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Review

Lampbrush chromosomes and associated bodies: new insights into principles of nuclear structure and function

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Abstract

The lampbrush chromosomes and assorted nuclear bodies of amphibian and avian oocytes provide uniquely advantageous and amenable experimental material for cell biologists to study the structure and function of the eukaryotic nucleus, and in particular to address the processes of nuclear gene expression. Recent findings discussed here include the molecular analysis of the actively elongating RNA polymerase complexes associated with lampbrush chromosome loops and of the association between loop nascent transcripts and RNA processing components. In addition, several types of chromosome structure that do not outwardly resemble simple extended loops and that may house novel nuclear functions have recently been studied in detail. Among these a type of chromosomal body that can also exist free in the oocyte nucleus, the Cajal body, has been shown to possess a range of characteristics that suggest it is involved in the assembly of macromolecular complexes required for gene expression. Homologous structures have also been described in somatic nuclei. Fundamental aspects of the looped organization exhibited by lampbrush as well as other chromosomes have also been addressed, most notably by the application of a technique for *de-novo* chromosome assembly.

Introduction

It is 120 years since the publication of the first account of the 'strange and delicate structures' found in the nuclei of amphibian oocytes (Flemming 1882). The results of the first century of extensive investigation into the structure and function of what later became known as lampbrush chromosomes have been collated and analysed authoritatively by H. G. Callan (1986). In this review, I shall attempt to describe what has been learnt about these most spectacular of chromosomes since the appearance of Callan's monograph. Lampbrush chromosomes (LBCs) are so called because of their resemblance to the 19th century equivalent of a test-tube brush and are highly extended diplotene bivalents found in the growing oocytes of most animals (Figure 1a). Their characteristic appearance derives from the fact that each homologue consists of a linear array of numerous compact chromatin granules or chromomeres, from which arise pairs of laterally-projecting loops that form the thousands of bristles of each set of lampbrushes (Figure 1b). The loops occur in pairs because they represent regions of intense transcriptional activity where

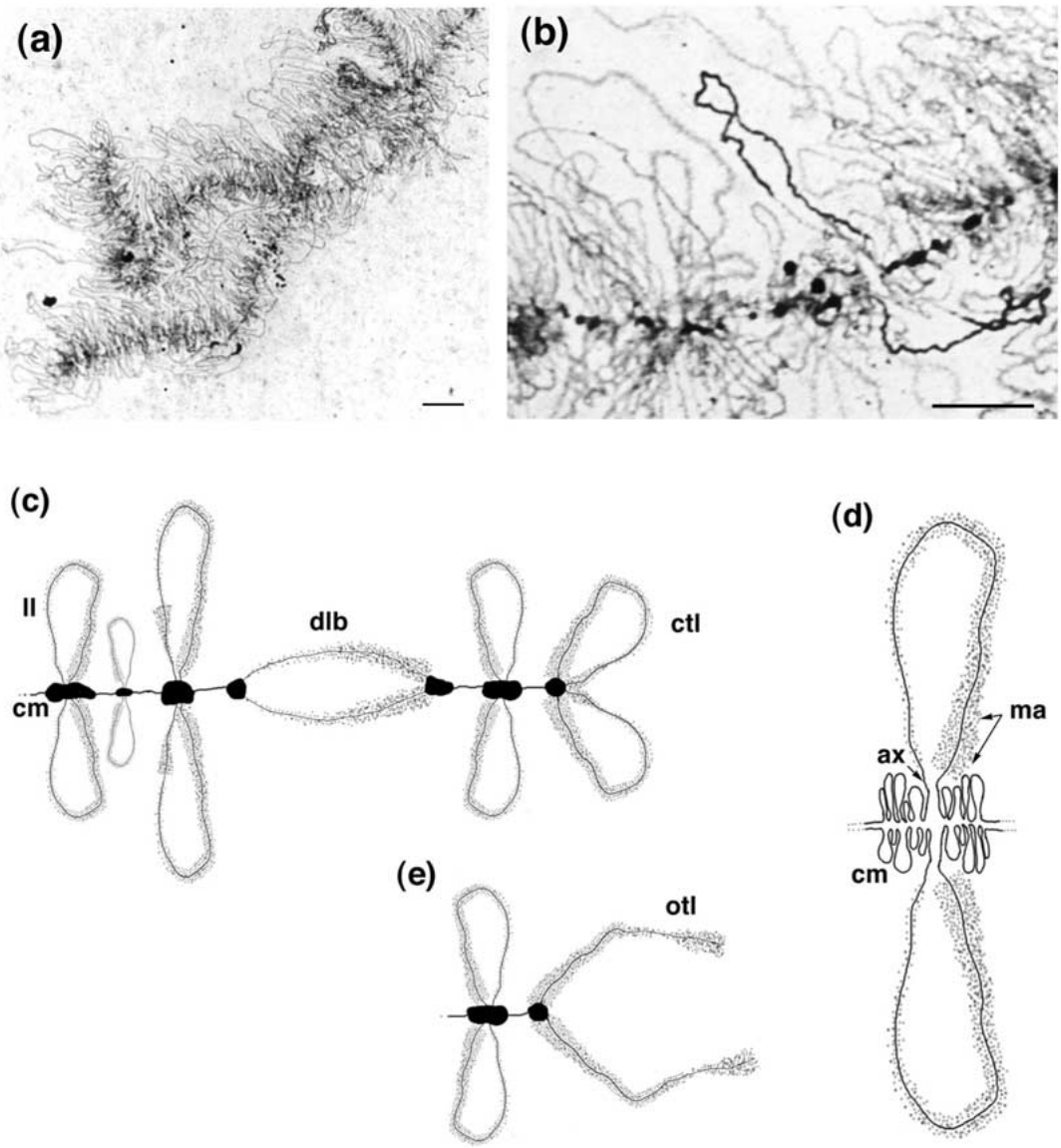

Figure 1. Summary of the main organizational features of lampbrush chromosomes. (**a**) Part of LBC X from *Triturus carnifex* after its attachment to a slide by centrifugation, followed by fixation and silver staining (from Varley & Morgan 1978). The two chiasmata formed between the homologues can be seen towards the upper right of the micrograph. Scale bar represents 20 μm. (**b**) A more highly magnified region of a silver-stained LBC that demonstrates the paired nature of loops by virtue of the more intense staining exhibited by one pair of sister loops. Highly stained chromomeres marking the chromosome axis are also visible (from Varley & Morgan 1978). Scale bar represents 10 μm. (**c**) Schematic depiction of part of a single homologue showing the paired lateral loops (ll) extended from highly condensed chromomeres (cm). The double-loop bridge (dlb) structure that results from the transverse breakage of a chromomere is indicated, as are the distinctive closed telomere loops (ctl) that characterize the ends of avian LBCs. (**d**) Diagram of the generally accepted view of the organization of the DNA strands comprising the paired sister chromatids of a single lampbrush homologue. Each line represents a single DNA duplex that, in an extended state, runs between chromomeres. It next becomes compacted to form part of a chromomere (cm), then in a more extended state forms the axis (ax) of a lateral loop before being recompacted into the remaining part of a chromomere and then reassuming its relatively extended interchromomeric path. The RNP matrix (ma) that coats the extended loop axis asymmetrically is also indicated. (**e**) A depiction of an alternative situation that has been observed at the ends of avian LBCs in which the terminal loops are open ended (after Solovei *et al.* 1994). The open telomere loops (otl) emerge from but do not return to the terminal chromomere and form a structure that is basically analogous to the double loop bridge shown in (**c**). (**a**) and (**b**) reproduced by permission of Bertelsmann-Springer.

sister chromatids are completely unassociated. Hence each loop is formed from a thin extended chromatin axis that typically is surrounded by a relatively bulky matrix of RNP. Even at the light microscope level it can be seen that the matrix of some loops is not distributed uniformly but rather appears as one or more regions in which the amount of RNP progressively increases in thickness along the loop axis. By electron microscopy it can be shown that each thin-to-thick matrix gradient represents a single transcription unit, with the matrix asymmetry reflecting the presence of tightly packed nascent RNP fibrils that increase in length in the direction of transcription. The precise structural relationship between chromomeres, loops and transcription units is illustrated by the appearance of LBCs when they are stretched to breaking point. The first breaks appear transversely across chromomeres and, rather than the chromosomes becoming fragmented, the gaps are bridged by the conversion of the paired sister loops into two parallel strands each displaying the same matrix asymmetry (Figure 1c). The resultant 'double-loop bridge' thus maintains the linear integrity of the chromosome axis and this phenomenon was a key element in the reasoning behind the accepted model of basic LBC organization. According to this model the DNA strand of each chromatid runs continuously from chromomere to chromomere where it is alternately condensed and extended out into lateral loops before passing on to the next chromomere (Figure 1d).

The unique molecular organization and levels of transcriptional activity exhibited by LBCs have meant that they have been studied as much for their potential to aid an understanding of the basic mechanisms and logistics of gene expression as they have for investigating those aspects of organizing, transmitting and recombining genomes with which chromosome studies are normally associated. In addition the LBCs that have been most intensely studied are those from amphibian oocytes, and the key contribution that the nucleus or germinal vesicle (GV) of such large yolky oocytes makes to the provision of maternal RNAs for the developing embryo has provided another key focus for the investigation of LBCs (reviewed in Davidson 1986). Indeed the nature of LBC transcription units (TUs) and the sequence identity of their transcripts were major subjects of Callan's monograph (Callan 1986), subjects that were addressed principally by the application of two experimental approaches. One was the examination of spread preparations by electron microscopy and led to the major conclusions that there could be multiple TUs per loop and that TUs could be surprisingly long, up to several hundred kilobases. The second approach relied on *in-situ* hybridization of labelled probes, usually non-coding repetitive sequences, to nascent loop transcripts and these studies consistently showed that even highly repetitive sequences could be intensively transcribed on LBC loops. However, exhaustive investigation of histone gene clusters in the newt *Notphthalmus viridescens* carried out by J. G. Gall and colleagues (Gall *et al.* 1983, Diaz & Gall 1985) suggested that there might be another unexpected feature of transcriptional control on LBC loops. These results were encapsulated in a model (Diaz & Gall 1985) based on the occurrence of readthrough or 'overrun' transcription (Varley *et al.* 1980). In its original, specific formulation the readthrough model held that transcription initiated at any of the promoters in a histone gene cluster, proceeded without interruption through the rest of the genes in the cluster and through satellite DNA sequences flanking the cluster, and that termination did not occur until either another active TU or the end of the histone loop was reached. When applied more generally the tenets of this model for the control of transcription on LBCs, namely accurate initiation at normal promoters, highly efficient elongation and a lack of response to expected termination sequences, provided a compelling explanation for the large size and high repetitive sequence content found for many LBC TUs in a variety of species.

Fittingly, the final short chapter of Callan's monograph (Callan 1986) was concerned with the beginnings of the study of the proteins associated with LBCs because it is with the protein rather than nucleic acid components that many recent investigations of LBCs have been concerned. The reasons have in part been technical; the widespread availability of highly-specific antibodies and the ready application of technologies for creating and expressing epitope-tagged fusion proteins in amphibian oocytes have enabled the

detailed study of LBC structures with regard to steady-state protein composition and to the targeting of proteins, respectively. A second impetus has been an increased emphasis on studying features of LBCs other than typical lateral loops, and in understanding the structure and function of such objects, knowledge of their protein components has proved central. The occurrence of objects with distinctive morphologies that are present repeatedly at specific LBC loci was initially of great value in the establishment of working maps that allowed individual chromosomes to be reliably identified. Such 'landmarks' are of two main types, those that have an underlying loop-like organization and those essentially globular objects that do not. Examples of both types are shown in Figure 6. Landmark loops are often unusually large but their distinctive appearance is primarily brought about by the possession of a bulkier, more refractile matrix than is found on typical loops and that often obscures any loop-like derivation. A variety of morphologies has been described for such matrices and they are thought largely to be determined by the types and amounts of proteins that accumulate there, proteins that are probably additional to those directly associated with nascent transcripts. I have used the term 'complex loops' to refer to those lateral loops, both landmarks and related examples that do not serve for chromosome identification, that possess a matrix with a complex morphology. Conversely I have termed as 'simple loops' the vast majority that have a fine fibrous type of matrix, sometimes with clearly discernible asymmetry and always with a clearly loop-like form.

The second type of landmark structure includes objects such as those originally referred to as spheres or granules that bear no obvious resemblance to or derivation from loops. I have referred to them here as 'chromosomal bodies'. This nomenclature is to emphasize the fact that some of these objects also exist in oocytes in an extrachromosomal form as free 'GV bodies' and, most importantly, to stress that for at least one type of LBC body a clear homology to nuclear bodies of somatic cells has emerged (reviewed by Gall 2000). The latter advance resulted largely from the development in Callan's and Gall's laboratories of reliable techniques for working with LBCs of the anuran *Xenopus laevis* (Callan *et al.* 1987, Gall *et al.* 1991). The use of *Xenopus* has meant that, in contradistinction to the urodele amphibians that had previously been used for most LBC studies, a broad array of nucleic acid and immunological reagents are available and molecular genetic analyses are now more feasible. Lest I give the impression that this categorization of LBC structures into simple loops, complex loops and chromosomal bodies is all encompassing, it should be pointed out that, in some organisms, there exist enigmatic landmark objects that appear completely novel (e.g. Solovei *et al.* 1992, 1996) and that promise to add currently unsuspected principles of LBC structure and function to those discussed below.

In the sections that follow I will discuss some of the new findings and outstanding questions regarding the underlying structure and composition of LBC loops and chromomeres and then consider the transcription apparatus and nascent transcripts of simple loops. This will be followed by a review of recent findings concerning the organization and composition of loops with complex morphology and finally by a consideration of the structure and function of four distinctive types of chromosomal body.

Lateral loops and the fundamentals of lampbrush organization

Formation of extended loops: loop fastening and the requirement for transcription

We still know little of the molecular details that underlie the defining structural characteristic of lampbrush chromosomes, namely the pairs of extended, transcriptionally-active DNA loops that emanate from compact, transcriptionally inert chromomeres. As an indication of the relative proportions of a genome that exist in these two conditions, a recent estimate for a typical urodele LBC obtained by scanning electron microscopy suggested that between 0.4% and 2.4% of the DNA is found in loops (Leon & Kezer 1990). The nature of the boundary between chromomeric and loop DNA is unknown, as is the manner in which the bases of a loop are fastened in order that closed loops are the default state rather than open double-loop bridges (Figure 1c). Since double-

loop bridges have long been observed in studies of LBCs, clearly under some circumstances the loop fastening mechanism either does not form or can fail, such as during mechanical stretching. A further striking example of this phenomenon has been described recently in avian LBCs. In most bird species examined it has been found that all LBCs end in a terminal chromomere from which a conspicuous pair of telomere loops emerge (Solovei *et al.* 1994, 1995). In many cases telomere loops are open ended, the loop having only one insertion into the chromomere while its other end hangs free. Structurally this condition is fundamentally the same as a double loop bridge (Figure 1e) and the disruption of the loop fastening mechanism that causes it is thought not to be due to mechanical breakage during preparation but to reflect the natural state of the chromosome ends. This finding also leads to the important conclusion that in at least bird LBCs each chromatid ends in loop DNA rather than in chromomeric or granular structures as described for amphibian LBCs (Callan 1986). One difference to the double loop bridge structure is the absence from open telomere loops of a detectable downstream chromomeric segment. This raises the possibility that in closed telomere loops the loop fastening function involves a relatively small and exclusively loop-derived DNA sequence.

Is sister chromatid cohesion required for the formation of the looped organization or do the loop/chromomere units of a chromatid form independently of its sister? A recent innovative approach based on the induction of LBCs in a controlled experimental system has allowed this question to be addressed directly. In a remarkable series of experiments, Gall & Murphy (1998) demonstrated that the chromatin from the demembranated sperm heads of a variety of vertebrates could form typical transcriptionally active LBCs just hours after their injection into oocyte GVs (Figure 2a). These sperm LBCs closely resembled in overall structure the endogenous LBCs of the injected GV except that each was clearly derived from a single chromatid rather than from the pair of sisters of a normal lampbrush half-bivalent. Hence loops with a characteristic morphology or staining pattern were always observed singly on sperm LBCs and single-loop rather than double-loop bridges spanned gaps in the chromosome axes (Figure 2b). Since sperm LBCs are formed *de novo* by the decondensation and subsequent transcription of previously highly-compacted sperm chromatin, this finding shows that the formation of LBC loops is dependent solely on intrachromatid associations and does not require interchromatid interactions. While this principle was previously inferred from the existence in the LBCs of some species of short 'double

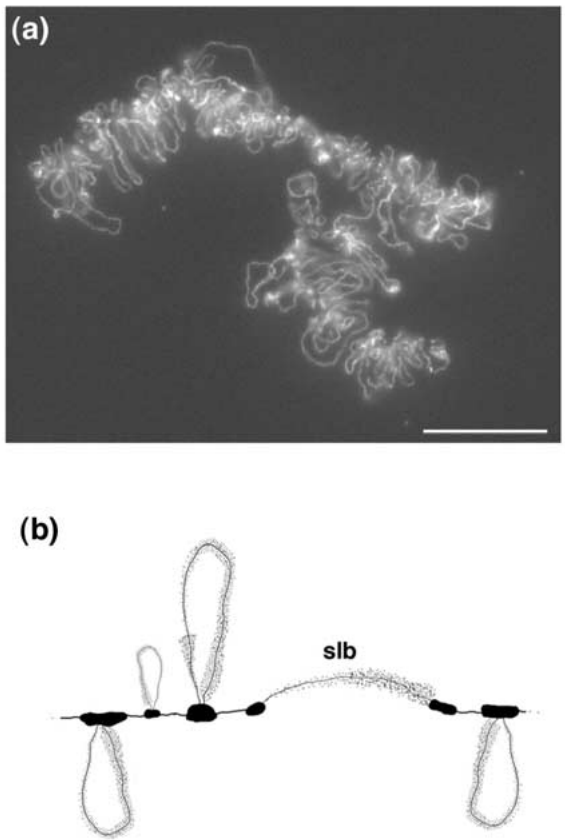

Figure 2. LBCs derived from sperm chromatin (Gall & Murphy 1998). (**a**) Two LBCs assembled from demembranated *Xenopus* sperm heads that had been injected into the nucleus of a newt oocyte two days previously. Immunofluorescent staining is for RNA polymerase II and therefore reveals the extended transcriptionally-active loop axes. (Image kindly provided by Professor J. G. Gall.) Scale bar represents 20 μm. (**b**) Schematic representation of the organization of sperm LBCs which, in contrast to the endogenous oocyte LBCs depicted in Figure 1c, are composed of only a single chromatid. Accordingly, the occurrence of a transverse chromomere break in these LBCs leads to the formation of a single-loop bridge (slb) rather than a double-loop bridge. (**a**) is reproduced from *Trends in Cell Biology* (1998) vol. 8, p. 207 with permission of Elsevier Science Ltd.

axis' regions caused by the premature separation of sister chromatids, it is now clear that it is generally applicable in the formation as well as the maintenance of a looped organization in all LBCs.

A further important conclusion regarding the mechanisms of loop formation comes from considering in more detail the assembly of sperm LBCs described by Gall & Murphy (1998). Upon injection, the sperm heads initially became swollen and then continued to expand with further incubation until the first indications of individual chromatids were seen. During these stages the chromosomes stained more and more intensely with an antibody directed against RNA polymerase II (pol II). Later, when definitive LBCs appeared, loop axes were also stained intensely with this antibody and the incorporation of labelled RNA precursors into the loop matrix confirmed that transcription was ongoing. Although incubation with the transcriptional inhibitor actinomycin D did not affect the early stages of decondensation and pol II accumulation in chromatin, it did prevent the formation of LBCs. It is well established that actinomycin D inhibits transcription by LBCs and leads to the retraction of existing loops, and also that recovery from such inhibition allows re-extension of loops (Callan 1986). These earlier findings clearly show that loop maintenance requires ongoing transcription but the blocking of sperm LBC assembly by actinomycin D (Gall & Murphy 1998) may suggest that the formation of loops *de novo* also requires transcription. However, because of the difficulty of detecting such structures, it is difficult to rule out an alternative explanation, namely that transcription is actually a consequence of the prior formation of extended chomatin loops during decondensation.

A role for the RNP matrix in actually preserving the extended form of lampbrush loops, perhaps *via* some kinds of intermolecular repulsive forces (e.g. Marko & Siggia 1997), is supported by numerous examples of the correlation between matrix release and loop retraction, such as found after transcriptional inhibition. However an absolute requirement for a bulky matrix to accomplish loop extension is at variance with observations in urodele LBCs of some extraordinarily thin loops that are close to the resolving power of the light microscope and that show no sign of a polar matrix (Callan 1986). Recent observations of the distribution of pol III in *Xenopus* LBCs have reinforced these earlier morphological observations. J. G. Gall (personal communication) has found that about 90 LBC sites are specifically stained with antibodies against four different pol III subunits and some of these sites are extended loops. Again, these loops can appear almost invisible when viewed in phase contrast whereas they are easily detectable as a continuous fine line of immunofluorescent staining. The identification of 'thin' loops as extended segments of DNA containing contiguous pol III transcription units provides a satisfying explanation for their unusual morphology. The TUs typically transcribed by pol III such as the 5S RNA and tRNA genes, are short and tandemly repeated. Provided that transcription termination is efficient in such arrays, each TU would be expected to produce correspondingly short transcripts, quite unlike the extended RNPs forming the bulkier visible matrix of TUs transcribed by pol II. Indeed unidentified transcription units exhibiting these characteristics have been seen by electron microscopy of spread preparations (Scheer 1981, 1982).

Composition of lampbrush chromatin

Recent studies of the chromatin composition of LBCs have established that they exhibit several unusual features. With respect to core histone proteins, the hyperacetylation of histone H4 in transcriptionally active LBCs has been shown by immunostaining with several antisera against H4 isoforms acetylated on different lysine residues (Sommerville *et al.* 1993). Core histone acetylation has long been associated with transcriptional activity (Turner 1991) and hence it was somewhat unexpected that several of the antisera stained the chromomeric axes of LBCs as well as, more faintly, the loop axes. However, individual chromomeres stained unevenly, with distinct foci of staining occurring close to the site of loop insertion. It has been suggested that in the loop attachment regions as well as at particular regions in the loops themselves, H4 is hyperacetylated in order to produce chromatin in a relaxed or accessible state for interaction with transcriptional proteins (Sommerville *et al.* 1993). Interestingly,

when mature oocytes were treated with butyrate, which stimulates histone acetylation, an increase in antibody staining of the contracted LBCs was seen followed by the appearance of short active loops. Histone hyperacetylation may therefore be a key step that precedes and could even induce loop formation. In agreement with this possibility, the experimental overexpression of histone deacetylase in oocytes that have transcriptionally active LBCs causes loop retraction (Ryan *et al.* 1999). LBC chromatin is also unusual with regard to the linker histone, H1, which is regarded as a general repressor of transcription and is normally required for the formation of the higher-order chromatin structures of inactive chromatin. Transcriptionally active LBCs are devoid of H1 and the maternal H1 variant, H1M (B4), and this is true for the condensed chromomeric DNA as well as the loops (Hock *et al.* 1993). Moreover, injection of histone H1 into oocytes causes loop retraction, chromosome shortening and transcriptional inactivation (R. Hock, personal communication). Clearly both of these histone modifications are explicable as adaptations to the persistent state of high transcriptional activity typical of LBCs.

Another chromatin component, a pair of HMG (high mobility group) proteins termed HMGN1/2 (previously known as HMG14/17) is also absent from LBCs of vitellogenic oocytes (R. Hock, personal communication). HMGN proteins are ubiquitous in somatic cell nuclei and are thought to bind to nucleosomes and enhance transcription and other DNA-dependent nuclear activities by modifying the structure of chromatin (Bustin 2001). While the absence of endogenous HMGN proteins from LBCs may be due to the pre-emptive effect of the changes to core histones described above, exogenously added HMGN does bind to LBC loops (R. Hock, personal communication). A further eccentricity of LBC chromatin is the absence of detectable DNA topoisomerase II in *Xenopus* LBCs and its confinement to scattered axial granules in *Pleurodeles* LBCs (Fischer *et al.* 1993, Hock *et al.* 1996). Since topoisomerase II is a major component of the scaffold of metaphase chromosomes, where it is thought likely to serve as a loop anchor, it is interesting that its distribution in LBCs appears to rule out a similar role for topoisomerase II as a fastener for lampbrush loops. Given the unusual composition of LBC chromatin, it will clearly be important to ascertain the distributions of recently-discovered chromosome structural proteins, such as the condensin and cohesin complexes (reviewed by Uhlman 2001), with regard to understanding basic features of the lampbrush structure. One approach to such investigations is suggested by recent success in localizing a centromere protein in *Xenopus* oocytes. It has been found that *myc*-tagged CENP-C is directed to a single well-defined spot on each LBC half-bivalent; in addition to indicating that endogenous and tagged chromosomal proteins are capable of quantitative exchange, this approach has also provided the means to identify the otherwise undetectable centromere regions of *Xenopus* LBCs (Z.Wu, personal communication).

Loop length, transcription units and sequence content

Comparisons of the LBCs of different amphibians have repeatedly demonstrated that lateral loops appear markedly longer in species with larger C-values (Macgregor 1980). It has been generally assumed that this correlation solely reflects differences in the sequence organization of the underlying genomes. One explanation for the C-value effect on loop length is based upon the there being substantial differences in the lengths and distributions of transcribed sequences relative to chromomeric sequences in genomes of different sizes (Davidson 1986), while another suggests an overall increase in the length of loop transcription units due to the (readthrough) transcription of the longer intergenic spacers of large genomes (Gall *et al.* 1983). However, experiments carried out by Gall & Murphy (1998) as part of their work on the assembly of LBCs from sperm chromatin have offered a fundamental new insight into the species-specificity of loop length. In these experiments demembranated sperm from *Xenopus* were injected into GVs of a newt, *Notophthalmus viridescens*, which has about a ten-fold higher C-value and correspondingly longer LBC loops. Unexpectedly, the *Xenopus* sperm LBCs resembled the endogenous newt LBCs far more closely than they did either the endogenous or sperm LBCs assembled in *Xenopus* GVs. Not only were the loops much

longer than normally expected for *Xenopus* LBCs, some measuring 30–40 μm, but also their abundant RNP matrices and the more intense immunostaining of their axes with a pol II antibody were both characteristic of newt LBCs. The use of newt-specific antibodies demonstrated that newt proteins had been used in the assembly of the *Xenopus* sperm LBCs, reinforcing the conclusion that the morphological characteristics of loops, including their overall length, can be dependent in large degree on the physiological and molecular features of the host GV rather than on the LBC genome itself. This conclusion was supported by another set of heterologous injections in which sperm of the leopard frog, *Rana pipiens* were injected into *Xenopus* GVs (Gall & Murphy 1998).

Although it is not proven that the short loops of endogenous *Xenopus* LBCs and the extended loops of *Xenopus* sperm LBCs in newt oocytes represent the same genomic sequences, there are other situations in which given loops can be said to exhibit different lengths; i.e. in the context of the general loop retraction that occurs towards the end of the lampbrush stage or after transcriptional inhibition (Callan 1986). Observation of lampbrush loops in electron microscope spreads have shown that, in shortening loops, the transcribing polymerases are less-tightly packed than usual and that there is a concomitant condensation into nucleosomes of the unoccupied regions of the loop between the polymerases (Scheer 1978, Scheer 1987). It is thought that increased nucleosome packing, together with the formation of higher-order chromatin structures, account for loop shortening (Callan 1986). Conversely then, the increased length of *Xenopus* loops seen in newt oocytes may be indicative of higher levels of polymerase packing, and correspondingly fewer nucleosomes, than would be the case for the same loops in standard *Xenopus* LBC spreads. The enhanced pol II immunostaining found for sperm LBCs in newt oocytes (Gall & Murphy 1998) is consistent with this explanation.

It is important to note, however, that differential transcription rates may not totally explain the longer LBC loops of newt oocytes. Although *Xenopus* sperm LBCs in newt oocytes have much longer loops than endogenous *Xenopus* LBCs, on average they are not as long as typical newt LBC loops (Gall & Murphy 1998). Hence, it is still likely that a feature of the sequence organization of large genomes can affect the length of LBC transcription units to some degree. It is interesting in this regard that genomic sequence has been obtained for part of a *Notophthalmus* myosin gene and it provides limited evidence for the presence of much longer introns in a newt gene than in the corresponding genes from mammalian species with similar C-values to *Xenopus* (Casimir *et al.* 1988). However DNA sequence data from large urodele genomes are so scarce that, at present, it is unclear how generally applicable any of the sequence-based explanations for a correlation between high C-value and loop length could be.

These uncertainties regarding the determination of loop length are indicative of what remains the major outstanding question concerning LBC structure and function, namely the identity, organization and control of loop transcription units. The readthrough transcription model initially provided a basis for understanding the many reported occurrences of highly repetitive sequences in LBC transcription units, with properly-initiated pol II transcription elongation complexes envisaged as failing to react to termination signals at the ends of genes and therefore continuing to transcribe repeat-filled flanking regions lying downstream. However a further analysis of transcripts of the histone loops in *Notophthalmus* (Bromley & Gall 1987) demonstrated that many histone TUs do not initiate at the histone gene promoters predicted by the original readthrough model, rather it appears that initiation occurs upstream of the histone genes, perhaps at random sites. The contribution of *bona fide* gene promoters to initiating readthrough transcription may also be difficult to reconcile precisely with the transcript patterns seen on the telomere loops of chicken LBCs. The clusters of short tandemly repeated sequences that form the telomeres of most eukaryotic chromosomes are arranged on each chicken LBC as a single small transcription unit that is transcribed from a subtelomeric location towards and up to the very end of the chromosome (Solovei *et al.* 1994). Since there appears to be little space in the telomere transcription unit for additional genes, it has been argued that readthrough from the promoter of

an upstream gene is unlikely and that, at least in LBCs, this repetitive sequence TU is transcribed in its own right from a dedicated promoter (Solovei *et al.* 1994).

Another explanation for the transcription of extensive repetitive regions by amphibian LBCs is suggested by recent work on the lampbrush loop-like structures found on the Y chromosome in *Drosophila hydei* spermatocytes (Reugels *et al.* 2000). Although there may be structural or functional distinctions between oocyte LBC loops and Y loops, the production and packaging of abundant complex nascent transcripts is common to both and can likewise result in morphologically complex matrices (see below). In addition both types of loop transcribe extensive repetitive sequences. It now appears that the Y loops called *Threads* each comprise a 5 megabase transcription unit that contains a single gene encoding an axonemal dynein within whose introns have accumulated huge clusters of satellite repeats. In this case, the generation of a microscopically visible loop structure can be understood as being wholly due to the transcription of massive regions of intragenic satellite DNA and the concomitant production of complex nascent RNPs. Whether this kind of genomic organization also applies to some of the larger repeat-containing transcription units of oocyte LBCs will require far more detailed genomic sequence information than is currently available even for *Xenopus*, as well as a means to correlate genome sequences cytologically with a given LBC transcription unit. Some progress has been made in applying *in-situ* hybridization techniques to amphibian LBCs such that probes for several single copy genes have been localized to transcripts of specific loops (Weber *et al.* 1989, Angelier *et al.* 1996). Unfortunately, the use of cDNA probes and other technical factors prevented a detailed analysis of underlying TU sequence organization.

The transcription apparatus and RNP matrices of simple loops

Although the general principles of the sequence organization of loops and their RNA transcripts remain obscure, a great deal of progress has been made in identifying many of the other components of the axes and matrices of simple loops. This has also allowed important features of the molecular processes taking place on loops to be addressed. Beginning with transcription of loop axes, it has been clear for some time from the results of studies using transcriptional inhibitors that the vast majority of LBC TUs are transcribed by RNA pol II. However, immunostaining approaches using a panel of highly specific antibodies have recently allowed a more direct demonstration of the involvement of pol II and of its particular subpopulations in transcription. Biochemical studies in a range of eukaryotes have repeatedly shown that the largest subunit of pol II can exist as either highly phosphorylated or relatively unphosphorylated isomers (reviewed by Dahmus 1996). These changes in phosphorylation state occur in the subunit's carboxy terminal domain (CTD) and are thought to be important in controlling various stages of transcription and transcript processing. In particular, it appears that phosphorylation of the CTD accompanies the conversion of pol II from an initiating form to an elongating form. The CTD consists of up to 52 tandem repeats of the heptapeptide consensus, YSPTSPS, with serine being the predominant *in-vivo* phosphoacceptor. Phosphorylation of serines either at position 2 or 5 of the heptapeptide creates phosphoepitopes that are recognized specifically by monoclonal antibodies (mAbs) H5 and H14, respectively. Another mAb, 8WG16, specifically recognizes unphosphorylated CTD repeats and its binding is actually inhibited by phosphorylation.

All three of these pol II mAbs have been used successfully in immunostaining LBC spreads (Gall & Murphy 1998, Gall *et al.* 1999, Morgan *et al.* 2000). In agreement with the predictions made from biochemical studies, both mAbs H5 and H14 give an intense uniform axial staining of the vast majority of simple loops (Figure 3) and this staining overlaps precisely with the location of RNA synthesis in such loops (Gall *et al.* 1999). Therefore, these staining patterns are thought to reflect the tightly packed linear arrays of elongating polymerases that are demonstrated in electron microscope spreads of lampbrush transcription units. As illustrated in Figure 3a for mAb H5, the intensity of axial staining appears not to alter markedly along the length of the loops,

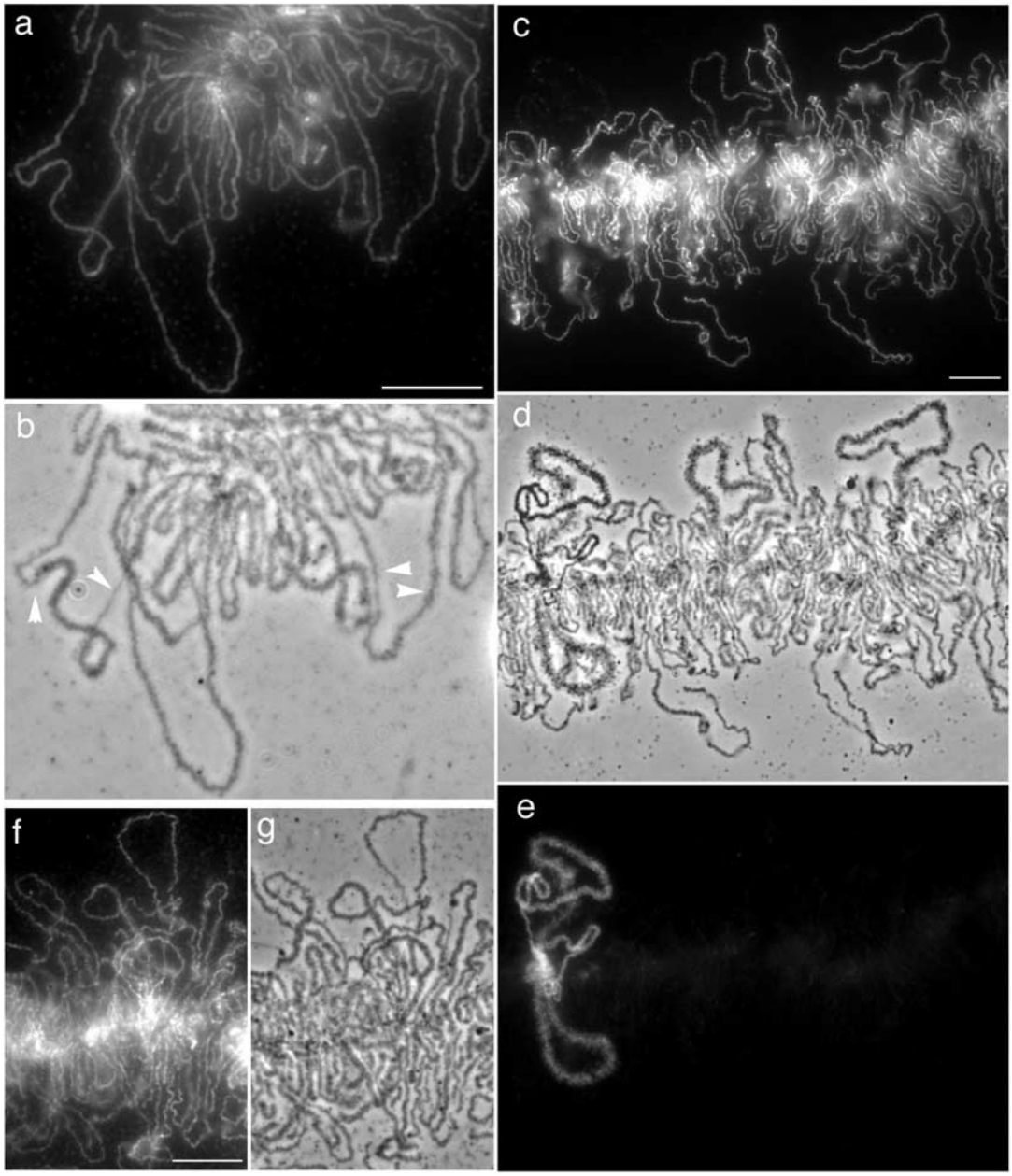

Figure 3. Immunolocalization of RNA pol II phosphoisomers on the loops of *Triturus vulgaris* LBCs. (**a** & **b**) Immunostained and phase contrast images, respectively, showing intense axial staining by mAb H5, which recognizes a phoshphorylated pol II CTD epitope. Note that the staining pattern is almost continuous throughout the loop axes and is not correlated with variations in matrix thickness (arrowheads). Scale bar represents 10 μm. (**c**, **d** & **e**) LBC immunostained with mAb H5 (**c**) and mAb 8WG16 (**e**) and viewed in phase contrast (**d**). The intense staining of most loop axes with mAb H5 contrasts with the lack of detectable axial staining with mAb 8WG16, which is specific for non-phosphorylated pol II CTD epitopes. Note that a small number of loops show intense matrical staining with mAb 8WG16, presumably because a matrix component shares an epitope with the pol II CTD. Although the significance of this behaviour is unclear, it serves to provide a positive control for the absence of axial staining. Scale bar represents 10 μm. (**f** & **g**) Immunostained and phase contrast images, respectively, from an LBC preparation that has been treated with calf alkaline phosphatase and then stained with mAb 8WG16. Under these conditions, convincing axial staining by mAb 8WG16 is obtained, indicating that non-phosphorylated CTD epitopes are detectable in transcribing pol II if present in sufficient quantity. Scale bar represents 10 μm.

suggesting that widespread changes in CTD phosphorylation levels do not occur during transcription elongation in oocytes. This conclusion is supported by the observation that mAb 8WG16 does not show specific staining of any part of the loop axis (Morgan *et al.* 2000 and Figure 3e), which suggests that dephosphorylated CTD repeat units are not produced during elongation, at least at levels that can be detected by immunofluorescence. That mAb 8WG16 staining is capable of detecting unphosphorylated CTDs in loop transcription units can be demonstrated in newt GV spread preparations that have been treated with calf alkaline phosphatase. As predicted, phosphatase treatment abolishes loop staining by mAb H5 and, conversely, allows detectable staining of loop axes by mAb 8WG16 (Figure 3f). In the light of this observation, the lack of specific axial staining obtained with 8WG16 in untreated lampbrush preparations further suggests that, of the multiple heptad repeats in each pol II elongation complex engaged in loop transcription, the vast majority are phosphorylated. It is interesting that the *in-vivo* phosphorylated pol II fraction from yeast is recognized by 8WG16 in Western blots (Patturajan *et al.* 1998), suggesting that perhaps fewer of the repeats in each CTD are phosphorylated in these cells than in LBC TUs. The possibility that transcription by 'superphosphorylated' pol II occurs on newt LBCs could be relevant to unusual features of LBC transcriptional control, such as readthrough transcription.

I have dealt so far only with pol II transcription in LBC loops, but will reiterate here the recent finding of pol III transcription that was mentioned above in the context of thin loops that lack a visible matrix. Antibodies against pol III subunits and pol III transcription factors have been found to stain a number of loops in *Xenopus* LBCs and these proteins are also targeted to the same loops (J. G. Gall, personal communication). Moreover, as predicted, these loops do not stain with antibodies against pol II and they are insensitive to concentrations of transcriptional inhibitors that cause pol II loops to retract. Importantly, these data show convincingly that a basic form of transcriptional control, namely the selection of the appropriate transcription machinery, is operational on extended loops during the lampbrush stages of oogenesis. As discussed above, the very absence of extensive RNP matrices from thin loops also indicates that transcription termination is occurring normally in the repeated pol III transcription units that comprise them. If the occurrence of pol II readthrough transcription is due simply to the abrrogation of the normal pol II termination signals in LBC loops, then the search for a molecular explanation of the phenomenon could be focussed on those features of the termination mechanism that distinguish pol II and pol III, such as the nature of the link with transcript processing exhibited in the former case (reviewed in Proudfoot 2000).

Considering now the loop matrix, progress in identifying its RNP components came initially from the use of monoclonal antibodies directed against previously uncharacterized GV proteins. These studies showed that certain epitopes were generally distributed along and between loop matrices and therefore presumably were derived from proteins involved in processes occurring on most nascent transcripts. Firmer identification of matrix components and therefore of the events likely to be occurring on nascent transcripts have come more recently from the application of defined antibody probes and, in the case of snRNPs, *in-situ* hybridization probes, that detect functionally characterized macromolecules. Similarly the approach of following the distribution of exogenous, tagged derivatives of identified molecules has enabled targeting behaviour as well as the steady-state localization patterns of matrix components to be established (for an example, see Figure 4). The application of these approaches has provided abundant evidence that many splicing components occur in the matrices of simple loops. The presence of splicing snRNPs on the majority of loops has been shown using antibodies specific for the unique trimethylguanosine cap of mature snRNAs, for the Sm core snRNP proteins and for snRNP-specific proteins, and by *in situ* hybridization with all five splicing snRNAs (Wu *et al.* 1991 Gall *et al.* 1999). Similarly, exogenously supplied snRNAs and tagged snRNP proteins such as the U1 C protein, have been shown to be targeted to loop matrices (Jantsch & Gall 1992 and see Figure 4). The

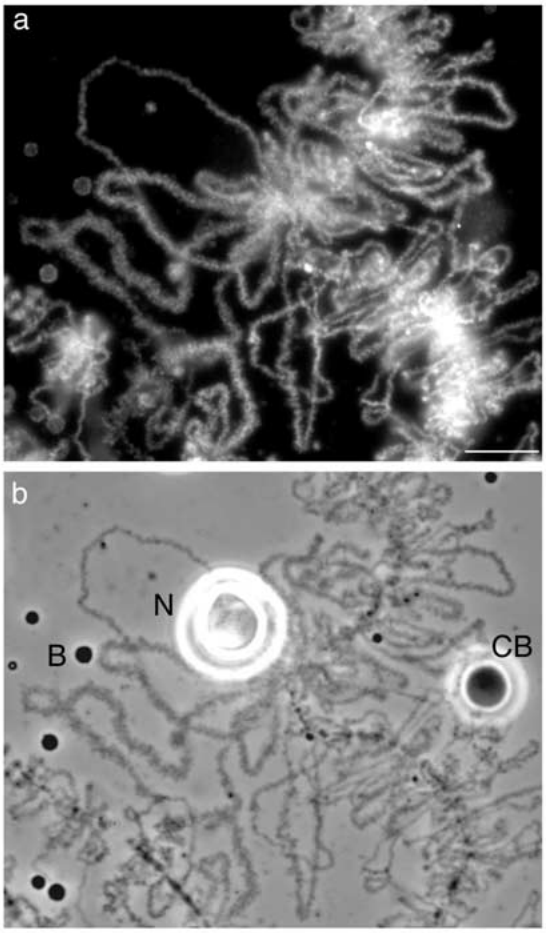

Figure 4. Targeting of an epitope-tagged protein to the matrices of LBC loops. (**a** & **b**) Immunostained and phase contrast images, respectively, of LBCs prepared from a *T. vulgaris* oocyte expressing a *myc*-tagged fusion of the C protein from the U1 snRNP. As originally shown by Jantsch and Gall (1992), oocytes injected with transcripts encoding the tagged protein show an accumulation of this snRNP component in the loop matrix, in this example by 24 h after injection. The intensity of immunostaining obtained with mAb 9E10 against the *myc* tag is roughly proportional to the quantity of matrix possessed by each loop. In these preparations B-snurposomes (B) were also stained as were, much more weakly, Cajal bodies (CB) whereas nucleoli (N) were unstained. Scale bar represents 10 μm.

non-snRNP splicing factors of the SR-family are also present in loop matrices as shown both by staining with antibodies, such as mAb SC35 (Gall *et al.* 1999) and mAb 104 (Roth *et al.* 1990), and by the targeting of SRp55 to lateral loops (Roth *et al.* 1991). Interestingly, mAb104, which was initially generated during an investigation of GV proteins, proved to be a key element in the discovery of this important class of splicing factor in a wide variety of organisms (Mayeda *et al.* 1992).

Components of another pre-mRNA processing pathway, that which generates the 3′ end of mature mRNAs, are also widely distributed in loop matrices. Antibodies against the 3′-processing proteins, CstF77 and CPSF100 stain most loops, and epitope-tagged CstF77 is likewise targeted to loop matrices (Gall *et al.* 1999). It should be noted that, for all of the RNA processing proteins mentioned above and particularly in the context of 3′-processing, which can only occur once the end of the TU has been transcribed, loop TUs were immunostained throughout their length. I shall return to this point below. Another general component of the loop matrix has proven to be the hnRNP proteins that are responsible for forming the basic hnRNP packaging particle. Antibodies against hnRNPs A and B and against hnRNP L stain the matrices of most simple loops along the entire length of TUs (Wu *et al.* 1991). Similar patterns were also found for other less well-characterized proteins, PwA33 (Lacroix *et al.* 1985, Pyne *et al.* 1994) and SE5 (Roth & Gall 1987), which nonetheless have sequence characteristics and distribution patterns suggestive of non-splicing RNA-binding proteins. The behaviour of exogenous tagged PwA33 (Bellini *et al.* 1993) and SE5 (Roth & Gall 1989) suggested that they are indeed generally targeted to nascent transcripts. Another class of protein found in most simple loop matrices is represented by the double-stranded RNA-binding proteins, Xlrpba (Eckmann & Jantsch 1997) and ADAR1 (Eckmann & Jantsch 1999), an RNA editing enzyme.

The widespread occurrence of splicing, 3′-processing and RNA packaging proteins in loop matrices clearly provides evidence of their association with nascent transcripts and therefore suggests that the molecular processes that they are involved in occur cotranscriptionally. A similar conclusion has recently been reached from completely different experimental approaches (reviewed in Bentley 1999) but the LBC observations provide simple and compelling evidence for the general occurrence of cotranscriptional

events *in vivo*. Another conclusion can be drawn from the distribution of these proteins on LBC loops. The continuous presence of both snRNP and hnRNP proteins on transcription units from their very beginnings suggests that these components can be loaded onto transcripts at the time transcription initiates. Such a scenario is in agreement with models emerging from biochemical and genetic studies that suggest a physical connection between transcript proteins and the transcription machinery that is mediated by the pol II CTD during initiation and promoter clearance (reviewed in Hirose & Manley 2000).

It is also relevant in this regard that preliminary observations of the distribution of proteins involved in general translational repression have shown that FRGY2 and Xp54 are associated with LBCs (Sommerville & Ladomery 1996, Ladomery *et al.* 1997) and this may indicate that other processes regulating oocyte gene expression at the RNA level are also predetermined during transcription. It might also be expected that proteins involved in gene-specific post-transcriptional regulatory events and therefore associated with the RNP matrix of a small subset of loops should also be identifiable by these approaches. For instance, the imposition during transcription of distinctive patterns of splicing, of translational activation/repression or of mRNA stability could be reflected in the restricted distribution among lampbrush transcription units of the proteins regulating such behaviour. In fact, there has been little further success in identifying such proteins beyond the early indications of loop-specific or loop-restricted staining patterns obtained from histochemical (Varley & Morgan 1978) and immunocytochemical investigations (Sommerville *et al.* 1978, Lacroix *et al.* 1985) in which the identity of the proteins was uncertain.

Loops with complex morphology

This section is concerned with those LBC structures that comprise loops with a complex matrix caused by the accumulation of large amounts and/or morphologically distinctive types of RNP. The basic feature of complex loops that distinguishes them immediately from chromosomal bodies such as 'spheres' and 'granules' is the clear presence of a chromatin loop at the heart of the structure. This was originally demonstrated by the observation of complex loops that had been disrupted either by dissolution of their RNP matrix in low-salt salines or by double-loop bridge formation. The recent demonstration of the utility of pol II CTD mAbs as highly specific and sensitive stains for loop axes in sperm-derived LBCs (Gall & Murphy 1998) has also allowed some detail of the axial organization of undisrupted complex loops to be visualized (Figure 5). The two examples shown of complex loops from newt LBCs are what would classically be termed 'beaded loops' and 'lumpy loops' (Callan 1986) because of the form that the matrix takes. The essentially looped organization of these structures is apparent in regions where matrix has not accumulated, but, in those regions where large amounts of matrix material are present, the details of any underlying looped organization are not observable in phase contrast or DIC. However, the fluorescent signal derived from binding of pol II mAbs clearly resembles the axes of simple loops shown in Figure 3. The signal appears as an intense thin line of staining that extends continuously throughout the structures (although, in the images shown, it passes through different focal planes and so exhibits some breaks in continuity). In addition to emphasizing that the same axial organization underlies even morphologically complex loops as underlies simple loops, these observations also make the point that such loops are being actively transcribed by phosphorylated pol II packed at about the same axial density as simple loops. What does appear distinctive in these examples though is that rather than the transcribed DNA being arranged as a linear extended loop axis in the manner of a simple loop, it follows a contorted path of coils and turns. In the two examples shown in Figure 5, this path is either within the mass of the matrix (Figure 5c) or around the outside of a globular matrix (Figure 5a). Since these contorted loop axes are actively transcribed, they presumably exist in a state of chromatin decondensation somewhere between that of a nucleosomal fibre and fully extended B-conformation DNA. The spatial organization exhibited by the transcribed DNA strand in complex loops therefore suggests the existence of a previously unsuspected mechanism contributing to chromo-

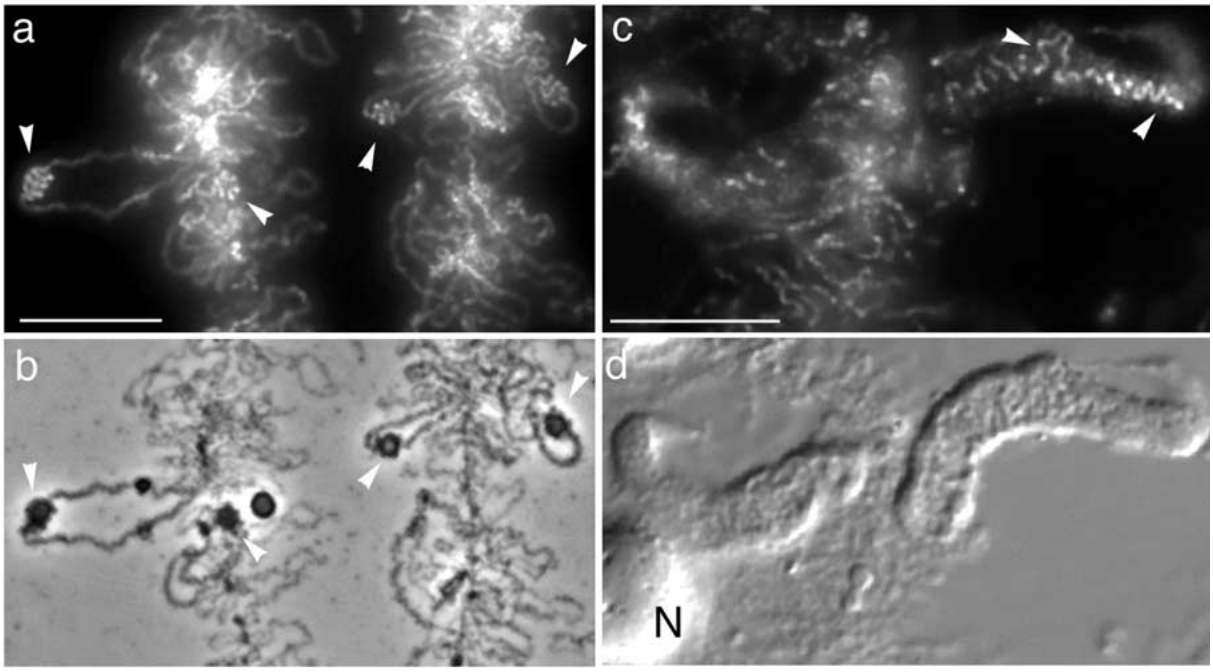

Figure 5. Conformation of the DNA axis in loops with complex matrix morphology. *T. vulgaris* LBCs viewed after immunostaining for pol II (**a** & **c**) or by phase contrast (**b**) or DIC (**d**). In (**a**) the path of the loop axis that is picked out by mAb H5 staining can be seen to follow a spiral around the sporadic bead-like condensates (arrowheads) that are formed by the matrices of these loops. Both homologous loci are visible, although, for one of them, one of the sisters has fallen onto the chromosome axis. In (**d**) can be seen the much larger mass of matrix forming a 'lumpy loop' and it is clear by immunostaining (**c**) that the matrix surrounds the loop axis, which follows a highly contorted path (arrowheads). N, nucleolus. Scale bars represent 10 μm.

some structure, a sort of 'RNP-chromatin' effect. It would be interesting to know whether regions of active chromatin in somatic nuclei can also be spatially constrained by interactions involving their transcripts.

There are many fundamental mysteries surrounding the organization of complex loops, such as the relationship between their nascent transcripts and the bulk of the RNP matrix, and whether some of the matrix RNA is transported from other loops rather than being transcribed *in situ*. There is little known about even the RNA sequence content of complex loops beyond the fact that the giant loops of chromosome II of *Notophthalmus viridescens* produce highly repetitive transcripts. A role for repetitive sequences in the generation of complex transcript morphologies has been convincingly demonstrated for structures that closely resemble the complex loops of amphibian LBCs, namely the massive transcription units that form the Y-chromosome loops of *Drosophila* spermatocytes. As described above, it has recently been found that the loops known as *Threads* are associated with megabase-sized highly-repetitive transcripts. More specifically, these transcripts contain three consecutive arrays of distinctive satellite sequences which form 'coaxial shells' by clustering around each other concentrically in the nascent RNA, with the most recently transcribed region on the inside (Kurek *et al.* 1996). However, there are many examples of the transcription of long repetitive transcripts in vertebrate LBCs that do not lead to complex loop morphologies, so a general dominant role for repetitive sequences in the generation of complex matrices seems unlikely.

Complex RNP matrices can exhibit distinctive protein compositions. For instance, some generally distributed proteins are present at particularly high concentrations in complex matrices. One example is the RNA-editing enzyme ADAR1 (Eckmann & Jantsch 1999), which is enriched in

a morphologically unusual loop pair on *Xenopus* LBC III. Other examples are the SR protein(s) detected by mAb 104, which stains a set of landmark loops on *Xenopus* LBC XIV (Roth *et al.* 1990), and hnRNP L, which is enriched in the giant loops on LBC II of *Notophthalmus* (Pinol-Roma *et al.* 1989). It is notable too that many widely distributed RNPs are specifically absent from certain types of complex loop. An example here is the SLL locus in *Notophthalmus* which lacks hnRNP L and a range of snRNP antigens (Wu *et al.* 1991). Examples of proteins that could be characteristic of complex matrices are limited to those containing the epitopes recognized by mAbs UF6 and TH2, which stain almost exclusively the complex matrix domain of the SLL (Roth & Gall 1987). It is possible that certain types of complex matrix may simply reflect the further organization of the basic RNP packaging particles found in simple loop matrices (reviewed in Callan 1986). Alternatively, there might be totally novel molecular processes correlated with the acquisition of complex matrix morphology. However, the only convincing evidence that complex matrices are indicators of loop-specific RNP interactions and/or novel molecular processes comes, paradoxically, from the study of oocyte pol I transcription units.

In the amphibian species commonly used to study LBCs, namely newts and *Xenopus*, the chromosomal rRNA genes are inactive in oocytes (Morgan *et al.* 1980, Callan *et al.* 1988) and so the processes that occur at the nucleolus organizer regions (NORs) of somatic cells and that induce the formation of nucleoli are absent from these LBCs. Instead, nucleolar activities are directed by extrachromosomal, amplified rRNA genes that generate many hundreds of free nucleoli. Extrachromosomal nucleoli normally exhibit a rather compact morphology and might seem, therefore, to have little relevance to considerations of LBC loop structure and function. However, under certain conditions, extrachromosomal nucleoli exhibit a far more extended, ring-like configuration in which the circular molecule of amplified rDNA in each nucleolus forms the connecting thread of a structure resembling a beaded necklace. Such an extended structure could be considered as corresponding to a loop without chromosomal insertions, and the periodic condensates that form its beads as a morphologically complex matrix. Moreover, in the LBCs of certain salamanders, the NORs appear as active structurally complex regions in which multiple ring nucleoli are attached periodically along an extended chromosome axis (Callan 1966, Kezer & Macgregor 1973). Indeed, in these lampbrush NORs, beaded nucleoli occasionally appear to have broken open at the point of their attachment to the chromosome, giving rise to a structure that is equivalent to the double loop bridges produced from lateral loops. In this context, therefore, the composition of one type of morphologically complex loop matrix is clear, given the existing detailed knowledge of the molecular architecture of nucleoli in general and of amplified nucleoli in particular (Mais & Scheer 2001). In addition, such nucleolar loops support the principle that a complex loop morphology can be correlated with the existence of a unique set of molecular events; in this case, these are specialized nucleolar functions, such as snoRNA-directed rRNA processing and ribosome assembly (reviewed in Olson *et al.* 2000). It remains to be seen whether such extreme compositional and functional novelty is a general feature of those complex loops transcribed by pol II. However, recent work on chromosomal bodies suggests that unexpected and complicated functions are very much a possibility for this second type of LBC landmark structure.

Chromosomal and GV bodies

I have used the term 'chromosomal bodies' to refer to objects that are regularly associated with LBCs at particular sites but for which there is no evidence that loop DNA ever forms an integral part of their structure. Perhaps because of the absence of extended DNA, such bodies have globular or even spherical morphology, although DNA sequences are thought to play a direct or indirect role in their attachment at defined chromosomal loci. Association with a chromosome is not actually obligatory for at least some of these bodies since large numbers of essentially the same objects can also be found as free GV bodies. The most significant advances in understanding the composition and function of any LBC structure have

come from detailed studies by J. G. Gall and colleagues of objects originally referred to as spheres.

Sphere loci and Cajal bodies

Spheres or sphere organelles are one of the most useful characteristics for the identification of individual lampbrush chromosomes (Figure 6). As their name implies they are almost spherical objects 2–10 μm in diameter that are attached singly to LBCs at a small number of specific loci, usually 2–4 according to the species. Objects identical in appearance to spheres are present in much higher numbers (50–100 in *Xenopus*) free in the GV. It was primarily through a detailed cytochemical analysis of the free spheres that the equivalence of spheres and the Cajal bodies (CBs) of somatic cells was established (Gall *et al.* 1995).

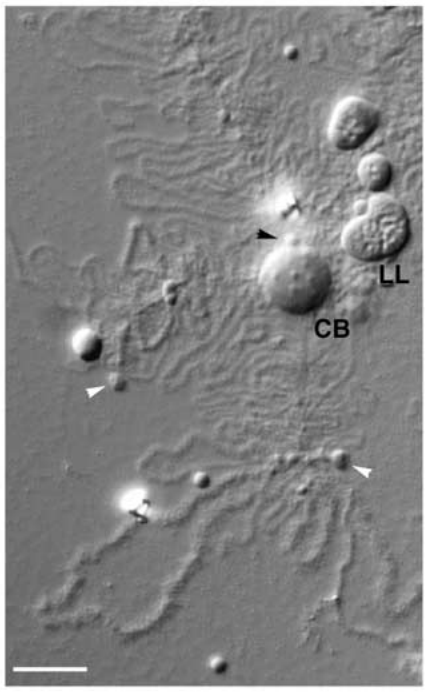

Figure 6. A sphere locus of *T. vulgaris* LBCs. This DIC image shows a single large CB formed by fusion of homologous chromosomal CBs near the ends (white arrowheads) of LBC VI. Multiple B-snurposomes are visible either embedded within or attached to the surface (black arrowhead) of the CB. The adjacent lumpy loops (LL) serve with the spheres as landmarks for chromosome VI in the working map of these LBCs (Barsacchi *et al.* 1970). Scale bar represents 10 μm.

Since the composition and function of oocyte and somatic CBs have recently been the subject of extensive and authoritative review (Gall 2000), I shall only briefly summarize some of the main features of oocyte CBs before concentrating on those aspects with the most direct relevance to lampbrush chromosomes *per se*.

Detailed morphological studies of CBs have shown them often to be closely associated with a smaller (1–4 μm diameter) type of particle, termed a B-snurposome; these are present in their thousands free in the nucleoplasm but one or more B-snurposomes can be embedded in the surface of a CB or completely enclosed within it (Figure 6). The numbers and sizes of CB-associated B-snurposomes can vary widely, but particularly large chromosomal CBs can be associated with several dozen. Strictly speaking, it is the non-B snurposome component of a sphere, referred to as the matrix, that is thought to be the actual equivalent of the somatic Cajal body. The CB matrix can be defined operationally by its possession of coilin, a marker protein that does not occur in B-snurposomes. Other components of the oocyte CB matrix that were identified initially and that were also found in somatic CBs were predominantly molecules involved in the processing of pre-mRNAs. These included the splicing snRNAs and associated proteins as well as components of pre-mRNA 3′ processing machineries (Abbott *et al.* 1999, Gall *et al.* 1999). Also, as in somatic CBs, a number of nucleolar proteins have been identified in the oocyte CB matrix, namely fibrillarin, NO38 (B23) and Nopp140 (Gall *et al.* 1999).

Recently, it has emerged that various components of the transcriptional machinery also occur in oocyte CBs. For example a combination of immunostaining with CTD mAbs and the rapid and specific targeting obtained with the epitope-tagged subunits, RPB6 and RPB9, has shown that core pol II is a component of the CB matrix (Gall & Murphy 1998, Gall *et al.* 1999, Morgan *et al.* 2000). Moreover the specific CB staining obtained with mAb H14, whose binding requires serine-5 in the CTD heptads to be phosphorylated, can be abolished by incubation of oocytes for 2–3 h with DRB, an inhibitor of several CTD kinases. CB staining by H14 is restored when DRB is removed and, taken together with the rapid

targeting of pol II subunits, these observations support a model in which pol II enters the CB, becomes phosphorylated and leaves the CB over a period of a few hours. Interestingly, the staining patterns obtained with other CTD mAbs are the reverse of those described above for loops, with mAB 8WG16 giving intense CB staining and mAb H5 poor staining. This suggests that, for the population of pol II in CBs, serine-2 of most CTD repeats is unphosphorylated with some or all repeats being phosphorylated on serine-5, whereas for the pol II population engaged in loop transcription, both serines are phosphorylated.

Oocyte CBs have been shown to contain other proteins involved in pol II transcription, namely a subunit of the general transcription factor TFIIF (Gall *et al.* 1999), the TBP subunit of TFIID (Gall 2000), the TBP-associated factor, TAFII70 (Bucci *et al.* 2001) and the pol II-binding transcription elongation factor, TFIIS (Smith, Ling & Morgan, in preparation). Somatic CBs are similarly loaded with components of the pol II transcriptional machinery (Grande *et al.* 1997, Schul *et al.* 1998) but so far only oocyte CBs have been shown to stain strongly with antibodies against pol I and pol III subunits and for pol III transcription factors (Gall *et al.* 1999). The occurrence of a complex array of transcriptional proteins and RNA processing components in Cajal bodies together with the demonstration for some of these components of rapid CB targeting have provided two of the strands of evidence for a wide-ranging model of Cajal body function recently proposed by Gall (Gall *et al.* 1999). In brief, the model is that CBs are sites for the assembly of complex and diverse molecular machines, 'transcriptosomes', that carry out all the processes of nuclear gene expression from transcription initiation through RNA processing and 3′ end formation. Hence, each of the three types of polymerase would associate with their requisite transcription factors and RNA processing factors in CBs prior to their transport directly or indirectly to sites of transcriptional activity. As well as providing a unifying theme for Cajal body function in all types of cell, this model is very much in accord with recent findings from biochemical and genetic approaches that suggest the pre-assembly of huge transcriptional holoenzymes and the association of the pol II holoenzymes with the various RNA-processing activities (reviewed in Myer & Young 1998, Hirose & Manley 2000).

In the context of this review, the transcriptosome model of Cajal body function demonstrates again how a morphologically distinctive feature of LBCs can be understood as resulting from the occurrence of complex and novel molecular activities, just as nucleoli are ultimately a morphological consequence of the manifold molecular events that produce ribosomes. However, the absence of DNA argues against the existence of a simple equivalent to a nucleolus organizer, namely a 'sphere organizer' DNA sequence, for determining the localization of CBs at particular chromosomal sites. A first clue as to what might determine the locus specificity of chromosomal CBs was provided some time ago by *in-situ* hybridization experiments that showed for the LBCs of several newts and *Xenopus* (Gall *et al.* 1981, Callan *et al.* 1991) that each CB was attached to the chromosome axis near a set of loops on which histone gene sequences were transcribed. Although there is no evidence that histone genes or transcripts are directly associated with chromosomal CBs, the subsequent discovery that the U7 snRNP, which is required for the 3′ processing of histone pre-mRNA, is found in all oocyte CBs (Wu & Gall 1993, Wu *et al.* 1996) provided at least a rationale for CB localization. Hence, the presence of CBs near the histone loops of LBCs could ensure the provision of high concentrations of the components needed for the efficient processing of the large number of pre-mRNAs produced by the repeated histone genes. The fact that CBs containing U7 snRNA can also be found adjacent to histone gene loci in HeLa cells (Frey & Matera 1995) suggests that this association is conserved and therefore likely to be functionally significant. A variety of possible explanations for the biogenesis of chromosomal and free CBs have been considered (Roth 1995) and recently the finding that additional free CBs are induced in GVs after injection of U7 RNA supports a model in which this RNA nucleates CB formation (Tuma & Roth 1999). Therefore locally high concentrations of U7 snRNP that are initially brought about by the processing of histone gene transcripts, might nucleate CB formation nearby and hence perpetuate high local U7 snRNP levels. However, this explanation for

CB biogenesis does not address the nature of the physical attachment between a chromosomal CB and its locus that is presumably required for such a precise and invariant localization to survive the production of LBC spread preparations.

Terminal and axial granules

Like chromosomal Cajal bodies, LBC granules have been invaluable for chromosome recognition purposes and recently they have also provided evidence of an unexpected molecular composition. Axial and terminal granules occur on the chromosome axis either at interstitial positions or at the very ends of chromosomes, respectively, and they appear in phase contrast or DIC simply as very large spherical or near-spherical chromomeres around 1–2 μm in diameter (Figure 7). However, cytochemical analyses show that the bulk of each granule does not contain DNA, although it is clearly attached to a small DNA-containing chromomere. The DNA sequences associated with many granules have been identified for *Xenopus* LBCs and recently some of the proteins comprising the granules themselves were revealed by immunostaining and targeting experiments. The teminal granules found at one end of 15 of the 18 *X. laevis* LBCs are associated with oocyte-type 5S rRNA genes (Callan *et al.* 1988), and of the five axial granules noted as landmarks (Callan *et al.* 1987), one is associated with U1 snRNA genes (Abbott *et al.* 1999), one with U2 snRNA genes (Abbott *et al.* 1999) and one with the inactive nucleolus organizer (Callan *et al.* 1988). Both the terminal and axial granules of *Xenopus* LBCs are intensely stained by the anti-CTD antibodies mAb H14 and mAB 8WG16 (Gall & Murphy 1998 and Figure 7) and tagged pol II subunits RPB6 and RPB9 are specifically targeted to granules (Morgan *et al.* 2000), suggesting that the pol II transcriptional machinery is a granule component. In agreement with this, we have recently found by immunostaining and targeting approaches that TFIIS is present in granules (Smith, Ling & Morgan, in preparation) and the same has been found for TBP (J. G. Gall, personal communication). Axial and terminal granules also contain the histone pre-mRNA processing factor, SLBP1 (Abbott *et al.* 1999). Similar axial granules are prominent in LBCs of the newt *Pleurodeles* and, although the underlying gene loci have not yet been identified, immunostaining with several CTD antibodies has indicated that pol II is also a

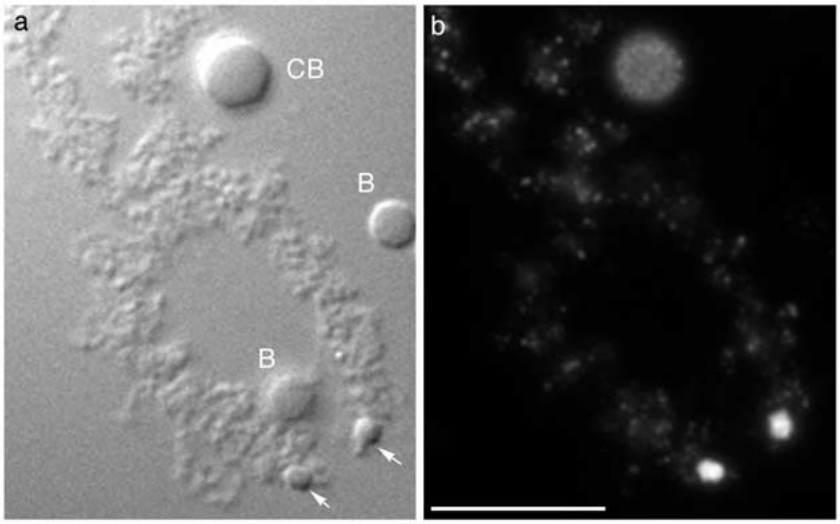

Figure 7. Terminal granules of *Xenopus laevis* LBCs. (**a**) DIC image of one end of a bivalent possessing terminal granules (arrows), which mark the location of the oocyte-type 5S rRNA genes in this species. Nearby are B-snurposomes (B) and a Cajal body (CB). (**b**) Immunostaining with mAb H14 shows intense staining of the terminal granules relative to weaker CB and chromosome staining, while B-snurposomes appear vitually unstained. This intense staining suggests the presence in granules of a high concentration of pol II CTD repeats phosphorylated on Ser 5. Scale bar represents 10 μm.

component of these granules (U. Scheer, personal communication). In addition, as mentioned above, topoisomerase II is a prominent component of the *Pleurodeles* granules (Hock *et al.* 1996) and, intriguingly, they are also stained by two antisera directed against the Cajal body marker protein, p80 coilin (U. Scheer, personal communication).

The compositional and targeting data have two intriguing features relevant to considerations of the functional significance of LBC granules, particularly in *Xenopus*. The first is that the 5S rRNA genes associated with terminal granules in this species would not be expected to be asssociated with the pol II transcriptional apparatus. Indeed only weak staining of terminal granules has been observed with antibodies against subunits of pol III (J. G. Gall, personal communication). The explanation here may be similar to that advanced to account for the occurrence of all three RNA polymerases in CBs (Gall *et al.* 1999); namely that, because certain subunits are common to pol I, II and III and all utilise the transcription initiation factor TBP, multiple types of transcriptional machinery could accumulate in a single structure due to a shared assembly pathway. Hence, even a low-level or even transient presence of pol III in terminal granules might be sufficient to nucleate the assembly of elements of the pol II transcription machinery. Alternatively, pol II may play an as yet unsuspected role in transcription of genes associated with granules.

The second intriguing feature is that all of the granule components for which we have evidence in *Xenopus* also occur in, and/or are targeted to, oocyte Cajal bodies. Axial granules and CBs share other properties such as their presence at specific LBC loci, a general resemblance in appearance, a tendency to undergo fusion with like objects (Callan 1986) and sometimes, as noted above for *Pleurodeles*, the apparent presence of coilin. However, there are some obvious differences between these two types of chromosomal body. Above all, there is no evidence that granules exist as free GV bodies, whereas most oocyte CBs occur in the nucleoplasm. Furthermore, oocyte CBs are usually much larger than granules, show an intimate association with B-snurposomes and always contain coilin as well as a wide array of pre-mRNA processing components that are not found in granules. However, the repeated 5S, rRNA, U1 and U2 genes with which granules are known to be associated produce transcripts that do not require splicing nor the types of 3′-processing exhibited by pre-mRNAs. Indeed, the unexpected presence of SLBP1 at such loci has led to the suggestion that perhaps this protein is involved in hitherto unexpected RNA processing activities (Abbott *et al.* 1999). Hence it is conceivable that terminal and axial granules are CB-related bodies that are assembled exclusively around transcriptional components (and for unknown reasons, SLBP1) but without regularly sequestering either the RNA-processing factors or coilin found in archetypal CBs. Intriguingly, in somatic cells it appears that CBs can be localized to the U1 and U2 snRNA gene loci just as are granules in *Xenopus* oocytes. Further experimental analyses of the composition of granules and the molecular basis of their protein targeting are needed before any role akin to that of CBs can be considered for them, but, given their association with identifiable genetic loci they offer an attractive subject for the further study of LBC structure/function relationships.

B-snurposomes

Although characterized primarily as extrachromosomal GV bodies, B-snurposomes can in some circumstances be considered as chromosomal bodies. As described above, B-snurposomes can be intimately associated with CBs and, in that context, they regularly occur at sphere loci (Figure 6). In addition, B-snurposomes are commonly found one on either side of telomere granules in *Xenopus* LBCs (Figure 6 in Abbott *et al.* 1999) and one or more can be associated with other specific loci, although they have not been noted as landmarks. Interestingly, in oocytes treated with the transcriptional inhibitor DRB, numerous additional small B-snurposomes were found attached to the chromosome axes (Morgan *et al.* 2000). The basis of their localization to particular chromosome regions, the nature of the attachment and their potential functional significance at these sites are all unknown at present. However, the composition of B-snurposomes has been studied in detail by the Gall laboratory (Wu *et al.* 1991, Gall *et al.* 1999), and this has allowed some suggestions as to their function. Immunofluorescence, *in-situ*

hybridization and targeting approaches have shown that B-snurposome contains the major splicing snRNPs (also known as 'snurps', and hence the term snurposome), SR proteins and the RNA 3′-processing factors, CstF77 and CPSF100. Initial immunostaining experiments also suggested that components of the pol II transcriptional apparatus might also be present in B-snurposomes (Gall *et al.* 1999) but certain anomalies in the staining and the lack of targeting exhibited by pol II subunits left some uncertainty as to this interpretation (Morgan *et al.* 2000). Recently, a careful study using a variety of antibodies has led to the conclusion that pol II is not a component of B-snurposomes (O. Doyle and J. G. Gall, personal communication). On the basis of their apparent composition and of their fine structure revealed by electron microscopy, it has been proposed that B-snurposomes could participate in the storage and transport of some of the macromolecular complexes previously assembled in Cajal bodies (Gall *et al.* 1999). This would explain the intimate relationship of B-snurposomes and CBs and would also be consistent with the associations observed between LBCs and B-snurposomes

In addition to RNA processing components, another set of proteins that are detected in loop matrices have also been found in association with newt B-snurposomes. These are the A and L hnRNP proteins (Wu *et al.* 1991) and the RNA-binding proteins PwA33 (Pyne *et al.* 1994) and SE5 (Wu *et al.* 1991) referred to above. These proteins all show an interesting distribution pattern in that they are concentrated in small patches on the surface of B-snurposomes rather than being homogeneously distributed like the bulk of the splicing components. This peripheral patchy labelling might be due to the concentration of such proteins in novel submicroscopic granules about 100 nm in diameter that have been observed by electron microscopy attached to the surface of *Pleurodeles* B-snurposomes (Pyne *et al.* 1994). Clearly, since these proteins are all associated with the nascent RNP of loop matrices and since hnRNP A and hnRNP L are known to be generally involved in packaging of hnRNA, the association of B-snurposomes with LBCs could also be important in supplying these proteins to the required locations. Indeed since B-snurposomes contain both snRNPs and hnRNPs in abundance, another suggestion for B-snurposome function is to allow the interaction of snRNPs with hnRNPs to form unitary particles that are supplied to loops or stored for future use (Wu *et al.* 1991).

Centromeric protein bodies

A final type of chromosomal body for which molecular detail is accumulating has been described attached to the LBCs of various groups of birds (Gaginskaya 1972). These so-called protein bodies (PBs) are perfectly spherical objects of variable size that are regularly attached to the chromosome axis of each LBC at a heterochromatic region that has been proven to be centromeric in pigeon (Solovei *et al.* 1996) and in chaffinch (E. Gaginskaya, personal communication). Although they resemble Cajal bodies in morphology, recent immunocytochemical investigations have shown that PBs do not contain p80 coilin nor other indictors of the CB matrix such as fibrillarin and the Sm and trimethylguanosine epitopes characteristic of splicing and U7 snRNPs (E. Gaginskaya, personal communication). The specification of PB position is thought to involve the characteristic highly repetitive sequences that surround the centromere. Although the centromere regions of most bird LBCs are marked by short loops, transcription of centromeric repeats in those loops may not be a regular occurrence since it has been found in one species of pigeon but not in another (Solovei *et al.* 1996). The biogenesis of PBs and their persistent association with centromeric LBC loci would not then seem to be due to a specific RNA nucleation event as suggested for amphibian oocyte CBs (Tuma & Roth 1999). It would clearly be of interest to examine the potential roles of proteins associated with centromeres and centromeric heterochromatin in the biogenesis and localization of these fascinating structures. Their distinctive composition suggests a completely different functional role from that of CBs and they may be involved in co-ordinating the spatial arrangement of meiotic chromosomes (E. Gaginskaya, personal communication). However, it is notable that, despite their apparently distinctive compositions and functions, PBs and CBs have very similar morphologies and this might reflect the existence of a common set of phys-

icochemical parameters governing the size and shape of chromosomal and GV bodies.

Summary and perspectives

Lampbrush chromosomes have long offered unique opportunities to integrate the study of nuclear processes with that of nuclear structure, and to combine the morphological with the molecular level of analysis. The increasingly sophisticated molecular investigations of LBCs that have been carried out of late and that I have summarized above have also confirmed other attributes of LBCs that are perhaps less widely appreciated. Namely that the conclusions drawn from LBCs are widely applicable to other eukaryotic cells and that, given their unique advantages, the study of LBCs can provide early evidence for the existence of novel general principles of nuclear structure and function. Nowhere is this more apparent than in the intensive investigation of oocyte Cajal bodies carried out by J. G. Gall and colleagues. These studies were not only able to address the composition and structure of a novel LBC and GV body and to show its homology with a class of nuclear bodies found in somatic cells, but they also led to the proposal of a function for such bodies. This function, namely the assembly of huge transcriptional and RNA-processing machines ('transcriptosomes'), links a novel nuclear structure with a novel nuclear process and sets the agenda for the investigation of CBs in other systems. Moreover, the CB paradigm also raises the question of whether the many other less well-understood LBC structures listed above are also indicative of the existence of novel nuclear functions. Since a wide range of enigmatic nuclear bodies is being increasingly documented in somatic cells (reviewed by Matera 1999), it is also clearly important to establish whether these might be related to any of the complex loops or chromosomal bodies of LBCs. The advantages for experimental investigation offered by LBCs are amply illustrated by the study of CBs, and among the most useful of these is the opportunity directly to access the chromosomal or genomic context in which such bodies arise.

A second example that shows how recent studies of LBCs have advanced more general concepts of nuclear function has been in the characterization *in situ* of elongating pol II and of the components of nascent transcripts. The accessibility of these stages of gene expression to cytological analysis is one of the greatest strengths of the lampbrush system. It has enabled the investigation of the phosphorylation state of the pol II CTD during elongation and the realization that splicing factors, 3′-processing factors and hnRNP proteins are deposited on nascent transcripts throughout the elongation phase. The latter observation has been a key one in establishing the co-transcriptional nature of RNA processing and should be a fruitful area for further investigation, especially with regard to the identification of transcript proteins that are specific or restricted to particular transcription units. Similarly, the recent demonstration of the targeting of pol III subunits to their cognate transcription units on LBC loops, shows that direct investigation of the *in-vivo* composition and function of transcription complexes is now very much a possibility.

Most LBC investigators are initially driven by a fascination with these intriguing objects in their own right, and, in this respect, the main recent advance has been the appreciation that, as just indicated, many of the molecular components expected to be present on *bona fide* gene transcripts can be detected in the matrix of most simple loops. This, together with the fact that a small number of loops are not so endowed (the pol III loops being an example of some that are not), is consistent with the majority of LBC transcription units containing genes. However, an alternative explanation is that pol II transcription of most LBC loops is carried out by pre-assembled transcriptosomes that transcribe even non-genic transcription units. Therefore, it is still conceivable that RNA-processing components could be deposited on inappropriate nascent transcripts, such as those composed of repetitive sequences. Our continuing ignorance of the organization of any LBC TU with respect to unique coding sequences remains a 'humiliating and tantalizing fact' (Callan 1982), the resolution of which is required for a full understanding of the functional significance of nascent transcript components as well as the wider question of the precise contribution of LBCs to embryonic development. However, it is to be hoped that hybridization of defined genomic probes using

novel labelling procedures may soon finally allow the fine-scale mapping of single-copy oocyte-expressed genes with respect to their LBC transcription units. In addition, it seems likely that the most startling recent development concerning LBCs, namely the discovery that sperm chromatin can be assembled into LBCs *de novo* upon injection into oocytes (Gall & Murphy 1998), will facilitate renewed investigation of this and many other fundamental questions regarding the lampbrush condition. Two applications of the approach that were discussed by Gall and Murphy (1998) are especially relevant to future prospects for LBC research. First, there is the possibility of forming LBCs from the sperm of organisms that have well-characterized genomes but that either do not naturally form LBCs, such as mammals, or produce LBCs that are technically difficult to handle. Secondly, it may be feasible to develop a cell-free system for LBC assembly that would enable components potentially required for chromatin decondensation, loop formation, transcription and RNA processing to be identified and their activity investigated.

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