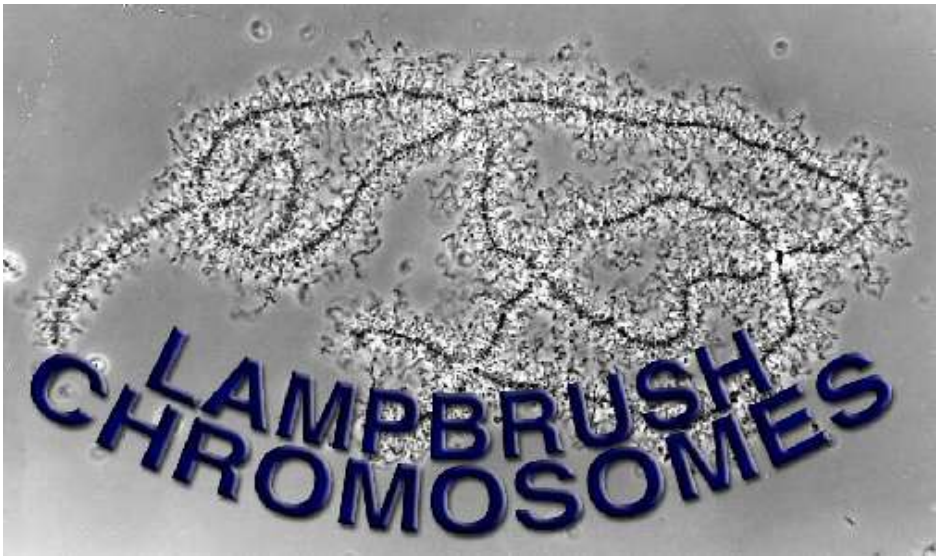
Revised and updated (2011) edition of Chapter 9 "Lampbrush Chromosomes" in *INTRODUCTION TO ANIMAL CYTOGENETICS*, by H.C.Macgregor, Chapman & Hall, 1993.



History and technology

Lampbrush chromosomes are something special for the cytologist. On the one hand they are big and they look quite different from any other form of chromosome. On the other hand they have offered a successful medium through which it has been possible to draw valid conclusions at the molecular level on the basis of observations and experiments carried out at the level of the light microscope. People have not only looked at lampbrush chromosomes very carefully indeed, they have also been able to do things to them and actually watch the consequences as they are happening.

Of course, the total volume of published research that has been carried out on polytene chromosomes from things like *Drosophila* and *Chironomus* far exceeds that on lampbrush chromosomes. Nevertheless, lampbrush chromosomes have proved uniquely valuable in two respects. First, they are transitory structures that exist during an extended diplotene of the first meiotic division. The chromosomes go from a compact telophase form at the end of the last oogonial mitosis, become lampbrushy and then contract again to form perfectly normal first meiotic metaphase bivalents. Secondly, their most conspicuous

feature is widespread RNA transcription from hundreds, and in some cases thousands, of transcription units that are arranged at short intervals along the lengths of all the chromosomes. In these senses it has been possible to exploit lampbrush chromosomes in the study of chromosome organisation and gene expression during meiotic prophase and in studies of the molecular and supramolecular morphology of RNA transcription.

Lampbrush chromosomes were first seen in sections of salamander oocytes by Flemming in 130 years ago. Ten years later they were described in the oocytes of a dogfish by Ruckert. The name lampbrush comes from Ruckert, who likened the objects to a 19th Century lampbrush, equivalent to the 20th Century test-tube brush. The lampbrush type of chromosome is now known to be characteristic of growing oocytes in the ovaries of most animals, vertebrates and invertebrates, with the exception of mammals and certain insects. The chromosomes are greatly elongated diplotene bivalents, sometimes reaching lengths of a millimeter or more.

Lampbrushes are exceedingly delicate structures and no further progress beyond the pioneer studies of Flemming and Ruckert was possible until a technique could be devised for dissecting them out of their nuclei and examining them in a life-like condition, separated from the remainder of the nuclear contents. That's not as difficult as it might sound. The largest lampbrush chromosomes are to be found in growing oocytes of newts and salamanders. These urodeles have big genomes, big chromosomes and big cells, so it is scarcely surprising that they have good lampbrushes. The best oocytes for lampbrush studies are the ones that make up the bulk of the ovary of a healthy adult female at the time of year when the eggs are actively growing in preparation for ovulation in the following Spring. They are about 1 mm in diameter. They have nuclei that are between a third and a half a millimeter in diameter, big enough to see with the naked eye. These nuclei are really not hard to isolate by hand and it is not much more difficult to remove their nuclear envelopes and spill out their chromosomes. Such a technique was introduced by Dr. Joseph Gall in 1954 (Figure 1), working in the University of Minnesota.

Figure 1. Joe Gall in 2001

The oocyte is submerged in a suitable saline solution, it is punctured with a needle, the nucleus is gently squeezed out of the hole, picked up in a Pasteur pipette and transferred to fresh saline in a chamber constructed by boring a hole through a microscope slide and then sealing a coverslip across the bottom of the hole with wax. The nuclear envelope is then removed and the nuclear contents, including the lampbrush chromosomes, come to lie flat, and hopefully unbroken and well displayed, on the bottom of the chamber (Figures 2 and 3). Then, by using a phase contrast microscope with an inverted optical system, the chromosomes can be examined in a fresh and unfixed condition with the highest resolution and magnification obtainable with a light microscope.

The reason for using an inverted optical system is simple. If the chromosomes are dissected onto a slide in a drop of saline and then covered with a coverslip and looked at with a normal microscope, the very act of placing a coverslip on top of them, a combination of movement, turbulence and surface tension, completely destroys them. So we have to dissect them into a flat bottomed chamber. When they are lying at the bottom of the chamber they remain undisturbed when we place a coverslip over the top of the chamber. If we then try to look at them from the top with an ordinary microscope, we are looking through

the entire depth of the chamber, which will be a distance equal to the combined thickness of the microscope slide and the top coverslip. That distance is greater than the working distance of most high power microscope objective lenses. It will therefore be impossible to bring the chromosomes into focus. If, on the other hand we look at them through the bottom of the chamber then we are looking through a distance equal only to the thickness of the coverslip that forms the floor of the chamber, well within the working distance of all objectives

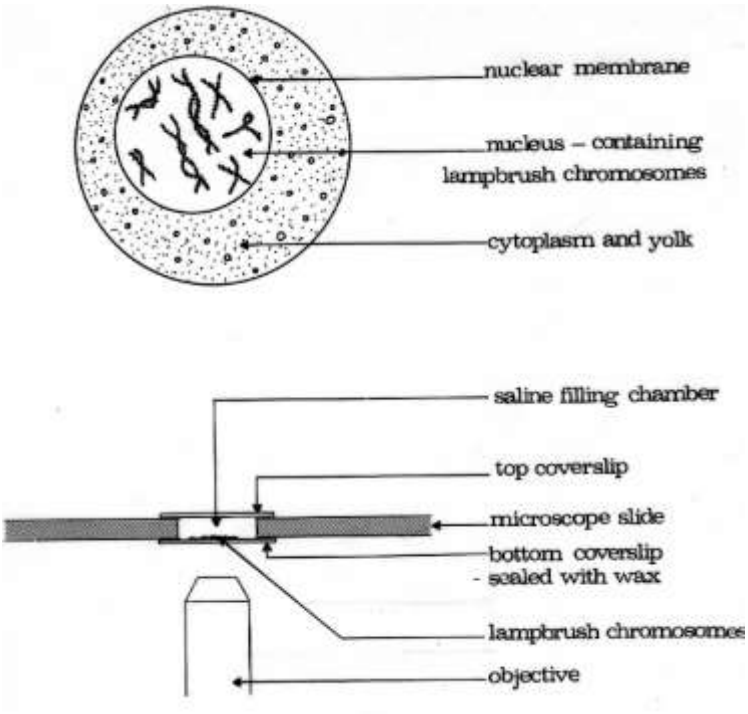


Figure 2. An egg oocyte (growing ovarian egg) showing the relative dimensions of the egg, its nucleus and its lampbrush chromosomes, and the system for visualising the chromoisomes using an inverted microscope and a chamber constructed from a slide with a hole bored through it.

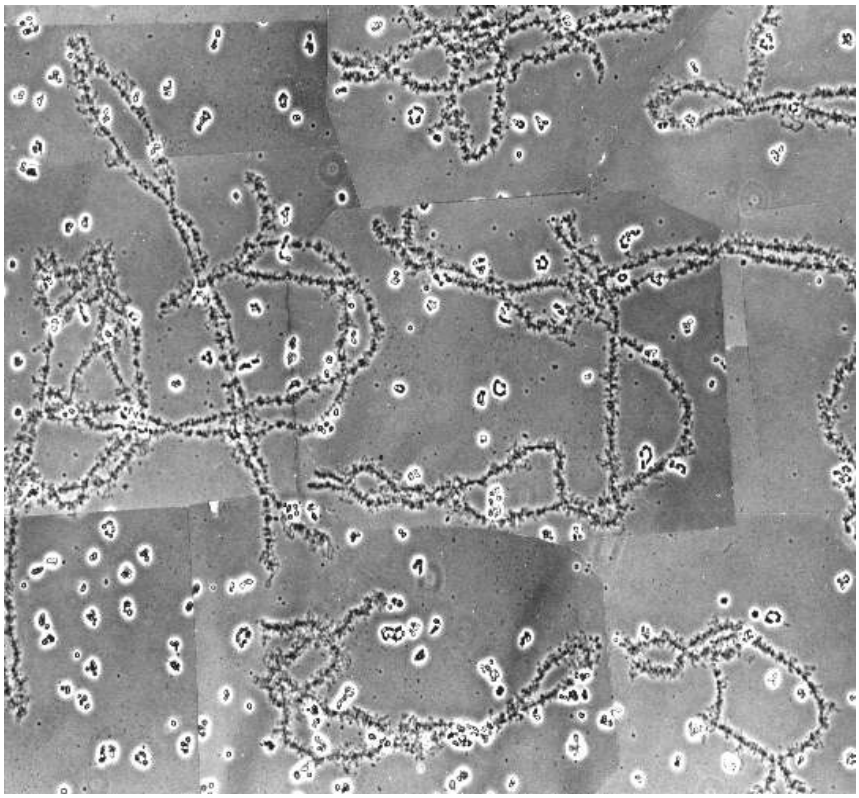


Figure 3. A composite of 15 phase-contrast micrographs covering the entire area of over 1 square millimeter occupied by a full set of lampbrush bivalents freshly isolated from an oocyte of a plethodontid salamander, *Plethodon cinereus*. The numerous small bright dots and rings are the many nucleioli that are the products of ribosomal gene amplification in the early stages of oogenesis. Note how each lampbrush bivalent consists of two half-bivalents stitched together at several points along their lengths by chiasmata.

Basic organisation

The most important factor to keep in mind in relation to the structure of a lampbrush chromosome is that it is a meiotic half-bivalent. This means that it must consist of two chromatids. The entire lampbrush bivalent, of course, will have a total of 4 chromatids. The chromosome appears as a row of granules of deoxyribonucleoprotein (DNP), the chromomeres, connected by an exceedingly thin thread of the same material.

Before we even start to think about the structure and functional organisation of a lampbrush chromosome, however, just take a careful look at the following micrograph (Figure 4).

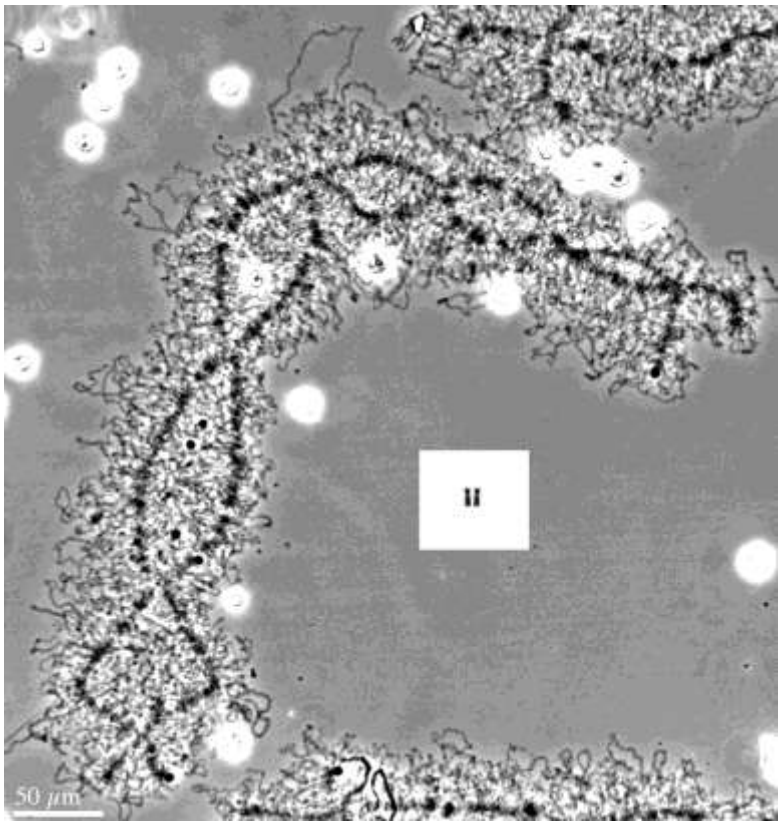


Figure 4. This photograph shows a medium sized lampbrush bivalent freshly isolated from an axolotl (*Ambystoma mexicanum*) oocyte and viewed in its life-like form with phase contrast microscopy. As a comparison, the inset shows **AT THE SAME MAGNIFICATION** a pair of medium sized mitotic metaphase chromosomes from an axolotl larva.

It shows a medium sized lampbrush bivalent freshly isolated from an axolotl (*Ambystoma mexicanum*) oocyte and viewed in its life-like form with phase contrast microscopy. As a comparison, the inset shows **AT THE SAME MAGNIFICATION** a pair of medium sized mitotic metaphase chromosomes from an axolotl larva. That the relatively minute volume of chromatin represented by the metaphase chromosome should be so transformed and rearranged as to constitute the DNA axis of a lampbrush chromosome that is nearly a millimetre long and all its lateral loops signifies that something very special must be happening here: first a 100x extension in length, then the formation of discrete chromomeres – tiny packages of condensed chromatin - separated from one another by an almost invisible thread, then the growth of loops and then the population of each loop with all the enzymatic machinery and accessories needed for transcription and the packaging of transcribed RNA. This has to be the most spectacular genomic sartorial

transformation in the entire living world.

Chromomeres are 1/4 to 2µm in diameter and spaced 1 - 2 µm centre to centre along the chromosome. Each chromomere has 2 or some multiple of 2 loops associated with it. The loops have a thin axis of DNP surrounded by a loose matrix of ribonucleoprotein (RNP). The loops are variable in length, and during the period of oogenesis when they are maximally developed, they extend from 5 to 50 µm laterally from the chromosome axis, which means that the longest loops in such a case would be 100 µm long. The loops are also variable in appearance (Figure 5). Loops of the same appearance always occur at the same locus on the same chromosome, from one animal to another within a species. Accordingly, some loops with particularly distinctive appearances can be used reliably for chromosome identification. Very importantly, "sister" loops, arising from the same chromomere, have the same appearance and usually, but not always, are of the same length.

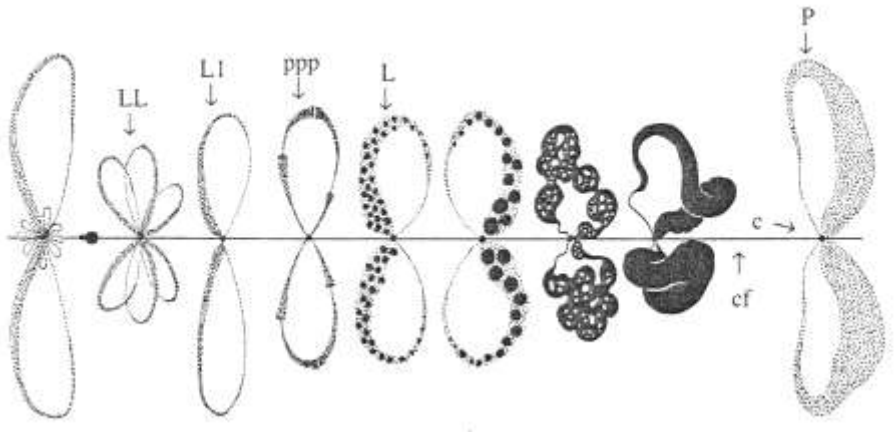


Figure 5. A region of a lampbrush chromosome showing the following features (1) interchromomeric axial fibre (cf) connecting small compact chromomeres (c); (2) chromomeres bearing pairs (L) or multiples of pairs (LL) of lateral loops; (3) loops of widely different morphologies; (4) sister loops of the same or different lengths (Ll); (5) chromomeres without loops; (6) polarization of thickness along loops (P); loops consisting of a single unit of polarization (P); (8) loops consisting of several units of polarization with the same or different directions of polarities (ppp).

In the European crested newt (*Triturus cristatus*) or the North American newt (*Notophthalmus viridescens*), the two animals on which most lampbrush studies were carried out in the early years of lampbrush research, the chromosomes are quite short and contracted at the end of pachytene in the female. They then assume the lampbrush form and they remain like that for several months. As the oocyte nears

maturity, the loops and chromosomes quite suddenly become shorter, the chromomeres become larger, and eventually the chromosomes come to look like normal condensed diplotene bivalents. The general pattern of events is one of extension followed by retraction of the lampbrush loops and a clear inverse relationship between loop length and chromomere size. The longer the loop, the smaller the chromomere, and *vice versa* .

Most lateral loops have an asymmetrical form. They are thin at one end of insertion into the chromomere and become progressively thicker towards the other end (Figure 5).

When one stretches a lampbrush chromosome, either deliberately or accidentally, breaks first happen transversely across the chromomeres in such a way that the resulting gaps are spanned by the loops that are associated with the chromomeres (Figure 6).

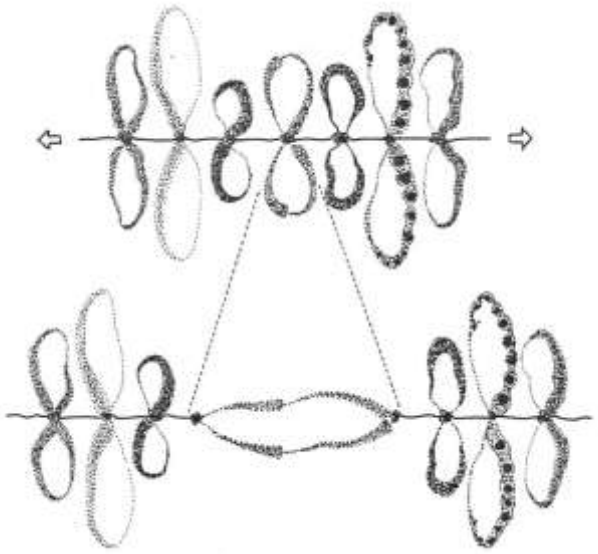


Figure 6. The formation of a double bridge break brought about by a characteristic fracture of a stretched lampbrush chromosome across a line of weakness in the chromomere, such that the loops associated with that chromomere come to span the gap created by the fracture: an important observation providing evidence of the two-chromatid structure of the chromosome, with chromatids closely fused into one fibre in the interchromomeric axis but separated from one another in the regions of the loops.

Breaks of this kind are referred to in the lampbrush literature as "double bridge breaks". Clearly, double bridge formation indicates that there must be a line of weakness separating the two halves of a

chromomere and, more importantly, it indicates a structural continuity between the main axis of the chromosome - the interchromomeric fibre - and the axes of the loops, in which connexion we should remember again that these chromosomes are 2-chromatid meiotic half-bivalents.

The last 2 basic points that need to be made about lampbrush chromosomes and their loops are very important ones indeed, since they serve as foci for the major questions that have been intensively investigated from 1954 right through to the present day. First the loops are sites of active RNA synthesis, and in the vast majority of cases RNA is being transcribed simultaneously all along the length of the loop. In the crested newt there are more than 20,000 RNA-synthesizing loops per oocyte. Secondly, it has become clear that within a species, particular loops may be present or absent in homozygous or heterozygous combinations, and if one examines the frequency of combinations within and between bivalents with respect to presence or absence of particular loops, then we find that these characters assort and recombine like pairs of Mendelian alleles. In other words, there appears to be an element of genetic unity in a loop/chromomere complex.

All the facts that I have given so far were known by 1960, just 6 years after Gall first inverted his phase contrast microscope and made the detailed study of lampbrushes possible. Most of the information comes from a monumental study by Mick Callan (Figure 7) and his assistant Lydia Lloyd who, over the course of 5 years or more, described and documented everything about the lampbrush chromosomes of European crested newts. Their work was published in 1960 in the Philosophical Transactions of The Royal Society.

Figure 7. H.G. (Mick) Callan, photographed in 1965

What then emerged was a model and two interesting and related hypotheses. The model depicted a lampbrush chromosome as 2 DNA duplexes running alongside one another in the interchromomeric fibre, compacted into chromomeres at intervals and extending laterally from a point within each chromomere to form loops where RNA transcription takes place. Each duplex represents one chomatid (Figure 8).

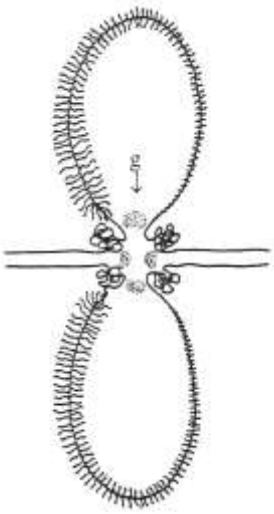


Figure 8. The currently accepted model of lampbrush organization, emphasizing the two-chromatid structure of the chromosome, a chromomere that must comprise 4 parts held together by some kind of glue proteins (g) without intimate intertwining of the chromatin fibres in each part, and sister loops that have similar, though not necessarily identical lengths and the same directions of thickness polarity.

Two famous hypotheses

The two hypotheses that followed this model have not stood the test of time or experiment, but both have stimulated a lot of thought and research from which there has been a remarkable spin-off of truth and understanding. In this sense we are reminded of the encouraging fact that a hypothesis does not have to be correct to be useful. In the sequence in which they were developed, the first hypothesis took account of

(1) the asymmetric shape of the loops
(2) the inverse relationship between chromomere size and loop length
(3) RNA synthesis on the loops.

It was referred to as the "spinning out and retraction hypothesis". It said that during the lampbrush phase of oogenesis all DNA is progressively spun out from one side of the chromomere and the loop extends to become longer and longer. Subsequently, and at times simultaneously, loop axis DNA is retracted back into the other side of the chromomere, at which point it ceases to support RNA transcription. According to this hypothesis, all chromosomal DNA is likely to be involved in transcription at some time during the lampbrush phase. Loop asymmetry is accounted for by supposing that the portion of the loop that has been involved in transcription for the longest time will have the most material associated with it, and that will be the thick end of the loop; whereas the portion of the loop that is newly released from the chromomere in the spinning out process will have the least material associated with it, and that will be the thin end of the loop. This hypothesis, as we shall soon see, was later proved to be completely wrong.

The second hypothesis, which in modern terms was much more fundamental, made the point that the spinning out and retraction explanation for loop asymmetry included the assumption that there was no genetic diversity within an individual lampbrush loop/chromomere complex, and that the information carried in one of these complexes, an average one of which contains as much DNA as in the entire genome of the common colon bacillus (*E. coli*), is serially repeated along the entire length of the DNA that is located in the loop and its half-chromomere.

As we shall see later, this assumption was unnecessary. At the time it was believed that the notion of serially repeated DNA sequences was incompatible with the fact that many phenotypically expressed mutations resulted from changes in only a few nucleotides, and it was hard to see how a mutation could possibly be expressed if it were not simultaneously imprinted in all copies of a repetitive gene complex. To overcome this dilemma, the then famous "Master/Slave hypothesis" was produced, in which the Master sequence imprinted itself on all the slaves once per generation, and what better time to do it than during meiotic prophase.

These wonderful hypotheses emerged at what has to have been the most exciting and progressive era of chromosome research of the 20th Century. People talked about them, worried about them, designed experiments to test and extend them, tried to apply them to other situations, built models around them, and then finally - demolished them. The instruments of demolition were one straightforward observation and one simple series of experiments, both were products of the new technologies of the late 1970s. The observation came with the application of the Miller spreading technique in the study of the amplification of the ribosomal DNA sequences in oocyte nuclei The experiments involved the technique of *in-situ* nucleic acid hybridisation.

Lampbrushes under the electron microscope

The same nuclei as were used for Miller spreads of ribosomal DNA from oocyte nuclei also, of course, had lampbrush chromosomes in them. The lampbrush loops that appeared in these spreads looked just like very long transcription units. These transcription units, like the ones formed by ribosomal DNA, consisted of a thin DNA axis with RNA polymerase molecules lined up and closely packed along its entire length (Figure 9).

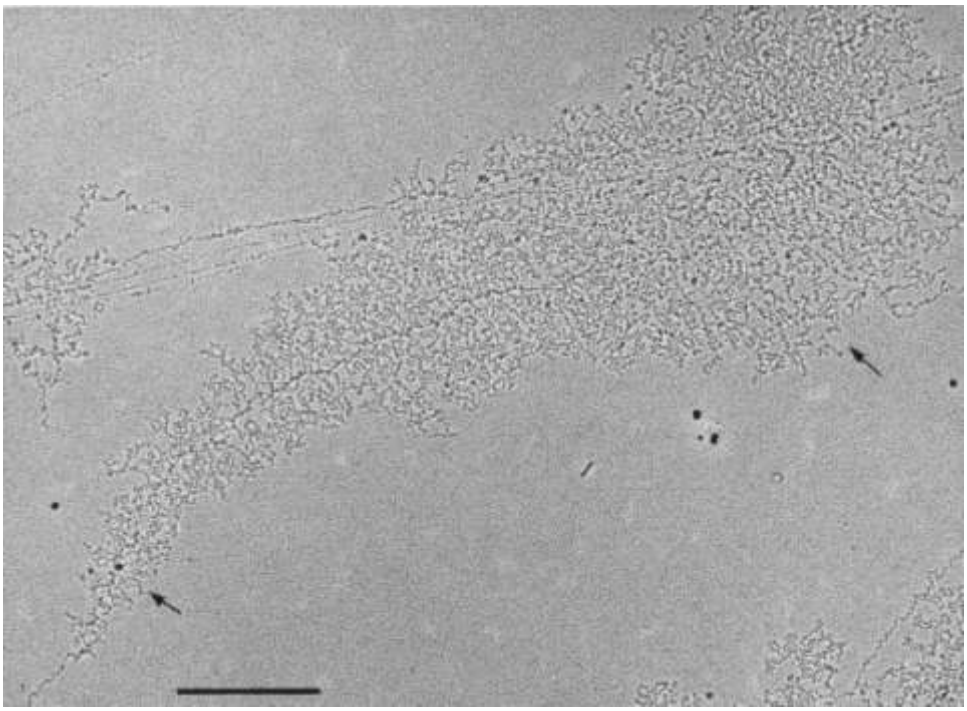


Figure 9. An electron micrograph of a single transcription unit from a lampbrush loop prepared by the Miller spreading technique in which most of the chromosomal protein is removed by treatment in pH9 detergent water, leaving only the DNA axis and its associated RNA polymerase and nascent RNA transcripts. The unit starts at the bottom left hand corner of the picture (lower arrow). Its overall length is 5.2μm. The axis of the unit is studded with RNA polymerase molecules.

Each polymerase molecule carried a strand of RNP. At one end of the transcription unit the RNP strands were short. At the other end they were much longer and they showed a smooth gradient in size from the one end to the other. In essence, the entire transcription unit was polarised, asymmetric, in the same sense as a loop as seen with the light Microscope is asymmetric. The DNA axis before the start of the transcription unit and beyond its end showed the structure that would be expected of non-transcribing chromatin. The average lengths of chromosomal transcription units were about the same as the average lengths of loops as seen and measured with light microscopy.

At the time these observations were first made, it was well known that RNA transcription from a DNA template involved attachment of RNA polymerase to the DNA and movement of the polymerase along the template, feeding off nascent RNA as it went. The farther the polymerase travelled, the longer the piece of nascent RNA attached to

the template by the polymerase. This was the mechanism that was held to account for the transcription of ribosomal RNA from the amplified rDNA in those same oocyte nuclei that had lampbrush chromosomes. This mechanism alone was sufficient to account for the asymmetry of lampbrush loops, and in relation to loops, it was inconsistent with the idea of a moving loop axis. Surely it was the polymerase that moved on a stationary axis, not the loop axis itself. Accordingly, it seemed simpler to suppose that a loop formed by an initial "spinning out" process, probably powered by the continuing attachment of more and more polymerases to a specific region of the chromomeric DNA. It then remained and was transcribed as a permanent structure throughout the lampbrush phase. Towards the end of the lampbrush phase, transcriptive activity would decline, polymerases would detach from loop axes, and loops would regress and disappear. In the sense of the old hypothesis, there was no continuous spinning and and retraction. The vast majority of the chromomeric DNA was never transcribed and a loop represented a short specific part of the DNA in a loop/chromomere complex.

In situ hybridisation

In situ nucleic acid hybridisation was originally invented as a means of locating specific gene sequences on chromosomes. It has a very special usefulness in relation to the study of lampbrush chromosomes. Let us suppose that each loop represents "a gene". The RNA that makes up the loop matrix, the attached nascent transcripts, will all be or include transcripts of that "gene". In effect, the loop represents a very large object, consisting of hundreds of RNA copies of the gene, all clustered at one position on the chromosome set. If we can isolate and purify the DNA of that gene and label it with a radioisotope or a fluorochrome, then it will be easy to make it single stranded, and bind it specifically to the complementary single stranded RNA attached to the lampbrush loop. The technique is known as DNA/RNA transcript *in situ* hybridisation (DR/ISH).

So how did it help to demolish the spinning out and retraction and the Master/Slave hypotheses? The end product of an experiment involving DR/ISH using a radiolabelled or fluorochrome labeled nucleic acid probe is an autoradiograph or a fluorescence micrograph showing one

or more pairs of loops with silver grains or fluorochrome distributed along their lengths (Figure 10).

Two particular observations were of crucial importance. First, it is not uncommon in DR/ISH experiments to find loops that are labelled over only part of their lengths (Figure 10).

This can be interpreted as evidence that the DNA sequence of a loop axis

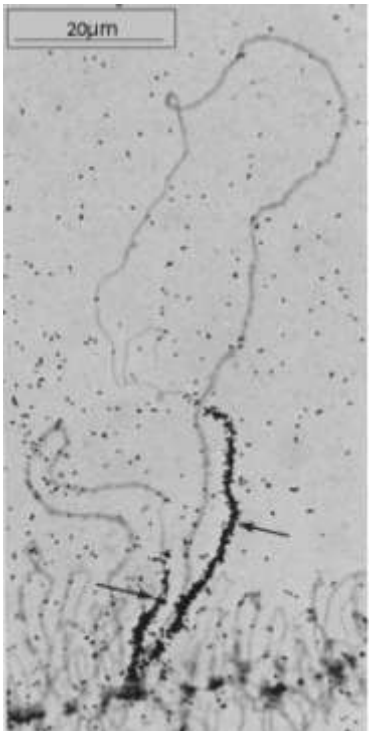


Figure 10. A tritium autoradiograph of part of a lampbrush chromosome following *in-situ* hybridisation of a single gene probe to RNA transcripts associated with lampbrush loops. The two largest loops in this micrograph are labelled part way along their lengths from one end. They are sister loops, arising from the same chromomere, yet they are of different lengths and their labelled segments are correspondingly different in length.

can and does change from place to place along the length of the loop. Evidence of that kind is incompatible with the Master/Slave hypothesis which is based on the principle that entire loop/chromomere complexes consist of multiple tandem repeats of the same sequence. Second, wherever we find partially labelled loops in a DR/ISH experiment, it is

usual to find the same partially labelled loops, with precisely the same pattern of labelling, in every oocyte over quite a wide span of size and stage.

Such evidence cannot be reconciled with a dynamic spinning out and retraction of loop axis throughout the entire lampbrush phase.

The two classic hypotheses were replaced in the late 1970s and early 1980s by a much clearer understanding of the mechanism of action of lampbrush chromosomes and their loops. One of the first and most surprising discoveries that emerged from DR/ISH experiments was that highly repeated short DNA sequences, commonly referred to as "satellite" DNA, sequences that could not possibly serve as a basis for transcription and translation into functional polypeptides, were abundantly transcribed on lampbrush loops along with more complex sequences that were definitely translated into functional proteins.

The read-through hypothesis

Following this discovery, a strong new hypothesis quickly evolved. At the thin base of each loop or the start of each transcription unit there is a promoter site, a start singnal, for a functional gene sequence. RNA polymerase molecules attach to this site and proceed to move along the DNA transcribing the sense strand of the gene and generating messenger RNA molecules that remain attached to the polymerase (Figure 11).

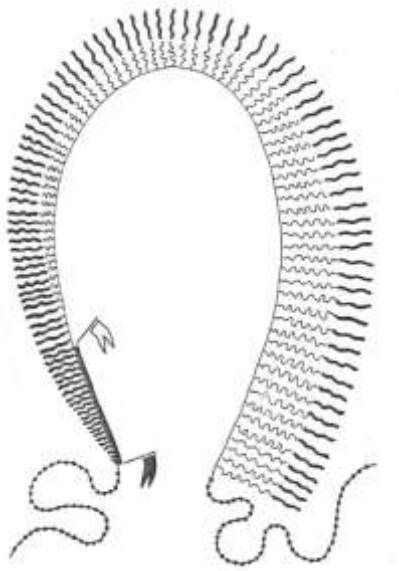


Figure 11. This diagram depicts the situation in a lampbrush loop where a gene is transcribed from a promoter (black flag) through to and past its normal stop signal (white flag) and into the normally non-transcribed DNA that lies downstream, thus generating very long transcription units with long transcripts that include RNA complementary to the sense strand of the gene (thick parts of the transcripts) and the non-sense DNA that lies downstream of the gene (thin parts of the transcripts).

In the lampbrush environment there are no definitive stop signals for transcription, so the polymerases continue to transcribe past the end of the functional gene and into whatever DNA sequences lie "downstream" of the gene. The consequences are very long transcription units, very long transcripts, mixing of gene transcripts with nonsense transcripts in high molecular weight nuclear RNA - and lampbrush loops. This "read-through" hypothesis predicts that the number of functional genes that are expressed to form translatable RNAs may be expected to equal the number of transcription units that are active in a lampbrush set. It is a good hypothesis and it is strongly supported by evidence from a series of *in-situ* hybridisation experiments on the transcription of histone genes and their associated highly repeated satellite DNA on lampbrush loops in *Notophthalmus viridescens.* It says, in effect, that the only unusual feature of a lampbrush chromosome , and the very reason for the lampbrush form, is that once transcription starts it cannot stop until the polymerase meets another promoter that is already initiated or some condensed chromomeric chromatin that is physically impenetrable and untranscribable.

Three simple experiments with enzymes

Is there any direct evidence in support of the read-through hypothesis for lampbrush chromosomes? Yes indeed there is, and it is of a nature that epitomises the supreme advantages of working with these truly remarkable objects and the manner in which it is possible to do the simplest of experiments in order to obtain uniquivocal information at the molecular level. This particular story goes back to 1958 when Mick Callan and the author showed that loops had DNA axes by dissecting lampbrush chromosomes directly into a solution of the enzyme deoxyribonuclease-I (DNase-I) and then watching what happened to them. Within a few minutes, all the loops broke into thousands of little pieces (Figure 12). The same effect was not obtained with ribonuclease nor with proteolytic enzymes.

A short time later Joe Gall had the bright idea of timing the breakage of loops and chromosome axis by DNase-I and plotting number of breaks against time on a log scale. If, as was then rightly supposed, the chromosome axis had two chromatid strands (2 double helices or 4 half-helices) and the loop axis had just one chromatid strand (1 double helix or 2 half-helices) then a log plot of time against breaks for the axis should have a slope of 4 and a corresponding plot for loops should have a slope of 2 (Figure 12). An incredibly easy experiment, requiring only a microscope and a stopwatch, and once again, *Q.E.D!*

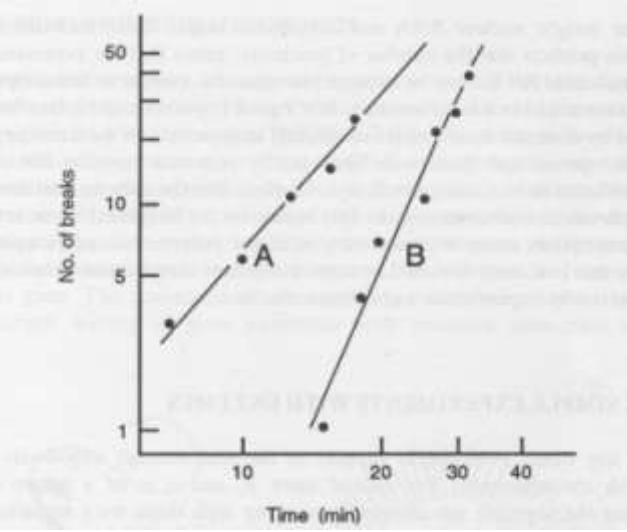


Figure 12

Much later, restriction endonucleases were discovered that were site-specific in their cutting action on DNA. Significantly, the first availability of restriction enzymes more or less coincided with the peak time of controversy over the two classic lampbrush hypotheses. It did not take long to realise that if a loop did consist entirely of identical tandemly repeated DNA sequences all of which possessed a particular restriction enzyme recognition site, then the loop would be destroyed by that enzyme. If, on the other hand the DNA sequences all lacked the enzyme recognition site then the loop would be totally unaffected and would remain intact. The experiment was set up using 5 enzymes and the lampbrush chromosomes from *N. viridescens*. The control enzyme was deoxyribonuclease-I. It destroyed all loops. Three of the other enzymes were recently isolated and only partially characterised restriction endonucleases with unknown site specificities: one of them was almost certainly *Hind* III and another *Eco*RI. The fifth enzyme was the one we refer to today as *Hae* III. Four of these enzymes destroyed all loops. Hae III did likewise, except that it left one set of loops completely intact. Just imagine what a remarkable observation this must have been for the investigator seated at his inverted microscope his eyes glued to a set of lampbrush chromosomes disintegrating into smaller and smaller fragments, and then suddenly realising that amongst this soup of destruction there was a little cluster of loops that seemed totally impervious to the enzyme's action. The loops were big ones and they mapped to the middle region of the second longest chromosome.

They had a curious arrangement in the sense that in some newts they regularly formed a cluster of several pairs of long loops associated with just one chromomere, whereas in other animals (of the same species) they took the form of a single immensely long loop that incorporated several tandem transcription units along its length. They came to be known as the Hae III-resistant loops on chromosome II of *N. viridescens.*

Here was strong direct evidence that at least one set of loops consisted of tandemly repeated short sequence DNA. A nucleotide sequence such as the recognition site for Hae III should occur by chance once in every 256 nucleotides. Here it was entirely lacking in pieces of loop axis measuring up to 100µm, equivalent to at least 300,000 nucleotides. In a sense this was weakly encouraging news for advocates of the

Master/Slave hypothesis, but it left the difficult matter of explaining the total susceptibility to digestion of all the other thousands of loops.

Much later again, the effects of a modern, purified and well characterised sample of *Hae* III were tested, with appropriate controls, on the resistant loops of *N. viridescens* chromosome II. If the loops were present as a bunch, they detached individually from the disintegrating chromosome and thereafter remained intact. If they were present as a single long loop consisting of several tandem transcription units (thin-to-thick segments), then breaks occurred precisely at the thin beginnings of each transcription unit, but the remainder of the loops remained intact. This is, of course, precisely as would be predicted on the basis of the currently accepted read-through hypothesis. The start of the transcription unit would be characterised by a long complex gene sequence that would almost inevitably include the *Hae* III recognition site. The remainder of the loop would consist entirely of repeat sequences that lacked the *Hae* III site.

Some more questions about lampbrushes

Are there any DNA sequences on lampbrush chromosomes that are never transcribed. Almost certainly yes. There are certainly some large blocks of tandemly repeated short DNA sequences that condense into large lumps of heterochromatic material with no associated loops. However, careful investigation usually reveals some parts where even the most nonsense-like sequences are transcribed, presumably by read-through from interspersed functional gene sequences. Only a small fraction of the entire DNA of a loop/chromomere complex forms the transcription unit that makes the loop. What about the rest of the DNA? How many potential promoter sites are there in a chromomere? Is the part that makes the loop preferentially or randomly selected? Can we liken the process to specifically picking out a particular stretch of chromomeric DNA for transcription, the same piece at the corresponding locus in every egg of every individual of a particular species. Or should we liken it to inserting a crochet hook into a ball of wool and pulling it out with a random loop on the end of it? We still, in 2011, do not know the answers to any of these questions, but it would not be hard to design experiments in search of them. Why do loops have

different morphologies that are heritable, locus specific and sometimes species-specific? Their basic organisation is, for the most part, the same, consisting of different levels of folding and secondary structure imposed on the initial nascent RNA transcript. The loop matrix is a site of processing, cleaving and packaging of nuclear RNA, so most of the variation in gross structure may be expected to reflect different modes of binding and interaction involving quite a wide range of proteins and RNAs. Callan and Lloyd's classic 1960 paper in the Philosophical Transactions of The Royal Society should be examined to see just how variable loops can be, whilst at the same time being faithfully recognisable from one preparation to another within an entire population of animals.

Do lampbrush chromosomes look the same in all animals? Naturally not. The lengths of lampbrush chromosomes at the time of their maximum development is in quite strict agreement with the relative lengths of the corresponding mitotic metaphase chromosomes from the same species. The overall lengths of lampbrush chromosomes are broadly related to genome size and chromosome number. Urodeles, with genomes of between 20 and 80pg have much longer lampbrushes than frogs with genomes of 3 to 20pg.

Some lampbrush chromosomes have long loops and others have very short ones. We have seen that the transcription units of lampbrush chromosomes are unusually long because they include interspersed repetitive elements of the genome. Structural genes in large genomes are more widely spaced, interspersed with non-coding DNA, than in small genomes. One might therefore expect lampbrush chromosomes from large genomes to have longer loops (transcription units) than those of smaller genomes, and this is precisely what has been observed. That is not, however, the whole story. It has been known for some time that some very large genomes, like that of the North American mud puppy (*Necturus maculosus*) with a genome that is at least 4 times as large as the crested newt and nearly 30 times as large as that of *Xenopus laevis* has lampbrush chromosomes with very short loops. It is also known that whereas *Xenopus* lampbrush chromosomes have small, stumpy and generally uninspiring loops in normal circumstances, if we do something to inhibit post-transcriptional processing of the loop RNA, then the loops become much longer and the general appearance of the

entire lampbrush chromosome set changes dramatically.

One final point in relation to loop morphology serves, yet again, to illustrate how we can extract molecular information from lampbrushes simply by looking at them with a microscope. Many of the very long loops that we see in lampbrushes from animals with large genomes show multiple, tandemly arranged thin-thick segments (transcription units). The remarkable thing about these situations is that the individual transcription units within one loop can have the same or opposite polarities and can be of the same or different lengths (Figure 13) .

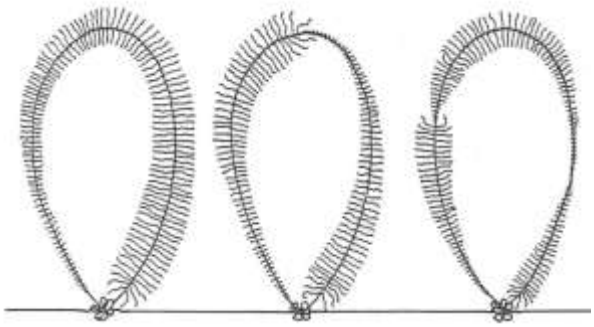


Figure 13. The various arrangements of transcription units that actually occur on lampbrush chromosomes. On the left is a loop consisting of a single transcription unit. In the middle is a loop consisting of two transcription units of the same size and with the same polarity. On the right is a loop with 4 transcription units of different sizes and different directions of polarity.

This observation, perhaps better than any other tells us that it is really the transcription unit that is the ultimate genetic unit in a lampbrush chromosome and not the loop/chromomere complex as was once thought.

Indeed, if there is any aspect of LBCs that remains a mystery it is the lampbrush chromomere. Here are a few facts to think about. Chromomeres are real: we can see them at light and electron microscope levels. They normally, but perhaps not always, have loops associated with them. They can be split to produce the double bridge effect that was described earlier. There are some regions of the lampbrush chromosomes of certain animals in which the chromosome axis is double and each component half has an identical chromomeric pattern with only one loop arising from each chromosome - and these

double axis regions are always so: they are an inherited karyotypic character, which says that there is something about these "half-chromomeres" that precludes them from sticking to their sister halves to make up a normal lampbrush chromomere. Lastly, whereas one might expect that closely related species within the same genus would have similar, if not identical numbers of chromomeres in their lampbrush chromosomes, they do not. The plethodontid salamanders of North America, belonging to the genus *Plethodon*, include certain species that are so morphologically and behaviourally similar that it's hard to tell them apart. They have identical chromosome numbers and identical karyotypes – apart from one thing: the genome of one species is twice as big as that of the other. The species with the big genome has twice as many chromomeres in its lampbrush chromosomes. Could there be more compelling evidence to suggest that the loop chromomere complex is not a feature of genetic significance!

In one rather special but nonetheless highly significant case, specific chromomeres are known to be made up largely of specific families of repeated DNA sequences. This is the W sex chromosome of birds, which is described a little later in this chapter.

To be sure, there is lots more to find out about chromomeres and they will probably be one of the foci of future studies of chromatin and DNA/protein interactions in the formation of lampbrush chromosomes.

The lampbrush chromosomes of birds

Most of what has been said so far is based on studies of the large and very beautiful lampbrush chromosomes of the big-genomed urodeles - newts, salamanders and axolotls. Those of birds, on the other hand, despite being smaller and much more difficult to isolate offer some unique opportunites as well as some special challenges. The pioneering work and subsequent research of Elena Gaginskaya (Figure 14) and her team of Russian lampbrushers in St.Petersburg is especially noteworthy in this regard.

Figure 14. Elena Gaginskaya in 2000.

Birds have small genomes, well characterised karyotypes, and workable lampbrush chromosomes. Moreover, commercially motivated molecular research on the chicken genome has generated a useful genetic map and made available a wide range of gene sequences that can be used as probes for *in-situ* hybridisation. And supplies of domestic chickens are unlimited. What more could one ask!

Perhaps most interesting of all is the fact that birds have well differentiated and strongly heteromorphic sex chromosomes and, because the female is the heterogametic sex, both these chromosomes (Z and W) can be studied at relatively high resolution in the lampbrush form(Figure 15) . In no other group of animals is this possible.

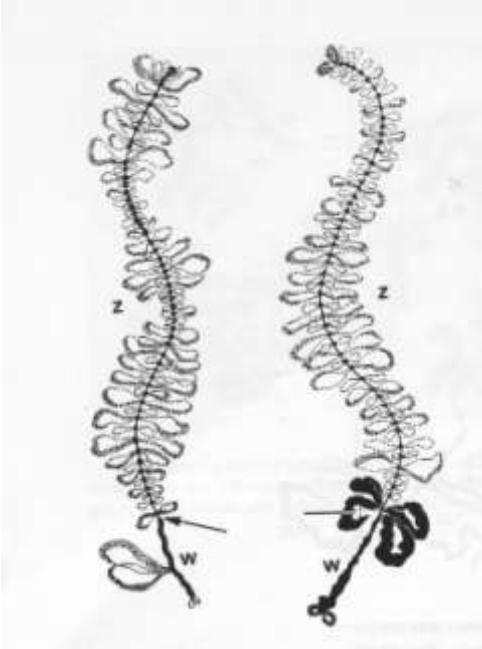


Figure 15. The ZW sex lampbrush bivalents of two species of bird. The mostly condensed and loopless W chromosome (w) can be seen at the bottom end of each bivalent attached to the Z (z) chromosome by a terminal chiasma (arrows).

The ZW lampbrush bivalent, when seen by phase contrast microscopy in a freshly made and unfixed lampbrush chromosome preparation, looks like a univalent. Most of it has a typical lampbrush organization but the terminal one fifth consists of a relatively thick condensed axis carrying only a very few small lateral loops and, often no discernible loops at all. The region with normal lampbrush appearance is the Z chromosome: the short thick loopless region is the W. All kinds of exciting things about these sex chromosome have been discovered just by looking at them in their lampbrush form and strategically employing specific DNA probes for *in-situ* hybridisation experiments. Some DNA sequences associated with sex determination have been mapped on the W. Conserved DNA sequences have been identified and have provided clues to the evolution of sex chromosomes in birds.

One particular feature of bird lampbrush chromosomes deserves special mention. Unlike the situation described in lampbrush chromosomes of amphibians, the lampbrush chromosomes of birds end in a terminal chromomere with conspicuous loops emerging from it. The fine scale morphology of the ribonucloprotein of these terminal loops is different from that of the majority of loops elsewhere on the chromosomes. In many cases the loops associated with the terminal chromomere are

open ended, emerging from the chromomere but not returning to it at the other end. The distal end of a terminal open-ended loop is therefore the true end of one of the chromatids that make up the lampbrush half-bivalent (Figure 16). Carefully controlled *in-situ* hybridisation experiments showed that a short extreme terminal transcription unit on the telomere loops represents transcription exclusively from the C-rich strand of the telomeric TTAGGG repeat unit. What is more, evidence suggests that this transcription of strictly terminal clusters of telomeric repeats is an obligatory event and not indiscriminate read-through from proximally located gene elements. What does it mean?

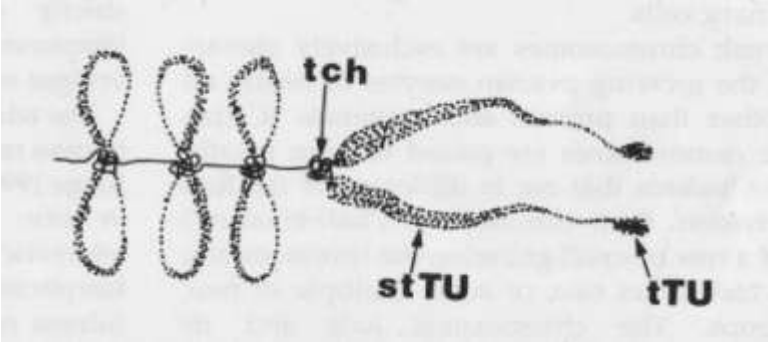


Figure 16. Diagramatic representation of the end region of a chicken lampbrush chromosome showing a terminal chromomere (tch)with its loops hanging free, each loop consisting of two transcription units. The sub-terminal transcription unit (stTU) is long and has distal-proximal polarity. The terminal transcription unit (tTU) is small and has proximal-distal polarity and represents transcription of the C-rich strand of a cluster of telomeric (TTAGGG/AATCCC) DNA sequences.

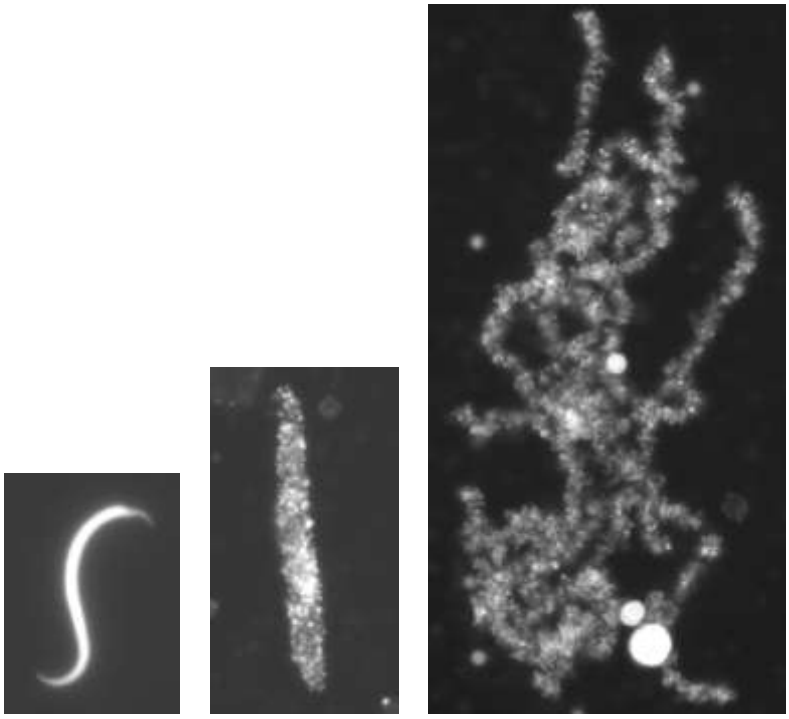
These are just a few examples of the emergence of the "bird era" of lampbrushology. Undoubtedly, the chromosomes are quite difficult to handle and killing birds and dissecting them for their ovaries is not a business that appeals to many modern laboratory scientists. But in this case the rewards have far outweighed the technical challenge of just getting a complete and well preserved set of chicken lampbrushes onto a slide and under a microscope.

Human lampbrush chromosomes!

Perhaps the most exciting thing that has happened in lampbrush

chromosome research in recent years was carried out, befittingly, by one of the original pioneers of the field, Professor Joe Gall. It's not entirely clear why he did the experiment other than just to have a look and see. The question: what happens to the chromosomes of other kinds of cell if they are placed in an oocyte nucleus containing fully developed lampbrush chromosomes? The ultimate test, of course, would be the nucleus of a mature spermatozoon, where all the chromosomes are complexed with special proteins and packed together about as closely as they can possibly be. Besides which, it's relatively easy to get some sperm and inject them into an oocyte nucleus and, if you want to extend the experiment and try putting chromosomes from a foreign species into an oocyte nucleus, then using sperm introduces an element of control into the experiment which would be difficult if nuclei from other types of somatic cell were used.

The results were spectacular. The sperm swell, their chromosomes become diffuse and with a few hours become absolutely normal lampbrush chromosomes except, of course, that a sperm chromosome consists of only one chromatid. So each of the lampbrush chromomeres has only one loop emerging from it (Figure 17). A simply beautiful result!



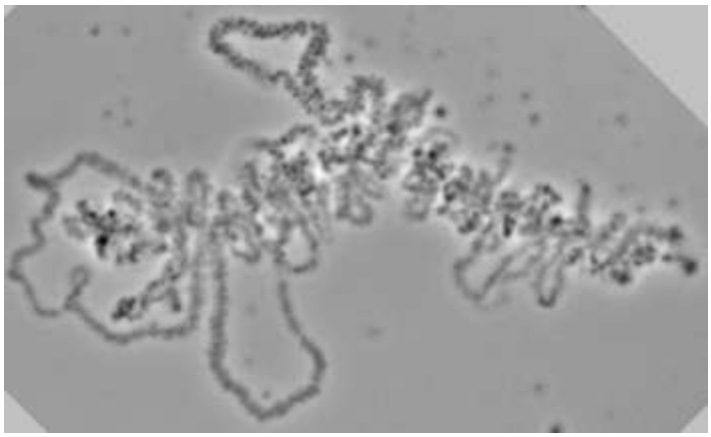


Figure 17. *Xenopus* sperm heads injected into *Xenopus* oocyte nuclei swell immediately and within hours begin to stain with an antibody against RNA polymerase II. Each sperm head becomes a loose mass of chromosome like threads, which by 24–48 h resolve into individually recognizable lampbrush *c*hromosomes. Although lampbrush chromosomes derived from sperm are unreplicated single chromatids, their morphology and immunofluorescent staining properties are strikingly similar to those of the endogenous lampbrush bivalents. They display typical transcriptionally active loops extending from an axis of condensed chromomeres, as well as locus-specific "landmarks." The figure at the bottom shows one such sperm-derived lampbrush chromosome (Gall and Murphy 1998).

Even more astonishing and portentious was the observation that human sperm nuclei injected in the oocyte nuclei of the frog *Xenopus laevis* make human lampbrush chromosomes (Figure 18). And here we are talking about chromosomes that never, ever, become lampbrushy in their normal human environment.

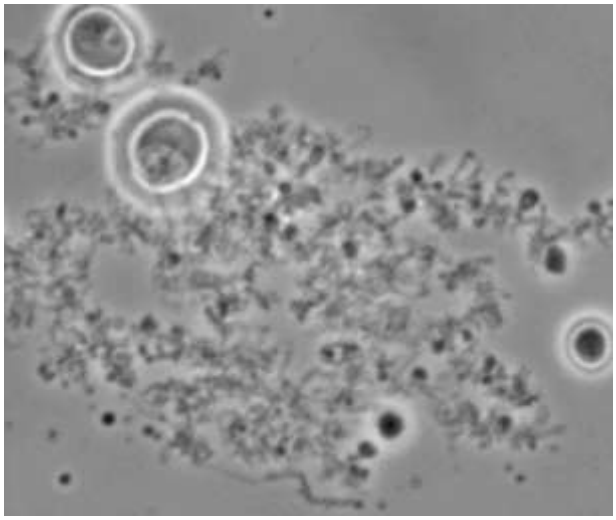


Figure 18. Phase contrast micrograph of a group of lampbrush chromatids derived from the nucleus of a human spermatozoan 24 hours after injection into a *Xenopus* oocyte nucleus.

So lampbrush is a chromosomal form that develops in the environment of the nucleus of the growing ovarian egg. More questions follow, of course. Do, for example, species-specific landmark objects, loops of species-specific appearance, appear in their right places on lampbrush chromosomes that have developed and are transcribing RNA in a foreign nucleoplasm? What happens if we place a somatic cell nucleus in an oocyte nucleus? A safe guess would be that its chromosomes, too, would quickly become lampbrushy – and that's an experiment waiting for somebody to do!

Why do lampbrush chromosomes exist at all?

They are characteristic of eggs that develop rather quickly into complex multicellular organisms independently of the parent. A frog's or a bird's egg, for example is fertilized, deposited by the mother and then develops into a complex tadpole within a few days. Much of the information for this process in the form of polyadenylated messenger RNA, most of the ribosomes for protein synthesis and all of the nutrient raw materials are laid down during oogenesis through activity of lampbrush chromosomes and amplified ribosomal genes and the accumulation of yolk proteins imported from the liver. Lampbrushes may therefore be regarded mainly as an adaptive feature that has evolved to pre-programme the egg for rapid early development. The fact that they are not developed in mammalian eggs could be regarded as a primitive feature that is consistent with the relatively slow pace of mammalian development. A frog's egg, for example, will have completed gastrulation and be well advanced in the differentiation of its central nervous system by the time a human egg has reached the 8 cell stage. But can this be the whole story?

Recommended further reading

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For full references of all publications on lampbrush chromosomes see www.projects.exeter.ac.uk/lampbrush/pubs.htm