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Localized co-transcriptional recruitment of the multifunctional RNA-binding protein CELF1 by lampbrush chromosome transcription units

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Abstract

The highly-extended transcription units of lampbrush chromosomes (LBCs) offer unique opportunities to study the co-transcriptional events occurring on nascent transcripts. Using LBCs from amphibian oocytes, I investigated whether CELF1, an RNA binding protein involved in the regulation of alternative splicing, mRNA stability and translation, is localized to active transcription units. Antibodies raised against mammalian (CUG-BP1) and amphibian (EDEN-BP) CELF1 were used to immunostain LBC spreads prepared from several species, including *Xenopus laevis* and the axolotl *Ambystoma mexicanum*. Up to about 50 separate LBC loci were convincingly immunostained and it was clear that CELF1 was present in the nascent RNPs of lateral loops. Furthermore, *myc*-tagged CUG-BP1 expressed in microinjected axolotl oocytes was specifically targeted to nascent transcripts of loops that recruit endogenous CELF1. In many active transcription units CELF1 was distinctly localized, being first recruited by nascent transcripts only far downstream of the transcription start site and remaining associated until the end of transcription. Overall it appears possible that the multiple functions of CELF1 in regulating posttranscriptional gene expression could all be predetermined during transcription by virtue of a region-specific binding to the nascent transcripts of target genes.

Abbreviations

CUG, oligoribonucleotide; 5′CUG 3′; CUG-BP1, CUG binding protein 1; ETR-3, embryonic lethal abnormal vision type RNA-binding protein 3; CELF1, CUG-BP1 and ETR-3 like factor 1; EDEN-BP, embryo deadenylation element binding protein; MBNL1, muscleblind-like protein 1; GV, germinal vesicle; LBC, lampbrush chromosome; pol II, RNA polymerase II; TU, transcription unit

Introduction

A major advance in understanding the structure and function of active genes has been the recent realization that many of the steps required for eukaryotic gene expression may be temporally and mechanistically coupled (reviewed by Bentley 2002, Guthrie & Steitz 2005). There is growing evidence that the processes required to convert primary transcripts into functional mRNAs may normally occur within ‘factories’ comprising the RNA polymerase II (pol II) transcription complex and various RNA-modifying machines that function co-transcriptionally on nascent transcripts (reviewed by Bentley 2005). The best understood example of the coupling of transcription and other nuclear activities is that of pre-mRNA processing,

for which a variety of approaches have shown that capping, splicing and 3′end formation are often co-transcriptional (reviewed by Neugebauer 2002, Proudfoot *et al.* 2002, Bentley 2005). Some of the subsequent nuclear processes such as those required for mRNA export and surveillance are also likely to be coupled to transcription (Andrulis *et al.* 2002, reviewed by Aguilera 2005, Guthrie & Steitz 2005). In addition it is intriguing that in a number of cases the regulation of gene expression in the cytoplasm with regard to the stability, movement, localization and translation of mRNAs is affected by prior nuclear events (reviewed by Kloc & Etkin 2005), raising the possibility that such regulatory events might also be predetermined during transcription.

Early evidence for the existence of co-transcriptional coupling was provided by the properties of members of a major class of nuclear proteins, the heterogeneous nuclear ribonucleoproteins (hnRNPs). These proteins were first identified as a group of chromatin-associated RNA-binding proteins (reviewed by Dreyfuss *et al.* 1993) and it has become apparent that as well as being involved in transcript packaging they function in a wide variety of nuclear activities (Krecic & Swanson 1999). Many hnRNPs, although mainly nuclear in their localization, shuttle between the nucleus and cytoplasm and a given hnRNP can affect several aspects of gene expression in both compartments. Some hnRNPs are abundant and present on transcripts of essentially all genes, and for this class there is already good evidence that their first association is with nascent transcripts (Dreyfuss *et al.* 1993). Many putative hnRNPs however, are less abundant and although most are poorly characterized some of them show a degree of transcript specificity that suggests they may well function in regulating the expression of restricted subsets of genes (Krecic & Swanson 1999).

A well characterized example of a minor hnRNP is CELF1, a member of the CELF/Bruno-like family of RNA binding proteins that is named after its founder members, mammalian CUG-BP1 and ETR-3 like factors (Ladd *et al.* 2001) and the *Drosophila* protein Bruno (Good *et al.* 2000). In vertebrates, CELF1 is a widely expressed protein found in the cytoplasm as well as the nucleus that functions in both translational regulation and the control of alternative splicing. Regulation of translation by CELF1 is effected by two different mechanisms (reviewed by Barreau *et al.* 2006): in the first, control of the translation/stability of maternal mRNAs in *Xenopus* eggs and early embryos depends on the binding of CELF1 (originally known as EDEN-BP; Paillard *et al.* 1998) to sequence elements in the 3′ UTR. These elements, known as embryo deadenylation elements, target mRNAs such as those encoding c-*mos* and Aurora-A kinase for deadenylation in the cytoplasm and for subsequent RNA degradation (reviewed by Paillard & Osborne 2003). Another mechanism of translational control has been described for mammalian CELF1, which shares 88% amino acid identity with EDEN-BP and was originally known as CUG binding protein 1 (CUG-BP1) because of its ability to bind specifically to CUG oligonucleotides (Timchenko *et al.* 1996). CELF1/CUG-BP1 also binds to G/C rich elements in the 5′ UTR of certain mRNAs and stimulates translation or the choice of initiation codon (Timchenko *et al.* 1999). The regulation of alternative splicing by CELF1 has been examined in detail for CUG-BP1 which, by binding to U/G rich sequences in pre-mRNAs, can control the inclusion or exclusion of regulated exons from a variety of mRNAs (reviewed by Barreau *et al.* 2006). Changes in the levels of CELF1 are thought to alter splicing patterns of cognate genes during normal development. Moreover, aberrant increases in the levels and activity of CUG-BP1 and consequent RNA missplicing are implicated in the disease process leading to the neuromuscular disorder myotonic dystrophy (Savkur *et al.* 2001, Timchenko *et al.* 2001, Dansithong *et al.* 2005). Knowledge of the initial site of action of CELF1 during gene expression is therefore likely to be useful in understanding the detailed mechanism of myotonic dystrophy pathogenesis.

The absence of straightforward molecular assay systems is a major difficulty in determining whether an RNA-binding regulator such as CELF1 binds nascent RNA. For instance, *in vitro* transcription systems that can reconstitute co-transcriptional RNA transactions are not currently available and there are inherent difficulties in investigating nascent RNA at the single gene level *in vivo* because it represents such a small proportion of a given mRNA and its progenitors. Although approaches based upon chromatin immunoprecipitation techniques that are targeted at nascent-RNA binding proteins have been carried out successfully (Gilbert *et al.* 2004), there are a number of complexities that complicate their detailed interpretation (Kotovic *et al.* 2003, Bentley

2005). An alternative, simpler approach that has been relatively unexploited is to adopt methods that allow analysis *in situ* of active transcription units and their nascent transcripts. For certain morphologically-distinctive genes the processes occurring on nascent transcripts were first analysed by electron microscopy and these observations have provided compelling evidence for the occurrence of RNA processing on nascent transcripts and for the co-transcriptional assembly of mRNP particles (Osheim *et al.* 1985, Daneholt 2001). The giant polytene chromosomes of the dipteran *Chironomus tentans* provided material particularly amenable for these electron-microscopic studies and recently they have also been analysed at the light-microscope level in order to identify RNA-associated proteins that bind to transcriptionally active regions in a gene-specific manner (Sun *et al.* 2004, Singh *et al.* 2006). Two such proteins, one a member of the SR family and one a transcriptional repressor, were successfully identified, although distinguishing their binding to nascent transcripts rather than to the template has to be inferred rather than being directly observable in this material.

In another type of giant chromosome, the lampbrush chromosome, the level of morphological detail does allow the resolution of co-transcriptional events within transcription units. In addition, since these chromosomes are typically obtained from the oocytes of vertebrates such as amphibians and birds, they appear well suited to study the *in vivo* site of action of CELF1. Lampbrush chromosomes (LBCs; reviewed in Callan 1986, Morgan 2002), are relatively decondensed diplotene bivalents from which extend loops of DNA that are transcribed at far greater rates than most genes in somatic nuclei. Individual transcription units and their transcriptional polarity can be observed by light microscopy because their densely-packed nascent transcripts can give rise to a visible, asymmetric ribonucleoprotein (RNP) matrix that surrounds the transcribed DNA of the loop axis. Studies of the composition of the RNP matrix have identified a variety of macromolecules such as snRNPs and hnRNPs in nascent transcripts of lampbrush loops (Pinol-Roma *et al.* 1989, Wu *et al.* 1991, Solovei *et al.* 1995) and targeting of exogenous RNA-binding proteins to nascent RNP has also been demonstrated (Roth & Gall 1989, Jantsch & Gall 1992, Bellini *et al.* 1993, Eckmann & Jantsch 1999). In most cases these proteins are fairly generally associated with the nascent RNP of the majority of the lampbrush transcription units, although there are examples of uncharacterized proteins that are specific to the transcripts of one or a small number of loops (Sommerville *et al.* 1978, Roth & Gall 1987). The latter observations suggested therefore, that if CELF1 functions co-transcriptionally, then the specific association of this protein with transcripts of certain transcription units should be demonstrable in LBCs.

The results of the experiments described here using LBCs from amphibian oocytes show that indeed CELF1 can be detected immunologically on nascent transcripts of a small number of transcription units. Epitope-tagged human CELF1/CUG-BP1 produced by translation of synthetic transcripts injected into oocytes was also specifically targeted to loops that normally recruit endogenous amphibian CELF1, suggesting that the mechanism of recruitment and the co-transcriptional mode of action is conserved in vertebrate CELF1. Most striking was the observation that in about half of the transcription units that recruited CELF1 the protein was not detectable in nascent transcripts until a considerable distance beyond the site of transcription initiation. The implications of these findings for the molecular mechanisms of CELF1/CUG-BP1 action in normal and aberrant mammalian cells and for understanding the functional organization of LBC transcription units are discussed.

Materials and methods

Preparation of GV spreads

Separated oocytes from *Xenopus laevis* or *Triturus vulgaris* (diameter 0.8–1.1 mm) or from axolotls (1.3–1.7 mm) were obtained by manual dissection of ovary fragments in OR2 saline (82.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM Na_2HPO_4, 5 mM Hepes; pH 7.4). GVs were then manually dissected from oocytes in isolation medium (83 mM KCl, 17 mM NaCl, 6.5 mM Na_2HPO_4, 3.5 mM KH_2PO_4, 1 mM $MgCl_2$, 1 mM DTT; pH 7.0–7.2). Spread preparations of *Xenopus* GV contents were made using the procedure of Gall (Gall 1998) and essentially the same procedure was used for axolotl GVs except that removal of the GV envelope was done in the observation chamber rather

than in the dissection dish. *Triturus* GV spreads were also made in the latter manner, although the medium used to disperse the nuclear gel contained paraformaldehyde at 0.01% instead of 0.1% and centrifugation to attach the GV contents to the base of the observation chamber was at 2500 *g* rather than 5000 *g*. After centrifugation, preparations were fixed for a minimum of 1 h and a maximum of 16 h in 2% paraformaldehyde made up in phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10.2 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) containing 1 mM $MgCl_2$.

Expression of myc-tagged CUG-BP in oocytes

An expression construct encoding human CUG-BP that was tagged at its N-terminus with the *myc* epitope was made by transferring the CUG-BP coding region from an existing GFP-fusion construct (Fardaei *et al*. 2001) into the vector pcDNA3.1/His/6myc (Ling *et al*. 2006). This vector was derived from pcDNA3.1/HisC (Invitrogen) by cloning into its *Bam*HI site a *Bgl*II/*Bam*HI PCR fragment from MT-6D (Tuma *et al*. 1993) that encodes six tandem copies of a 13-amino-acid peptide containing the *myc* epitope. Capped sense-strand transcripts were prepared from the fusion construct using a mMessage mMachine kit (Ambion Inc.) to transcribe the linearized plasmid DNA with T7 RNA polymerase according to the manufacturer's instructions. The RNA was resusupended in RNase-free H_2O and its concentration and sizes were estimated by agarose gel electrophoresis.

Tagged CUG-BP was expressed from synthetic transcripts that were injected into separated axolotl oocytes of 1.3–1.8 mm diameter. Oocytes were prepared from small ovary fragments either by manual dissection or by treatment with 1 mg/ml collagenase (Type II; Sigma) in MBS saline (88 mM NaCl, 1 mM KCl, 0.8 mM $MgSO_4$, 0.4 mM $CaCl_2$, 0.3 mM $Ca(NO_3)_2$, 2.4 mM $NaHCO_3$, 10 mM Hepes; pH 7.4) for about an hour, followed by rinsing in MBS. Oocytes were injected with 20 ng of RNA (1 μg/μl) into the cytoplasm and incubation at 19°C continued for the periods stated below.

Immunostaining and fluorescence microscopy

Fixed preparations were rinsed in PBS and blocked by incubation in 5% normal goat serum (NGS; Jackson Immunoresearch Laboratories) in PBS for 15 min. The spreads were then incubated for 1 h at room temperature with primary antibodies, rinsed briefly with 5% NGS and then incubated for 1 h with secondary antibodies diluted in PBS. Preparations were rinsed with PBS, with the penultimate rinse containing 4′,6-diamidino-2-phenylindole (DAPI) at 0.5 μg/ml), and then mounted in 50% glycerol/PBS. Primary antibodies were diluted as follows: rabbit polyclonal antiserum 83 against *Xenopus* CELF1/EDEN-BP (Paillard *et al*. 1998), 1:400 dilution; mAb 3B1 against human CELF1/CUG-BP1 (Timchenko *et al*. 1996 and Abcam plc), 1:500 dilution; mAb 9E10 (Calbiochem), 1 μg/ml; mAb H5 (Warren *et al*. 1992), culture supernatant 1:50 dilution; rabbit polyclonal antiserum anti-RPC15 (Murphy *et al*. 2002), 1:1000 dilution; mAb SE5 (Roth & Gall 1987) undiluted culture supernatant. Secondary antibodies, which were used at dilutions of 1–5 μg/ml, were Alexa 488-conjugated goat anti-mouse IgG, Alexa 594-conjugated goat anti-mouse IgM or goat anti-rabbit IgG (all obtained from Molecular Probes), and Cy2-conjugated goat anti-mouse Fc (gamma) fragment (Jackson Immunoresearch Laboratories).

Phase-contrast and fluorescence observations were made with an Olympus BX-60 microscope as described previously (Smith *et al*. 2003). Images were captured with a Princeton Instruments digital CCD camera (Roper Scientific) using IPLab imaging software (Scanalytics, Inc.) and processed with iVision-Mac (BioVision Technologies) and Adobe Photoshop (Adobe Systems, Inc.).

Results

CELF1 is localized to a small number of loop loci in amphibian LBCs

In human cultured cells CELF1/CUG-BP1 has a predominantly nuclear distribution when assayed by immunofluorescence staining using monoclonal antibody (mAb) 3B1 (Timchenko *et al*. 1996). Since proteins immunologically related to CELF1/CUG-BP1 are detected by mAb 3B1in immunoblots of cell extracts from a variety of vertebrates, including *Xenopus* (Timchenko *et al*. 1996), I first determined whether CELF1 was also detectable in nuclear structures of *Xenopus* oocytes. The giant nucleus or germinal vesicle (GV) of an amphibian oocyte contains lampbrush chromosomes and a variety of

nuclear bodies (reviewed by Gall *et al*. 2004) suspended in a relatively low-viscosity nucleoplasm that can be readily dispersed to produce spread preparations of nuclear structures. When GV spreads from oocytes of *Xenopus laevis* were immunostained with mAb 3B1, 20–30 chromosomal structures were stained distinctly but to varying extents in each spread. Some brightly-stained loci had a clearly

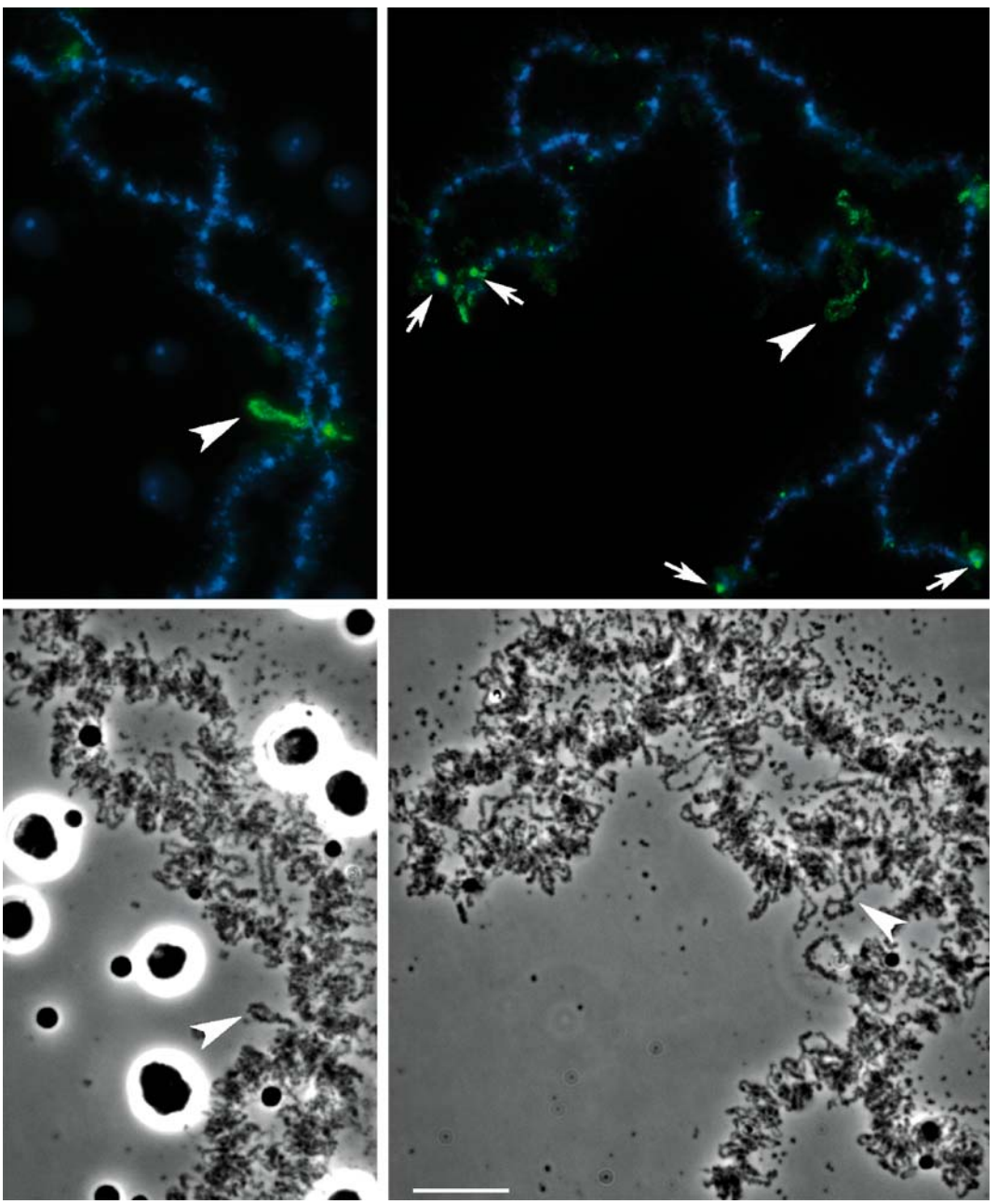


Figure 1. Immunostaining of CELF1 in *Xenopus laevis* LBCs. Two LBC bivalents immunostained with mAb 3B1 (green) and counterstained with DAPI (blue) to show the chromosome axes. The matching phase-contrast images are shown below. Loop-like stained loci are indicated by arrowheads and spot-like foci of staining by arrows. Where the loop loci are clear in the phase-contrast image at this magnification they are indicated by arrowheads. Scale bar represents 10 μm.

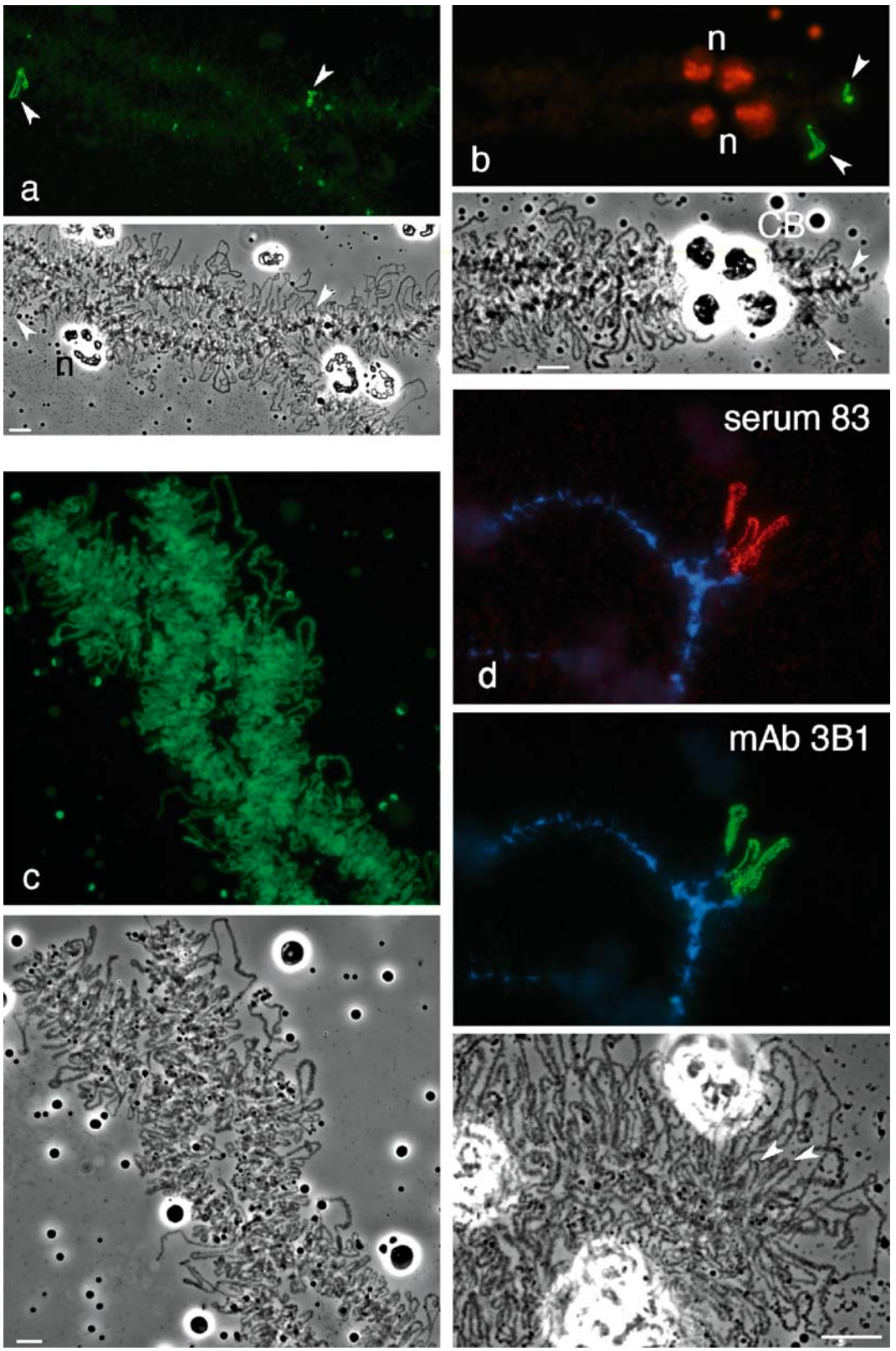
loop-like morphology while others appeared as small fluorescent specks or blobs that could have been collapsed or very short loops or perhaps non-loop structures (Figure 1). This highly restricted pattern of immunostaining was confirmed in LBCs from *X. tropicalis* and by using another CELF1 antibody, antiserum 83 (Paillard *et al.* 1998), raised against *Xenopus* CELF1/EDEN-BP.

In order to examine CELF1-stained loci in more detail I used LBCs from oocytes of urodele amphibians because their loops are more extended than those of *Xenopus*. As with the latter, LBC preparations from *Ambystoma mexicanum* (axolotl) and *Triturus vulgaris* (common newt), exhibited small numbers of loci in each spread that were brightly immunostained by mAb 3B1 and antiserum 83 (Figure 2). I estimate that up to about 50 different loci were convincingly stained by mAb 3B1 in preparations of well-extended LBCs, with somewhat fewer being detectable in the more contracted LBCs obtained from larger oocytes, presumably because of the reduced mass of many of their loops. Up to 30 distinct loci were also stained by antiserum 83, slightly fewer than the maximum obtained with mAb 3B1; antiserum 83 identified the same loops as mAb 3B1, although some mAb 3B1-stained loops exhibited weak or unremarkable antiserum 83 staining because of the somewhat lower signal-to-noise ratio exhibited by the latter antibody. The conservation of this highly specific immunostaining in both urodele and *Xenopus* LBCs in itself suggests that it gives a reliable indicator of the distribution of CELF1. Such a highly restricted pattern of CELF1 distribution among LBC loci is also clearly different from the widespread distribution across most LBC loops usually found for RNA-binding proteins. For example, Figure 2c shows that mAb SE5 (Roth & Gall 1987), an antibody prepared against a newt protein that is targeted generally to LBC nascent transcripts (Roth & Gall 1989), brightly immunostains the RNP matrices of most loops of axolotl LBCs.

In order to confirm the reproducibility of the immunostaining by CELF1 antibodies, I used axolotl LBCs and the working map of these chromosomes produced by Callan (1966). This map allows the unambiguous identification of many LBCs on purely morphological grounds, with LBC3 in particular being readily identifiable because, in addition to its relative length, it exhibits (1) the frequent occurrence of nucleoli at the nucleolus organizer region (NOR), which lies close to the right telomere (Figure 2b); (2) the common presence of a chiasma between the NOR and the telomere; (3) the presence in the right arm of a single, usually small Cajal body. In all preparations examined, the right arm of LBC3 bore one of the most prominent examples of CELF1 immunostaining, exhibiting a small but brightly stained loop or derived object in an almost telomeric location (Figure 2b) that was reproducibly stained by both CELF1 antibodies (Figure 2d).

Most of the convincingly stained chromosomal structures observed in optimal axolotl LBC preparations could clearly be recognized as lateral loops or their derivatives but were unremarkable with regard to their length or matrix morphology in phase contrast (Figures 1 and 2). For many loops it was also obvious that, as would be predicted from the known properties and functions of CELF1, immunostaining was predominantly localized to the nascent RNP matrix cloaking the central loop axis rather than being confined to the axis itself. Other LBC landmark structures such as terminal, axial or suspended granules (*sensu* Callan 1966) were not reproducibly immunostained, and although some extrachromosomal bodies were immunostained I have not analysed these systematically. Where the pairs of sister loops were well displayed, both were stained similarly, but in a

Figure 2. Immunostaining of CELF1 loci in axolotl LBCs. (a) A low-magnification survey of part of LBC6 immunostained with mAb 3B1. The arrowheads indicate two brightly-stained loop loci that are apparently heterozygous with respect to the level of activity shown by the homologous loci. The positions of the stained loops are indicated in the phase-contrast image below (*n*=extrachromosomal nucleolus). (b) Low-magnification survey of the end of the right arm of LBC3 immunostained for CELF1 with mAb 3B1 (green) and for pol I and pol III with anti-RPC15 serum (red). The characteristic CELF1 small loop loci at the end of both half bivalents are indicated by arrowheads and the pol I-containing cores of the nucleoli attached at the NOR are indicated (n). In the matching phase-contrast image below, the positions of the CELF1 loops are shown by arrowheads and two Cajal bodies, which are also stained by anti-RPC15 serum, are indicated (CB). (c) Low-magnification survey of an LBC stained with mAb SE5, which recognizes a general nascent transcript protein and immunostains most loops. Phase-contrast image below. (d) More detailed image of CELF1-positive loops close to the right telomere of LBC3 co-immunostained with antiserum 83 (red) against CELF1/EDEN-BP and mAb 3B1 (green) against CELF1/CUG-BP1 and counterstained with DAPI (blue). Where visible in the phase-contrast image below, the corresponding loops are indicated by arrowheads. Scale bars represent 10 μm.


a
n
b
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n
CB
serum 83
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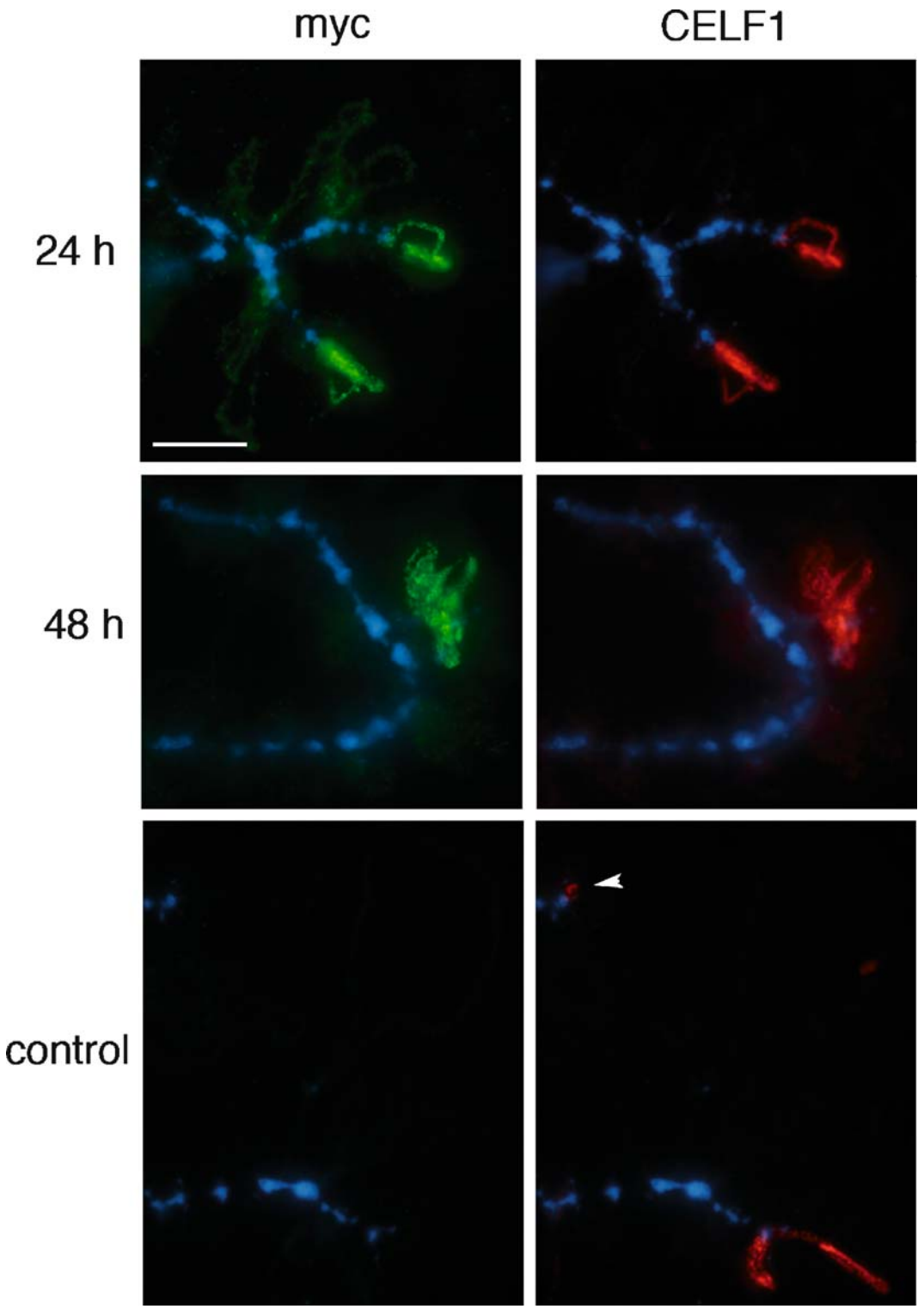
c
mAb 3B1


myc
CELF1
24 h
48 h
control

number of instances (e.g. Figures 2a and 3 control) homologous loci had such a distinctively different appearance that it seems that very different levels of transcriptional activity rather than technical factors such as differential spreading are the cause. Such morphological heterozygosity is a well-established feature of certain LBC loci that appear as unusual and/or complex 'marker' loops, but it clearly also is a feature of the less obvious but more typical loops identified by CELF1 immunostaining.

Targeting of human CELF1/CUG-BP1 to specific nascent transcripts in LBCs

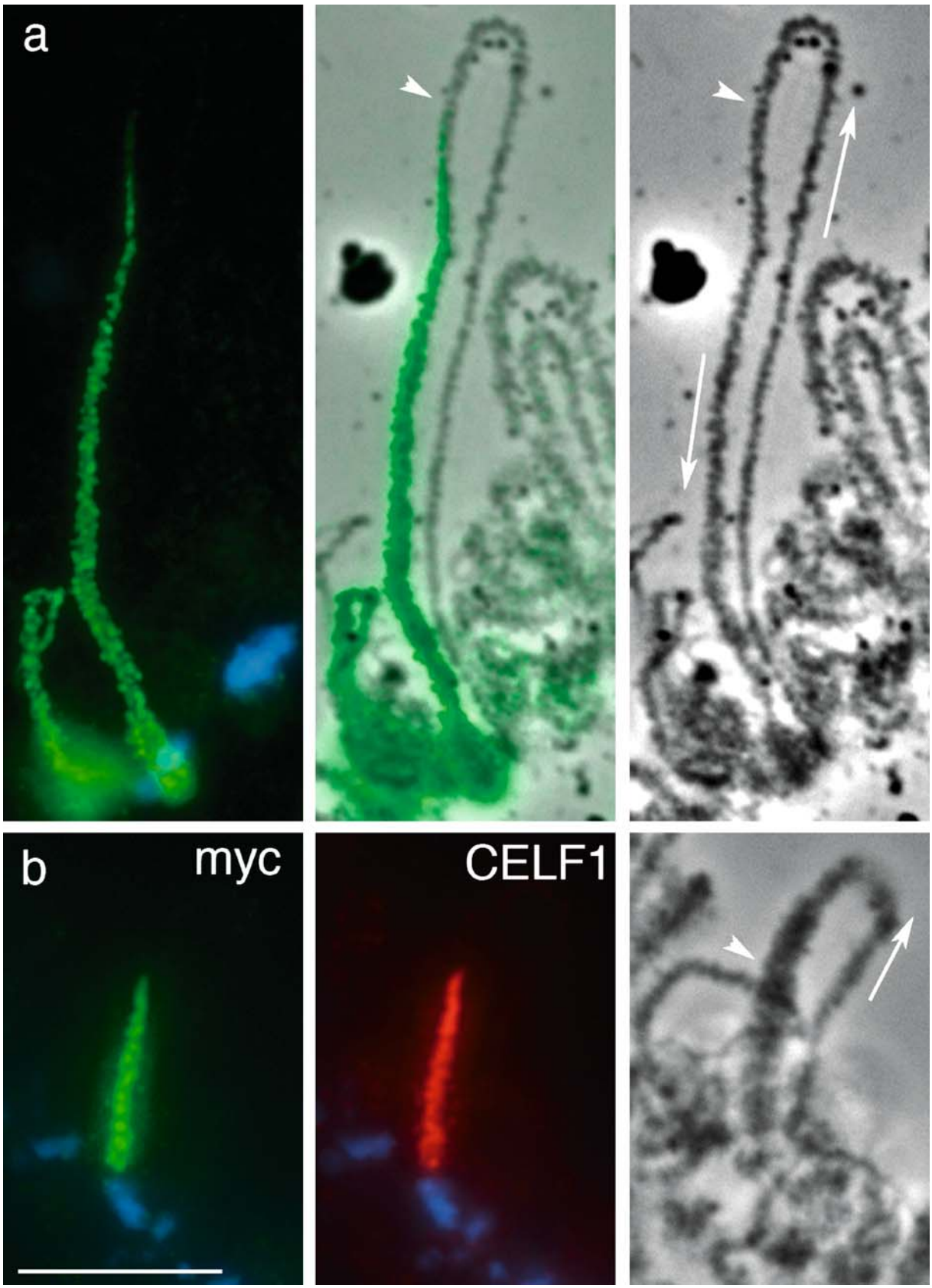
To confirm that the conserved and highly reproducible immunostaining patterns described above are a result of *bona fide* cross reaction between the antibodies and CELF1 in nascent RNP, I examined the targeting behaviour of exogenous CELF1 translated from synthetic transcripts injected into the cytoplasm of oocytes. This approach has been used successfully to examine the localization in amphibian GVs of nuclear proteins ranging from transcription factors to the general components of nascent transcripts (e.g. Roth & Gall 1989, Jantsch & Gall 1992, Bellini *et al*. 1993, Morgan *et al*. 2000, Smillie & Sommerville 2002, Smith *et al*. 2003). Also, in order to determine whether nascent transcript localization is a conserved feature of CELF1 function or an amphibian-specific property, the expression construct consisted of a *myc*-tagged fusion of human CELF1/CUG-BP1. GV spread preparations were made from injected and uninjected oocytes after incubation for 1–2 days to allow for expression of the tagged protein.

LBC preparations made from injected and uninjected oocytes were immunostained with mAb 9E10 to detect the *myc* epitope. The RNP matrices of a small number of loops were brightly-stained by mAb 9E10 only in preparations from injected oocytes, indicating the specific targeting of the CELF fusion protein to nascent transcripts. Moreover, the loops targeted included those previously identified as containing endogenous CELF1; Figure 3 shows examples of *myc*-CUG-BP1 targeting to the loops at the right end of LBC3. Some preparations were also co-stained with antiserum 83 (Figure 3) in order to confirm that it was not simply loops neighbouring the endogenous CELF1 loci that had bound *myc*-CELF1. In addition, the antiserum 83 co-staining demonstrated that the bright mAb 9E10 staining observed in injected oocytes was not the result of spurious cross-reaction, a necessary control since weak staining of a small number of other loops by mAb 9E10 can occur in axolotl and other LBC preparations (Bellini *et al*. 1993). The general pattern of mAb 9E10 staining in LBCs from injected oocytes was remarkably similar to that found for uninjected oocytes immunostained with the anti-CELF1 antibodies, although in general fewer loop loci were brightly-stained for *myc*-CELF. The high selectivity of CELF1 targeting to specific nascent transcripts stands in contrast to that observed for other RNA-binding proteins examined in oocytes in which tagged proteins become generally distributed among virtually all loops (Roth & Gall 1989, Jantsch & Gall 1992, Bellini *et al*. 1993, Eckmann & Jantsch 1999, Smillie & Sommerville 2002). The highly specific nature of CELF1 targeting is emphasized by the occurrence of loops in which the tagged protein is confined to only part of their length (Figure 4c); this unusual targeting pattern resembles immunostaining patterns that were obtained with both CELF1 antibodies and is considered in detail in the next section.

CELF1 recruitment can be highly localized within transcription units and within nascent transcripts

Instead of the almost loop-like track of immunostaining apparent in well-spread examples of, for example, the terminal loops of LBC3, in about half of the CELF1 loci in axolotl LBCs the immunostaining appeared as an unlooped straight line with only one end attached to the chromosome axis (Figure 4). Closer examination of both endogenous and tagged CELF1 immunostaining patterns revealed that this appearance was due to localized staining of only part

Figure 3. Targeting of *myc*-tagged CELF1/CUG-BP1 to axolotl LBC3. LBC spreads prepared from oocytes injected with transcripts encoding *myc*-CELF1/CUG-BP and incubated for about 24 h or 48 h. Preparations were co-immunostained with mAb 9E10 directed against the *myc* tag (green) and antiserum 83 (red) against CELF1/EDEN-BP, and counterstained with DAPI (blue) to show the chromosome axis. The characteristic small loop-locus at the right telomere of LBC3 is shown for each of the injected preparations and for the uninjected control preparation at the bottom. Note in the control preparation that the CELF1 locus on one half bivalent (arrowhead) appears virtually inactive. Scale bar represents 10 μm.


a
b
myc
CELF1

of the loop rather than to mechanical breakage of a completely stained loop producing a linear loop fragment. Moreover it was clearly due to staining of only part of the RNP matrix that comprised a single transcription unit (TU) rather than to complete staining of a single TU within a loop that contains multiple TUs. One of the crucial advantages of LBC loops for studying gene activity *in situ* is that for some loops it is possible to discern a thin-to-thick asymmetry in the distribution of the RNP matrix along their length; this asymmetry reflects the gradually increasing length of the transcripts in an array of nascent transcripts that results from continuous transcription. Therefore, units of matrix asymmetry not only define a transcription unit, they also allow the direction of transcription within that TU to be deduced. In cases where only part of a TU was immunostained, the portion containing CELF1 was always the downstream (promoter-distal) part of the TU rather than the promoter proximal one. Moreover, where the distal region of a loop could be followed back to its re-insertion into the DAPI-stained chromomeric axis of the LBC (e.g. Figure 4a), it appeared that CELF 1 persisted in the RNP matrix of a TU until its presumptive termination site. The portion of a TU associated with CELF1 was often less than 50% of total TU length, as in the examples in Figure 4. In calculating the absolute length of template in a given length of loop chromatin, it is widely assumed that nucleosomes, and their concomitant compaction of DNA, do not form in lampbrush loops because of the maximal pol II packing densities characteristic of these TUs. So, for example, it appears that the best spread loop in Figure 4a contains a TU comprising about 70 μm, or 210 kb, of B-form DNA of which only the promoter-distal 90 kb is associated with CELF1.

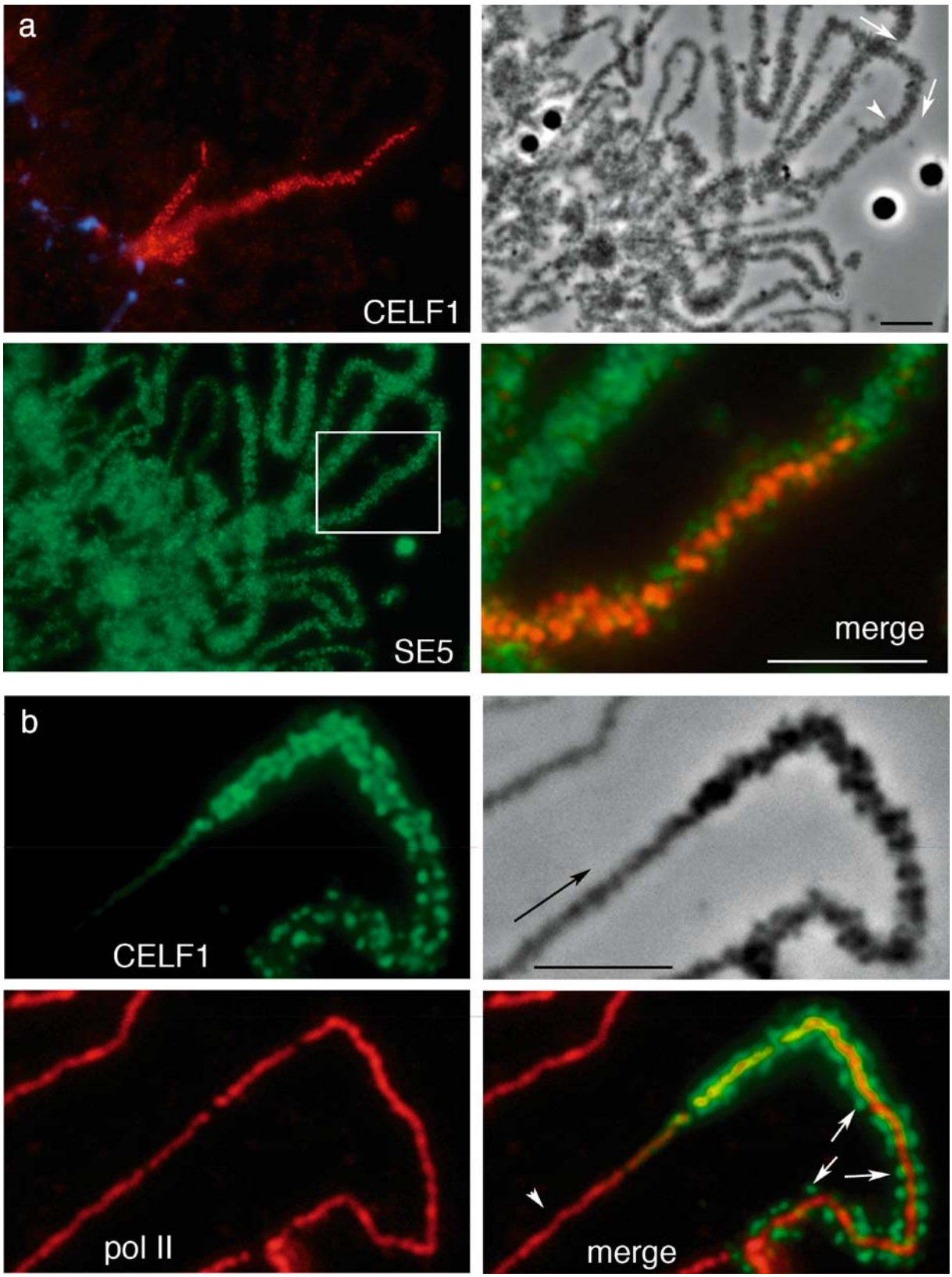
The underlying molecular explanation for localized TU staining is suggested by detailed consideration of nascent transcript arrays in loops co-stained for CELF1 and a second loop constituent, as shown in Figure 5. At the point at which CELF1 is first detectable in the RNP matrix it appears to be confined to the central region of the nascent transcript array rather than decorating the full width of the array in the manner of a more general transcript-binding protein such as SE5 (see inset in Figure 5a). Indeed, so far as the resolution limit of the light microscope allows, it seems that at its initial site of recruitment CELF1 is co-localized with the transcription complexes of the loop axis since CELF1 and pol II staining are coincident (Figure 5b). In more downstream regions of the array, CELF1 appears displaced into non-axial regions of the RNP matrix and is no longer co-localized with the diffraction-limited line of pol II staining (Figure 5b, merge). As discussed below, this pattern presumably reflects the gradual outward movement of transcript-bound CELF1 as the nascent transcripts lengthen due to progressive chain elongation during transcription. It is also apparent that in these distal regions of a transcription unit the CELF1 staining pattern often becomes distinctly punctate (Figures 4a, 5b) although the RNP matrix does not appear particularly granular in phase contrast.

Overall, these patterns of CELF1 localization within loop matrices suggest a mode of association with nascent transcripts in which there is a single defined entry point into the transcript array followed by continued association of CELF1 with nascent RNA until the end of the TU. The resultant partial TU-staining patterns provide intuitive evidence for the dynamic and highly specific co-transcriptional recruitment of sequence-specific RNA-binding proteins to nascent RNA within active eukaryotic genes.

Discussion

Evidence for the co-transcriptional binding of the multifunctional protein CELF1 to nascent transcripts of amphibian lampbrush chromosomes (LBCs) was obtained by immunostaining and from the targeting exhibited by epitope-tagged human CELF1. Similar immunolocalization patterns were obtained for *Xenopus, Triturus* and axolotl LBCs and in the latter

Figure 4. Localized recruitment of CELF1 to axolotl lampbrush loops. (a) A pair of lateral loops immunostained for endogenous CELF1 with mAb 3B1 (green) and counterstained with DAPI (blue) to show the chromomeres of the chromosome axis. In the phase-contrast image (right) the more extended of the two sister loops demonstrates the asymmetric distribution of the loop RNP matrix from which the direction of transcription can be deduced (arrows). The central panel is a blend of the CELF1 fluorescent image and the phase-contrast image to indicate the point in the RNP gradient at which CELF1 becomes detectable (arrowhead). (b) Lateral loop from an oocyte expressing *myc*-tagged CELF1/CUG-BP1 co-immunostained with mAb 9E10 directed against the *myc* tag (green) and antiserum 83 (red) against CELF1/EDEN-BP and counterstained with DAPI (blue). In the phase-contrast image (right) the direction of transcription in this transcription unit (arrow) and the point at which CELF1 immunostaining is first detected (arrowhead) are indicated. Scale bar represents 10 μm.


a
CELF1
SE5
merge
b
CELF1
pol II
merge

case up to 50 loci were estimated to recruit CELF1. Since this is less than 1% of the likely total number of lateral loop loci, CELF1 appears to be targeted to nascent transcripts in a highly specific manner, as would be predicted from its functions as a regulator of a restricted subset of genes. However, even the relatively small number of loops identified as CELF1 targets suggests that the number of maternally-expressed RNAs that are regulated posttranscriptionally by this protein could be far higher than the several mRNA substrates for CELF1/EDEN-BP so far identified in *Xenopus* (Paillard & Osborne 2003). The co-transcriptional nature of the binding of CELF1 is clear from its presence in the RNP matrices that surround the DNA axes of targeted loops. Two different types of CELF1 localization within the matrix were found; for some loops the matrix appeared stained around the whole length of the loop, while in others CELF was localized to only a part of the matrix that started at an internal point in the loop and continued to the apparent site of transcription termination. A definitive explanation for the molecular basis of these two patterns would require a detailed understanding of the DNA sequence organization of the underlying transcription units. For instance, it could be argued that such patterns result from the presence at appropriate locations in rare transcription units of CUG-containing repetitive sequences. However, CELF1 is known to interact with either the 5′ UTR or the 3′ UTR of various mRNAs as well as with internal exons or introns in its role as an alternative splicing factor. Binding to the 5′ UTRs of a given nascent transcript array would be consistent with CELF1 being present in the RNP matrix all along a TU, while, as explained below, the other interaction sites would result in partially stained loops. Partially immunostained loops are unusual in LBCs and have two possible origins: one, for which there are known examples, occurs when there are two or more transcription units in a loop but the nascent RNP of only one is associated with a given protein (see examples in Lacroix *et al*. 1985, Gall & Murphy 1998). Partial staining might also be found in loops that comprise a single transcription unit if transcripts in only part of the transcript array were able to recruit the protein. The latter explanation clearly applies to the partial loop staining observed for CELF1 and to my knowledge this is the first example of this type of protein localization in a typical LBC loop.

In some loops the partially stained nascent RNP was so well displayed that some details of the mechanisms involved in the co-transcriptional recruitment of CELF1 to active genes can be inferred (summarized in Figure 6). One obvious feature is that CELF1 does not enter a transcription unit until after a defined point, which can be many kb downstream of the presumptive site of transcription initiation. It seems most likely that this point of entry marks the place in the transcription unit where a binding site for CELF1 is first transcribed into RNA and extruded from pol II. At this point the CELF1 immunostaining appears abruptly and overlies the DNA axis of the loop but it does not extend into the surrounding RNP matrix (see for example Figure 5b). The initial co-localization of CELF1 and pol II seem most simply explained by the newly-transcribed CELF1 interaction sites in nascent RNA not yet being a sufficient distance from the transcription complex for the two to be resolved by the light microscope (Figure 6). Another possible explanation for their co-localization is that CELF1 first associates with pol II before being 'handed off' onto the transcript when a binding site appears as a result of further transcription (Bentley 2005). However, if there were a period of prior association of CELF1 with pol II it would be short in comparison with the period during which pol II transcribes the extensive promoter proximal regions that precede the point at which CELF1 is first detected in many TUs.

Figure 5. Localization of CELF1 within nascent transcript arrays. (a) A pair of lateral loops from axolotl LBCs co-immunostained for CELF1 using antiserum 83 (red) and for a general nascent transcript protein using mAb SE5 (green), and counterstained with DAPI (blue in upper panel). In the phase-contrast image (right) the matrix asymmetry in the more extended transcription unit indicates the direction of transcription (arrows) and the point at which CELF1 is first detectable is indicated with an arrowhead. An enlarged merged image of CELF1 and SE5 co-staining shows the initial axial localization of CELF1 in contrast to the matrix-wide distribution of SE5. The region selected for enlargement is indicated by the box in the mAb SE5-stained image. (b) Part of a transcription unit from LBCs of the newt, *Triturus vulgaris*, co-immunostained for CELF1 using mAb 3B1 (green) and for pol II using mAb H5 (red). In the phase-contrast image (right) the matrix asymmetry indicates the direction of transcription (arrow). In the merged image of CELF1 and pol II immunostaining the point at which CELF1 is first detectable is indicated (arrowhead) and in more downstream regions of the nascent transcript array arrows indicate where CELF1 is not co-localized with pol II. Scale bars represent 5 μm.

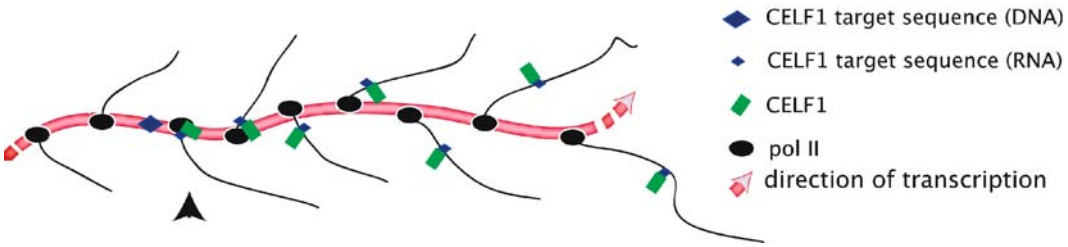


Figure 6. Localized recruitment of CELF1 by nascent transcripts. Interpretation of partial loop immunostaining patterns of the type shown in Figures 4 and 5. The interaction of CELF1 protein with nascent transcripts (arrowhead) begins shortly after the DNA sequence specifying its binding site is transcribed and the corresponding RNA sequence becomes extruded from pol II as a result of continued transcription elongation. As the nascent transcripts grow even longer during extended transcription elongation, CELF1 remains attached to the RNA binding site and so is moved away from the pol II-coated DNA axis of the transcription unit. Although a single molecular interaction per transcript is drawn here for the sake of simplicity, it is also possible that there are multiple binding sites and bound CELF1 molecules at a single region in each transcript. Similarly, potential protein–protein interactions between CELF1 and other transcript-binding proteins that could play a role in initiating and/or maintaining CELF1 recruitment by nascent transcripts are not included.

Another feature of the CELF1 recruitment is revealed by the increasing distance between the CELF1-stained regions of the RNP matrix and the loop axis as transcription continues, as can be seen most clearly in the pol II co-stained example shown in Figure 5b. The simple explanation for this increasing displacement of CELF1 away from the transcription complexes, as indicated in the diagram in Figure 6, is that as nascent transcripts are extended from their 3′ ends by transcription, the CELF1 binding site and the attached protein move farther away from the template. It also appears from this pattern of localization that after an initial period of recruitment CELF1 ceases to be recruited to transcripts in the more downstream regions of the TU, otherwise the nascent RNP matrix would be immunostained in axial and non-axial regions alike. In this respect the recruitment of CELF1 is highly localized within each and every nascent transcript of the array rather than exhibiting the continuous recruitment or accretion over the length of a transcript that is suggested by the homogeneous distribution in matrix RNP of other RNA-binding proteins (e.g. SE5 in Figure 5a). This high degree of localization within the transcript presumably results from the more specific nature of CELF1 target sequences compared to other nascent transcript-binding proteins examined previously, and could be a distinguishing feature of regulatory proteins compared with those involved in constitutive processes.

Localization of CELF1 in nascent transcript arrays appears to persist until transcription termination and transcript release. Given the distances transcribed in some lampbrush loops after CELF1 recruitment and a rate of RNA chain elongation of 1–2 kb/min, CELF1 can be associated with individual nascent transcripts for at least an hour. Such a prolonged interaction could be an essential property of regulators that are required to preprogram the cytoplasmic fates of mRNAs such as their stability, localization and translation. However, without knowing the identity of the underlying transcription units it is possible that the recruitment of CELF1 to LBC nascent transcripts observed here actually reflects the functions of this protein in controlling alternative splicing patterns rather than later events. At the molecular level the apparent persistence of CELF1 in nascent transcripts could mask a continuous dynamic exchange of individual CELF1 molecules onto and off transcripts, as appears to be the case for many other nuclear processes and structures (reviewed in Misteli 2001). Dynamic exchange of nascent transcript proteins as well as *de novo* incorporation into nascent transcripts during transcription presumably underlie the precise targeting observed for *myc*-CUG-BP1 to loops that normally associate with endogenous CELF1. Overall, the success of this targeting approach suggests that the expression of tagged proteins in oocytes has the specificity, sensitivity and resolution to provide a robust method for assessing the potential involvement of known or suspected RNA-binding regulators in co-transcriptional processes at the single gene level.

The finding that human CELF1/CUG-BP1 is targeted to LBC transcription units has implications for understanding the missplicing of certain transcripts that is a feature of the neuromuscular disease myotonic dystrophy (DM). Current models for the molecular basis of splicing misregulation in DM invoke the increased levels of CUG-BP1 and the decreased levels of another splicing regulator, MBNL1, that is its normal antagonist (Cooper 2006, Mahadevan *et al*. 2006). It has recently been shown that by manipulating the relative amounts of CUG-BP1 and MBNL1 in affected cells so that they more closely resemble normal levels, missplicing effects can be reversed (Kanadia *et al*. 2006, Mahadevan *et al*. 2006). Given that CUG-BP1 is recruited co-transcriptionally to LBCs, it may be that the competition between CUG-BP1 and MBNL1 that apparently controls splicing patterns in normal and DM cells also occurs on nascent transcripts. Recent evidence suggests that one consequence of the occurrence of splicing on nascent transcripts is the existence of a 'kinetic coupling' between transcription and splicing such that the relative frequencies of the alternative splicing products exhibited by a pre-mRNA can be affected by rates of transcription elongation (reviewed by Bentley 2005). It is possible then that due to the co-transcriptional nature of their recruitment, the effect of CUG-BP1/MBNL1 competition on alternative splicing outcomes may be different for different genes according to their local transcription elongation rates. Another implication from the finding that, at least in LBCs, an mRNA-binding protein like CELF1 is also present in nascent transcripts, is that such proteins clearly should be regarded as components of chromatin. Therefore they, and the genes they target, may be amenable to study using the wide array of chromatin-based analytical approaches.

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References

Aguilera A (2005) Cotranscriptional mRNP assembly: from the DNA to the nuclear pore. *Curr Opin Cell Biol* **17**: 242–250.

Andrulis ED, Werner J, Nazarian A, Erdjument-Bromage H, Tempst P, Lis JT (2002) The RNA processing exosome is linked to elongating RNA polymerase II in *Drosophila*. *Nature* **420**: 837–841.

Barreau C, Paillard L, Mereau A, Osborne HB (2006) Mammalian CELF/Bruno-like RNA-binding proteins: molecular characteristics and biological functions. *Biochimie* **88**: 515–525.

Bellini M, Lacroix JC, Gall JG (1993) A putative zinc-binding protein on lampbrush chromosome loops. *EMBO J* **12**: 107–114.

Bentley D (2002) The mRNA assembly line: transcription and processing machines in the same factory. *Curr Opin Cell Biol* **14**: 336–342.

Bentley DL (2005) Rules of engagement: co-transcriptional recruitment of pre-mRNA processing factors. *Curr Opin Cell Biol* **17**: 251–256.

Callan HG (1986) *Lampbrush Chromosomes*. Berlin: Springer-Verlag.

Callan HG (1966) Chromosomes and nucleoli of the axolotl, *Ambystoma mexicanum*. *J Cell Sci* **1**: 85–108.

Cooper TA (2006) A reversal of misfortune for myotonic dystrophy? *N Engl J Med* **355**: 1825–1827.

Daneholt B (2001) Assembly and transport of a premessenger RNP particle. *Proc Natl Acad Sci USA* **98**: 7012–7017.

Dansithong W, Paul S, Comai L, Reddy S (2005) MBNL1 is the primary determinant of focus formation and aberrant insulin receptor splicing in DM1. *J Biol Chem* **280**: 5773–5780.

Dreyfuss G, Matunis MJ, Pinol-Roma S, Burd CG (1993) hnRNP proteins and the biogenesis of mRNA. *Annu Rev Biochem* **62**: 289–321.

Eckmann CR, Jantsch MF (1999) The RNA-editing enzyme ADAR1 is localized to the nascent ribonucleoprotein matrix on Xenopus lampbrush chromosomes but specifically associates with an atypical loop. *J Cell Biol* **144**: 603–615.

Fardaei M, Larkin K, Brook JD, Hamshere MG (2001) *In vivo* co-localisation of MBNL protein with DMPK expanded-repeat transcripts. *Nucleic Acids Res* **29**: 2766–2771.

Gall J (1998) Spread preparation of *Xenopus germinal* vesicle contents. In: Spector D, Goldman R, Leinwand L eds. *Cells. A Laboratory Manual*, vol. 1. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp 52.51–52.54.

Gall JG, Murphy C (1998) Assembly of lampbrush chromosomes from sperm chromatin. *Mol Biol Cell* **9**: 733–747.

Gall JG, Wu Z, Murphy C, Gao H (2004) Structure in the amphibian germinal vesicle. *Exp Cell Res* **296**: 28–34.

Gilbert C, Kristjuhan A, Winkler GS, Svejstrup JQ (2004) Elongator interactions with nascent mRNA revealed by RNA immunoprecipitation. *Mol Cell* **14**: 457–464.

Good PJ, Chen Q, Warner SJ, Herring DC (2000) A family of human RNA-binding proteins related to the *Drosophila* Bruno translational regulator. *J Biol Chem* **275**: 28583–28592.

Guthrie C, Steitz J (2005) Nucleus and gene expression. Coordinated nuclear events regulate mRNA synthesis, processing, export and turnover. *Curr Opin Cell Biol* **17**: 239–241.

Jantsch MF, Gall JG (1992) Assembly and localization of the U1-specific snRNP C protein in the amphibian oocyte. *J Cell Biol* **119**: 1037–1046.

Kanadia RN, Shin J, Yuan Y, *et al*. (2006) Reversal of RNA missplicing and myotonia after muscleblind overexpression in a mouse poly(CUG) model for myotonic dystrophy. *Proc Natl Acad Sci USA* **103**: 11748–11753.

Kloc M, Etkin LD (2005) RNA localization mechanisms in oocytes. *J Cell Sci* **118**: 269–282.

Kotovic KM, Lockshon D, Boric L, Neugebauer KM (2003) Cotranscriptional recruitment of the U1 snRNP to intron-containing genes in yeast. *Mol Cell Biol* **23**: 5768–5779.

Krecic AM, Swanson MS (1999) hnRNP complexes: composition, structure, and function. *Curr Opin Cell Biol* **11**: 363–371.

Lacroix JC, Azzouz R, Boucher D, Abbadie C, Pyne CK, Charlemagne J (1985) Monoclonal antibodies to lampbrush chromosome antigens of *Pleurodeles waltlii*. *Chromosoma* **92**: 69–80.

Ladd AN, Charlet N, Cooper TA (2001) The CELF family of RNA binding proteins is implicated in cell-specific and developmentally regulated alternative splicing. *Mol Cell Biol* **21**: 1285–1296.

Ling Y, Smith AJ, Morgan GT (2006) A sequence motif conserved in diverse nuclear proteins identifies a protein interaction domain utilised for nuclear targeting by human TFIIS. *Nucleic Acids Res* **34**: 2219–2229.

Mahadevan MS, Yadava RS, Yu Q, *et al*. (2006) Reversible model of RNA toxicity and cardiac conduction defects in myotonic dystrophy. *Nat Genet* **38**: 1066–1070.

Misteli T (2001) Protein dynamics: implications for nuclear architecture and gene expression. *Science* **291**: 843–847.

Morgan GT (2002) Lampbrush chromosomes and associated bodies: new insights into principles of nuclear structure and function. *Chromosome Res* **10**: 177–200.

Morgan GT, Doyle O, Murphy C, Gall JG (2000) RNA polymerase II in Cajal bodies of amphibian oocytes. *J Struct Biol* **129**: 258–268.

Murphy C, Wang Z, Roeder RG, Gall JG (2002) RNA polymerase III in Cajal bodies and lampbrush chromosomes of the *Xenopus* oocyte nucleus. *Mol Biol Cell* **13**: 3466–3476.

Neugebauer KM (2002) On the importance of being co-transcriptional. *J Cell Sci* **115**: 3865–3871.

Osheim YN, Miller OL Jr, Beyer AL (1985) RNP particles at splice junction sequences on *Drosophila* chorion transcripts. *Cell* **43**: 143–151.

Paillard L, Osborne HB (2003) East of EDEN was a poly(A) tail. *Biol Cell* **95**: 211–219.

Paillard L, Omilli F, Legagneux V, Bassez T, Maniey D, Osborne HB (1998) EDEN and EDEN-BP, a cis element and an associated factor that mediate sequence-specific mRNA deadenylation in *Xenopus* embryos. *EMBO J* **17**: 278–287.

Pinol-Roma S, Swanson MS, Gall JG, Dreyfuss G (1989) A novel heterogeneous nuclear RNP protein with a unique distribution on nascent transcripts. *J Cell Biol* **109**: 2575–2587.

Proudfoot NJ, Furger A, Dye MJ (2002) Integrating mRNA processing with transcription. *Cell* **108**: 501–512.

Roth MB, Gall JG (1987) Monoclonal antibodies that recognize transcription unit proteins on newt lampbrush chromosomes. *J Cell Biol* **105**: 1047–1054.

Roth MB, Gall JG (1989) Targeting of a chromosomal protein to the nucleus and to lampbrush chromosome loops. *Proc Natl Acad Sci USA* **86**: 1269–1272.

Savkur RS, Philips AV, Cooper TA (2001) Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. *Nat Genet* **29**: 40–47.

Singh OP, Visa N, Wieslander L, Daneholt B (2006) A specific SR protein binds preferentially to the secretory protein gene transcripts in salivary glands of *Chironomus tentans*. *Chromosoma* **115**: 449–458.

Smillie DA, Sommerville J (2002) RNA helicase p54 (DDX6) is a shuttling protein involved in nuclear assembly of stored mRNP particles. *J Cell Sci* **115**: 395–407.

Smith AJ, Ling Y, Morgan GT (2003) Subnuclear localization and Cajal body targeting of transcription elongation factor TFIIS in amphibian oocytes. *Mol Biol Cell* **14**: 1255–1267.

Solovei I, Macgregor H, Gaginskaya E (1995) Single stranded nucleic acid binding structures on chicken lampbrush chromosomes. *J Cell Sci* **108**: 1391–1396.

Sommerville J, Crichton C, Malcolm DB (1978) Immunofluorescent localization of transcriptional activity on lampbrush chromosomes. *Chromosoma* **66**: 99–114.

Sun X, Zhao J, Kylberg K, *et al*. (2004) Conspicuous accumulation of transcription elongation repressor hrp130/CA150 on the intron-rich Balbiani ring 3 gene. *Chromosoma* **113**: 244–257.

Timchenko LT, Miller JW, Timchenko NA, *et al*. (1996) Identification of a (CUG)n triplet repeat RNA-binding protein and its expression in myotonic dystrophy. *Nucleic Acids Res* **24**: 4407–4414.

Timchenko NA, Welm AL, Lu X, Timchenko LT (1999) CUG repeat binding protein (CUGBP1) interacts with the 5′ region of C/EBPbeta mRNA and regulates translation of C/EBPbeta isoforms. *Nucleic Acids Res* **27**: 4517–4525.

Timchenko NA, Cai ZJ, Welm AL, Reddy S, Ashizawa T, Timchenko LT (2001) RNA CUG repeats sequester CUGBP1 and alter protein levels and activity of CUGBP1. *J Biol Chem* **276**: 7820–7826.

Tuma RS, Stolk JA, Roth MB (1993) Identification and characterization of a sphere organelle protein. *J Cell Biol* **122**: 767–773.

Warren SL, Landolfi AS, Curtis C, Morrow JSW (1992) Cytostellin: a novel, highly conserved protein that undergoes continuous redistribution during the cell cycle. *J Cell Sci* **103**: 381–388.

Wu ZA, Murphy C, Callan HG, Gall JG (1991) Small nuclear ribonucleoproteins and heterogeneous nuclear ribonucleoproteins in the amphibian germinal vesicle: loops, spheres, and snurposomes. *J Cell Biol* **113**: 465–483.