RECENT DEVELOPMENTS IN THE STUDY OF LAMPBRUSH CHROMOSOMES

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My brief in writing this essay is to provide a state-of-the-art review about research into the organisation and function of the lampbrush chromosomes that are found in the growing oocytes of amphibians. The task is likely to be pleasurable, since lampbrushology is a field of research that offers wide horizons and innumerable challenges. Within this field, the questions remain clear and the challenges manageable. As from the very beginning all we have to do is sort out our reasoning, take advantage of what Nature has to offer, steady our hands, and get on with the job.

My starting point is a paper written in 1975 (Macgregor, 1977) in which I attempted to summarise the history of lampbrush studies and focused on two major questions. The first concerned the functional significance of a lampbrush loop. The second question concerned the nature of a lampbrush chromomere and its associated loops in molecular terms. At the time, the answers were difficult to find. In the matter of function I concluded that there was widespread transcription of many DNA sequences on the lateral loops of lampbrush chromosomes. The primary transcript was large. Only a small part of it was retained, and apart from clearly repetitive sequences such as the ribosomal, 5S, and histone genes, the retained material was transcribed from single copies. This explanation fitted with most of the available evidence in 1975. It did not tell us anything about regulation of transcription, and it did not explain the fact that a set of lampbrush chromosomes may have as many as 5000 chromomeres, all of which bear loops that are transcriptionally active at the same time. In particular, I concluded that Callan's spinning out and retraction hypothesis (Callan and Lloyd, 1960; Gall and Callan, 1962; Snow and Callan, 1969) in which he sought to explain the shape of an average lampbrush loop, its relationship with its chromomere and its extension and retraction during oogenesis, was open to question and further experimentation. Essentially, the problem of movement of the DNA loop axis *and* its associated RNP product, or movement of the RNP transcript over the stationary loop axis was unresolved. As we shall see later, the clear solution of this particular problem represents one of the major steps forward of the past 5 years.

With regard to chromomeres, the scarcity of hard data imposed rather strict limits on speculation. I suggested in 1975 that chromomeric material was essentially heterochromatic in the same general sense that a chromocentre is heterochromatic, and that it owed its compactness to a commonness in the sequences of its DNA. I represented an average chromomere as a gathering together of a stretch of DNA that was rich in a particular repetitive

sequence and its near descendants, together with such single copy sequences as may have found their way into the repetitive cluster. On account of such repetitive commonness, this DNA was supposed to clump and complex with protein to form a granule that was visible as a chromomere. Where there were clusters of conserved repetitive sequences, such as at the ribosomal or 5S loci, then chromomeres would form whose DNA was highly uniform. Where selection pressure favouring conservation of a particular sequence was low and the sequence was relatively non-functional in the transcriptive/translative sense, then chromomeric DNA was supposed to be less uniform.

Two techniques have dominated the lampbrush scene over the past 5 years, and in what follows I shall examine in detail the facts that have emerged from each of them. The first is the *in situ* hybridisation of certain tritium labelled DNAs to nascent RNA transcripts that are associated with the DNA axes of lampbrush loops. Henceforth I shall refer to this particular technique as "DNA/RNA-transcript hybridisation". The technique was first introduced and applied by Dr Pat Pukkila in 1975 in her studies of the 5S genes on the lampbrush chromosomes of *Notophthalmus viridescens*. Since then it has been a major source of excitement and trouble in the field. It has provided evidence that suggests transcription of satellite DNA sequences and of spacer sequences that are not normally transcribed in somatic cells, it has been used to search for single copy genes on lampbrush chromosomes, and it has shown up genes for histones and ribosomal RNAs in some extraordinary places. Therefore, before we examine any of the odd situations that have been uncovered, we should look very closely indeed at the methodological details.

In all experiments that have been carried out so far, lampbrush chromosomes have been isolated in the manner described by Callan and Lloyd (1960) using isolation media consisting of either 0.1 M K/NaCl + low concentrations of calcium (10^{-4} M CaCl_2) or a mixture of 0.1 M K/NaCl and dilute phosphate buffer to give a final molarity of around 0.06 and a pH of between 6.5 and 7. The preparations have then been allowed to stand, either at room temperature or on ice for up to 2 hours to allow the chromosomes to disperse over the bottom of the well slide chamber. They have been centrifuged, as described by Hennen *et al.* (1975) to flatten and stick the chromosomes on to the bottom of the chamber, and have then been immersed in either alcohol or 3:1 alcohol:acetic acid, passed through 95 per cent and 100 per cent alcohol, xylene and acetone, and air dried. As a rule, the preparations have been used for *in situ* hybridisations within a few days of making, but some investigators have stockpiled slides and stored them in the refrigerator for varying periods. In some cases, notably in experiments carried out by H. G. Callan and his colleagues, preparations have been made on cover glasses which have subsequently been sealed, preparation uppermost, to a slide with a plastic adhesive that required hardening at 37°C for several hours.

The dried preparations have then been incubated directly in hybridisation reaction mixtures containing tritiated or iodinated nucleic acid probes, and washed in various ways before coating with liquid nuclear track autoradiographic emulsion. Table 1 shows the fixatives, reaction mixtures, and washing procedures, as well as any special treatments used in the laboratories where this technique has been most extensively applied.

TABLE 1

Factor	Investigator			
	Pukila	Old <i>et al.</i>	Macgregor and Andrews	Morgan <i>et al.</i>
Isolation medium	—	5:1 K:NaCl 0.1 M + 10 ⁻⁴ M CaCl ₂	7 parts 5:1 K:NaCl 0.1 M to 3 parts 0.015 M phosphate buffer	5:1 K:NaCl 0.1 M + 10 ⁻⁴ M CaCl ₂
Fixation	70% ethanol overnight	30-100% ethanol series	70% ethanol	70% ethanol ice cold, 30m-2 h
Pretreatment	—	Xylene; Acetone; baking on hotplate 2-3 days	95% ethanol air dry	95% ethanol; 100% ethanol; Xylene; Acetone; air dry
Probe	<i>Xenopus laevis</i> 5S DNA. ^{12a,1}	6 Kb histone cluster + 6.5 Kb histone cluster + 4.5 Kb histone cluster in pSC 101	Cot 0.2-50 <i>Triturus cristatus</i> DNA	Cot = 0.2 and various purified satellite components from <i>Triturus cristatus</i>
Specific activity of probe	1.6 × 10 ⁸ dpm/μg	2 × 10 ⁴ ; 1.4 × 10 ⁴ ; 3 × 10 ⁶ cpm/μg	2 × 10 ⁴ cpm/μg	1.5 × 10 ⁶ cpm/μg
Concentration of probe in reaction mix	0.1 μg/ml	0.1 μg/ml	0.05 μg/ml	0.05 μg/ml
Method of denaturing probe	—	Heat	NaOH 0.1 N	NaOH 0.1 N
Incubation mixture	3 × SSC 40% formamide	3 × SSC 40% formamide	4 × SSC 50% formamide	4 × SSC 50% formamide
Incubation time	16 hours	Overnight	12 hours	12 hours
Wash	2 × SSC 40°C and room temperature	2 × SSC room temperature	2 × SSC 65°C and room temperature; cold 5% TCA	2 × SSC 65°C and room temperature; cold 5% TCA
Exposure time	1-3 months	4 weeks	4-27 days	1-7 days

The method has proved to be remarkably effective. In general, autoradiographic exposures have been usefully short and final preparations have shown some specific labelling patterns. To evaluate these specificities we need to be keenly aware of the reaction conditions. There are two components involved. The first is the RNA that is attached to the DNA axis of each lampbrush loop, and most of the loops that have been labelled with the DNA/RNA transcript hybridisation technique have been of more or less normal lampbrush form. While the *in situ* hybridisation reaction is in progress, the loop is effectively surrounded by the reaction mixture for a period of several hours. An average loop in *Triturus cristatus* (the European crested newt) is about 30 μm long and 1.3 μm wide, and has nascent RNA molecules attached to it by RNA polymerases and closely packed along its entire length. The nascent RNA is associated with loop proteins and is more or less compactly folded and coiled to reduce it to a small fraction of its actual length. The maximum degree of packing can be visualised by supposing that in cases where one 30 μm loop represents one transcription unit, then the associated RNA molecules towards the end of that unit may be nearly 30 μm long, yet the width of the loop at that point is only about 3 μm . So the loop matrix to which our labelled DNA probe is to hybridise is a folded meshwork of ribonucleoprotein fibres that has been pre-treated with 0.1 M saline, ethanol, xylene, acetone, and then air dried.

The reaction mixture that surrounds the loop contains, in addition to the chemicals specified in table 1, labelled DNA at concentrations ranging from 0.5–5 $\mu\text{g}/\text{ml}$. The DNA has been denatured with NaOH or by heating to 100°C for 5 min., mixed with the appropriate salts and other chemicals to make up the final mixture, neutralised with HCl and then applied to the lampbrush preparation within a matter of minutes. The preparation is then incubated at 37°C for periods ranging from 6 to 18 hours, washed in one of the ways specified in table 1, and then coated with liquid nuclear track emulsion for autoradiography.

All investigators who have used this technique have incorporated ribonuclease controls in their experiments and these controls have shown no autoradiographically detectable hybridisation of labelled DNA to nucleic acids on any part of a lampbrush chromosome. Furthermore, in my own experience, preparations treated with deoxyribonuclease are indistinguishable from untreated preparations with respect to their capacity to bind denatured labelled DNA. I have also established that native labelled DNA from *T. cristatus* and labelled denatured DNA from *Escherichia coli* or lambda phage do not hybridise detectably to any part of the lampbrush chromosomes of *T. cristatus*. I consider that controls of these kinds help to show convincingly that labelled denatured DNA binds to loops of lampbrush chromosomes only under the conditions in which it would normally be expected to form H-bonds with complementary sequences on the nascent RNA transcripts that are associated with the loop axes.

Having looked at the technique, now let us examine some of the experiments in which it has been applied and see what we can learn from them. The first study to be carried out with the DNA/RNA-transcript hybridisation technique involved lampbrush chromosomes from *N. viridescens* and 5S DNA from *Xenopus laevis* (Pukkila, 1975). The 5S probe was prepared by repeated Cs salt centrifugations. It is therefore reasonable to assume that it was not free from contamination with sequences other than those of the 5S

complex. The probe was labelled with iodine 125 to a specific activity of 1.5×10^8 d.p.m. Autoradiographic exposure times were of the order of 1-3 months. Parallel experiments were carried out in which 5S RNA labelled with ^{125}I was hybridised to the denatured DNA of lampbrush and mitotic chromosomes. In these, the autoradiographic exposure times were 1-3 days.

These RNA/DNA hybrids showed 4 out of the 11 bivalents with silver grains over the DNP "bars" that characterise the centromere regions of *N. viridescens*. One of these chromosomes (number 6) was labelled on one half bivalent but not on the other. Labelling was strictly confined to the centromere bars and a few adjacent chromomeres. No loops were labelled. Autoradiographic background labelling was low and the preparations were generally of good quality. This experiment, alongside a similar experiment with mitotic chromosomes gave good evidence of the presence of 5S genes in the centromeric or pericentric regions of chromosomes 1, 2, 6, and 7 in *N. viridescens*.

The DNA/RNA-transcript hybrids showed consistent labelling of lampbrush loops at the "base of the centromeres of chromosomes 1, 2, 6, and 7". The autoradiographs had a heavy background and labelling patterns were unclear. No observations were made regarding heterozygosity on chromosome 6. In essence, the Pukkila study showed that in DNA/RNA-transcript hybrids, specific loops label and chromomeres or centromere bars do not. The study pioneered the technique but gave no new fundamental information about lampbrush chromosomes. It purported to show the location of the 5S genes in *N. viridescens* and the expression of these genes on certain lampbrush loops. However, it is important to realise that the probe must have contained sequences other than the 5S genes, so that although the location of these genes was probably accurate, any conclusions regarding their expression or the significance of labelling of lateral loops may be suspect. More recent experiments carried out by Dr J. G. Gall, using cloned 5S genes from *N. viridescens* have shown labelling not of loops but of the centromere bars themselves in DNA/RNA-transcript hybrids (Gall, personal communication).

The next series of experiments were carried out by Old, Callan and Gross (1977), who employed purified histone genes from sea urchins labelled with ^3H . Before examining the information that can be obtained from these experiments, I wish to emphasise several very important points.

The probes used by Old, Callan and Gross were pure and well characterised, but not with respect to specific nucleotide sequence. Recent studies have shown that some of the histone genes of sea urchins contain short repeated sequences that may have contributed to the *in situ* hybrids (Schaffner *et al.*, 1978; Sures *et al.*, 1978). So it is perhaps best at this stage to consider these experiments as relating to certain specific DNA sequences, but not necessarily to the histone sequences themselves. This does not in any way affect the main conclusions that can be drawn from the experiments regarding the structure and function of a lampbrush loop/chromomere complex. It only means that at the time of writing we cannot be sure exactly what was hybridising to what. Other points that should be made are (a) the investigators did not know how many copies of the histone genes they might expect to find at each histone locus; (b) all evidence necessarily comes from DNA/RNA-transcript hybrids, and there is no "back-up" from DNA/DNA or RNA/DNA hybrids as in the Pukkila experiments; (c) there

was a high degree of specificity of labelling in all preparations, and (d) all the controls were satisfactory and labelling was obtained only under conditions that would normally be expected to permit the formation of a reasonably well-matched nucleic acid hybrid.

Old, Callan and Gross' preparations showed labelling of four or fewer pairs of long lateral loops on each of the long arms of bivalent 1 in the crested newt, *T. cristatus*. The long arm of chromosome 1 in this species is heteromorphic over most of its length with respect to the distribution of large lampbrush loops that have distinctive morphologies. The heteromorphic region is achiasmatic in male and female, it is strongly Giemsa-C-positive, and it is slightly longer on one chromosome 1 than it is on the other (Callan and Lloyd, 1960; Macgregor and Andrews, 1977; Mancino *et al.*, 1977; Morgan, 1978). The location of eight of the "histone-labelling" loops in the heteromorphic arms of chromosome 1 is of special interest, as we shall see later. Other labelled loops were located on chromosomes 6, 10 and 11.

It is when we come to examine the detailed labelling of individual loops in these histone hybrids that matters of real significance arise. The labelling patterns of sister loops arising from the same chromomere were always identical, as one would expect from the basic "rules" of lampbrushology laid down more than 20 years ago. Some labelled loops were heterozygous in the sense that they were present on one half bivalent but absent at the corresponding position on the other. The heterozygosity was either total, where no labelled structure was seen on one half bivalent, or partial, where one half bivalent had a heavily labelled pair of loops and the other had a lightly labelled pair. Loops at different loci showed quite widely different levels of labelling. Most lampbrush loops are thin at one end and become progressively thicker towards the other, a phenomenon called loop asymmetry. Most of the histone loops showed a pattern in which labelling began at a certain point a short way along the loop from its thin end. Silver grain density then rose steeply to a plateau where it was uniform. After that followed a region where the grains were irregularly clustered and of diminished overall density, leading into an unlabelled portion of the loop towards its thick end. The essential matter to note here is that the loop was not progressively more heavily labelled from its thin to its thick end, and in many cases the thick end was unlabelled even though it carried lots of RNP matrix.

Of all these observations, heterozygosity and the identity of sister loops are easiest to comprehend. The other observations seem to point to a mechanism in which transcription begins at an initiation site near the thin end of the loop but the RNA transcripts do not include histone messenger sequences until they enter the short histone sequence zone in the loop axis. Having traversed this zone, the transcripts enter a long region containing other sequences. Here each transcript includes the same length of histone message. Since the amount of histone message per transcript doesn't change from then on, the level of labelling over the loop in autoradiographs remains the same. Eventually the histone messages are excised from the transcripts, and since there is then nothing for the labelled probe to hybridise with, the remainder of the loop appears unlabelled in autoradiographs. The whole process is nicely summarised in Old, Callan and Gross's diagram which is reproduced in fig. 1.

FIG. 1.—Diagram to illustrate the organisation of a lateral loop that is transcribing histone sequences. The DNA duplex in the loop axis is represented as a single line shown thicker in the region that contains the histone sequences (HS). RNA transcripts project laterally from the loop axis. *I* indicates the initiation site for transcription and *T* the termination site. Thickened portions of the transcripts represent histone messenger RNA sequences. *P* indicates where processing of the transcript occurs, liberating the histone messenger RNA from the matrix of the loop. To the left is shown a portion of the main chromosome axis including a chromomere. This diagram is reproduced with the kind permission of Dr R. W. Old and Professor H. G. Callan (Old, Callan and Gross, 1977).

Two more sets of observations come from DNA/RNA-transcript hybrids, and I shall deal with these only insofar as they conflict with or add to the information obtained from the histone hybrids. The first involves *Xenopus laevis* pMB-9 ribosomal DNA that has been highly purified by cloning in a bacterial plasmid and then labelled with tritium by nick translation (Morgan, Coleman and Macgregor, 1979). This probe binds to several pairs of loops on at least three different lampbrush chromosomes in *Triturus cristatus*. The precise locations of the labelled loops are unimportant. What is remarkable is that in one particular case we have observed a pair of sister loops each of which appear to consist of two zones of transcription of opposite polarity meeting at their thin ends about one quarter of the way round the loop. The shorter zone was labelled and the longer one unlabelled. The labelled zone showed continuously increasing grain density from its thin to its thick ends. Most importantly, these so-called "sister loops" are of widely different lengths, and the lengths of the labelled regions were proportional to the overall lengths of the loops (fig. 2). In essence, these loops were twin sisters, but one was a miniature of the other. Yet they were supposedly homologous regions of two chromatids that were products of the same semi-conservative replication at pre-meiotic S phase.

I have seen the same phenomenon at another locus in *T. cristatus* in hybrids formed with a mixed probe of middle repetitive (Cot 0.2-50) DNA. How, we might ask, can sister loops differ in this way? The only possibility worth mentioning now is that a chromomere may contain several copies of

some long transcription complex, that these copies may differ widely in length, perhaps according to the number of internal repeats that they contain, and that different representatives of these complexes may be exposed on different "sides" of the chromosomes. For any other explanation we must suppose that the two chromatids are different in the region of the unequal loops or that the regulation of their transcription is different.

Some of the most unexpected results obtained with DNA/RNA transcript hybridisation were those from experiments in which highly repetitive "satellite" DNAs from *T. cristatus* were reacted with lampbrush chromosomes from the same species (Macgregor and Andrews, 1977; Macgregor, 1979; Varley *et al.*, 1979, in prep.). These experiments involved first a Cot 0.2 fraction, and then two satellites isolated by Cs salt centrifugation and one isolated as a restriction fragment after digestion of whole DNA with Bgl II. The results were similar but not identical, with all four probes. We need not trouble ourselves at this point with the exact locations of the labelled loops. Suffice it to say that most of the labelled loops lay in the heteromorphic region occupying most of the long arm of the longest chromosome, and DNA/DNA *in situ* hybrids using mitotic and lampbrush chromosomes have shown this region to be rich in highly repetitive DNA (Macgregor, 1979; Varley *et al.*, 1979, in prep.).

So far, four points of significance to lampbrush chromosomes have come out of the highly repetitive/satellite studies. The first is the indication that a DNA fraction that is in every accepted sense a "satellite" seems to be transcribed on some lampbrush loops. What becomes of the transcript, of course, we cannot say. Secondly, many of the labelled loops showed a continuously increasing gradient of silver grain density, without the "plateau" described by Old, Callan and Gross (1977) which would seem to indicate that most of the transcript on the "satellite" loops is complementary to the labelled probe, and therefore the sequence of the probe must be represented many times along most of the length of the loop axis. Thirdly, all kinds of labelling patterns have been seen, with loops labelled over their thin or thick halves or over long or short segments part way round their lengths.

Finally comes a very important point that has direct bearing on the manner in which a lampbrush loop functions in relation to its form, and it is a point that has been made convincingly by Old, Callan and Gross (1977) and amply confirmed by studies with repetitive DNA probes (Macgregor and Andrews, 1977). It is that where one can identify a loop that is partially labelled at a particular position on a lampbrush chromosome after DNA/RNA-transcript hybridisation, then that loop is *always* partially labelled and always shows the same *pattern* of labelling in different oocytes from the same and from different animals. According to Gall and Callan (1962) one

major lampbrush phase of oogenesis, but remain stationary and constant with respect to the DNA sequences that are available for transcription in the loop axis and with respect to the form of the loop.

All of these observations, considered alongside the histone hybrids, serve to confirm the general hypothesis put forward virtually simultaneously and independently by Macgregor and Andrews (1977) and by Old, Callan and Gross (1977). Essentially, this hypothesis says that each loop begins to take form following the initiation of RNA synthesis from a point that is later identifiable as the thin insertion of the loop into a chromomere. As the RNA polymerase molecules move along the transcription zone, so the lampbrush loop emerges by unfolding at its thick end from the main body of the chromosome. The loop transcribes all along its length. The DNA sequence in the axis of the loop may be tandemly repetitive and uniform, or there may be points along the length of the loop at which the DNA sequence changes and the transcript changes accordingly, and these will show in an *in situ* hybrid as places where labelling starts on loops that are labelled only over their thick ends or only over an intercalary region part-way round their lengths. There may also be points where parts of the transcript are processed and released from the loop in a region where the DNA sequence that made the transcript is no longer represented in the axis or where there is an abrupt change in the DNA sequence of the axis. These points show in loops that are labelled only over their thinner sides but have an uninterrupted gradient of RNP matrix from one end to the other.

What, then, has DNA/RNA-transcript *in situ* hybridisation taught us about lampbrush chromosomes? First and foremost it has played a major role in finally demolishing the two major lampbrush hypotheses of the 60's. On the basis of the *in situ* hybridisation evidence alone it seems most unlikely that the loop spins out at its thin end and retracts at its thick end with a moving axis and a relatively stationary ribonucleoprotein matrix. When this evidence is coupled with the visualisation of lampbrush transcription units by Miller technology, as we shall see in the next section of this review, the spinning out and retraction hypothesis becomes quite untenable. The Master/Slave hypothesis as it related to lampbrush chromosomes required that each loop consists of uniform tandem repeats (slaves) of a master sequence that resided either in the chromomere or at the base of the loop. The *in situ* hybridisation evidence tells us that the DNA sequence of the loop axis can and does change from place to place along the length of a loop, and so the second historic hypothesis, which has already taken many damaging blows from fine structural and molecular studies, must finally be discarded.

It should be said that these two famous lampbrush hypotheses have proved to be immensely useful and have for many years provided clear targets for talented investigators in the field of lampbrushology. It is with regret that I witness their demise, since at the moment there is nothing to put in their place.

So far I have studiously avoided the question of what binds to what in a DNA/RNA-transcript hybridisation, and I have done so for good reasons. At the time of writing it has to be admitted that in none of the experiments carried out so far has it been possible to say with certainty that the labelled probes that have been used have specifically bound to the genes or sequences that they were intended to represent. What can be said is that all investigators have applied the proper controls in their experiments and all have been

fully satisfied with the outcome of these controls. So at least we can be quite sure that provided the appropriate conditions for nucleic acid hybridisation have been applied, then hybrid molecules will be formed and their locations will be faithfully represented in autoradiographs. The real problem arises when we begin to look at the probes themselves.

The 5S DNA used by Pukkila (1975) undoubtedly contained sequences other than the 5S genes, and although we can be reasonably sure that in *N. viridescens* the 5S genes are located near the centromeres on chromosomes 1, 2, 6 and 7, we cannot be sure of the significance of wholly labelled loops in the region of the 5S loci. The 5S genes are repetitive and have substantial non-transcribed spacers (Carroll and Brown, 1976). How, then, can we reconcile their expression in a loop that is labelled over its entire length and shows an uninterrupted gradient of ribonucleoprotein matrix along its length? Does this mean that in the lampbrush form the 5S genes are read right through, spacer and all?

The histone genes used by Old, Callan and Gross (1977) label loops, 8 out of 12 of which are situated in the heteromorphic regions of the long arms of chromosome 1. These regions also have many large loops that label heavily with highly repetitive ($Cot = 0.2$) DNA and with three specific "satellite" DNAs isolated by Cs salt and restriction enzyme methods. Some of the histone genes have been shown to contain repeats of the simple sequence CT/GA (Schaffner *et al.*, 1978; Sures *et al.*, 1978). What, we must ask, was hybridising to what? Can the histone hybrids really tell us where the histone genes are located? Right now, we cannot be sure. Precisely the same argument applies to the genes for ribosomal RNA. Bgl II fragments B 3050 and B 1750, each representing highly specific, definable and untiled regions of the ribosomal DNA of *X. laevis*, both bind to loops on the long heteromorphic arms of chromosome 1 in *T. c. carnifex*, just like the repetitive, satellite, and histone probes, though not necessarily to the same loops (Morgan, Coleman and Macgregor, 1979). Does this mean that there are ribosomal genes in this region that are expressed during the lampbrush phase, or does it mean that our G+C-rich ribosomal probes are binding non-specifically to short repetitive sequences that may also be capable of binding satellites, GC-tailed histone genes, or histone inserts? Hopefully, all these kinds of questions will soon be resolved, perhaps even before this article appears in print.

In the late 1960's, O. L. Miller and his associates introduced and developed an ingenious and exceedingly powerful technique for visualising the transcription of amplified ribosomal DNA in amphibian oocytes (Miller and Beatty, 1969*a, b* and *c*). A germinal vesicle from an amphibian oocyte has 20-30 pg of ribosomal DNA, in the form of 1000 or more circular molecules, most of which have been produced during early meiotic prophase by a process of rolling circle replication from the primary extrachromosomal descendants of a chromosomal nucleolus organiser template (see reviews by Macgregor, 1972; Tobler, 1975). These circles include variable numbers of tandem repeats of the entire nucleolar DNA sequence, comprising the 28S and 18S transcribed sequences, the small internal transcribed spacers that lie between and just outside the 18S and 28S sequences, and the major non-transcribed spacer that separates the 18S/28S pairs. Altogether this amplified nucleolar DNA represents, in an oocyte from *Xenopus laevis*, more than 60 per cent of the entire nuclear DNA. In terms of transcriptive activity it

is a truly fantastic situation which is capable of manufacturing the RNA for 300,000 ribosomes per sec.!

The technique for visualising this transcriptional phenomenon is, like many other things associated with studies on germinal vesicles, simple in principle, but technically challenging (Miller and Bakken, 1972). The germinal vesicle is isolated manually in an isotonic saline. It is then washed in and transferred to a drop of alkaline water. Most of the protein associated with the nucleoli and chromosomes is removed and passes into solution, leaving only the chromosomal and nucleolar DNA, and the nascent RNA molecules still attached to their DNA templates by water-resistant granules that contain RNA polymerase. This microsuspension of chromosomal and nucleolar material is then picked up with a pasteur pipette and placed in a microcentrifugation chamber at the bottom of which lies an electron-microscope grid coated with a thin supporting film of carbon. The top half of the chamber contains alkaline water, and the bottom half contains a solution of sucrose and formalin. The chamber is then put in a centrifuge and spun for 15-20 min., during which time the chromosomal and nucleolar DNA, stripped of most of its protein but still with its transcripts attached, is driven down to lie flat and firm on the supporting film of the electron-microscope grid. Staining and shadowing techniques are then applied to make these components suitably electron dense, and at the end of the day we are in a position to see genes that have been caught in the act of transcription, with the products of transcription faithfully represented *in situ* on their templates.

The technique, as I have outlined it, and as it was applied for the first 5 years of its use was ideal for the study of transcription of the amplified genes for ribosomal RNA. These exist as relatively short circles, most of which are quite unaffected by the physical rigours of the isolation procedures. The shorter DNA circles are 10-20 μm long, the individual transcribing regions are only 2-3 μm long, and the transcripts are closely packed and rarely project more than $\frac{1}{2}$ μm at right angles to the DNA template strand: all in all, rather a compact and robust structure that is quite likely to, and indeed does, survive intact through the entire preparative sequence. Small wonder that the vast majority of studies employing the Miller technique over the past 10 years have concentrated on the transcription of ribosomal DNA.

The general picture that has emerged from studies of transcribing nucleolar DNA in organisms ranging from unicellular algae to water beetles and amphibians is as follows. The nucleolar DNA is divided up into quite strictly alternating sections that are transcribed and ones that are not transcribed. The lengths of both transcribed and non-transcribed regions is species-specific, the transcribed regions showing much less variability in length from one species to another than the non-transcribed "spacer" regions, as might be expected. The transcribed regions are always polarised in the sense that the nascent RNA molecules at one end—the start—are short and grow progressively longer towards the other end, the whole transcriptional or "matrix" unit presenting an appearance akin to a Christmas tree. In dynamic terms relating to the amplified ribosomal DNA of *Xenopus laevis*, a granule containing RNA polymerase associates with the start of a transcription unit every 2 sec., travels along the transcribed portion of the gene for 4 min., and then detaches, releasing a fully formed ribosomal precursor

RNA molecule that contains 18S and 28S sequences and the transcribed parts of the internal and external spacers. This completed transcript is complexed with protein as it is synthesised and it acquires some secondary structure, such that the finished article is appreciably shorter than the DNA template upon which it was made. The best sources for reference with regard to the organisation of ribosomal transcription complexes as seen in Miller preparations are Franke and Scheer (1978) and Franke *et al.* (1978).

What happens to lampbrush chromosomes in Miller preparations? The answer, I think, is simple. First, the alkaline water has the same effect on the chromosomes as it has on the nucleoli. Much chromosomal protein is lost, the entire lampbrush structure is disorganised, chromomeres fall apart into long tangled masses of fibrillar nucleoprotein, and transcribing regions retain their organisation only in so far as the nascent transcripts remain attached to their DNA templates by granules that contain RNA polymerase. Secondly, when the microsuspension of germinal vesicle material is pipetted into the upper layers of the microcentrifugation chamber, turbulence and shearing forces literally tear the remaining chromosomal material to pieces. Thirdly, during centrifugation the longer stretches of chromosomal material are subjected to still more stretching and shearing, so that the best that we can hope for on our electron-microscope grid is random bits and pieces of chromosomal material liberally intermixed with nucleolar material whose uniformity and abundance makes it by far the most conspicuous and attractive feature of the final preparation. Whatever happens to the chromosomal material in a normal Miller spread, it certainly does not all reach the grid. If it did, then in a preparation from one *Triturus* germinal vesicle there would be up to 10 metres of fibrous nucleoprotein on the grid, at least 50 centimetres of which would be clothed with nascent RNA transcripts, and all in addition to at least 150 centimetres of nucleolar DNA. This simply does not happen. Consequently anyone who has first hand experience of Miller technology is likely to agree that, whilst immensely useful and informative in certain important respects, it is none the less an extremely inefficient and clumsy way of looking at chromosomes.

Before considering what we have learned about lampbrush chromosomes from Miller technology, let us first remind ourselves of what lampbrush loops look like through a good light microscope when they are in a life-like condition, and then summarise some of the basic concepts that have emerged from light-microscope studies.

It has been claimed that the chromomeres of lampbrush chromosomes are Feulgen positive and the loops Feulgen negative (Macgregor and Callan, 1962). That the loop axis consists of DNA and depends upon that DNA for its integrity was shown many years ago when loops were shattered into countless tiny pieces specifically by the action of deoxyribonuclease I (Callan and Macgregor, 1958; Macgregor and Callan, 1962). Loops were measured, average loop lengths were estimated, loops were counted and total genome size was determined. From these data it was said that if the DNA of all loop axes was in the simple extended duplex form then 95 per cent of the chromosomal DNA was in the chromomeres and 5 per cent was in the loops (Callan, 1963). This seemed entirely reasonable, particularly since it was later found that all the RNA synthesised in the germinal vesicle and conserved for use during early embryonic development was transcribed from little more than 5 per cent of the genome. This situation has been exten-

FIG. 2.—Autoradiograph of part of a lampbrush chromosome from *Triturus cristatus carnifex* following *in situ* hybridisation of ^3H pMB9-ribosomal DNA to loop transcripts. Autoradiographic exposure 58 days. The two longest loops in this photograph are each labelled part way along their lengths from one end. They are sister loops arising from the same chromomere, yet they are of different lengths, and their labelled segments are correspondingly different in length. Note also that the labelled segments show a gradient of grain density inwards towards the chromosome axis, whereas the regions of the loops immediately distal to the labelled segments show a gradient of thickness in the opposite direction. This micrograph is shown here with the kind permission of Dr. G. T. Morgan.

FIG. 3.—Phase contrast micrograph of part of a lampbrush chromosome from *Triturus cristatus carnifex*. The long loop in this picture shows multiple asymmetry, going from thin to thick three times along its length. Two of the starting points or thin ends of asymmetrical segments are indicated by arrows.

sively documented and discussed in a recent review by Sommerville (1977).

In the light microscope many, though not all, loops have a polarised appearance, being thin at one end and becoming progressively thicker towards the other. This observation, taken with the notion that loop length and chromomere size were inversely related, formed the basis of the long-standing, but now discredited, spinning-out and retraction hypothesis (Callan and Lloyd, 1960; Gall and Callan, 1962). The hypothesis was strongly supported by autoradiographic observations on the pattern of incorporation of tritiated uridine into the giant granular loops on chromosome XII of *Triturus cristatus cristatus*, in which labelling first appeared at the thin end of the loop and then progressively "moved" round towards the thick end of the loop over a period of about 14 days (Gall and Callan, 1962). It should be said once and for all that this loop, and its counterpart in *Notophthalmus viridescens* are exceptional in their pattern of incorporation of labelled uridine, and we do not yet understand their extraordinary behaviour. Most importantly, we should bear firmly in mind that all other loops label with tritiated uridine all along their lengths.

Of particular interest is the fact that in animals with large genomes, lampbrush loops showing multiple tandem asymmetries are commonplace. A clear example of such a loop is shown in fig. 3.

Most lampbrush loops start with a thickness approaching the limits of resolution of the light microscope and are perhaps 2 or 3 μm wide at their thick ends. These dimensions apply to loops ranging in length from 10 to 50 μm or more. The loop matrix is generally uncompacted and fluffy or finely granular in appearance, and appears uniform with respect to phase contrast throughout the entire length of the loop. Some loops have highly distinctive morphologies and textures of matrix, but the vast majority of the 5000 or more (haploid) loops in *T. cristatus* have the same general qualitative appearance.

As to the relationship between loops and chromomeres, we cannot, in all honesty, say anything with certainty. Loops seem to arise from chromomeres, but the number of loops per chromomere and the relationship, if any, between loop length and chromomere size, are matters that are entirely open to investigation.

It is at this point that we can usefully turn to Miller technology with some objective questions in mind. First, what is a lampbrush loop in terms of transcriptive function? Second, what happens to the loop transcripts and how does this relate to the morphology of the average loop as we see it with a phase contrast microscope? Thirdly, how much of the chromosomal DNA becomes exposed for transcription during the entire lampbrush phase of oogenesis? It will not escape the reader's attention that this last question strikes at the very roots of meaning of the lampbrush phenomenon, for if indeed only 5 per cent of the genome is ever exposed for transcription then it signifies something rather special and selective about the whole process. But what if we have miscalculated? What if the loop DNA is not in an extended duplex form? What if we have placed too much credence on the exceedingly faint and sometimes almost imaginary Feulgen positivity of the lampbrush chromomere?

It is now abundantly clear that a lampbrush loop is not necessarily a unit of transcription. The experiences of three separate sets of investigators are worth citing separately in this context. First, we should consider the

study of Angelier and Lacroix (1975), who described certain features of chromosomal transcription units that were scattered amongst nucleolar and other chromatin complexes in Miller preparations from oocytes of the European newt, *Pleurodeles poireti*. Angelier and Lacroix's preparations were made according to the method described by Miller and Bakken (1972) in which a microsuspension of germinal vesicle contents is deliberately stirred by sucking in and out of a micropipette before transferring it to the microcentrifugation chamber. It is therefore largely fortuitous that any meaningful observations whatever can be made with regard to the organisation of chromosomal material, and Angelier and Lacroix confine their remarks to short stretches of transcriptionally active chromosomal DNA. They observed chromosomal matrix units ranging in length from 3-30 μm . Most matrix units were polarised, but in some, polarisation was not obvious, and most were much shorter than the average lengths of lampbrush loops in *Pleurodeles*. Stretches of chromosomal DNA were seen where several matrix units followed one another, separated by short untranscribed spacer regions: the spacer shown in one of Angelier and Lacroix's illustrations is a little over 2 μm in length. Adjacent matrix units were of different lengths and frequently of opposite polarities. All of these observations tend to strengthen the notion that although some lampbrush loops may be single transcription units, with long stretches of non-transcribing chromomeric DNA in between them, there must also be other rather common arrangements of transcriptionally active DNA in a lampbrush chromosome. We shall soon see to what extent these may be related to the lampbrush phenomenon as we see it with a phase-contrast microscope.

The next study that has real significance in relation to the transcriptive activity of lampbrush loops is that of Scheer *et al.* (1976). Once again, these investigators made their Miller preparations in a manner that was bound to produce extensive disorganisation of the chromosomes. Nonetheless, their paper has 10 good and broadly representative illustrations of a diversity of lampbrush matrix units supporting a usefully detailed description and discussion of situations that they encountered. The thin and thick ends of many loops can be distinguished at their insertion sites on the chromosome axis, they say, but a gradual increase in the thickness of the matrix layer is usually only recognised at the thin end, most of the remaining loop axis being covered by a more or less uniform coat of matrix material. This is a simple but very important observation to which we shall return when we come to think about what happens to the loop transcripts. Scheer and his colleagues, like Angelier and Lacroix, observe that the majority of chromosomal matrix units are shorter than the average loop, and adjacent units have short spacers between them. They reasonably conclude that many loops may be complexes of several tandemly arranged and often dissimilar matrix units, and they go on to classify the arrangements that they have identified in their Miller preparations. Their system of classification is shown in fig. 4 which is extracted from their paper with their kind permission. Another hypothetical but none the less useful classification of transcription units is given by Sommerville (1977). However, it is essential to stress that Scheer and his colleagues did not see lampbrush loops that showed these kinds of arrangements of matrix units. When looking at fresh lampbrush chromosomes with a phase-contrast microscope they did see some lampbrush loops that had multiple asymmetries like that shown in

FIG. 4.—A diagram showing the various arrangements of transcription units within individual loops of lampbrush chromosomes, as proposed by Scheer *et al.* (1976). The numbers 1-1''' denote units of equal length, 1-5 of different lengths. Only one loop is shown emerging from each chromomere in this diagram: sister loops have been omitted. Reproduced with the kind permission of Dr U. Scheer.

fig. 3, and in their Miller preparations they came across situations showing each of the arrangements of matrix units that are represented in fig. 4. They *conclude* that where there are several tandemly arranged and closely spaced matrix units having like or unlike polarities and sizes, then these together must represent part or all of a lampbrush loop that would be visible as such in a fresh and intact chromosome, and some loops must therefore be "truly multigenic". We should bear in mind that this conclusion is based on low resolution studies of whole chromosomes on the one hand, and high resolution studies of their exploded and deproteinised remnants on the other. Without controlling the effects of the Miller technique, we cannot hope to do better.

The third set of investigations that have concerned relationships between lampbrush loops and transcription units come from my own laboratory and have been carried out by Dr Stephen Hill (Hill, 1979) and Dr G. T. Morgan. Unlike most previous Miller technologists, they adopted a method in which an entire germinal vesicle is rinsed in alkaline water and transferred whole and intact to a microcentrifugation chamber containing the usual alkaline water layered on to formalin/sucrose. The germinal vesicle is then allowed to burst of its own accord. An intermediate approach that has been used by certain other investigators is to place the entire germinal vesicle in alkaline water, with or without added detergent, remove its nuclear envelope manually without disturbing the contents, and then transfer the ball of nucleoplasm containing chromosomes and nucleoli very carefully to the same medium in a microcentrifugation chamber. In both cases, the nucleoplasm is allowed to disperse and then is centrifuged and prepared for electron microscopy in the usual way. For success, these gentler versions of the Miller technique must be carried out very quickly indeed, the whole process from isolation of the germinal vesicle to completion of transfer of the germinal vesicle or nucleoplasmic ball usually taking less than 30 sec. The great advantage of this method is that it eliminates the first of the major

destructive phases of the Miller technique, *viz.*, the dispersal and mixing of nuclear contents in a drop of alkaline water before transfer to the centrifugation chamber. The chromosomal material is therefore very much better preserved and much more of it, in the form of longer stretches of active and inactive chromatin fibres, is represented in the final preparation. Hill and Morgan worked with material from *Xenopus laevis* and *Triturus cristatus* respectively. Their observations tie in nicely with those of previous investigators. In *X. laevis*, chromosomal matrix units have been seen ranging in length from 0.65-18.4 μm (fig. 5). Some units longer than 18.4 μm were undoubtedly present in Hill's preparations, but they could not be measured because they became intermeshed with other transcribing and non-transcribing chromatin. In one specific instance in *Xenopus*, there were five separate units ranging from 2.5-10.6 μm in length within one continuous 73 μm stretch of chromatin. In *Triturus*, Morgan has described matrix units ranging from 2-30 μm long, and he supposes that much longer matrix units actually exist and could be measured if methods could be devised for gentler isolation and better dispersal of chromosomal material.

The question of the loop and the transcription unit, then, is not one that can be fully resolved, but at least we now have some rules and measurements to go by. There are many matrix units that are much shorter than average loops. There are some matrix units that are quite as long as some of the longest loops. Adjacent matrix units are not all separated from one another by long stretches of non-transcribing chromatin, such as would be expected if each loop were a transcription unit and were separated from its neighbour by a long stretch of nontranscribing chromomeric DNA. The gaps between matrix units are often very short indeed. There is a wide variability in the lengths of adjacent matrix units.

At this stage I would like to suggest three possibilities, without implying that these are exhaustive or mutually exclusive. First, each transcription unit is potentially a loop in its own right. The gaps between transcription units vary widely. Where the gap between two units is long, then the intervening non-transcribed DNA will form a chromomere. If the gap is short, then the two adjacent units will either form a complex loop or two short loops with the intervening DNA stitched back into the main bulk of the chromosome axis. In this scheme, long loops with multiple asymmetries reflect an unstitching of short non-transcribed spacer regions from the chromosome axis, adjacent transcription units do not necessarily have any functional relationship, and the ratio of loops to chromomeres is not necessarily 1:1.

Secondly, loops with multiple asymmetries may be complexes of transcription units that are related in function. We would then suppose that these complexes were separated from their neighbouring complexes by longer stretches of non-transcribed chromatin that form chromomeres, and we might reasonably anticipate a ratio of one chromomere to one loop. The notion of grouping of transcription units with related functions is of special interest in view of recent observations on the distribution of globin and ovalbumin genes in man and in birds. The genes for β - and δ -globins in man are closely linked, transcribed from the same strand, and show extensive sequence homologies, and the 2 γ -globins likewise lie close together in the same region of the genome (Flavell *et al.*, 1978; Lawn *et al.*, 1978). In chickens the complete ovalbumin gene is closely linked with two other genes of unknown function. The three are orientated in the same direction,

they share some sequence homologies, and their expression is under a common hormonal control in the chick oviduct (Royal *et al.*, 1979).

Both these first two possibilities imply a static situation in which the loop forms and remains as a more or less constant feature throughout oogenesis. However, there is some evidence to suggest that this is not the case, at least with respect to certain specific loops (Callan and Lloyd, 1960; Macgregor and Andrews, 1977). So we may take another view and suppose that new transcription units in the form of new loops or parts of loops emerge from time to time from clumps of chromomeric DNA, that this emergence can happen at either or both ends of the loop, and that the process may be compensated by an inactivation and regression of other parts of loops. In such a scheme, polarised transcription units would be regarded as emergent or transcriptionally active, and unpolarised ones as transcriptionally regressive.

Next we come to the question of the nature and fate of the RNA transcripts on lampbrush loops, and in this context the very first thing that we have to do is to admit that we know nothing about the function of the transcripts, so we will not concern ourselves with idle speculation on the matter. In the same sense we know very little about the proteins that become associated with the RNA transcripts on loops. What is known is outlined and adequately discussed by Maundrell (1975), Sommerville (1977), and Sommerville, Malcolm and Callan (1978). We do know that more than 95 per cent of the matrix of a lampbrush loop consists of protein (Sommerville, Malcolm and Callan, 1978). The extended primary transcript can be reduced to a string of beads of non-basic protein each about 30 nm in diameter and spaced 10-20 nm apart, connected by a thin fibre of RNA. Consider a loop 50 μm long, with associated RNA transcripts emerging from its DNA axis at a frequency of 20 per μm , and each of these becoming associated with non-basic protein granules 30 nm in diameter and packed at 50 per 1.7 μm (the packing density shown in fig. 2 of Sommerville, Malcolm and Callan, 1978). Assume also, no loss of any part of any of the RNA transcripts, so that the RNA of the last and longest transcript would be 50 μm long, and that of the transcript half-way round the loop would be 25 μm long. In such a situation the entire volume occupied by all the primary transcript and its associated non-basic protein granules would be of the order of 10 μm^3 , which is very substantially less than the likely volume of the whole loop as one might measure it with a light microscope. This is not to say, however, that we should expect a lampbrush loop to be a compact mass of nucleoprotein, and I have already made the point that the vast majority of loops appear faint, diffuse, and exceedingly finely granular when examined in a life-like condition with the highest magnification of a good phase-contrast microscope. When lampbrush chromosomes are carefully fixed in such a way as to preserve their fine structure as faithfully as possible, and then embedded in plastic and sectioned, the loop matrix appears in the electron microscope as a loose and spacious network of ribonucleoprotein fibres and granules of varying sizes that together account for the fine scale morphologies of loops as we see them with a light microscope (Mott and Callan, 1975).

There are just two more points that we should bear in mind when considering what happens to loop transcripts. First, we have seen from DNA/RNA-transcript *in situ* hybrids that in some loops, at any rate, certain portions of the RNA transcripts that were made from DNA in the thin half or

in an intermediate part of the loop axis are no longer present in the loop matrix farther along the loop towards its thicker end. This implies that certain parts of the RNA transcript have been excised while the transcript was still on the loop, leaving other parts of the transcript still associated with the loop and still elongating as the polymerase moves along the loop axis towards the thick end.

Secondly, I think it's worth taking note of the straightforward dimensions and densities of loops as we see them in a light microscope. In most loops, the optical density of the matrix in phase contrast is quite uniform all along the loop. If one can assume that most loops are more or less round in transverse section at any point along their lengths, that there is an uninterrupted gradient of associated transcript-length from one end of the loop to the other and that the nascent transcript is distributed evenly over the whole cross-sectional area of the loop at any point, then one would predict a linear relationship between cross-sectional areas of a loop at any two points and the distance separating these points. On this basis a loop that is 0.2 μm wide at a point *A* near its thin end will be 0.88 μm wide at a point *B* near its thick end if the distance from *A* to *B* is 20 μm , it will be 1.4 μm wide at *B* if *A* to *B* is 50 μm , and it will be 2.44 μm wide at *B* if *A* to *B* is 150 μm . This is more or less what one observes in any lampbrush preparation from any animal, and it represents crude but none the less useful light-microscope evidence suggesting that in most loops with normal matrix texture and asymmetry there is transcription all along the length of the loop and no substantial loss of transcript before the thick end. It follows that if loops have uniform density and no asymmetry then transcript must be lost along the way. Conversely, if the relationship between cross-sectional area and length is obviously greater than linear, then either transcript must be accumulating on the loop after it has been detached from the axis or, as seems more likely, the transcriptively active loop axis must be much longer than the actual contour of the loop.

Now we come to the facts as we find them in Miller preparations, and it is mainly upon these that we shall have to base our current views regarding the fate of loop transcripts. First, the transcripts can be and often are very long indeed. The longest that Dr Hill, in my laboratory, has measured in *Xenopus laevis* were about 10 μm long, which is equivalent to at least 30 kilobases. Secondly, the spacing between polymerases on the loop axis DNA varies over at least an order of magnitude. In one transcription complex Hill has identified tandem matrix units in which polymerase spacings range from 7-20 per μm , and in material from one oocyte the range identified was from 2.4-20 polymerases per μm , the most common spacing being in the range 15-20 per μm . Thirdly, the transcripts on some matrix units appear as relatively simple structures, without extensive folds, loops, or overlaps. In other cases they appear to be extensively contorted and looped. Some investigators have attached significance to the looped appearance of transcripts and have suggested that the loops may be parts of the transcript that are in process of being nipped out or excised, an event that might explain the discontinuities of labelling in some DNA/RNA-transcript hybrids. However, we have evidence to suggest that the straight or contorted appearances of transcripts are largely the results of different methods of preparation. Essentially, rough preparation and vigorous centrifugation seem to produce a preponderance of straight-looking transcripts, and gentle isolations yield

a preponderance of contorted and looped ones. So the presence of loops on transcripts, as described by Angelier and Lacroix (1975) and Sommerville *et al.* (1978), may have nothing whatever to do with excision or processing of the primary transcript molecule while it is *in situ* on the loop. In this context it is certainly also important to take note of matrix units reported by Scheer *et al.* (1979) where transcripts become progressively longer up to a point, and then quite abruptly appear shorter, producing a visible "step-down" in the unit's polarisation. This kind of situation would be most easily explained by supposing that a terminal or interstitial excision of part of the transcript occurred at the point of step-down. Finally, in a careful survey of five transcription units all of different lengths and in different chromosome regions, Hill (1979) has shown that the ratio of RNA transcript length to distance of the transcript's polymerase from the start of the unit remains constant at between 0.38 and 0.54 from beginning to end of the unit. Perhaps the most crucial point about this ratio is that it is remarkably similar for all the transcription units that Hill has measured.

What then can we conclude about the fate of the RNA transcripts on lampbrush loops? Certainly we can say that loop RNA is transcribed from the DNA of the loop axis as a series of continuous molecules. The RNA is complexed with protein and becomes more or less packed as it is made, so that each transcript never appears as long as the DNA upon which it is made. In many, perhaps most, cases transcription proceeds uninterrupted from one end of the loop or transcription unit to the other, and then detaches. In some cases there is excision of part of the transcript at a specific point before the polymerase reaches the end of the transcription unit. Step-down in polarisation or looping of transcripts may be indications of this kind of event. What happens to the RNA transcripts after they have detached from the loops is not known at the present time.

The last matter in which Miller technology has proved helpful is the question of the amount of chromosomal DNA that is exposed for transcription during oogenesis. Let us look at the background once again. Estimates of the numbers of lateral loops present on whole sets of lampbrush chromosomes have been based on estimates of the number of chromomeres from which these loops arise. The kind of figure that recurs in the literature is between 3000 and 6000 loop pairs per haploid set of chromosomes. Continuous spinning out and retraction of the DNA loop axes has been largely discredited, and it seems that most loops are probably stationary but some may appear or regress at specific stages of oogenesis (Macgregor and Andrews, 1977). All loops are said to reach a maximum extension, remain in this state for a significant proportion of the oogenetic period, and then retract quite quickly towards the end of oogenesis. Estimates of average loop length at the stage of maximum extension have normally been made by measuring the contours of discernible loops as seen in the fresh condition with a good phase-contrast microscope. If, therefore, we accept about 5000 loops, each 30 μm long, are fully extended for most of the lampbrush phase of oogenesis, and if we suppose that the DNA of the loop axis is in the B form, then in a genome of 30 pg all of the extended loop DNA will account for less than 2 per cent of the genome, and it follows that there must be something rather special about this material in the functional sense. But to what extent could this 2 per cent be an underestimate? One serious source of error could be our estimate of the number of loops in a haploid set of lampbrushes, and I

believe that most experienced lampbrushologists would now agree that chromomere number is not a reliable indicator of loop number, and on average there are likely to be at least two or three pairs of loops per chromomere. So on this basis we may confidently double our 2 per cent estimate.

Another source of error and possibly a very important one indeed concerns the reliability of putative correspondence between a "polarisation unit" in a loop as seen with a light microscope, and a matrix unit as seen in a Miller preparation. After all, it must be admitted that no person has ever identified a polarised lampbrush loop with a light microscope, then treated that loop in such a way as to remove most of its protein, and subsequently visualised *the same loop* as a single matrix unit in a Miller preparation with an electron microscope. To be sure, the apparent and striking likenesses in form between small lampbrush loops that show a single gradient of polarity and large Miller matrix units with continuous gradients of polarity is highly persuasive (Scheer *et al.*, 1976), but on the other hand, intact, carefully fixed, and critical point dried loops (fig. 6) have little in common with Miller matrix units, and it is sometimes hard to see how the latter can be derived from the former simply by the removal of substantial amounts of protein.

Could it be that some, perhaps many, loops contain much more DNA than we suppose on the basis of their length, and that the loop asymmetry and Miller matrix asymmetry are features that respectively represent different levels of structural organisation? One striking case in point is the giant granular loop (GGL) in *T. c. cristatus* that acquired notoriety following the studies of Gall and Callan (1962) and Snow and Callan (1969), and provided the firmest evidence in support of the spinning out and retraction hypothesis. This loop is large, about 60 μm long and 5-10 μm wide at its thick end, and it is strikingly polarised. However, it incorporates tritiated uridine only in one short region 10-15 μm long near its thin end, and its axis is thick enough to be resolved easily with a light microscope (Snow and Callan, 1969). What is more, the axis of the GGL can be stripped of virtually all its ribonucleoprotein matrix and still resolved with a light microscope (Macgregor and Callan, 1962). Under certain experimental conditions (Snow and Callan, 1969) the first 10-15 μm of the loop which normally forms a dense conical tip and which is the site of incorporation of ^3H -uridine can be persuaded to unwind and expose 100 μm or more of delicate loop axial filament. So how much DNA does the axis of the GGL contain, and how many other loops are more or less similar in their form and behaviour to the *cristatus* GGL? We simply do not know, but the answer to that question could make an appreciable difference to our estimates of the proportion of chromosomal DNA that is extended into the loops at any one time in the lampbrush phase of oogenesis.

The final matter that needs close attention is the form of the DNA in the axes of Miller matrix units and in the intervening regions that do not have polymerases associated with them. Expressly, is this DNA straight, or is it complexed with histones into a nucleosomal organisation? The answer to this question could again make a substantial difference in our estimate of the length of DNA in a lampbrush loop. In citing a number of statements that have been made on the subject over the past 3 years, the trend in our thinking will soon become obvious. In 1978 Franke and Scheer stated that "... under conditions which preserve the nucleosomal organisation in con-

FIG. 5.—An electron micrograph of a single transcription unit from a lampbrush loop of *Xenopus laevis* prepared by the Miller technique. The unit starts at the bottom right-hand corner of the picture and ends near the middle of the top of the picture. The overall length of this unit is $5.2 \mu\text{m}$. The axis of the unit is studied with RNA polymerase granules. Note the complex form of the transcripts. This picture may be compared with fig. 6 which shows a pair of stereo electron micrographs of a small loop from *Triturus cristatus carnifex*, prepared in such a way as to minimise the loss of material from the loop and preserve its fine scale life-like morphology. Fig. 5 was kindly provided by Dr R. S. Hill.

FIG. 6.—A stereoscopic pair of electron micrographs of a small lampbrush loop from *Triturus cristatus carniifex*. The chromosome set of which this loop was a part was isolated in 0.07 M K/NaCl (5 : 1) lightly buffered with phosphate to pH 6.5. The chromosomes were fixed in vapour from 50 per cent neutral formaldehyde followed by vapour from 50 per cent ethanol. They were then placed in 0.5 per cent uranyl acetate for a few minutes, washed in water, dehydrated in an ethanol series and critical point dried through liquid CO₂. Immediately before critical point drying the chromosomes had an appearance that was considered to be as close to life-like as is possible with fixed lampbrush material. This pair of photographs are best looked at with a stereo viewer such as is usually obtainable from departments of Geography. The full three-dimensional aspect of the loop can then be visualised.

FIG. 7.—A phase contrast micrograph of part of a freshly isolated and unfixed lampbrush chromosome from *Taricha granulosa*. About one-third of the length of the whole chromosome is represented here. The preparation was centrifuged to lay all the lateral loops down in one plane on the bottom of the lampbrush observation chamber. This micrograph is shown here with the kind permission of Dr James Kezer.

FIG. 8.—Part of a polytene chromosome from *Chironomus thummi*. This chromosome was from one of the largest cells of a late larva. It has been fixed in ethanol/acetic acid, squashed in 0.5 per cent orcein in 45 per cent acetic acid, and photographed in phase contrast.

FIG. 9.—Part of a set of chromosomes from the Central American salamander *Oedipina uniformis*. These chromosomes are in pachytene of the first meiotic division in the male. Note the indistinct chromomeric organisation and the finely fuzzy material projecting from the chromosomes' axis. The chromosomes in this preparation were fixed in ethanol/acetic acid, stained with the Feulgen reaction, and squashed in 45 per cent acetic acid. (Figures 7, 8 and 9 are all shown at the same magnification. They are intended to show three entirely different grades of organisation that have been called "chromomeric".)

FIG. 11.—Phase contrast micrographs of two lampbrush chromosomes. The larger one (A) is from a maximum lampbrush stage oocyte of the Central American salamander *Bolitoglossa subpalmata* which has a C value of 87 pg DNA ($n = 13$). The smaller one (B) is from the amphibiaean repile *Bipes canaliculatus* which has a C value of 2.0 pg ($n = 11$ macrochromosomes + 11 microchromosomes). Only half of the *Bolitoglossa* chromosome is shown. These two chromosomes are shown at the same magnification and together they provide a clear demonstration of the way in which lampbrush chromosome size, and particularly loop length, varies according to C value.

densified chromatin studied in parallel, nucleosomes are not recognised in transcriptionally active chromatin. This holds for the transcribed regions as well as for the apparently untranscribed (*i.e.*, fibril-free) regions interspersed between ("spacer") and/or adjacent to transcribed genes and for fibril-free regions within transcription units of reduced fibril density." Later in the same year Franke *et al.* (1978) stated that "... the DNA in transcriptionally active chromatin regions is in an extended state and is not packed in nucleosomal particles." However, in the same publication, they add that: "Most of our observations have been made in chromatin material that has been dispersed briefly in media of very low ionic strength, *i.e.*, conditions known to be favourable to the unfolding of nucleosomal structure. . . . So we cannot rule out the possibility that the low salt treatment has selectively altered the nucleosomal arrangement in transcribed chromatin, but not in non-transcribed chromatin." In a still later and generally very useful publication from the same laboratory (Scheer *et al.*, 1979) it is stated that "... during mid-oogenesis the lateral loops of lampbrush chromosomes of amphibia are densely covered with transcriptional complexes in an almost maximal packing density . . . no nucleosomal particles can be recognised in between the basal particles of the lateral fibrils, *even in regions of somewhat reduced lateral fibril density*" (my italics). However, the same authors also state that in the course of inactivation of the genes present in the chromosome loops "... spacious transcriptionally inactive regions within an initiated transcriptional unit can assume the nucleosomal configuration, *i.e.*, nucleosomal packing is reformed between two transcriptional events that are separated by extended periods of time and/or space."

The most recently published statement regarding nucleosomes and lampbrush loops comes from Osheim *et al.* (1978) and it emphasises how very important it is to take account of the rigours of the Miller technique when interpreting what one sees in the preparations. Osheim *et al.* state that using "... extremely gentle spreading procedures for electron microscopy on *X. laevis* oocytes ..." they have found that "... highly active units lie adjacent to nucleosomal DNA and minimally and moderately active loci exhibit nucleosomes between polymerases".

Our findings in the Leicester laboratory agree with those of Osheim *et al.*, and we now have no doubt that wherever there is room for nucleosomes to associate with loop DNA they do so.

In the last section of this review I wish to examine and discuss some of the more elusive and open-ended matters relating to lampbrush chromosomes: matters that have been with us for a very long time and that have not yet yielded to conventional approaches. What are lampbrush chromosomes? What initiates the formation of a lampbrush loop? Why is there such a striking positive correlation between genome size and the average loop length at the stage of maximum lampbrush development? In what formal and functional sense are loops related to the chromomeres from which they arise?

All these questions can be reduced to one major outstanding problem in lampbrushology. Essentially, we do not yet understand how a lampbrush chromosome forms. However, before tackling this problem with what little evidence we have to hand, I think it is particularly important that I state my views about chromomeres.

There are three kinds of chromosomes that have been described as having a chromomeric organisation: the individual chromonemata of polytene

chromosomes from larvae of dipteran flies, zygotene and pachytene chromosomes from spermatocytes of various organisms, and lampbrush chromosomes from germinal vesicles of animal oocytes. Examples of each of these three grades of organisation are shown in figs. 7, 8 and 9. In my opinion, the three have little, if anything, in common and it is not useful to attempt comparisons of one with the other. A similar view with respect to polytene and lampbrush chromomeres was recently stated by Callan in his introductory remarks to a Royal Society discussion on functional units in eukaryotic chromosomes (Callan, 1978). Therefore in what follows I shall consider lampbrush organisation as a phenomenon in its own right, representing an adaptive feature specific to cells that undergo an extended meiotic prophase, during which there is substantial cell and nuclear growth and amplification of the genes for ribosomal RNA.

How, then, do lampbrush chromosomes form? The useful evidence that can be cited in this context is of three kinds. It comes from sectioned oocytes covering all stages of oogenesis, from studies of incorporation of ^3H uridine into oocyte RNA, and from observations on the dimensions of chromosomes and loops in various species at certain stages of oogenesis. Of course, the one thing we do not have is a means of seeing freshly isolated, unfixed and spread chromosomes from young oocytes, this being an important technical problem that has yet to be overcome. Early or pre-lampbrush oocytes from most animals are just too small to handle successfully, or the nucleoplasm is too stiff and the chromosomes too entangled to permit enough spreading for clear visualisation.

Pachytene nuclei from an amphibian ovary show the usual compact chromosomes, with rough outlines and some coarse linear differentiation. In newts and salamanders the chromosomes are clearly arranged in a "bouquet" with their ends all pointing in the same direction and attached to the inner surface of the nuclear envelope. In general, pachytene in oocytes resembles pachytene in spermatocytes, apart from differences that are a consequence of oocyte-specific ribosomal gene amplification. In *Xenopus* the arrangement of the pachytene chromosomes is much less clear mainly because they are smaller and crowded into one half of the nucleus by the relatively large "cap" of nucleolar DNA (Macgregor, 1968).

After pachytene the oocyte nucleus grows and the chromosomes become longer and progressively more finely "chromomeric" in appearance. Wherever there are thickenings of the chromosome axis they are surrounded, not flanked, but surrounded, by a finely fuzzy material that sometimes seems to include thin, loop-like formations. This fuzz extends from 2-4 μm from the chromosome axis in an early diplotene oocyte with a nuclear diameter of 50 μm in *T. cristatus*, and it closely resembles in every respect the lateral fuzz that is associated with chromosomes at a corresponding stage from spermatocytes. It is very important to realise that at this stage we may be looking at a lampbrush-like chromosome, but what we are seeing is an order of magnitude smaller *in every respect* than the lampbrushes that we shall see in the same oocyte in early or mid-vitellogenic stages. As the oocyte enlarges the chromosomal material becomes progressively finer in structure, and then, quite soon, our evidence runs out, and it becomes exceedingly difficult, and in some species impossible, to see what is happening to the chromosomes until the oocyte reaches a size at which we can manually remove its germinal vesicle and apply the standard techniques of lampbrushology.

The questions that remain to be answered are these. Does the lateral fuzz that we see in early diplotene represent the future lampbrush loops, as yet unclothed in the large masses of RNP that later accumulate as a consequence of transcriptional activity? We do not know. Is the lateral fuzz of early diplotene transcriptionally active? Probably it is, but at a much lower level than we find in fully fledged lampbrushes. Is there a further, perhaps total, disorganisation of the chromosomes before the definitive lampbrushes form? Perhaps so. In spermatocytes pachytene is followed by a diffuse diplotene during which it is impossible to trace chromosome axes either in squashes or sections. Perhaps the same happens in oocytes. We do not know.

From the extensive literature on gene amplification, ribosomal RNA synthesis, the structure and function of the 28S, 18S and 5S genes, and the transcription and processing of high molecular weight RNAs of different kinds, mostly in amphibian oocytes, one might suppose that we should know a great deal about RNA synthesis during oogenesis. Unfortunately, this is not the case, mainly because although oocytes have been widely exploited as systems in which to study specific aspects of nucleic acid metabolism, the details of oogenesis itself have been largely disregarded. Persons interested in amplification of ribosomal DNA concentrated on early oogenesis. Those interested in patterns of RNA synthesis worked only with lampbrush-stage oocytes that could be sized, classified, and the classes separated from one another in large numbers. Those interested in transcription and translation have used only yolky oocytes. The evidence that we have on RNA synthesis during oogenesis is therefore fragmentary, and because different investigators have used different conventions for defining the stages and sizes of the cells with which they worked, and have used different species, it is extremely difficult to piece that evidence together into any kind of useful framework.

The most up-to-date and comprehensive review of nucleic acid synthesis in oogenesis is given by Sommerville (1977). Out of the many papers that he reviews in this context, Sommerville gives three conclusions that are of particular significance for those interested in the function and activity of lampbrush chromosomes. First, in pre-vitellogenic oocytes ribosomal RNA synthesis proceeds at a low level that is about 0.01 per cent of that found in mid-vitellogenic oocytes. This is to say that although early oocytes do have their full quota of amplified nucleolar (ribosomal) DNA, it is not yet transcriptionally active. Second, the synthesis of informational messenger RNA occurs exclusively during the definitive lampbrush stage of oogenesis. Thirdly, the patterns of synthesis of 5S and transfer RNAs largely follow the course of lampbrush chromosome activity. The picture that emerges from most of the biochemical work, therefore, is that nothing much happens in the way of RNA synthesis until the lampbrush chromosomes form. There is just one problem: all the biochemical studies began with oocytes that already had lampbrush chromosomes, and they can tell us nothing whatever about the changing patterns of RNA synthesis that must occur in those very small oocytes of the larval and post-metamorphic gonad where the pachytene-diplotene-lampbrush transformations are taking place.

The cytological approach using ^3H -uridine, squashes and sections, and autoradiography, for all its shortcomings, is probably the only way in which we can usefully investigate RNA synthesis during early lampbrush transformations. Unfortunately, no studies of this kind have been published,

although several have been carried out in different lampbrush laboratories from time to time. Our own attempts involved ovaries from *Xenopus* froglets 3-6 weeks after metamorphosis. These ovaries have cells in all stages of early oogenesis ranging from leptotene through to late diffuse diplotene where the germinal vesicle is over $50\text{ }\mu\text{m}$ in diameter and lampbrush transformations have almost certainly taken place. The aim of our studies was to see if RNA synthesis increased gradually and smoothly throughout early oogenesis, or if there was at some point a rapid acceleration of synthesis such as might signify the beginning of lampbrush loop activity. Our method was to incubate pieces of ovary in amphibian culture medium with added ^3H -uridine, wash, fix, embed in plastic, section, and autoradiograph. We then counted the number of silver grains over nuclei of different stages and sizes and plotted silver grains per unit area against nuclear area, beginning with oocytes that could be recognised as pachytene on account of their large "cap" of amplified nucleolar DNA. Our results are summarised in fig. 10. We have interpreted them as signifying that in *Xenopus* the level of RNA synthesis increases quite sharply between pachytene and the early germinal vesicle stage. Once the elevated synthetic rate has been attained it continues while the oocyte increases in size. We think that the increase in RNA synthesis happens as the chromosomes are decondensing but before definitive lampbrush loops and chromomeres have formed. Precisely the same phenomenon has been observed in spermatocyte nuclei from plethodontid salamanders (Owen, 1973) and although levels of incorporation of uridine have not been quantified in these cells, it is none the less clear that there is a sharp increase in the level of RNA synthesis as the nuclei enter the diffuse diplotene stage.

FIG. 10.—A graph constructed from data obtained from autoradiographs of sections of *Xenopus* oocytes that had been incubated in ^3H uridine. Points on the graph represent the number of silver grains per μm^2 plotted against the maximum area of the nucleus in section (d = diameter). Pachytene nuclei ▲, diffuse diplotene ○, early germinal vesicle ●. The continuous line of the graph is drawn arbitrarily to join the means (■) for each kind of nucleus. I am indebted to Miss Ruth Challenger for permission to publish this diagram.

When we come to consider evidence from the dimensions of chromosomes and loops in different organisms, some of the most tantalising problems of lampbrushology emerge. Two matters are particularly worth thinking about in this context: the stage of oogenesis at which maximum lampbrush development is attained, and the average length of the lateral loops when they are maximally extended. Both these matters relate to genome size.

In all organisms that have been studied, lampbrush chromosomes with long loops in relation to chromosome size have been recognised in the smallest oocytes from which they could be manually isolated. In *Triturus cristatus*, for example, Callan and Lloyd's (1960) study began with oocytes of 0.6 mm diameter, although they state that lampbrushes are certainly present in oocytes of 0.3 mm diameter. The great majority of their observations were made on oocytes of 0.9-1.4 mm diameter. In this species the stage of maximum loop extension is probably in oocytes of around 0.7-0.9 mm diameter, and the oocytes mature at around 1.7 mm diameter. All oocytes over 0.6 mm diameter are yolky. The haploid genome of *Triturus cristatus* has 29 pg of DNA.

In the amphisbaenian reptile, *Bipes canaliculatus*, on the other hand, the situation is rather different (Macgregor and Klosterman, 1979). Here our study began with an oocyte that is small and yolkless by reptilian standards, shaped as a blunt, oblate spheroid, measuring 0.58 mm and 0.77 mm along its short and long axes respectively. This contained lampbrush chromosomes that had loops extending about 1.5 μm sideways from the chromosomes' axes. There were no clearly distinguishable chromomeres. In early vitellogenic oocytes measuring about 0.7×1.0 mm there were normal lampbrushes with loops extending 2.5 μm from the chromosomes' axes and clearly definable chromomeres. The lampbrush stage was completed and the loops and chromosomes contracted soon after the start of the major phase of vitellogenesis when the oocytes were still less than one thousandth of their final volume. The genome size for this animal is 2.0 pg.

Comparable data can be gleaned from the literature for other species including *Amphystoma mexicanum* (Callan, 1966), certain species of *Plethodon* (Vlad and Macgregor, 1975), *Xenopus laevis* (Muller, 1974), and *Pleurodeles poireti* (Lacroix, 1969), and the general impression that seems to emerge is that in animals with small genomes, lampbrush chromosomes and their loops become maximally extended and then contract much earlier in oogenesis than in animals with large genomes. Essentially, the larger the genome, the longer the lampbrush phase.

With respect to average lengths of normal lateral loops when they are maximally extended, the data are much harder to extract from the literature. Table 2 shows the fruits of my efforts along these lines, although I should say immediately that some of my data have been obtained by measuring loops shown in published photomicrographs rather than from definitive statements made by authors. I might add that in more than one case the statements made by authors did not agree with their photomicrographs! None the less the message is crystal clear. Animals with large genomes have very long lampbrush loops, and those with small genomes have short ones. The point is perhaps best made by reference to fig. 11 which shows middle-sized lampbrush chromosomes at the same magnification from two species, one of which has 87 pg of DNA in its genome, the other 1.84 pg.

Any synopsis of the events that take place during early lampbrush

TABLE 2

Species	DNA/haploid chromosome set (C value) $\text{g} \times 10^{-12}$	L factor μm	Reference
<i>Bolitoglossa subpalmata</i>	87	22	Personal observation
<i>Ambystoma mexicanum</i>	35	12	Callan (1966)
<i>Triturus cristatus</i>	29	11	Callan and Lloyd (1960)
<i>Plethodon cinereus</i> *	20	8.35	Vlad and Macgregor (1975)
<i>Plethodon dunni</i> *	38.8	17	Vlad and Macgregor (1975)
<i>Asaphus truei</i>	8.2	4	Macgregor and Kezer (1970)
<i>Xenopus laevis</i>	3	5-10	Muller (1974)
			Hill (unpublished observations)
<i>Bipes canaliculatus</i> *	2.0	2-3	Macgregor and Klosterman (1979)
<i>Sepia officinalis</i>	All less than 10	2-3	Callan (1957)
<i>Scyllium catulus</i>			
<i>Anilocra physoides</i>			

In species labelled * the chromosomes had been flattened on to the base of the lampbrush observation chamber by centrifugation. This tends to stretch the loops slightly, so that L factors may be correspondingly higher than they might appear in uncentrifuged preparations.

The value given for *Xenopus laevis* and quoted from Muller (1974) is cited from the text of this paper. Muller's photomicrographs, on the other hand, suggest a higher L factor of around 12.5 μm . C values are not known for the three species studied by Callan in 1957, but it seems likely that all are well below 10 pg, and probably less than 5 pg.

L factor is defined by Vlad and Macgregor (1975) as the average distance between each loop inflexion and the chromosome axis over a chromosomal distance of 50 μm .

development is bound to be speculative but I, none the less, wish to put forward three separate views in the hope that they will at least spark off some discussion and suggest foci for future experiments.

My first scheme is the one that is widely accepted at the present time. It says simply that chromomeres form and loops unfold from them as transcription proceeds from specific initiation points. It was introduced, outlined and discussed in general terms by Old *et al.* (1977), by Macgregor and Andrews (1977) and by Callan (1978). I have no objection to the scheme except that it takes too little account of detail and it seems inadequate for certain situations that we have encountered recently, especially in the very small lampbrush chromosomes of some reptiles (Macgregor and Klosterman, 1979).

In my second scheme I would propose that the fuzz that we see surrounding the small axial condensates in early diplotene chromosomes is made up of diffuse chromatin that will later develop into the lateral loops of definitive lampbrush chromosomes. Accordingly, the earliest lampbrush loops are short, thin, and exceedingly fine in texture, and they do not have much RNP associated with them. Gradually the loop chromatin starts to transcribe RNA, and as the polymerases become more and more closely packed along the transcription complexes, RNP matrix accumulates and the loops become bulkier and more coarsely granular in texture. Then quite suddenly there is a general shut-down of transcription and the loops shorten and disappear. The chromomeres begin as exceedingly small, almost indistinguishable condensates that are closely packed along the chromosomes' axes. Later they grow by addition of some new protein and are stretched apart from one another as the chromosome elongates to accommodate the

relatively massive amount of loop transcript that forms as more and more loops become maximally active. This scheme requires that the whole lengths of chromatin that form lampbrush loops may be predetermined, both with respect to their beginnings and their ends before transcription commences. It also requires that loop DNP have a different composition and structure from chromomeric DNP in the earliest pre-lampbrush stage, and it implies that most of the growth of a lampbrush chromosome is a consequence of increase in the synthetic activity of the loops and growth of the chromomeres. Larger genomes would supposedly give longer loops because the distances between the start and stop signals on the loop DNA are further apart, but it is not clear why animals with larger genomes should have longer lampbrush phases.

In my third scheme I would propose that there is a progressive and extensive disorganisation of chromosomes following pachytene, leaving them as little more than linearly integrated strings of nucleosomes. The axial condensates and lateral fuzz that we see at early diplotene would therefore bear no relation whatever to the lampbrushes that we see in germinal vesicles later on. Once this fully diffuse condition has been attained, either locally or generally throughout the whole chromosome set, definitive lampbrush loops begin to form through the displacement of nucleosomes by polymerases at specific places on the chromosomal DNA and the initiation of RNA synthesis along transcription units. This event coincides with the start of condensation of neighbouring chromatin that is not to be transcribed during the lampbrush phase of oogenesis. Transcription and condensation proceed together until the typical loop pair/chromomere pattern becomes distinguishable. There are no specific stop signals for transcription. It just proceeds until the polymerases hit chromatin that is untranscribable. In animals with large genomes the start signals for transcription are spaced further apart, and there is more chromatin to be condensed between them. Condensation takes more time and in the early stages of lampbrush development the polymerases move further along the DNA, establishing longer transcription complexes before they are stopped by condensed and untranscribable material; so both the loops and the lampbrush phase are longer. This scheme allows for the transcription of anything that is accessible to RNA polymerase, whether it be sense or nonsense, translatable or no. It can account for the inverse relationship between loop length and chromomere size: long loops are regions where transcription progressed further along the DNA before it met the condensation block, and correspondingly less material would be available for condensation into a chromomere.

In assessing each of these schemes it is essential to bear in mind that RNA polymerase molecules moving along a transcription unit at a rate comparable to that which has been demonstrated in other animal systems (see Anderson and Smith, 1978) can traverse the length of the longest known loop (700 μm , Mancino *et al.*, 1969) and generate the full amount of RNP matrix in just 2-3 days, and an average 20 μm loop can be formed in less than 2 hours. So in all three schemes the limiting factors to the rate at which definitive lampbrushes form are likely to be the rate at which maximum transcriptive activity is attained, as determined by the packing densities of polymerases, and/or the rate of chromomere condensation and enlargement. I think it is also important to remember that at least some loops do always seem to be of the same length relative to their neighbours, and at some stage

we will have to try and decide whether such loops have predetermined start *and* stop signals, or have lengths that are rather uniformly proportional to the distances between neighbouring starts.

The last thing that I wish to do in this article is to consider what lampbrush chromosomes accomplish in the general sense. In my opinion, the following points are fundamentally important:

(1) Lampbrush chromosomes antedate the evolution of coelomates so they have been around for a very long time indeed, and they must surely have a quite important role in oogenesis.

(2) Lampbrush chromosomes are peculiar to oocytes, with a very few special exceptions that have not so far proved useful in establishing new principles.

(3) The lampbrush phase lasts for a long time, estimated at 1-6 months in vertebrates, and there is intensive RNA synthesis throughout.

(4) Lampbrush chromosomes are absent from meroistic ovaries of insects, *i.e.*, where there are nurse cells the chromosomes of the germinal vesicle do not assume the lampbrush form.

(5) In organisms that have lampbrush chromosomes, all the chromosomes assume the lampbrush form, including such things as microchromosomes and supernumeraries.

(6) Extra lampbrush chromosomes seem to confer no special advantage upon an oocyte. Triploid gynogenetic females of *Ambystoma jeffersonianum* have hexaploid oocytes with three times as many lampbrush chromosomes as in the oocytes of their normal diploid kin (Macgregor and Uzzell, 1964), and the oocytes of the Pacific tailed frog, *Ascaphus truei*, have 8 and sometimes even 16 germinal vesicles each with a full set of lampbrushes (Macgregor and Kezer, 1970). Yet in so far as limited observations have been made on the biology of these species, they have nothing special to show for their extra lampbrush endowment.

(7) There seems to be no obligatory relationship between the number of lampbrush loops and/or chromomeres and the number of transcription units. In other words, the absolute length of DNA in the loops of a set of lampbrush chromosomes and the way this DNA is divided up among loops bears no relationship to the number of sites that are active and functional in the transcriptive sense.

(8) Many "genes" are active in the lampbrush phase of oogenesis.

(9) Loops are specific manifestations of DNA sequences in the genome in the sense that at least those with distinctive morphologies have constant positions in the chromosome set and seem to behave as simple Mendelian factors with respect to their presence or absence in a normal lampbrush preparation (Callan and Lloyd, 1960; Callan, 1963; Sommerville, Malcolm and Callan, 1978).

(10) There are substances present and synthesised in the germinal vesicle that are essential at specific stages of early embryonic development. The "O+" substance in the axolotl is perhaps the best known case in point (Briggs and Cassens, 1966; Brothers, 1976).

(11) Unusual and certainly untranslatable sequences are transcribed on lampbrush loops (Hartley and Callan, 1978).

(12) There is evidence that at least 97 per cent of the 5S genes are transcribed in *Xenopus* oocytes, whereas only a small percentage of the same

class of genes are transcribed in somatic cells from the same organism (Brownlee *et al.*, 1974). So that at least with respect to these particular genes transcription in the germinal vesicle seems to be more widespread than it is in other cells.

(13) There are messenger RNAs that are made during oogenesis from single copy genes and stored for use in early embryonic development; but they are made early in oogenesis, before the main lampbrush stage (Rosbash and Ford, 1974).

(14) There are transcripts of repetitive DNA sequences that are made during the lampbrush stage and conserved, but their function is unknown (see Davidson, 1976).

(15) There is reasonable correspondence, subject to problems arising from different genome sizes and uncertainty about the amount of DNA in the axis of a lampbrush loop, between the complexity of conserved transcripts and the proportion of the genomic DNA that is in the loops at the stage of maximum lampbrush development (Sommerville and Malcolm, 1976).

With these points in mind, let us now look briefly at some of the major hypotheses that have been offered to explain these remarkable chromosomes.

The "classical" hypothesis for lampbrush function essentially says that lampbrush chromosomes make many kinds of RNA that are needed for maintenance and programming of early embryonic development. The notion was first introduced with specific regard to lampbrushes by Duryee in 1950 and later by Gall in 1955. It has most recently been ably and critically examined by Davidson (1976), and it remains valid and interesting in principle.

The first "genetic" hypothesis of lampbrush function was the famous 'Master/Slave Hypothesis' introduced by Callan in 1967. It drew attention to evidence that suggested that "... each unit of information encoded as a DNA base sequence is serially repeated" and "... among such serially repeated sequences a terminal unit serves as a 'Master' sequence, within which recombinational events can occur, followed by 'slave' sequences which are not directly involved in recombination but which are made congruent to the master sequence once per life cycle" (Callan, 1967). The formation of lampbrush loops was claimed to represent the outcome of the master/slave matching process. The hypothesis was immensely attractive until we realised that many, indeed perhaps most, functionally important DNA sequences are not serially repeated but present in just one copy per genome.

The other genetic role that had been proposed, again with a careful mustering of the facts available at the time of its publication, presented the lampbrush loops as regions where the chromosomal DNP was undergoing a preprogramming for development, such as might involve exchange or enzymatic modification of regulatory molecules, including perhaps certain non-histone proteins (Léon, 1975).

The most recent proposal for lampbrush function (Cavalier-Smith, 1978) gives them a role that is shockingly menial, at least to one who has spent 20 years studying these chromosomes and feasting his eyes upon their aesthetic virtues! Cavalier-Smith's idea is none the less extremely interesting and very cleverly presented. In essence he proposes that the function of lampbrush synthetic activity and the production of large amounts of hnRNA

from a wide range of sense and nonsense sequences is to swell the germinal vesicle, so increasing the area of its nuclear envelope and the number of nuclear pores available for export of the large amount of ribosomal material that is produced by amplified nucleolar DNA. Upon reflection, and with careful reference to the 15 points that I have made earlier in this section, Cavalier-Smith's hypothesis has much in its favour, and in principle it fits better than any other hypothesis that has been offered so far, particularly as it is discussed in the wider context of the role of nucleoskeletal DNA and genome evolution. I would none the less like to take this opportunity to make three rather specific comments in relation to Cavalier-Smith's arguments. First, in an anuran such as *Xenopus laevis* the germinal vesicle contains approximately 42 pg of DNA, 12 pg of which is chromosomal and 30 pg nucleolar (Perkowska *et al.*, 1968). About 60 per cent of the nucleolar material but not more than 10 per cent of the chromosomal material is synthetically active. The synthetic activity of the nucleolar (ribosomal) DNA itself is therefore at least $10\times$ that of the chromosomal, so that with respect to swelling the nucleus and facilitating export through nuclear pores, the chromosomal role is unlikely to be significant, except perhaps in the earliest lampbrush stages before nucleolar activity is fully under way. My second point is related to the situation in *Ascapus truei* (Macgregor and Kezer, 1970) where each oocyte has eight germinal vesicles each with a full set of lampbrush chromosomes but only one-eighth of the expected level of amplified nucleolar (ribosomal) DNA. In these oocytes, each germinal vesicle is one-eighth of the *volume* that would be expected in the single germinal vesicle of a normal frog oocyte of the same size and stage. This again implies that the dimensions of the germinal vesicle are largely dictated by the amount of nucleolar DNA that is present and not by the level of chromosomal activity. Lastly, it seems significant that the oocytes of *Dytiscus marginalis*, an animal with a meroistic ovary and highly polyploid nurse cells, have large swollen germinal vesicles, a high level of ribosomal gene amplification, but no lampbrush chromosomes.

In a sense, I would have wished to end this article by offering some new and conclusive explanation of the lampbrush phenomenon, but it is perhaps just as well that I cannot do so, for lampbrushes are truly amazing objects and it would be a pity if they lost their challenge, so depriving young cytologists of the pleasure of handling and working with them in years to come.

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