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The arrangement and transcription of telomere DNA sequences at the ends of lampbrush chromosomes of birds

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This paper is dedicated to the memory of the late Professor H. G. Callan FRS, who inspired our interest in lampbrush chromosomes and taught us many successful ways of working with them.

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The arrangement of loops and chromomeres at the ends of lampbrush chromosomes in four species of bird is described with reference to chromomeres, loops and transcription units. 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 ribonuclear protein matrix 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 ends of terminal open-ended loops therefore represent the true ends of the chromatids that make up a lampbrush half-bivalent. The pattern of binding of three telomeric DNA sequence probes to the terminal regions of bird lampbrush chromosomes, under conditions of DNA/DNA and DNA/RNA transcript *in situ* hybridization has been investigated by fluorescence *in situ* hybridization. All three probes gave the same results. With DNA/DNA and DNA/RNA transcript hybridization, three classes of structure were labelled: the terminal chromomere, a small number of interstitial chromomeres and the terminal transcription unit on telomere loops. Labelling of telomere loops, but not of terminal or interstitial chromomeres, was eliminated by ribonuclease treatment before *in situ* hybridization. The labelled regions of telomere loops were spaced away from the labelled terminal chromomere by an unlabelled sub telomeric transcription unit. After DNA/DNA *in situ* hybridization, no labelled loops were seen. DNA/RNA transcript *in situ* hybridization with single-stranded hexamers of each strand of telomeric DNA showed that the terminal transcription unit on telomere loops represents transcription exclusively from the C-rich strand of the repeat outwards towards the end of the chromosome. It is concluded that transcription specifically of the C-rich strand of strictly terminal clusters of telomere repeats is an obligatory event on the lampbrush chromosomes of birds and is unlikely to represent indiscriminate readthrough from proximally located gene elements.

Key words: birds, FISH, lampbrush chromosome, telomere, transcription

Introduction

The chromosomes of most eukaryotes end in a series of tandem repeats of certain simple DNA sequences. The basic repeating unit varies from one organism to another but in every case it consists of a DNA duplex based on a G-rich strand orientated 5′–3′ towards the chromosome end. At the extreme end of the chromosome there is a 12–16 bp single-strand overhang of the G-rich strand. The complementary C-rich strand is thought, at least in its distal portion, to contain single-strand nicks. It has been suggested that the ends or 'telomeres' of chromosomes are maintained by a telomerase that prevents loss of DNA sequences during semiconservative replication (Grieder & Blackburn 1989, Blackburn 1992). The effective role of the telomerase depends on the specific properties of the terminal DNA repeats and on the single-strand overhang of the G-rich strand. The telomerase is an RNA–protein complex in which the RNA component, at least in *Tetrahymena*, is a complement to the G-rich strand of the telomeric DNA (Grieder & Blackburn 1989). The activity of telomerase is thought to be under the control of a structural protein that binds specifically to the G-rich overhang (Lustig *et al.* 1990).

In what follows the word telomere will be used in both the cytological and molecular sense. The matter is important in the context of this paper since lampbrush chromosomes actually have cytologically discrete telomeres (end bodies), and one of the objec-

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tives of this study was to determine the relationship between these structures and telomeric DNA.

The telomeres of human chromosomes consist of 10–15 kb of DNA. The repeating unit is TTAGGG, and this is thought to be a common telomeric sequence among vertebrates (Hastie & Allshire 1989, Meyne *et al.* 1990). In humans, there are more repeats of the telomere sequence in chromosomes from spermatozoa than there are in chromosomes from somatic cells (Allshire *et al.* 1989, Hastie *et al.* 1990), and it seems possible that telomerase may be more active and effective in preserving the chromosomes in germ cells than it is in somatic cells.

Lampbrush chromosomes are exclusively characteristic of the growing ovarian oocytes of nearly all animals other than protists and mammals (Callan 1986). The chromosomes are paired to form greatly elongated bivalents that are in diplotene of the first meiotic division. Each chromosome ('half-bivalent') consists of a row of small granules, the chromomeres, each of which bears two, or some multiple of two, lateral loops. The chromosome axis and its chromomeres represent regions where the 2 chromatids of the meiotic chromosome are closely apposed to one another, making up a visibly single structure; the loops represent regions where the two chromatids are separated. Each loop consists of a DNA duplex covered with densely packed RNA polymerase molecules, each of which bears an RNA transcript of part of the loop axis. The RNA transcripts are complexed with proteins of one kind or another. The entire loop makes up one or more transcription units. Transcription on lampbrush loops is known to follow a 'read-through' pattern in which a gene sequence and its promoter are located at the start of the unit and the polymerase reads through this gene and on into whatever other DNA sequences lie immediately downstream of the gene (Varley *et al.* 1980, Gall *et al.* 1983). All of this process is reflected in the shape of the majority of lampbrush loops, which are thin at the start of the transcription unit and grow progressively thicker towards the end of the unit, a feature that was described in some of the earliest studies of these structures (Gall 1954, Callan & Lloyd 1960). A wide range of DNA sequences, single copy and repeated, translatable or not, are transcribed on lampbrush loops, some of them apparently indiscriminately. The entire field of lamphrushology was expertly and comprehensively reviewed by Callan in 1986.

Loops are long structures in molecular terms. The shortest identifiable loops on the relatively small lampbrush chromosomes of chickens, for example, are several micrometres in length. One micrometre of B-form DNA includes about 3 kb (Watson & Crick 1953). The longest known loops on some lampbrush chromosomes from urodeles, consisting of a single transcription unit, may include up to half a million base pairs of DNA.

A very striking feature of the lampbrush chromosomes from birds is that each chromosome, macro and micro alike, ends in a chromomere with two lateral loops that have a quite distinctive appearance as compared with other loops along the lengths of the chromosomes' axes (Chelysheva *et al.* 1990). In many cases, the loops associated with the last chromomere are open ended, emerging from the chromomere but not returning to it at the other end (Figure 1). In such cases we can but conclude that the distal ends of the loops represent the true ends of the two chromatids. Open-ended terminal loops represent a situation that is strictly comparable, in terms of fundamental lampbrush chromosome organization, to the double bridges originally described by Callan (1963, 1986).

The telomeric DNA repeat in birds is identical to the human repeat (Moyzis *et al.* 1988, Meyne *et al.* 1990, de Jonge 1991). The opportunity exists, therefore, of using *in situ* nucleic acid hybridization with human telomeric DNA as a probe to investigate the nature of lampbrush telomeres in birds. The feasibility and usefulness of such an approach was originally demonstrated by Dr Nancy Hutchison in 1991 (unpublished observations) and, in a later study of the sex bivalents of birds in their lampbrush form, we showed that a human-derived telomeric sequence binds to the telomeric chromomeres of the Z and W chromosomes of chicken in fluorescence *in situ* hybridization (FISH) experiments (Solovei *et al.* 1993).

In this study we have concentrated on three questions: (1) Are the telomeres of bird lampbrush chromosomes situated in the telomeric loops? (2) If the telomeric loops include telomeric DNA sequences, are these sequences transcribed during the lampbrush phase of oogenesis? (3) If telomeric sequences are transcribed, which strand is transcribed? Each of these questions is uniquely approachable through a FISH–cytomolecular study of lampbrush chromosomes.

Materials and methods

The birds used in this study were chickens (*Gallus gallus domesticus*, Rhode Island cross), turkey (*Meleagris gallopavo*), Japanese quail (*Coturnix coturnix japonica*) and domestic pigeon (*Columba livia*).

Lampbrush chromosomes were isolated manually in the usual way as described by Macgregor & Varley (1988), with some minor modifications of technique as described by Solovei *et al.* (1993). A special type of isolation slide was used consisting of a square of glass, 25 × 25 × 1 mm with four 5-mm-diameter holes bored through it, sealed to an ordinary microscope slide with rubber cement. Such a device has two major advantages. First it allows four separate preparations, the same or different, to be placed on one microscope slide and therefore subsequently processed in exactly the same manner. Secondly, the use of rubber cement

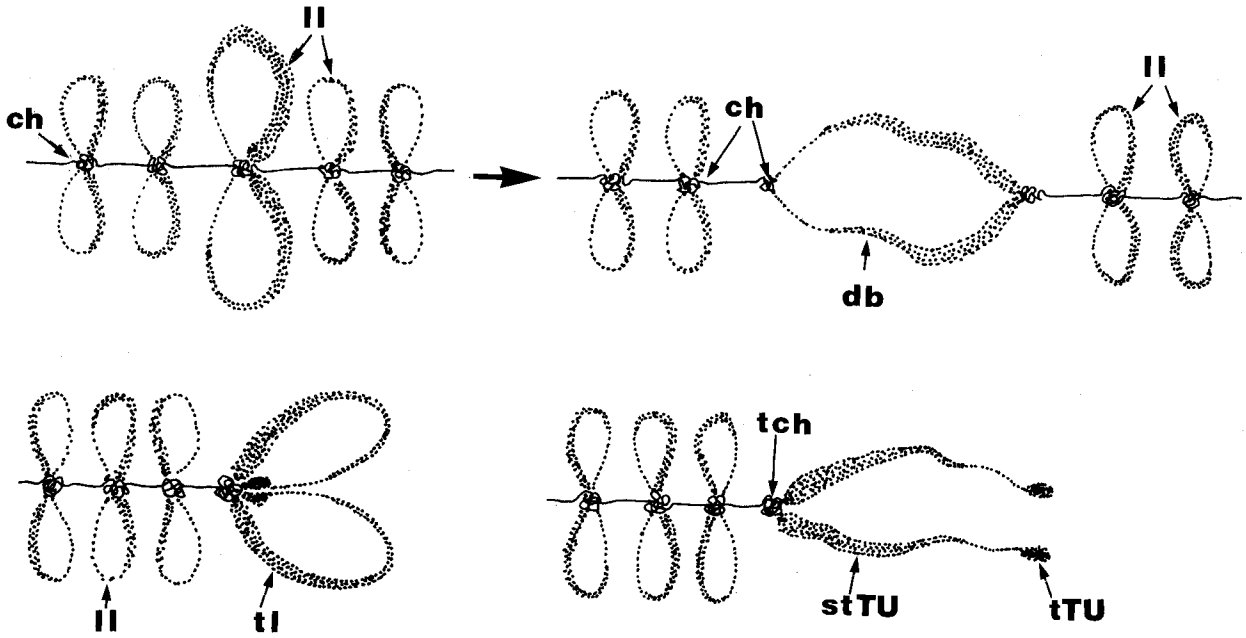


Figure 1. The structure of a double bridge formed by lateral loops (top). This situation can exist naturally or can be produced by mechanical longitudinal stretching of the chromosome. The two alternative situations at the ends of chicken lampbrush chromosomes are shown in the bottom pair of drawings. On the left, both terminal loops (TBLs) emerge from and return to the terminal chromomere. On the right the TBLs are open ended. Note the basic structural similarity of these different situations, db, double bridge formed by spanning lateral loops; **ll**, lateral loops; **tl**, telomere loops; **tTU**, telomere transcription unit; stTU, subtelomeric transcription unit; **ch**, chromomere; **tch**, terminal chromomere.

to seal the glass square to the slide allows the former to be removed from the slide absolutely cleanly, leaving only the chromosomes (or other experimental objects) remaining on the surface of the slide. Older lampbrush techniques employed single-chamber slides in which the components were sealed together with paraffin wax which has to be removed with xylene.

After isolation, chromosomes were attached to the slides by centrifugation. They were subsequently fixed in 2% paraformaldehyde for 5 min, post-fixed in 70% ethanol and then stored in 70% ethanol at 4°C. Most slides were prepared with four sets of lampbrush chromosomes from four oocytes. Some had sets of chromosomes in three of the chambers and erythrocyte nuclei from the same bird in the fourth chamber. Erythrocyte nuclei served as a convenient positive control for FISH experiments.

Three kinds of telomeric DNA sequence were used as probes for *in situ* hybridizations: (1) plasmid pHuR93 which has a 240-nucleotide insertion of the human telomere repeat TTAGGG (Moyzis *et al.* 1988); (2) a PCR-generated tolomere repeat, also TTAGGG, kindly provided by Dr Nicola Royle of the Department of Genetics, Leicester University (Royle *et al.* 1992); (3) the synthetic deoxynucleotide oligomers (TTAGGG) × 5 and (TAACCC) × 5. Double-stranded probes were labelled using a nick-translation kit and biotin-16-dUTP (Boehringer Mannheim). After labelling and ethanol precipitation, probes were dissolved at a concentration of 10 ng/μl in a hybridization mix consisting of 50% formamide, 2 × SSC, 10% dextran sulphate and yeast tRNA. Oligonucleotides were synthesized on an Applied Biosystems 394 DNA synthesizer and labelled with one molecule of biotin on the 5′ end. They were dissolved at 1 ng/μl in a hybridization mix of 25% formamide, 4 × SSC, and 10% dextran sulphate.

Fluorescence *in situ* hybridization (FISH) was carried out as follows. Five microliters of heat-denatured probe (3 min at 75°C) was put on a slide with either untreated or denatured (3 min at 75°C in 70% formamide, 2 × SSC, pH 7.0) chromosomes. The preparations were covered with a coverslip, sealed with rubber cement and incubated on a slide warmer at 37°C for 12–16 h. Preparations were subsequently washed in 50% formamide in 2 × SSC at 42°C, 2 × SSC at 42°C, and 2 × SSC at room temperature. Nucleic acid hybrids were detected with avidin DN conjugated with FITC (Vector Laboratories) with one round of amplification. Chromosomes were stained with DAPI and propidium iodide (PI), air dried and then mounted in antifade medium (1% DABCO, 50% glycerol, 1 × SSC).

Some control slides were treated with ribonuclease A (100 μg/ml in 2 × SSC, 1 h at 37°C) or with proteinase K (5 μg/ml in 2 × SSC, 15 min at 37°C) before hybridization.

In situ hybrids were visualized with a fluorescence microscope and imaged using a Biorad MCR600

confocal scanning laser attachment to a Zeiss Axiovert microscope. Grey-scale images were false coloured using the autumn look-up table prior to being hard copied with a Sony UP-300P colour video printer.

Observations

In this section we concentrate mainly on the results of experiments with the lampbrush chromosomes of chickens because they are better characterized than those of any other birds and identification maps of all the chicken macro-lampbrush chromosomes have already been published (Hutchison 1987, Chelysheva *et al.* 1990, Solovei *et al.* 1993). We would like to emphasize at the outset, however, that in all species of bird so far examined (chicken, turkey, pigeon, sparrow, chaffinch), except for quail, all the lampbrush chromosomes end in a terminal chromomere with conspicuous loops, TLs) emerging from it. TLs are distinguished on account of their appearance, their relatively large size and the fine-scale morphology of their ribonuclear protein (RNP) matrix.

Morphology

The telomere loops in chicken may be classified into three types.

1. Telomere bow-like loops (TBLs) (Chelysheva *et al.* 1990) are slightly larger than normal lateral loops in other parts of the chromosome. A major part of the TBL consists of one transcription unit (TU) with an RNP matrix having a fluffy appearance. At the start of this TU (thin end of the loop), there is a smaller TU with a dense RNP matrix (Figures 1–4). TBLs are present at the ends of the long arms of chromosomes 1, 2, 3 and Z and some micro-chromosomes.
2. Telomere giant loops (TGLs) are much bigger than any other loops in the entire chromosome set. They may be up to 100 μm long. They have a dense RNP matrix, on account of which they are conspicuous in phase contrast or after staining with Coomassie blue R250. The shape of the TGLs suggests that the greater part of each of these loops is occupied by a single enormously long TU (Figures 2, 5 & 6). TGLs are present at the ends of the long arms of chromosomes 4, 5 and 6, at the chiasmate ends of the Z and W sex chromosomes (Solovei *et al.* 1993) and at the ends of some microchromosomes.
3. Telomere small loops (TSLs) are usually smaller than normal lateral loops. The greater part of each of these consists of one TU with dense RNP matrix. TSLs are present at the ends of the short arms of macrochromosomes and microchromosomes (Figure 2).

The one common characteristic of all telomere loops is that they always consist of or terminate in a TU with dense RNP matrix.

By far the most striking and significant feature of telomere loops is that they are frequently open ended in the sense that only one end of the 'loop' is attached to the chromomere and the other end hangs free (Figures 1 & 2). These open-ended loops are not a consequence of accidental breakage during manual isolation of the chromosomes. They can be observed in intact germinal vesicles examined with a confocal microscope, and their orientation with respect to the main body of the chromosome, as determined from their polarized (thin to thick) shape, is always the same. The extreme ends of telomere loops must represent the true ends of the chromatids.

Fluorescence *in situ* hybridization

All three telomere repeat probes, pHuR93, PCR-generated telomere repeats and the combined synthetic complementary oligonucleotides (TTAGGG) × 5 and (TAACCC) × 5, produced the pattern of labelling summarized in Table 1. When the DNA probe was allowed to hybridize to both chromosomal DNA and loop RNA transcripts (denatured chromosomal DNA and no ribonuclease treatment), three distinct categories of labelling sites were distinguishable: (1) the chromomeres at the extreme ends of all chromosomes, (2) a number of interstitial chromomeres (the exact locations of these will be reported in a later publication by A.V. Rodionov *et al.*) and (3) The TUs with dense RNP matrix in the telomere loops. The labelled regions of the telomere loops were spaced away from the terminal chromomere by the large fluffy unlabelled TU of TBLs or, in the case of TGLs and TSLs, by a short unlabelled segment of loop without discernible polarization of RNA matrix (Figures 7–12).

Control slides, on which the chromosomal DNA had not been denatured and/or the chromosomes had been treated with ribonuclease to remove the majority of the loop RNA transcripts, helped to confirm that labelling of terminal and interstitial chromomeres resulted from probe DNA to chromosomal DNA complementary binding, whereas labelling of telomere loops resulted from probe DNA to loop RNA transcript complementary binding (Figures 10 & 11). No labelled loops were seen in interstitial regions. No loops were discernibly labelled in experiments that favoured only the binding of probe DNA to chromosomal DNA. We judge this to be because of the very small amount of extended DNA duplex in a loop axis and/or masking of the loop axis DNA by RNA polymerases.

In our opinion, the labelling of the RNP matrix of the terminal TU of all telomere loops is good evidence for transcription of telomeric DNA.

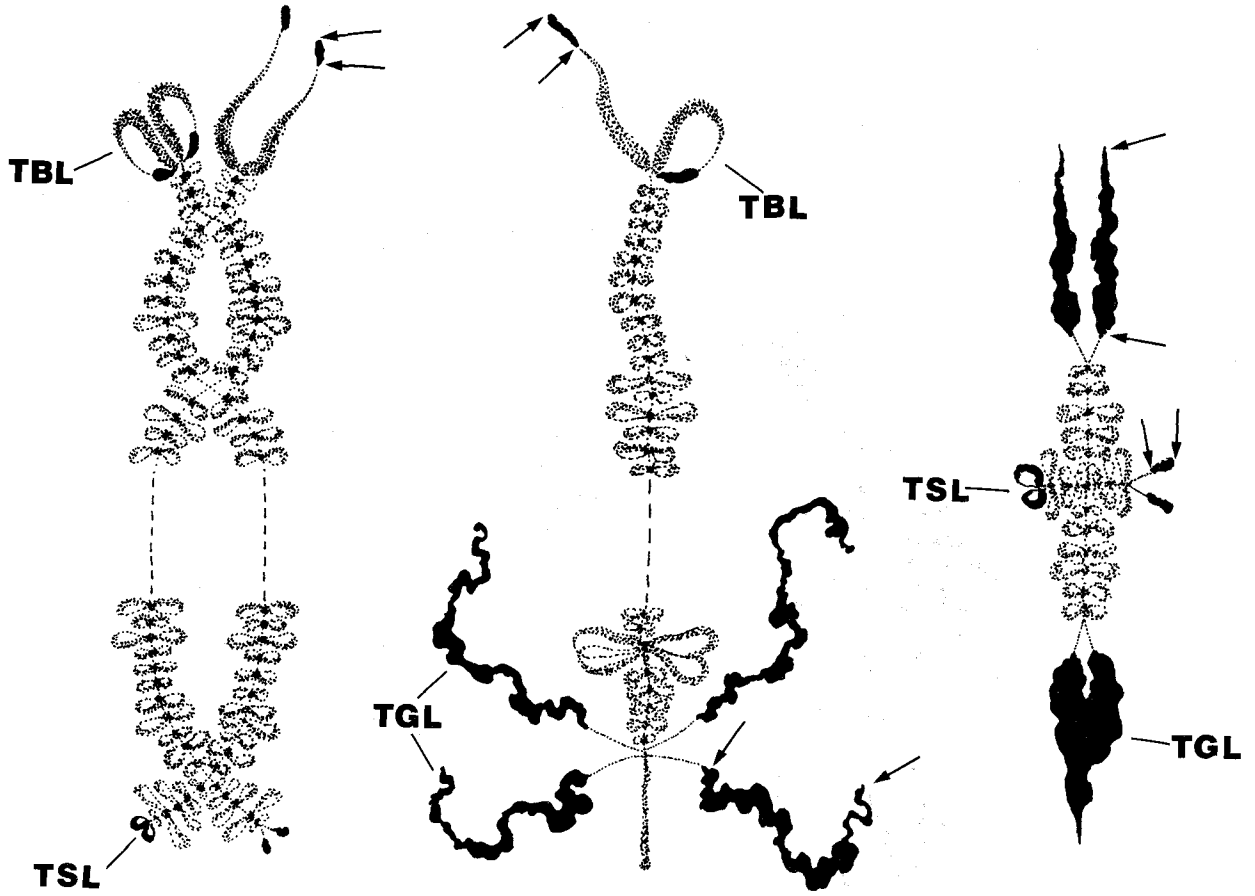


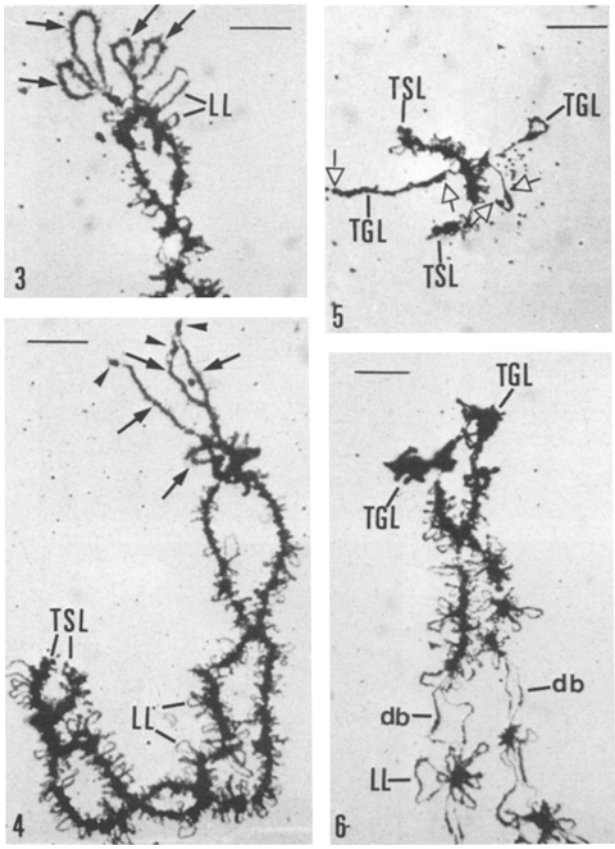
Figure 2. Schematic drawings of the first (left), sex (middle) and micro- (right) bivalents from chicken showing different types of terminal loops. Note that some terminal loops are open ended. **TBL**, telomere bow-like loop; **TGL**, telomere giant loop; **TSL**, telomere small loop. Arrows show the limits of the terminal transcription unit with dense RNP matrix.

To determine which strand of the telomeric DNA is transcribed in the terminal TUs, we carried out separate DNA/RNA transcript *in situ* hybridizations with (TAACCC) × 5 and with (TTAGGG) × 5. TAACCC gave strong labelling (Figures 13 & 14); TTAGGG gave none (Table 2). We conclude that the terminal TU is formed by the selective transcription of the C-rich strand of the telomere repeat and that transcription is always directed towards the end of the chromosome.

On TBLs, the relatively long subterminal TU with fluffy RNP matrix was always unlabelled, in contrast to the adjacent terminal TU, which was always labelled in experiments that favoured the binding of probe DNA to RNA transcripts (Figures 7, 8, 10, 11 & 12). Smaller TGLs, including those at the ends of the long arms of bivalent 4 and on some of the microchromosomes, were labelled throughout their lengths (Figures 9 & 19). The dense TUs of the largest TGLs, however, including those on chromosomes 6, Z and W and some microchromosomes, were only partially labelled: the intensity of fluorescence gradually diminished in a distal direction and the most distal portions of the TUs were unlabelled (Figures 12 & 19).

The sizes of TUs on TBLs are variable. Those at the ends of bivalent 1 are always the smallest; those on bivalent 3 are always the largest. There is also variation in the sizes of TUs on TBLs between the same chromosomes from different individual chickens and even between the two chromosomes of the same bivalent. The TUs on long arms of the two half-bivalents of a third chromosome are shown in Figures 10 and 11. One of these measures 1 μm; the other 15 μm. The TUs of sister chromatids, that is those located on loops that arise from the same chromomere, are always identical. A similar general pattern of variation with respect to lampbrush loop morphologies within and between individuals has been described by Callan & Lloyd (1960).

Results closely similar to those obtained with chicken chromosomes have been obtained with chromosomes from turkey and pigeon. All lampbrush chromosomes of turkey and pigeon were labelled on the terminal chromomere and the RNP matrix of terminal loops after DNA/DNA and DNA/RNA transcript *in situ* hybridization respectively with TAACCC (Figures 15 & 16). No other sites of DNA/RNA tran-



Figures 3–6. The three types of telomeric loops on chicken lampbrush chromosomes. Scale bars = 10 μm. **Figures 3** & **4**. Bivalent 2 with closed (Figure 3) and open ended (Figure 4) telomere bow-like loops (TBLs). Arrows, TBLs; solid arrowheads, small TUs with dense RNP matrix on the free ends of TBLs. **Figures 5** & **6**. Telomere giant loops (TGLs) on a microchromosome (Figure 5) and on bivalent 5 (Figure 6). TGLs are open ended on the microchromosome and closed on the bivalent 5; note the characteristically contorted lumpy appearance of the closed TGLs. LL, lateral loops; TSL, telomeric small loops; db, double-loop bridges; empty arrowheads show the limits of the terminal TUs with dense RNP matrix.

Table 1. FISH labelling with pHuR93 on chicken lampbrush chromosomes with different pretreatment combinations designed to discriminate between DNA/DNA hybridization and DNA/RNA transcript hybridization

Labelled chromosomal sites	Denatured chromosomal DNA		Native chromosomal DNA	
	No RNAse	RNAse	No RNAse	RNAse
End chromomere and some interstitial chromomeres	+	+	–	–
TU with dense RNP matrix on TLs	+	–	+	–

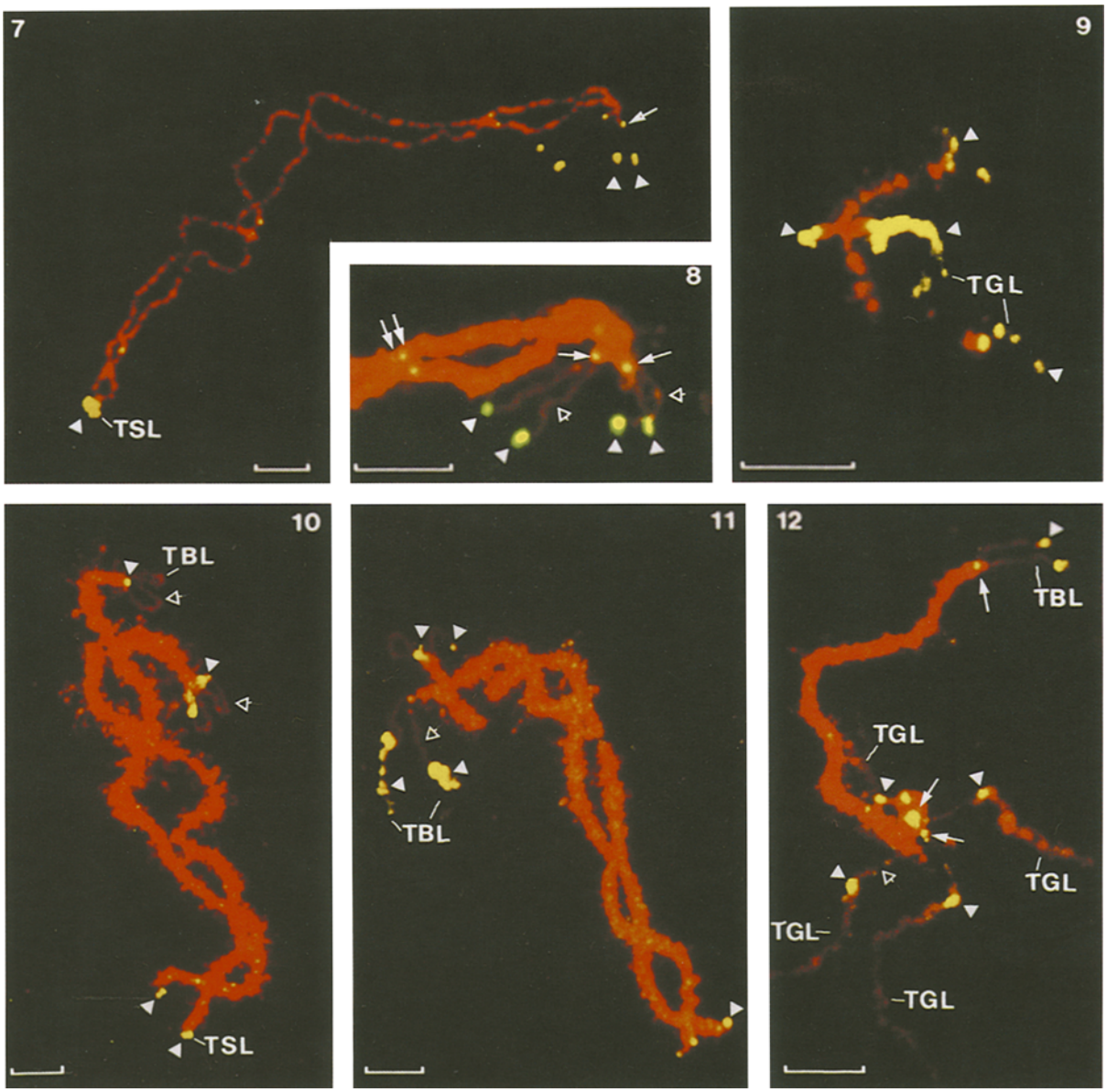
script *in situ* hybridization were seen. The telomere loops of pigeon are very large and generally similar in appearance to those of chicken. They showed label from a DNA/RNA transcript *in situ* hybrid only in their proximal portion, and the size and brightness of the labelled region were consistently less than in chicken. Quail differs from the other birds in this study in that it lacks telomeric loops on its lampbrush chromosomes: only DNA/DNA *in situ* hybridization to the terminal chromomeres was observed in this species (Figures 17 & 18).

Discussion

We have shown by fluorescence *in situ* hybridization that there are two distinct but adjacent regions consisting of repeated telomeric DNA sequences at the ends of chicken lampbrush chromosomes. The strictly telomeric cluster of these sequences is situated in the telomere loops. A subtelomeric cluster is incorporated into the compacted chromatin of the terminal chromomere (Figure 19). The arrangement of these two clusters is especially evident in the TBLs, where they are separated from each other by a relatively large transcription unit that does not bind either strand of the telomeric DNA probe under conditions of FISH (Figures 2 & 19). The length of the intervening transcription unit is approximately 15 μm. One micrometre of B-form DNA corresponds to about 3 kb. The molecular distance between the two clusters of telomeric DNA can therefore be said to be at least 45 kb, and this estimate does not include the length of intervening non-telomeric DNA that may be compacted into the terminal chromomere. Additional clusters of telomeric DNA repeats have also been demonstrated in subtelomeric positions on chromosomes of whales (Adegoke *et al.* 1993) and on human chromosomes, in the latter case at a distance of about 30 kb from the chromosome end (Wells *et al.* 1990, Weber *et al.* 1990, 1991).

True telomeres are composed entirely of the TTAGGG hexamer together with some minor variants of this motif that are usually located towards the proximal end of the repeat cluster (Brown *et al.* 1990, Cross *et al.* 1990). Subtelomeric sites may be a regular alternating array of the TTAGGG hexamer and other repeated sequences. For example, the subtelomeric heterochromatin of whale chromosomes includes 30–160 copies of TTAGGG and related hexanucleotides and a conserved 211/212 bp repeat (Adegoke *et al.* 1993).

In the mitotic metaphase chromosomes of chicken, the largest of which is about 10 μm long, telomeric and subtelomeric sites of *in situ* hybridization with TTAGGG cannot be separately resolved (Meyne *et al* 1990, Nanda & Schmid 1994, Rodionov *et al.* 1994) and the same is true of metaphase chromosomes from other organisms that have been studied in this regard



Figures 7–12. FISH with PCR-generated (Figures 7, 8 & 9) and pHuR93 (Figures 10, 11 & 12) telomere probes on chicken lampbrush chromosomes under conditions of DNA/DNA+RNA transcript (Figures 7, 8, 9 & 12) and DNA/RNA transcript (Figures 10 & 11) hybridization. **Figure 7** shows bivalent 1 with open ended TBLs. **Figure 8** is an enlargement of the TBL portion of the long arm of the same chromosome as in Figure 7. **Figure 9** shows a micro-bivalent with strongly labelled TGLs. **Figures 10** & **11** show bivalents 3 with closed (Figure 10) and open ended (Figure 11) TBLs. Note the different sizes of labelled TUs on the TBLs of the two chromosomes of the same bivalent. **Figure 12** shows the sex bivalent (ZW) with open ended TBL Z and TGLs Z and W; note that only the beginnings of the terminal TUs on these TGLs are labelled. Arrows show label on the terminal chromomeres of all lampbrush chromosomes. Double arrows show interstitial sites of telomere repeats on the long arm of bivalent 1. Solid arrowheads point to the labelled terminal TUs on TBLs, TGLs and TSLs. Empty arrowheads point to the unlabelled portions of the terminal loops.

[see Wells *et al.* (1990) for human metaphase chromosomes]. FISH as applied to lampbrush chromosomes, under the conditions of DNA/RNA transcript hybridization, offers the best currently obtainable level of cytomolecular resolution (Buckle & Kearney 1993).

Chicken lampbrush chromosomes also provide us with good cytological evidence of variation between individual chickens with regard to the length of the telomeric site in terms of numbers of repeats on the telomere DNA sequence. The length of the FISH (DNA/RNA transcript)-positive region on chromosome 3, for example, varies from about 0.5 μm to 15 μm, which is equivalent to 1.5 kb–45 kb. These measurements represent the actual lengths of TUs that consist entirely of telomeric repeats and that are not directly associated with a FISH (DNA/DNA)-positive

Table 2. FISH labelling of chicken lampbrush chromosomes with single-stranded hexamers of the telomeric DNA repeat.

Labelled chromosomal sites	(TTAGGG) × 5		(TAACCC) × 5		(TTAGGG) × 5+ (TAACCC) × 5	
	D	N	D	N	D	N
Telomeric and interstitial chromomeres	+	–	+	–	+	–
TU with dense RNP matrix on TLs	-	–	+	+	+	+

D, denatured chromosomal DNA; N, undenatured chromosomal DNA.

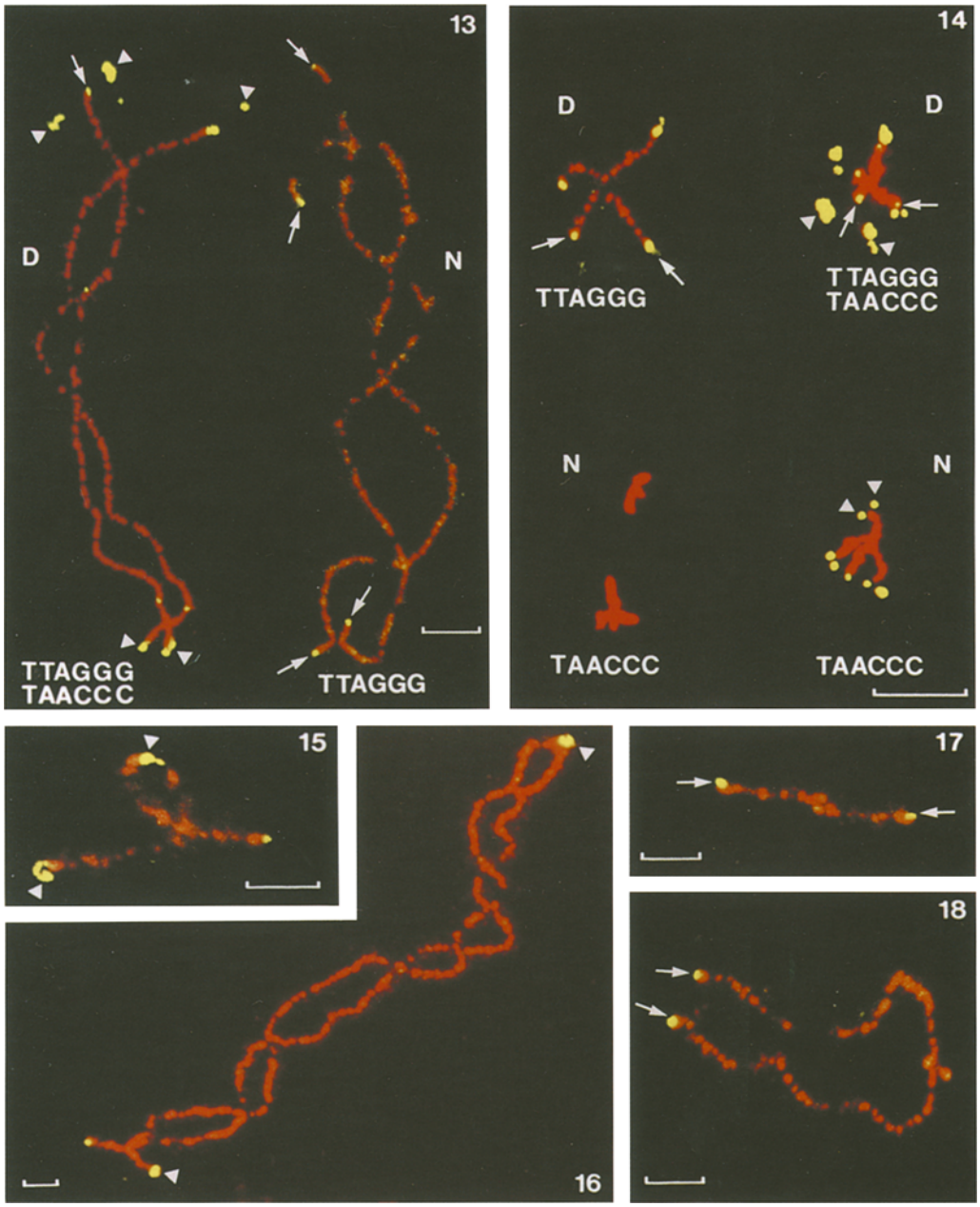
chromomere. Differences of this order are not uncommon between corresponding TUs on the two chromosomes of the same bivalent (Figures 10 & 11). A similar scale of variations between individuals with regard to telomere repeat number has been demonstrated in humans and mice (de Lange *et al.* 1990, Kipling & Cooke 1990).

Our experiments have shown that telomeric DNA repeats are transcribed, specifically from the C-rich strand, on telomeric loops of the lampbrush chromosomes of chickens and turkeys but not on those of quail. The latter has no telomeric loops and we can find no evidence of transcription of telomeric DNA. We cannot, at present, explain the absence of telomere loops and TUs in quail. We do know, however, that the lampbrush chromosomes of chaffinch, sparrow and pigeon all have telomeric loops, and we have demonstrated transcription of telomere repeats in pigeon. We therefore think it likely that transcription of telomere repeats is common in the oocytes of birds and that the lack of this feature in quail reflects some species-specific peculiarity of a kind that has often been encountered in studies of lampbrush chromosomes from birds and amphibians (Callan 1986).

Transcription of telomeric DNA repeats has previously been demonstrated by biochemical methods in six species of trypanosomes and related kinetoplastids (Rudenko & van der Ploeg 1989). In all but one, transcription was shown to proceed unidirectionally towards the chromosome end and was from the C-rich strand. In *Trypanosoma lewisi* about 40% of transcription was shown to be from the G-rich strand. The same authors demonstrated transcription of telomere repeats in *Plasmodium falciparum*. Rudenko & van der Ploeg (1989) suggest that transcription of telomere repeats is a consequence of readthrough from transcription of sub-terminal VSG (variable cell surface glycoprotein) genes. That does not account, however, for their own observation that telomeric DNA repeats are transcribed in the insect form of *T. brucei*, in which the VSG genes are not transcribed (Rudenko & van der Ploeg 1989).

As is always the case with lampbrush chromosomes, clues to fine structure come from both morphological and molecular observations. In this regard it is important to consider carefully the fine-scale arrangement of chromomeres, loops and transcription units at the ends of chicken chromosomes as well as the evidence from FISH experiments. In our view, the important considerations here are as follows.

1. There is no detectable FISH (DNA/DNA) labelling at the extreme tips of any of the open-ended terminal loops that we have examined. We can therefore say with some confidence that the transcribed telomeric sequences are right at the ends of the chromatids and represent the true telomeres.
2. In chicken there are telomeric TUs on all 39 bivalents. There are no TUs associated with the 39 × 4 subtelomeric chromomeric sites detected by DNA/DNA FISH, nor with any of the eight or more interstitial locations of TTAGGG repeats recently identified in chicken by Rodionov *et al.* (unpublished observations). This argues against the notion that transcription of telomere repeats is triggered indiscriminately by neighbouring genes.
3. In the open-ended TBLs, the thin-to-thick morphological polarity of the sub terminal TU, which does not contain telomeric DNA sequences, strongly suggests that transcription in this TU is proceeding in a distal-to-proximal direction (Figures 2 & 19). The terminal TU in the TBL, which does contain telomeric DNA sequences, is too small for its morphological polarity to be determined by light microscopy but, if it is transcribed only from the C-rich strand, then we know for certain that the polarity of transcription must be outwards towards the chromosome end (Figure 20).
4. There is no resolvable gap between the adjacent starts of the terminal and subterminal TUs. The length of the terminal TU corresponds roughly to the number of telomeric repeats found at the termini of mouse and human chromosomes (de Lange *et al.* 1990, Kipling & Cooke 1990). We suggest, therefore that there is little room in the terminal TU from anything other than telomere sequence repeats and little reason to suppose that anything



Figures 13–18. Arrows show label on the terminal chromomeres of lampbrush chromosomes after DNA/DNA FISH. Arrowheads show labelled terminal TUs on chicken and turkey telomeric loops after DNA/RNA transcript FISH. **Figures 13 & 14.** FISH with synthetic oligonucleotides on chicken bivalents 3 (Figure 13) and microbivalents (Figure 14). D, denatured chromosomal DNA; N, undenatured chromosomal DNA. Note that the TBLs of the third bivalent in Figure 13 and the TSLs of microbivalents in Figure 14 are open ended and their labelled regions are therefore spaced well away from the labelled terminal chromomere. **Figures 15 & 16.** Turkey micro- (Figure 15) and macro- (Figure 16) bivalents after FISH DNA/RNA transcript with (TAACCC) × 5. **Figures 17 & 18.** Quail micro- (Figure 17) and macro- (Figure 18) bivalents after FISH DNA/DNA hybridization with a mixture of both oligos (TTAGGG) × 5 and (TAACCC) × 5.

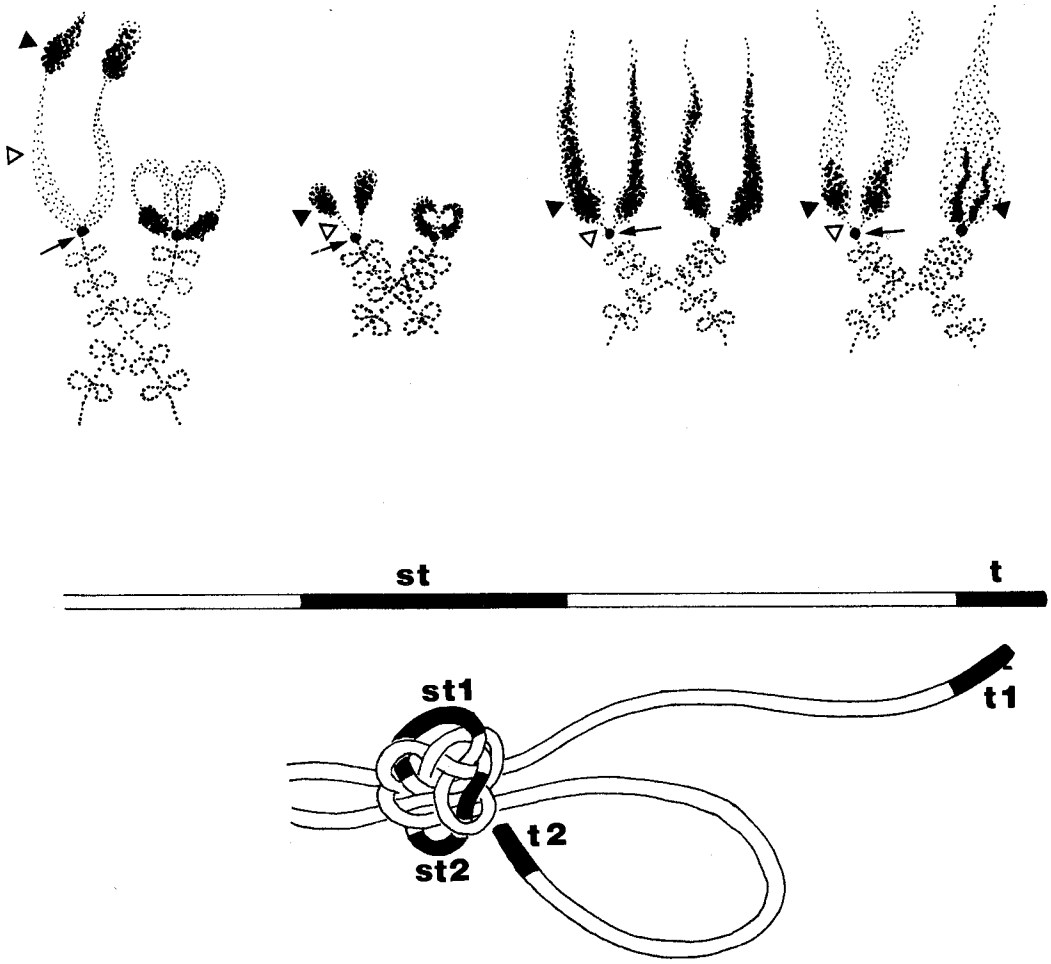


Figure 19. Scheme of labelling of the telomeric region of chicken lampbrush chromosomes after FISH (DNA/DNA + RNA transcript) with telomere probes. Top: Labelling of different types of telomeric loops. Heavy black shading signifies chromomere or loop labelling. Note the distance between the labelled terminal chromomeres (arrows) and the labelled terminal TUs (solid arrowheads). Empty arrowheads show the unlabelled portions of the telomeric loops. Note also the two different patterns of labelling of the TGLs. Bottom: Suggested pattern of distribution of the telomere repeats in the telomere region of chicken chromosomes. **t**, telomeric site (on the loops); **st**, subtelomeric site (in the terminal chromomere).

other than these sequences is being transcribed in this region.

It is on the basis of these observations that we suggest that transcription specifically of terminal clusters of telomere repeats from their C-rich strand is an obligatory event on the lampbrush chromosomes of birds and, in our view, it is unlikely to represent indiscriminate readthrough from proximally located gene elements.

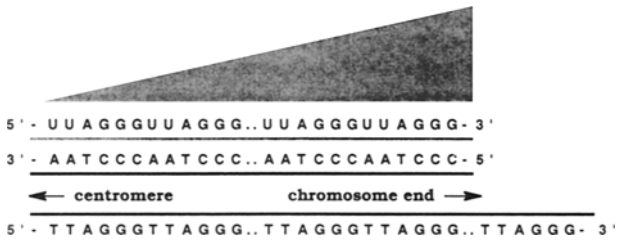


Figure 20. Sequence of the RNA transcript read from the C-rich strand of the telomere repeat with the thin–thick polarized shape of the TU shown above. Transcription from the other strand would proceed in the opposite direction.

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