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Chicken transferrin receptor gene: conservation 3′ noncoding sequences and expression in erythroid cells

Lee-Nien L.Chan*, Nikos Grammatikakis+, Janet M.Banks§ and Elizabeth M.Gerhardt

Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550

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ABSTRACT

Recombinant clones of the chicken transferrin receptor gene and cDNA have been isolated and sequenced. Two highly conserved regions have been identified in the 3′ noncoding sequence of the human and chicken TR gene. The conserved regions include sequences that have been shown to be involved in the iron-dependent regulation of human TR mRNA stability. These sequences can be modeled as two different types of RNA secondary structures, one containing stem-loop structures that are similar to the iron-responsive elements found in ferritin mRNA and the other being a stable, duplex/stem-loop structure. Both forms show considerable similarity between chicken and human mRNA. The expression of TR is developmentally regulated during erythroid maturation, and immature erythroid cells express exceptionally high levels of TR mRNA.

INTRODUCTION

The transport of iron into cells is accomplished by the receptor-mediated endocytosis of iron-transferrin complexes (1−8). The amount of iron transported is regulated by the number of transferrin receptors (TR) expressed by cells, which is in turn regulated in a feedback fashion by intracellular iron: excess free cellular iron leads to a decrease in the amount of TR, whereas a lack of iron results in an increase of TR (9−14). Certain sequences in both the 5′ and 3′ noncoding regions of the human TR gene have been shown to be necessary for the iron-dependent regulation of human TR mRNA levels (15,16). Parts of the TR 3′ noncoding sequence can be formed into two different types of RNA secondary structures: one type has stem-loops which share concensus with the iron-responsive element (IRE) found in the 5′ noncoding region of the ferritin gene and is implicated in translational control (17−20), and the other type has a duplex/stem-loop RNA structure which is thermodynamically stable (21). Both of these structures have been proposed to be involved in the iron-dependent regulation of human TR mRNA stability.

In this report, the isolation and sequencing of recombinant clones of the chicken TR gene and cDNA are described. Strong sequence homology between the 3′ noncoding sequences of the chicken and human TR genes is shown. These homologous sequences can be modeled to form RNA secondary structures which have been implicated in the iron-dependent posttranscriptional regulation of TR mRNA levels. Comparisons between the chicken and human TR mRNA secondary structure models show considerable similarity. The chicken TR transcripts are 4.9 kilobases (kb) in size, similar to that of human (23) and mouse (24). Also, the expression of TR genes in chick embryonic erythroid cells is developmentally regulated during erythroid maturation.

MATERIALS AND METHODS

Screening of DNA libraries

A chicken genomic DNA library (kindly provided by J.D. Engel) was screened by standard methods (25) using as probe the 5.0 kb BamHI fragment of pcDTR1, which is a full length human TR cDNA clone (kindly provided by F. Ruddle and A. McClelland, 23), containing both the coding as well as the 3′ noncoding sequences. The hybridization buffer contained 1M NaCl, 0.05M Tris.HCl pH7.4, 1mM EDTA, 5× Denhardt's solution (1× is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 150 μg/ml denatured salmon sperm DNA, 0.05% NaDodSO4, and 10^6 cpm/ml ^{32}P labelled probe. The filters were hybridized for 18 hours at 53°C. Washing was with 4 changes of 2×SSC (1× is 0.15 M NaCl, 0.015M trisodium citrate pH7) with 0.1% $NaDodSO_4$ at room temperature followed by 2−3 changes of 0.1×SSC with 0.1% $NaDodSO_4$ at 53°C for 30 minutes each. A total of 2.5×10^5 phage plaques were screened and three overlapping positive clones were isolated and designated as CTR1, CTR2 and CTR3.

One hundred and fifty thousand plaques of a λgt10 cDNA library prepared from a chicken B-lymphoma cell line, R2B (kindly provided by J.M. Bishop), was screened using a 1.2 kb BamHI/HindIII fragment of CTR1 which hybridized strongly with pcDTR1. Nine positive clones were obtained with sizes ranging from about 0.5 kb to 2.3 kb. The largest clone was designated as CTRcDNA8, and was subcloned using standard procedures (25) into M13 mp18 or mp19 phage for sequencing.

Sequencing and sequence analysis

Nests of overlapping fragments cloned in M13 phage were prepared using the Cyclone kit (IBI). Sequencing was accomplished by the dideoxy method (26) using sequencing kits from IBI, Pharmacia or US Biochemicals. Sequence data was analyzed by the IBI/Pustell

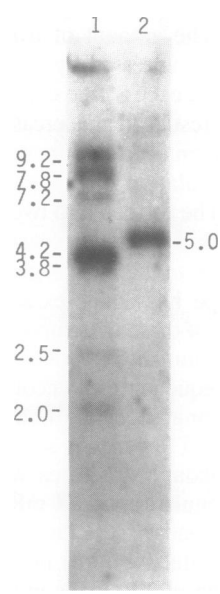

Fig. 1. Analysis of chicken and human genomic DNA digested with EcoRI. Transfer membranes were hybridized with ^{32}P-labeled BamHI fragment of pcDTRI, the full length human TR cDNA probe. 1, human DNA; 2, chicken DNA. Sizes of hybridizing bands are shown in kb.

DNA Sequence Analysis Program Version 1.0 (International Biotechnologies, Inc.) using an AT&T PC. For determining secondary structure of RNA, the PC Gene program (Intelligenetics) which is based upon that of Zucker and Stiegler (27) was used.

Analysis of chicken and human DNA

Chicken DNA was prepared from chick embryonic red blood cells using standard methods (25). Human DNA, isolated from TT1, a pancreatic tumor cell line, was kindly provided by T.-s. Chan. After digestion with restriction endonuclease EcoRI, the DNA was analyzed by electrophoresis in 1% agarose gels, transferred to nitrocellulose membranes and baked (25). Hybridization and washing were as described above.

Preparation and analysis of RNA

RNA was extracted from chick embryonic red blood cells at various stages of development. Red blood cells were obtained from the circulation of chick embryos, washed with sterile 1× PBS, resuspended in 6M guanidinium isothiocyanate, and were homogenized by passage through 18 gauge, then 23 gauge hypodermic needles. RNA was isolated by centrifugation through a cesium chloride cushion (28) and was quantified spectrophotometrically. Glyoxalated RNA was analyzed by electrophoresis in 1% agarose gels (25). 20 μg of total cellular RNA were loaded per lane. After the completion of electrophoresis, the gels were

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   1 TACCGAGCTC GAATTCTGAC AGTGCAAGCA CGTGGTTAAG GTACAGGGTT AGGGAAACCC
  61 ACTCCCTACC CTGGCGTTCT GTAGAGGGTG TTTTTTTTTT TTTTTTGGTA ACAAATCTTC
 121 TCTTGACAAA GTTGAGCGTA AAATTGAAAT CTCACCTTAC ACAAATTCAT AGAGCAGTTC
 181 TAAAGCAGTA CTAAAATTAG GATGAAATCT CTGACCAGGC TGCTGTCTGA CCACAATCCA
 241 AACCTGTCGC CTGCAGCTGC TTGTGGGGTT TGCTCTCTGG TGTACTCGAG AGCTAACAGC
 301 ACAAGGGAAT CCTGAACCAG TACCTTGAGT GGGGAGGTAC TGTGTCTATC TAGAATCTTC
 361 TTATTGTTCG CCTCCTAAAT GTAGTGACAG GGCAGGGAAC GGATTCCTCT AACACACTGA
 421 GGATCCCTTC TCTGGCCAGC GAGCTGCAGG CACAAAGCCT ATGGTTGAAG AATAAAAACA
 481 GCAGCCTGTA AGTGCTGGGT TATCAGCAAG CTCTGAAGTG CCGGCCTGCC TTTAGTCAGC
 541 AGCAGGGCTC CAATGACATC TTGGAGGCTT GCTGCTACAA GGCAGGCCTC AATACCCAGG
 601 AATGATAACC CAAAGACTGT CATGCGCCCC TTTTGTATTC CTAGCTGGCA CCTCCGCGAG
 661 TCCTGCAGCA TTAGAATCAT AGAATAGAAT CATTGAGAGC AGCTCTAGGC TGGCACTTCA
 721 GACCTTGCTG CGCTGGGGCC TTTTGCAAAG CAGAGTCTCT TGCTGGCCAA ACTCTGTTTG
 781 CTGATGCCAT TTTGCAGGAC GGCTCTTGTT TGACACTGAG ATATTTATTT GTTTATTTAT
 841 TTATCAGTGA CAGCGTTCAC TATAAATGGT GTGTGGGTTT TTTTTATAGA AGATAATTAT
 901 CGGAAGCAGT GCCTTCCATA ATTATGACAG TTATACTGTC GTTTTTGAAT AAAGCAGCAT
 961 CTGCTATTAC AATCAAACAT GATACTGGAA CTTTGCATTT AAAATAATCC AAACGAGCCC
1021 CCCCCTCCTC CCCCTAAATT TTATTTTTTA AACCTGGTAG CAAGTCTCTG GGTCGCACTT
1081 CCAAATCACC CTTGAAATGC TGAAAAGCAA CCCAAAATTA GGAAATTCTG GCTCCCTGAG
1141 GTGCCTTGTG CCCTTCTGTG GGCTTAGGAT TCTTCTCCCC TGTTTCTGGA AGCCTTCTGC
1201 TCGTGTGATG GCACTGCTCC ATGGATGGGC AGAGCACCCT GAGCAGGGCT CGCCGTGCAG
1261 CGCTCACTCC ATCCCACAGC GCTGCACACA AACTCATCCC TCGGATTGCA TTGGCCCCCA
1321 TTGGAAATGG CAGCTTCCTG CAGACTTCCA GTACCTTATG TATCATGAAA CCTAACTGAC
1381 ATTATCGGGG GCAGTGTCTT CCATAATGTG TAAAGAACAA GGTAGTTTTT CCTACCACAG
1441 TGTTATATCG GAGGCAGTGA CCTCCATATG TTGCACTATG GGTGTACGTA ATTATCGGGG
1501 ACAGTGTTTC CCATAATTGT TTTATGCTTA TCATGAAATG TCATCTGCAA AGCTTGATGG
1561 TTAGTATCTA ACATGGATCA ACTTCCTGCA GTCCTATTTT TTCCACTCTC CTGTGGTGAT
1621 GCAAATACGA ACTTGAATCT GTTCTGTACT TCTGTAGACT CTGTAGGCTG AACTCGTTCT
1681 AATAGCTGTG CTGTTGCGGA GTTGCTTCTT GCCCCAAGTA ACAGGACTGG GGTTGGGATG
1741 GTAGTGAAAG CTAGAGTAGT TAGCAGTAGT TTGTGTGTTC TGTGCTGTTG TTAACTCCCT
1801 TCAAACCGGT CACAAAGACG TGATGAACGC AAGGTGAATG GAGGGTTGGG GCTGACTGCA
1861 GGGCTCAGGC TGCTGCTCTG GGTGTGTACT GCTACAGAGA ACTCAGCCTG CTTTGGCTGA
1921 TGCTGGGGTG CACGCCTGGA GGGGCTGCTT GCTTCTGTCC TGGTGTTGCT GGAAACCTCA
1981 CTCTTTCCTT TTGGCTGTTT TTGGGAGAAA CACCTTCCTC ATGGTGCTGT GCAGCTCCCT
2041 GACCCGATGC TGAACACTGC AGATTGGTGT TTGACAGCTG CAGATTGCCA GGAGGAAGGC
2101 GTCAGTCAGC ATCTAGCTGT GTACTTGGTA TAAAAAAAAA AAAAATAGTG TAAAAATGAT
2161 GATGACTAAA TCAAGAGCCT TACCTGCGGC CCAGCAGTAA TGGCCTGAAG GATGTGCAGT
2221 TGGGTGGGAG CCTTAGCCCT CCACAAAGGT CAGCACGGAT GCCACTGCCT GTGCAGTGAG
2281 CTCAGCCCAT GTGAACGTTC ATGAGAACCT GGCAGCCTGC TGTCCAGGTC TGTGTCAGTC
2341 CCAGCGCTCA TCCTTAACTG CCCTTGG
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Fig. 2. Nucleotide sequence of the 3′ noncoding region of the chicken transferrin receptor cDNA. Numbering indicates nucleotide position from the 5′ most end of the 3′noncoding region. Sequences homologous to the 3′ noncoding sequence of the human transferrin receptor cDNA are underlined.

Chicken 809
Human 3406
Chicken 1339
Human 3848

stained with ethidium bromide to visualize the RNA bands. The RNA was transferred to nylon membranes in the presence of alkali (29). After transfer, the membranes were rinsed in 2×SSC and 0.1% $NaDodSO_4$ and baked for 1 hour at 80°C in a vacuum oven. Prehybridization and hybridization buffer contained 50% deionized formamide, 5× Denhardt's solution, 5× SSPE (1×SSPE is 0.18M NaCl, 10mM NaH_2PO_4 pH7.4, and 1mM EDTA pH7.4), 0.2% $NaDodSO_4$, and 250 μg/ml denatured salmon sperm DNA. Hybridization was at 42°C for 24 hours. The filters were washed with 2 changes of 6× SSPE and 0.5% $NaDodSO_4$ at room temperature for 15 minutes each, then with 2 changes of 1× SSPE, 0.5% $NaDodSO_4$ at 37°C for 15 minutes each, and finally with 1×SSPE, 0.5% $NaDodSO_4$ at 65°C until background radioactivity was removed as monitored by a Geiger counter. Before rehybridization, the membranes were repeatedly boiled in 0.01×SSC and 0.01% $NaDodSO_4$ to remove previously bound radioactive probe. Quantitation of the relative amounts of TR and globin transcripts was done by densitometry using a Helena QuickScan densitometer.

RESULTS AND DISCUSSION

The homology between human and chicken TR genes was analyzed by digestion of chicken DNA with EcoRI, transfer to nitrocellulose membranes, and hybridization with human TR cDNA probe. Full-length human TR cDNA (BamHI fragment isolated from pcDTR1) hybridized strongly with a single band of 5.0 kb (Fig.1), indicating that there is considerable sequence homology between the chicken and human TR genes.

The sequence of CTRcDNA8, the largest chicken TR cDNA clone, was analysed by subcloning into M13 phage and sequencing both strands of the DNA or sequencing the same strand two or more times (Fig.2). Homology matrix plots which compared the CTRcDNA8 sequence with that of the human TR cDNA (30) were obtained using the IBI/Pustell DNA Sequence Analysis Program. Further detailed comparisons show extensive homology in two regions of the 3′ noncoding sequence (Figs.2, 3). Essentially no homology was detected in the remaining sequences. The first homologous region extends from position 809 to 1001 of the chicken sequence and has 87% homology with human TR sequence nucleotides 3406 to 3592. The other region of homology includes nucleotides 1339 to 1587 of the chicken sequence, which shares 83% homology with human TR sequence nucleotides 3848 to 4081. Sequences and secondary RNA structures contained within these homologous regions are highly similar to those shown to be implicated in the iron-dependent regulation of ferritin translation (17−19) and human TR mRNA levels (20,21). Since these sequences are so highly conserved through evolution, posttranscriptional regulation by iron may be mediated by very similar mechanisms amongst various species.

The TR 3′ noncoding sequences involved in the posttranscriptional regulation of human TR mRNA levels have been presented as two different possible types of RNA secondary structures; as IREs (20), which share a concensus sequence and structure with that found in the 5′ noncoding region of the ferritin gene (18,19), or as a stable duplex/ stem-loop structure which includes only two of the IRE sequences as part of the duplex RNA backbone rather than as individual stem-loops (21). For comparison, the conserved chicken TR 3′ noncoding sequences are presented in both types of configurations.

Fig.3. Comparison of the nucleotide sequence of 3′ noncoding regions of the chicken and the human transferrin receptor genes. The homologous regions are aligned using the IBI/Pustell DNA Sequence Analysis System. Dots denote identical nucleotides; spaces represent missing nucleotides. The numbering of the human sequence is based upon that of Schneider et al.(30).

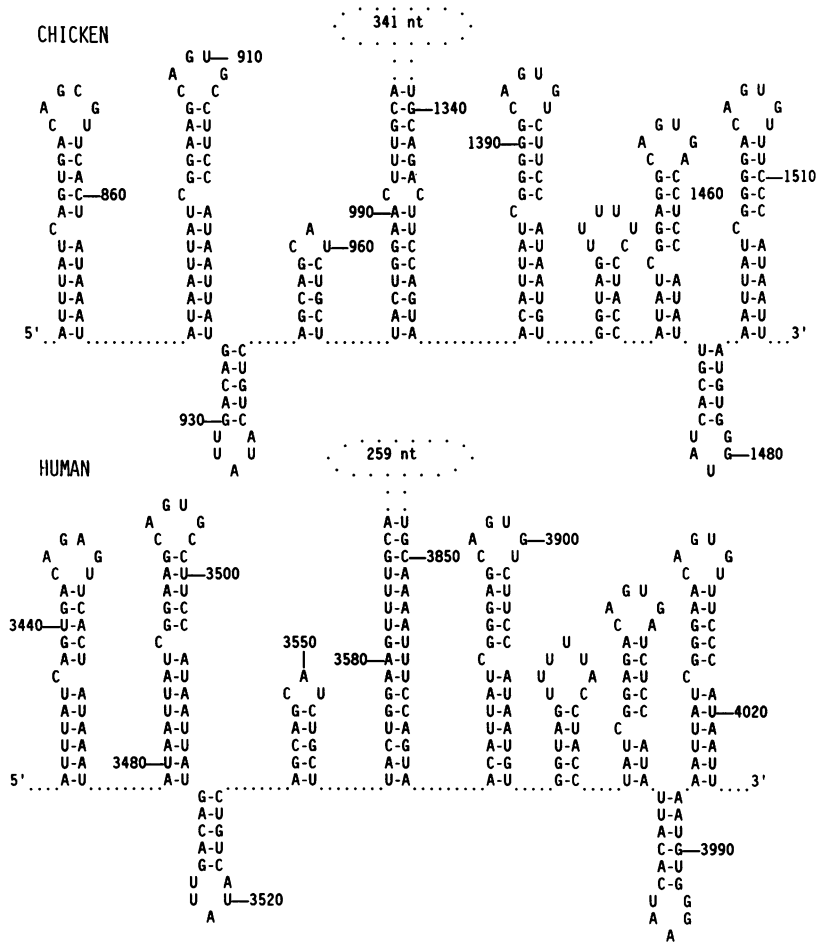

Fig. 4. Models of RNA secondary structures that contain IREs as formed by the conserved sequences in the 3′ noncoding regions of the chicken and human transferrin receptor mRNAs.

When modeled as IREs (Fig.4), the chicken and human TR RNA structures are almost identical, and involve all of the conserved sequences shown in Figure 3. These structures contain 8 stem-loops, 5 of which are IREs. Four of the stem-loops are separated from the other four by several hundred non-homologous nucleotides. However, due to the presence of palindromic sequences that span the intervening non-homologous sequences and can form a region of duplex RNA, these two groups of stem-loops can be brought into close proximity. The IREs have been shown to be necessary and sufficient for the iron regulation of ferritin translation as well as the regulation of human TR mRNA levels by iron (17−20). Furthermore, they can also confer iron regulation of translation if inserted into the 5′ noncoding region of indicator genes (20). IREs may confer different types of regulation depending upon where they are located within the transcript. Recently,

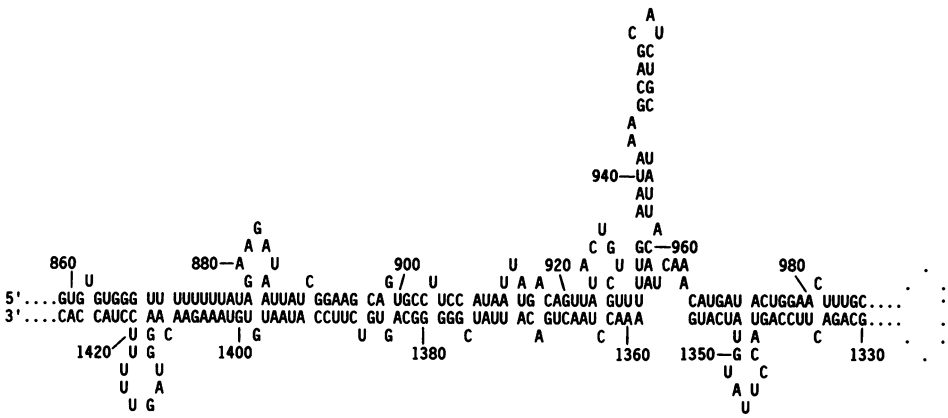

Fig. 5. Another possible secondary RNA structure formed by sequences of the 3′ noncoding region of the chicken transferrin receptor mRNA modeled after that of the human as proposed by Mullner and Kuhn (21). Nucleotides 893 to 925 and 1379 to 1409 correspond to the second and third IRE stem-loops shown in Fig.4. This structure was derived using the PC Gene program (Intelligenetics), which is based upon the program of Zucker and Stiegler (27).

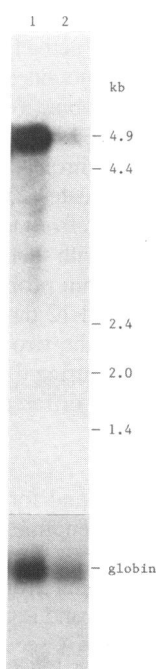

Fig. 6. Analysis of RNA from chick embryos and chick embryonic erythroid cells. Transfer membranes were hybridized with ^{32}P-labeled chicken TR cDNA and adult chicken β-globin probes. Lane 1, RNA from 9 day embryonic erythroid cells; 2, RNA from 12 day embryonic erythroid cells. Numbers denote RNA sizes in kb. A, hybridization with CTRcDNA8; B, hybridization with pβ1BR15, the adult chicken β-globin probe.

cytoplasmic protein factors that bind to the IRE of ferritin mRNA have been demonstrated, and iron regulation has been hypothesized to be mediated by these cytosolic factors (31,32).

A different RNA stem-loop structure has been proposed by Mullner and Kuhn to be responsible for iron regulation of human TR mRNA stability (21). This structure was predicted by the program of Zucker and Stiegler to be thermodynamically stable (27). Using a similar program, a comparable structure was constructed with corresponding chicken sequences (Fig.5). In this structure, only portions of the homologous sequences, as well as some flanking nonhomologous sequences, are included. The duplex RNA backbone portions of the structures are very similar between the two species, whereas the stem-loops show differences in sequence as well as arrangement. In addition, the chicken sequence contains a stretch of 16 U's close to the 5′ end which may interact with the poly A sequence (14 A's) located close to the 3′ end to result in the formation of a giant loop that encompasses almost all of the 3′ noncoding region of the chicken TR mRNA. To understand the mechanism of regulation of TR mRNA, determination of the RNA secondary structures that exist *in vivo* would be important.

The conserved 3′ noncoding sequences include a total of 442 nucleotides, with 193 nucleotides in the first region, and 249 nucleotides in the second region. Yet deletion studies have shown that only a select portion of these nucleotides are required for iron regulation of human TR mRNA stability (21). The function(s) of the remainder of the conserved sequences is not known.

The expression of TR is developmentally regulated in red blood cells during erythroid maturation (33−35). Erythroblasts that are actively synthesizing hemoglobin express high levels of TR in order to import adequate amounts of iron for the synthesis of heme, which in turn is used for the synthesis of hemoglobin. To determine whether the changes in TR is reflected in TR mRNA levels during red blood cell maturation, the relative amounts of TR transcripts in embryonic chick erythroid cells at different stages of development were measured (Fig. 6). The results indicate a 10 fold decrease in the amount of TR transcripts in erythroid cells from embryos from 9 days and 12 days of development. To correlate the expression of the TR genes with that of globin genes, the same RNA filters were hybridized with adult chicken β-globin probe. The results also showed a decrease in globin mRNA levels between 9 day and 12 day erythroid cells, except the difference in this case was only 1.4 times between the two stages of development (Fig.6). Thus, both TR and globin mRNA levels decline during the latter part of erythroid differentiation, but their rates of decrease are apparently different.

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*To whom correspondence should be addressed

Present addresses: [+]National Cancer Institute/Frederick Cancer Research Facility, Frederick, MD and [§]School of Nursing, University of Texas Medical Branch, Galveston, TX 77550, USA

REFERENCES

1. Hemmaplardh, D. & Morgan, E.H. (1977) Br. J. Haematol. 36, 85–96.
2. Karin, M. & Mintz, B. (1981) J. Biol. Chem. 256, 3245–3252.
3. Pearse, B. (1982) Proc. Natl. Acad. Sci. USA 79, 451–455.
4. Enns, C.A., Larrick, J.W., Suomabinen, H., Schroder, J., Sussman, H.H. (1983) J. Cell Biol. 97, 579–585.
5. Dautry-Varsat, A., Ciechanover, A., & Lodish, H.F. (1983) Proc. Natl. Acad. Sci. USA 80, 2258–2262.
6. Harding, C., Heuser, J., & Stahl, P. (1983) J. Cell Biol. 97, 329–337.
7. Hopkins, C.R. & Trowbridge, IS. (1983) J. Cell Biol. 97, 508–521.
8. Iacopetta, B.T., Morgan, E.H., & Yeoh, G. C. (1983) J. Histochem. Cytochem. *31*, 336–344.
9. Ward, J.H., Kushner, J.P. & Kaplan, J. (1982) J. Biol. Chem. 257, 10317–10323.
10. Pellicci, P.G., Tabilio, A., Thomopoulos, P., Titeus, M., Vainchenker, W., Rochant, H., & Testa, U. (1982) FEBS LETT. 145, 350–354.
11. Mattia, E., Rao, K., Shapiro, D.S., Sussman, H.H., & Klausner, R.D. (1984) J. Biol. Chem. 259, 2689–2692.
12. Bridges, K.R. & Cudkowicz, A. (1984) J. Biol. Chem. 259,12970–12977.
13. Rao, K.K., Shapiro, D., Mattia, E., Bridges, K., & Klausner, R.D. (1985) Mole. Cell Biol. 5, 595–600.
14. Rao, K., Harford, J.B., Rouault, T., McClelland, A., Ruddle, F.H. & Klausner, R.D. (1986) Mol. Cell Biol. 6, 236–240.
15. Casey, J.L., DiJeso, B., Rao, K., Klausner, R.D., & Harford, J.B. (1988a) Proc. Natl. Acad. Sci USA 85, 1787–1791.
16. Owen, D. & Kuhn, L. (1987) EMBO J. 6, 1287–1293.
17. Aziz, N. & Munro, H.N. (1987) Proc. Natl. Acad. Sci. USA 84, 8478–8482.
18. Hentz, M.W., Rouault, T.A., Caughman, S.W., Dancis, A., Harford, J.B., & Klausner, R.D. (1987a) Proc. Natl. Acad. Sci. USA 84, 6730–6734.
19. Hentze, M.W., Caughman, S.W., Rouault, T.A., Barriocanal, J.G., Dancis, A., Harford, J.B., & Klausner, R.D. (1987b) Science, 1570–1573.
20. Casey, J.L., Matthias, W., Hentze, W., Koeller, D.M., Caughman, S.W., Rouault, T.A., Klausner, R.D., & Harford, J.B. (1988b) Science 240, 924–928.
21. Mullner, E.W. & Kuhn, L.C. (1988) Cell 53, 815–825.
22. Chan, L.-N., Grammatikakis, N., Banks, J.M. & Gerhardt, E.M. (1987) J. Cell Biol. 105, 154a.
23. Kuhn, L.C., McClelland, A., & Ruddle, F.H. (1984) Cell 37, 95–103.
24. Stearne, P.A., Pietersz, G.A., & Goding, J.W. (1985) J. Immunol. 134, 3474–3479.
25. Maniatis, T., Fritsch, E.F., & Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).
26. Sanger, F., Nicklen, S. & Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
27. Zuker, M., & Stiegler, P. (1981) Nuc. Acid. Res. 9, 133–148.
28. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. & Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
29. Vrati, S., Mann, D.A. & Reed, K.C. (1987) Molec. Biol. Reports 1, 1–4.
30. Schneider, C., Owen, M.J., Banville, D., & Williams, J.G. (1984) Nature 311, 675–678.
31. Liebold, E.A. & Munro, H.N. (1988) Proc. Natl. Acad. Sci. USA 85, 2171–2175.
32. Rouault, T.A., Hentze, M.W., Caughman, S.W., Harford, J.B., & Klausner, R.D. (1988) Science 241, 1207–1210.
33. Sieff, C., Bicknell, D., Caine, G., Robinson, J., Lam., G., & Greaves. M.F. (1982) Blood 60, 703–713.
34. Iacopetta, B.J., Morgan, E.H., & Yeoh, G.C. 91982) Biochem. Biophys. Acta. 687, 204–210.
35. Horton, M.A. (1983) Exp. Cell Res. 144, 361–366.

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