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Efficient expression of a protein coding gene under the control of an RNA polymerase I promoter

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ABSTRACT

In mammalian cells, RNA polymerase I transcripts are uncapped and retain a polyphosphate 5′ terminus. It is probably for this reason that they are poorly translated as messenger RNA. We show in this report that insertion of an Internal Ribosome Entry Site (IRES) into the 5′ leader of an RNA polymerase I transcript overcomes the block to translation, presumably by substituting for the 5′ trimethyl G cap. Addition of an SV40 polyA addition signal also enhances protein production from the RNA polymerase I transcript. RNA Polymerase I driven expression vectors containing both elements produce protein at levels comparable to that produced from RNA polymerase II driven expression vectors which utilize a retroviral LTR. RNA Polymerase I driven expression vectors may have a variety of uses both for basic research and for practical expression of recombinant proteins.

INTRODUCTION

RNA polymerase I is required only for transcription of the precursor to the large RNAs of ribosomes. This has been definitively shown in yeast (1) and is probably true in mammals and most other eukaryotes. Possibly because of this specialization, transcription by RNA polymerase I appears to be subject to different controls than is transcription by RNA polymerase II or III. In rapidly growing cells RNA polymerase I transcription is limited by the number of stable promoter complexes that can be assembled (2). This is likely the consequence of one of the two factors composing the stable complex, UBF and SL1, being present in limiting amounts (of these two factors, it is likely to be SL1 that is limiting). For those promoters that are able to assemble stable initiation complexes, RNA polymerase I is in excess and each promoter with a stable complex loads RNA polymerase I to the maximum possible extent. The net result is that ribosomal RNA production in growing cells accounts for nearly half of all of the RNA that is transcribed. In addition to their high expression rate, RNA polymerase I promoters are not tissue specific and are expressed in essentially all cells of the body. When cells slow their growth and become quiescent, RNA polymerase I transcription also decreases. The decrease appears to be due to alteration in the activity of a factor that associates with RNA polymerase I (3). This factor changes the ability of the polymerase to interact with the stable promoter complex even though the stable complex itself is still present and is potentially active.

Transcription by RNA polymerase I is relatively species specific as compared to transcription by RNA polymerases II and III. It has been shown, for example, that a mouse RNA polymerase I promoter will not function in human cell extracts nor will a human RNA polymerase I promoter function in mouse extracts. Between these two species the major specificity determinant appears to reside within the SL1 fraction (4). Between more distantly related species, as between human and frog, the UBF factor also contributes to specificity (5).

For many purposes it might be useful to produce a protein product driven by a promoter with the characteristics of RNA polymerase I promoters. It might be a useful means of obtaining over expression of a protein in a tissue independent manner. Furthermore, it would be very useful to have selectable marker genes under control of an RNA polymerase I promoter in order to study the mechanism of RNA polymerase I transcriptional control in more detail.

There have been several previous attempts to drive protein expression from an RNA polymerase I promoter (6–11). From these reports, it seems clear that RNA polymerase I will produce abundant transcripts from essentially any chimeric gene connected to an RNA polymerase I promoter (7,8,11). However, in the cases where protein expression was quantitated, expression was at best 5–10 fold lower when transcription is driven by an RNA polymerase I promoter than when the chimeric gene is driven by a moderately active RNA polymerase II promoter (7,9). Because of the low efficiency of protein expression it becomes important to prove that protein expression actually is due to RNA polymerase I rather than due to cryptic RNA polymerase II promoters (8,11). An obvious reason for low protein expression is the fact that RNA polymerase I transcripts retain a triphosphate at their 5′ terminus (12) rather than receiving a trimethyl G cap as is the case for RNA polymerase II transcripts. In the absence of a trimethyl G cap ribosomes appear not to recognize the transcript as message and translation initiation is impaired.

Recent research has shown that eukaryotic cells have a mechanism for obtaining efficient translation in the absence of a trimethyl G cap. Picornavirus transcripts, for example, contain

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a region of secondary structure in the leader which stimulates cap independent initiation of translation (13,14). These have been called Internal Ribosome Entry Sites (IRES). It has been shown previously that use of an IRES element will allow protein production from the uncapped transcripts driven by a bacteriophage T7 promoter (15). We reasoned that the presence of an IRES element in the leader region of an RNA polymerase I transcript might, likewise, allow high level protein production under control of an RNA polymerase I promoter. Data presented in this paper shows that, in fact, use of an IRES element allows protein production from an RNA polymerase I promoter comparable to that obtained from a retroviral promoter that is efficiently transcribed by RNA polymerase II.

MATERIALS AND METHODS

Plasmids

pEN and pENA. An EcoRI to BamHI fragment that contains the entire coding region for the Neomycin resistance gene (*neo*) was subcloned from the plasmid pLNSX (16) into the EcoRI and BamHI sites of pBluescript SK$^+$ (Stratagene). The sequence at the translation start site of *neo* was mutated to an NcoI restriction site using the oligonucleotide 5′-AGGATCGTTTCCCATGGT-TGAACAAGAT-3′. Following mutagenesis, sequences upstream of the *neo* coding region, between the EcoRI site and the newly introduced NcoI site, were replaced with a 596bp EcoRI to NcoI fragment from the plasmid pCITE-1 (Novagen). This fragment contains the IRES element from encephalomyocarditis virus. The resulting plasmid was called pEN.

A 237 bp BamHI to BclII fragment that contains polyadenylation signals from SV40 was subcloned into the BamHI site downstream of the *neo* coding region in the plasmid pEN in an orientation such that an intact BamHI site is maintained between *neo* and the polyadenylation signal. This plasmid was called pENA.

pHENA and pΔHENA. The plasmid prHu3 contains the human rDNA promoter within a fragment that extends from an EcoRI site at −500 to a BamHI site at +1500 relative to the site of transcription initiation at +1 (17). prHu3 was cut at the unique BstEII site at +80, filled in, and EcoRI linkers (CGGAATTCCG) were added. Then the promoter fragment (−500 to +80) was cloned as an EcoRI fragment into pBluescript SK$^+$ in the orientation such that the −500 EcoRI site is adjacent to the T7 promoter in the vector. This plasmid was called pH.

Nucleotides −8 to +3 in the human rDNA promoter were deleted by oligo directed mutagenesis using the oligonucleotide 5′-GCATTTTGGGCCGCCGACACGCTGTCCTCT-3′. The resulting plasmid was called pΔH.

The EcoRI fragments containg the human rDNA promoters in pH and pΔH were subcloned into pENA in the orientation such that RNA polymerase I transcription is directed toward the IRES and *neo* elements. The resulting plasmids were called pHENA and pΔHENA.

pMENA and pΔMENA. The plasmid p5′ −2150 was a gift from Barbara Sollner-Webb (see 18 for sequence). It contains mouse rDNA promoter sequences from −2150 to +292. This plasmid contains a unique StuI site (−640) and a SmaI site (+155). p5′ −2150 was digested with StuI and SmaI and EcoRI linkers (CGGAATTCCG) were added. Then the −640 to +155 fragment was cloned as an EcoRI fragment into pBluescript SK$^+$ in the orientation such that the −640 EcoRI site is adjacent to the T7 promoter in the vector. This plasmid is called pM.

Nucleotides −8 to +3 in the mouse rDNA promoter were deleted using an oligonucleotide of the following sequence: 5′-CCTATTGGACCTGGGACACGCGGTCCTTTC-3′. The resulting plasmid was called pΔM.

EcoRI fragments containing the mouse rDNA promoter in pM and pΔM were subcloned into pENA in the orientation such that polymerase I transcription was toward the IRES and *neo* elements. The resulting plasmids were called pMENA and pΔMENA.

pΔENA, pHΔENA and pMΔENA. The plasmid pENA was cut at a unique PflMI restriction site in the EMC IRES. After blunt ending and ligation of EcoRI linkers (CGGAATTCCG) the plasmid was digested with EcoRI and recircularized, resulting in the deletion of 461bp fro the 5′ portion of the EMC IRES. This plasmid was called pΔENA. pHΔENA and pMΔENA were constructed by inserting the human and mouse promoter fragments from pH and pM respectively into the EcoRI site of pΔENA.

pHEN and pMEN. The SV40 polyadenylation signal present in pHENA and pMENA was deleted by cutting these plasmids at a unique BstBI restriction site 18 bp downstream of the translation stop codon of *neo*. Following blunt ending and addition of XbaI linkers (CTCTAGAG) the plasmids were digested with XbaI and recircularized. This resulted in deletion of sequences from immediately downstream of the *neo* coding region to the XbaI site in the polylinker of the pBluescript SK$^+$ vector. These plasmids were called pHEN and pMEN.

pLNX. This plasmid was constructed by removing the BstBI to StuI fragment from the retroviral construct pLNSX (16). The resultant construct uses a Moloney murine sarcoma virus promoter/enhancer LTR to drive pol II transcription of the *neo* coding region. A second copy of the LTR provides polyadenylation signals (see Figure 2).

pRSV-βgal. This was a gift from Y. Zhang (FHCRC, Seattle) and consists of a Rauscher sarcoma virus (RSV) promoter (376bp MluI-HindIII fragment from pRSV-neo) linked to the entire *E.coli* β-galactosidase coding sequence followed by an SV40 polyA addition signal (273bp BclI-BamHI fragment from SV40). The entire construct is cloned between the SacI and XhoI sites in pBluescript SK$^+$.

Cells and cell culture conditions

LSNV cells (SV40 transformed, HPRT$^-$, human fibroblasts; (19) and rat 208F fribroblasts (20) were cultured in 10% CO_2, 100% humidity, in Dulbecco's Modification of Eagle's Medium (DMEM) containing 4.5g glucose/liter and supplemented with 10% fetal bovine serum and penicillin/streptomycin.

DNA transfection

Cells were plated at a density of 10^6 per 10cm tissue culture dish one day prior to transfection and the medium was replaced with fresh medium immediately prior to transfection. Calcium phosphate-DNA precipitates, prepared as described (16,21) were added to each dish and the cells incubated for 24 hrs. The medium containing the precipitate was then replaced with fresh medium (without precipitate). After further incubation for 24 hrs, the cells

were either harvested for npt assays or passaged into medium containing G418 (0.5mg/ml active concentration) to select for expression of the *neo* gene. All transfection precipitates contained 10μg *neo* test plasmid and 2 μg pRSVβgal control plasmid.

In the transient transfection experiments, variations in transfection efficiency from one experiment to the next were adusted for by setting β-galactosidase activity in each experiment to unity and adjusting the npt activity accordingly.

Neomycin phosphotransferase assay

Cells growing in log phase were treated with trypsin and washed once with medium containing fetal bovine serum and twice with phosphate buffered saline. About 10^6 cells were resuspended in100μl of lysis buffer (50mM phosphate, pH 8.0, 10mM KCl, 1 mM $MgCl_2$, 50mM β-mercaptoethanol) and freeze/thawed three times (−70°). Cell lysates were then clarified by centrifugation and aliquots of the supernatants were asssayed for npt activity. For npt assay, 1−10μl of cell extract was mixed with 50μl of npt reaction mix (67mM tris, pH 7.1, 42mM $MgCl_2$, 400mM NH_4Cl_2, 1.2 μM ATP, 90 μg/ml neomycin sulfate, 20 μCi γ-^{32}P-ATP/ml). The reaction was incubated for 1 hr at 37° and then extracted with an equal volume of phenol/chloroform/2% isoamyl alcohol. Aliquots of the aqueous phase were spotted onto P81 ion-exchange paper (Whatman) and the paper was washed extensively in 50mM phosphate, pH 7.5. Npt mediated incorporation of ^{32}P into neomycin sulfate was determined by scintillation counting. Npt activity was expressed as CPM incorporated per μg of soluble cell protein per hour.

β-galactosidase assay

Activity was measured in clarified extracts (as prepared for npt assays) by adding aliquots to ONPG reaction mix (50mM phosphate, pH 8.0, 10mM KCl, 1mM $MgCl_2$, 0.4mg/ml 0-nitrophenyl-β-D-galactopyranoside). Reaction was at room temperature and the change in optical density at 410 nm was followed over a period of 0.5 to 2.0 hrs. β-galactosidase activity was expressed as the change in optical density per μg of cell protein per hr.

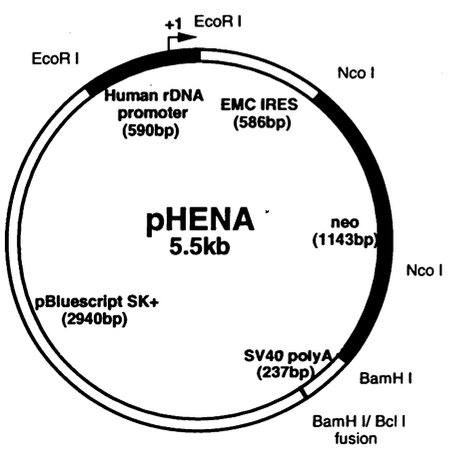

Figure 1. Structure of pHENA, a plasmid designed to express a functional protein under control of an RNA polymerase I promoter. This plasmid contains a human polymerase I promoter, an IRES element, the coding region of the neomycin phosphotransferase gene, and a poly A addition signal. These elements are linked in order and inserted into the vector, pBluescript SK$^+$. Details of the construction of this plasmid, and others related to it, are given in the Materials and methods.

RESULTS

Plasmid constructs

Figure 1 illustrates the structure of pHENA, the basic construct that was used to obtain protein production under control of an RNA polymerase I promoter. pHENA contains in order, **1)** a human RNA polymerase I promoter, **2)** an IRES element from encephalomyocarditis virus (EMCV), **3)** the protein coding region of the neomycin phosphotransferase gene (*neo*), and **4)** a poly A addition signal from SV40. This linear array of elements is inserted into the plasmid vector, pBluescript SK$^+$(Stratagene). We chose neo as a marker for protein production since neomycin phosphotransferase (npt) activity can be measured both by an enzymatic assay and by its abilty to confer resistance on cells to the neomycin analog G418. Thus we could measure neo activity in both transient and stable transfection assays.

As shown in Figure 2, we also constructed a systematic set of other constructs in which components of the basic pHENA plasmid were either deleted or replaced by other components. For example, pΔHENA is identical to pHENA except that it contains a 10bp deletion in the human RNA polymerase I promoter which makes it inactive for RNA polymerase I transcription (17). pHΔENA is identical to pHENA except that most of the IRES element is deleted (and thereby rendered nonfunctional). pHEN is identical to pHENA except that the polyA addition signal is omitted. pMENA, pΔMENA, pMΔENA and pMEN are analogous constructs in which the human RNA polymerase I promoter is replaced by the RNA polymerase I promoter from mouse. pENA has no RNA polymerase I promoter while pΔENA and pEN lack an RNA polymerase I promoter plus either the IRES element or the polyA signal. Finally, pLNX contains a strong RNA polymerase II promoter (the Moloney virus LTR) driving the *neo* gene (16).

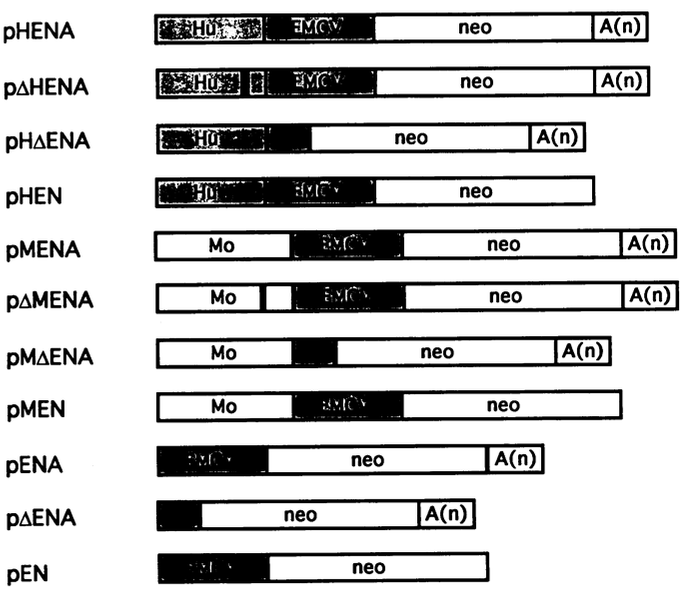

Figure 2. Schematic structure of RNA polymerase I expression contructs. The elements which were linked together to make these chimeric constructs were: Hu, human polymerase I promoter; Mo, mouse polymerase I promoter; EMCV, IRES element from encephalomyocarditis virus; neo, coding region from the neomycin phosphotransferase gene; A(n), poly A addition signals from SV40. Each chimeric construct was inserted into the vector, pBluescript SK$^+$ as described in Methods.

Transient transfection assays

Each of the constructs shown in Figure 2 was tested in transient transfection assays for the ability to produce npt. Npt activity was assayed by measuring the incorporation of ^{32}P into neomycin sulfate using extracts prepared from transfected cells. Table I shows the results when the constructs are transiently introduced into either human cells or rodent cells. In Table I the npt values have been normalized to adjust for variations in transfection efficiency (using β-galactosidase expression as the internal control, see Materials and methods). In Figures 3A and B the same data are presented as bar graphs in which the npt activity produced by pLNX, the RNA polymerase II driven construct, is set at 100% within each cell type and the npt values of the other constructs are compared to it.

As expected, the pLNX construct, containing a strong retroviral RNA polymerase II promoter, actively produces npt in both human and rodent cells. In human cells, pHENA, the complete construct driven by a human RNA polymerase I promoter, produced an amount of npt comparable to that produced by pLNX (Table 1 and Figure 3A). This indicates that the human RNA polymerase I promoter is capable of driving efficient protein production. Examination of the activity produced by the other constructs supports the conclusion that this high level of protein production requires each of the individual elements that go to make up pHENA. For example, npt enzyme production from pΔHENA and pENA is very low (30 fold and >60 fold down respectively), indicating that a functional RNA polymerase I promoter is required. Enzyme production from pHΔENA and pHEN is also low (~10 fold down), indicating that an intact IRES element and a polyA signal are important for expression. Further evidence that npt enzyme expression is being driven by an RNA polymerase I promoter is evidenced by the species specificity of the expression. All four of the constructs that are driven by a mouse RNA polymerase I promoter (pMENA, pΔMENA, pMΔENA and pMEN) show only marginal expression when introduced into human cells.

Each of the constructs was also tested for transient expression in a rat cell line (Table 1 and Figure 3B). The mouse RNA polymerase I promoter functions well in rat cells while the human promoter does not (discussed in 4). As expected from the known species specificity of RNA polymerase I transcription, the complete construct driven by the mouse RNA polymerase I promoter (pMENA) expressed the highest levels of npt in this set of assays. In fact, pMENA actually produced more enzyme activity on average than did the RNA polymerase II promoter driven construct (pLNX). As in Figure 3A, all of the other constructs produced significantly lower levels of enzyme activity supporting the conclusion that the majority of protein expression from pMENA is due to transcription from the mouse RNA polymerase I promoter and requires both the IRES and polyA elements.

In Figures 3A and B the results of the transient expression npt assays are expressed as a percentage of the npt level expressed by the control plasmid, pLNX, in either human or rat cells. When presented in this manner the data suggest that background expression of npt activity is higher in rat cells than in human cells. For example, in rat cells the construct with a debilitated mouse rDNA promoter, pΔMENA, expressed npt activity at 37% the level expressed by the control plasmid, pLNX. In human cells, by contrast, pΔMENA expressed npt at only 9% the level expressed by pLNX. However, examination of the absolute values for npt activity, in Table 1, leads to a different conclusion. In fact, background expression of npt activity is similar in both cell types (pΔMENA expression is actually lower in rat than in human cells). What is different is that overall transformation efficiency is lower in rat cells and thus expression of the control plasmid, pLNX, is 6.8 fold lower in rat than in human cells. Thus, while the signal to background ratio was lower in rat than in human cells, it appears that the various constructs behaved with similar specificity in both types of cells.

Stable transfection assays

We have also tested each of the constructs shown in Figure 2 for their ability to cause stable transformation of cells to resistance to the neomycin analog, G418. In Figure 4A experiments are summarized testing stable transfomation of human cells. The control RNA polymerase II promoter construct (pLNX) produced a total of 31,733 resistant colonies. The human RNA polymerase I promoter construct, pHENA, produced about 50−70% of the control number of resistant colonies. No other construct produced colonies above a low background except for pHEN. This indicates that the human RNA polymerase I promoter and the IRES elements were strictly required while the poly A signal was partly dispensable in human cells. A similar result was obtained when stable transformants were tested on rat cells (Figure 4B) except that even the poly A signal was required in the rat cells in order to detect activity above background. As was observed for transient expression (Table 1 and Figure 3B), transformation efficiency was lower on rat cells (4,506 total resistant colonies produced by pLNX) and the activity driven from the mouse RNA polymerase I promoter in rat cells was up to two fold higher than

Table 1. Npt activity in transfected cells.

		NPT Activity	
Promoter	**Plasmid**	**Human Cells**	**Rat Cells**
Human	**pHENA**	**15200 ± 3900**	**990 ± 350**
	pΔHENA	**1000 ± 100**	**550 ± 190**
	pHΔENA	**2700 ± 1300**	**510 ± 180**
	pHEN	**500 ± 300**	**40 ± 20**
Mouse	**pMENA**	**700 ± 200**	**4660 ± 1200**
	pΔMENA	**2200 ± 980**	**1360 ± 390**
	pMΔENA	**1300 ± 400**	**370 ± 50**
	pMEN	**200 ± 100**	**480 ± 150**
None	**pENA**	**400 ± 100**	**1170 ± 340**
	pΔENA	**1000 ± 400**	**290 ± 40**
	pEN	**200 ± 100**	**110 ± 20**
MoMLV LTR	**pLNX**	**24900 ± 5500**	**3670 ± 1280**
	none	**500 ± 100**	**150 ± 20**

mean ± s.e. CPM / hr / mg protein

Npt activity was measured as described in Methods. The values in the Table have been corrected for transfection efficiency by comparison to the amount of β-galactosidase activity produced from a co-transfected internal standard plasmid.

that obtained from the control RNA polymerase II construct (pLNX).

DISCUSSION

The results presented here lead us to conclude that it is possible to produce functional protein from a gene driven by an RNA polymerase I promoter at a level comparable to that produced from a highly active RNA polymerase II promoter. Requirements for such protein production include **1)** an RNA polymerase I promoter with the correct species specificity, **2)** an IRES element in the leader of the chimeric mRNA, and **3)** a polyA addition signal on the 3′ end of the mRNA.

Evidence that the protein production we observe actually is being driven by an RNA polymerase I promoter and not from cryptic polymerase II promoters is three-fold. First, efficient

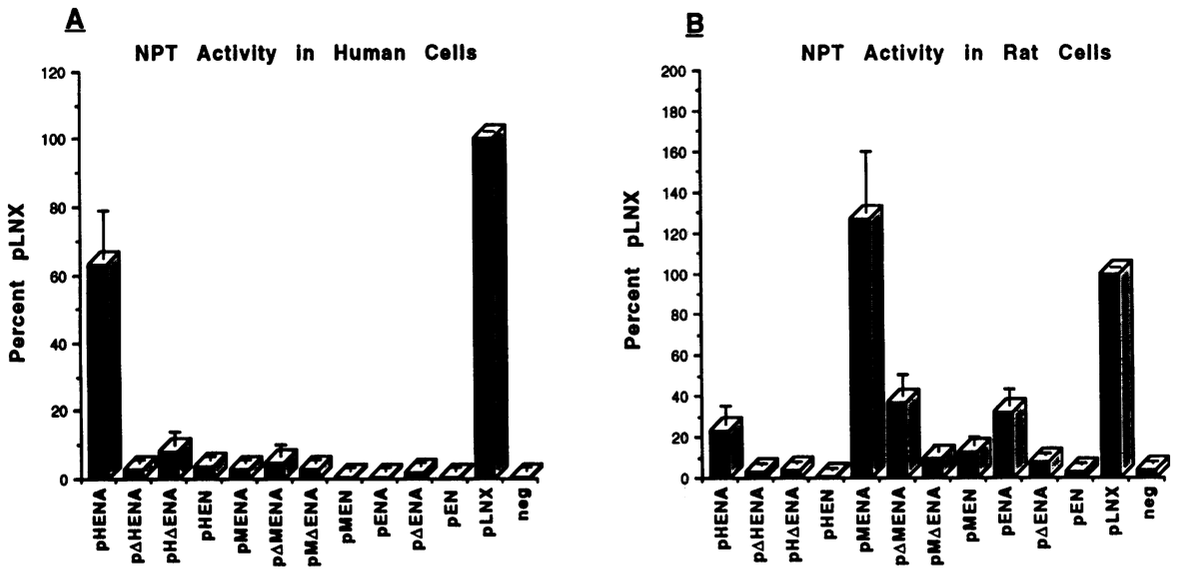

Figure 3. Protein expression of various polymerase I constructs as assayed by transient expression in cultured cells. Cells were transiently transformed with each of the individual constructs shown in Figure 2 as described in Methods. Cell extracts were then assayed for the expression of npt activity. Activity produced by each of the polymerase I driven constructs was normalized for transfection efficiency (by comparison to the amount of β-galactosidase produced from a co-transfected internal standard plasmid) and then was compared to the npt activity produced by pLNX, a construct in which a *neo* gene is driven by a polII transcribed retroviral LTR. **A.** Activity of constructs transfected into human cells. **B.** Activity of constructs transfected into rodent (rat) cells.

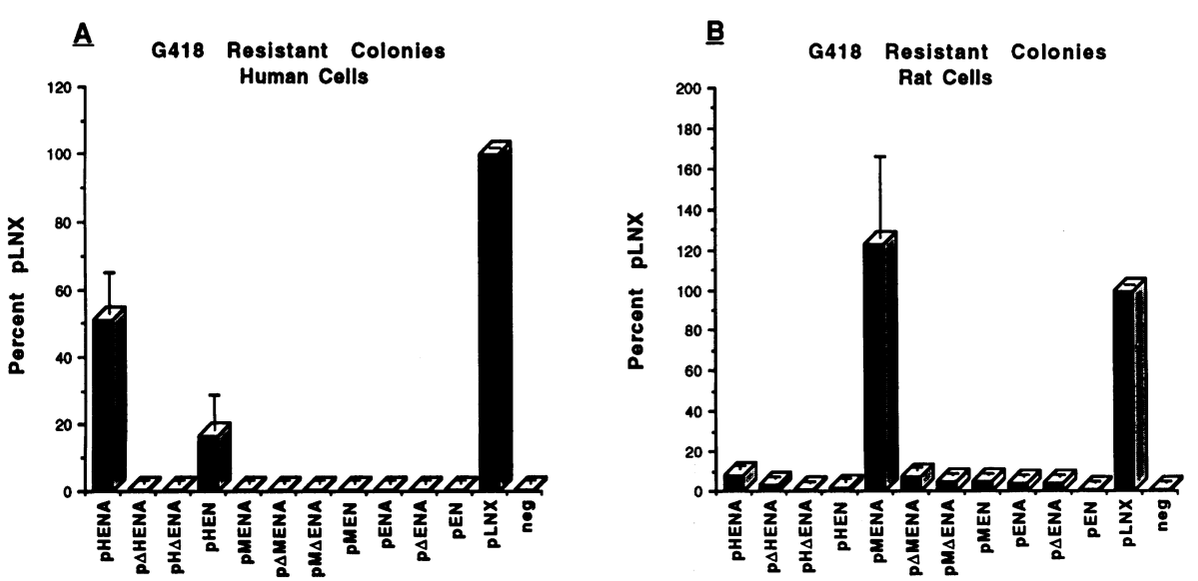

Figure 4. Ability of various constructs to cause stable transformation to G418 resistance. Each of the constructs shown in Figure 2 was tested for its ability to stably transform cells to G418 resistance. The number of stable colonies produced by the polymerase II driven construct, pLNX, was set at 100% and each of the polymerase I driven constructs was compared to that number. **A.** G418 resistant colonies produced in human cells. **B.** G418 resistant colonies produced in rodent (rat) cells.

production requires an intact RNA polymerase I promoter. Either deleting the RNA polymerase I promoter (pENA) or introducing a debilitating mutation (pΔHENA in human cells or pΔMENA in rat cells) caused a significant loss of protein expression. Second, protein expression exhibited the strong species specificity expected of promoters for RNA polymerase I (but not expected for RNA polymerase II). The human promoter construct (pHENA) worked poorly in rat cells while the mouse promoter construct (pMENA) functioned poorly in human cells. Finally, the strong requirement for the IRES element supports the conclusion that the transcripts produced from the RNA polymerase I driven plasmids were not capped (ie, were not produced by RNA polymerase II). Taken together, these results argue strongly that protein production was largely due to transcription by RNA polymerase I.

We agree, however, with earlier authors (8,11) that transcription from cryptic RNA polymerase II promoters can occur in some situations and may cause artifacts. In the transient expression assays shown in Figure 3B, the human promoter construct yielded anomalous expression in rat cells that ranged up to nearly half that obtained with the control RNA polymerase II promoter construct (pLNX). We suspect that this anomalous expression was due to cryptic RNA polymerase II transcription since it did not require a functional RNA polymerase I promoter. Fortunately, even this relatively low anomalous transcription appears to be absent when stable transformants are scored (Figure 4B). In the stable transformants the background expression is uniformly low and expression due to the RNA polymerase I promoters is still high relative to the RNA polymerase II promoter control. Indeed, in the stable transformation experiments the requirement for an intact RNA polymerase I promoter of the approriate species as well as an intact IRES element is more evident than in the transient transfection experiments.

Even after allowing for the possibility of expression from cryptic RNA polymerase II promoters, it is likely that previous workers also obtained some protein expression driven by an RNA polymerase I promoter. This can be deduced from the fact that expression in some of the earlier reports was also shown to follow species specificity (6,7,9). However, in the cases where expression levels were actually quantitated (7,9,11) protein production was at least five to ten-fold lower than that obtained with any of several RNA polymerase II promoters. We think we have reproduced these earlier results by using the pHΔENA and pMΔENA constructs which produce npt activity about ten-fold lower than that produced by the control RNA polymerase II promoter (Figure 2). Our major contribution in this work is to show that insertion of an IRES element into the mRNA leader of an RNA polymerase I driven gene can overcome this problem and lead to protein production equal to, or better than, that obtained with a highly active retroviral LTR promoter.

At the outset of this work we imagined a variety of possible reasons why protein expression from an RNA polymerase I promoter might be poor. The fact that simple insertion of an IRES element improves expression, indicates that a major problem is the inability of the ribosome to recognize most RNA polymerase I transcripts as messenger RNA. A similar conclusion can be reached from recent work in trypanosomes. In these organisms RNA polymerase I transcripts receive a capped 5′ terminus via an efficient transplicing mechanism. Such trans-spliced RNA polymerase I transcripts are efficiently translated (22–24), leading to the conclusion that lack of a proper 5′ terminus is probably the only thing that normally prevents RNA polymerase I transcripts from being treated as messenger RNA.

It is probable that additional improvements can be made in both the pHENA and pMENA constructs that would lead to even higher levels of RNA polymerase I driven protein production. Available evidence indicates that all of the RNA polymerase I promoters within a cell are in competition with each other for the factors needed to establish the stable promoter complex (see 2 for review). Only a fraction of RNA polymerase I promoters are successful in this competition indicating that at least one component of the stable complex is limiting in most cells. Addition of multiple RNA polymerase I enhancer elements in cis to a given promoter will give it an advantage in this competition. Thus it is possible that adding enhancer elements to the pHENA and pMENA constructs would lead to activation of larger numbers of the introduced promoters and thus to increased protein production.

Two independent studies report finding abundant transcripts from a chimeric gene in the nucleus with much smaller amounts being found in the cytoplasm (8,11). Thus it is also possible that improvements could be made in the efficiency of RNA polymerase I chimeric transcript transport to the cytoplasm. Since endogenous RNA polymerase I transcripts account for up to 50% of all the RNA synthesized in the nucleus it seems reasonable to think that eventually an RNA polymerase I driven chimeric gene could be engineered to produce much more protein product than any known RNA polymerase II driven gene in any cell type.

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REFERENCES

1. Nogi, Y., Yano, R. and Nomura, M. (1991) Proc. Nat. Acad. Sci. USA, **88**, 3962–3966.
2. Reeder, R. H. (1992) in K. Yamamoto and S. L. McKnight (eds.) *Transcriptional regulation* Cold Spring Harbor, New York, pp.315–347
3. Mahajan, P. B., Gokal, P. K. and Thompson, E. A. (1990) J. Biol. Chem., **265**, 16244–16247.
4. Bell, S. P., Jantzen, H.-M. and Tjian, R. (1990) Genes and Dev., **4**, 943–954.
5. Bell, S. P., Pikaard, C. S., Reeder, R. H. and Tjian, R. (1989) Cell, **59**, 489–497.
6. Fleischer, S. and Grummt, I. (1983) EMBO J., **2**, 2319–2322.
7. Grummt, I. and Skinner, J. A. (1985) Proc. Nat. Acad. Sci. USA, **82**, 722–726.
8. Smale, S. T. and Tjian, R. (1985) Mol. Cell. Biol., **5**, 352–362.
9. Surmacz, E., Ronning, O., Kaczmarek, L. and Baserga, R. (1986) J. Cell. Phys., **127**, 357–365.
10. Surmacz, E., Kaczmarek, L., Ronning, O. and Baserga, R. (1987) Mol. Cell. Biol., **7**, 657–663.
11. Lopata, M. A., Cleveland, D. W. and Sollner-Webb, B. (1986) Proc. Nat. Acad. Sci. USA, **83**, 6677–6681.
12. Reeder, R. H., Sollner-Webb, B. and Wahn, H. L. (1977) Proc. Nat. Acad. Sci. USA, **74**, 5402–5406.
13. Jang, S. K., Davies, M. V., Kaufman, R. J. and Wimmer, E. (1989) J. Virol., **63**, 1651–1660.
14. Sonenberg, N. and (1990) Curr. Top. Microbiol. Immunol., **161**, 23–47.
15. Elroy-Stein, O., Fuerst, T. R. and Moss, B. (1989) Proc. Nat. Acad. Sci. USA, **86**, 6126–6130.
16. Miller, A. D. and Rosman, J. (1989) Biotechniques, **7**, 980–990.
17. Learned, R. M. and Tjian, R. (1982) J. Mol. Appl. Gen., **1**, 575–584.
18. Kuehn, M. and Arnheim, N. (1983) Nucl. Acids Res., **11**, 211–224.

19. Miller, A. D., Jolly, D. J., Friedmann, T. and Verma, I. M. (1983) Proc. Nat. Acad. Sci. USA, **80**, 4709–4713.
20. Quade, K. (1979) Virol., **98**, 461–465.
21. Corsaro, C. M. and Pearson, M. L. (1981) Somatic Cell Genetics, **7**, 603–616.
22. Zomerdijk, J. C. B. M., Kieft, R. and Borst, P. (1991) Nature, **353**, 772–775.
23. Zomerdijk, J. C. B. M., Kieft, R., Shiels, P. G. and Borst, P. (1991) Nucl. Acids Res., **19**, 5153–5158.
24. Rudenko, G., Chung, H.-M. M., Pham, V. P. and Van der Ploeg, L. H. T. (1991) EMBO J., **10**, 3387–3397.