Different RNA patterns of globin and non-globin 40S heterogeneous nuclear RNA-protein complexes in chicken reticulocyte nuclei

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ABSTRACT

40s heterogeneous nuclear RNA-protein complex (HnRNP) was isolated from chicken reticulocyte nuclei after digestion of RNA by the endogenous nuclease. The size of the total RNA and β globin RNA was compared by agarose and high resolution acrylamide gel electrophoresis. The results show that size of the total RNA ranges between 20 to 650 bases and the most prominent sizes are between 20 and 70-80 bases. The size of the RNA increases at roughly 10 base intervals between 40 and 70 bases on an acrylamide gel. The size of the β globin RNA is smaller (25-50 bases) than the total RNA and also the RNA pattern is considerably different from that of total RNA. This suggests that, although the overall structure of 40s HnRNP might be the same, there is significant differences in the interaction of globin RNA with HnRNP protein and that of non-globin RNA with HnRNP protein in chicken reticulocyte nuclei.

INTRODUCTION

Eucaryotic precursor mRNAs (heterogeneous nuclear RNA) are complexed with proteins during transcription (1,2). Recent electronmicroscopic studies on the nascent HnRNP structure indicate that RNP particles are assembled at specific regions of precursor RNA, suggesting that the RNP may have important biological functions, such as RNA processing and splicing (3).

HnRNP is usually obtained from sonicated nuclei (4,5). Mild nuclease digestion of HnRNP (5,6) or digestion of nuclei by the endogenous nuclease (7,8,9) produces a HnRNP particle which has a sedimentation coefficient of 40s. The 40s HnRNP could be the basic unit structure of HnRNP analogous to nucleosomes. Purified 40s HnRNPs from various tissues and cells show a well defined protein composition (10). Sizes of the proteins range from 30,000 to 90,000 daltons (10) and the total HnRNP proteins crosslinked to RNA by UV in vivo show a protein pattern remarkably similar to that of the isolated 40s HnRNP, suggesting that most of the RNA in HnRNP is bound by the same proteins (11). However, the size pattern of RNA and the specificity of protein-RNA interaction in 40s HnRNP are not well defined.

The sequence complexity of nuclear precursor mRNA is much larger than the

complexity of the cytoplasmic mRNA (12,13). This suggests that much of the nuclear RNA is turned over and does not leave the nucleus. In the case of duck reticulocyte 40s HnRNP the globin RNA sequence is only 0.5% of the total RNA sequences in the 40s HnRNP (9). Therefore, most of the nuclear RNA turned over in the nucleus is also assembled into a similar HnRNP structure. In view of the uniqueness of the globin RNA, which is the major mRNA in the cytoplasm in reticulocytes, we examined the possible differences in the structure of the globin 40s HnRNP and that of non-globin 40s HnRNP in chicken reticulocytes. We examined the RNA fragment size of the total RNA and the globin RNA. We found that the size pattern of the total RNA and that of the β globin RNA are different and that the globin RNA fragments are much smaller than the total nuclear RNA fragments in the 40s HnRNP.

MATERIALS AND METHODS

White leghorn chicken (1-2 kg) were injected for 7 days with phenylhydrazine (10 mg/kg/day) intramuscularly, and blood was collected in 0.14 M NaCl, 5 mM KCl, 1.5 mM MgCl₂ containing 0.1 mg/ml heparin. The cells were washed free of plasma and buffy coat and lysed in TKM (10 mM Tris, pH 7.5, 30 mM KCl, and 2 mM MgCl₂). Isotonicity was restored by adding 0.1 vol. of 2.5 M sucrose. The lysed cells were centrifuged at 1000xg to pellet the nuclei. The nuclei were washed once with 1% Triton X-100 in 0.25 M sucrose-TKM and several times in 0.25 M sucrose-TKM.

Preparation of 40s HnRNP.

40s HnRNP was isolated from the reticulocyte nuclei by the procedure of Samarina et al. (7) with the modifications adopted for duck reticulocyte 40s HnRNP (9). Nuclei were extracted with 0.1 M NaCl, 1 mM ATP, 10 mM Tris, pH 8 at 37° C by gently shaking for 15 min. The nuclei were centrifuged at 5000 rpm in a Sorvall centrifuge (HB-4 rotor). The supernatant was saved. The nuclei were extracted two more times, and the supernatants were combined and clarified by centrifugation at 10,000 rpm. HnRNP was pelleted by centrifugation at 60,000 rpm in a Beckman 65 rotor for 4 hrs at 4°C over 1.5 ml of 20% sucrose cushion in 10 mM Tris, pH 8, 0.1 M NaCl, 1 mM MgCl₂ (STM). The pellet was resuspended in STM and centrifuged over a 15-30% sucrose gradient in STM in a SW27 rotor for 16 hrs at 25,000 rpm at 4°C. The gradient was withdrawn from the bottom of the tubes and monitored at 254 nm by an Isco UV monitor (model U5). Ehrlich ascites carcinoma (mouse) and chicken reticulocyte ribosome subunits were used as size markers. The 40s HnRNP peak was pooled, concentrated, and recentrifuged over another 15-30% sucrose gradient.

SDS gel electrophoresis.

The purified 40s HnRNP and ribosomal subunits were dissociated in 1% SDS (sodium dodecylsulfate), 10 mM Tris, pH 8, and 50 mM mercaptoethanol and electrophoresed through 8% acrylamide gel according to the procedure of Laemmli (14).

Isolation of RNA.

40s HnRNP was made 0.5% SDS, 10 mM Tris, pH 7.5, 1 mM EDTA and extracted with phenol-chloroform (1:1) and chloroform. The RNA sample was made 0.3 M sodium acetate and precipitated with 2 vol. of ethanol at -70°C for 30 min and centrifuged. The RNA sample was dissolved in 10 mM Tris, pH 7.5, 0.1 M NaCl, 1 mM MgCl₂ and digested with 10 units of RNase-free DNase I (15) for 30 min at 37°C. The RNA was reextracted with phenol-chloroform.

End labeling of the RNA extracted from 40s HnRNP.

The RNA extracted from 40s HnRNP was labeled with ³²P-γ-ATP (2900 Ci/mmol, New England Nuclear) in the presence of T4 polynucleotide kinase (Bethesda Research Laboratory) (16) without dephosphorylation of RNA. The radiospecific activity of the labeled RNA was usually 2-4 x 10⁶ cpm per μg of RNA.

Isolation of β globin RNA.

A pBR322 plasmid (pGβ4) containing the genomic chicken β globin gene (4.3 Kb EcoRI-BamHI fragment) was used for hybridization of the labeled RNA. The plasmid DNA (25 μg) in 50-100 μl of 0.3 M NaOH was heated at 100°C for 5 min and diluted with 3 ml of 0.5 M Tris, pH 7.5, and 1.5 M NaCl. The DNA sample was passed through a 24 mm nitrocellulose filter (Schleicher-Schull, BA-85). The filter was rinsed in 2x SSC (1xSSC = 0.15 M NaCl and 0.015 M sodium citrate) and baked at 80°C for 2 hrs. The filter was prehybridized and hybridized with the ³²P-RNA in the buffer system described by Wahl et al. (17) at 42°C. The filter was washed in 2x SSC for 5-10 min at room temp and with 0.1xSSC-0.1% SDS at 50°C for 15-20 min each for 4 times. RNA was eluted in 1 ml of H₂O at 100°C for 1 min. After second elution the 2 ml of eluants were mixed with 10 μg of yeast RNA and extracted with phenol-chloroform and precipitated with ethanol. About 1% of the labeled 40s HnRNP RNA binds to the β globin gene.

Polyacrylamide gel electrophoresis of RNA.

³²P-labeled RNA samples were precipitated in ethanol and rinsed with 70% ethanol and dried. The sample was dissolved in 10 μl of 80% formamide, 10 mM NaOH, 0.1% xylene cyanol, and 0.1% bromphenol blue, heated at 90°C for 1 min and quick cooled in ice water. About 10,000 cpm were applied on a 8% acrylamide gel (15 x 20 cm) in 8 M urea (16) and electrophoresed. ³²P-labeled Hinf I

digest of M13mp7 RF DNA (denatured) was used as size markers. The gel was exposed to a Kodak XAR film at -70°C in the presence of a DuPont intensifying screen.

Blot hybridization of 40s HnRNP RNA.

RNA and marker DNA were denatured in a mixture of formamide and formaldehyde and applied to a 2% agarose gel containing 2.2 M formaldehyde which was prepared according to the procedure of Lehrack et al. (18). After electrophoresis the gel was stained with ethidium bromide and photographed. The RNA was transferred to DBM paper as described by Wahl et al. (17). The paper was hybridized under the conditions described by Wahl et al. (17) with the plasmid pG β 4 which was labeled with 32 P by nick translation (19). The AluI digest of pBR322 plasmid DNA was used as size markers.

RESULTS

The 40s HnRNP isolated by extraction of nuclei is the product of endonucleolytic digestion of HnRNP (7-9), and Maundrell and Scherrer (9) showed that the RNA fragments present in duck reticulocyte 40s HnRNP range from 100 to 300 bases. The content of globin RNA in the duck 40s HnRNP is about 0.5% (9). Since globin RNA is the major cytoplasmic mRNA in the reticulocyte, the bulk of the pre-mRNA in the nucleus is non-globin RNA which is turned over. Maundrell and Scherrer reported that about 50% of the nuclear globin RNA can be extracted in the form of HnRNP (9). Since we were interested in comparing the size of non-globin and globin RNA in 40s HnRNP we had to be sure that the 40s HnRNP preparation is not contaminated by other RNP. We found that the reticulocyte nuclei are contaminated with a significant amount of ribosomes (unpublished results). Therefore, ribosomal subunits could be the major contaminants of our 40s HnRNP preparation. Fortunately the chicken and duck reticulocyte ribosome subunits have sedimentation coefficients of 30s and 50s (9) which are considerably different from that of 40s HnRNP. As shown in Fig. 1 the 40s HnRNP has a sedimentation coefficient distinctly different from those of the chicken reticulocyte ribosomal subunits. The size of 40s HnRNP was further confirmed by using mouse ribosomal subunits which have sedimentation coefficients of 40s and 60s (not shown here). The 40s HnRNP peak was pooled and recentrifuged on a sucrose gradient to insure the purity of the particle.

The 40s HnRNP obtained after the second sucrose gradient centrifugation are enriched in the major HnRNP proteins whose molecular weights range from 32K to 44K daltons (9,10, Fig. 2). The overall pattern of proteins is clearly different from that of 30s and 50s ribosomal subunits. Therefore, if there is

Fig. 1. Sucrose gradient centrifugation of chicken reticulocyte 40s HnRNP. The HnRNP extracted from reticulocyte nuclei was loaded on a 15-30% sucrose gradient and centrifuged. Chicken reticulocyte ribosome subunits were used as size markers. —, HnRNP; ---, ribosome subunits.

any contamination by ribosomal subunits it must be minor. In addition intact ribosomes and polysomes migrate faster than 40s HnRNP, and the globin mRNA-protein complex originally associated with polyribosomes and the post-ribosomal globin mRNA-protein complex have sedimentation coefficient of 15s and 20s, respectively (20). Therefore, it is unlikely that our 40s HnRNP preparation is contaminated by ribosomes or any cytoplasmic globin mRNA-protein complex. As far as we can determine there is no other well defined globin RNA-protein particle in both the cytoplasm and the nucleus.

To determine the size of total and globin RNA sequences in the 40s HnRNP the RNA isolated from the 40s HnRNP was denatured in formamide and formaldehyde and electrophoresed through a 2% agarose gel in the presence of formaldehyde (18). Staining of the RNA with ethidium bromide shows a smeared pattern which ranges between 50 to 650 bases. However, when the RNA was transferred from the gel to DBM-paper (17) and probed with labeled β globin DNA, the size of the β globin RNA was much smaller than the total RNA even after a long exposure of X-ray films (Fig. 3). The smaller size of β globin RNA is not due to the loss of RNA during hybridization since the RNA was crosslinked to the DBM paper. Also it is highly unlikely that the RNA was degraded during the transfer of the RNA. To resolve the RNA better the 40s HnRNP RNA was labeled at the 5' end with ^{32}P - γ -ATP in the presence of T4 polynucleotide kinase. To obtain the globin RNA the labeled RNA was hybridized to genomic β globin DNA fixed on a nitrocellulose filter and was eluted from the filter as described in Materials and Methods. About 1% of the labeled HnRNP RNA hybridized to the

Fig. 2. Protein pattern of HnRNP and ribosome subunits. The 40s HnRNP after first and second sucrose gradient centrifugation and the ribosome subunits were dissociated in SDS and separated by SDS gel electrophoresis. Lane 1, 50s ribosome subunit; 2, 30s ribosome subunit; 3, 40s HnRNP after 2nd centrifugation; 4, 40s HnRNP after 1st centrifugation.

globin gene. This amount is in good agreement with the amount of the duck globin RNA sequences present in the duck 40s HnRNP (9). The labeled total RNA and globin RNA were resolved by electrophoresis through a 8% acrylamide gel in 8M urea as shown in Fig. 4. The HnRNP RNA was efficiently labeled at 5' ends without dephosphorylation. Therefore, the RNA was cleaved by endonuclease which most likely produces a 3' phosphate in the RNA. The labeled RNA should have phosphates at both the 5' and 3' ends, and this will retard the mobility of small RNAs to some extent. Mobility of the RNA with one phosphate at one end is the same as the mobility of single-stranded DNA fragments under denaturing conditions (21). However, the main purpose of our work is to compare the size of the total and the globin RNA and we should be able to estimate the approximate size of the RNA fragments. Electrophoresis through a 0.4 mm thick gel shows a rather complex pattern of total and globin RNA (Fig. 4). The size of total RNA ranges from 20 bases up to 300 bases (upper limit of the gel system). The most prominent sizes appear to be between 20 and 70-80 bases. The fragments do not appear to be the product of completely random cutting of the 40s HnRNP since some bands are more intense than others. As will be discussed later we can see some regularity in the size of RNAs using a lower resolution gel (See Fig. 5). The size of the β globin RNA is smaller than the total RNA fragments in this high resolution gel system. The most prominent sizes are from 25 to 50 bases (Fig. 4, lane 2). The patterns of intense bands in the total RNA and in the globin RNA are not the same. The globin RNA left in the nucleus after extraction of HnRNP is also in the range of 25 and 50

Fig. 3. Agarose gel electrophoresis of the 40s HnRNP RNA. The RNA extracted from the 40s HnRNP was denatured in formamide and formaldehyde and run on a 2% agarose gel in formaldehyde. The RNA was stained with ethidium bromide (lane 1) and photographed. The RNA was then transferred to DBM paper and hybridized with ^{32}P -globin plasmid (lane 2). The size markers (lane M) used in this experiment was the Alu I digest of pBR322 plasmid. The DNA was denatured before electrophoresis.

bases, but the band pattern is somewhat different from that of the β globin RNA in 40s HnRNP. For example, the strong 45 base band in the 40s HnRNP (lane 2) is missing in the globin RNA left in the nucleus (lane 3). In contrast the 40 base band in the nuclear globin RNA is missing in the 40s HnRNP globin RNA.

The smaller size of the globin RNA in the 40s HnRNP could be due to faster rate of digestion of the globin HnRNP than non-globin HnRNP. To examine this possibility the isolated 40s HnRNP was treated with micrococcal nuclease for varying lengths of time. RNA was extracted from each HnRNP sample and labeled with ^{32}P . The labeled RNA was separated on a 1.5 mm thick 8% acrylamide gel in 8M urea (Fig. 5A). The resolution of RNA in a thicker gel was lower than that with the 0.4 mm gel shown in Fig. 4 but we can see a regular pattern of RNA bands probably due to the clustering of similar sizes of RNA fragments. The RNA in lane 1 and 2 were from two different preparations of 40s HnRNP and the pattern is remarkably similar (Fig. 5A). The same RNA shown in lane 1 of Fig. 5A was used in the experiment shown in Fig. 4. Therefore,

Fig. 4. Acrylamide gel electrophoresis of the ^{32}P labeled 40s HnRNP RNA. The RNA extracted from the 40s HnRNP was labeled with ^{32}P - γ -ATP in the presence of T4 polynucleotide kinase and the RNA was hybridized to the β -globin gene fixed to a nitrocellulose filter. The total RNA (lane 1) and the globin RNA (lane 2) were separated by 8% acrylamide gel (15 x 20 cm; 0.4 mm thick) in 8 M urea. Lane 3 shows the β -globin RNA remaining in the nucleus after extraction of HnRNP. As size markers the M13mp7 phage DNA (replicative form), digested with *Hinf*I, was used after labeling with ^{32}P at 5' end. The DNA was denatured before electrophoresis.

the different appearance of the RNA pattern in the two gel systems (compare Fig. 4 and 5A) is due to higher resolution of similar sizes of RNA in a thinner gel. Of particular interest is the 10 base intervals of intense RNA bands from 40 to 70 bases as marked by dots in Fig. 5A. This could indicate a basic structural organization of the 40s HnRNP. Digestion of the 40s HnRNP with 20 units/ml of micrococcal nuclease for 60 min does not change the RNA pattern significantly. Even digestion with ten times more enzyme for 60 min does not change the overall pattern except for the reduction of larger bands (Fig. 5A, lane 7). This suggests that the RNA in our 40s HnRNP preparation was digested close to the limit by the endogenous nuclease during extraction from nuclei. The globin RNA in the 40s HnRNP which was digested for 60 min with 20 units/ml micrococcal nuclease still shows the major 45 and 25 bases RNA remaining (Fig. 5B).

Fig. 5. Digestion of the 40s HnRNP with micrococcal nuclease. The 40s HnRNP was digested with 20 units/ml of micrococcal nuclease at 37°C for varying lengths of time, and the RNA was extracted and labeled with ^{32}P as in Fig. 4. (A), Lane 1 and 2 show the total RNA from two different preparations of 40s HnRNP. Please note that the RNA used in Fig. 4 was the same RNA shown in Lane 1 of this figure. Lane 3, 1 min.; lane 4, 5 min.; Lane 5, 30 min.; lane 6, 60 min. Lanes 7 shows the RNA from the sample digested for 60 min. with 200 units/ml micrococcal nuclease. Lane M shows the labeled DNA standards. The numbers indicate the size of DNA fragments in bases. The RNA samples were separated on a 1.5 mm thick 8% acrylamide gel (15 x 20 cm) in 8 M urea. (B). The globin RNA in the 40s HnRNP digested for 60 min with 20 units/ml micrococcal nuclease (lane 1); markers, lane 2.

DISCUSSION

The 40s HnRNP released from chicken reticulocyte nuclei by the endogenous nuclease digestion of RNA was purified by two successive sucrose gradient centrifugations. The size of the RNA fragments present in our 40s HnRNP preparation ranges from 20 bases up to 650 bases. However, the predominant size of the RNA fragments is in the range of 20 and 70-80 bases. Acrylamide gel electrophoresis shows that there is a certain regularity in the band

pattern (see Fig. 5). For example, the bands between 40 and 70 increase in ten base increments. However, these bands are mixtures of RNA fragments of similar sizes since a high resolution acrylamide gel (0.4 mm thick) resolves these bands further (see Fig. 4). This is reminiscent of the cutting pattern of nucleosomes by DNase I (22). DNase I produces DNA fragments of multiples of 10 base pairs. However, a high resolution gel shows that this is in fact due to clustering of bands of similar size with varying intensity, the bands in the middle being most intense. Our result certainly is not as striking as that with nucleosomes, but it does show that the bands generated by the endogenous nuclease digestion of HnRNP reflect a certain basic structural organization of 40s HnRNP. The 10 base increments are most likely not due to partial digestion of the RNA by the endogenous nuclease. Further digestion of isolated 40s HnRNP with micrococcal nuclease does not change the overall RNA patterns (see Fig. 5).

The RNA fragments of 40s HnRNP are mixtures of many different RNA sequences in the nucleus. About 10% of the genome is transcribed in duck reticulocytes (23) and approximately 1% of the total RNA of 40s HnRNP is that of the globin RNA. Therefore, most of the nuclear RNA is assembled into a similar HnRNP structure as indicated by the regular RNA patterns of the total nuclear RNA in the 40s HnRNP preparation (see Fig. 5). However, the smaller size of the β globin RNA and different band patterns of the globin and non-globin RNA suggest that there are significant differences in the interaction of the globin RNA and non-globin RNA with the HnRNP proteins. It is highly unlikely that the different RNA patterns are due to the possible sequence specificity of the endogenous nuclease since this will produce smear pattern of the non-globin RNA due to its high sequence complexity. The significance of our finding is not clear at the present time since little is known about the function and structural organization of HnRNP. At the present time the only significant difference between the globin RNA and the non-globin RNA in the reticulocyte nuclei is that the globin RNA is the major RNA transported into the cytoplasm. It is possible that there are subtle differences in the organization of HnRNP between the RNA exported into the cytoplasm and the bulk of the RNA degraded in the nucleus. We plan to examine more RNA sequences to answer this question.

We (14) and others (25, 26) reported that both of the precursor and processed RNA are tightly bound to the nuclear matrix. This suggests the possibility that RNA processing occurs in association with the nuclear matrix. The tight binding of RNA to the matrix appears to be due to the high affinity of two of the HnRNP proteins (27). The minor differences in the pattern of

the globin RNA in the 40s HnRNP and the globin RNA remaining in the nucleus after extraction of the HnRNP could be due to the RNA fragments associated with the HnRNP which remains bound to the matrix.

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REFERENCES

1. Hamkalo, B.A., Miller, O.L.Jr. and Bakken, A.H. (1973) *Cold Spring Harbor Symp. Quart. Biol.* 38, 915-919.
2. Malcolm, D.B. and Sommerville, J. (1974) *Chromosoma* 48, 137-158.
3. Beyer, A.L., Miller, O.L.Jr. and McKnight, S.L. (1980) *Cell* 20, 75-84.
4. Pederson, T. (1974) *J. Mol. Biol.* 83, 163-183.
5. Stévenin, J., Gattoni, R., Keohavong, P. and Jacob, M. (1982) *J. Mol. Biol.* 155, 185-205.
6. Stévenin, J., Gattoni, R., Divilliers, G. and Jacob, M. (1979) *Eur. J. Biochem.* 95, 593-606.
7. Samarina, O.P., Lukanidin, E.M., Molnar, J. and Georgiev, G.P. (1968) *J. Mol. Biol.* 33, 251-263.
8. Beyer, A.L., Christensen, M.E., Walker, B.W. and Lestourgeon, W.M. (1977) *Cell* 11, 127-138.
9. Maundrell, K. and Scherrer, K. (1979) *Eur. J. Biochem.* 99, 225-238.
10. Lestourgeon, W.M., Lothstein, L., Walker, B. and Beyer, A.L. (1981) in *The Cell Nucleus*, Busch, H., Ed., Vol. 9, pp. 49-87 Academic Press, New York.
11. Economidis, I.V. and Pederson, T. (1983) *Proc. Nat. Acad. Sci. USA* 80, 1599-1602.
12. Bantle, J.A. and Hahn, W.E. (1976) *Cell* 8, 139-150.
13. Wold, B.J., Klein, W.H., Hough-Evans, B.R., Britten, R.J. and Davidson, E.H. (1978) *Cell* 14, 941-950.
14. Laemmli, U.K. (1970) *Nature (Lond.)* 227, 680-685.
15. Wang, D. and Moore, S. (1978) *J. Biol. Chem.* 253, 7216-7219.
16. Maxam, A.M. and Gilbert, W. (1980) *Methods Enzymol.* 65, 499-560.
17. Wahl, G.M., Stern, M. and Stark, G. (1979) *Proc. Natl. Acad. Sci. USA* 76, 3683-3687.
18. Lehrach, H., Diamond, D., Wozney, J.M. and Boedtker, H. (1977) *Biochemistry* 16, 4743-4751.
19. Rigby, P.J.W., Dieckmann, M., Rhodes, C. and Berg, P. (1977) *J. Mol. Biol.* 113, 237-251.
20. Civelli, O., Vincent, A., Maundrell, K., Buri, J.-F. and Scherrer, K. (1980) *Eur. J. Biochem.* 107, 577-585.
21. Maniatis, T., Jeffrey, A., and Van de Sande, H. (1975) *Biochemistry* 14, 3787-3794.
22. Lutter, L.C. (1979) *Nucleic Acid. Res.* 6, 41-56.

23. Imaizumi-Scherrer,M.-T., Maundrell,K., Civelli,O. and Scherrer,K. (1982) *Develop. Biol.* 93, 126-138.
24. Ross,D.A., Yen, R.-W. and Chae, C.-B. (1982) *Biochemistry* 21, 764-771.
25. Mariman,E.C.M., van Eekelen,C.A.G., Reinders,R.J., Berns,A.J.M., and van Venrooij,W.J. (1982) *J. Mol. Biol.* 154, 103-119.
26. Ciejek,E.M., Nordstrom,J.L., Tsai,M.-J. and O'Malley,B.W. (1982) *Biochemistry* 21, 4945-4953.
27. van Eekelen,C., and van Venrooij,W.J. (1981) *J. Cell Biol.* 88, 554-563.