Specific Interactions of the Autoantigen L7 with Multi-zinc Finger Protein ZNF7 and Ribosomal Protein S7*

(Received for publication, March 31, 1997)

Stephan Witte and Ulrich Krawinkel
From the Fakultät für Biologie, Universität Konstanz, Postfach 5560, 78434 Konstanz, Germany

The eucaryotic protein L7, which associates with the large subunit of ribosomes, has been shown to be a major autoantigen in systemic autoimmune arthritis. The N terminus carries a sequence motif that is similar to the leucine zipper domain of eucaryotic transcription factors. This domain promotes the homodimerization of protein L7 through β-helical coiled-coil formation and binds to distinct mRNAs, thereby inhibiting their cell-free translation. Using a yeast two-hybrid selection, we have identified from a Jurkat T lymphoma cDNA library ribosomal protein S7 and the multi-zinc finger protein ZNF7 as proteins that interact with protein L7. A fragment of L7 carrying the leucine zipper-like domain is fully sufficient to mediate these interactions. Their potential biological significance is indicated by low apparent dissociation constants of S7-L7 (15 × 10−9 M) and, respectively, ZNF7-L7 (2 × 10−9 M) complexes and co-immunoprecipitation of proteins S7, ZNF7, and L7 from a cell lysate with an anti-L7 antibody. We also show that ZNF7-like L7 and S7 can exist in a ribosome-bound form. This study provides further evidence suggesting that L7 is involved in translational regulation through interactions with components of the translational apparatus.

Eucaryotic protein L7 associates in the cytoplasm with the large ribosomal subunit (1, 2). The N-terminal region of human and rodent protein L7 carries a sequence motif that is similar to the basic region leucine zipper (BZIP)1 domain characteristic of some eucaryotic transcription factors (3, 4). The BZIP-like domain mediates the formation of L7 homodimers (3, 5), which interact with cognate sites on mRNA (3, 4), thereby inhibiting their cell-free translation (6). Constitutive expression of human protein L7 in Jurkat T lymphoma cells suppresses the synthesis of at least two nuclear proteins, arrests the cell cycle in G1, and induces apoptosis (7). Thus, protein L7 is one of the growing number of ribosomal proteins that seem to have extraribosomal functions (8). Like some other riboproteins, protein L7 is a major autoantigen in rheumatoid autoimmune diseases, such as systemic lupus erythematosus. This suggests that autoimmunogenic L7 is released during such phases and stimulates normally silent B-lymphocyte clones expressing low affinity antigen receptors (12). In this context, we study the functions of protein L7 with the intention to elucidate potentially autoimmunogenic mechanisms (13, 14).

To understand the biological function of protein L7 we employed yeast two-hybrid selection (15) to identify ribosomal protein S7 and the multi-zinc finger protein ZNF7 as proteins that specifically interact with L7. Complexes of L7 with S7 and, respectively, ZNF7 apparently exist in lymphoid cells, as demonstrated by co-immunoprecipitation. These and previous findings (3–7) suggest a function of protein L7 in translational regulation.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid System—The yeast two-hybrid system used in this study was kindly donated by E. A. Golemis (Fox Chase Cancer Center, Philadelphia, PA) and has been described previously (15, 17). We employed yeast strain S. cerevisiae EGY48, which contained for selection the reporter plasmid pkL103 (15).

Construction of Bait Plasmids for the Yeast Two-hybrid Screen—pEG202 (15) was used as an expression vector for constructing LexA-fused bait proteins. pLexA-L7 expresses LexA fused to the full-length coding sequence of human ribosomal protein L7. pLexA-L71–124 and pLexA-L7125–248 express the 124 N-terminal amino acids or, respectively, the 124 C-terminal amino acids of L7, fused to LexA. PCR was used to prepare DNA fragments that contained 5′ EcoRI and 3′ XhoI sites. These fragments were treated with EcoRI and XhoI and were ligated into pEG202. Recombinant plasmids were amplified in E. coli, and the integrity and orientation of their cDNA inserts were verified by nucleotide sequencing.

Construction of a Jurkat T Lymphoma cDNA Library—Approximately 2 × 109 Jurkat cells (RNeasy, QIAGEN, Hilden, FRG), 7 μg of poly(A)1+ RNA were obtained from total RNA of 1 × 109 Jurkat cells (RNeasy, QIAGEN, Hilden, FRG). 7 μg of poly(A) RNA were obtained from total RNA (Oligotex, QIAGEN), and cDNA was prepared (cDNA Synthesis kit, Stratagene, Heidelberg, FRG) according to the instructions of the suppliers. The resulting cDNA was size-fractionated using a Sephacryl S-500 spin column. Fractions containing cDNA molecules larger than 700 base pairs were pooled, unidirectionally ligated into pJG4–5 (15), cut with EcoRI and XhoI, and used to transform E. coli XL2-blue MRF (Stratagene, Heidelberg, FRG). Ampicillin-resistant colonies were pooled, resulting in a cDNA library comprising 2 × 106 independent clones. Inserts ranged from 700 to 3500 base pairs with an average length of 1200 base pairs.

Construction of a Jurkat T Lymphoma cDNA Library—S. cerevisiae EGY48, containing pJK103 and pLexA-L7, was transformed with the library. Approximately 2 × 107 transformants were plated on synthetic medium lacking uracil, histidine, tryptophan, and leucine (17). After 3 days, colonies appeared. These were tested in a filter assay (18) for activation of the β-galactosidase reporter gene (lacZ). From colonies that activated both the lexA2 reporter gene (required in the biosynthetic pathway for leucine) and the lacZ reporter gene, library plasmids were rescued (17). Inserts were amplified by PCR, followed by restriction mapping of the product with enzymes AluI and HaeIII, and assigned to classes according to their restriction map pattern. At least two cDNAs from each class were partially sequenced.

* This work was supported by the Deutsche Forschungsgemeinschaft through Sonderforschungsbereich 156. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This paper is dedicated to Professor Rudolf Pichlmayr.

1 The abbreviations used are: BZIP, basic region leucine zipper; PAGE, polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography; PCR, polymerase chain reaction.
**L7 Interactions with ZNF7 and S7**

**Specificity of Interaction—Rescued library plasmids of S7 and ZNF7**

were retransfected into EGY48/pJK103, containing as baits either pRPM1 (15), which encodes the homeodomain of boid, or pLexA-myc (19), which encodes the carboxyl-terminal 176 amino acids of human c-Myc. Transformants were assayed for leu2 and lacZ activity.

**DNA and Protein Sequence Analysis—DNA sequences were determined by the dideoxy-mediated chain termination method using α-35SdATP and Sequenase DNA polymerase version 2.0 (U.S. Biological Corp.).** Sequence analysis and homology searches were performed using the GCG program package (Genetics Computer Group, Madison, WI). Secondary structure predictions of proteins were performed using the PSAT algorithm (20). Predictions of probabilities for putative coiled coil regions were performed with the program Coils (21). The width of the chosen window was 14 amino acids.

**Expression and Purification of Hexahistidine-tagged Fusion Proteins—Full-length HisL7 was prepared as described (5) and further purified by HPLC on a C4 reversed-phase column (Vydac, Hesperia, CA). To produce HisS7, the full-length human S7 cDNA was amplified by PCR using primers designed to provide BamHI and XhoI sites for subcloning (5′-GGCCCTTCGAGTTACAATGAAACTTCGGA-3′ for sense primer and 5′-GGCCCTTCGAGTTACAATGAAACTTCGGA-3′ for antisense primer). To produce HisZNF71–687, the human ZNF7 cDNA inserted in the screen was amplified by PCR using primers again providing BamHI and XhoI sites for subcloning (5′-AAATGTCGACAGTGACGACGCTGCTGAGGACA-3′ for sense primer and 5′-GGCCCTTCGAGTTACAATGAAACTTCGGA-3′ for antisense primer).** To produce HisS7 and HisZNF71–687, the cDNAs were purified from bacterial lysates as described (5). HisS7 was further purified by HPLC on a C4 reversed-phase column. Protein concentrations were determined photometrically (22). Proteins were analyzed by SDS-PAGE and Coomassie Brilliant Blue staining to determine their purity. The purity of the preparations of HisL7 and HisS7 was >95%, and in the case of HisZNF71–687 it was >85%.

**Determination of Dissociation Constants—Apparent dissociation constants (Kd) of L7-ZNF7 and L7-S7 dimers were determined essentially as described previously (5) by measuring the binding of [35S]cysteine-labeled ZNF71–687 to immobilized HisL7 or by measuring the binding of [35S]methionine-labeled L7 to immobilized HisS7.** S-Labeled protein was produced using the TNT coupled transcription/translation kit (Promega, Madison, WI) according to the instructions of the supplier. Plasmids used here were phumL7–14 (3), for radiolabeled L7, and pSK-ZNF71–687. To produce pSK-ZNF71–687, the BamHI and XhoI fragment from pHisZNF71–687 was subcloned into vector pSK II (Stratagene, Heidelberg).

**Polyclonal Antibodies—Antibodies against human proteins were generated using standard immunization protocols (20). Anti-HisL7 antibodies were raised in chickens, and the antibodies were purified from egg yolk (24). In immunoblot analyses, this antisemur recognized a protein band that displayed the electrophoretic mobility of L7. Antibodies against HisZNF71–687 and against the ovalbumin-coupled peptide sequence CGRHHTGKEFKYRG (anti-ZNF71–687) that represents the conserved spacer sequence between zinc finger domains in ZNF7 and many other human zinc finger proteins were raised in rabbits. Both antisera recognized immunoblotting of cell lysates a band with the predicted electrophoretic mobility of ZNF7 (78 kDa) and two additional bands of 55 and 57 kDa, respectively. Polyclonal rabbit anti-S7 antibody was a gift from A. Hohlbaum (Universita¨t Konstanz, Konstanz, FRG). Polyclonal mouse anti-HisL7 antibodies were a gift from E. A. Golemis. Polyclonal mouse anti-LexA antibodies were a gift from J. Horwath, Universita¨t Konstanz, Konstanz, FRG, which were treated as described above.

**RESULTS**

**Isolation of Proteins Interacting with Autoantigen L7—To identify proteins, which potentially interact with protein L7 in vivo, we performed interaction screening using the yeast two-hybrid system.** The yeast reporter strain EGY48 containing the lacZ reporter plasmid pJK103 was transfomed with the plasmid pLexA-L7, which encodes full-length protein L7 fused to the LexA DNA-binding domain. Fig. 1 verifies the correct expression of fusion proteins containing the LexA DNA-binding domain fused to protein L7 and fragments thereof. Immunoblots of yeast homogenates transformed with the indicated plasmids and probed with anti-L7 and anti-LexA antibodies showed bands of the expected size (52 kDa for LexA-L7, 38 kDa for LexA-L71–124, and 37 kDa for LexA-L7125–248). None of the bait plasmids alone activated the leu2 or the lacZ reporter gene, respectively (data not shown). To confirm that the LexA-L7 fusion proteins were synthesized in yeast and that the LexA domains are fully functional to bind to LexA operator sequences, we performed a repression assay (17) (data not shown).

To screen the Jurkat T lymphoma cell cDNA library, the EGY48/pJK103/pLexA-L7 cells were transformed with the library plasmids. Approximately 2 × 109 yeast transformants were screened. 173 colonies grew on leucine-free galactose plates and displayed β-galactosidase activity. Of these, 61 showed strong galactose-inducible activity of both reporter genes. Library plasmid DNA was prepared from these positive colonies. Their inserts were amplified by PCR followed by restriction mapping of the product with endonucleases AluI and HaeIII (not shown). Inserts isolated independently at least twice were assigned to seven groups according to the size of the insert and its restriction site pattern. The inserts of at least two cDNAs from each group were then partially sequenced. The two most abundant cDNAs could be matched to known sequences in the data base; 12 independently isolated cDNAs (restriction group 1) coded for the multizinc finger protein ZNF7 (26), with the longest cDNA encoding amino acids 91–687, comprising the C-terminal domain of 15 subsequent zinc finger domains. The full-length cDNA coding for ribosomal protein S7 (27) was independently isolated seven times (restriction group 2). Re-
TABLE I
Interaction of protein L7 with proteins S7 and ZNF7 using a yeast two-hybrid system

| pEG202 insert ("bait") | pG4–5 insert ("prey") | Growth on leucine minus medium | Colony color in filter assay |
|------------------------|-----------------------|-------------------------------|-----------------------------|
| L7                     | S7                    | +                             | Blue White                  |
| Bicoid                 | S7                    | –                             | White White                 |
| c-Myc                  | S7                    | –                             | White White                 |
| L7                     | ZNF7                  | +                             | Blue White                  |
| Bicoid                 | ZNF7                  | –                             | White White                 |
| c-Myc                  | ZNF7                  | –                             | White White                 |
| L71–124                | S7                    | +                             | Blue White                  |
| L71–124                | ZNF7                  | +                             | Blue White                  |
| L7125–248              | S7                    | –                             | White White                 |
| L7125–248              | ZNF7                  | –                             | White White                 |

Interaction of the leu2 reporter gene was monitored by growth on leucine-deficient medium, and the activity of the lacZ reporter gene was monitored using a filter assay (blue colonies). Expression of the prey protein was induced by adding galactose to the medium.

Restriction group 3 (six isolated cDNAs) turned out to be the cDNA for ribosomal protein S5 in a wrong reading frame. The remaining four restriction groups, consisting of 2–4 cDNAs, coded for as yet unidentified C2H2 zinc finger proteins, which could not be assigned to any sequence in the data base. This study focuses on the characterization of the abundant cDNAs of proteins S7 and ZNF7 as potential L7 interactors.

Specificity of the Interaction—To confirm the specificity of the interactions of S7 and ZNF7 with protein L7, we retransformed the isolated library plasmids into EGY/pJK103, containing bait plasmids that encoded LexA fused to protein L7, to the homeodomain of drosophila bcd, to the carboxyl-terminal domain of the human c-Myc protein. We then assayed for the activation of both reporter genes. The interaction of S7 and ZNF7,91–687 with protein L7 turned out to be specific, in that only cotransformation of the L7 bait lead to the activation of both reporter genes (Table I).

The L7 Region That Mediates the Interaction—To determine the region of L7 mediating the interaction of L7 with S7 or ZNF7, we transformed the library plasmids of the latter proteins into EGY/pJK103 containing bait plasmids coding for LexA fused to full-length protein L7 (pLexA-L7), the N-terminal half of protein L7 (comprising residues 1–124 (pLexA-L71–124)), or the C-terminal half of L7 (comprising residues 125–248 (pLexA-L7125–248)). As shown in Table I, the N-terminal region of protein L7 is fully sufficient to activate both the leu2 reporter gene and β-galactosidase activity. No activation of the reporter genes was observed with the pLexA-L7125–248 bait. The interface on protein L7 that interacts with S7 and ZNF7 thus resides between residues 1 and 124.

Apparent Dissociation Constants—The interaction of S7 and ZNF7 with L7 was further characterized by determination of the dissociation constants of the S7-L7 and the ZNF7-L7 complexes. Dissociation constants were determined utilizing a binding assay employing oligohistidine-tagged proteins immobilized on a Ni2+–chelate column at different concentrations. Immobilized HisS7 was incubated with 35S-labeled ZNF791–687 at a starting concentration of 3.5 × 10−5 M. Immobilized HisS7 was incubated with 35S-labeled L7 at the same starting concentration. For both immobilized proteins, we utilized radiolabeled luciferase as a control ligand. The columns were washed with a total of 15 batch volumes of binding buffer, and the fractions of column-bound and eluted ligand were determined for increasing dilutions of HisL7 and HisS7 (Fig. 2). For both proteins a lowest concentration of approximately 25 × 10−5 M was found that provided nearly complete retention of liquid phase radiolabeled ligand; hence, Kd values of 2 nM for the L7-ZNF7 interaction (Fig. 2B) and of 15 nM for the L7-S7 interaction (Fig. 2A) were estimated. No retention of radiolabeled luciferase as an irrelevant protein was observed on the HisL7 or HisS7 column.

Binding of S7 and ZNF7 to L7 in Vivo—Having demonstrated the interaction of ribosomal protein S7 and ZNF7 with ribosomal protein in vitro as well as in the yeast two-hybrid system, we further examined the association of these proteins in a lymphoid cell line. We immunoprecipitated the total cell lysate of MOLT-4 cells with an immobilized polyclonal chicken anti-L7 antibody. The precipitate was fractionated by SDS-PAGE and analyzed by immunoblotting using for detection mouse anti-L7 antibodies, rabbit anti-S7 antibodies, and rabbit anti-ZNF7 antibodies and an unrelated antibody as a control. As shown in Fig. 3A, protein bands with the predicted electrophoretic mobility of proteins L7 (lane 1), S7 (lane 2), and ZNF7 (lanes 3 and 4) could be detected in the precipitate, thus indicating co-precipitation with ribosomal protein L7. No signal was found either with the unrelated control antibody (lane 5) or in a control precipitation with immobilized preimmune serum (lanes 6–10).

Binding of ZNF7 to Ribosomes—The association of ZNF7 with ribosomes was examined by immunoblotting of Jurkat T lymphoma ribosomes and visualization of ribosome-bound ZNF7 by anti-ZNF7 antibodies (Fig. 3B). Anti-L7 (lane 1) and anti-S7 antibodies (lane 2) served as a control for the preparation of ribosomal fractions. Lanes 3 and 4 show that a protein band with the predicted molecular mass of ZNF7 could be clearly detected by the two available anti-ZNF7 antibodies in preparations of ribosomes. The anti-ZNF7 antibody (lane 3), raised against recombinant ZNF7, detected an additional cross-reactive protein band with a molecular mass of 25 kDa, which
L7 Interactions with ZNF7 and S7

In this study, we have used the yeast two-hybrid system to identify from a Jurkat T lymphoma cDNA library ribosomal protein S7 and multi-zinc finger protein ZNF7 as proteins that interact with ribosomal protein L7. Co-immunoprecipitation of both proteins with an anti-L7 antibody from the lysate of the MOLT-4 cell-line and dissociation constants of 15 nM for the interaction of these proteins. Our results show that the N-terminal half of L7, comprising residues 1–124 is fully sufficient to mediate the interaction with both S7 and ZNF7, respectively.

Protein S7 consists of 194 amino acids, has a predicted molecular mass of 22 kDa, and is, like protein L7, very basic (pI of S7: 10.6) (27, 28). Protein S7 has been shown to be located at the back lobe of the small 40 S subunit of eukaryotic ribosomes (24). Together with ribosomal proteins S3, S3a, S14, and S15, it plays an important role in the biogenesis of the 40 S small ribosomal subunit (29). Ribosomal protein L7 is located at the surface of the large 60 S ribosomal subunit (30, 31), to which it associates in the cytoplasm (32). The N-terminal BZIP-like domain of protein L7 is thought to be exposed to the cytoplasm due to its ability to bind mRNAs with high affinity (3, 4) and because of cross-linking and labeling experiments (31, 33). This domain also carries the immunodominant autoepitope of protein L7 (12). These findings and the data presented in this study suggest that the interaction of protein S7 with the N-terminal half of protein L7 is important for the interaction of the small 40 S and the large 60 S ribosomal subunit during the translation of mRNAs. This is supported by the finding that ribosomal protein L7 could be in situ chemically cross-linked to both the 28 S rRNA of the large ribosomal subunit and to the 18 S rRNA of the small ribosomal subunit (34). Secondary structure predictions performed on the amino acid sequence of protein S7 suggest that it has, like protein L7, mainly an α-helical conformation (Fig. 4A). A prediction of putative coiled-coil regions in S7 shows a high probability (>90%) for such a domain between amino acids 29 and 50 of protein S7 (Fig. 4B). This region shows also a high probability for an α-helical conformation, which is required for a coiled-coil structure. Within this predicted amphipathic helix are two leucine repeats in the D position of the helix (residues 36 and 43) and hydrophobic residues in the A position of the helix (residues 40 and 47). In a previous study, we have shown that a putative coiled-coil region, located in the first N-terminal α-helix of protein L7, is capable of forming leucine zipper-like homodimers (5). We therefore suggest that the interaction of proteins L7 and S7 is mediated by the BZIP-like domain of L7 and the putative leucine zipper domain of protein S7.

ZNF7 is a transcription factor-like multi-zinc finger protein consisting of 687 amino acids and has a predicted molecular mass of 78 kDa. It is widely expressed in various cell lines. The protein consists of 15 Cys-His zinc finger domains that comprise nearly 70% of the protein (26). The finger domains share 70% sequence similarity and conform almost exactly to the consensus sequence of DNA- and RNA-binding zinc fingers (35). In addition to the nucleic acid binding properties of zinc finger domains, zinc finger-mediated protein interactions have been reported (36), including the interaction of a zinc finger domain with a leucine zipper motif (37). However, the interfaces responsible for the interaction of L7 and ZNF7 remain to be specified. The cellular localization of ZNF7 is unknown, although a nuclear localization has been suggested (26). However, a localization in the cytoplasm has not been excluded, and we show in this study that ZNF7 co-purifies with eucaryotic ribosomes. This suggests a ribosomal localization of ZNF7, where it may act as translational regulator. In addition, ZNF7 has been recently shown to be a substrate of mitogen-activated protein kinase in vitro (38), suggesting a role in signal transduction pathways. However, it is unknown whether mitogen-activated protein kinase-mediated phosphorylation of ZNF7 influences the binding of ZNF7 to L7 in vivo.

In summary, we have shown in this and previous studies that protein L7 can interact with various components of the translational apparatus (i.e. mRNA, 28 S rRNA, ribosomal...
protein S7, and ZNF7). This hints at a complex role of L7 in translational regulation. It is clear, however, that the functional significance and the molecular details of these interactions remain to be analyzed.

Acknowledgments—We thank Erica A. Golemis for providing plasmids and strains and for valuable help in setting up the yeast two-hybrid system. We are also grateful to Joachim Stahl for providing antibodies, to Andreas Hohlbaum and Jörg Horwath for providing reagents and helpful discussions, to Rikiro Fukunaga for communicating data prior to publication, and to Katja Aviszus and Elli Neu for comments on the manuscript.

REFERENCES
1. Tsurugi, K, Collatz, E., Wool, I. G., and Lin, A. (1976) J. Biol. Chem. 251, 7940–7946
2. Lin, A., Chan, Y.-L., McNally, J., Peleg, D., Meyuhas, O., and Wool, I. G. (1978) J. Biol. Chem. 253, 12665–12671
3. Hemmerich, P., von Mikecz, A., Neumann, F., Sozeri, O., Wolff-Vorbeck, G., Zobelein, R., and Krawinkel, U. (1993) Nucleic Acids Res. 21, 223–231
4. Hemmerich, P., Bosbach, S., von Mikecz, A., Krawinkel, U. (1997) Eur. J. Biochem. 245, 549–556
5. Witte, S., Neumann, F., Krawinkel, U., and Prezybiski, M. (1996) J. Biol. Chem. 271, 18171–18175
6. Neumann, F., Hemmerich, P., von Mikecz, A., Peter, H. H., and Krawinkel, U. (1995) Nucleic Acids Res. 23, 195–202
7. Neumann, F., and Krawinkel, U. (1997) Exp. Cell. Res. 230, 252–261
8. Wool, I. G. (1996) Trends Biol. Sci. 21, 164–165
9. von Mikecz, A., Hemmerich, P., Peter, H. H., and Krawinkel, U. (1994) Immunobiology 192, 137–154
10. Neu, E., von Mikecz, A., Hemmerich, P., Peter, H. H., Fricke, M., Deicher, H., Genth, E., and Krawinkel, U. (1995) Clin. Exp. Immunol. 100, 198–204
11. von Mikecz, A., Hemmerich, P., Peter, H. H., and Krawinkel, U. (1995) Clin. Exp. Immunol. 100, 205–213
12. Neu, E., Hemmerich, P., Peter, H. H., Krawinkel, U., and von Mikecz, A. (1997) Arthritis Rheum., in press
13. Tan, E. M. (1989) Adv. Immunol. 44, 93–151
14. Tan, E. M. (1991) Cell 67, 841–842
15. Gyuris, J., Golemis, E., Chertkov, H., and Brent, R. (1993) Cell 75, 791–803
16. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1991) Current Protocols in Molecular Biology, Wiley Interscience, New York
17. Golemis, E. A., Gyuris, J., and Brent, R. (1993) in Current Protocols in Molecular Biology (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds) Vol. 3, pp. 20.1.1–20.1.28, Wiley Interscience, New York
18. Breeden, L., and Nasmyth, K. (1985) Cold Spring Harbor Symp. Quant. Biol. 50, 543–550
19. Estejaj, J., Brent, R., and Gollemis, E. (1995) Mol. Cell. Biol. 15, 5820–5829
20. Stultz, C. M., White, J. V., and Smith, T. F. (1993) Protein Sci. 2, 305–314
21. Lupas, A., van Dyke, M., and Stock, J. (1991) Science 252, 1162–1164
22. Gill, S. C., and von Hippel, P. H. (1989) Anal. Biochem. 182, 319–326
23. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
24. Gassmann, M., Tohmme, P., Weiser, T., and Huber, U. (1990) FEBS Lett. 252, 257–2635
25. Lutsch, G., Stahl, J., Kargel, H.-J., Noll, F., and Bielka, H. (1990) Eur. J. Cell Biol. 51, 140–150
26. Lania, L., Danti, E., Pannuti, A., Pasucci, A., Pengue, G., Feliciello, I., La Mantia, G., Lanfrancone, L., and Pelici, R. (1990) Genomics 6, 333–340
27. Annilo, T., Laan, M., Stahl, J., and Metspalu, A. (1995) Gene (Amst.) 165, 297–302
28. Wool, I. G., Chan, Y., and Gluck, A. (1995) Biochem. Cell Biol. 73, 933–947
29. Mundus, D. A., Bulygin, K. N., Venyaminova, A. G., Vladimirov, S. N., Vratskikh, L. V., Repkova, M. N., Yamkovoi, V. S., and Karpova, G. G. (1993) Mol. Biol. 27, 91–95
30. Kislevsky, R., and Gore, J. (1987) Biochim. Biophys. Acta 910, 282–291
31. Marion, M.-J., and Marion, C. (1987) Biochim. Biophys. Res. Commun. 149, 1077–1083
32. Hadjipavlov, A. A. (1985) The Nucleolus and Ribosome Biogenesis, Cell Biology Monograph 12, Springer Verlag, Wien
33. Lin, A. (1991) FEBS Lett. 287, 121–124
34. Niyogi, O., and Nika, H. (1982) EMBO. J. 1, 357–362
35. Mattaj, I. W. (1993) Cell 74, 837–840
36. Sun, L., Liu, A., and Georgopoulos, K. (1996) EMBO J. 15, 5358–5369
37. Diaz-Meco, M., Municio, M. M., Frutos, S., Sanchez, P., Lozano, J., Sanz, L., and Moscat, J. (1996) EMBO J. 16, 777–786
38. Fukunaga, R., and Hunter, T. (1997) EMBO J. 16, 1921–1933