Accurate and Efficient Transcription of Human *c-myc* Genes Injected into *Xenopus laevis* Oocytes

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We investigated the expression of the cloned human *c-myc* gene in *Xenopus laevis* oocytes microinjected with different recombinants. We found that microinjected plasmid DNA carrying an intact human *c-myc* gene directs efficient and faithful transcription from its own two promoters in *X. laevis* oocytes. This active transcription was unaffected by the presence of previously identified enhancing elements such as simian virus 72-base pair repeats or mouse immunoglobulin heavy-chain gene enhancer sequences in the construct *in cis*. This suggests that all necessary DNA sequences for accurate and faithful transcription recognized by the transcription machinery of the frog oocyte are self-contained. In addition, we have found that human *c-myc* transcripts synthesized in oocytes are properly polyadenylated at either one of two sites and also that the transcripts are spliced correctly but with low efficiency.

The structure of human and mouse *c-myc* genes is now known in detail (10, 15, 59, 62, 63). The gene is encoded in three exons separated by two introns. The first exon, about 550 base pairs (bp) in length, is entirely non-coding. It contains two TATA sequences, such as commonly found at the 5' end of eucaryotic genes transcribed by RNA polymerase II (12), and two initiation sites, separated from one another by about 165 bp. The unusually long noncoding exon sequence is tightly conserved between human and mouse genes (7, 10), suggesting a possible role for this portion of the gene in transcriptional or translational control (38, 55). The remaining part of the *c-myc* gene contains two coding exons that direct the synthesis of a protein 439 amino acids in length (63).

Several different alterations in the *c-myc* locus, such as gene amplification (3, 16, 21, 40), retroviral promoter (34, 46) and enhancer (17) insertion, and chromosomal translocation (1, 2, 19, 22, 32, 57, 59, 61), have been discovered in tumor cells. Activation of the *c-myc* genes involved in such drastic changes has been reported in most of these tumors. The importance of temporal regulation during the cell cycle and cell differentiation has been also reported (14, 36). Study of the cell cycle dependency of *c-myc* gene expression in fibroblasts and lymphocytes has indicated that *c-myc* is an inducible gene regulated by specific growth signals and also that the expression starts and peaks in the early G1 phase of the cell cycle (14, 36). The effect of *in vitro*-induced cell differentiation on *c-myc* expression has also been investigated. During the differentiation of teratocarcinoma stem cells to endoderm cells (14), *c-myc* gene expression is diminished markedly. Thus, a relaxation of normal regulation of the cell cycle-dependent and cell differentiation-restricted expression of *c-myc* may be sufficient for partial loss of growth control. In Burkitt lymphoma cells, transcription of the normal, untranslocated *c-myc* allele remains inactive, whereas the translocated and rearranged *c-myc* is constitutively expressed at a high level (4, 10, 33, 48). Similar observations have been reported for mouse plasmacytomas (2, 59). These results have suggested that the normal *c-myc* gene may be either autorepressed directly or indirectly by *c-myc* gene products derived from the rearranged allele (38)

or suppressed by a differentiation-specific mechanism from which the altered *c-myc* gene escapes (47, 48).

To acquire some insights into the control mechanism of *c-myc* gene expression, we have studied the expression of *c-myc* genes in *Xenopus laevis* oocytes microinjected with various human *c-myc* gene recombinants. Protein-coding genes, such as the simian virus 40 (SV40) early and late gene (64), the herpesvirus thymidine kinase gene (44), and the sea urchin histone gene (23, 52), transcribed by RNA polymerase II have been shown to be expressed efficiently. However, some of the cell type-specific genes such as rabbit β -globin (54) and the chicken ovalbumin gene (65) have been shown to generate only inaccurate transcripts initiating from the plasmid parts, suggesting selectivity of this system in faithful expression of the foreign genes microinjected (30). Here we show that the cloned intact human *c-myc* gene is transcribed very efficiently and accurately from its own promoters in *X. laevis* oocytes after microinjection. This active transcription does not require any modifications of the gene such as fusion of the virus promoter or enhancing sequences. The transcripts are properly polyadenylated, and some of them are correctly spliced.

MATERIALS AND METHODS

Plasmid constructions. pLcMycHE was constructed by excising the *Hind*III/*Eco*RI 8.2-kilobase (kb) fragment carrying the human genomic *c-myc* sequences from the original λ clone, λ MC41 (20), and inserting it at the *Hind*III and *Eco*RI sites of pML2 (41), a derivative of pBR322. pSV2cMycHE was constructed as follows. First, we excised the *Hind*III/*Eco*RI 8.2-kb DNA fragment from plasmid pLcMycHE. Then this DNA fragment was inserted into the *Hind*III and *Eco*RI sites of the pSV2CAT (26) vector after removing the CAT gene and a part of the SV40 sequences from this vector. pHEa5'myc and pHEs5'myc recombinants were constructed as follows. The mouse immunoglobulin H (IgH) enhancer sequence previously identified between J and C μ genes (6, 25) was excised as the *Xba*I/*Eco*RI 680-bp fragment from the pJ3J4 clone, and its ends were filled in with DNA polymerase I (Klenow) and then *Bam*HI linked. The resulting DNA fragment was inserted at the *Bam*HI site within the pBR322 portion of plasmid pLcMycHE. After bacterial transformation, individual colonies were screened

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FIG. 1. Structure of the human *c-myc* gene. Schematic diagram of the human *c-myc* gene. The *c-myc* gene consists of three exons, one noncoding (E1) and two coding (E2 and E3) (15, 62), as shown. The 8.2-kb *Hind*III/*Eco*RI restriction fragment was subcloned into the derivative of pBR322, pML2 (41), resulting in the pLcMycHE clone. The location of the genomic fragment used for subcloning is indicated.

for the insert and its orientation to the *c-myc* gene. In the pHEa5'myc recombinant, the orientation of the immunoglobulin enhancer is opposite the *c-myc* gene, whereas it orients in the same direction as *c-myc* in the pHEs5'myc recombinant. λ MC41 and pJ3J4 were obtained from C. M. Croce. pSV2CAT was a generous gift from B. H. Howard.

Oocyte injection and RNA preparation. Typically, a set of 20 oocytes was injected for each sample as described by Gurdon (29). Oocytes were homogenized in proteinase K solution, and total RNAs were extracted with phenol-chloroform and then precipitated with ethanol as described previously (49). The RNAs were further purified by an oligodeoxythymidylate selection procedure (5).

S1 nuclease mapping. RNA was analyzed by the S1 nuclease mapping procedure (9). End-labeling was performed by the method of Maxam and Gilbert (43), using polynucleotide kinase (5' end) or *Escherichia coli* DNA polymerase I Klenow fragment (3' end). The uniformly labeled DNA probe was prepared according to Ley et al. (39). Typically, the polyadenylated [poly(A)⁺] RNAs from the equivalent of one oocyte or 20 μ g of cytoplasmic RNAs from tissue-cultured cells were used for each assay. The ³²P-labeled DNA probes were heat denatured, hybridized in 80% formamide to poly(A)⁺ or cytoplasmic RNAs at 56.5°C for 10 h, digested with 80 U of nuclease S1, and analyzed by electrophoresis on a 7 M urea-4% polyacrylamide gel (42).

RESULTS

Construction of human *c-myc* recombinants. In this study, various human *c-myc* gene recombinants were constructed to test gene expression in the *X. laevis* oocyte system. The structure of the human *c-myc* gene is shown in Fig. 1. In some of the recombinants, sequences from the SV40 genome or mouse IgH gene are also added to the *c-myc* gene. All *c-myc* gene recombinants used in this study are summarized in Table 1 and are schematically presented in Fig. 2.

Human *c-myc* genes are transcribed from their own two

promoters in *X. laevis* oocytes after microinjection. First, we microinjected the human genomic *c-myc* recombinant pLcMycHE (Fig. 2A) DNAs into *X. laevis* oocytes. After 24 h, the poly(A)⁺ RNA was prepared from the oocytes and analyzed for the presence of *c-myc* transcripts by the S1 mapping procedure (9). The *c-myc* gene contains two TATA sequences and two initiation sites separated by 165 bp at the 5' side of the first noncoding exon (7, 62). We first determined whether any of the human *c-myc*-specific transcripts initiated from their two cap sites were synthesized in oocytes. Two S1 nuclease-resistant DNA fragments, 515 and 350 nucleotides in length, were detected in the oocytes microinjected with pLcMycHE (Fig. 3A, lane 4). As we have previously shown (4, 62), these two lengths correspond to the distance from the 3' *Pvu*II site to the two initiation sites of the *c-myc* gene (Fig. 3B). As controls (Fig. 3A, lanes 2 and 3), we used the RNAs prepared from Burkitt lymphoma BL2 (19) and also from promyelocytic leukemia cell line HL60 (21). It has been shown that these cells synthesize elevated levels of *c-myc* mRNAs (about 50 copies per cell; see reference 10) and also that two *c-myc* promoters are utilized to a different extent in these cells (62). Our results clearly indicate that the two promoter and initiation sites of the human *c-myc* genes are accurately recognized and utilized for *c-myc* RNA synthesis in the *Xenopus* oocytes. Since coinjection of α -amanitin at 5 pg per oocyte with the recombinant abolished the S1 nuclease-resistant DNA products of 515 and 350 nucleotides in length (data not shown), we concluded that the *c-myc*-specific RNAs initiating from the two sites were transcribed by RNA polymerase II (31).

Various enhancer sequences have no effect on *c-myc* transcription from its own two promoters in *Xenopus* oocytes. We also microinjected pSV2cMycHE, pHEa5'myc, or pHEs5'myc clone DNAs, which contain the replication origin of the SV40, with the enhancer or the mouse IgH enhancer, in addition to human *c-myc* genes (Fig. 2A and B). The total level of the *c-myc* gene transcription from pSV2cMycHE DNAs (Fig. 3A, lane 5), pHEa5'myc (lane 6), or pHEs5'myc (lane 7) was almost unchanged in comparison with that from pLcMycHE (lane 4).

To assess the amount of *c-myc* RNAs synthesized in the *Xenopus* oocytes microinjected with pLcMycHE, we scanned the autoradiogram and compared the integrated intensities of the two bands (515 and 350 nucleotides) with those for the controls (Burkitt lymphoma BL2, Fig. 3A, lane 2; and promyelocytic leukemia HL60, Fig. 3A, lane 3). We estimate that each oocyte microinjected with the cloned *c-myc* genes (1 ng or 5×10^7 copies of the templates) synthesizes 2.5×10^8 copies of the *c-myc* RNAs, equivalent to the amount of *c-myc* mRNAs synthesized by 5×10^6 BL2 or HL60 cells. From these results on S1 mapping analysis of the human *c-myc* transcripts, we conclude that, without additional *cis*-acting elements, the 5'-flanking sequence region of the human *c-myc* gene containing the two TATA sequences can direct accurate and active transcription of *c-myc* RNA in the *Xenopus* oocyte after microinjection.

TABLE 1. Summary of *c-myc* gene recombinants used in this study

Plasmid	Salient features
pLcMycHE.....	Contains the intact human genomic <i>c-myc</i> DNA
pHEa5'myc.....	Same as pLcMycHE; also contains the mouse IgH chain gene enhancer in the opposite orientation to the <i>c-myc</i> gene
pHEs5'myc.....	Same as pLcMycHE; also contains the mouse IgH chain gene enhancer in the same orientation as the <i>c-myc</i> gene
pSV2cMycHE	In addition to the intact human genomic <i>c-myc</i> DNA, also contains the SV40 replication origin, early gene promoter with the 72-bp repeat enhancer

Two poly(A) sites of the *c-myc* genes are utilized in *Xenopus* oocytes. We next analyzed the 3' ends of the *c-myc* RNAs synthesized in *X. laevis* oocytes microinjected with various human *c-myc* recombinants. The S1 probe, the *Clal/EcoRI* fragment, ^{32}P labeled at the 3' end of the *Clal* site, used for the poly(A) site analysis is described in Fig. 4B. Although the human *c-myc* gene contains two potential poly(A) addition sites separated by 165 bp in the 3'-flanking region (7), the second site (site 2, indicated in Fig. 4B) is dominantly utilized for *c-myc* mRNA synthesis in various Burkitt lymphoma and human lymphoblastoid cells (4). The two poly(A) addition sites were utilized equally for the formation of 3' ends of the *c-myc* RNAs transcribed from pLcMycHE (lane 5) and pSV2cMycHE (lane 6) recombinants (Fig. 4A), whereas the RNAs synthesized in three Burkitt lymphoma cells have their 3' ends predominantly at the second sites as expected. Since RNAs prepared from oocytes were selected for the poly(A)⁺ fraction by the oligodeoxythymidylate column three times, the 3' ends of the *c-myc* RNAs, detected by S1 mapping analysis, are most likely polyadenylated at these sites. Therefore, we conclude that in oocytes the injected *c-myc* genes direct the synthesis of poly(A)⁺ *c-myc* RNAs whose 3' ends are at one of the two poly(A) addition sites.

Splicing of the human *c-myc* gene transcripts also occurs in *Xenopus* oocytes. Splicing of the *c-myc* gene transcripts was also analyzed. First, we assayed for RNA splicing between the second and third exons of the *c-myc* gene transcripts, using a 5'-end-labeled human *c-myc* cDNA probe (Fig. 5B). The correctly spliced *c-myc* RNA produced in oocytes protected a full-length DNA probe (1,034 nucleotides) from S1 nuclease digestion (lanes 5 and 6) corresponding to the distance between the *BclI* site in the 3' untranslated region and the 5' end of the *c-myc* cDNA insert of the pRyc7.4 probe (48). These S1 nuclease-resistant DNA products were observed in control lanes for the *c-myc* RNAs prepared from various Burkitt lymphomas (lanes 2 to 4). The unspliced precursor type *c-myc* RNAs protected a 637-nucleotide fragment of this cDNA probe, corresponding to the distance between the *BclI* site and the 5' end of the third exon (Fig. 5B). It has been previously reported that *Xenopus* oocytes can correctly but inefficiently splice microinjected precursor RNAs (27) or the transcripts synthesized from injected DNAs (64). Our results on analysis of splicing between E2 and E3 exons of *c-myc* gene transcripts also indicate that the oocytes accurately splice the human *c-myc* transcripts but with low efficiency. By comparing the two S1 nuclease-resistant DNA products of 1,034 and 637 nucleotides, we estimate that up to 30% of the *c-myc* transcripts were properly spliced. These results were also confirmed for splicing between E1 and E2 exons of the *c-myc* transcripts produced in *Xenopus* oocytes by using another cDNA clone spanning the two exons (data not shown).

DISCUSSION

Expression of human *c-myc* gene in *Xenopus* oocytes. Although accurate and efficient transcription initiation of microinjected 5S rRNA (13) and tRNA (18, 37) genes by RNA polymerase III or rRNA genes (45, 53) by RNA polymerase I occurs in *Xenopus* oocytes, protein-coding genes, transcribed by RNA polymerase II, have been reported to be poorly expressed in oocytes except for a few cases (30). Such exceptional cases are herpesvirus thymidine kinase genes (44), SV40 early and late genes (64), and sea urchin histone genes (23, 52). However, chicken ovalbumin genes (65) and rabbit β -globin genes (54) have been shown to be

FIG. 2. Maps of various recombinants containing the human *c-myc* gene. The structure of the *c-myc* gene recombinants used in this study is schematically presented. The stippled boxes represent human *c-myc* gene exons. The narrower black boxes represent 5'- and 3'-flanking regions and introns of *c-myc* gene. The black boxes represent the SV40 replication origin region containing the enhancer (72-bp repeat) (8, 24, 28) and the early gene promoter, in addition to the replication origin. The thinner line represents pBR322. The orientation of the *c-myc* gene (*c-myc*) of the β -lactamase gene (Amp^r) is shown by an arrow. The triangle indicates the mouse IgH chain gene enhancer (6, 25) and its insertion position. B, *Bam*HI; E, *Eco*RI; H, *Hind*III; P, *Pvu*II restriction sites. (A) Map of pLcMycHE, pHEa5'myc, and pHEs5'myc. (B) Map of pSV2cMycHE. Details are discussed in the text.

FIG. 3. Detection of specific 5' ends of the human *c-myc* RNAs from *Xenopus* oocytes microinjected with cloned *c-myc* gene plasmids. The poly(A)⁺ RNAs, prepared from one microinjected oocyte, were subjected to S1 nuclease mapping analysis (9). The uniformly ³²P-labeled DNA probe was heat denatured, hybridized in 80% formamide to the various RNA samples at 56.5°C for 10 h, digested with S1 nuclease, and analyzed by electrophoresis on a 7 M urea-4% polyacrylamide gel as described previously (50). (A) S1 mapping analysis of the RNAs from oocytes microinjected with pLcMycHE (lane 4), pSV2cMycHE (lane 5), pHEa5'myc (lane 6), and pHEs5'myc (lane 7). Yeast-soluble RNAs (lane 1) and cytoplasmic RNAs (20 µg or prepared from 2 × 10⁶ cells) from Burkitt lymphoma cell line BL2 (lane 2) and promyelocytic leukemia cell

transcribed randomly, without fidelity, after microinjection into *Xenopus* oocytes. We have found that transcription of mouse β-globin (major) and human α2 globin genes mainly initiates from the plasmid DNA, but not from their own promoters (K. Nishikura, unpublished data). Expression of cell type-specific genes usually seems to produce only heterogeneous transcripts initiating from various regions of the plasmid in the microinjected oocytes, whereas virus genes, designed to function efficiently in various host cells, are expressed efficiently in amphibian oocytes (30).

The cellular oncogene *c-myc* is believed to play important roles in regulation of the growth and development of normal cells, as do other *c-oncogenes* (11, 35). In that sense, the *c-myc* gene is not a cell type-specific gene such as ovalbumin or globin genes, but perhaps should be considered as one of the housekeeping genes such as histone, tubulin, or actin genes. However, it was still surprising to find that the human genomic *c-myc* gene is actively and faithfully transcribed from its own promoters in *Xenopus* oocytes, which are completely undifferentiated and arrested in the prophase of meiosis. It appears as if this cellular gene is one of the genes programmed to be expressed in oocytes to carry out some fundamental functions necessary for oocytes themselves or for embryos during early development after fertilization. In supporting this possibility, our recent studies on endogenous transcription of the frog *c-myc* gene by dot blot analysis have indicated that oocytes express almost equivalent levels of *c-myc* RNAs as that of rapidly dividing early embryos (24-h embryos) (data not shown). Persson et al. (51) have also recently shown that *c-myc* proteins can be detected in *Xenopus* oocytes by using *c-myc*-specific antisera. Interestingly, we have found that the human cellular oncogene *c-H-ras* (56, 60) only generated heterogeneous transcripts, initiating from various sites randomly in *Xenopus* oocytes, after microinjection of the cloned plasmid p344 carrying the normal human *c-H-ras* gene (56), suggesting that not all cloned *c-oncogenes* are competent for active and accurate transcription in amphibian oocytes (K. Nishikura, S. Goldflam, and G. Vuocolo, unpublished data).

The human *c-myc* gene contains all control regions required for active and accurate transcription in *Xenopus* oocytes. Our results clearly show that neither the previously identified IgH enhancer (6, 25) nor SV40 72-bp repeats (8, 24, 28) increase accurate and efficient transcription initiation of the *c-myc* gene in *Xenopus* oocytes. In other words, the intact human *c-myc* gene fragment inserted into the pLcMycHE recombinant (Fig. 1 and 2A) is self-sufficient with respect to all signal sequences necessary for active transcription from the correct initiation sites. This result was unexpected, since there is a commonly accepted impression that in various tumors the *c-myc* gene is activated only by drastic alterations of the *c-myc* locus such as retroviral promotor insertion, retrovirus enhancer insertion, gene amplification, or chromosomal translocation. Accumulated evidence has suggested the existence of a delicately balanced control mechanism which operates on normal *c-myc* genes in a cell differentiation-specific manner (48) or in a cell cycle-depen-

line HL60 (lane 3) were also analyzed as controls. Lane M, Size marker: φX174 digested with *Hae*III and 5'-³²P labeled. (B) The expected S1 nuclease-resistant DNA products are shown in the diagram. DNA fragment sizes of 515 and 350 nucleotides in length correspond to the distance from the *Pvu*II site to the two transcription initiation sites as described previously (62). Sm, *Sma*I (*Xma*I); Pv, *Pvu*II restriction sites.

dent way (36). The target of such a mechanism has been considered to be located at the first noncoding exon and its 5'-flanking sequences (38, 58), where putative suppressor molecules may bind and keep the *c-myc* gene expression

FIG. 4. S1 nuclease mapping analysis for polyadenylation sites of human *c-myc* transcripts synthesized in *Xenopus* oocytes. (A) Detection of two 3' ends of the *c-myc* RNAs from oocytes microinjected with pLcMycHE (lane 5) and pSV2cMycHE (lane 6). The S1 probe used was the genomic *c-myc* DNA fragment *Clal*/*EcoRI* (1.4 kb), 3'-end-³²P labeled at the *Clal* site. Yeast-soluble RNAs (lane 1) and cytoplasmic RNAs (20 μ g or prepared from 2×10^6 cells) from Burkitt lymphoma cell line BL2 (lane 2), Daudi (lane 3),

at relatively low levels. However, the results presented herein strongly indicate that the promoters of the intact *c-myc* gene are active ones when tested in the *Xenopus* oocyte system. Furthermore, no extensive alteration to the *c-myc* gene seems to be necessary for active and accurate transcription. Our best quantitation from S1 mapping autoradiograms for the stable *c-myc* RNAs transcribed from two authentic promoters in oocytes microinjected with pLcMycHE is 2.5×10^8 copies of RNAs per 24 h. This is probably an equivalent or even higher level of transcriptional activity than those for SV40 late genes and the herpesvirus thymidine kinase gene, which are the genes most actively transcribed by RNA polymerase II in *Xenopus* oocytes (30, 44, 64).

Thus, one is led to wonder if the low level of *c-myc* expression in normal cells is due to the effects of some repressor-like control factor which suppresses or turns off (in some cases) *c-myc* transcription. Such a negative control may be consistent with the difference between the rapid transcription of cloned *c-myc* genes microinjected and a low level of transcription of normal cellular *c-myc* genes. In the former, the number of genes microinjected into oocytes (1 ng or 5×10^7 copies per oocyte) may be far in excess of the physiological concentration of the putative factor, whereas in the latter a single copy of the *c-myc* gene may be tightly regulated by such a regulatory molecule. Alternatively, the level of *c-myc* transcription in injected oocytes may indicate the release of the genes from regulatory factors present in somatic cells but absent in these undifferentiated germ cells, oocytes.

3'-End formation, polyadenylation, and splicing of *c-myc* gene transcripts in *Xenopus* oocytes. In this study, we have demonstrated that not only accurate and efficient transcription but also some post-transcriptional processing of *c-myc* transcripts take place in *Xenopus* oocytes microinjected with cloned human *c-myc* genes. It has been shown previously that *Xenopus* oocytes are able to carry out some of the post-transcriptional steps required for modifications of mRNA precursors synthesized by polymerase II from injected genes (64). In this study transcripts of *c-myc* genes were found to have been properly polyadenylated at either one of the previously identified two poly(A) addition sites (4, 7). The utilization ratio of the two sites for 3'-end formation was quite different in oocytes and in various Burkitt lymphoma cells. Namely, the promoter-distal site (site 2 in Fig. 4B) was favored over the proximal site (site 1 in Fig. 4B) in *c-myc* mRNAs of all Burkitt lymphoma cells (20 to 1 ratio judged by scanning of the autoradiogram), whereas in oocytes the two sites were utilized almost equally. Since two poly(A) addition sites are located closely within the 3'-flanking region, two *c-myc* mRNAs having different 3' ends will give rise to a single *c-myc* protein structure. The biological significance, if any, of the difference between two such mRNAs, for instance, stability of the message or translational efficiency, is not yet known.

and ST486 (lane 4) were analyzed as controls. Conditions for S1 nuclease analysis were described in the legend to Fig. 3. S1 nuclease-resistant DNA products were fractionated on a 4% denaturing polyacrylamide gel. Lane M, Size marker: ϕ X174 digested with *HaeIII* and 5'-³²P labeled. (B) The expected S1 nuclease-resistant DNA products are shown in the diagram. DNA fragment sizes of 845 and 1,010 nucleotides in length correspond to the distance from the *Clal* site to the two 3'-end sites as described previously (4). Cl, *Clal*; E, *EcoRI* restriction sites.

FIG. 5. S1 nuclease mapping analysis for splicing of human *c-myc* transcripts synthesized in *Xenopus* oocytes. (A) Detection of the spliced *c-myc* RNAs from oocytes microinjected with pLcMycHE (lane 5) and pSV2cMycHE (lane 6). The S1 probe was prepared from the pRyc7.4 clone, which carries a human *c-myc* cDNA 1.2-kb insert in pBR322 (63). pRyc7.4 plasmid DNA was digested with *Bcl*I, 5'-end-³²P labeled, and used as a probe. Yeast-soluble RNAs (lane 1) and cytoplasmic RNAs (20 μ g or prepared from 2×10^6 cells) from Burkitt lymphoma cell line BL2 (lane 2), ST486 (lane 3), and Daudi (lane 4) were analyzed as controls. Conditions for S1 nuclease analysis were described in the legend to Fig. 3. S1 nuclease-resistant DNA products were fractionated on a 7 M urea-4% polyacrylamide gel. Lane M, Size marker: ϕ X174 digested with *Hae*III and 5'-³²P labeled. (B) The expected S1 nuclease-resistant DNA products are shown in the diagram. The 1,034-nucleotide S1 nuclease-resistant DNA fragment is protected by the correctly spliced *c-myc* RNAs, whereas the 637-nucleotide product is generated by the unspliced or precursor-type *c-myc* RNAs. Bc, *Bcl*I; Ps, *Pst*I restriction sites.

We have also shown that accurate splicing of the *c-myc* transcripts synthesized from the injected genes occurs in *Xenopus* oocytes. Although the efficiency of splicing is not necessarily high, the inefficiency of this RNA processing step in *Xenopus* oocytes has been reported for several other gene transcripts (27, 64). Regardless of efficiency, our results demonstrate that the *c-myc* RNAs transcribed from their own promoters are polyadenylated at one of two potential 3'-end sites, that some of them are spliced correctly, and that they give rise to a significant amount of mature functional human *c-myc* mRNAs. It will be interesting to know whether any of these *c-myc* mRNAs are translated into functional *c-myc* proteins in *Xenopus* oocytes. The possibility is presently being investigated.

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