The β4 Integrin Interactor p27<sub>BBP/eIF6</sub> Is an Essential Nuclear Matrix Protein Involved in 60S Ribosomal Subunit Assembly

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Abstract. p27<sub>BBP/eIF6</sub> is an evolutionarily conserved protein that was originally identified as p27<sub>BBP</sub>, an interactor of the cytoplasmic domain of integrin β4 and, independently, as the putative translation initiation factor eIF6. To establish the in vivo function of p27<sub>BBP/eIF6</sub>, its topographical distribution was investigated in mammalian cells and the effects of disrupting the corresponding gene was studied in the budding yeast, Saccharomyces cerevisiae. In epithelial cells containing β4 integrin, p27<sub>BBP/eIF6</sub> is present in the cytoplasm and enriched at hemidesmosomes with a pattern similar to that of β4 integrin. Surprisingly, in the absence and in the presence of the β4 integrin subunit, p27<sub>BBP/eIF6</sub> is in the nucleolus and associated with the nuclear matrix. Deletion of the IIH S. cerevisiae gene, encoding the yeast p27<sub>BBP/eIF6</sub> homologue, is lethal, and deletion of the corresponding gene product is associated with a dramatic decrease of the level of free ribosomal 60S subunit. Furthermore, human p27<sub>BBP/eIF6</sub> can rescue the lethal effect of the iihΔ yeast mutation. The data obtained in vivo suggest an evolutionarily conserved function of p27<sub>BBP/eIF6</sub> in ribosome biogenesis or assembly rather than in translation. A further function related to the β4 integrin subunit may have evolved specifically in higher eukaryotic cells.

Key words: epithelial cells • yeast • nucleolus • hemidesmosomes • intermediate filaments
cytodomain of β4 exerts its function through the interaction with cytoplasmic molecules led us to search for protein interactors of the β4 cytodomain. Through an extensive two hybrid analysis, a previously unknown peptide named p27BBP (BBP for beta4 binding protein) that binds the β4 cytodomain was discovered. p27BBP directly binds, in vitro and in vivo, a 300-aa amino acid long stretch of β4 integrin cytodomain, a region required for targeting β4 to the hemidesmosomes and to the intermediate filament cytoskeleton as determined by genetic studies. In addition, p27BBP was found to be present at high levels in the submembrane region of epithelial cells containing β4. Finally, the biochemical association of p27BBP with keratin intermediate filaments, suggested that p27BBP might be the molecular link between β4 and the cytoskeleton (Biffo et al., 1997). The precise ultrastructural localization of p27BBP, in vivo hemidesmosomes was not yet defined.

The finding that p27BBP homologues exist both in yeast and Drosophila, in which p4 integrin homologues are absent (Biffo et al., 1997) suggested that p27BBP might also have a β4-independent function. Consistently, the cloning of a human cDNA encoding a protein named elF6 (identical to p27BBP) was almost concomitantly obtained by Si et al. (1997). The biological assay used to clone elF6 was based on its in vitro ability to inhibit the association between the 40S and the 60S ribosomal subunits, and was not related to integrin function. On the basis of its in vitro determined properties, it was suggested that elF6 might act as a translation initiation factor. The cloning and sequencing of elF6 unequivocally indicate that elF6 and p27BBP are the same protein (Biffo et al., 1997; Si et al., 1997). Much more recently, elF6/p27BBP has also been identified by another group as a gene induced in mast cells by allergic reaction (Cho et al., 1998). To acknowledge the independent identification of p27BBP and elF6, the protein will be denoted as p27BBPeIF6 throughout this work.

Both studies left a set of unresolved questions: (a) Which is the precise cellular localization of p27BBPeIF6 and is it modulated by the presence of β4 integrin?; (b) Is the association of p27BBPeIF6 with the intermediate filament cytoskeleton a unique feature of cells that contain β4?; (c) Is p27BBPeIF6 present in hemidesmosomes?; and (d) Which is the general, evolutionarily conserved function of p27BBPeIF6? To address these questions, we used integrated approaches. First, the fine localization of p27BBPeIF6 was studied in cell lines, either containing β4 integrin or not. Our studies show that p27BBPeIF6 is a nuclear matrix-associated protein present in the nucleolus of all cells analyzed and enriched at the basal membrane of β4 expressing epithelial cells. Second, the function of p27BBPeIF6 was addressed in Saccharomyces cerevisiae by constructing and characterizing a null mutant. The yeast p27BBPeIF6 homologue is essential for cell viability and its depletion results in an abnormal ribosomal profile, with a dramatic reduction of the levels of free 60S ribosomal subunits. Taken together these data indicate that the conserved role of p27BBPeIF6 is linked to 60S ribosome subunit metabolism, and that this process may be linked to the nuclear matrix. In higher organisms, novel functions of p27BBPeIF6 may have appeared that link this molecule to epithelial adhesion.

Materials and Methods

Antibodies and Cell Lines

The rabbit polyclonal antiserum against the COOH-terminal peptide of p27BBPeIF6 (NH2-CTIA TSM R DSLI DSLT-COOH) was tested for its specificity by Western blotting and immunoprecipitation both on the recombinant protein and on cellular lysates (Biffo et al., 1997). Integrin β4 was detected with the rat mAb b3E1 (10 μg/ml; Chemicon International, Inc.), or with the mouse mAb b A A 3 (Kajiiji et al., 1989) at 10 μg/ml (gift of Vito Quaranta, Scripps Research Institute, La Jolla, CA). The human autoantibodies against fibrillarin (Ochs and Smetana, 1991) were a generous gift of R. Shibb Orchs (Scripps Research Institute) and were diluted 1:300. Cytokeratins were detected either with mouse monoclonal anticytokeratin 8/18, IgG2a (Dianostika) at 1:200, or with mouse monoclonal anticytokeratin 7/17 IgG1, according to the manufacturer’s protocol (C46; Euro-Diagnostica). Secondary antibodies were rhodamine- and fluorescein-tagged swine anti–rabbit IgGs (1:50; DAKO Corp.), rhodamine-tagged goat anti–human IgGs (10 μg/ml; Chemicon International, Inc.), rhodamine-tagged goat anti–mouse IgGs (7.5 μg/ml; Molecular Probes Europe) and fluorescein-tagged goat anti–mouse IgGs (1:50; A nibodies Inc.). In control experiments, primary antibodies were replaced by preimmune sera or irrelevant mAbs. In addition, the p27BBPeIF6 antiserum was preadsorbed with the peptide used for its generation (1 μM, overnight, 4°C), or with the bacterially produced human recombinant full length protein (at 10 μg/ml, 2 h at 4°C) purified by ion exchange chromatography.

The cell lines and primary cells used in this study, as well as the conditions for their propagation, are described in the American Type Culture Collection cell line catalogue or in the references between parentheses. They are as follows: mouse NIH/3T3 fibroblasts, human A 431 epidermoid carcinoma, human HeLa epithelioid carcinoma, human pancreatic carcinoma, human glioma cells SU6606 (gift of Robert Ochs, Scripps Research Institute) and were diluted 1:300. Cytokeratins were detected either with mouse monoclonal anticytokeratin 8/18, IgG2a (Dianostika) at 1:200, or with mouse monoclonal anticytokeratin 7/17 IgG1 (gift of V. Gianiotti (Memorial Sloan-Kettering Cancer Center, New York) and has been described in Spinardi et al. (1993). Mouse resting splenocytes, human fibroblasts from the umbilical cord, and Xenopus oocytes were gifts of A. Cabibbo, E. Bianchi, and E. Pannese (all at DIBIT, Milano, Italy) and obtained by standard procedures.

Actinomycin Treatment

Cells were treated with actinomycin D (Boehringer Mannheim G mbH) at the final concentration of 5 μg/ml for 1, 4, and 12 h, washed, and fixed as described. In some experiments cells were allowed to recover after treatment by switching them to their normal medium.

Electron Microscopy on Human Aminion

Human fresh amniotic membrane (obtained immediately upon delivery from the Department of Obstetrics, San Raffaele Hospital, Milano, Italy) was dissected and pieces of tissue were fixed with 4% paraformaldehyde and 0.25% glutaraldehyde in 125 mM sodium phosphate buffer, pH 7.4, for 45 min at 4°C. The samples were infiltrated with polyvinylpyrrolidone and frozen in a 3:1 (vol/vol) mixture of propane and isopentane cooled with liquid nitrogen. Ultrathin cryosections (50–100 nm thick) were obtained using an LIII autocut ultramicrotome equipped with a Reichert FC4 cryosectioning apparatus. The cryosections were processed as described in Villa et al. (1993) using the rabbit anti-p27BBPeIF6 antiserum and the mouse monoclonal anticytokeratin 7/17 IgG1. Cryosections were examined in an electron microscope (Hitachi H-7000).

Western Blot Analysis

A II samples were denatured before loading in Laemmli buffer (Laemmli, 1970) and run on denaturing 12% SDS-acrylamide gel, transferred to Immobilon P membranes (Millipore Corp.), and blotted with the rabbit...
p27BPRF1 antiserum at 1:10,000 dilution as previously described (Bilbao et al., 1997). In the control of the fractionation experiment, a mouse monoclonal anticytokeratin B/35 (G2a) at 1:200 was used. Detection was always performed by the commercially available chemiluminescence detection system (ECL) technique (Nycomed A. mersham).

**Extraction of Nuclear Matrix and Ribosomal Proteins**

Intermediate filaments/nuclear matrix filaments fractions were prepared exactly according to He et al. (1990). Briefly, all the soluble proteins, the nonintermediate filament cytoskeleton, DNA associated proteins, and proteins loosely associated with the nuclear matrix proteins were removed by sequential washes in buffers (Triton X-100, 250 mM ammonium sulfate, D Nase I, and 2 M NaCl). At the end of this procedure, a cytoplasmic and nuclear intermediate filament network containing keratin, lamin, and intermediate filament-associated proteins was left. The efficiency of the extraction was routinely controlled by DNA staining, or by immunostaining for keratins.

Preparation of ribosomes was performed through established procedures and exactly as described in M adjar (1994). 80A4 clone A cell line monolayer was washed and scraped with cold PBS. The pellet was resuspended in cold buffer A (0.25 M sucrose, 25 mM KCl, 5 mM MgCl2, 50 mM Tris-HCl, pH 7.4), stirred slowly with a vortex, while adding NP-40 to a final concentration of 0.7%, and kept on ice for 10 min. The suspension was centrifuged at 750 rpm, 10 min at 4°C. The low-speed supernatant was added to 0.32 vol of Tris-HCl, pH 7.4, and centrifuged at low speed (600 g for 5 min). The pellet was included in diethylene glycol distearate as described by Nickerson et al. (1994). Resinless sections were examined in a H-itachi H-7000 electron microscope.

**Database Searches**

Homology searches were performed with the Blast programs available through http://www.ncbi.nlm.nih.gov, or by the alerting system of EMBL (http://www.bork.embl-heidelberg.de/AIerling). The accession number of the sequences retrieved are: Homo sapiens, Y13435; S. cerevisiae, Z99919; Caenorhabditis elegans, Z97907; A. rabidopsis thaliana, A003000; M. ethanococcus jannaschii, U76463; S. acidocaldarius, P38619; M. ethanolubacte rium thermoautotrophicum, A000920; P. bokmioshi, A8009481; and A. rhaeaceobius fulgidus, A000961. The alignment was created using the CLUSTAL W algorithm (Thompson et al., 1994). The phylogram of the aligned proteins were produced with the GROWTREE program in GCG with the Jukes-Cantor distance matrix and neighbor-joining method.

**Yeast Strains and Media**

All strains used are derivatives of W303 (MATa, ade2-1, trpl-1, leu2-3, 112, his3-11,15, ura3, can-100). Cells were grown in YEP medium (1% yeast extract, 2% bactopeptone, 50 µg/liter adenine) supplemented with either 2% glucose (YEPD) or 2% raffinose and 1% galactose (YEPG). Yeast strains and media were obtained from the American Type Culture Collection or from gift of V. Zappavigna, DIBIT. The oligonucleotides YSTAG (5'- AATCAACAATAATGTACCCATACGAGCTTCCA -3') and oSP46 (5'- TTACAAGAAGCAATACGACAG -3') were synthesized at the Institute for Biotechnology Research (IBR). Transformants carrying the kanMX4 cassette were selected on YEPD plates containing 400 µg/ml G418 (US Biological).

**Plasmid Construction and Genetic Manipulations of Yeast Strains**

Standard techniques were used for genetic crosses (Rose et al., 1990) and DNA manipulations (Sambrook et al., 1989). The yeast integrin interacting homologue (IIH1) gene was cloned by PCR using as a template the genomic DNA of strain W303 and oligonucleotides oSP44 (5'- CGA-GAAATGTCGGAGAAGCGGAC -3') and oSP45 (5'- GTAAGGTGCA- AAGTACAACTGTAAACAGACTTGA -3') and oSP46 (5'- TTACAAGAAGCAATACGACAG -3') as primers. The amplification product contained the entire open reading frame of human IIH1 (200 bp). The PCR product was ligated into the HindIII and XhoI sites of YEG1 as a template and sequenced. The plasmid pSP43 was sequenced by the dideoxy chain termination method (Sanger et al., 1980). The plasmid pIIH1 was sequenced in frame with a 10–amino acid HA tag in the N-terminus (the original gift of V. Zappavigna, DIBIT). The oligonucleotides YSTAG (5'- AATCAACAATAATGTACCCATACGAGCTTCCA -3') and oSP46 (5'- TTACAAGAAGCAATACGACAG -3') were synthesized at the Institute for Biotechnology Research (IBR).

The plasmid was transformed into W303 by the lithium acetate method (Gietz and Sugino, 1988) that carried the BamHI–EcoRI GAL 1–10 promoter fragment (pC21). To construct the GAL–IIH1 fusion (pSP43), the III 1 X 1 balal/Sigl fragment was cloned in XbaI (SalI) of a Y plaq21–derived plasmid (Gietz and Sugino, 1988) that carried the BamHI–EcoRI GAL 1–10 promoter fragment (c21). To construct the GAL–p27BPRF1 fusion (pSP45), a PCR fragment containing a hemagglutinin (HA)–tagged version of p27BPRF1 was amplified using a PCR as a template the p565-p27 expression plasmid in which the entire open reading frame of human p27BPRF1 gene was cloned in frame in a 10–amino acid HA tag in the N-terminus (the original vector is described in Green et al., 1988, the HA-modified vector was a gift of V. Zappavigna, DIBIT). The oligonucleotides YSTAG (5'- CGG- AATTCACAAATATAGTACCACTAGGACCCACATCCGTTAC- CTGAGTCTG-3') and oSP46 (5'-GCTCCATCTCCCTGCTGTCTTATAGTAAATTACGAGCAGCCAGGCTGTTAC-3') were synthesized at the Institute for Biotechnology Research (IBR). The plasmid pIIH1 was sequenced in frame with a 10–amino acid HA tag in the N-terminus (the original vector is described in Green et al., 1988, the HA-modified vector was a gift of V. Zappavigna, DIBIT). The oligonucleotides YSTAG (5'- CGG- AATTCACAAATATAGTACCACTAGGACCCACATCCGTTAC- CTGAGTCTG-3') and oSP46 (5'-GCTCCATCTCCCTGCTGTCTTATAGTAAATTACGAGCAGCCAGGCTGTTAC-3') were synthesized at the Institute for Biotechnology Research (IBR).
were used as primers. The obtained PCR product was cut with E.colI and cloned in the EcoRI site of pC2139. Both GAL-IHH1 and GAL-H-sp27BBP/eIF6 fusions were integrated at the ura3 locus of ySP478 by cutting pSP43 and pSP40 with A pal before transformation. As a result, strains ySP650 (GAL-IHH1 single copy), ySP653 (GAL-H-sp27BBP/eIF6 single copy), and ySP652 (GAL-H-sp27BBP/eIF6 multiple copies), respectively were generated. The copy number of the integrated plasmids was checked by Southern analysis. Strain ySP661 (MATa, iih1::kanMX4, and ura3::URA3::GAL-IHH1) was obtained after sporulation and tetrad dissection of ySP650, whereas ySP664 (MATa, iih1::kanMX4, and ura3::URA3::GAL-H-sp27BBP/eIF6) was obtained after sporulation and tetrad dissection of ySP653.

**Polysomal and Western Blot Analysis of Ribosomal Fractions**

Polyribosome preparation and polysome analysis were done exactly according to Foiani et al. (1991). Briefly, cell cultures of W303 (wt), ySP661 (iih1Δ, GAL-IHH1), and ySP664 (iih1Δ, GAL-H-sp27BBP/eIF6) were grown in YEPDG medium and shifted to YEPD at time 0 to repress the GAL promoter. Yeast extracts were prepared from 300 ml of cell culture at OD = 0.5–1 (Foiani et al., 1991), layered on a 7–47% sucrose gradient in 50 mM Tris-acetate, pH 7.0, 50 mM NH4Cl, 12 mM MgCl2, and 1 mM dithiothreitol and centrifuged at 4°C in a SW41 Beckman rotor for 2 h at 39,000 rpm. Gradient analysis was performed with a gradient collector with continuous monitoring at A260.

For protein analysis, the collected fractions were precipitated with TCA to a final concentration of 10% and left on ice for 30 min. Fractions were centrifuged at 15,000 g, for 15 min at 4°C, and resuspended in Laemmli buffer. Equal amounts of extracts were run on denaturing 12% acrylamide gels and blotted as described above.

**Results**

**p27BBP/eIF6 Is Present in All Cell Lines, at Early Developmental Stages and Is Associated with the Cytoskeleton**

It was previously observed that p27BBP/eIF6 mRNA was highly expressed during mouse embryonic development and that highly conserved homologues were present in the unicellular organism S. cerevisiae (Biffo et al., 1997). These data suggested that p27BBP/eIF6 might have a general role in cellular processes that is not limited to epithelial cells expressing β4 integrin only. To test this hypothesis, the expression of p27BBP/eIF6 was first measured by Western blot analysis with a polyclonal antiserum directed against the COOH terminus of p27BBP/eIF6 on total protein lysates from immortalized cell lines of various origin (see Materials and Methods for original references). So far, p27BBP/eIF6 has been detected in all cell lines analyzed. Fig. 1 (left) shows the levels of p27BP/eIF6 in the immortalized cell lines NIH/3T3 (nontransformed mouse fibroblasts), Jurkat (human T cells), SK-N-MC (human neuroblastoma), A431, HeLa, HaCaT, and FG2 (transformed human epithelial cell lines), and Rin2A (human insulinoma). Constitutive expression of p27BBP/eIF6 was also detected in two out of two primary cultures tested, respectively, human primary fibroblasts, and mouse resting splenocytes.

It was previously observed that p27BBP/eIF6 mRNA was abundant during embryonic development and declined in the adult, where it was mainly retained in epithelial tissues (Biffo et al., 1997). To test the hypothesis that p27BBP/eIF6 protein may be present already at early phases of development, its onset was studied in embryos. The protein was found to be expressed from the earliest developmental stage and later on. Fig. 1 (right) shows p27BP/eIF6 in the Xenopus egg, between fertilization and the beginning of segmentation. Comparable results were obtained in mice. As previously observed for p27BP/eIF6 mRNA, in the adult, high levels of p27BP/eIF6 protein were retained mostly in epithelial tissues, and testis (not shown).

In epithelial cells containing β4 integrin, ~50% of p27BP/eIF6 was associated with the intermediate filament cytoskeleton (Biffo et al., 1997). The association of p27BP/eIF6 with the cytoskeleton was analyzed in cell lines not containing β4 integrin. For this purpose, cells were first extracted with detergent containing buffers (Materials and Methods) and the various fractions were analyzed by Western blot. Part of p27BP/eIF6 was always found in the cytoskeletal fraction. However, the extent of the association varied according to the cell line (not shown). Fig. 1 (right) shows the results of the fractionation experiments in Xenopus eggs, where at least half of p27BP/eIF6 was found to be resistant to detergent extraction and associated with the cytoskeleton.

**Nucleolar Localization of p27BBP/eIF6**

The topographical distribution of p27BP/eIF6 was studied in detail by immunofluorescence, immunocytochemistry, and electron microscopy. To summarize our findings, as shown in Figs. 2–4, p27BP/eIF6 was present in the nucleus, with a clear nucleolar pattern. The nucleolar staining of p27BP/eIF6 was present in all the organisms analyzed so far (from worms to humans) and in all cell lines. In addition, in some cell lines containing β4 integrin, p27BP/eIF6 was...
clearly evident in the cytoplasm (see Fig. 5). All the immunoreactivity described is specific, since both the nuclear and the cytoplasmic staining could be routinely abolished by preincubating the antiserum with either the peptide used for immunization or with the recombinant protein (Fig. 2, A and B). In addition, a similar nucleolus-enriched staining pattern could be seen on NIH/3T3 fibroblasts transfected with a HA-tagged version of p27BBP/eIF6, followed by immunofluorescence with a mouse anti-HA mAb (not shown).

The nuclear staining of p27BBP/eIF6 and its dynamic features will be described using the FG2 cell line as a model. In the interphase nucleus, p27BBP/eIF6 was clearly concentrated in the nucleolus (Fig. 2 A). This pattern was similar to the one obtained with an antiserum recognizing the nucleolar protein, fibrillarin (Fig. 2 E). The nuclear staining of p27BBP/eIF6 was always present in the nucleolus. The experiments were performed on the FG2 cell line. Identical results are obtained with all cell lines. FG2 cells were stained with the specific p27BBP/eIF6 antiserum, revealed by immunoperoxidase (A and B) or immunofluorescence (C and D) and with a human antiserum recognizing the nucleolar marker fibrillarin (nucleoantiserum; E and F) followed by immunofluorescence. A strong p27BBP/eIF6 nuclear staining, as well as a weaker cytoplasmic staining are visible in A. The staining was completely eliminated by pre-adsorption of the antiserum with the pure recombinant p27BBP/eIF6 protein. (B) The immunofluorescence pattern of p27BBP/eIF6 in normal untreated cells is shown where one to three intensely labeled dots are present in nuclei (C). By comparison, the pattern of the nucleolar antigen fibrillarin is shown (E); the large labeled cell in the middle is in early prophase. The effects of treatment with actinomycin D for 4 h on the pattern of both p27BBP/eIF6 (D) and fibrillarin (F) are shown. This treatment results in the loss of the nucleolus: note that both p27BBP/eIF6 and fibrillarin largely redistribute within the nucleus. (A–F), 5 μm. Immunoelectron microscopy localization of p27BBP/eIF6 within the nucleolus of epithelial cells (G). Cells were sequentially treated with p27BBP/eIF6 antiserum followed by 5-nm gold-conjugated secondary antibodies. Note the high number of gold particles scattered throughout the nucleolus (n). Bar, 0.1 μm. Proteins from the 804G cell line were separated in the following fractions: soluble, nuclear (also containing highly insoluble intermediate filament-associated proteins), mitochondrial, and ribosomal as described in the Materials and Methods.
colocalization was supported by double immunofluorescence studies with fibrillarin and p27\textsubscript{BBP/eIF6} (not shown).

To establish whether p27\textsubscript{BBP/eIF6} was dynamically associated with the nucleolus, epithelial cells were treated with low doses of actinomycin D and p27\textsubscript{BBP/eIF6} localization was analyzed after 1, 4, and 12 h. This treatment caused the collapse of the nucleolus and the redistribution of nucleolar-associated proteins (Schofer et al., 1996). In actinomycin D–treated cells, both p27\textsubscript{BBP/eIF6} (Fig. 2 D) and the nucleolar antigen, fibrillarin (Fig. 2 F), reversibly weakened their association with the nucleolus and became mostly diffuse in the cell’s nucleus. Importantly, no effect on p27\textsubscript{BBP/eIF6} localization was seen when cells were treated with the protein synthesis inhibitors, cycloheximide and puromycin (not shown). Nucleolar localization of p27\textsubscript{BBP/eIF6} was confirmed by immunoelectron microscopy, using the anti-p27\textsubscript{BBP/eIF6} antiserum, followed by 5-nm gold-labeled secondary antibodies. 5-nm gold beads were strongly concentrated within the nucleolus (Fig. 2 G).

Next, we tested whether p27\textsubscript{BBP/eIF6} was stably associated with ribosomal proteins in the cytoplasm of the 804G-clone A epithelial cell line. For this purpose, ribosomes and ribosomal proteins were separated from all of the following: mitochondria, nuclear matrix/intermediate filaments, and soluble proteins. Afterwards, the different fractions were tested for the presence of p27\textsubscript{BBP/eIF6} by Western blot analysis. As shown in Fig. 2 H, most of the protein was present in the nuclear matrix/intermediate filament cytoskeleton fraction. A faint band was associated with the ribosomal fraction.

\textbf{p27\textsubscript{BBP/eIF6} Redistributes during Mitosis}

The strong nucleolar-associated pattern of p27\textsubscript{BBP/eIF6} was visible in all cell lines during interphase, as well as in various normal and neoplastic tissues (Sanvito, F., manuscript in preparation). Therefore, it was expected that during mitosis, when the nucleolus disappears, the protein would be redistributed. Indeed, during cell division p27\textsubscript{BBP/eIF6} dra-
matically changed its topographical pattern. At prophase, the immunoreactivity tended to become more dispersed at first. Later, it became associated with the periphery of condensed chromosomes (Fig. 3, A and B). At metaphase, p27<sub>BP/eIF6</sub> was enriched in the central mass of chromatin formed by the condensed chromosomes of the metaphasic plate (Fig. 3, C and D) and this pattern was even more noted at anaphase (Fig. 3, E and F). With the onset of telophase and the reappearance of the nuclear organization, p27<sub>BP/eIF6</sub> first scattered and then regained its association with the nucleolus (Fig. 3, G and H). The redistribution of p27<sub>BP/eIF6</sub> during the mitotic phases was not associated with its proteolytic degradation. Furthermore, no obvious physical association of p27<sub>BP/eIF6</sub> with tubulin was observed (not shown). A similar redistribution was observed for some nuclear antigens, chromosome passengers, which redistribute around chromosomes during mitosis (Earnshaw and Bernat, 1991), as well as for some nuclear matrix-associated antigens, whose immunoreactivity become more dispersed during mitosis (Nickerson et al., 1992).

**p27<sub>BP/eIF6</sub> Is Associated with the Nuclear Matrix**

To investigate whether the nucleolar p27<sub>BP/eIF6</sub> was associated with the nuclear matrix, FG2 cells were extracted with a sequential treatment by means of detergents, DNAse, RNase, and high salts (He et al., 1990), and then analyzed by immunofluorescence and electron microscopy. This treatment removed >90% of the proteins, and 95% of the DNA. In addition, the treatment uncovered a nuclear matrix consisting of a nuclear lamina connected to the cytoplasmic intermediate filaments and of an internal meshwork of polymorphic fibers connecting the lamina to masses within the nucleus. In conditions that lead to the complete loss of DNA (Fig. 4 D), the p27<sub>BP/eIF6</sub> staining, associated with the nucleolus was clearly retained (Fig. 4, C–E). Also, the nuclear staining of p27<sub>BP/eIF6</sub> was unaffected after digestion of residual RNA with RNAse (not shown).

To establish whether the residual staining of p27<sub>BP/eIF6</sub> was present in specific structures, extracted cells were examined by immunoelectron microscopy. By this analysis, immunoreactivity of p27<sub>BP/eIF6</sub> was always found to be associated with the residual thick filaments of the nuclear matrix (Fig. 4 F). Together these data show that in the nucleolus and in the nucleus a relevant part of p27<sub>BP/eIF6</sub> is tightly associated with the nuclear matrix.

**Topographical Relationships of p27<sub>BP/eIF6</sub> and β4 at Hemidesmosomes in Epithelial Cells**

In epithelial cells containing the β4 integrin, the pattern of immunoreactivity of p27<sub>BP/eIF6</sub> was slightly different and is briefly described using the epithelial cell line 804G clone A. This cell line contains human β4 integrin, clustered in rosettes of hemidesmosomes (Spinardi et al., 1993). A result, when stained with antibodies against β4, these cells exhibit a typical Swiss cheeselike pattern in which intense β4 staining surrounds cytoplasmic areas devoid of integrin (Fig. 5, A and D). Confocal laser scanning microscopy analysis in the horizontal section (x, y) of p27<sub>BP/eIF6</sub> immunolocalization in these cells clearly showed a cytoplasmic staining partially superimposable to the one for β4 integrin (Fig. 5, A and D; β4; B and C, p27<sub>BP/eIF6</sub>; E, β4–p27<sub>BP/eIF6</sub>). Most importantly, in the vertical (x, z) and horizontal (x, y) sections, both β4 and p27<sub>BP/eIF6</sub> stainings were excluded from the small circular areas forming the holes of the Swiss cheeselike pattern (Fig. 5, A, A’ and D; B’ and C). However, staining with the labeled actin-binding drug, phalloidin, showed that these holes contained other cytoskeletal components such as actin and actin-binding proteins (data not shown; Spinardi, L., manuscript in preparation). These data suggest that in epithelial cells that require β4 to form hemidesmosomes, p27<sub>BP/eIF6</sub> can be specifically recruited in the intermediate filament’s cytoskeleton converging on these adhesion structures.

To extend these observations, the presence of p27<sub>BP/eIF6</sub> was analyzed by immunoelectron microscopy on cryosections of human amnion, a tissue that contains hemidesmosomes clustered at the basal cell surface. Consistent with the pattern observed in the 804G clone A cells, p27<sub>BP/eIF6</sub> was detected at the level of inner plaque of the hemidesmosome, where it seemed associated with a thin filament network (Behzad, 1995) running between the intermediate filaments and the hemidesmosomal dense plaque (5-nm gold beads; Fig. 5, F and H, arrowheads). A specific immunolabeling was also noticed in the cytoplasm associated with filamentous structures (e.g., the area indicated by the arrow in Fig. 5, G and H), and also at the inner face of desmosomes (Fig. 5 J). In agreement with the association with the intermediate filament cytoskeleton, p27<sub>BP/eIF6</sub> immunolocalization was resistant to high salt extraction (not shown). However, the p27<sub>BP/eIF6</sub> positive structures (5-nm gold beads) were within intermediate filament bundles, as shown by a double staining with antikeratin antibodies (15-nm gold beads; Fig. 5, G and H, arrows).

**p27<sub>BP/eIF6</sub> Is Essential for Yeast Cell Viability**

To gain more insights into p27<sub>BP/eIF6</sub> function, several approaches were taken, but our efforts to manipulate the levels of p27<sub>BP/eIF6</sub> in mammalian cell lines were not successful. Briefly, the expression of p27<sub>BP/eIF6</sub> antisense mRNA in NIH/3T3 cells led only to a small decrease of protein levels and established clones could not be derived (Sanvito, F., unpublished observations). Furthermore, transient expression of several mutated constructs in COS cells led in some cases to accumulation of p27<sub>BP/eIF6</sub> either in the nucleus or in the cytoplasm, and was toxic to the cells (Sanvito, F., unpublished observations). These observations, together with the nucleolar localization and the fact that the protein is conserved from yeast to humans (Biffo et al., 1997; Si et al., 1997), might suggest a conserved function for this protein, which should be independent of β4 integrin (S. cerevisiae does not have β4 homologues). The possibility that p27<sub>BP/eIF6</sub> has an ancestral function is further supported by the finding that putative genes encoding peptides homologous to human p27<sub>BP/eIF6</sub> are present in the genome of different Archaea and are also found in plants (Fig. 6).

The analysis of the conserved amino acid sequences does not provide any insight into p27<sub>BP/eIF6</sub> function. However, the fact that S. cerevisiae contains a p27<sub>BP/eIF6</sub> homologue, 80% identical to the human protein, allowed

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Figure 4. p27\textsuperscript{BBP/eIF6} is associated with the nuclear matrix. FG2 cells were stained for p27\textsuperscript{BBP/eIF6} (A, C, and E) and counterstained with Hoechst dye for nuclear DNA (B and D) before (A and B) or after (C, D, and E) extraction of all soluble proteins to reveal the nuclear matrix/intermediate filament cytoskeleton, according to the procedure of He et al. (1990). B (before) and D (after) show the complete loss of nuclear DNA provided by this treatment. In contrast, A (before) and C (after) show that a conspicuous part of p27\textsuperscript{BBP/eIF6} is retained in the nucleolus. E shows a p27\textsuperscript{BBP/eIF6} immunoperoxidase staining of cells, after extraction. Note that although the strongest cytoskeleton associated staining is in the nucleolus (large black dots), a clear residual staining is also visible in the nucleus. (F) To reveal the intimate association of p27\textsuperscript{BBP/eIF6} with the nuclear matrix, immunoelectron microscopy was performed on resinless sections of extracted cells, exposing the polymorphous nuclear matrix filaments (as in Nickerson et al., 1992). Cells were first treated with p27\textsuperscript{BBP/eIF6} antibodies, followed by gold-conjugated antibodies. The electron micrograph shows a nucleus surrounded by the nuclear lamina (L), which anchored the intermediate filaments of the cytoskeleton (IF). The fibers of the nuclear matrix are strongly decorated with 5-nm gold particles indicating the location of p27\textsuperscript{BBP/eIF6}. Intense gold labeling was seen throughout the nuclear matrix filaments. Bars: (A–E) 4 \mu m; (F) 0.15 \mu m.
us to analyze the functional role of the protein in the yeast model. The yeast protein is encoded by a single copy gene, which we called \( \text{IIH1} \). We disrupted one chromosomal copy of the \( \text{IIH1} \) gene in a diploid strain (see Materials and Methods), followed by sporulation of the obtained \( \text{IIH1/iih1} \) heterozygous strain. Tetrad dissection and analysis showed that all tetrads contained only two viable spores (Fig. 7), none of which carried the disruption marker \( \text{KanMX4} \), indicating that deletion of \( \text{IIH1} \) was lethal. Spores carrying the \( \text{iih1} \) allele were able to germinate, but arrested cell division either in the first or the second cell cycle.

Figure 5. In epithelial cells \( \text{p27BBP/eIF6} \) distribution is similar to the one of \( \beta 4 \) integrin and is present in hemidesmosomes and desmosomes. (A–E) The 804G clone A cell line that expresses human \( \beta 4 \) integrin and forms hemidesmosomes in vitro was double-labeled for \( \beta 4 \) integrin (A, A’, and D) and \( \text{p27BBP/eIF6} \) (B, B’, and C), and the results were analyzed in the confocal microscope (E). In horizontal sections (x, y), \( \beta 4 \) integrin staining is concentrated in the Swiss cheese pattern formed by hemidesmosome rosettes (A and D). The labeling generated by the \( \text{p27BBP/eIF6} \) antiserum yields a pattern similar and superimposable to that of \( \beta 4 \) (B and C). In vertical sections (x, z) part of \( \text{p27BBP/eIF6} \) is observed at the basal surface of epithelial cells, where \( \beta 4 \) is localized. Note that both \( \beta 4 \) and \( \text{p27BBP/eIF6} \) stainings are excluded from the small circular areas characterizing the Swiss cheese pattern (A’ and B’). Bar, 10 \( \mu \)m. (F–I) Electron microscopy studies were performed on ultracytosections of human amnion immunolabeled with \( \text{p27BBP/eIF6} \) antiserum (F and I) or double-immunolabeled with both anticytokeratins (15-nm gold beads) and \( \text{p27BBP/eIF6} \) antiserum (5-nm gold beads) (G and H). The basal cytoplasm of amnion epithelial cells contains a large number of hemidesmosomes, especially in the distal portions of basal cell foot processes. As shown in F and H, \( \text{p27BBP/eIF6} \) is localized in the innermost plaque of hemidesmosomes (arrowheads) in a region composed of a discrete network of thin filaments between the dense plaque and the intermediate filaments. \( \text{p27BBP/eIF6} \) immunolabeling is also detected throughout the cytoplasm associated with thin filaments, which are adjacent to intermediate filaments, stained with anticytokeratin (G and H, arrows). \( \text{p27BBP/eIF6} \) is also present in the cytoskeletal filament network that converges upon desmosomes (I). Bars, 0.125 \( \mu \)m (F); 0.1 \( \mu \)m (G and H); and 0.08 \( \mu \)m (I).
We asked whether the human protein could rescue the lethality caused by deletion of the IIH1 gene. For this purpose, we constructed fusion genes where the yeast or the human p27BBP/eIF6 coding sequences were expressed under control of the yeast galactose inducible GAL1-10 promoter. These fusions were integrated in either single or multiple copies at the yeast URA3 locus of IIH1/iih1 heterozygous diploid strains. Subsequently, these integrated fusions underwent induced sporulation to analyze viability of their meiotic segregants under galactose-induced conditions. As shown in Fig. 7, most tetrads derived from any of these diploid strains contained either three or four viable spores, as expected if expression of human p27BBP/eIF6 (Hsp27BBP/eIF6) was able to rescue the lethality caused by the iih1Δ allele. These data indicate that human and yeast
p27\textsuperscript{BP/eIF6} share a common function. However, expression of human p27\textsuperscript{BP/eIF6} seems to complement the defect less efficiently than its yeast counterpart; as indicated by the slower growth of the clones derived from spores expressing a single copy of the human gene and the iih\textsuperscript{1} allele (Fig. 7). This might be due to inefficient translation of the human mRNA gene in yeast (CAI-S.c. = 0.076); consistently with this hypothesis, the slow growth phenotype was substantially abolished when multiple copies of the GAL-Hsp27\textsuperscript{BP/eIF6} fusion were integrated at the ura3 locus (Fig. 7).

Depletion of p27\textsuperscript{BP/eIF6} Causes Accumulation of G1 Cells

To study the function of p27\textsuperscript{BP/eIF6} in yeast cells, we characterized the phenotype caused by its depletion. For this purpose, wild-type and iih\textsuperscript{1} cells, carrying either the GAL-\textsuperscript{IIH1} or the GAL-Hsp27\textsuperscript{BP/eIF6} fusion and logarithmically growing in galactose, were transferred to glucose-containing medium, to switch off the GAL promoter. The switch to a glucose-containing medium resulted in the progressive loss of the p27\textsuperscript{BP/eIF6} protein (not shown). Since the shut-off of the GAL-Hsp27\textsuperscript{BP/eIF6} fusion caused a much quicker arrest of cell division than that of the GAL-\textsuperscript{IIH1} fusion, we used the GAL-Hsp27\textsuperscript{BP/eIF6} fusion-expressing strain for all the described depletion experiments. As shown by the FACS\textsuperscript{®} profiles in Fig. 8, yeast cells depleted of p27\textsuperscript{BP/eIF6}, progressively stopped growing and accumulated as G1 cells with 1C DNA content. This phenotype is consistent with a role of p27\textsuperscript{BP/eIF6} in protein synthesis since yeast cells need to grow in cell mass and reach a critical size before they can enter the S phase.

p27\textsuperscript{BP/eIF6} Depletion Correlates with the Loss of Free 60S Ribosomal Subunit

The arrest of p27\textsuperscript{BP/eIF6} depleted cells in G1, the fact that p27\textsuperscript{BP/eIF6} has been independently identified as a putative translation initiation factor (Si et al., 1997) and our observation that p27\textsuperscript{BP/eIF6} is detected in the nucleolus of all cell lines, suggested that this protein might be involved in protein synthesis and/or ribosome assembly. To understand the relevance of p27\textsuperscript{BP/eIF6} in one of these processes in yeast, the polysome profiles of wild-type- and p27\textsuperscript{BP/eIF6}-depleted cells were analyzed. For this purpose, wild-type and iih\textsuperscript{1} strains carrying the GAL-Hsp27\textsuperscript{BP/eIF6} were grown in galactose-containing medium, and then shifted to glucose-containing medium to switch off the GAL promoter. As a control, a strain where the iih\textsuperscript{1} allele lethality was rescued by the GAL-\textsuperscript{IIH1} fusion was also used.

As shown in Fig. 9, the polysomal profiles of wt and iih\textsuperscript{1} GAL-\textsuperscript{IIH1} strains were very similar at time 0, whereas iih\textsuperscript{1} GAL-Hsp27\textsuperscript{BP/eIF6} cells, consistently with their slow growth phenotype, already showed a marked decrease in the amount of both the 60s subunit and the polysome fraction at the same time point. In contrast, the levels of the free 40s subunit seemed unaffected or slightly increased. This phenotype was even more dramatic 6 h after shifting to the glucose-containing medium of iih\textsuperscript{1} GAL-Hsp27\textsuperscript{BP/eIF6} cells (Fig. 9). Furthermore, an accumulation of half-mer polysomes (i.e., 80S + 60S) was detectable under these conditions. These data suggest that...
p27BBP/eIF6 might have a primary function in the correct assembly of the 60S ribosomal subunit in yeast.

Cofractionation of human p27BBP/eIF6 in yeast cells was analyzed in parallel. For this end, fractions from the ribosomal gradients were precipitated with TCA and analyzed by Western blot using antibodies against the human protein. As shown in Fig. 10, p27BBP/eIF6 was detected in the 80S and in the free 60S fractions, but absent from polysomes.

Discussion

p27BBP/eIF6 was simultaneously identified by two laboratories using two different approaches. It was isolated in our laboratory as a cytoplasmic interactor of the β4 integrin subunit, and we have shown that it can specifically bind the cytodomain of β4 in vitro (Biffo et al., 1997). However, the discovery of p27BBP/eIF6 homologues in organisms that do not contain β4 indicated that this protein might have a function independent of β4. Among this line, p27BBP/eIF6 was independently identified by Si et al. (1997) as a putative translation initiation factor, able to inhibit the association between the 60S and the 40S ribosomal subunits.

In this study, we have shown that although in epithelial cells p27BBP/eIF6 is coresident with β4 at hemidesmosomes, its association with the cytoskeleton is not a unique feature of epithelial cells. Indeed the protein is in the nuclear matrix of all growing cells. Consistently with its conserved nucleolar expression pattern and sequence, p27BBP/eIF6 is necessary for growth in yeast cells where its loss correlates with a reduced level of the free 60S ribosomal subunit. The in vivo findings were unexpected because they were consistent with a role of p27BBP/eIF6 in ribosomal biogenesis rather than in mRNA translation. In addition, the association of p27BBP/eIF6 with the nuclear matrix suggested that this process was linked to the nuclear cytoskeleton. The ability of the human protein to complement yeast mutation further suggested a conserved function for p27BBP/eIF6.

An Evolutionarily Conserved Function for p27BBP/eIF6 in 60S Metabolism

Database analysis indicates that p27BBP/eIF6 is a very ancient, evolutionarily conserved protein. It is striking to note that the homology is not restricted to a particular domain of the protein, and that even the length of the protein is constant among different species (246 amino acids in C. elegans; 245 in humans, fly, yeast, and A. thaliana; and 215–222 in different Archibacteria). These data suggest that p27BBP/eIF6 may have a critical and conserved function. Indeed, we have shown that the deletion of the S. cerevisiae H11 gene, encoding the p27BBP/eIF6 homologue, is lethal to yeast cells, and that the human protein can complement the yeast-null mutation. Some lines of evidence suggest that also in mammalian cells, p27BBP/eIF6 may be required for growth because of the following: (a) the inability to produce stable p27BBP/eIF6 mRNA antisense expressing mammalian cells (not shown); (b) the ubiquitous p27BBP/eIF6 expression in all immortalized cell lines so far analyzed; (c) and the presence of a single p27BBP/eIF6 gene in the human genome (Sanvito et al., 1998). The generation of p27BBP/eIF6-null mice will help to understand whether p27BBP/eIF6 is also necessary for growth in higher vertebrates. Unfortunately, extensive sequence analysis did not yield significant clues to understand p27BBP/eIF6 function.

To gain some insights into this problem, we used two complementary approaches: the depletion of p27BBP/eIF6 in the genetically manipulable yeast model, and the study of its topographical localization and biochemical properties in mammalian cell lines and tissues. Yeast cells depleted of p27BBP/eIF6 are progressively arrested in G1, a phenotype consistent with a defect in either protein synthesis or ribosomal biogenesis. This fact, and the localization of p27BBP/eIF6 in nucleioli prompted us to analyze the effect of its depletion on the polysome profile. These experiments provide useful information about how p27BBP/eIF6, based on its in vitro ribosomal anti-association activity, could be a translation initiation factor (Si et al., 1997). Polysome profiles of p27BBP/eIF6-depleted yeast cells showed a dramatic reduction in the peak of free 60S subunits and the appearance of half-mer polysomes. Similar polysome profiles have been observed for mutants defective in ribosomal proteins of the 60S ribosomal subunit (M. Ortiz et al., 1991; D. Eshmuk et al., 1993; V. Vilardell and Warner, 1997), or for components involved in pre-rRNA processing and 60S ribosomal subunit assembly (Ripmaster et al., 1992; Sun and Woolford, 1994; H. Ong et al., 1997; Weaver et al., 1997; Zanchin et al., 1997; K. Ressler et al., 1998). Thus, the primary function of p27BBP/eIF6 in yeast is likely related to the 60S ribosomal subunit metabolism.

Polysome profiles of yeast cells, defective in translation initiation factor proteins, are generally characterized by the reduction of the rate of polysomes accompanied by the gradual accumulation of both the free 40S and 60S subunits. Therefore, the polysome profile of p27BBP/eIF6-depleted yeast cells does not support its primary function as a translation initiation factor. However, on the basis of the in vitro data of Si et al. (1997), and in view of the presence of p27BBP/eIF6 also in the cytoplasm of some human cells, the possibility that this protein might have a function also as a cytosolic initiation factor cannot be ruled out, as such ac-
activity could be masked by the predominant defect in 60S metabolism.

The polysome profile does not enlighten the precise role played by p27BBP/eIF6 in 60S metabolism. The protein may be necessary for ribosome assembly/transport, or may act as a structural ribosomal protein. On the basis of the available data, this last possibility is less likely to be true. In fact, the amount of p27BBP/eIF6 sedimenting with the ribosomal fraction in several cell lines represents only a minor fraction of the total p27BBP/eIF6 content. Furthermore, no p27BBP/eIF6 was detected in the polysome fraction.

p27BBP/eIF6 accumulates in the nucleolus of all the analyzed cell lines, where its pattern follows nucleolar evolution (redistribution at mitosis, when the nucleolar organizing region disappears, and redistribution after actinomycin D treatment). Since the nucleolus is the site where ribosommal subunits are assembled, it seems plausible to speculate that p27BBP/eIF6 might be involved in 60S ribosomal biogenesis. The process of ribosome biogenesis is complex and involves several factors (for review see Woolford and Warner, 1991; Eichler and Craig, 1994) including proteins with diverse functions such as RNA helicases, transcription factors, and nucleases. Further studies will address the precise role that p27BBP/eIF6 might play in this process.

Finally, it is possible that p27BBP/eIF6 may be involved in the transport of the 60S subunit from the nucleus to the cytoplasm. To date, very little is known about this process (for review see Shaw and Jordan, 1995), and only a few nuclear proteins have been found to shuttle between the nucleolus and the cytoplasm. In this context, three observations are particularly intriguing: (a) the presence of p27BBP/eIF6 both in a soluble pool and in a cytoskeletal bound compartment; (b) the existence of trace amounts of soluble cytoplasmic p27BBP/eIF6 in all cells; and (c) the ability of p27BBP/eIF6 to bind also the mature 60S subunit (Si et al., 1997).

It is also worth noting that the nucleolar localization of p27BBP/eIF6 is observed in the absence of a consensus nuclear localization signal. Therefore, either p27BBP/eIF6 carries an unknown sequence for nuclear targeting or it is targeted into the nucleus by binding an additional factor in the cytoplasm. The second hypothesis is supported by the fact that even in its most soluble form, p27BBP/eIF6 partitions in gel filtration as a high molecular weight complex (unpublished observation). The molecular dissection of this high molecular weight complex may shed light on the mechanism by which p27BBP/eIF6 is transported to the nucleus.

**p27BBP/eIF6 in the Nuclear Matrix/Intermediate Filaments Fraction**

Our study shows that a relevant fraction of p27BBP/eIF6 is highly insoluble in vivo and is associated both with the nuclear matrix and with the intermediate filament pool. In the cytoplasm, electron microscopy studies have detected p27BBP/eIF6 on thin cytoplasmic filaments of unknown composition that are spatially separated from the classical keratin intermediate filaments, and converge both upon hemidesmosomes and desmosomes. To our knowledge, beside keratins, only another intermediate filament associated protein, IFAP 300, has been described both in hemidesmosomes and desmosomes (Skalli et al., 1994). In this context, it is interesting to note that a recent thorough electron microscopy analysis of human hemidesmosomes has shown the presence of a novel filamentous structure in the proximity of the inner plaque of the hemidesmosome (Behzad et al., 1995).

Nuclear matrix consists of both thick polymorphous filaments and of thin filaments known as core filaments (He et al., 1990). In the nucleus, p27BBP/eIF6 is associated with polymorphous thick filaments, and is absent from the core filaments. This observation is fully consistent with the notion that core filaments may be formed by nuclear RNA, and that p27BBP/eIF6 distribution is resistant to RNA digestion (He et al., 1990). The localization of p27BBP/eIF6 in the nuclear matrix is of extreme interest in the context of ribosome biogenesis. Our data provide an intriguing link between the nuclear cytoskeleton and the process of ribosome assembly.

In recent years growing evidence has indicated that most nuclear and cytoplasmic processes including transcription, DNA replication, and protein synthesis are spatially organized in association with the cytoskeleton. The combined roles of p27BBP/eIF6 protein in 60S assembly, its association with the cytoskeleton, and its ability to bind β4 integrin (Biffo et al., 1997) and the mature 60S ribosome subunit (Si et al., 1997) belong to an integrated view of cell regulation that encompasses structure as well as biochemical processes (Chicurel et al., 1998).

**p27BBP/eIF6 and β4 Integrin**

We have previously shown that p27BBP/eIF6 binds specifically to the cytodomain of β4 integrin in vitro and in yeast (Biffo et al., 1997). Our previous data, and specifically the association of p27BBP/eIF6 with keratin intermediate filaments, strongly suggested that this interaction could occur also in vivo and be necessary for targeting β4 to hemidesmosomes and intermediate filaments. Since intermediate filament-associated proteins can be solubilized only upon SD S treatment, rendering the maintenance of biochemical interactions impossible, an association between β4 and p27BBP/eIF6 in tissues could not be proved. We now provide two further elements suggesting that p27BBP/eIF6 is functionally associated to the β4 integrin in vivo: (a) its peculiar Swiss cheese distribution is superimposable to that of β4 in cells that form hemidesmosomes; and (b) the presence of the protein, in vivo, in hemidesmosomes of the human amnion. Further experiments are needed to clarify the functional significance of β4-p27BBP/eIF6 interaction, and specifically whether p27BBP/eIF6 may direct β4 to hemidesmosomes. Alternatively, as it has been recently suggested, on the basis of in vitro evidence and yeast two-hybrid assays, the crucial step in targeting β4 to hemidesmosomes is the interaction with the large intermediate filament-associated protein, H D-1 (Nissen et al., 1997; R ezniczek et al., 1998). If this is the case also in vivo, then the role of p27BBP/eIF6 binding to β4 may be related to a nonstructural function of β4 integrin, similar to that shown in the case of the recruitment of shc and grb2 (Mainiero et al., 1995) or of PI3 kinase (Shaw et al., 1997).

In the absence of further evidence, we may reasonably suggest that p27BBP/eIF6 has an evolutionarily conserved...
function linked to 60S ribosome biogenesis, and one acquired during evolution in epithelial cells containing b4 integrin. At least one precedent of a protein with a dual function acquired during evolution, i.e., b2-catenin/arma-dillo, has already been reported. This remarkable protein can be found both at sites of cell-cell adhesion in connection to cadherins and in the nucleus where it can signal in conjunction with LEF-1 (for review see Willert and Nusse, 1998).

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