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Molecular cloning and regulated expression of the human c-*myc* gene in *Escherichia coli* and *Saccharomyces cerevisiae*: Comparison of the protein products

(oncogenes/expression vectors/posttranslational modification)

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ABSTRACT **mRNA from human HL-60 cells was used to prepare a cDNA library, from which two full-length clones that encompass the complete c-*myc* coding region were isolated. One clone, pM1-11, contains all three exons of human c-*myc*. The second clone, pM4-10, represents a relatively rare transcript that initiated in the first intron and includes the coding exons 2 and 3. The cDNA insert in pM1-11 was used to express the human c-myc protein in both prokaryotic and eukaryotic cells. Insertion of the coding sequences in exons 2 and 3 into the appropriate expression vectors yielded detectable c-myc protein in *Escherichia coli* lacking the Lon protease and in *Saccharomyces cerevisiae* upon induction. The protein produced in *E. coli* has an apparent size of 60 kDa and appears to be unmodified, as it is identical in size to the protein synthesized in an *in vitro* system. In contrast, yeast cells synthesize two myc proteins, of 60 kDa and 62 kDa. The difference in apparent molecular mass between the two proteins appears to be due, in part, to phosphorylation. Subcellular fractionation of yeast cells showed that the c-myc phosphoprotein is located predominantly in the nuclear fraction.**

Activation of the cellular *myc* gene has been associated with neoplasia in a variety of species. In transformed cells, the c-*myc* gene can be altered by several mechanisms that result in abnormal or elevated expression of c-*myc* RNA (1, 2) and, presumably, in increased levels of c-myc protein. Analysis of genomic clones of the human c-*myc* gene shows that it contains three exons (3). Exon 1 represents a long 5′ noncoding leader region, whereas all of the coding sequences are located in exons 2 and 3. The coding sequences are highly conserved among species and with v-*myc*, the viral oncogene homolog harbored by avian myelocytomatosis virus (4, 5). Immunoprecipitation studies show that myc-specific antisera recognize proteins of ≈60 kDa in human cells (6–8). The highly conserved nature of the c-myc protein in many vertebrate species suggests that c-myc must supply a crucial function in eukaryotic cells. Such a function has not been elucidated, although studies on v-myc and c-myc indicate that this protein is found predominantly in the nucleus and binds to double-stranded DNA (9–13).

Studies on the structure and function of the c-*myc* gene product have been hampered by the difficulty in obtaining sufficient amounts of protein for biochemical analyses from human cells that express elevated levels of c-*myc* RNA (unpublished observations). In this report, we describe the isolation and characterization of cDNA clones containing the complete coding region of human c-*myc*. The cDNA clones were used to express the c-*myc* gene in heterologous cells under regulated conditions. The c-*myc* gene products synthesized in *Escherichia coli* and in *Saccharomyces cerevisiae* were compared and found to exhibit host-cell-specific modifications.

MATERIALS AND METHODS

Cell Strains. *E. coli* strain KRR123 was derived from strain RR1 (14) by phage P1 transduction of the *lon9* allele from strain RGC103 (15). KRR124 was also derived from RR1 through the same steps, except that the wild-type *lon* allele was selected. These strains were transformed with the low-copy-number-compatible plasmid pRK248*c*Its, which carries the gene for a temperature-sensitive repressor (16). RR1(pRK248*c*Its) was used to propagate plasmids containing the P_L promoter of bacteriophage λ. The yeast vectors pYE7(+*Bam*HI) and pCM14 were propagated in *E. coli* MC1061 (17). *S. cerevisiae* 20B-12 (*MATα trp1 pep4-3*) (18) was used for c-*myc* expression.

Molecular Cloning of Human c-*myc* cDNA. Total RNA was prepared from HL-60 cells by the proteinase K/NaDodSO$_4$ procedure (19) and was fractionated by oligo(dT)-cellulose column chromatography and sucrose gradient centrifugation. Fractions containing c-*myc* sequences were detected by "dot blot" hybridization with a chicken c-*myc* probe (20). These fractions were used to construct a cDNA library in pBR322 by the method of Gubler and Hoffmann (21). The cDNA library of 40,000 recombinants was screened with the chicken c-*myc* probe. Positive clones were analyzed further by restriction endonuclease digestion, and two clones with the largest inserts were characterized further. Nucleotide sequence analysis was as described by Maxam and Gilbert (22).

Construction of Recombinant Expression Plasmids. The expression vector pRC23 (23) has been described. The yeast expression vector pYE7(+*Bam*HI) is a derivative of pYE4 (24) with an additional *Bam*HI site. Synthetic oligodeoxyribonucleotides were prepared as described (25). Methods used for transformation of *E. coli* (14) and yeast (26) were also as described.

Induction of c-*myc* Synthesis. Bacterial cells containing c-*myc* expression vectors were grown at 30°C to early logarithmic phase (OD$_{600}$ 0.5) in M9 medium (14). Cultures were induced as described (27). For *in vitro* expression, the *E. coli* DNA-directed coupled transcription/translation system was used as recommended by the supplier (Amersham). Yeast transformants were cultured for expression as described (24). For labeling experiments, cultures were induced for expression by transfer to phosphate-free medium for 3–5.5 hr and then were incubated with [^{32}P]phosphate (car-

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Abbreviation: bp, base pair(s); kb, kilobase(s).

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rier-free, Amersham) for 1–4 hr before harvest and NaDodSO$_4$/10% polyacrylamide gel analysis as described (24).

Immunological Detection of c-myc Protein. The c-myc-specific monoclonal antibody IF7 was prepared by immunization of mice with a synthetic peptide corresponding to a portion of the human c-myc protein. Details of the preparation of this antibody will be described elsewhere. Immunoblot analysis and immunostaining of c-myc protein were performed by a modification of the procedure of Towbin *et al.* (28).

Fractionation of Yeast Cells. After induction and metabolic labeling, yeast cells were separated into cytoplasmic and nuclear fractions by a modification of the method of Udem and Warner (29). Each fraction was analyzed by immunoprecipitation and NaDodSO$_4$/PAGE.

RESULTS

Isolation and Characterization of Human c-myc cDNA Clones. A cDNA library, prepared from size-fractionated mRNA isolated from HL-60 cells, was screened with a probe prepared from a molecular clone of the chicken c-*myc* gene (20). Twelve *myc*-related clones were identified, and two clones with the largest inserts were characterized further by restriction endonuclease mapping and partial nucleotide sequence analysis. The structures of the two cDNA clones and their relationship to a human c-*myc* genomic clone are summarized in Fig. 1.

The two cDNA clones both contain inserts 2.2–2.3 kilobases (kb) long but differ markedly in their 5′ regions. By restriction mapping, we determined that the insert in pM1-11 is composed of sequences from exons 1, 2, and 3. Nucleotide sequence analysis reveals that this cDNA begins at nucleotide 812, using the coordinates of Battey *et al.* (3). This would correspond closely to a full-length cDNA copy of an mRNA transcribed from initiation site no. 2, identified as nucleotide 811 (3). This initiation site is used preferentially in HL-60 cells, as shown by nuclease S1 mapping studies (30). Analysis of the sequence of exon 1 and the beginning of exon 2 in pM1-11 shows it is identical to the previously determined sequence of the human c-*myc* gene (3). In contrast, pM4-10 contains a cDNA insert with sequences from intron 1 fused to exons 2 and 3. This cDNA, which begins at position 2344 in the first intron, may represent a rare transcript that initiates 3′ of the more commonly used initiation sites. Such transcripts have been detected in cells where the c-*myc* translocation removed exon 1 and allows the use of cryptic promoters and initiation sites in intron 1 (1, 31, 32). Sequence analysis has revealed (3) that the translation initiation ATG codon of

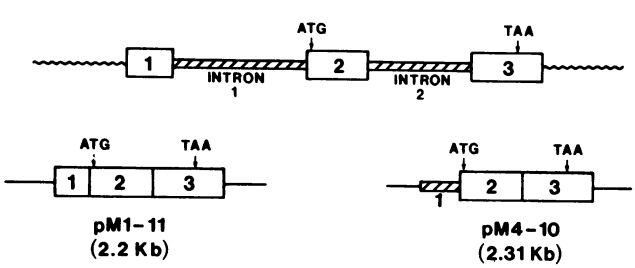

FIG. 1. Relationship between the human c-*myc* gene and two HL-60 c-myc cDNA clones. The locations of the initiation (ATG) and termination (TAA) codons for translation are indicated. Exon sequences are represented by open bars; intron sequences, by hatched bars; flanking cellular DNA, by wavy lines; and pBR322 DNA, by straight lines. (*Upper*) Genomic organization of the human c-*myc* gene. The arrangement of the c-*myc* gene is shown as determined by Battey *et al.* (3). (*Lower*) Organization of two cDNA clones of HL-60 c-myc poly(A)$^+$ RNA. The structures of the two cDNAs are shown, along with the approximate size of the inserts.

c-*myc* is located in exon 2 (Fig. 1) and that exon 1 represents a long 5′ noncoding leader region. Although the inserts in pM1-11 and pM4-10 have different 5′ noncoding regions, both cDNAs encompass the complete protein coding region of the c-*myc* gene.

Expression of the Human c-*myc* Gene in *E. coli*. For the production of c-myc protein in *E. coli*, we made use of the expression vector pRC23, which contains the strong, tightly regulated bacteriophage λ P_L promoter and a synthetic ribosome-binding site (23). Details of the construction of the c-*myc* expression vector are shown in Fig. 2*A*. The resultant vector pCM8 contains the complete coding sequence of the human c-*myc* gene under the transcriptional control of the P_L promoter.

Expression of c-*myc* was determined in several *E. coli* strains transformed with pCM8. After induction of the P_L promoter by incubation at 42°C, cell extracts were analyzed by polyacrylamide gel electrophoresis and immunoblot analysis with c-myc-specific monoclonal antibody IF7, which was prepared against a synthetic peptide corresponding to a region of the c-myc protein (see *Materials and Methods*). Initial attempts to detect c-myc in induced *E. coli* RR1 containing pCM8 were unsuccessful (data not shown), suggesting that the c-myc protein was unstable in these cells. To test this possibility, pCM8 was transferred to the protease-deficient (Lon$^-$) strain KRR123. Induction of these cells at 42°C for 2 hr resulted in the production of a c-myc protein with an apparent size of ≈60 kDa (Fig. 3*A*, lane 7). Small amounts of c-myc-related proteins of ≈58 kDa and ≈42 kDa were also detected; these may represent degradation products. The Lon$^+$ strain KRR124 (which is isogeneic with KRR123) was also unable to produce detectable myc protein (Fig. 3*A*, lanes 4 and 5). The absence of c-myc protein accumulation in the Lon$^+$ strains is probably due to proteolytic degradation.

Antibody IF7 is clearly specific for c-myc, as shown by immunostaining of the protein in induced KRR123 (pCM8) cells (Fig. 3*A*, lane 7). No such protein was present in these cells under conditions where the P_L promoter was uninduced (Fig. 3*A*, lane 6). Control KRR123 cells containing the parental expression vector pRC23 without c-*myc* sequences also did not produce any crossreacting proteins detectable with antibody IF7 (Fig. 3*A*, lanes 2 and 3). In addition, reactivity of this antibody with the pCM8 product demonstrated the integrity of the coding sequences in our myc cDNA clone.

The identification of the 60-kDa protein as the product of the c-*myc* coding sequences was confirmed by analysis of the pCM8 expression vector in an *E. coli*-derived *in vitro* coupled transcription/translation system. [^{35}S]Methionine incorporation into the *in vitro* reaction followed by gel electrophoresis showed that pCM8 in this system directed the synthesis of a protein that migrated at 60 kDa and comigrated with the protein synthesized *in vivo* in *E. coli* (Fig. 3*B*, lane 8). Several less prominent bands were also detectable and may represent degradation products (the 30-kDa protein is the product of the β-lactamase gene). No c-*myc*-related proteins were synthesized in the presence of the parental expression vector pRC23 (data not shown). Since posttranslational modifications are not known to occur in the *in vitro* system, the comigration of the predominant c-myc proteins in the *in vivo* and *in vitro* systems suggests that the c-*myc* gene product does not undergo any extensive modifications in *E. coli*.

Expression of the Human c-*myc* Gene in *S. cerevisiae*. To characterize the properties of c-myc protein made in eukaryotic cells, we chose to study the expression of c-*myc* in *S. cerevisiae*. The yeast expression vector pYE4 contains the regulated acid phosphatase *PHO5* promoter (24). This promoter is repressed in medium with a high concentration of phosphate but is induced in cells grown in medium lacking

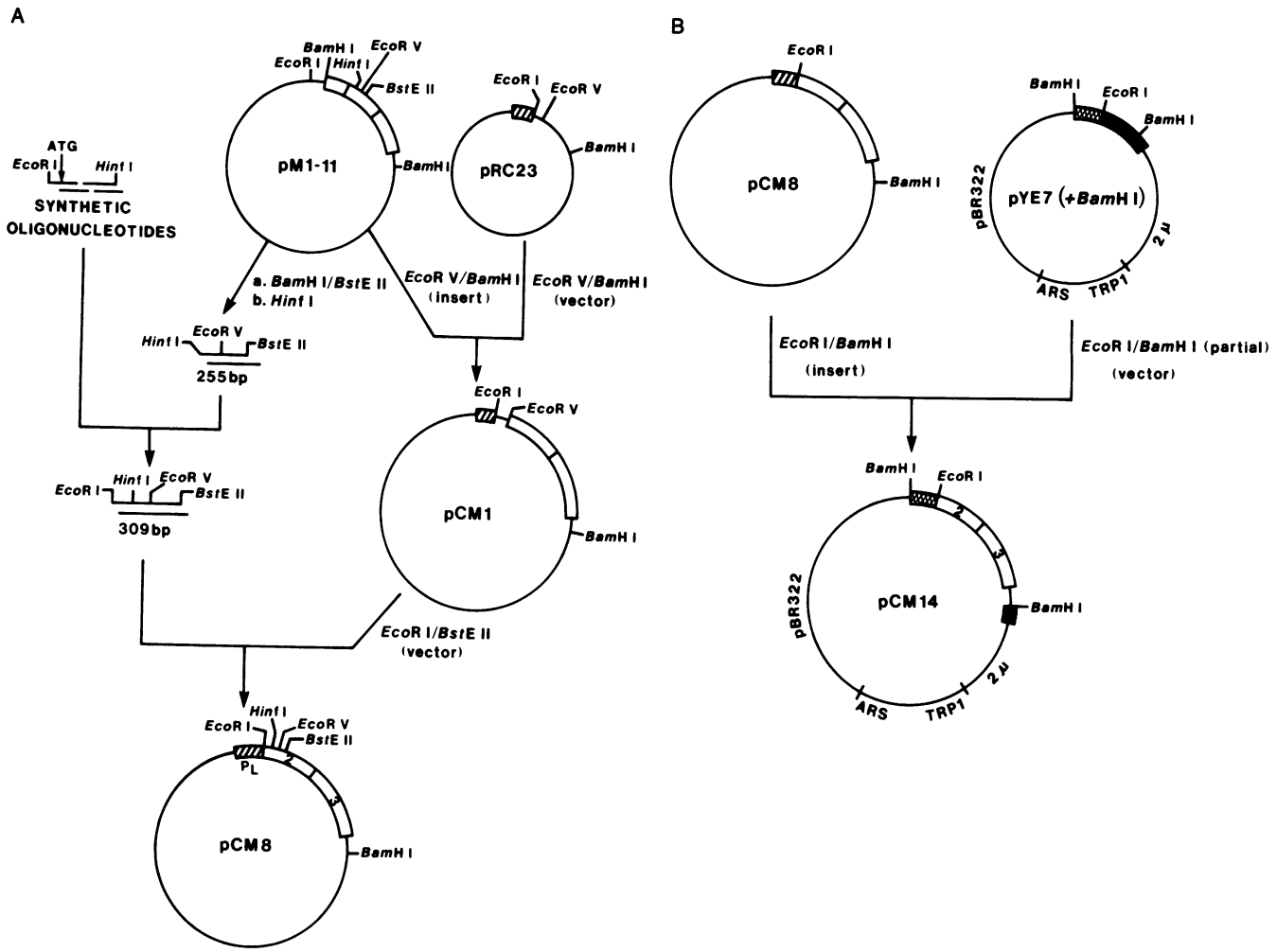

FIG. 2. Construction of expression vectors for c-*myc* synthesis. (*A*) *E. coli* expression vector pCM8. Open bars represent c-*myc* exons. The 1.84-kb *Eco*RV–*Bam*HI fragment from pM1-11 was isolated and ligated into *Eco*RV/*Bam*HI-digested pRC23, which contains the inducible P_L promoter (hatched bar) of phage λ, to yield pCM1. Four synthetic oligonucleotides were synthesized to create a 54-bp fragment flanked by *Eco*RI and *Hin*fI sites. The 54-bp fragment encodes an initiation ATG codon followed by sequences coding for the next 15 amino acids of the c-*myc* gene (5). The 54-bp *Eco*RI–*Hin*fI synthetic DNA fragment then was ligated to the 255-bp *Hin*fI–*Bst*EII fragment from pM1-11, to yield a 309-bp *Eco*RI–*Bst*EII fragment that contains the 5′ translated region of c-*myc*. This 309-bp fragment then was inserted into *Eco*RI/*Bst*EII digested pCM1 to yield pCM8. pCM8 contains the reconstructed c-*myc* coding region (exons 2 and 3) under the control of the P_L promoter. (*B*) *S. cerevisiae* expression vector pCM14. The yeast vector pYE7(+*Bam*HI), a derivative of the expression vector pYE4 (26), contains the inducible acid phosphatase *PHO5* promoter (cross-hatched bar), an additional *Bam*HI site for cloning, and sequences required for stable replication and selection in yeast. The *Eco*RI–*Bam*HI fragment from the *E. coli* expression vector pCM8, which includes an initiation ATG and the entire c-*myc* coding region, was excised and inserted into the pYE vector to yield the c-*myc*/yeast vector pCM14.

phosphate (35). The c-*myc* coding sequences from pCM8 were inserted in the appropriate orientation into a derivative of pYE4 (Fig. 2*B*). The c-*myc* expression plasmid pCM14 was then introduced into *S. cerevisiae* cells. Transformants were assayed for c-myc protein synthesis after induction in phosphate-free medium (Fig. 4*A*, lanes 2 and 3). Immunoblot analysis of extracts of yeast (pCM14) cells showed that these cells synthesized c-myc proteins with two apparent molecular masses, a major component at 62 kDa and a less intense band at 60 kDa. The smaller protein comigrated with the major myc protein synthesized in *E. coli in vivo* (Fig. 4*A*, lane 1).

The time course of synthesis and accumulation of the c-myc protein in yeast cells was analyzed by polyacrylamide gel electrophoresis and immunoblotting. Yeast transformants containing pCM14 were grown under conditions in which the *PHO5* promoter was repressed (high-phosphate medium) or induced (phosphate-free medium). Under induced conditions, yeast cells synthesized c-myc proteins over a 24-hr period (Fig. 4*B*). Maximal levels were accumulated at 6.5 hr and 9 hr of induction (lanes 3 and 5) and decreased by 24 hr (lane 1). No protein was detectable by the c-myc-specific antibody at any time when the *PHO5* promoter was not induced (lanes 2, 4, 6, and 8).

Phosphorylation of the c-myc Protein in *S. cerevisiae*. It has been shown that the endogenous c-myc proteins in human and mouse cells are phosphorylated (7, 8, 12). To determine whether such modification occurs in cells that normally do not produce human c-myc protein, yeast cells containing pCM14 were induced for 3 hr in phosphate-free medium, then grown in the presence of [^{32}P]phosphate for 4 hr. Total cell extracts then were prepared and myc protein was immunoprecipitated with the myc-specific antibody IF7. $NaDodSO_4$/PAGE and subsequent autoradiography of the precipitated material showed that the major phosphoprotein migrated at ≈60–62 kDa (Fig. 5*A*, lane 2). The immunoprecipitated phosphoprotein appeared as a diffuse band that was difficult to resolve in comparison to the proteins detected by immunoblotting. A phosphoprotein of 38 kDa was also immunoprecipitated but was not detectable by immunoblotting (see Fig. 4*B*). The autoradiographic smear in the region above 92.5 kDa may represent the precipitation of complexes

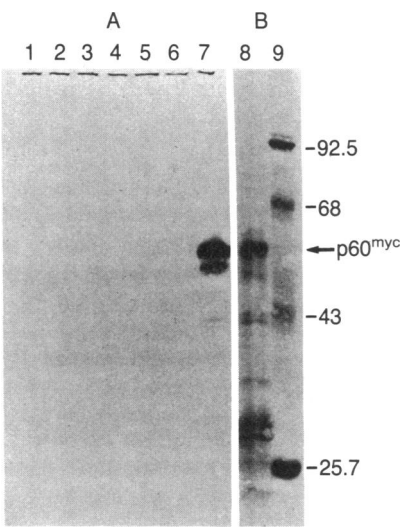

FIG. 3. (*A*) Immunoblot detection of c-myc protein synthesized in *E. coli*. Cultures were grown in M9 medium at 30°C to an OD_{600} of 0.5. The cultures were then divided and either maintained at 30°C (uninduced) or transferred to 42°C (induced) for 2 hr. Samples were harvested, resuspended in sample buffer, and analyzed by electrophoresis in $NaDodSO_4$/10% polyacrylamide gels. After electrophoresis, the protein was transferred to nitrocellulose membranes. c-myc protein was detected by immunostaining using the monoclonal antibody IF7. Lanes: 1, KRR123 induced; 2, KRR123(pRC23) uninduced; 3, KRR123(pRC23) induced; 4, KRR124(pCM8) uninduced; 5, KRR124(pCM8) induced; 6, KRR123(pCM8) uninduced; 7, KRR123(pCM8) induced. (*B*) pCM8 *in vitro* translation product. Radiolabeled proteins were separated in the same gel as represented in *A* and transferred to nitrocellulose membranes, but this portion of the membrane was removed, impregnated with fluor (Enlighten, New England Nuclear), dried, and exposed to x-ray film at −80°C. Lane 8: [^{35}S]methionine-labeled proteins synthesized in pCM8-directed *E. coli in vitro* transcription/translation system. Lane 9: ^{14}C-labeled protein standards (kDa at right).

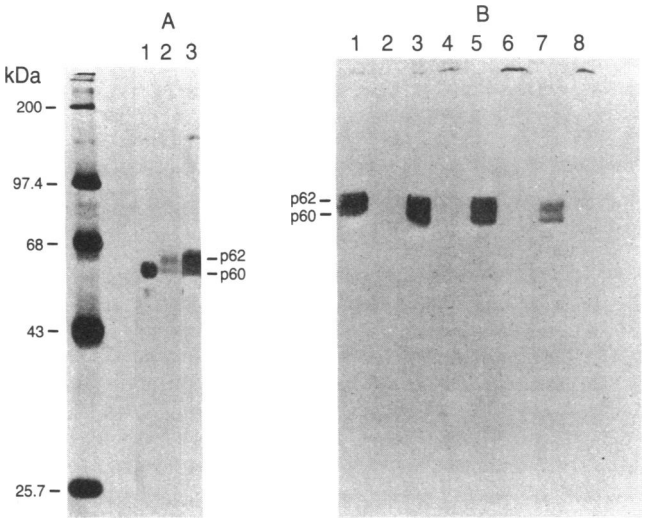

FIG. 4. Characterization of c-myc protein synthesis in *S. cerevisiae*. Yeast cells transformed with pCM14 were grown at 30°C in either high-phosphate medium (uninduced) or phosphate-free medium (induced). *E. coli* (KRR123) cells transformed with pCM8 were induced as described in the legend to Fig. 3. Cells were collected at various times after induction, pelleted, and lysed in sample buffer (27). After boiling, samples were analyzed by electrophoresis in $NaDodSO_4$/10% polyacrylamide gels and transferred to nitrocellulose paper. c-myc protein was detected by immunostaining with monoclonal antibody IF7. (*A*) Comparison of c-myc proteins synthesized in yeast and *E. coli*. Lane 1: *E. coli* KRR123(pCM8) transformants induced for 2 hr at 42°C. Lanes 2 and 3: yeast (pCM14) transformants induced for 7 hr in phosphate-free medium (lane 3 represents 6-fold more protein than lane 2). At left is a fluorograph of the region of the blot containing ^{14}C-labeled standard proteins. (*B*) Time course of c-*myc* protein synthesis in yeast. Uninduced: lane 2, 24 hr; lane 4, 9 hr; lane 6, 6.5 hr; lane 8, 4 hr. Induced: lane 1, 24 hr; lane 3, 9 hr; lane 5, 6.5 hr; lane 7, 4 hr.

between c-myc and ^{32}P-labeled high molecular weight macromolecules, either proteins or possibly DNA.

Subcellular Localization of the c-myc Protein. It has been reported that the protein products of v-*myc* and c-*myc* are associated with the nucleus of the host cell (9–13). It was of interest to determine the intracellular distribution of the c-myc protein in a heterologous system, namely the yeast cells. After induction and labeling in the presence of [^{32}P]phosphate, cells were separated into nuclear and cytoplasmic fractions. Aliquots of each fraction were analyzed by immunoprecipitation and gel electrophoresis. The c-myc phosphoprotein was found entirely in the nuclear fraction of the transformed yeast cells (Fig. 5*B*, lane 1). No c-myc phosphoproteins were present in the cytoplasmic fraction of cells containing pCM14 (lane 2). The c-myc antibody did not detect any labeled protein in yeast cells lacking the c-*myc* expression vector (lanes 3 and 4).

DISCUSSION

We have isolated and characterized two cDNA clones that contain the complete coding sequence of the human c-*myc* gene. The coding region from one of these clones was inserted into expression vectors designed for the regulated synthesis of proteins in heterologous cells. Recombinant c-myc protein was produced in both *E. coli* and yeast cells, as detected by a monoclonal antibody directed against the human c-*myc* gene product. Watt *et al.* (36) have also reported on the expression of the human c-myc protein in *E. coli*.

The cDNA clones that we have analyzed represent two alternative modes of *myc* transcription in human cells. The predominant class of transcripts (characterized by the insert in pM1-11) initiates at sites near or at the beginning of exon 1, as identified by nuclease S1 mapping studies (3, 30–32). The second class (characterized by the pM4-10 cDNA) is composed of transcripts that initiate at multiple sites within intron 1 (1, 31, 32). The latter class of mRNAs have arisen as a result of a variety of DNA rearrangements that activate the c-*myc* gene in neoplastic cells and cause the displacement of exon 1 from the body of c-*myc*. The origin of the pM4-10 transcript from HL-60 cells is unclear. It is possible that the gene amplification described in HL-60 cells (33, 34) could result in a rearranged copy of the c-*myc* gene. Alternatively, this template may have resulted from an incomplete splicing event.

Studies on the structure and function of the c-*myc* gene product would be facilitated by the availability of substantial amounts of the protein. To this end, we have synthesized recombinant human myc protein in both prokaryotic and eukaryotic cells. In *E. coli*, myc-specific antibody recognizes a major protein product of 60 kDa. The molecular mass of the human c-myc protein is predicted to be 48 kDa, as deduced from its nucleotide sequence (5). The anomalous behavior of the *E. coli* product in $NaDodSO_4$/polyacrylamide gels seems to be an inherent property of the unmodified protein, whether synthesized *in vivo* or *in vitro* (see Fig. 3). Similar results have been observed for an *in vitro* reticulocyte lysate translation product of mouse c-*myc* (6). This characteristic of myc—i.e., the anomalous migration—is shared with the protein products of other nuclear transforming genes (37).

Yeast cells transformed with a *myc* expression vector synthesize myc proteins of 60 kDa and 62 kDa that are modified, in part, by phosphorylation. Two sizes of c-myc phosphoproteins are also detected in human cells, described

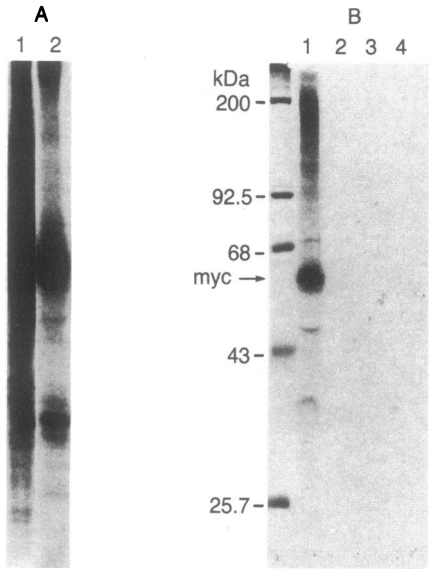

FIG. 5. Modification and subcellular localization of c-myc protein in yeast. (*A*) Phosphorylation of c-myc protein. After 3 hr of induction in phosphate-free medium, yeast (pCM14) cells were labeled for 4 hr at 30°C with [^{32}P]phosphate (4 mCi/5 ml, 1 Ci = 37 GBq). Spheroplasts were prepared and then lysed in 1% $NaDodSO_4$ by boiling for 10 min. Aliquots of total cell lysates were analyzed by immunoprecipitation and $NaDodSO_4$/PAGE. Lane 1: total cell lysate. Lane 2, immunoprecipitate obtained with myc-specific antibody IF7. (*B*) Intracellular distribution of c-myc protein. After induction for 5.5 hr, yeast cells were labeled with [^{32}P]phosphate (0.5 mCi/6.5 ml) for 1 hr at 30°C. Cells then were separated into cytoplasmic and nuclear fractions. Aliquots of each fraction were analyzed by immunoprecipitation and $NaDodSO_4$/PAGE. Yeast (pCM14) cells: lane 1, nuclear fraction; lane 2, cytoplasmic fraction. Yeast cells without pCM14: lane 3, nuclear fraction; lane 4, cytoplasmic fraction. Lane at left shows marker proteins.

by various investigators as 65 and 68 kDa (6), 64 and 67 kDa (7), and 62 and 66 kDa (8). Biochemical characterization of the modifying groups will clarify the relationship between these different c-myc species. The yeast cell product does not appear to be as extensively modified as myc proteins synthesized by use of a recombinant expression vector in insect cells (39). Thus, the extent of posttranslational modification may be a reflection of the host-cell environment. Alternatively, the conditions of induction in yeast (phosphate-free medium) may limit the available phosphate and thus the extent of modification.

Both expression systems may prove useful for the analysis of the function of the c-myc protein. The use of microorganisms for the production of large amounts of modified and unmodified c-myc protein represents a significant advantage over endogenous sources of the c-*myc* gene product. Using the inducible phosphatase promoter, one can study the regulated expression and modification of c-myc in yeast cells, which represent a defined eukaryotic genetic system. Studies on ras proteins in genetic mutants of yeast have yielded valuable information on the function of the *ras* oncogene (38). The variety of forms of the c-myc protein made in *E. coli*, yeast, and higher eukaryotic cells will help to define the biochemical modifications required for c-myc functions (specific DNA binding, localization to the nuclear matrix, and, possibly, transformation).

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1. Bernard, O., Cory, S., Gerondakis, S., Webb, E. & Adams, J. M. (1983) *EMBO J.* **2**, 2375–2383.
2. Nishikura, K., ar-Rushdi, A., Erikson, J., Watt, R., Rovera, G. & Croce, C. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4822–4826.
3. Battey, J., Moulding, C., Taub, R., Murphy, W., Stewart, T., Potter, H., Lenoir, G. & Leder, P. (1983) *Cell* **34**, 779–787.
4. Watson, D. K., Psallidopoulos, M. C., Samuel, K. P., Dalla-Favera, R. & Papas, T. S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3642–3645.
5. Colby, W. W., Chen, E. Y., Smith, D. H. & Levinson, A. D. (1983) *Nature (London)* **301**, 722–725.
6. Persson, H., Hennighausen, L., Taub, R., DeGrado, W. & Leder, P. (1984) *Science* **225**, 687–693.
7. Hann, S. R. & Eisenman, R. N. (1984) *Mol. Cell. Biol.* **4**, 2486–2497.
8. Ramsey, G., Evan, G. I. & Bishop, J. M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7742–7746.
9. Donner, P., Greiser-Wilke, I. & Moelling, K. (1982) *Nature (London)* **296**, 262–266.
10. Abrams, H. D., Rohrschneider, L. R. & Eisenman, R. N. (1982) *Cell* **29**, 427–439.
11. Alitalo, K., Ramsay, G., Bishop, J. M., Pfeifer, S. O., Colby, W. W. & Levinson, A. D. (1983) *Nature (London)* **306**, 274–277.
12. Hann, S. R., Abrams, H. D., Rohrschneider, L. R. & Eisenmann, R. N. (1983) *Cell* **34**, 789–798.
13. Persson, H. & Leder, P. (1984) *Science* **225**, 718–721.
14. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
15. Gayda, R. G., Yamamoto, L. T. & Markovitz, A. (1976) *J. Bacteriol.* **127**, 1208–1216.
16. Bernard, H. & Helinski, D. R. (1979) *Methods Enzymol.* **68**, 482–492.
17. Casadaban, M. J. & Cohen, S. N. (1980) *J. Mol. Biol.* **138**, 179–207.
18. Jones, E. (1976) *Genetics* **85**, 23–33.
19. Hall, L., Craig, R. K. & Campbell, P. N. (1979) *Nature (London)* **277**, 54–56.
20. Neel, B. G., Gasic, G. P., Rogler, C. E., Skalka, A. M., Ju, G., Hishinuma, F., Papas, T., Astrin, S. M. & Hayward, W. M. (1982) *J. Virol.* **44**, 158–166.
21. Gubler, U. & Hoffmann, B. J. (1983) *Gene* **25**, 263–269.
22. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
23. Crowl, R., Seamans, C., Lomedico, P. & McAndrew, S. (1985) *Gene*, in press.
24. Kramer, R. A., DeChiara, T. M., Schaber, M. D. & Hilliker, S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 367–370.
25. Kumar, G. & Poonian, M. S. (1984) *J. Org. Chem.* **49**, 4905–4912.
26. Hinnen, A., Hicks, J. B. & Fink, G. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1929–1933.
27. Lacal, J. C., Santos, E., Notario, V., Barbacid, M., Yamazaki, S., Kung, H.-F., Seamans, C., McAndrew, S. & Crowl, R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5305–5309.
28. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
29. Udem, S. A. & Warner, J. R. (1973) *J. Biol. Chem.* **248**, 1412–1416.
30. Watt, R., Nishikura, K., Sorrentino, J., ar-Rushdi, A., Croce, C. M. & Rovera, G. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6307–6311.
31. Saito, H., Hayday, A. C., Wiman, K., Hayward, W. S. & Tonegawa, S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7476–7480.
32. Taub, R., Moulding, C., Battey, J., Murphy, W., Vasicek, T., Lenoir, G. M. & Leder, P. (1984) *Cell* **36**, 339–348.
33. Collins, S. & Groudine, M. (1982) *Nature (London)* **298**, 679–681.
34. Dalla-Favera, R., Wong-Staal, F. & Gallo, R. C. (1982) *Nature (London)* **299**, 61–63.
35. Toh-e, A., Ueda, Y., Kakimoto, S. & Oshima, Y. (1973) *J. Bacteriol.* **113**, 727–738.
36. Watt, R., Shatzman, A. R. & Rosenberg, M. (1985) *Mol. Cell. Biol.* **5**, 448–456.
37. Van Beveran, C., Van Straaten, F., Curran, T., Muller, R. & Verma, I. M. (1983) *Cell* **32**, 1241–1255.
38. Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsumoto, K. & Wigler, M. (1985) *Cell* **40**, 27–36.
39. Miyamoto, C., Smith, G. E., Farrell-Towt, J., Chizzonite, R., Summers, M. D. & Ju, G. (1985) *Mol. Cell. Biol.* **5**, in press.