorter than normal, though well labelled; others gave preparations with loops in the normal size range, but these poorly labelled. Oocytes sampled after 2 days of recovery gave preparations in all of which the loops were within the normal size range, and well labelled, apart from an occasional loop labelled only in the region adjacent to its thinner insertion (Fig. 12).

Table 1. Number of silver grains per 100 μ lateral loop length in autoradiographs over lampbrush chromosomes prepared from oocytes which had [3H]uridine available in vitro for 4 h (preparations exposed for 12 days)

<table>
<thead>
<tr>
<th>Oocyte sample</th>
<th>Grain counts per preparation</th>
<th>Mean grain counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>67</td>
</tr>
<tr>
<td>Control, RNase-digested</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Immediately after treatment with actinomycin D</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td>One day after treatment with actinomycin D</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Two days after treatment with actinomycin D</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>54</td>
</tr>
<tr>
<td>Four days after treatment with actinomycin D</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>66</td>
</tr>
<tr>
<td>Eight days after treatment with actinomycin D</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>55</td>
</tr>
</tbody>
</table>

Data obtained from T. c. cristatus Act C, whose ovaries were exposed to 20 μg actinomycin D per ml frog Ringer for 4 h without interruption of the blood circulation.

The results of the experiments in vivo so far described show that partial inhibition of RNA synthesis on the lateral loops of lampbrush chromosomes by actinomycin D, when not accompanied by matrix stripping, is reversible. Recovery may not be complete a few days after the inhibitor has been removed, but is nearly so. Bearing
this in mind, the significance and interpretation of partially labelled loops observed during recovery becomes problematic. If polarized extension of loop axis from the parent chromomere does occur, then one must assume that the inhibition of RNA synthesis on some few loops is irreversible, but that loop axis emerging after the inhibitor has been removed is or becomes free from bound actinomycin D, and thus able to engage in RNA synthesis. However, an alternative explanation is not excluded: assuming that loop axes remain stationary, occasional loops may be exceptional in that, after the inhibitor has been removed, actinomycin D remains bound to parts of these loops, but disengages from other parts (in all cases the region adjacent to the thinner insertion), there allowing RNA synthesis to proceed. The problem remained in this unsatisfactory state until we investigated the possibility that lateral loops might recover from a totally stripped condition, and concentrated our attention on to the giant granular loops of chromosome XII.

The last experiment described, in which a newt (Act E) had been exposed for 2 h to actinomycin D at 50 µg/ml, suggested that recovery after matrix stripping might indeed occur. However, there was too much variability. Some but not all oocytes sampled at the end of treatment showed matrix stripping; all oocytes sampled 2 days later showed loops with normal amounts of matrix. Although there were abnormally large numbers of free granules in some of these latter preparations—that is, evidence that matrix stripping had earlier occurred—there remained the suspicion that by accident all of the oocytes sampled 2 days after actinomycin D treatment might have been amongst those not showing matrix stripping had they instead been sampled immediately after treatment. It was thus clearly necessary to establish conditions of treatment under which matrix stripping could be assured.

Two newts (Act F and Act G) with their ovaries exposed were treated for 4 h with actinomycin D in frog Ringer at 100 µg/ml; a few small incisions were made in the ovary walls to ensure penetration of the inhibitor, but the incisions were placed so as to interfere minimally with blood circulation. Samples of oocytes were removed at the end of treatment. Variation was still apparent in the freshly made chromosome preparations from both animals, some showing extensive matrix stripping, others little or none. Another newt (Act H) was treated for 4 h with actinomycin D at 150 µg/ml, which is close to the solubility limit of the drug. It too gave preparations showing great variability in the degree of matrix stripping.

By this time it had become apparent that matrix stripping under the action of actinomycin D must depend in some way on blood circulation. The question was investigated by ligaturing the blood supply to the newt ovary.

A newt (Act J) was anaesthetized, its body wall slit lengthwise and an ovary dragged out through the slit. The newt was submerged in frog Ringer. A noose was made in 3/0 gut, using a double knot; the noose was slipped over the entire ovary and drawn tight around the peritoneal fold by which the ovary is suspended in the body cavity and in which run the multiple ovarian arteries and veins. Tightening of the gut ligature was controlled under a binocular microscope, and was continued until circulation ceased. The ovary wall was sliced in a few places, avoiding damage to major blood vessels, and the newt was now placed in frog Ringer, pH 7, containing
Movement of oocyte chromosomes' lateral loops

Actinomycin D at 100 μg/ml and sufficient MS222 to maintain anaesthesia. The newt was examined at intervals to ensure that the blood circulation remained shut off and portions of ovary were removed after one and 2 h. The tourniquet was now released and the blood circulation seen to re-establish. The newt was thoroughly washed in frog Ringer, and the body wall stitched with gut.

The portions of ovary excised after 1 and 2 h immersion in actinomycin D were rinsed several times in frog Ringer, and lampbrush chromosome preparations from oocytes of standard size were made at once. The preparations were examined fresh, unfixed. Those from oocytes which had received 1 h treatment showed loops which were not fully stripped, but the loops were unusually ragged, with missing segments of matrix, and they were irregularly twisted. The preparations from oocytes treated for 2 h showed more severe stripping, though it was still not complete. The remainder of the 2-h sample of oocytes was stored in frog Ringer for 3 h, at which time further preparations were made. These uniformly showed complete stripping of matrix from all but the lumpy loops of chromosome II, the giant fusing and giant granular loops of chromosome XII and very occasional large normal loops on chromosome I. In the meantime a further sample of oocytes had been removed from the newt for comparison with those stored in vitro for 3 h after actinomycin D treatment. These oocytes, treated for 2 h with actinomycin D followed by 3 h in vivo, were also uniformly and severely stripped.

Seven days later another sample of oocytes was removed from the treated ovary of newt Act J, after establishing that ovarian blood circulation was still functioning. All preparations from the oocytes of standard size showed lampbrush chromosomes which had returned to a normal condition, except that the giant granular loops of chromosome XII were unusually small and of low refractility. The outcome of this and several repeat experiments conclusively established that most lateral loops can recover within a few days their normal size and morphology after having been stripped of matrix. We do not know why the treatment with actinomycin D is uniformly effective only if it is accompanied by interruption of the blood circulation—particularly since most of the newts while being treated were totally immersed in solution containing the inhibitor. A degree of anoxia may be the responsible co-factor; it is certainly not the primary factor, for oocytes from an ovary ligatured for 4 h but not treated with actinomycin D gave entirely normal preparations of lampbrush chromosomes.

If enzymes such as RNase or trypsin are applied to preparations of lampbrush chromosomes lying in saline at pH 7, matrix is stripped from the lateral loops, loop axes retract to the chromosome axes and appear to amalgamate with the chromomeres, and neighbouring chromomeres coalesce with one another (Macgregor & Callan, 1962). The question arises whether, as an outcome of matrix stripping induced by actinomycin, the loop axes denuded of matrix remain extended inside oocyte nuclei and retract only when the chromosomes are isolated, or whether instead the bare loop axes retract in situ in the oocytes. The observation that most loops are extremely short in preparations of the chromosomes isolated from oocytes which have been allowed to recover for 1 day after matrix stripping is not conclusive, for a long loop axis with
scanty matrix might very well contract during isolation of the chromosomes, while the nuclear sap disperses. The question has, however, been answered decisively by studying sections through fixed oocytes. Sections cut through control standard oocytes fixed in Sanfelice's fluid, embedded in wax and stained in iron haematoxylin show the loops extending for long distances through the volume of the nucleus (Fig. 18). The chromomeres are minute, and it may even be difficult to identify chromosome axes. In sections through actinomycin-treated oocytes, in which matrix has been stripped from the loops, loops can no longer be seen but chromosome axes with abnormally large chromomeres are clearly visible (Fig. 19).

In sections through oocytes allowed 1 day to recover from matrix stripping the chromosome axes are still evident, but the chromomeres are individually smaller and short lateral loops (some 20 \mu in length) can be seen (Fig. 20). We can therefore be sure that when lateral loops lose their matrix as a result of actinomycin treatment their axes do retract to the parent chromomeres, and neighbouring chromomeres coalesce. We have estimated that this leads to a 40% reduction in chromosome axis length. During recovery, neighbouring chromomeres separate, and loop axes sprout out afresh as they accumulate RNP matrix.

**Observations on the giant granular loops of chromosome XII.** In the course of the experiments described in the preceding section we had noticed peculiar morphological responses of the giant granular loops to inhibition of RNA synthesis by actinomycin D, and recovery thereafter. Unlike most lateral loops, the giant granular loops do not at once lose their RNP matrix after treatment which causes other loops to lose theirs. And during the recovery of oocytes from conditions of treatment which, though partially inhibitory, do not produce severe stripping of matrix from the general run of loops, whereas the latter have recovered their normal lengths and RNA-synthesizing capacities within 2 days of treatment, the giant granular loops are at this time, and for several days thereafter, abnormally short (Figs. 16, 17), though otherwise of normal morphology. Furthermore, in the last experiment described, involving newt Act J, where stripping had preceded recovery, the giant granular loops 7 days after treatment were not only small, but of reduced refractility and morphologically abnormal. These casual observations prompted us to repeat, with minor modifications, the treatment given to Act J on several more newts, and to examine in greater detail what happens

---

**Fig. 2.** Camera lucida drawings of the giant granular loops of chromosome XII of *T. c. cristatus*. The drawings illustrate the morphological consequences of exposure of a newt ovary for 4 h to actinomycin D at 100 \mu g/ml, with the blood supply shut off during treatment; and subsequent recovery with the circulation re-established.

A, Control. B, Immediately following exposure to actinomycin. Note the blunted dense tips and the short filaments which connect them to the parent chromomere. C and D, One day of recovery. Note the long filaments which connect the dense tips to the parent chromomeres, devoid of matrix at both ends. E, 2 days, and F, 4 days, of recovery. Note the bare portions of filament still visible adjacent to the dense tips. G, 7 days, and H, 14 days, of recovery. The original giant granular loops have entirely disappeared, and have been replaced by new loops formed on the loop axis which extended after actinomycin treatment.
Fig. 2. For legend see opposite page.
to the giant granular loops immediately after treatment with the inhibitor, and during
the following days while the majority of loops return to normal.

Normal giant granular loops are shown in Figs. 2A and 6. A thin filament 3-10 μ long
connects the parent chromomere with the pointed, highly refractile (hereafter termed
‘dense’) tip of the loop. This dense tip starts as a solid cone, but within a few microns
of its origin it splits into several refractile bars angled obliquely with respect to the
long axis of the loop; these bars wind a spiral course for a few microns, and then give
way to a granular region which extends throughout the rest of the loop. The granules
are larger (1-2 μ diameter) close to the bars, and become progressively smaller as the
other end of the loop is approached. The granules are embedded in a matrix of lower
refractility. A loop axis can often be seen in the granular region but is not visible within
the dense zone. In normal circumstances the giant granular loop is some 60 μ long.

If T. c. cristatus oocytes are treated with actinomycin D so as to produce total
stripping of matrix from most of the loops (i.e. 2 h in vitro at 20 μg/ml, or 4 h in vivo
at 100 μg/ml with the blood circulation interrupted), the morphology of the giant
granular loop (Figs. 2B, 21) is altered in 3 respects: the tip of the dense region
becomes blunt and rounded off; the filament between the parent chromomere and the
tip of the dense region shortens to about 3 μ; and the granular region becomes more
compact and refractile. Overall, the length of the giant granular loop is reduced some
5-10%, but there is no sign that matrix has stripped from the loop axis.

On removal of the inhibitor and re-establishment of blood circulation, while most of
the loops extend and return to their normal shapes and sizes, significant morphological
changes are observable in the giant granular loops. A filament gradually appears
between the parent chromomere and the blunt-ended dense region of the loop. Given
36 h of recovery this filament has reached a length of some 25 μ; thereafter estimates
of length are not very meaningful, for the filament becomes contorted.

The morphology of this long filament is peculiar, and consistently so in all the
newts we have studied. After 24 h of recovery, where the filament emerges from
the dense tip it is very thin, close to the resolving limit of the light microscope for the
first 5 μ or so, and without discernible matrix. Fine-textured matrix is attached to the
rest of the filament, increasing in amount as the filament approaches the chromomere
for all but the final 3 μ or so. Here the matrix ends abruptly. Figs. 2C, D, 22, 23 show
the appearance of the filament in giant granular loops from oocytes allowed to recover
for 24 h. At this time the matrix associated with the long filament is similar to that of
the ordinary loops, though the manner of its distribution, more towards the chromo-
mere, less towards the dense tip of the giant granular loop, appears opposite in
polarity to the distribution of matrix on the original giant granular loop. By this time
some shedding of granular matrix in beaded strings from the other (thick) end of
giant granular loop has occurred (the shed material can often be observed near its site
of origin in preparations examined before the nuclear sap has had time to disperse), and
there are signs of the original dense conical tips of the giant granular loops breaking up
into refractile bars. Both these processes continue as time passes, so that in oocytes
sampled 4 days after actinomycin D treatment little remains of the giant granular loop
originally present when the inhibitor was administered.
Meanwhile changes occur in the filament connecting the former dense tip (itself progressively transforming into granular region) and the chromomere. Close to the former dense tip the filament remains thin and without matrix, but in the region where it is covered with matrix the filament becomes thicker, more refractile, and it is thrown into a loose coil. Figures 24 and 2E show the condition in oocytes sampled after 42 and 48 h of recovery. At this time the matrix texture is still like that of neighbouring ordinary loops but when oocytes are sampled after 4 days of recovery the matrix is distinctly coarser and identification of the filament is no longer difficult (Fig. 2F). By now there are signs of a new dense tip forming on the filament close to its insertion in the axial chromomere; at the other end the filament remains devoid of matrix where it links on to the remnants of the original giant granular loop.

In oocytes sampled 7 days after actinomycin D treatment all trace of the original giant granular loop has disappeared. In its place there is a tiny new granular loop 20–30 µ long (Figs. 2G, 2H), built up in association with the filament which appeared during recovery from inhibition. The polarity of this little loop is the same as that of the loop it has come to replace, but it is looser in texture than a normal giant granular loop, its granules are less refractile and its dense tip tapers more gradually towards the filament leading to the chromomere.

We have not had much success following further stages towards recovery. Mortality becomes high 7 days and more after actinomycin D treatment. The inhibitor interferes in some way with tissue regeneration and wound healing, and a large ulcer invariably develops around the original incision in the body wall. Moreover, in those newts which did survive for several weeks after treatment the operated ovary developed adhesions to the peritoneal lining of the body cavity, and defects arose in its blood circulation which caused many oocytes to become moribund. In later experiments we attempted to improve the prospects for recovery of each newt treated with actinomycin D by placing the animal on a plastic bridge with only its exposed ovary projecting through a hole in the bridge and bathed in the actinomycin solution. One newt handled in this way yielded seemingly healthy oocytes 14 days after treatment, and 2 newts 21 days after treatment. In all 3 animals the granular loops were smaller, 35–40 µ long, and of lower refractility, than the giant granular loops of untreated newts, and their dense ends still tapered less abruptly than normally towards the filament leading to the chromomere (Fig. 2H).

The filament which makes its appearance during the early stages of recovery of the giant granular loop from poisoning by actinomycin D may have emerged from the parent chromomere; that is, it may be newly extended loop axis; or it may have been liberated from the original dense tip as a result of matrix shedding, or of matrix moving around a stationary loop axis. Since the axis of a lateral loop contains a continuous DNA fibre, and since no synthesis of chromosomal DNA occurs during the lampbrush phase of oogenesis, there appear to be no other alternatives.

We have recently established that loop axis is tightly wound up in the dense conical tip of the normal giant granular loop, i.e. in the region which supports RNA synthesis. There is certainly sufficient axis in this short region to generate the length of filament observed. If T. c. cristatus lampbrush chromosomes are dispersed in dilute saline
(0.02–0.04 M) all the lateral loops lose refractility, and the giant granular loops especially so. The granules break up into smaller and smaller particles and this region of the loop 'fluffs out'. The dense tip, while maintaining its evident polarity, uncoils and extends, and its matrix too fluffs out until it comes to resemble the matrix of the generality of loops. The original boundary between the region of refractile bars and the granular region is often marked by a distinct shoulder in giant granular loops fluffed out in this manner; there may be some 100 μ of loop between the parent chromomere and this shoulder, and this length has been generated from a dense end, including the terminal cone and refractile bars, which is normally only 10–15 μ long.

Fig. 3. Camera lucida drawing of the giant granular loops taken from a preparation of lampbrush chromosomes dispersed in dilute (0.0375 M) saline. The narrower, outlined regions of these loops are derived from the normally dense tips.

We can exclude the possibility that the 'post-actinomycin' filament appears as a result of matrix shedding from the dense tip of the giant granular loop during the first day of recovery from treatment. Two hundred μc of [3H]uridine were injected into a newt, and 12 h later 1 ovary, with its blood supply ligatured, was exposed for 4 h to actinomycin D at 100 μg/ml. A sample of oocytes was examined to establish that matrix stripping had occurred, the ligature was removed, the newt was washed in Ringer and its ovary re-inserted into the body cavity. One day later lampbrush chromosome preparations were made and processed for autoradiography. In all preparations where the giant granular loops were conveniently disposed for observation (as in Fig. 26), their dense tips were labelled to the extent that is characteristic of preparations made after 12 h availability of [3H]uridine. If matrix had been shed from the dense tips, less than the normal extent of labelling should have resulted. Silver grains over the dense tips register [3H]uridine that was already incorporated before inhibition of RNA synthesis by actinomycin D. There are also a few silver grains over part of the filament connecting the dense tip of the giant granular loop to the parent chromomere, the part labelled being regularly that closer to the chromomere. These grains register [3H]uridine incorporated during the day of recovery.
DISCUSSION

We are thus left with two possible explanations for the origin of the 'post-actinomycin' filament. Either it extends from the parent chromomere, as 'new' loop axis, or it is released while old matrix moves on towards the thicker end of the giant granular loop.

The most telling evidence against matrix movement being responsible for exposure of the post-actinomycin filament is morphological: the persistence for at least 4 days of the bare region of filament adjacent to what remains of the original giant granular loop. If the loop axis remained stationary, movement of matrix would certainly expose loop axis, but without adherent material to hinder its progress one might reasonably expect that new matrix accumulating nearer to the chromomere would quickly close the gap and obliterate the bare region. This does not occur. As long as part of the original giant granular loop remains, so too does the adjacent bare region.

The alternative explanation for the sequence of morphological changes which have been described, namely that loop axis and matrix move together, appears much the more likely. The persisting bare patch of filament originally connected the dense tip of the giant granular loop directly with its chromomere at the time when RNA synthesis was suppressed by actinomycin D. Thereafter this region of the loop axis did not regain its capacity to direct RNA synthesis, just as axis of the normal giant granular loop loses its RNA-synthesizing capacity once it has passed beyond the dense conical tip. Meanwhile loop axis newly emerging from the chromomere is progressively subjected to less and less inhibition as the concentration of actinomycin D in the oocyte falls, this accounting for the apparent inverse polarity of matrix accumulating on the filament during the first days of recovery.

Moreover, the idea that loop axis and matrix move together readily explains the origin of the partly labelled ordinary loops described earlier in this paper. For such loops a theory of matrix movement alone provides no explanation.

It is not disputed that during transcription newly synthesized RNA progressively peels off from the loop axis as nucleotides are added, and thus the filamentous RNP matrix described by Miller (1965) must be presumed to move its point of attachment to loop axis as RNA synthesis proceeds. Miller interprets his observations on the assumption that the loop axis is stationary, the synthesis of RNA molecules being initiated repeatedly at the thin insertion of an ordinary loop, with the molecules peeling off as synthesis progresses right round towards the thick insertion. The distribution of matrix on most ordinary loops is not in accord with Miller's proposal, at least as judged by observations made with the light microscope, for the maximum spread of matrix about loop axis is attained well before the thicker end of the loop inserts into its parent chromomere. However, be this as it may, loop axis movement and the steady transport of matrix along with the axis is not incompatible with contemporaneous movement of matrix relative to the axis. Indeed, movement of matrix relative to axis could well be opposite to the direction of loop axis movement, with many points of initiation of RNA synthesis scattered over the loop's length. There is insufficient evidence to warrant further speculation along these lines at the present
Our intention has been merely to set out the evidence for loop axis movement, which if proved correct has important implications regarding the organization of genetic material (Callan, 1967) in all eukaryotic organisms.

REFERENCES


(Received 3 December 1968)

Figures 4-8 are all at the magnification indicated by the scale on Fig. 4, and all were photographed using phase contrast.

Fig. 4. An exceptionally long, but in other respects ordinary, lateral loop on chromosome I of T. c. cristatus, showing the typically polarized distribution of matrix attached to the loop's axis.

Fig. 5. Autoradiograph of a loop such as that to be seen in Fig. 4. [3H]Uridine was provided by injection, and the preparation was made 18 h later.

Fig. 6. Left end of the longer arm of chromosome XII of T. c. cristatus, including the pair of giant granular loops. The dense tip of one of these loops is indicated by an arrow.

Fig. 7. Autoradiograph including the giant granular loops. [3H]Uridine was provided in vitro and the preparation was made 4 h later. Arrows point to silver grains over the dense tips.

Fig. 8. Autoradiograph including the giant granular loops. [3H]Uridine was provided by injection, and the preparation was made 6 days later. Arrows point to the unlabelled portions of these loops.
Movement of oocyte chromosomes' lateral loops
Figures 9 and 10 are at the magnification indicated by the scale on Fig. 9. Figs. 11–15 are all at the magnification indicated by the scale on Fig. 11. All figures are of autoradiographs photographed using phase contrast. [3H]Uridine was in all cases provided in vitro, and the preparations were made 4 h later.

Fig. 9. Part of a control preparation from Act D. The pair of giant granular loops is indicated by arrows.

Fig. 10. Part of a preparation from an oocyte of Act D exposed for 1 h in vitro to actinomycin D at 20 µg/ml. Extensive stripping of matrix from all but the giant granular loops, indicated by arrows, has occurred, and little [3H]uridine has been incorporated.

Fig. 11. Differentially labelled pair of normal loops from Act A. Oocyte exposed in vivo for 1 h to actinomycin D at 20 µg/ml, then allowed 3 days to recover.

Fig. 12. Differentially labelled (on the left) and uniformly labelled (on the right) normal loops from Act E. Oocyte exposed in vivo for 2 h to actinomycin D at 50 µg/ml, then allowed 4 days to recover.

Fig. 13. Differentially labelled normal loop from Act B. Oocyte exposed in vivo for 2 h to actinomycin D at 20 µg/ml, then allowed 2 days to recover.

Fig. 14. Differentially labelled normal loop, flanked by uniformly labelled loops, from Act C. Oocyte exposed in vivo for 4 h to actinomycin D at 20 µg/ml, then allowed 2 days to recover.

Fig. 15. Differentially labelled (on the left) and uniformly labelled (on the right) normal loops from Act C. Oocyte exposed in vivo for 4 h to actinomycin D at 20 µg/ml, then allowed 4 days to recover.
Movement of oocyte chromosomes' lateral loops
Figures 16–20 are all at the magnification indicated by the scale on Fig. 16. Figs. 16 and 17 are phase-contrast photographs of freshly isolated lampbrush chromosomes lying in saline. Figs. 18–20 are photographs of 8-μ sections through oocyte nuclei fixed in Sanfelice's fluid and stained with iron haematoxylin.

Fig. 16. Double bridge formed by abnormally short giant granular loops from Act G. Oocyte exposed in vivo for 4 h to actinomycin D at 100 μg/ml, without interruption of the blood circulation, and allowed 2 days to recover.

Fig. 17. Abnormally short giant granular loops from Act F. Oocyte exposed in vivo for 4 h to actinomycin D at 100 μg/ml, without interruption of the blood circulation, and allowed 5 days to recover.

Fig. 18. Section through a control oocyte nucleus, for comparison with Figs. 19 and 20. The field is full of long lampbrush loops cut in various planes, and no one loop is visible throughout its length. An arrow points to a row of neighbouring chromomeres which happen to lie in the plane of the section.

Fig. 19. The oocyte from which this section was taken was exposed in vivo for 4 h to actinomycin D at 100 μg/ml, and during treatment the blood supply to the ovary was shut off; the oocyte was fixed immediately after treatment. Rows of chromomeres are visible, but there is no sign of lateral loops.

Fig. 20. As Fig. 19, but the blood supply to this oocyte was restored after actinomycin treatment, and 1 day allowed for recovery. Notice that all the loops are short; several lie wholly within the plane of the section, and within the depth of focus of a ×100 apochromatic objective.
Movement of oocyte chromosomes' lateral loops
Figures 21–26 are all at the magnification indicated by the scale on Fig. 22. Figs. 21–25 are phase-contrast photographs of freshly isolated lambrush chromosomes lying in saline. Fig. 26 is a phase-contrast photograph of an autoradiograph. All figures include the giant granular loops of chromosome XII of *T. c. cristatus*. All these preparations were made from oocytes which had been exposed to actinomycin D for 4 h at 100 µg/ml, and during treatment the blood supply to the ovary was shut off.

Fig. 21. Immediately following treatment. Notice the blunt dense tips of the giant granular loops lying very close to the chromosome axis. Matrix from the other loops has been sloughed off.

Fig. 22. The oocyte from which this preparation was made was allowed to recover for 24 h, with its blood circulation restored. Notice the filament connecting the dense end of one giant granular loop to the chromosome axis. An arrow points to the region devoid of matrix.

Fig. 23. Just as Fig. 22.

Fig. 24. As Figs. 22 and 23, but after 42 h of recovery.

Fig. 25. The oocyte from which this preparation was made was allowed to recover for 7 days. The giant granular loop originally present has been replaced by a tiny new granular loop; its sister loop is out of focus.

Fig. 26. The newt from which this preparation was made was first injected with [3H]uridine. Twelve hours later one of its ovaries was exposed for 4 h to actinomycin D. The preparation was made after allowing the oocyte 1 day to recover. The silver grains over the dense tip of the giant granular loop register [3H]uridine which was incorporated before actinomycin treatment. The grains over the lower half of the filament connecting the dense tip to the chromosome axis, indicated by an arrow, register incorporation after treatment.
Movement of oocyte chromosomes' lateral loops