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Translation of the Human C3b/C4b Receptor mRNA in a Cell-Free System and by *Xenopus* Oocytes

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ABSTRACT: The C3b/C4b complement receptor (CR1) is a large, single-chain integral membrane glycoprotein present on erythrocytes, leukocytes, glomerular podocytes, and splenic dendritic-reticular cells that mediates the binding of complement-coated particles and immune complexes. CR1 is unusual in that it is polymorphic in size with the four allelic variants having molecular weights of 190 000, 220 000, 250 000, and 280 000 (SDS-PAGE, reducing conditions). The in vitro translation of the common (M_r 220 000) allelic variant CR1 has been achieved by using mRNA in lysates of rabbit reticulocytes and in *Xenopus* oocytes. HL-60, a promyelocytic human leukemic cell line, was treated with DMSO to induce differentiation and synthesis of CR1. Poly(A+) RNA was purified from these cells by column chromatography on oligo(dT)-cellulose. In the rabbit reticulocyte system, no CR1 was detected unless the translation mixture was denatured. In the presence of methylmercuric hydroxide, the CR1 translation product, unlike most translation products, had the same molecular weight in gel electrophoresis as the high-mannose-containing pro-CR1 and was 15-20K larger than nonglycosylated CR1. This suggests that a cotranslational modification of CR1 structure occurs, probably involving a proteolytic cleavage event. When poly(A+) RNA was translated in *Xenopus* oocytes, CR1 could be detected by treatment of oocytes with anti-CR1 monoclonal antibody followed by fluorescein-conjugated goat anti-mouse IgG. CR1 was diffusely distributed but preferentially localized to the vegetal surface. The molecular weight of this product, identified in immunoprecipitates of lysates of [^{35}S]methionine-labeled oocytes, was identical with that of CR1 of HL-60.

Complement and receptors for complement activation fragments play an important role in the identification and processing of microbes (Fearon & Wong, 1983; Ross & Medof, 1985; Reid, 1986; Muller-Eberhard, 1988). The third component of complement (C3) occupies a central place in this host defense system. During complement activation, C3

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is cleaved by the C3 convertases to yield the large C3b fragment that can attach to substrates covalently via ester linkages and the small C3a fragment that has anaphylactoid properties. The bound C3b serves as a ligand for the C3b/C4b receptor (complement receptor type 1 or CR1) which is found on peripheral blood cells and tissue monocytes. In addition to this receptor function, CR1 has regulatory activity in that it can inhibit activity of the complement cascade by disassociating the C3 convertases and by serving as a cofactor for proteolytic degradation of C3b (Fearon, 1979; Iida et al., 1983).

CR1 is a single-chain integral membrane glycoprotein that exhibits an unusual size polymorphism. The four codominantly inherited allelic variants have molecular weights of 190 000, 220 000, 250 000, and 280 000, as assessed by SDS–PAGE under reducing conditions (Dykman et al., 1983, 1984, 1985; Wong et al., 1983). CR1 is expressed on erythrocytes, granulocytes, monocytes, natural killer cells, B lymphocytes, a subpopulation of T lymphocytes, dendritic–reticular cells, and glomerular podocytes (Fearon & Wong, 1983; Ross & Medof, 1985; Fearon, 1980; Wilson et al., 1983; Reynes et al., 1985; Gelfand et al., 1975, 1976; Kazatchkine et al., 1982). Work at the protein (Lublin et al., 1986; Nickells et al., 1986), mRNA (Wong et al., 1985; Holers et al., 1987), and DNA (Wong et al., 1986; Klickstein et al., 1987) levels suggests that variation in the number of highly homologous repeating units accounts for this unusual polymorphism. CR1 is a member of a multigene family of complement regulatory proteins (de Cordoba et al., 1984; de Cordoba, 1985; Holers et al., 1985; Reid et al., 1986) whose structural genes are tightly linked on human chromosome 1 (Weis et al., 1987; de Cordoba, 1987; Rey-Campos, 1988; Carrol et al., 1988; Campbell et al., 1988). These proteins are also related functionally in that they bind C3b and/or C4b (Holers et al., 1985) and structurally in that they are composed, beginning at their amino terminus, of an approximately 60 amino acid cysteine-repeating motif (Reid et al., 1986). For example, CR1 is composed of 30 of these tandomly arranged repeats before ending at its carboxyl terminus in a transmembrane hydrophobic domain followed by a cytoplasmic tail (Hourcade et al., 1988; Klickstein et al., 1987, 1988).

The human promyelocytic cell line (HL-60) synthesizes the most common variant of CR1 after exposure to dimethyl sulfoxide (DMSO), vitamin D, and other compounds (Lublin et al., 1986; Atkinson & Jones, 1984). The acquisition of CR1 during the differentiation of HL-60 has served as a model system for the analysis of the biosynthesis of CR1 (Lublin et al., 1986). Also, B cell lines derived from donors expressing each of four allelic variants have been utilized to compare the biosynthesis of each variant (Lublin et al., 1986). Thus, a high-mannose pro-CR1 form for each size variant was identified that was ~10 000 lower in molecular weight than the mature form. In tunicamycin-treated cells, the aglycosylated precursor of each size variant was ~30 000 less in molecular weight than the mature form. Although glycosylation was shown to be important in receptor metabolism and binding activity, these data indicated that posttranslational modifications such as glycosylation did not play a role in determining the remarkable size differences of the allelic variants.

In the case of the common CR1 size variant, approximately equal quantities of two mRNA species of 9 and 11 kb have been detected on Northern blots of differentiated HL-60 or tonsillar cell poly(A+) RNA (Wong et al., 1985; Holers et al., 1987). For the other three polymorphic variants, two mRNA species are also present, and variations in mRNA correlate with the size differences of the mature protein (Holers et al., 1987). However, if surface-labeled or biosynthetically labeled cells homozygous for the two common variants are solubilized and then immunoprecipitated with monoclonal or polyclonal antibodies to CR1, only one mature molecule is observed (Dykman et al., 1983; Fearon, 1980; Lublin et al., 1986). Further, the size of these mRNAs is considerably larger than is necessary to translate the CR1 protein. Therefore, to analyze the in vitro translated products in relationship to these issues and as a prelude to the characterization of the structure–function relationships of this receptor, the translation of human CR1 was assessed in cell-free and oocyte systems.

Materials and Methods

Materials. [^{35}S]Methionine and [^{3}H]glucosamine were obtained from Amersham Corp., Arlington Heights, IL. Methylmercuric hydroxide (CH_3HgOH) was from Alfa Alfa Chemicals, Los Angeles, CA. Guanidinium isothiocyanate and ultrapure cesium chloride were products of Bethesda Research Laboratories (BRL), Bethesda, MD. The rabbit reticulocyte lysate system was obtained from Promegabiotec, Madison, WI, and BRL. Female *Xenopus leavis*, injected with human chorionic gonadotropin 2 weeks prior to shipping, were obtained from Wasco, Fort Atkinson, WI. Phenylmethanesulfonyl fluoride, iodoacetamide, pepstatin, ethylenediaminetetraacetic acid (EDTA), β-mercaptoethanol, and trisodium citrate were obtained from Sigma Chemical Co., St. Louis, MO. HL-60, a human promyelocytic leukemic cell line, was maintained in RPMI-1640 with 10% fetal calf serum (Lublin et al., 1986; Atkinson & Jones, 1984). Goat anti-rabbit and rabbit anti-mouse antibodies were obtained from DAKO Immunologicals, Westbury, NY. Staphylococcal protein A was from Calbiochem, La Jolla, CA.

Isolation of RNA. Total cellular RNA was isolated by the guanidinium isothiocyanate method (Chirgwin et al., 1979). Differentiated (see below) or undifferentiated HL-60 were suspended in 5 M guanidinium isothiocyanate, 5 mM sodium citrate (pH 7.0), and 100 mM β-mercaptoethanol. Following a 60 s disruption in a mechanical tissue disrupter, the lysate was layered on a cushion of 5.7 M cesium chloride in 0.01 M sodium acetate (pH 7.0) and centrifuged in an ultracentrifuge at 32000g for 16 h using an SW41 Beckman rotor. In some experiments, the RNA was also prepared by the hot phenol method (Nikolaeve et al., 1974). The RNA pellet was resuspended in sterile water and precipitated with 2.5 volumes of absolute ethanol in the presence of 0.15 M sodium acetate. Poly(A+) RNA was prepared from total RNA by using oligo(dT)–cellulose chromatography (Edmonds et al., 1971).

In Vitro Translation of mRNA in Rabbit Reticulocytes. Ten to twenty-five micrograms of poly(A+)-containing RNA was treated with different concentrations of CH_3HgOH for 5 min at room temperature and then diluted such that the final concentration of CH_3HgOH in the reaction mixture was less than 0.3 mM. Translation in the rabbit reticulocyte lysate was performed in a final volume of 50 μL in the presence of 5 μCi of [^{35}S]methionine at 37 °C for 90 min (Pelham & Jakson, 1976; Komaroff et al., 1974). The lysate was solubilized in 0.5 mL of phosphate-buffered saline (pH 7.4) (PBS) containing 1% NP-40, 5 mM PMSF, 0.5 mM pepstatin, 15 mM EDTA, and 1 mM iodoacetamide. Ribosomes in the translation mixture were removed by centrifugation (105000g × 1 h). The supernatant was used for direct immunoprecipitation or applied to monoclonal antibody affinity chromatography (Holers et al., 1986). The monoclonal antibodies to CR1 were 5C11, 2B6, 3D9 [see Holers et al. (1986)], and 57F, a generous gift of Victor Nussenzweig (New York

University School of Medicine). One polyclonal antibody to CR1 was raised in our laboratory (Holers et al., 1986), and the other was a gift of M. Edward Medof (Case Western University).

In Vitro Translation of mRNA in Xenopus Oocytes. The general procedure has been described (Brown et al., 1986; Valle et al., 1981). After the mechanical separation of the oocytes from the frog ovary, ~450 viable oocytes were selected, and each was injected with ~50 nL of 0.3 mg/mL poly(A+) RNA prepared from HL-60 cells previously induced for 60 h with DMSO. Injected and control oocytes were incubated for 20 h at 20 °C in groups of 10 in 100 μL of MBS [modified Barths' saline: 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 15 mM Hepes, 0.3 mM $CaNO_3$ ($4H_2O$), 0.41 mM $CaCl_2$ ($6H_2O$), 0.82 mM $MgSO_4$ ($7H_2O$), 10 μg/mL sodium penicillin, and 10 μg/mL streptomycin sulfate] in a 96-well ELISA plate. At the end of this incubation, the medium was removed along with damaged eggs, and new medium containing 5 μCi each of [^{35}S]methionine and [^{35}S]cysteine in 50 μL of medium and 1 μg/mL BSA was added to each well. The incubation was continued for an additional 5 h. Twenty eggs from both the control and RNA-injected eggs were then set aside for immunofluorescence, and the remaining eggs were homogenized in 1% NP-40 in PBS containing the protease inhibitors listed above. The extract was centrifuged, and the supernatant was used for immunoprecipitation.

Immunofluorescent Analysis of CR1 on Xenopus Oocytes. This procedure was similar to that previously reported (Liu & Orida, 1984). Twenty RNA-injected and 10 uninjected oocytes were washed in RPMI containing 1% fetal calf serum and 0.5% BSA (RPMI–FCS–BSA) and preincubated with collagenase (2 μg/mL) for 30 min. Following this incubation, the eggs were washed 3 times with 3 mL of RPMI–FCS–BSA containing 0.002% sodium azide and then incubated with the monoclonal antibody to CR1 or a control mouse monoclonal antibody in 500 μL of the same medium for 1 h on ice. The eggs were washed 3 times and resuspended in 500 μL. Fluorescein-conjugated rabbit anti-mouse IgG antibody was added, and the incubation was continued for an additional 1 h. The eggs were washed 3 times, and then the fluorescence of RNA-injected and uninjected oocytes was compared.

Biosynthetic Labeling of HL-60. A total of 30–(50×10^6) HL-60 cells, induced to differentiate for 60 h by 1% DMSO, were suspended in 10 mL of methionine-free RPMI medium containing 10% dialyzed fetal calf serum; 30 μCi/mL [^{35}S]-methionine was added, and the cells were incubated for 120 min at 37 °C with constant shaking. At the end of the incubation, the cells were centrifuged and resuspended in media containing 2 mM cold methionine. After a 2-h incubation, the cells were washed 3 times with PBS and lysed in PBS containing 1% NP-40 and the protease inhibitors at the concentrations listed above. In some experiments, cold methionine was not added in order to visualize the pro form of CR1. The solubilized preparation was centrifuged in a microfuge (Beckman) at 10000*g* for 5 min. The supernatant was subjected to immunoprecipitation. To obtain nonglycosylated CR1, the incubation medium containing 5 μg/mL tunicamycin (Lublin et al., 1986).

Immunoprecipitation. Immunoprecipitation was performed as previously described (Dykman et al., 1983) in a volume of 0.5 mL containing 0.475 mL of diluted translation mix and 25 μL of antibody. The mixture was left on ice for 1 h, and 50 μL of the second antibody (rabbit anti-mouse or goat anti-rabbit) was added, and the incubation was continued on ice for an additional 1 h. The immune complex was incubated with 60 μL of 0.5% staphylococcal protein A suspension for 15 min. This preparation was washed 3 times with PBS containing 1% NP-40, and the radioactive antigen was released by boiling in sample buffer [125 mM Tris-HCl (pH 6.8), 15% glycerol, 2.5% SDS, and 0.005% bromophenol blue] for PAGE.

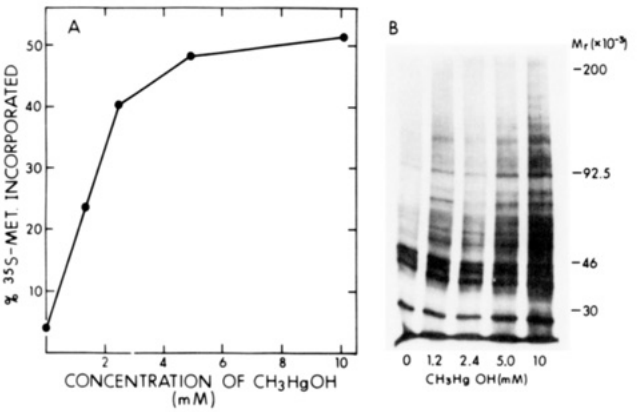

FIGURE 1: Effect of CH_3HgOH on in vitro translation. Poly(A+) RNA from DMSO-induced HL-60 was treated with the specified concentrations of CH_3HgOH. Following translation, aliquots were precipitated with TCA (A) or electrophoresed on 10% PAG (nonreducing conditions) and subjected to autoradiography (B).

Affinity Chromatography. Purification of the in vitro translated CR1 was also achieved by affinity chromatography on anti-CR1 Sepharose (mouse monoclonal antibody 5C11) (Holers et al., 1986). The translation mixture was suspended in 50 mM Na_2HPO_4/270 mM NaCl/0.2% NP-40 (pH 7.5) and then applied to the affinity column. The column was then successively washed with 0.6 M NaCl/12.5 mM K_2HPO_4/0.2% NP-40 (pH 7.4), 0.01 M Tris-HCl/0.2% NP-40 (pH 7.4), and 1.0 M KSCN/0.2% NP-40, (pH 6.7). The antigen was released from the column by 0.1 M triethylamine/0.2% NP-40 (pH 11.0). The sample was neutralized with 1 M Tris-HCl/0.2% NP-40/0.5 mM PMSF (pH 7.0).

Polyacrylamide Gel Electrophoresis. Polyacrylamide gels (10%) were constructed as described by Laemmli (1970) using a 30:0.8% acrylamide/bis(acrylamide) solution.

Results

Initial attempts to immunoprecipitate CR1 from a mixture of rabbit reticulocyte translated proteins using either polyclonal or monoclonal antibodies were unsuccessful, despite a systematic variation in incubation conditions, the use of two translation kits, and size selection of RNA on sucrose density gradients. However, if a strong denaturing agent such as CH_3HgOH was used, there was a general increase in the quantity of translated proteins and the appearance of higher molecular weight products (Figure 1). For the representative experiment shown, 10 μg of poly(A+) RNA was treated with the specified concentrations of CH_3HgOH. Following translation, an aliquot was precipitated with TCA, and washed, and the pellets were counted (Figure 1A). A 10-μL aliquot was also analyzed by SDS–PAGE (Figure 1B). Relative to the concentration of CH_3HgOH, there was an increase in the incorporation of [^{35}S]methionine into acid-precipitable material that was linear from 0 to 2.4 mM, reached a plateau at 5–10 mM, and decreased at >10 mM (not shown).

To determine if CR1 was translated in the experiments employing CH_3HgOH, the in vitro translation products of total RNA or the poly(A+) RNA were immunoprecipitated. In the representative (one of five) experiment shown in Figure 2A, a specific band with a molecular weight of approximately 200K was obtained from the poly(A+) material with poly-

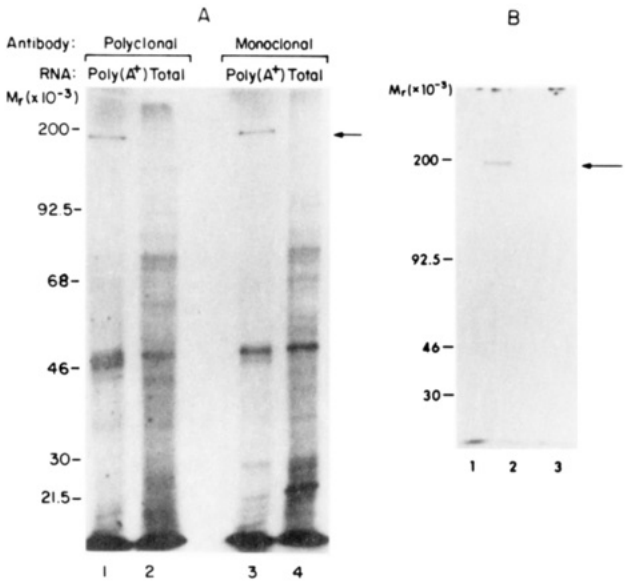

FIGURE 2: In vitro translation of CR1 by total or poly(A+)-selected RNA. Twenty micrograms of total RNA or 10 μg of poly(A+) was translated after treatment with CH_3HgOH. (A) Aliquots were then precipitated with polyclonal or monoclonal antibodies to CR1. Arrow indicates the position of CR1 on the autoradiograph (10% PAG, nonreducing). Immunoprecipitations with normal rabbit serum and a mouse monoclonal antibody of the same isotype but of unknown specificity (not shown) did not precipitate the band with a molecular weight of ~200K. The bands in lanes 1 and 4 with a molecular weight of <50K are also present in lanes 2 and 3 and to a variable degree in control (nonspecific antibodies or Staph cowan protein A alone) immunoprecipitations (not shown). (B) Analysis of translation products eluted from anti-CR1 Sepharose. Three hundred microliter fractions were collected; 150 μL was counted, and 150 μL was pooled, lyophilized, and electrophoresed (10% PAG, nonreducing) and subjected to autoradiography. Lanes 1 and 2 represent the eluates of the CR1 affinity column fractions of poly(A+) RNA translation products of uninduced and DMSO-induced cells, respectively. Translation products of DMSO-induced cells were also applied to a BSA–Sepharose column (lane 3). Arrow indicates the position of CR1.

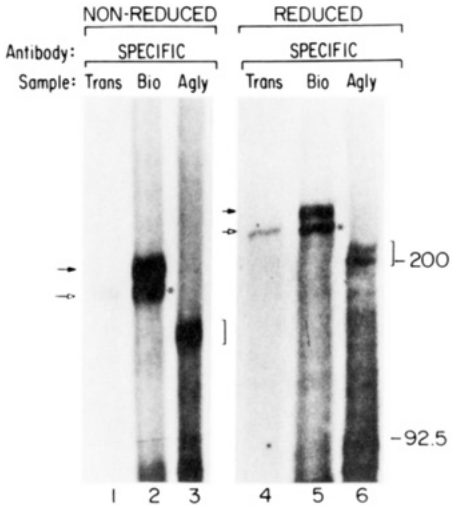

FIGURE 3: Comparison of in vitro translated and biosynthetically labeled mature and aglycosylated forms of CR1. Translation products equivalent to 40 μg of poly(A+) RNA from DMSO-induced cells were immunoprecipitated with a monoclonal antibody to CR1 and compared to the mature and aglycosylated forms of CR1 under nonreducing (lanes 1–3) and reducing (lanes 4–6) conditions. Closed and open arrows designate the mature and in vitro translated forms of CR1, respectively. The asterisks and the brackets mark the pro-CR1 and aglycosylated molecules, respectively. Trans, in vitro translated material; Bio, biosynthetically derived material; Agly, biosynthetically derived material in which tunicamycin was used to inhibit glycosylation.

clonal (lane 1) and monoclonal antibodies (lane 3) to CR1. This protein was not immunoprecipitated with control polyclonal or monoclonal antibodies. If total RNA (up to 20 μg) was used in the translation mix, no CR1-specific band was precipitated (lanes 2 and 4). The molecular weight of the translated product under nonreducing conditions was 185K–195K, somewhat larger than anticipated on the basis of the molecular weight of a high-mannose-containing precursor of 173K as assessed here (see below) and in other experiments (Lublin et al., 1986). Several other prominent bands in the poly(A+) track are present with molecular weights of 10K–50K. Species of about the same molecular weights are also observed in the immunoprecipitates of total RNA (tracks 2 and 4) as well as in a variety of control immunoprecipitates (see legend to Figure 2A).

To further establish the specificity of the band at ~200K, the in vitro translated products were characterized on an antibody affinity column. The translation mixture of poly(A+) RNA from induced and uninduced cells was incubated with Sepharose to which a monoclonal antibody to CR1 was bound. The eluate from the induced but not that of uninduced cells gave a peak of radioactivity. Also, the eluate of the poly(A+) RNA translation mixture applied to a BSA–Sepharose column did not give a similar peak of radioactivity. The eluates were also analyzed by SDS–PAGE (Figure 2B). The product isolated from the poly(A+) RNA of induced cells (lane 2) was of the same molecular weight as that obtained by immunoprecipitation (Figure 2A), and no lower molecular weight species were observed. No such species was observed from the translation products of poly(A+) RNA of uninduced cells (lane 1), or if the translation mixture of the induced cells was applied to a BSA–Sepharose column (lane 3).

A comparative analysis was performed of the molecular weight of the biosynthetically labeled CR1 with that of the in vitro translated CR1 (Figure 3). Under nonreducing conditions, the in vitro translated CR1 (lane 1) is 10K smaller than the mature molecule (lane 2) and aligns with the pro form (lane 2, asterisk) of CR1 of HL-60. A comparison was also made in this same experiment of the translated product and aglycosylated form (lane 3) of CR1. The molecular weight of the aglycosylated form of CR1 was ~20K less than the translated product. Under reducing conditions, similar results were obtained. The translated product (lane 4) again aligns with pro-CR1 (lane 5). The mature molecule (lane 5, upper species noted by a solid arrow) has a molecular weight of 235K. The aglycosylated molecule (lane 6) is a doublet, probably reflecting partial glycosylation. The lower species has a molecular weight of 173K and represents the CR1 with no attached oligosaccharides (Lublin et al., 1986).

To further investigate the translation products of CR1 poly(A+) RNA, the *Xenopus* oocyte system was employed (Figure 4). The oocytes were injected with poly(A+) RNA from DMSO-induced HL-60 cells. After the incubation, the oocytes were treated with anti-CR1 monoclonal antibody followed by a fluorescein-conjugated rabbit anti-mouse antibody. The fluorescence of the oocytes injected with RNA was considerably higher than uninjected oocytes, and none of the 20 uninjected oocytes exhibited immunofluorescence comparable to the poly(A+) RNA injected eggs. A diffuse staining pattern, primarily of the vegetal side of the egg, was observed. None of the eggs showed a clustered type of staining pattern. Of the 20 eggs treated with anti-CR1, 8 showed minimal fluorescence while 12 exhibited moderate to strong immunofluorescence. Three out of 12 showed very intense fluorescence. Figure 4A shows the vegetal part of an egg to

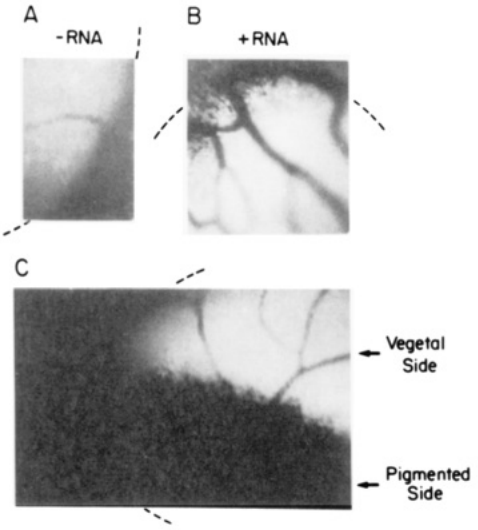

FIGURE 4: Comparison of the immunofluorescence for CR1 of the *Xenopus* oocytes uninjected or injected with poly(A+) RNA. Oocytes were treated with a monoclonal anti-CR1 followed by a fluorescein-conjugated second antibody. Photographs were taken with a fluorescent microscope (10× magnification). Three eggs are shown. Panel A (upper left), vegetal part of an egg that was uninjected; panel B (upper right), vegetal part of an egg that was injected with poly(A+) RNA. Panel C, comparison of the immunofluorescence on the vegetal (yellow) and pigmented (dark) side of an egg strongly positive for CR1 fluorescence. The dashed lines delineate the contour of the egg.

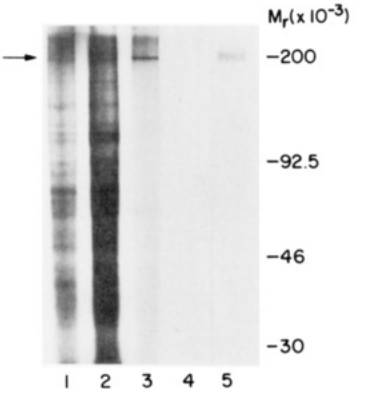

FIGURE 5: Translation of CR1 poly(A+) RNA by *Xenopus* oocytes. RNA-injected or uninjected oocytes were homogenized and immunoprecipitated with monoclonal antibody to CR1 and subjected to electrophoresis (10% PAG, nonreducing). An autoradiograph is shown. Lanes 1 and 2, the total translation products of RNA-uninjected and injected eggs, respectively; lane 3, immunoprecipitate of the eggs that received poly(A+) RNA; lane 4, immunoprecipitate of the eggs that did not receive the poly(A+) RNA; lane 5, immunoprecipitate of the mature form of biosynthetically labeled CR1 from HL-60 cells. Arrow designates CR1.

which no mRNA was injected. Figure 4B shows the same side of an egg injected with poly(A+) RNA. The egg shown in Figure 4C exhibits strong immunofluorescence and demonstrates how the fluorescence was almost entirely restricted to the yellow (vegetal) side of the egg.

Figure 5 shows the immunoprecipitation of extracts of poly(A+) mRNA injected oocytes. The total translation products of RNA-injected and uninjected eggs are shown in lanes 1 and 2, respectively. A species of the same molecular weight as that of the mature CR1 molecule of HL-60 cells could be detected upon immunoprecipitation of the extracts from the poly(A+) RNA injected eggs (Figure 5, lane 3). No such species was immunoprecipitated from eggs that did not receive poly(A+) RNA (lane 4). This CR1 species (lane 3) aligned with biosynthetically labeled mature CR1 (lane 5).

Discussion

In our initial experiments, poly(A+) RNA of DMSO-treated HL-60 was translated in vitro in a rabbit reticulocyte system, but CR1 was not detected. However, if an RNA denaturing agent, CH_3HgOH, was added to the culture system, CR1 was easily detected. Methylmercuric hydroxide increased the quantity of all translation products but especially appeared to facilitate the synthesis of larger proteins. Methylmercuric hydroxide may denature the RNA sufficiently to allow the ribosomes to read through the complete mRNA (Payvar & Shimke, 1979). It may also inactivate reducing agents that are present in the commercially available reticulocyte lysates. Both of these factors could be playing a role in our experiments. For example, conalbumin mRNA was shown to aggregate and thus become a poor template for in vitro translation and cDNA synthesis (Buell et al., 1978; Haines et al., 1974; Stumph et al., 1984). This problem was corrected by CH_3HgOH, and we would suggest that this nucleoside binding agent had a similar effect in our system. CR1 mRNA may be prone to aggregate due to the presence of large stretches of internally repeated highly homologous (as much as 99%) sequences (Hourcade et al., 1988; Klickstein et al., 1987, 1988). Second, reduced CR1 is not recognized by either the polyclonal or the monoclonal antibodies in a direct immunoprecipitation analysis or in Western blotting (unpublished observation). Consequently, small amounts of a reducing agent in an in vitro translation system could also affect the immunoprecipitation, and these could have been neutralized by CH_3HgOH.

The quantity of CR1 that was immunoprecipitated in our in vitro translation system seems low. For example, poly(A+) RNA obtained from 1×10^8 cells resulted in approximately 100-fold less CR1 than could be isolated in biosynthetic studies from 3×10^7 cells with a 20-min pulse label. These results suggest that in vitro translation in rabbit reticulocytes is much less efficient for CR1 compared to the biosynthetic system. A differential rate of synthesis for different or even similar proteins like α- and β-globins in in vitro systems is known (Lodish, 1971; Lodish & Jacobsen, 1972). Wide differences in the in vitro synthesis with 10–20 orders of magnitude were reported for monogenic retroviruses (Jochlik, 1973) and bacteriophage RNAs (Steitz, 1975). The nucleotide sequences near the initiation codon or the tertiary structure of the mRNA may affect the efficiency of the translation (Steitz, 1969; Lodish, 1970).

Although approximately equal quantities of two RNA molecules of 9 and 11 kb exist in cells homozygous for the common polymorphic variant of CR1 (Wong et al., 1985; Holers et al., 1987), only one membrane form of CR1 is identifiable (Dykman et al., 1983; Fearon, 1980; Lublin et al., 1986). Similarly, only one mature molecule was detected in the two translation systems. In the rabbit reticulocyte system, the molecular weight of the translated CR1 was only ~10K smaller than the mature (membrane) form of CR1, similar to the high-mannose-containing pro-CR1 (Lublin et al., 1986; Atkinson & Jones, 1984), and ~20K larger than the aglycosylated form of CR1. There is no glycosylation of an in vitro translated molecule by the rabbit reticulocyte system, and the explanation for the synthesis of a larger than expected molecule is unknown. However, these results suggest that there is a proteolytic processing event that occurs cotranslationally to reduce the molecular weight of the primary CR1 transcript by 20K.

We are unaware of an example in eukaryotes in which there is such a large discrepancy in molecular weight between an

aglycosylated precursor and the in vitro translation product. Three possibilities that we have considered are a long signal peptide, a read-through of the termination signal, or an activation peptide that interacts with the CR1 transcript. The first possibility is unlikely since the length of the signal peptide for CR1 has recently been determined by two groups (Holers et al., 1988; Klickstein et al., 1988), and it is not unusually long. With regard to the second possibility, translation products larger than the precursors or even the mature molecule are known in viruses but are unusual (Maeshima et al., 1987). In our case, a read-through of the termination signal might be occurring similar to that reported in bacteriophage Qβ or λ (Horiuchi et al., 1971; Yates et al., 1971), several filamentous phages (Engelberg-Kulka et al., 1979), and Mul and Ms viral RNAs (Philipson et al., 1978). Considerable evidence for a role of sequences near the 3′ end of the termination signal in the suppression of a termination codon has also accumulated in prokaryotes (Sasler, 1969; Yahata et al., 1970; Bosi & Roth, 1980, Bosi, 1983; Murgola et al., 1984). Release and chain termination factors also play a yet unknown role in the liberation of the polypeptide from the ribosome complex (Murgola et al., 1984; Ryoji et al., 1985). In our translation system, if the polypeptide was not released at the first termination signal but was terminated at the second one (D. Hourcade and J. Atkinson, unpublished results), the size of the translated polypeptide would be ~8K larger than expected. This combined with approximately 2.5K of the signal peptide could account for the size difference that we have observed. Lastly, a covalent association of a factor or factors to the translated product from the translation mix is also possible (Small et al., 1987).

Further analysis of the translation products was performed in the *Xenopus* oocyte system. The translated CR1 molecules migrated to the surface of the egg and were detected by immunofluorescence. The CR1 processing in these eggs seems to be similar to that in HL-60 cells as the CR1 produced had an almost identical molecular weight with CR1 of HL-60. Of interest, CR1 migrated to the vegetal pole of *Xenopus* oocytes where it was diffusely distributed. Specific translation of maternal mRNA to the vegetal pole of *Xenopus* oocytes has been described (Melton, 1987). The author suggested that the protein synthesized by this mRNA may be localized to this region and in turn play a role in the development of the vegetal cells. Also of interest in this regard is that yolk platelets are synthesized diffusely in the cytoplasm of the immature egg but then, as the egg matures, a gradient along the animal (pigment)–vegetal axis (Melton, 1987; Gerhart, 1980) forms, possibly in association with cytoskeletal elements. CR1 may also localize to the vegetal axis because of an association of this side of the egg with cytoskeletal elements. Evidence has been presented that CR1 is in association with such structural proteins (Jack et al., 1986) and an affinity of part of CR1 for cytoskeletal proteins could dictate its movement and localization to this region of the egg.

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