Preparation and Characterization of Antibodies against Human Ribosomal Proteins: Heterogeneous Expression of S11 and S30 in a Panel of Human Cancer Cell Lines

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Mutants of model eukaryotic organisms have revealed that most ribosomal proteins are essential for cell viability. However, the precise functional role of each ribosomal protein is largely unknown. Recent reports on the involvement of ribosomal proteins in various genetic diseases and studies on the extraribosomal functions of these proteins have cast some light on their localization and functions. Here we prepared rabbit polyclonal antibodies against 26 human ribosomal proteins; each of these reagents recognized a single band in immunoblots of the purified ribosome. We used these antibodies to evaluate a panel of human cancer cell lines. Although no deficiency of ribosomal proteins was observed, the abundance of S11 and S30 varied substantially among the cell lines, but the difference did not affect the biogenesis or composition of the ribosome. Therefore, the heterogeneity may be related to extraribosomal functions of S11 and S30. The antibodies described here are powerful tools for research into the molecular mechanisms of protein translation, cell-biological and medical studies on the ribosomal proteins, and ultimately a comprehensive understanding of all ribosomal proteins (“ribosomics”).

Key words: Proteomics — Ribosomal proteins — Antibodies — Expression profiles — Human cancer

All living organisms have ribosomes, which are a protein complex that is essential for protein synthesis. Bacterial ribosomes are an excellent in vivo model system that has been studied for a long time, and much information on the roles of the ribosome in translation has accumulated. Therefore a conception prevalent among many scientists is that most of the important questions about the ribosome and its components have been resolved already. However, the following points relating to ribosomal components in higher organisms argue against this notion. Abnormality of ribosomal proteins causes diseases of specific tissues. For example, haploinsufficiency of S19 is associated with Diamond-Blackfan anemia. The absence of a ribosome-associated protein causes fragile X syndrome, which is characterized by mental retardation. In addition, mutation of the gene encoding a protein essential for the processing of pre-ribosomal RNA leads to dyskeratosis congenita. Ribosomal proteins appear to have roles in addition to those in the translation machinery (extraribosomal functions). In Jurkat T-lymphoma cells, overexpression of L7 suppresses the expression of nuclear proteins and arrests the cell cycle in G1. L32 may have bone-promoting potential. L29 is expressed on the cell surface, binds to heparin, and may be involved in embryo implantation. S19 is released as an oligomer from apoptotic cells into the extracellular space; this protein functions as a monocyte chemotactic factor, which is likely to be important in vivo for phagocytic clearance of apoptotic cells. In addition, the regulation of ribosome-interacting proteins seems to be tightly associated with the stress response, apoptosis, and carcinogenesis. Collectively, various ribosomal and ribosome-interacting proteins as well as changes in their expression and localization seem to participate in and modulate a wide variety of cellular activities.

To investigate the expression and roles of ribosomal proteins in the cell, specific probes are essential. Detection of the mRNA of ribosomal proteins by using their cDNA sequences has revealed that these proteins are associated with cell differentiation and malignant tumorigenesis. Because the expression of ribosomal proteins is controlled translationally and post-translationally, detection of translation products is very important. However, in contrast to those analyses of mRNA, few reports on analysis at the protein level have appeared. The paucity of specific probes for ribosomal proteins (that is, antibodies against them) has limited such biochemical analysis.

In the present study, we have prepared and characterized rabbit polyclonal antibodies against human ribosomal pro-
teins. For the initial application of these reagents, we evaluated the expression of ribosomal proteins in a number of cancer cell lines. This analysis revealed an obvious variation in the expression of at least two ribosomal proteins.

**MATERIALS AND METHODS**

**Antibody production and purification** We obtained complete amino acid sequences of human ribosomal proteins from a BLAST homology search of the NCBI database and the review by Wool et al. Using this information, we chemically synthesized oligopeptides (10–13 amino acids) to these human ribosomal proteins; we conjugated the oligopeptides with 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide-HCl and keyhole limpet hemocyanin to prepare the immunogens. Each immunogen was injected into two pathogen-free rabbits (six injections per rabbit). The fractions of immunoglobulin G were isolated from the antisera of the rabbits by using column chromatography with protein A-Sepharose (Amersham Pharmacia Biotech, Uppsala, Sweden); the collected fractions were aliquoted and stored at −80°C until use.

**Cells and culture** The cell lines used in this study were obtained from the Japanese Collection of Research Biological Sources (JCRB; Tokyo), maintained according to the supplier’s instructions, and grown in an atmosphere of 5% CO₂ at 37°C. These lines are A549 (lung cancer, catalog no. JCRB0076), Caki-1 (renal cancer, JCRB0801), C32TG (amelanotic melanoma, JCRB0227), HuO-3N1 (osteosarcoma, JCRB0413), LoVo (colon adenocarcinoma, JCRB0983), L999 (lung carcinoma, JCRB0080), ME-180 (cervical cancer, JCRB0810), MRK-nu-1 (mammary carcinoma, JCRB0628), NEC8 (testicular germ cell tumor, JCRB0250), NH-6 (neuroblastoma, JCRB0832), NH-12 (neuroblastoma, JCRB0833), NY (osteosarcoma, JCRB0614), PA-1 (ovarian teratocarcinoma, JCRB9061), RPMI1788 (peripheral blood, JCRB0035), SBC-3 (small cell carcinoma, JCRB0808), YMB-1 (breast cancer, JCRB0823), and YMB-1-E (breast cancer, JCRB0825). These cell lines have no mutation in the p53 tumor suppressor gene.

**Ribosomes and polysomes** Rat liver ribosomes were isolated by ultracentrifugation as described previously. Briefly, the liver from a Sprague-Dawley rat was homogenized in 50 mM Tris-Cl, pH 7.5, containing 0.1 M KCl, 5 mM MgCl₂, 10 mM 2-mercaptoethanol, and 0.25 M sucrose. We collected the post-mitochondrial fractions by using a brief centrifugation. The ribosomal fractions were resuspended in buffer A. Using the conversion factor of 13 absorbance units/ml at 260 nm equals a ribosome concentration of 1 mg/ml, we calculated the concentration of the isolated ribosomes and polysomes.

**Immunoblotting** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli. Cells (1×10⁶) were washed with PBS, lysed in 0.5 ml of 1× SDS-PAGE sample buffer, and immediately boiled for 10 min. After electrophoresis of the lysates, proteins were transferred to Immobilon membrane (Millipore, Bedford, MA) at 25 mA. The blots were probed with antibodies against ribosomal proteins by using one of the two following methods. For the Tween-plus method, the blots were blocked in PBS containing 5% skim milk (Difco, Sparks, MD) and 0.1% Tween 20 for 1 h at room temperature then incubated for 1 h at room temperature with primary antibodies diluted in 0.5% milk in PBS. After five 5-min washes with 0.5% Tween 20 in PBS, the blots were incubated for 30 min at room temperature with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Bio-Rad) diluted in 0.5% milk in PBS and washed five times with 0.5% Tween 20 in PBS. The Tween-plus method was suitable for all antibodies listed in Table I except the anti-L32 antibody. In addition, the blots were stained with monoclonal anti-α-tubulin antibody (clone B-5-1-2; Sigma, Saint Louis, MO) by using the Tween-plus method.

For the Tween-minus method, the blots were blocked overnight at 4°C in 10% skim milk in H₂O and incubated for 1 h at room temperature with primary antibodies diluted with 50 mM Tris-Cl, pH 7.5, containing 0.5 M NaCl and 0.5% milk skim milk. After five 15-min washes with PBS, the blots were incubated for 30 min at room temperature with the secondary antibody diluted in 0.5% skim milk in PBS then washed five times with PBS. The signal...
RESULTS

Preparation of polyclonal antibodies against human ribosomal proteins According to a recent review,\textsuperscript{18} the human ribosome (80 S) comprises a large subunit (60 S) of 46 proteins and a small subunit (40 S) of 33 proteins. To accomplish our final goal of surveying all the ribosomal proteins in the cell, we started by preparing antisera against all 79 ribosomal proteins.\textsuperscript{24} For each ribosomal protein, we conjugated an appropriate oligopeptide with keyhole limpet hemocyanin then used the conjugated protein to immunize two rabbits. According to a BLAST search, the selected oligopeptide sequences showed no significant homology to other proteins, including other ribosomal proteins. The reactivity of the 158 antiserum samples thus obtained was evaluated by immunoblotting using the purified ribosome. We used rat liver ribosomes for this primary screening because we can obtain a large amount of very pure ribosomes by using an established method. The antigen peptide structures of human ribosomal proteins are identical or very similar to the corresponding structures of their rat homologs (70 of 79 structures were identical; Table I and data not shown). In light of this evaluation, we selected antibodies against 26 ribosomal proteins (Table I). Immunoblotting with any of these antibodies gave a single band (Fig. 1), whose mobility was close to that expected from the calculated molecular weight of the rat protein.\textsuperscript{18} Therefore, these antibodies can be used to specifically identify the target proteins in the ribosome fraction isolated by using a simple, conventional method.

Evaluation of ribosomal proteins in human cancer cell lines by using the polyclonal antibodies We next examined whether our antibodies were useful for detecting ribosomal proteins in crude samples, lysates of human cancer cells. To confirm the detection of ribosomal proteins in various tissues, we used cell lines derived from distinct organs and cells. Lysates of 17 human cancer cell lines were prepared by using 1× Laemmli sample buffer, electrophoresed in 12% gels, blotted onto membrane, and sub-

| Antibody name | Target protein | Accession no. | Mol. weight | Sequence | Homology to rat (%) |
|---------------|---------------|---------------|-------------|----------|---------------------|
| HSa1 S4a      | J03799        | 32 852        | RMRGTISREHP | 100      |
| HS4X1 S4X     | M58458        | 29 596        | DKLRAAKQSSG | 100      |
| HS82 S8       | X67247        | 24 204        | GGTKKPYHKKR | 100      |
| HS111 S11     | X06617        | 18 430        | DYLHYRKYNR  | 100      |
| HS121 S12     | X53505        | 18 489        | RRRGGRRGLR | 100      |
| HS141 S14     | M13934        | 17 039        | QRRRLNRGLRR | 100      |
| HS152 S15     | J02984        | 17 718        | RGRVTGVSKKK | 100      |
| HS182 S18     | P25232        | 17 608        | RMRGTISREHP | 100      |
| HS242 S24     | U12202        | 15 068        | KRRANKRRNKRMK | 100 |
| HS302 S30     | X65921        | 14 389        | FGKNNPNANS  | 100      |
| HL52 L5       | U14966        | 34 446        | HSTKRFPYLDSES | 100  |
| HL71 L7       | X52967        | 29 224        | DAGRREDQINR  | 100      |
| HL7a2 L7a     | M36072        | 29 994        | TNYNDYDEIR  | 100      |
| HL81 L8       | Z28407        | 28 023        | RGTQTVQKEKEN | 100     |
| HL92 L9       | D14531        | 21 826        | RLRVDKKWGNR  | 100      |
| HL13a2 L12a   | X56932        | 23 576        | KHYRKKQKLQ  | 100      |
| HL142 L14     | D87735        | 23 802        | QKYVRQAWQKA  | 100      |
| HL151 L15     | L25899        | 23 927        | DTQWITKPDHK  | 100      |
| HL171 L17     | X53777        | 21 396        | AKQWGWTQGRW  | 100      |
| HL182 L18     | L11566        | 21 633        | RSKRKRKFERAR | 100     |
| HL281 L28     | U14969        | 15 761        | RKRTRPTKKSS  | 100      |
| HL322 L32     | X03342        | 15 859        | KRNAWKPRPGID | 100     |
| HL341 L34     | P49207        | 13 304        | KTRLSRTPGNR  | 100      |
| HL35a2 L35a   | X52966        | 12 494        | KRGLNQREHT  | 100      |
| HL36a2 L36a   | M15661        | 12 468        | KQQHPTQKVYQK | 100     |
| HL392 L39     | L05096        | 6 292         | KQQQNPQIPQW  | 100      |
jected to staining with the antibodies. Because samples of normal human tissues are difficult to obtain, we included a lysate of RPMI1788 cells, which are derived from normal peripheral blood leukocytes, as a normal control. Of the 26 antibodies, the 19 antibodies against S4X, S11, S12, S14 (Fig. 2A), S15, S18, S30, L5, L7, L8, L9, L13a (Fig. 2B), L14, L15, L17, L18, L34, L35a, and L36a showed a single strong band that co-migrated with the rat homolog. The remaining seven antibodies (anti-SA, S8, S24, L7a, L28, L32, and L39) each yielded a putative positive band in the midst of a high background, which probably resulted from nonspecific binding of the primary antibodies. These results suggest specific binding of the 19 antibodies to ribosomal proteins in crude cell extracts.

In addition, we evaluated the amounts of the 19 ribosomal proteins in the 18 cell lines. We used α-tubulin, which is a main component of microtubules that is expressed in all cells, as an internal standard (Fig. 3D). None of the 19 ribosomal proteins was missing, a finding consistent with the essential roles of ribosomal proteins as demonstrated by mutant analysis (see “Discussion”). Most of the ribosomal proteins showed high, uniform band intensities among the cell lines (for instance, Figs. 2 and 3C)—a finding that agrees with the commonly held opinion of ubiquitous, abundant ribosomal proteins. However, the expression levels of ribosomal proteins S11 (Fig. 3A) and S30 (Fig. 3B) obviously differed among these cell lines. More specifically, the expression of S11 in HuO9 osteo-

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![Figure 1](image-url)

**Fig. 1.** Reactivity of antibodies to proteins in the ribosome. Immunoblotting of rat liver ribosomes (10 µg/lane) by using antibodies against 26 ribosomal proteins was performed as described in “Materials and Methods.” At the top of each panel, the target protein of the antibody is indicated. CBB, blot stained with Coomassie Brilliant Blue. The migration positions of the protein markers (Daichi Pure Chemicals, Tokyo) are shown in the far left panel.
sarcoma cells (Fig. 3A, lane 7) was lower than that in RPMI1788 (Fig. 3A, lane 4), whereas the amounts of S14, L13a, and S4X were equal in these two cell lines (lanes 7 and 4, Figs. 2 and 3C). Leukemic blast cells have been reported to have independent uncoordinated mRNA levels of S11 and S14. These findings suggest that the expression of ribosomal proteins is distinctly regulated in various cancers.

Because the samples here were cells lysed with Laemmli sample buffer, ribosomal proteins in all cellular compartments were solubilized. To examine whether the observed differences in the expression of ribosomal proteins affect the amount and composition of the ribosome involved in protein synthesis, the polysome fractions from ten cell lines were isolated and analyzed by immunoblotting. Although HuO9 weakly expressed S11 (as described in the preceding paragraph), the concentration of polysomes in this cell line was not lower than that of other cell lines (Table II). This result suggests that the low expression of S11 did not compromise the biogenesis of ribosomes involved in protein synthesis. Further, the amounts of S11 and S30 in the polysomes were equal among the ten cell lines (Fig. 4) like S4X, which is uniformly expressed among the cell lines (Fig. 3C). This result suggests that the stoichiometry of the ribosomal proteins in these ribosomes is identical. Therefore, the differential expression of S11 and S30 does not seem to affect ribosome complexes; rather, this difference appears to be related to the role of these proteins in an extraribosomal localization.

DISCUSSION

A deficiency in the gene encoding a ribosomal protein undermines cell viability. This view is supported by a growing body of evidence obtained by using eukaryotic model organisms. For example, analysis of yeast mutants has shown that 30 of 32 ribosomal proteins play essential roles in cell growth (Briones et al. and references cited therein). Even a reduced dose of these proteins impairs the
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viability of a living organism. Haploinsufficiency of more than ten Drosophila ribosomal proteins (the Minute mutants) leads to malformation of various tissues.28) In contrast, hemizygotes of ribosomal protein S6 showed overgrowth of the lymph glands (the hematopoietic organs) in the fly,29, 30) demonstrating that reduced expression of ribosomal proteins can promote cell growth in vivo. To our knowledge, in vivo manipulation of the expression of mammal ribosomal proteins has not been reported. Here, we have prepared anti-human ribosomal protein antibodies to detect ribosomal proteins with high sensitivity and specificity. Specificity of these antibodies was also confirmed by direct binding of every antibody to its antigenic protein fused with glutathione-S-transferase, but not to glutathione-S-transferase itself (our unpublished data). Because the primary structures of ribosomal proteins are well conserved in eukaryotes,18) the antibodies likely will cross-react with ribosomal proteins from other, non-human eukaryotic species. Therefore, our antibodies may be widely applicable to studying the biology and genetics of ribosomal proteins in model animals and organisms.

Table II. Amounts of Polysome in Human Cell Lines

| Cell line       | Polysome (µg/10⁶ cells) |
|-----------------|-------------------------|
| YMB-1           | 5.43                    |
| RPMI1788        | 1.08                    |
| HuO-3N1         | 4.17                    |
| HuO9            | 4.63                    |
| NEC8            | 19.3                    |
| MRK-nu-1        | 21.4                    |
| A549            | 6.45                    |
| Caki-1          | 7.62                    |
| LoVo            | 9.39                    |
| NH-6            | 7.11                    |

Fig. 3. Heterogeneous expression of ribosomal proteins S11 and S30 in cancer cell lines. The lysates of 18 human cell lines were subjected to immunoblotting using antibodies against S11 (panel A), S30 (panel B), S4X (panel C), and α-tubulin (panel D). Proteins from about 1×10⁶ cells were loaded into each well of the gels. Lane 1, YMB-1; 2, YMB-1-E; 3, LU99; 4, RPMI1788; 5, NH-12; 6, HuO-3N1; 7, HuO9; 8, NEC8; 9, MRK-nu-1; 10, ME-180; 11, A549; 12, SBC-3; 13, C32TG; 14, NY; 15, PA-1; 16, Caki-1; 17, LoVo; 18, NH-6.

Fig. 4. The proteins in the ribosomes of cancer cell lines. Polysome fractions were isolated from the various cell lines and subjected to immunoblotting with antibodies against S11 (panel A), S30 (panel B), and S4X (panel C). We loaded 9 µg of polysomes into each well. Lane 1, YMB-1; 2, RPMI1788; 3, HuO-3N1; 4, HuO9; 5, NEC8; 6, MRK-nu-1; 7, A549; 8, Caki-1; 9, LoVo, 10, NH-6.
The papers described in the introduction and our data suggest abnormal expression of ribosomal proteins in mammalian tumor cells. How can a change in the expression of ribosomal proteins be connected with tumor progression and development? Only a few plausible links at a molecular level have been described. The ribosomal protein L18 is an inhibitor of double-stranded RNA-activated protein kinase (PKR). Because an active form of PKR autophosphorylates, phosphorylates eukaryotic translation initiation factor-2, and eventually inhibits protein synthesis, up-regulation of PKR activity seems to suppress translation of initiation factor-2, and eventually inhibits protein synthesis. Therefore, overexpression of L18 has been implicated in the unregulated growth of colon carcinoma cells through the protein’s inhibition of PKR. Another indicator of the connection between ribosomal proteins and tumorigenesis is the association of ribosomal L5 with the tumor suppressor p53, mdm-2, and protein kinase CK2 in the cell.

In the present study, we identified heterogeneous expression of ribosomal S11 and S30 by using our antibodies (Fig. 3). Because neither the biogenesis of the poly-some (Table II) nor the protein makeup of the ribosomes (Fig. 4) differed as a result of the heterogeneity, the heterogeneity may be related to extraribosomal functions of S11 and S30. No extraribosomal function of S11 has been established as yet, and HuO9 cells with their low endogenous S11 will be useful for pursuing this question. One approach to elucidating the function of proteins is the production of null mutants. However, because ribosomal proteins are essential for cell growth, S11-null mutants likely are lethal. Manipulation of S11 expression by using the HuO9 cell line, such as overexpression of S11 in stable transfectants, may reveal the role of S11 in the cell. Dramatic but controlled change in the expression level of ribosomal protein S3a in culture cells has revealed the protein’s pro-apoptotic nature. Moreover, the unbalanced levels of S11 and L13a gene products in HuO9 cells are obvious (Fig. 3A and Fig. 2B, respectively). Coordinated regulation of the transcription of the genes for S11 and L13a has been proposed because they are located close to each other on chromosome 19 and have similar promoter regions. Analysis of the regulation of these two genes in the HuO9 cell line may reveal a new mechanism underlying the aberrant expression of ribosomal proteins.

The heterogeneous expression of S30 is interesting because the gene encoding this protein is a putative tumor suppressor gene, the fau gene; the initial gene product is a ubiquitin-like protein that is fused with S30. The fau gene is the cellular homolog of the fox sequence in the Finkel-Biskis-Reilly murine sarcoma virus; this virus contains the inverse of the mouse fau cDNA sequence. Expression of fox as the antisense of the fau gene increases the transforming capacity of the virus, probably by inactivating expression of the fau gene product. In addition, low expression of the Fau protein may sensitize cells to arsenite-associated genotoxicity and carcinogenesis. Further, the Fau protein is an immune suppressor. The signaling pathways in these various phenomena remain unclear. Further analysis of cancer cells with our anti-S30 antibody may uncover a connection between S30 and carcinogenesis. Although the exact biological significance of the heterogeneous expression of S11 and S30 is unclear at this stage, it is considered that the expression level of these ribosomal proteins is intimately linked to a specific intracellular environment. Actually, S11 has been found to be specifically down-regulated in apoptosis of human breast carcinoma cell line, MCF7, induced by staurosporine (our unpublished data). The relationship between ribosomal proteins and apoptosis is under investigation in our laboratory.

Although our antibodies will be used for basic research on translational machinery, they also are potentially powerful tools for medical and clinical applications, for example, for “ribosomopathy” and “ribosomics” (genomics, transcriptomics, and proteomics of the ribosome). The evaluation of cancer cell lines in the present report suggests that our antibodies are applicable to analyses (e.g., immunohistochemistry) of tissue samples from patients, and the resulting information will complement message-level data. Further, our antibodies will make it possible to screen ribosomal proteins as a new marker, promoter, or suppressor of malignant neoplasms and other diseases. This process will improve our understanding of extraribosomal functions.

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REFERENCES

1) Nomura, M., Tissieres, A. and Lengyel, P. “Ribosomes” (1974). Cold Spring Harbor Laboratory Press, New York.
2) Draphtchinskaia, N., Gustavsson, P., Andersson, B., Pettersson, M., Willig, T.-N., Dianzani, I., Ball, S., Tchernia, G., Klar, J., Matsson, H., Tentler, D., Mohandas, N., Carlsson, B. and Dahl, N. The gene encoding ribosomal
protein S19 is mutated in Diamond-Blackfan anaemia. *Nat. Genet.*, **21**, 169–175 (1999).

3) Eberhart, D. E., Malter, H. E., Feng, Y. and Warren, S. T. The fragile X mental retardation protein is a ribonucleoprotein containing both nuclear localization and nuclear export signals. *Hum. Mol. Genet.*, **5**, 1083–1091 (1996).

4) Lazzotto, L. and Karadimitris, A. Dyskeratosis and ribosomal rebellion. *Nat. Genet.*, **19**, 6–7 (1998).

5) Wool, I. G. Extraribosomal functions of ribosomal proteins. *Trends Biochim. Sci.*, **21**, 164–165 (1996).

6) Neumann, F., Hemmerich, P., von Mikecz, A., Peter, H.-H. and Krawinkel, U. Human ribosomal protein L7 inhibits cell-free translation in reticulocyte lysates and affects the expression of nuclear proteins upon stable transfection into Jurkat T-lymphoma cells. *Nucleic Acids Res.*, **23**, 195–202 (1995).

7) Neumann, F. and Krawinkel, U. Constitutive expression of human ribosomal protein L7 arrests the cell cycle in G1 and induces apoptosis in Jurkat T-lymphoma cells. *Exp. Cell Res.*, **230**, 252–261 (1997).

8) Ito, Y. Purification and partial identification of bone-inducing protein from a murine osteosarcoma. *Biochem. J.*, **284**, 847–854 (1992).

9) Liu, S., Smith, S. E., Julian, J., Rohde, L. H., Karin, N. J. and Carson, D. D. cDNA cloning and expression of HIP, a novel cell surface heparan sulfate/heparin-binding protein of human uterine epithelial cells and cell lines. *J. Biol. Chem.*, **271**, 11817–11823 (1996).

10) Nishiura, H., Shibuya, Y., Matsubara, S., Tanase, S., Kambara, T. and Yamamoto, T. Monocyte chemotactic factor in rheumatoid arthritis synovial tissue. Probably a cross-linked derivative of S19 ribosomal protein. *J. Biol. Chem.*, **271**, 878–882 (1996).

11) Dever, T. E. Translation initiation: adept at adapting. *Trends Biochim. Sci.*, **24**, 398–403 (1999).

12) Clemens, M. J. and Bommer, U.-A. Translational control: the cancer connection. *Int. J. Biochem. Cell Biol.*, **31**, 1–23 (1999).

13) Curcic, D., Gliticic, M., Larson, D. E. and Sells, B. H. GA-binding protein is involved in altered expression of ribosomal protein L32 gene. *J. Cell. Biochem.*, **65**, 287–307 (1997).

14) Barnard, G. F., Staniunas, R. J., Mori, M., Puder, M., Jessup, M. J., Steele, G. D., Jr. and Chen, L. B. Gastric and hepatocellular carcinomas do not overexpress the same ribosomal protein L32 gene. *Cancer Res.*, **53**, 4048–4052 (1993).

15) Starkey, C. R. and Levy, L. S. Identification of differentially expressed genes in T-lymphoid malignancies in an animal model system. *Int. J. Cancer*, **62**, 325–331 (1995).

16) Mager, W. H. Control of ribosomal protein gene expression. *Biochim. Biophys. Acta*, **949**, 1–15 (1988).

17) Warner, J. R. The economics of ribosome biosynthesis in yeast. *Trends Biochim. Sci.*, **24**, 437–440 (1999).

18) Wool, I. G., Chan, Y. L. and Gluck, A. Structure and evolution of mammalian ribosomal proteins. *Biochem. Cell Biol.*, **73**, 933–947 (1995).

19) Maloy, W. L., Coligan, J. E. and Paterson, Y. Production of antipeptide antisera. In “Current Protocols on Immunology,” ed. J. E. Coligan, A. M. Kruijsbeek, D. H. Margulies, E. M. Shevach and W. Strober, pp. 9.4.1–9.4.11 (1991). John Wiley & Sons, New York.

20) Bommer, U. A., Burkhardt, N., Junemann, R., Spahn, C. M. T., Triana-Alonso, F. I. and Nierhaus, K. H. Ribosomes and polysomes. In “Subcellular Fractionation. A Practical Approach,” ed. J. M. Graham and D. Rickwood, pp. 271–301 (1997), Oxford University Press, New York.

21) Sabatini, D. D. Subcellular fractionation of rough microsomes. In “Cells: A Laboratory Manual,” ed. D. L. Spector, R. D. Goldman and L. A. Leinwand, pp. 37.31–37.22 (1998). Cold Spring Harbor Laboratory Press, New York.

22) Wettstein, F. O., Staehelin, T. and Noll, H. Ribosomal aggregate engaged in protein synthesis: characterization of the organelle. *Nature*, **197**, 430–435 (1963).

23) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685 (1970).

24) Sato, T., Aoki, C., Ishihara, G. and Nadano, D. Systematic preparation of antibodies against all human ribosomal proteins. In “The Proceedings of the Ribosome: Structure, Function, Antibiotics and Cellular Interactions,” ed. R. A. Garrett, S. R. Douthwaite, A. Liljas, A. T. Matheson, P. B. Moore and H. F. Noller, p. 70 (1999). Helsinger.

25) Huang, C. C. and Moore, G. E. Chromosomes of 14 hematopoietic cell lines derived from peripheral blood of persons with and without chromosome anomalies. *J. Natl. Cancer Inst.*, **43**, 1119–1128 (1969).

26) Ferrari, S., Manfredini, R., Tagliafico, E., Rossi, E., Donelli, A., Torelli, G. and Torelli, U. Noncoordinated expression of S6, S11, and S14 ribosomal proteins in leukemic blast cells. *Cancer Res.*, **50**, 5825–5828 (1990).

27) Briones, E., Briones, C., Remacha, M. and Ballesta, J. P. G. The GTPase center protein L12 is required for correct ribosomal stalk assembly but not for *Saccharomyces cerevisiae* viability. *J. Biol. Chem.*, **273**, 31956–31961 (1998).

28) Lambertsson, A. The *Minute* genes in *Drosophila* and their molecular functions. *Adv. Genet.*, **38**, 69–134 (1998).

29) Watson, K. B., Konrad, K. D., Woods, D. F. and Bryant, P. *J. Drosophila* homolog of the human S6 ribosomal protein is required for tumor suppression in the hematopoietic system. *Proc. Natl. Acad. Sci. USA*, **89**, 11302–11306 (1992).

30) Stewart, M. J. and Denell, R. Mutations in the *Drosophila* gene encoding ribosomal protein S6 cause tissue overgrowth. *Mol. Cell. Biol.*, **13**, 2524–2535 (1993).

31) Kumar, K. U., Srivastava, S. P. and Kaufman, R. J. Double-stranded RNA-activated protein kinase (PKR) is negatively regulated by 60S ribosomal subunit protein L18. *Mol. Cell. Biol.*, **19**, 1116–1125 (1999).

32) Proud, C. G. PKR: a new name and new roles. *Trends Biochim. Sci.*, **20**, 241–246 (1995).

33) Marechal, V., Elenbaas, B., Piette, J., Nicolas, J.-C. and...
Levine, A. J. The ribosomal L5 protein is associated with mdm-2 and mdm-2-p53 complexes. *Mol. Cell. Biol.*, 14, 7414–7420 (1994).

34) Guerra, B. and Issinger, O.-G. p53 and the ribosomal protein L5 participate in high molecular mass complex formation with protein kinase CK2 in murine teratocarcinoma cell line F9 after serum stimulation and cisplatin treatment. *FEBS Lett.*, 434, 115–120 (1998).

35) Naora, H., Takai, I., Adachi, M. and Naora, H. Altered cellular responses by varying expression of a ribosomal protein gene: sequential coordination of enhancement and suppression of ribosomal protein S3a gene expression induces apoptosis. *J. Cell Biol.*, 141, 741–753 (1998).

36) Higa, S., Yoshihama, M., Tanaka, T. and Kenmochi, N. Gene organization and sequence of the region containing the ribosomal protein genes RPL13A and RPS11 in the human genome and conserved features in the mouse genome. *Gene*, 240, 371–377 (1999).

37) Michiels, L., van der Rauwelaert, E., van Hasselt, F., Kas, K. and Mierregaert, J. *fau* cDNA encodes a ubiquitin-like-S30 fusion protein and is expressed as an antisense sequence in the Finkel-Biskis-Reilly murine sarcoma virus. *Oncogene*, 8, 2537–2546 (1993).

38) Rossman, T. G. and Wang, Z. Expression cloning for arsinite-resistance resulted in isolation of tumor-suppressor *fau* cDNA: possible involvement of the ubiquitin system in arsenic carcinogenesis. *Carcinogenesis*, 20, 311–316 (1999).

39) Nakamura, M., Xavier, R. M., Tsunematsu, T. and Tanigawa, Y. Molecular cloning and characterization of a cDNA encoding monoclonal nonspecific factor. *Proc. Natl. Acad. Sci. USA*, 92, 3463–3467 (1995).