A 69-kD Protein That Associates Reversibly with the Sm Core Domain of Several Spliceosomal snRNP Species

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Abstract. The biogenesis of the spliceosomal small nuclear ribonucleoproteins (snRNPs) U1, U2, U4, and U5 involves: (*a*) migration of the snRNA molecules from the nucleus to the cytoplasm; (*b*) assembly of a group of common proteins (Sm proteins) and their binding to a region on the snRNAs called the Sm-binding site; and (*c*) translocation of the RNP back to the nucleus. A first prerequisite for understanding the assembly pathway and nuclear transport of the snRNPs in more detail is the knowledge of all the snRNP proteins that play essential roles in these processes.

We have recently observed a previously undetected 69-kD protein in 12S U1 snRNPs isolated from HeLa nuclear extracts under non-denaturing conditions that is clearly distinct from the U1-70K protein.

The following evidence indicates that the 69-kD protein is a common, rather than a U1-specific, protein, possibly associating with the snRNP core particles by protein-protein interaction. (*a*) Antibodies raised against the 69-kD protein, which did not cross-react with any of the Sm proteins B'–G, precipitated not only U1 snRNPs, but also the other spliceosomal snRNPs U2, U4/U6 and U5, albeit to a lower extent. (*b*) U1, U2, and U5 core RNP particles reconstituted in vitro contain the 69-kD protein. (*c*) *Xenopus laevis* oocytes contain an immunologically related homologue of the human 69-kD protein. When U1 snRNA as well as a mutant U1 snRNA, that can bind the Sm core proteins but lacks the capacity to bind the U1-specific proteins 70K, A, and C, were injected into *Xenopus* oocytes to allow assembly in vivo, they were recognized by antibodies specific against the 69-kD protein in the ooplasm and in the nucleus.

The 69-kD protein is under-represented, if present at all, in purified 17S U2 and in 25S [U4/U6.U5] tri-snRNPs, isolated from HeLa nuclear extracts. Our results are consistent with the working hypothesis that this protein may either play a role in the cytoplasmic assembly of the core domain of the snRNPs and/or in the nuclear transport of the snRNPs. After transport of the snRNPs into the nucleus, it may dissociate from the particles as for example in the case of the 17S U2 or the 25S [U4/U6.U5] tri-snRNP, which bind more than 10 different snRNP specific proteins each in the nucleus.

EUCARYOTIC cells contain a group of small, uridyl-rich nuclear RNAs, designated U1, U2, U4, U5 and U6. While the snRNAs U1, U2, and U5 are organized as separate ribonucleoprotein particles, the U4 and U6 snRNAs reside in the same RNP complex, forming the U4/U6 snRNP particle (Reddy and Busch, 1988). All four major snRNPs are essential *trans*-acting factors in the splicing of pre-mRNA (Steitz et al., 1988; Lamond et al., 1990; Lührmann et al., 1990). Owing to the introduction of improved preparation techniques and the application of more sensitive analytical procedures, the number of well-characterized snRNP proteins has increased considerably during the last few years (Lührmann et al., 1990). Generally, the snRNP proteins fall into two classes. The first class comprises the so-called common proteins, denoted B', B, D1, D2, D3, E, F, and G. These are shared by the snRNP particles U1, U2, U5, and U4/U6 (Lührmann, 1988; Lehmeier et al., 1990). Because they all react with the anti-Sm autoantibodies from patients with systemic lupus erythematosus, the common proteins are also termed Sm proteins (Lerner et al., 1981; Pettersson et al., 1984).

The second class comprises proteins that bind to the snRNPs in a particle-specific manner. For example, U1 snRNPs purified on Mono Q contain, in addition to the common proteins, three distinct U1-specific proteins termed 70K, A, and C (Bach et al., 1989). The U2-specific proteins can be divided into two groups, according to the conditions under which they bind. Under high-salt conditions, U2 snRNPs are isolated in a 12S form; these particles contain, in addition to the common proteins, two U2-specific proteins that are termed A' and B''. However, in splicing active nuclear extracts, which are prepared under low-salt conditions, the

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U2 and snRNPs have a Svedberg value of 17 (Black and Pinto, 1989; Behrens et al., 1993*b*) and contain another nine U2-specific proteins with molecular weights ranging from 35 to 160 kD (Behrens et al., 1993). This group of proteins dissociates from the U2 snRNP at salt concentrations higher than 200 mM (Behrens et al., 1993).

Similarly, the 20S U5 snRNP, prepared at moderate salt concentrations, contains eight U5-specific proteins, which are absent from the 12S U5 snRNP prepared under high-salt conditions (Bach et al., 1989).

Particularly noteworthy is a group of snRNP proteins that is specifically associated with the 25S [U4/U6.U5] tri-snRNP complex in nuclear extracts. The purified tri-snRNP complex contains, in addition to the common proteins and the 20S U5 specific proteins, a group of polypeptides characterized by molecular weights of 15.5, 20, 27, 60 and 90 kD, which are termed the tri-snRNP-specific proteins (Behrens and Lührmann, 1991). These proteins dissociate from the 25S [U4/U6.U5] tri-snRNP complex at salt concentrations higher than 300 mM which is accompanied by dissociation of this RNP complex into its constituent particles. Because the proteins specific to the 17S U2 snRNP and to the 25S tri-snRNP dissociate easily when the salt concentration is raised, these proteins eluded detection until recently.

The biogenesis of the snRNPs is a complex process that occurs in the nuclear and the cytoplasmic compartments of the cell and consequently requires nucleocytoplasmic transport events (Mattaj, 1988; Zieve and Sauterer, 1990). The newly transcribed m^7G-capped snRNAs U1, U2, U4, and U5 are initially exported to the cytoplasm. In the cytoplasm, the common proteins B′ to G assemble on the Sm site of the snRNAs to form the core RNP domain (Mattaj and De Robertis, 1985; Hamm et al., 1990). The Sm site of the U1, U2, U4, and U5 snRNAs consists of a conserved single-stranded region rich in uridylic acid and flanked by two hairpin loops (Branlant et al., 1982). As has been shown previously, binding of the common proteins to the Sm site is a prerequisite for hypermethylation of the m^7G cap to give the caryophilic m_3G cap (Mattaj, 1986). Both the m_3G cap and another caryophilic signal on the Sm core particle, of as yet unknown identity, function as a bipartite nuclear localization signal for the snRNPs (Fischer and Lührmann, 1990; Fischer et al., 1993; Hamm et al., 1990).

The location in the cell at which the particle-specific snRNP proteins associate with their respective snRNP particles is not yet known. For some of these proteins, such as the U1-specific 70K, A, and C, there is experimental evidence that they can in principle be imported into the nucleus independently of the U1 core RNP (Kambach and Mattaj, 1992; Jantsch and Gall, 1992; Feeney and Zieve, 1990). Consequently, they may recombine with the cognate core RNP to form a native U1 snRNP particle inside the nucleus. Nothing is yet known about the mechanism of binding of the proteins specific for the 17S U2 and 20S U5 snRNPs. In the case of the U4 snRNP particle, no particle-specific proteins have yet been identified. There is strong evidence, however, that the assembly of the U4/U6 snRNP occurs in the nucleus (Wersig et al., 1992; Vankan et al., 1992), which is consistent with the fact that U6 snRNA, after transcription, does not leave the nucleus (Vankan et al., 1990). Consequently, the assembly of the 25S[U4/U6.U5] tri-snRNP complex should also be a nuclear event. The [U4/U6.U5] tri-snRNP is also believed to recycle after each round of splicing (for review see Moore et al., 1993), which again argues strongly for nuclear assembly.

The pathway by which the various Sm core proteins assemble to form the cytoplasmic core snRNP structure is not fully understood. It appears to involve at least two steps, with the formation of RNA-free protein hetero-oligomers being followed by the assembly of these complexes on the Sm site of the various snRNAs. In particular, it has been reported that the snRNP proteins E, F, and G along with one or more of the D proteins form RNA-free 6S hetero-oligomeric protein complexes (Fisher et al., 1985; Sauterer et al., 1990), which apparently bind as such to the Sm site. In this connection, it may be relevant that the small nuclear ribonucleoprotein G binds directly to the Sm site of U1 snRNA (Heinrichs et al., 1992). In addition, there is evidence that some of the core proteins, such as B, B′, and one or more of the D proteins, form 20S homo-oligomeric protein particles in the cytoplasm (Sauterer et al., 1990; Zieve and Sauterer, 1990). The questions of whether there is an ordered pathway by which the various proteins form the Sm core RNP domain and, if so, which of the proteins participate in early assembly intermediates, have yet to be investigated.

Here we describe a novel 69-kD protein that associates with the Sm core domain of the various spliceosomal snRNPs. This was shown by reconstitution of the snRNPs in vitro and by immunoprecipitation of snRNPs with anti-69-kD antibodies from *Xenopus* oocytes after injection of snRNAs into the ooplasm. The 69-kD protein is, however, under-represented in native snRNPs such as the 17S U2 snRNP and the 25S [U4/U6.U5] tri-snRNP complex, purified from HeLa nuclear extracts, and this under-representation indicates that the 69-kD protein interacts in a reversible manner with the Sm core domain. We propose that the 69-kD protein may play a part in the assembly and/or cytoplasmo-nuclear transport of the snRNPs.

Materials and Methods

Preparation of HeLa nuclear Extracts

Hela cells (S3) were grown in suspension culture as described by Bringmann et al. (Bringmann et al., 1983) or obtained from the Computer Cell Culture Centre (Mons, Belgium). Nuclear extracts were prepared by the method of Dignam et al. (1983).

Anti-m_3G Immunoaffinity Chromatography of snRNPs

The affinity purification of snRNPs U1 to U6 was performed with monoclonal antibody H20 bound to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) as described previously (Bochnig et al., 1987). Before being loaded onto the affinity column, nuclear extracts were dialyzed against buffer C-5 (20 mM Hepes/KOH, pH 7.0, 250 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA-NaOH, pH 8.0, 0.5 mM DTE, 0.5 mM PMSF, 4 mg/ml leupeptin and 5% vol/vol glycerol. They were subsequently passed over an anti-m_3G column that had been equilibrated in the same buffer. Washing and elution with m^7G were carried out as described previously (Bringmann and Lührmann, 1986), with buffer C-5 used in all steps.

Preparative Gradient Centrifugation

Centrifugation of native snRNP particles U1 to U6 in glycerol gradients was carried out essentially as described by Behrens and Lührmann (1991). The eluate from the anti-m_3G column containing native snRNP particles U1 to U6 was dialyzed against buffer G (20 mM Hepes/KOH pH 7.9, 150 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA-NaOH, pH 8.0, 0.5 mM DTE, 0.5

mM PMSF, 4 mg/ml leupeptin, and 5% vol/vol glycerol) before sedimentation on a linear glycerol gradient (10–30% vol/vol) in buffer G. The gradients were centrifuged in a Beckman SW40 Ti rotor (Beckman Instruments, Palo Alto, CA) at 29,000 rpm for 18 h. After fractionation of the gradients, each fraction was extracted with phenol/chloroform. To obtain good separation, the proteins were analyzed on high-TEMED SDS gels as described by Lehmeier et al. (1990). The snRNAs were fractionated on 10% urea-TBE-acrylamide gels (Bringmann and Lührmann, 1986).

Preparation of Protein-deficient snRNP Particles by Mono Q Chromatography

Ion-exchange chromatography of snRNPs U1 to U6 on a Mono Q column under standard conditions yielded U1, U2, U4/U6, and U5 snRNP particles (Bach et al., 1989). When the Mono Q column was eluted at 37°C, core RNP particles, containing the common proteins B′ to G, came off the column (Bach et al., 1990; Kastner et al., 1992). Most of the particle-specific proteins, including the 69-kD protein, thus dissociate from the snRNP particles on the column. We found that they could be recovered from the flow-through of the Mono Q column (details not given here).

Preparation of Intact U1 and U5 snRNP Particles

Isolated U1 and U5 snRNP particles, containing the particle-specific proteins and the 69-kD protein in addition to the common proteins B′ to G, were desorbed from an anti-m^7G column at 420 mM KCl. Subsequently, they were fractionated by centrifugation on a glycerol gradient (Behrens and Lührmann, 1991). The fraction collected from the 12S region and from the 20S region of the gradient were then submitted to immunoaffinity chromatography on a column bearing monoclonal antibody H386. The fact that H386 interacts with both the U1-specific 70K protein and the U5-specific 100K protein (Behrens and Lührmann, 1991) enabled us to affinity purify both of these snRNPs from their respective gradient fractions. Bound U1 and U5 snRNP particles were eluted with buffer G containing 50 μg of a 32-residue synthetic oligopeptide that comprises the H386 epitope and thus competes for the binding of this antibody to the U1 70K and the U5 100K proteins, respectively (Behrens and Lührmann, 1991).

Separation of snRNP Proteins by HPLC

The total protein mixture of intact snRNP particles U1 to U6 was separated from the snRNAs and extracted according to the method of Lehmeier et al. (1990). Dried total protein was solubilized with 0.1% (vol/vol) trifluoroacetic acid (TFA)[1] in water prior to chromatography by reversed-phase HPLC (Separation System A120; Applied Biosystems, Foster City, CA). Fractionation was performed on an Applied Biosystems microbore RP-300 column (C8); the column was eluted with a step gradient at a rate of 200 ml/min (eluent A, 0.1% [vol/vol] TFA in water; eluent B, 80% acetonitrile/0.085% [vol/vol] TFA).

Two-dimensional Protein Analysis

In the first dimension, the proteins were analyzed on isoelectric-focusing gels as described previously by Woppmann et al. (1990). In the second dimension, proteins were separated by standard techniques on discontinuous SDS-polyacrylamide gels (Laemmli, 1970). The proteins were stained with Coomassie brilliant blue (Serva).

Immunoprecipitation

Protein G-Sepharose (Pharmacia Fine Chemicals) was pre-swollen in PBS buffer (130 mM NaCl, 20 mM $NaPO_4$, [pH 8.0]). 20-μl aliquots of a 50% suspension of beads were mixed with 20 μl serum or 5 μl concentrated monoclonal antibody in a total volume of 500 μl. The mixture was incubated overnight at 4°C with continual end-over-end rotation of the vessel. After the beads had been washed five times with 500 μl PBS containing 1.5 mM $MgCl_2$, the antigen was added. Immunocomplex formation was allowed to take place for 4 h at 4°C in the same buffer. The protein G-Sepharose was then washed five times with IPP150 (10 mM Tris/HCl, pH 8.0, 150 mM NaCl, 0.1% NP-40, 0.1% sodium azide). Bound RNA was extracted with phenol/chloroform, precipitated with ethanol, and 3′ end labeled with [^{32}P]pCp (Amersham Corp., Arlington Heights, IL) by the method of England and Uhlenbeck (1978). The RNA was finally analyzed on 10% urea-TBE-polyacrylamide gels (Bringmann and Lührmann, 1986).

1. *Abbreviation used in this paper*: TFA, trifluoroacetic acid.

Reconstitution In Vitro of the Sm Core RNP Domain

Reconstitution in vitro of core RNP particles from isolated proteins and native snRNA was carried out essentially as described by Sumpter et al. (1992). A standard reconstitution mixture contained a 10-fold molar excess of snRNP proteins over wild-type snRNA in 20 μl. Reconstitution was allowed to proceed in a buffer containing 20 mM Hepes/KOH (pH 7.9), 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTE and 0.5 U/ml RNasin. This reaction mixture was incubated for 30 min at 30°C followed by 15 min at 37°C. ^{32}P-labeled snRNAs used for reconstitution of snRNPs were transcribed in vitro as described previously (Fischer et al., 1993).

Antibodies and Immunoblotting

Antisera specific for the 69-kD protein were raised in rabbits according to standard immunization protocols. Additionally, the following monoclonal antibodies were used: H20 (Bochnig et al., 1987), H111 and H386 (Reuter and Lührmann, 1986), and Y12 (Lerner et al., 1981). For immunological investigation, proteins pre-fractionated on high-TEMED SDS gels were transferred to nitrocellulose by a method similar to that of Towbin et al. (1979), as modified by Lehmeier et al. (1990). Specific immunocomplex formation with the cognate antigens was made visible by the alkaline-phosphatase reaction (Lehmeier et al., 1990).

CsCl Gradient Centrifugation

CsCl was added to buffer G to a final concentration of ca. 1.4 g/cm^3. A preparative amount of reconstituted particles was centrifuged in polycarbonate tubes for 16 h at 90,000 rpm and 4°C in a Beckman TLA-100.3 rotor. 200-μl fractions were collected manually from the top of the tube. The protein and RNA content of each fraction were analyzed as described previously (Lehmeier et al., 1990; Bringmann and Lührmann, 1986).

Oocyte Injections

3′ end-labeled U1 snRNA (England and Uhlenbeck, 1978) as well as [^{32}P]CTP-labeled SmII RNA synthesized in vitro were mixed in suitable ratios to give similar intensities of radioactive signals on autoradiographs. Approximately 20 nl were injected into the vegetal half of the oocyte. Oocytes were dissected manually after transfer into buffer J (70 mM NH_4Cl, 7 mM $MgCl_2$, 0.1 mM EDTA, 2.5 mM DTT, 20 mM Tris/HCl at pH 7.5, 10% vol/vol glycerol), and RNA was extracted from the oocyte fractions as described previously (Fischer et al., 1993). The RNAs were finally analyzed on denaturing TBE–polyacrylamide gels that contained 8% wt/vol urea.

Results

A Previously Undetected 69-kD Protein in Immunoaffinity-purified snRNPs

SnRNPs U1 to U6 were purified from HeLa cell nuclear extracts by anti-m_3G immunoaffinity chromatography at a salt concentration of 250 mM KCl. Under these conditions, the m^7G eluate of the affinity column contains the 12S U1 snRNP, the 12S U2 snRNP and the 25S [U4/U6.U5] tri-snRNP complex (Fig. 1 *a*). The majority of free proteins characteristic of the 17S form of the U2 snRNP, however, have under these conditions dissociated from the U2 RNP particle and consequently are not present in such an snRNP preparation (Behrens and Lührmann, 1991; Behrens et al., 1993). As shown in Fig. 1 *a*, native snRNP particles, which have been eluted from the affinity column with m^7G, contain the expected common proteins and the previously described particle-specific proteins as well as significant amounts of a previously undetected protein migrating above the U1 70K protein (lane *I*). On account of the somewhat slower migration of this protein as compared with the 66-kD BSA standard, we shall refer to it as the 69-kD protein.

To investigate whether the 69-kD protein is associated

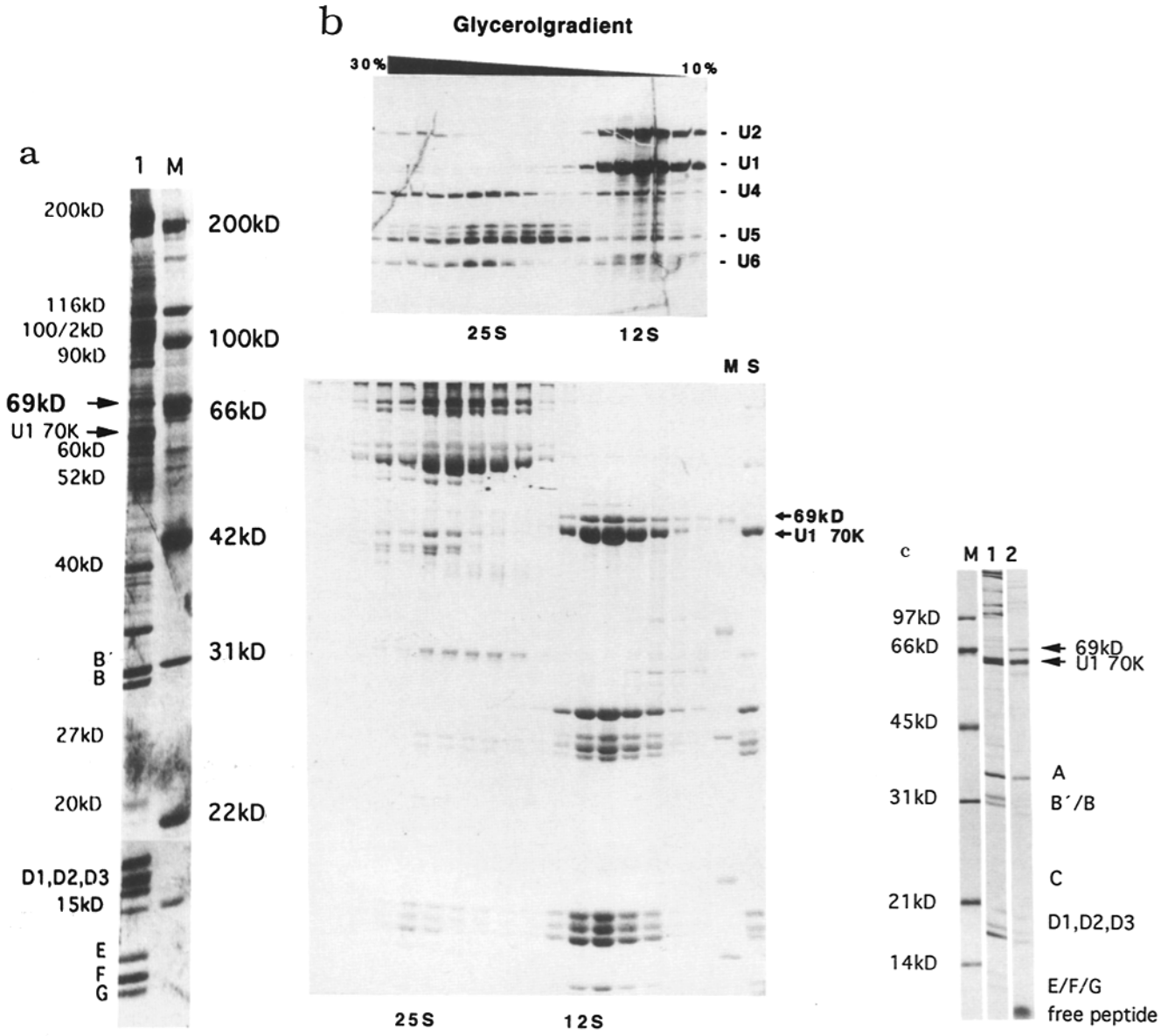

Figure 1. Detection of a novel 69-kD protein in immunoaffinity-purified snRNPs prepared at low-salt concentrations. (*A*) Separation of total proteins from anti-m_3G affinity-purified snRNPs on a 12% high-TEMED SDS gel (Lehmeier et al., 1990). The proteins were stained with Coomassie brilliant blue. Lane *M*, protein markers in kD; lane *1*, protein composition of purified snRNPs U1 to U6 (20 μg). The U1 70K and the 69-kD proteins are indicated by arrows. Lefthandside labels indicate the common proteins (Lührmann, 1988), the 20S U5 specific proteins (Bach et al., 1989), and the 25S [U4/U6.U5] tri-snRNP specific proteins (Behrens and Lührmann, 1991). (*B*) Sedimentation of snRNPs U1 to U6 on glycerol gradients. Anti-m_3G affinity-purified snRNPs U1 to U6 were applied to a linear 10–30% (vol/vol) glycerol gradient, centrifuged, and fractionated as described in Materials and Methods. The RNA and protein content of each fraction was analyzed on a 10% urea-TBE gel (*top*) and a 12% high-TEMED SDS gel (*bottom*), respectively. The sedimentation coefficients of the various snRNPs, determined by calibration using 16S and 23S rRNA standards in a parallel gradient, are given at the bottom. The arrows indicate the 69-kD protein and the U1 70K. (*C*) Protein composition of immunoprecipitated 12S U1 snRNP. Gradient fractions containing 12S U1 and 12S U2 snRNPs (*b*) were passed over an immunoaffinity column containing covalently bound mab H386 (Behrens and Lührmann, 1991). Elution of the 12S U1 snRNP particles immobilized on the column was performed by competition with a synthetic 32-mer oligopeptide comprising the H386 epitope (Behrens and Lührmann, 1991). The proteins were separated on a 12% high-TEMED SDS gel. Lane *M*, protein markers (mol wt in kD); lane *1*, total proteins (10 μg) from 12S U1 snRNPs prepared by ion-exchange chromatography on a Mono Q column (Bach et al., 1989); lane *2*, total proteins (8 μg) of affinity-purified 12S U1 snRNP. The U1 specific 70K protein and the 69-kD protein are indicated by arrows at the right. At the bottom of lane *3*, the free 32-mer oligopeptide used for elution can be seen.

specifically with snRNPs, we submitted the m^{7}G eluate to density-gradient centrifugation in a glycerol gradient. Analysis of the proteins and the RNA along the gradient shows that the bulk of the 69-kD protein co-migrates with U1 and U2 snRNPs in the 10S-12S region (Fig. 1 *b*, *bottom*).

Gradient fractions containing the 12S U1 and 12S U2 snRNPs (Fig. 1 *b*) were passed over an immunoaffinity column containing covalently bound monoclonal anti-U1 70K antibodies. Native 12S U1 snRNPs were then eluted with an excess of peptide competing with the H386 epitope (Behrens and Lührmann, 1991). Subsequent analysis of the U1 proteins on an SDS gel revealed that affinity-purified U1 particles, in contrast to Mono Q–purified U1 particles, contained significant amounts of the 69-kD protein (Fig. 1 *c*, lanes *1* and *2*). The absence of the 69-kD protein from Mono Q–purified U1 snRNPs is apparently due to its having dissociated from the particles on account of non-specific interaction with the Mono Q resin; it could be recovered from the flowthrough (details not shown). These findings indicate that the 69-kD protein, at least under the moderate conditions of affinity chromatography, is stably associated with the 12S U1 snRNP particle.

The 69-kD Protein Is Distinct from the U1 70K Protein

In view of the fact that the U1 70K is highly phosphorylated (Woppmann et al., 1990), it was important to ascertain that the 69-kD protein really is distinct from the U1 70K and is not merely a posttranslationally modified variant of it. The former possibility was supported indirectly by the migration behavior of the 69-kD protein in SDS–polyacrylamide gels as compared with that of U1 70K. While the migration of 70K varies considerably in different gel systems with respect to the BSA marker (data not shown), the 69-kD protein consistently co-migrates with BSA (see for example Fig. 1 *a*, lane *1*). The U1 70K and the 69 kD are best resolved on the 12% high-TEMED SDS gels described by Lehmeier et al. (1990). More direct evidence for a fundamental difference between the 69 kD and the U1 70K proteins came from the following experiments.

We were further able to purify the 69-kD protein to near homogeneity by subjecting the protein fraction from affinity-purified snRNPs to HPLC reversed-phase chromatography (Lehmeier et al., 1990). As shown in Fig. 2 *a* (*left*), the 69-kD protein is the first snRNP protein to be eluted from the column, after about 12 min and before the more hydrophobic U1 70K.

When the two proteins were compared by two-dimensional gel analysis with isoelectrofocusing in the first dimension, the 69-kD protein exhibited a basic pI value, well separated from the more acidic phospho-variants of U1 70K (Fig. 2 *b*). While the bulk of the HPLC-purified 69-kD protein focuses at pI 10,0 (fig. 2 *b*, *bottom*), a minor fraction of the 69-kD protein exhibited a more acidic pI value. Given that the more acidic variant was not observed, when total snRNP protein from anti-m^{7}G affinity-purified snRNPs were directly fractionated by two-dimensional gel electrophoresis (Fig. 2 *b*, *top*), we cannot exclude the possibility that the more acidic variant observed with the HPLC-purified 69-kD protein is artificially generated by chemical modification of basic amino acid residues during the purification procedure.

That the U1 70K and the 69-kD protein are immunologically unrelated proteins was demonstrated by immunoblotting studies with U1 70K-specific and 69-kD–specific antibodies. Anti-69-kD antisera were obtained from rabbits which were immunized with purified 69-kD protein or fragments thereof. As shown by the western blots in Fig. 3 *a*, one

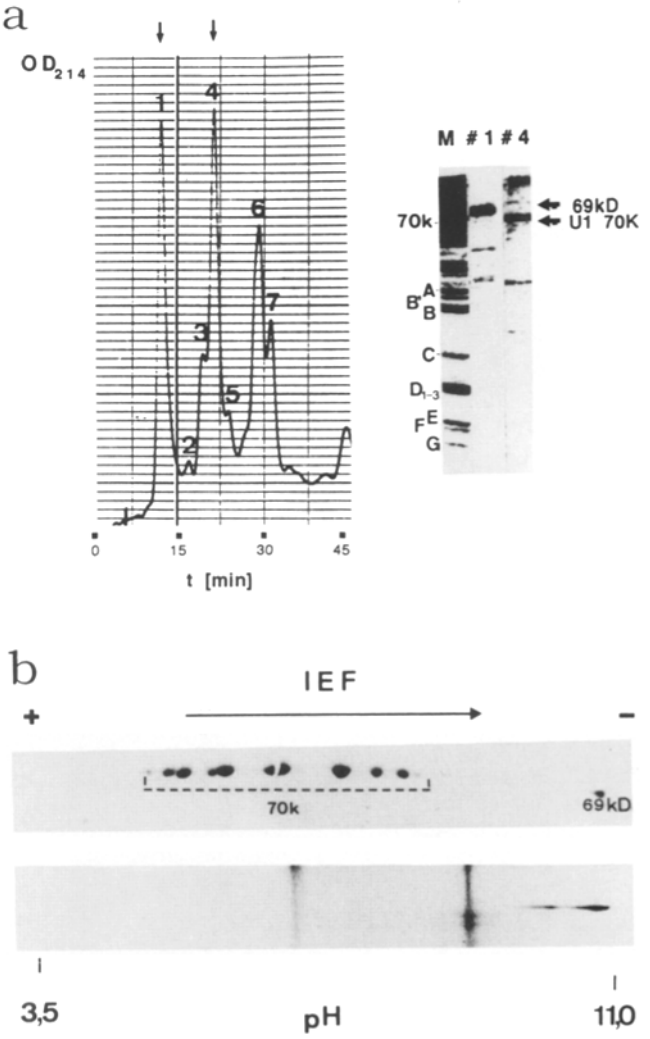

Figure 2. Physico-chemical characterization of the 69-kD protein. (*A*) (*Left*) Proteins isolated from immunoaffinity purified snRNPs U1-U6 prepared at high salt concentrations were fractionated by reversed-phase HPLC (see Materials and Methods). The figure shows a section of the elution profile, in which fractions 1 and 4, indicated by arrows, contain the 69-kD and the U1 70K protein, respectively. (Right) Protein analysis of fractions from the HPLC. The protein from each fraction was recovered by drying in a speed-vac rotor and was subsequently separated on a 12% high-TEMED SDS gel. Lane *M*, protein (15 μg) from snRNP U1-U6; lane *1*, 69-kD protein (1 μg) from fraction 1; lane *4*, U1 70K protein (1 μg) from fraction 4. (*B*) Two-dimensional analysis of the 69-kD protein. 30 μg purified snRNP proteins U1-U6 (*top*) or 3 μg of HPLC-purified 69-kD protein (*bottom*) were separated by isoelectric focusing in a gradient from pH 3 to 11 for 4,000 V-h. The pH gradient was calibrated by use of pI markers. The second dimension was run in 15% polyacrylamide with SDS. For details, see Materials and Methods. Only the 70-kD regions of the second-dimension gels are shown. While the bulk of the HPLC-purified 69-kD protein focuses at pI 10,0, a minor fraction of the 69-kD protein exhibited a more acidic pI value.

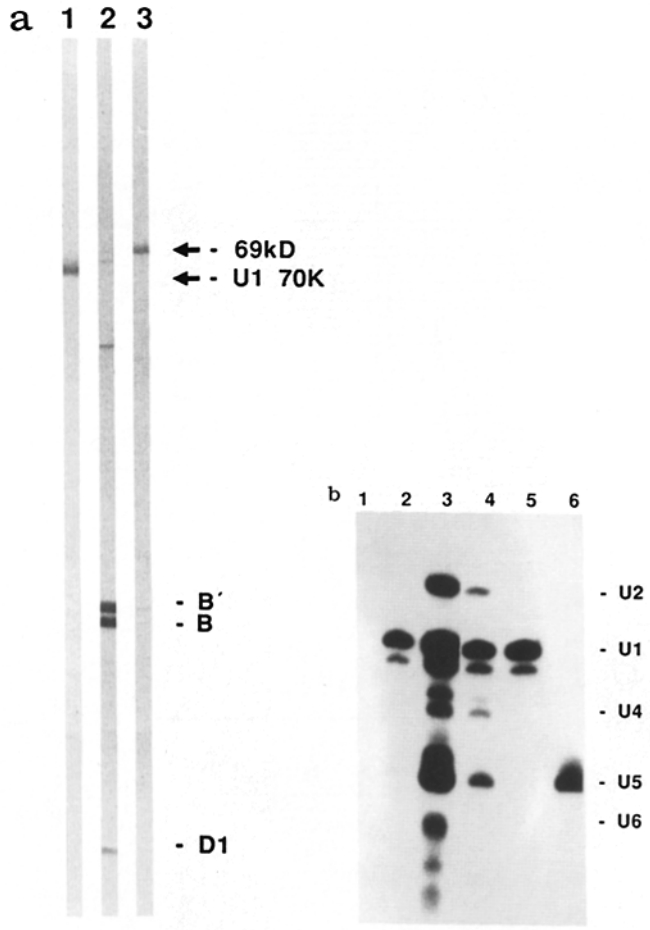

Figure 3. Immunoprecipitation of snRNPs with antibodies against the 69-kD protein. (*A*) Characterization of an antiserum reacting specifically with the 69-kD protein. Nitrocellulose strips containing 8 μg snRNP U1-U6 protein (Fig. 1 *a*) were incubated with monoclonal anti-U1 70K antibody H111 (1:100) (lane *1*), monoclonal anti-Sm antibody Y12 (1:500) (lane *2*), and rabbit anti-69-kD antiserum (1:5,000) (lane *3*). (*B*) Immunoprecipitation assays were carried out essentially as described in Materials and Methods. An autoradiograph of the gel-fractionated [^{32}P]pCp-labeled snRNAs from the various immunoprecipitates is shown. The RNP-specific mab H111 (Reuter and Lührmann, 1986) and the Sm-specific mab Y12 (Lerner et al., 1981) are compared with the 69-kD-specific antibodies in respect of their ability to precipitate snRNP particles from splicing-active HeLa nuclear extracts and from samples of purified 12S U1 and 12S U5 RNP particles. Typically, 20 μg of nuclear extract and 2 μg of the purified snRNPs were used. Lane *1*, splicing-active extract and pre-immune serum; lane *2*, splicing-active extract and mab H111; lane *3*, splicing-active extract and mab Y12; lane *4*, splicing-active extract and 69-kD-specific antibodies; lane *5*, purified 12S U1 particles and 69-kD-specific antibodies; lane *6*, purified 12S U5 particles and 69-kD-specific antibodies.

immune serum out of three reacted specifically with the 69-kD protein on nitrocellulose strips, which contained the total proteins from affinity-purified snRNPs U1 to U6 (compare Fig. 1 *a*, lane *1*), but failed to react with the U1 70K or any of the other snRNP proteins (lane *3*). Conversely, the U1 70K-specific mab H111 failed to react with the 69-kD protein (Fig. 3 *a*, lane *1*).

Finally, partial sequence analysis of tryptic peptides derived from purified 69-kD protein in a gas-phase sequenator provided conclusive evidence that the 69-kD and the U1 70K are definitely unrelated proteins (data not shown).

Immunoprecipitation of snRNPs with Antibodies against the 69-kD Protein

The failure of the anti-69 kD antibodies to cross-react with any of the other snRNP proteins except the 69-kD protein made this serum a useful tool for the direct re-investigation of the association of the 69-kD protein with snRNPs by immunoprecipitation.

Interestingly, the anti-69-kD antibodies precipitated not only U1 snRNPs from nuclear extracts, but U2, U4/U6, and U5 snRNPs as well (Fig. 3 *b*, land *4*). To exclude the possibility that the precipitation of all spliceosomal snRNPs by anti-69-kD antibodies was due to co-precipitation of snRNPs with larger aggregates in the nuclear extract, we further used purified U1 snRNPs and U5 snRNPs (in both cases avoiding Mono Q chromatography, see Materials and Methods) for immunoprecipitation assays. As shown in lanes *5* and *6* of Fig. 3 *b*, both U1 and U5 snRNPs were independently precipitated by anti-69-kD antibodies. It should be noted, however, that precipitation of the snRNPs with anti-69-kD antibodies is always significantly weaker than precipitation with the mab Y12 (Fig. 3 *b*, lane *3*). None the less, precipitation of the snRNPs by anti-69-kD antibodies is highly specific, as no reaction occurred with the pre-immune serum (Fig. 3 *b*, lane *1*). These data support our conclusions drawn above that the 69 kD is a snRNP protein. However, they go further to suggest that the 69-kD protein may associate with all spliceosomal snRNP particles and not only with the U1 snRNP particle.

Binding of the 69-kD Protein to the Sm Core Domain Occurs for All snRNA Species

We have recently established the reconstitution in vitro of structurally intact snRNPs from native snRNP proteins and isolated snRNPs (Sumpter et al., 1992). The snRNP proteins used for the reconstitution are prepared from isolated snRNPs by the DEAE/EDTA method (Sumpter et al., 1992; Walter and Blobel, 1983). A typical mixture of snRNA-free snRNP proteins contains all of the common proteins B′ to G, while most of the particle-specific proteins, such as the U1 70K, and most of the [U4/U6.U5] tri-snRNP-specific proteins and the 17S U2-specific proteins, are missing. Thus, incubation of isolated snRNA with a tenfold excess of native snRNP proteins under reconstitution conditions allows the efficient reconstitution in vitro of the Sm core RNP domain of the various snRNP particles. Such reconstituted core RNP particles exhibit the same biochemical and biophysical properties as do isolated snRNPs. For example, as has been shown recently, core RNPs reconstituted in vitro are translocated to the nucleus of *Xenopus* oocytes with an efficiency similar to that of in situ assembled core RNPs (Fischer et al., 1993).

Interestingly, the 69-kD protein is usually retained in significant amounts in the DEAE/EDTA protein fraction used for reconstitution in vitro. This has been shown by Coomassie staining of an SDS-gel onto which a fraction of snRNP proteins was loaded (Sumpter et al., 1992), as well as by immunoblotting with anti-69-kD antibodies (data not shown).

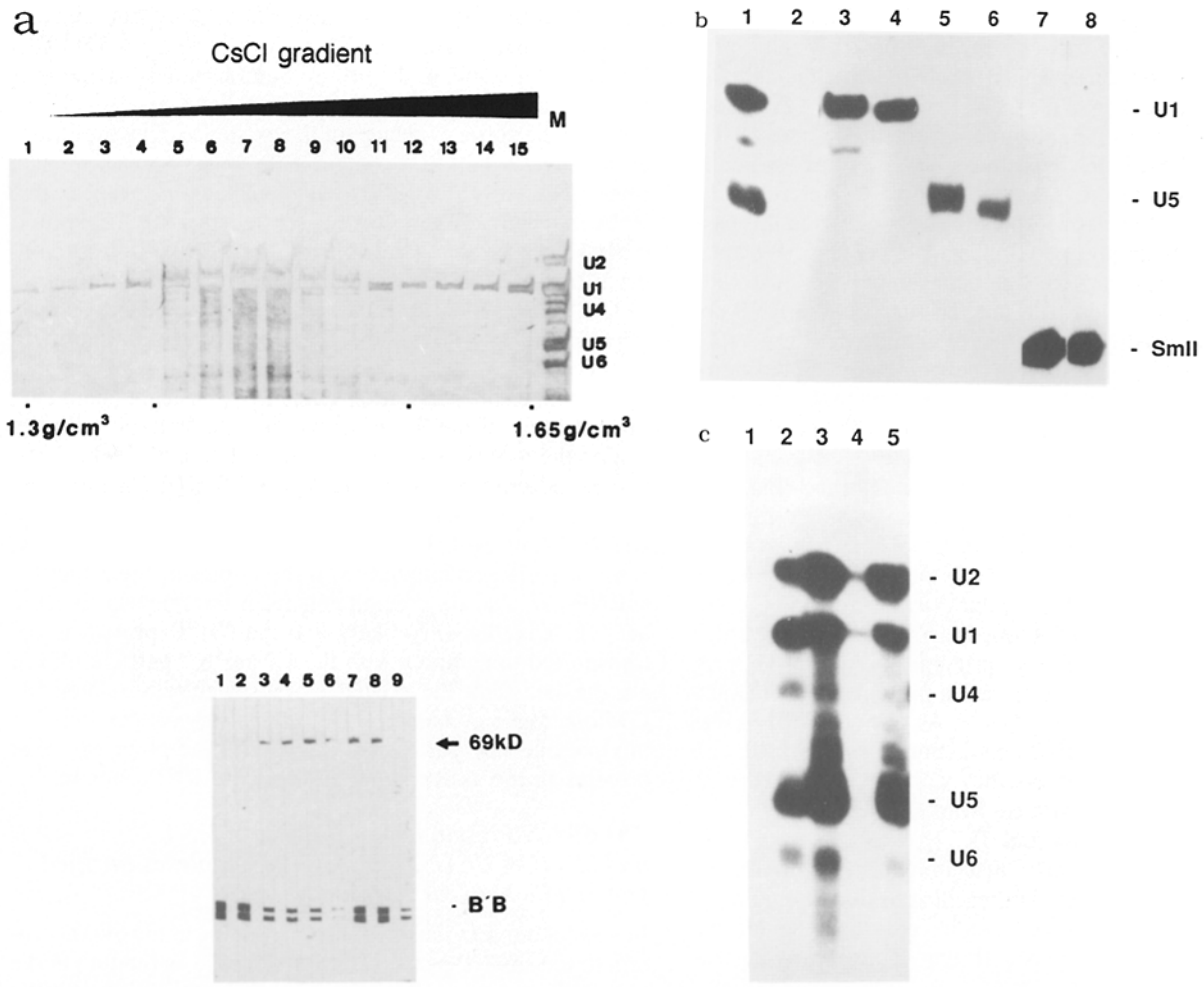

Figure 4. Binding of the 69-kD protein to the Sm core domain in a manner independent of the RNA sequence. (*A*) Immunoblotting analysis of U1 RNP core particles reconstituted in vitro after CsCl-gradient centrifugation. Reconstitution of the particles and subsequent CsCl-gradient centrifugation were performed as described in Materials and Methods. Gradient fractions, collected by hand, were analyzed for RNA content (*top*) and for protein content by immunoblotting (*bottom*). The numbers at the tops of the lanes correspond to the fraction numbers. (*Top*) Lane *M*, snRNAs from snRNPs U1-U6. (*Bottom*) Nitrocellulose strips containing the proteins from the respective gradient fractions, incubated with a mixture of the anti-Sm antibody Y12 (1:500) and anti-69-kD serum (1:5,000). (*B*) Immunoprecipitation assay of U1, U5 and SmII core RNP particles reconstituted in vitro. After reconstitution, the particles were incubated immediately with Sepharose-coupled antibodies. Immunoprecipitation was carried out essentially as described in Materials and Methods. Lane *1*, U1 and U5 core RNPs incubated with anti-cap antibodies; lane *2*, U1 and U5 core RNPs incubated with pre-immune serum; lanes *3* and *4*, U1 core RNP particles with mab Y12 and with anti-69-kD antibodies, respectively; lanes *5* and *6*, U5 core RNP particles with mab Y12 and with anti-69-kD antibodies, respectively; Lanes *7* and *8*, SmII core RNP particle with mab Y12 and with anti-69-kD antibodies, respectively. (*C*) Immunoprecipitation assay of Mono Q-purified core RNPs with mab Y12 and 69-kD-specific antibodies. Immunoprecipitation of core RNPs (lanes *1–4*) and core RNP plus 69-kD protein (lane 5) was carried out as described in Materials and Methods. An autoradiograph of the gel-fractionated, [^{32}P]pCp-labeled snRNAs from the various precipitates is shown. Lane *1*, pre-immune serum; lane *2*, antibodies against the m^{7}G cap; lane *3*, mab Y12; lane *4*, anti-69-kD antibodies; lane *5*, 4 μg Mono Q-purified core RNPs U1-U6 were incubated for 30 min at 30°C with 1 μg native 69-kD protein, isolated from the flowthrough of a Mono Q column, and subsequently immunoprecipitated with 69-kD-specific antibodies.

The reconstitution in vitro of snRNPs with various snRNA species has enabled us to investigate the structural requirements of the binding of the 69-kD protein to snRNPs in more detail. Initially, we reconstituted U1 snRNP core particles and subjected them to centrifugation on a caesium chloride gradient. Proteins were recovered from the fractions collected along the respective gradient and used for immunoblotting studies. As shown in Fig. 4 *a*, the bulk of the 69-kD protein fractionated together with U1 snRNA and the other Sm core proteins at a density of about 1.4 g/ml in the gradient, suggesting that the 69-kD protein specifically interacts with the U1 core RNP domain. It further indicates that the association of the 69-kD protein is not dependent on the presence of the U1-specific proteins 70K, A, and C.

Similar results were obtained when U1 and U5 core RNP particles as well as the SmII core RNP, reconstituted in vitro, were analyzed in an immunoprecipitation assay with 69-kD specific antibodies. The latter RNP contains a truncated U1 snRNA molecule, which consists of the 3′-terminal 42 nucleotides of the U1 snRNA (including the single-stranded Sm site and the 3′-terminal E-loop) and, for reasons of stability, an artificially designed 5′-terminal stem–loop structure 22

nucleotides in length (Fischer et al., 1993). However, since this mutant lacks the capacity to bind the U1-specific proteins, any contribution of snRNP proteins other than the Sm proteins to the binding of the 69-kD protein can be excluded. As shown in Fig. 4 *b*, in vitro reconstituted U1 (lane *4*), U5 (lane *6*), and SmII RNP particles (lane *8*) are efficiently precipitated by 69-kD-specific antibodies. Interestingly, precipitation by the 69-kD-specific antibodies is in this case about as efficient as precipitation by mab Y12 (Fig. 4 *b*, lanes *3*, *5*, and *7*), suggesting that significant amounts of the 69-kD protein were associated with the various Sm core RNP domains under in vitro reconstitution conditions.

As an important control for the immunoprecipitation of snRNPs with anti-69-kD antibodies, we had to show that the antibodies did not fortuitously cross-react with one of the Sm core proteins B′ to G. The absence of such a cross-reaction with these proteins, as shown by immunoblotting (Fig. 3 *a*, lane *3*), does not necessarily exclude cross-reactivity with the proteins when they are in their native state as part of the intact core RNP structure. For this purpose, we subjected U1-U6 snRNPs from HeLa nuclear extracts to Mono Q chromatography at 37°C, to obtain core snRNP particles which lacked all the particle-specific proteins and the 69-kD protein, but still contained the common proteins B′ to G (Kastner et al., 1992; Bach et al., 1990). As demonstrated in Fig. 4 *c*, the core snRNP particles could not be precipitated with anti-69-kD antibodies unless they were pre-incubated with native 69-kD protein isolated from the flowthrough of a Mono Q column (lanes *4* and *5*).

These data show clearly that immunoprecipitation of snRNPs by anti-69-kD antibodies must be due to binding of the antibodies to the 69-kD protein, and they exclude the possibility of cross-reaction with any of the Sm core proteins.

The data further suggest that the 69-kD protein can bind to a high-affinity binding site available on native core snRNP domains, even in the absence of any particle-specific proteins and irrespective of the nature of the snRNA molecules. In conclusion, these data support the idea that the 69-kD protein may associate with the core RNP domain by protein-protein interactions with one or more of the common proteins B′ to G.

The Xenopus Homologue of the Human 69-kD Protein Binds to the snRNP Core Domain in the Ooplasm

So far we have shown that the 69-kD protein may bind to apparently any Sm core particle in vitro. Given our evidence that the 69-kD protein most probably associates with the Sm core domain by protein-protein interactions and the fact that in vivo the Sm core proteins B′ to G assemble onto the Sm site of an snRNA molecule in the cytoplasmic compartment of the cell, we were interested to know whether the 69-kD protein may also associate with snRNPs in the cytoplasm. We have investigated this question in *Xenopus laevis* oocytes, since in this system nuclei and ooplasm can easily be analyzed independently of each other. However, a prerequisite for these studies was that the 69-kD protein be evolutionarily highly conserved among species. Initial evidence that this is likely came from immunoblotting studies with proteins from *Xenopus* oocyte extracts. As shown in Fig. 5 *a* (lane *4*), the antibodies raised against the human 69-kD protein react strongly with a protein from *Xenopus* oocytes that migrates with an apparent molecular weight of about 64 kD. This reaction is specific, as the pre-immune serum from the same rabbit did not give a signal (Fig. 5 *a*, lane *3*).

Given the above evidence for the existence of an evolutionarily conserved 69-kDa protein in *X. laevis* oocytes we next injected U1 snRNA together with the above-mentioned SmII RNA into the ooplasm of *Xenopus* oocytes. We have previously shown that upon injection of naked SmII RNA into oocytes the RNA is capable of binding the Sm proteins B′ to G. The RNP particle, however, remains entirely in the ooplasm and is not transported to the nuclear compartment (Fischer et al., 1993). This is most likely due to the fact that a proper nuclear localization signal is not brought about during assembly of the Sm proteins onto the heterologous Sm site on the SmII RNA. Fig. 5 *b* shows that anti-69-kD antibodies indeed precipitate both U1 and SmII RNA from the cytoplasmic compartment of the oocytes (Fig. 5 *b*, lanes *2* and *4*). While SmII RNA, owing to its transport deficiency, is exclusively precipitated from the ooplasm, the intact U1 snRNP can also be precipitated from the nucleus (lanes *1* and *3*). It is thus very likely that the 69-kD protein is co-transported in complex with the U1 snRNP particle. All in all, the data show the existence of a structurally related 69-kD homologue in *Xenopus* oocytes and support the idea that this protein may participate along with the other Sm core proteins in the assembly of the Sm core RNP domain.

The 69-kD Protein Is Not Present in 17S U2 and 25S [U4/U6.U5] Tri-snRNP Complexes Purified from HeLa Nuclear Extracts

In view of our previous finding that binding of the 69-kD protein to the snRNP particles is not as stable as binding of the Sm proteins and given our previous observation that the total proteins of purified 25S[U4/U6.U5] tri-snRNPs and 17S U2snRNPs, when fractionated on SDS gels, failed to contain significant amounts of the 69-kD protein (Behrens et al., 1993; Behrens and Lührmann, 1991) (see also Fig. 1 *b*, this manuscript), we re-investigated the content of 69-kD protein in purified 17S U2 snRNPs and 25S [U4/U6.U5] tri-snRNP complexes by immunoblotting with 69-kD-specific antibodies. The various particles were isolated by immunoaffinity chromatography as described previously (Behrens et al., 1993; Behrens and Lührmann, 1991) and were subsequently subjected to a final purification step by glycerol-gradient centrifugation. This step was included in order to ensure that only stably bound proteins are retained on the various snRNPs. Fig. 6 shows the respective immunoblots, which were probed with 69-kD-specific antibodies. As a control we have also probed total proteins from immunoaffinity purified U1 snRNPs. As expected, a strong signal in the 69-kD region was observed with 12S U1 snRNPs (Fig. 6, lane *3*). However, no reaction was observed between the antibodies and the 25S [U4/U6.U5] tri-snRNP complex (Fig. 6, lane *5*). Likewise, the 17S U2 snRNP also failed to react (Fig. 6, lane *4*). Although the amount of total proteins bound to nitrocellulose strips was not stoichiometric for all three particles tested, as shown by the reaction with anti-B′/B specific antibodies (Fig. 6, compare lanes *3–5*), the data clearly show the absence of the 69-kD protein from the 17S U2 and the 25S [U4/U6.U5] tri-snRNPs.

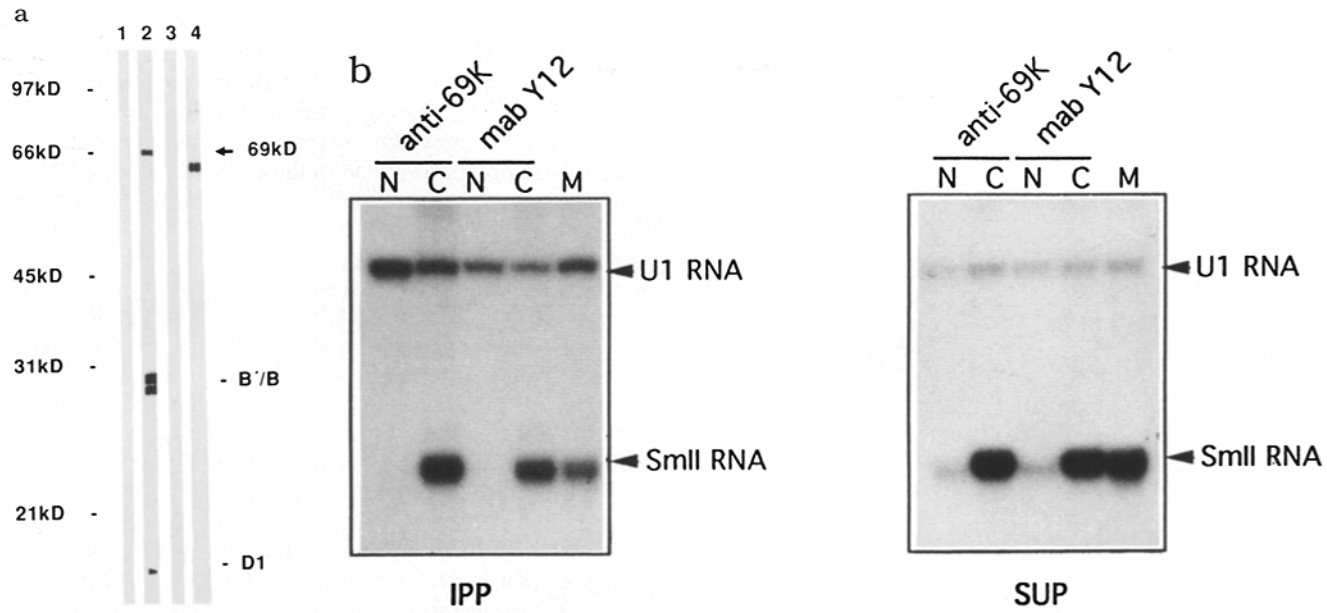

Figure 5. Detection of a 69-kD homologue in *X. laevis* oocytes. (*A*) Immunoblot analysis of extracts from oocytes with anti-69-kD antibodies. Nitrocellulose strips containing 10 μg snRNP U1-U6 protein (lanes *1* and *2*) and 40 μg extract from *X. laevis* oocytes (lanes *3* and *4*) were incubated with pre-immune serum (lanes *1* and *3*), mab Y12 (1:500) plus anti-69-kD antibodies (1:5,000) (lane *2*), and anti-69-kD antibodies (1:500) (lane *4*). (*B*) Co-injection of [^{32}P]pCp-labeled U1 snRNA as well as [^{32}P]CTP-labeled SmII RNA synthesized in vitro, into oocytes and subsequent immunoprecipitation with the nuclear and cytoplasmic extracts. The amount of each of the snRNAs on the gel was adjusted so as to give signals of similar intensity on the autoradiograph. Microinjection and immunoprecipitation were performed as described in Materials and Methods. (*IPP*) Immunoprecipitates; (*SUP*) supernatants. Lane *M*, microinjected U1 snRNA and SmII RNA precipitated from oocytic extracts using the mabY12. Samples from nuclear extracts are indicated by an *N* at the top of the respective lanes and samples from ooplasm by a *C*. To allow efficient assembly in vivo, oocytes were incubated for 12 h at 20°C. The antibodies used for precipitation are indicated at the top of the lanes.

Discussion

The correct assembly of the Sm proteins in the cytoplasm onto the Sm site of an m^{7}G-capped snRNA is a prerequisite not only for the hypermethylation of the m^{7}G- to the m$_3$G cap (Mattaj et al., 1986), but also for translocation of the spliceosomal snRNPs into the nucleus. In fact, there is good evidence that the Sm core domain provides binding sites or signal structure(s) that interact respectively with the methyltransferase involved in the cap-hypermethylation and with the transport factor that recognizes the "Sm core NLS" (Fischer et al., 1993). The protein(s) contributing to these signal structures on the Sm core domain have not yet been identified, and only little is known about the pathway by which the Sm proteins are assembled into a functional core RNP (see Introduction).

It is somewhat surprising that the 69-kD protein has not been observed before. However, in conventional gel systems this protein is completely obscured by the U1-specific 70K protein, with which it co-migrates exactly. It is well resolved by the improved electrophoresis that has been in use in our laboratory for some time (see Materials and Methods), but in previous work we routinely purified U1 snRNPs by Mono Q chromatography (Bach et al., 1990), under which conditions the 69K protein dissociates completely from the U1 particle (see Results).

It is nevertheless clear that the 69-kD protein differs from all the snRNP proteins characterized up to now, and in particular from the 70K protein. This view is supported by the following findings: (*a*) It has a highly basic pI value, in contrast to the relatively neutral values characteristic of the isoelectric variants of the U1 70K protein. (*b*) As shown by immunoblotting, all the isoelectric variants of 70K reacted with a U1-specific anti-RNP antibody, while the basic protein failed to react (Woppmann et al., 1990). (*c*) Antibodies against the 69-kD protein failed likewise to react with 70K or with any other of the common or specific snRNP proteins (Fig. 3 *a*). (*d*) No cross-reaction took place between 69-kD specific antibodies and the common proteins B′ to G, even for the native proteins as demonstrated by radioimmunoprecipitation assays (Fig. 4 *c*).

The 69-kD protein was originally found as a component of snRNP particles that were isolated under non-denaturing conditions by anti-m$_3$G or anti-70K immunoaffinity chromatography when chromatography with Mono Q was avoided. This protein associates by far the most strongly with the 12S U1 particle, as isolated by affinity chromatography that employed a H386 column. It is under-represented in comparison with the other particle-specific and common proteins, but a more accurate assessment of its copy number cannot be made at present.

The 69-kD protein does not belong to the category of particle-specific proteins, and there is a good deal of evidence that it is a common protein, as follows: (*a*) The antibody against the 69-kD protein precipitated not only U1 snRNPs from the HeLa nuclear extract, but also U2, U4/U6, and U5 particles, although the yield of the other particles was a good deal lower than that of U1 (Fig. 3 *b*). (*b*) When reconstitution was carried out in vitro with native protein mixtures that had been isolated from affinity-purified snRNPs and that

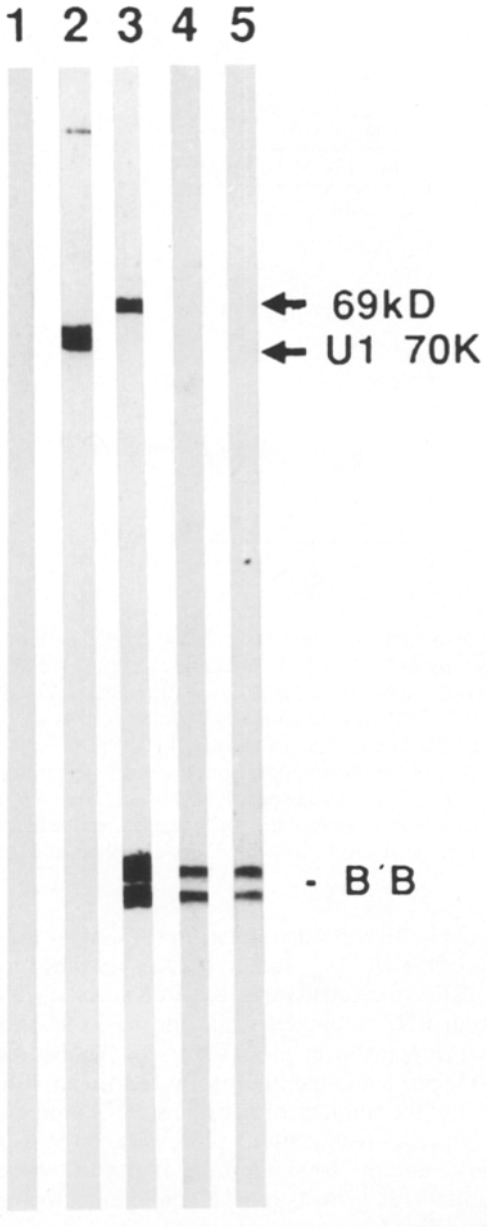

Figure 6. Analysis of the protein composition of the 17S U2 and the 25S [U4/U6.U5] tri-snRNP complexes. Detection of the 69-kD protein in 12S U1, 17S U2 and 25S[U4/U6.U5] tri-snRNP particles by immunoblotting. In order to achieve good separation, the total proteins were fractionated on a high-TEMED SDS-gel that measured 30 cm in length. However, for immunostaining we blotted the proteins with molecular weights ranging from 20 to 100 kD. This explains why the D-proteins were not visualized by mab Y12. Lane *1*, snRNP U1-U6 protein (15 μg) probed with pre-immune serum; lane *2*, 12S U1 snRNP protein (10 μg) probed with anti-U1 70K mAb H111; lane *3*, 12S U1 snRNP protein (10 μg) probed with mab Y12 and 69-kD-specific antibodies; lane *4*, 17S U2 snRNP protein (15 μg) with mAb Y12 and 69-kD-specific antibodies; lane *5*, [U4/U6.U5] tri-snRNP protein (15 μg) with mAb Y12 and 69-kD-specific antibodies.

contained significant quantities of the 69-kD protein, the 69-kD protein was incorporated into all snRNP particles. It is important to note here that, on account of the lack of specific proteins in the reconstitution mixture, most of the snRNPs formed were intact core particles lacking their particle-specific proteins. Thus the 69-kD protein behaves in a manner more typical of the common than of the specific proteins (Fig. 4 *a*). (*c*) The binding of the 69-kD protein to the Sm core occurs not only during total reconstitution but also when 69-kD protein is added to the cores of native snRNPs that have been stripped of their specific proteins by Mono Q chromatography at raised temperatures (Fig. 4 *c*). This shows (*i*) that there is a strong binding site for the 69-kD protein on the intact core, and (*ii*) that the 69-kD protein can bind to the core of any snRNP particle. The fact that this binding is independent of the identity of the snRNA makes it seem likely that it comes about by direct interaction between the 69-kD protein and the other common proteins, a conclusion that is strengthened by the observation of binding between the 69-kD protein and the SmII RNP particle, which does not bind specific proteins (Fischer et al., 1993), so that these cannot determine the binding of the 69-kD protein. However, it is still not known whether the 69-kD protein interacts at least in part with the Sm site of the snRNAs. (*d*) Finally, the experiments with *Xenopus* oocytes also confirm the assignment of the 69-kD protein to the common proteins. The clearest result is the finding in the frog ovum of a protein showing apparent structural homology with the 69-kD protein of HeLa (Fig. 5 *a*). This at once suggests that this protein may have a central function. Like the other core proteins B' to G, this homologue appears to be present in the cytoplasm in sufficient quantities to attach itself to newly assembled core RNP particles. Core particles containing the 69-kD protein were also found in the nucleus (by immunoprecipitation; Fig. 5 *b*), so it appears that the 69-kD protein is co-transported to the nucleus. However, we cannot at present exclude the possibility that there is a pool of 69-kD protein in the nucleus, ready to associate with the cores once they have arrived there.

It must be emphasized that in some respects the 69-kD protein differs considerably from the established common proteins B' to G. Its binding to the snRNP particle is less stable, so that it dissociates under the conditions of Mono Q chromatography. It also appears generally to be underrepresented in comparison with the other proteins, which may be connected with its lower affinity for the snRNP. However, it appears in especially low quantities, if at all, in the purified larger particles, 17S U2 and the 25S [U4/U6.U5] tri-snRNP as demonstrated by immunoblotting studies (Fig. 6 *a*). It is of course possible that the 69-kD protein is bound to the high-molecular-weight particle in a dynamic equilibrium in the cell. Yet our data still show clear differences between the binding to U1 snRNPs, which contain substantial quantities of the 69-kD protein, and native 17S U2 and the 25S [U4/U6.U5] tri-snRNP complexes. At present we do not know whether the affinity of the 69-kD protein to the core RNP domain differs to some degree dependent upon the snRNA species. The underrepresentation of the 69-kD protein in the 17S U2 and 25S [U4/U6.U5] tri-snRNP complex could, however, also be due to competition between the 69-kD protein and particle specific proteins for the same binding site on the Sm core RNP domain. Consequently, the 69-kD protein would be displaced during the later stages of assembly.

In summary, our data strongly suggest that the binding of the 69-kD protein to the Sm core domain is transient in na-

ture. This could in turn indicate a role for it in assembly or transport. One possibility is that it acts in the cytoplasmic morphogenesis of the Sm core domain by interacting with one or more of the other established core proteins. In fact, we have preliminary evidence that 69-kD protein can interact with B/B' and one of the D proteins in the absence of snRNA (Hackl, W., unpublished observation). Whether such a hetero-oligomeric protein complex containing the 69-kD protein indeed functions as an early assembly intermediate in vivo has yet to be established. Whatever the role of the 69-kD protein in the assembly of the Sm core domain may turn out to be, we believe for the following reasons that it does not function as a molecular chaperon. First, we have as yet no evidence that the binding of the 69-kD protein to the Sm core is an event that requires ATP. Second, the finding that the 69-kD protein is also present, at least in part, in assembled snRNPs in the nucleus does not support this idea. However, our data do not exclude the possibility that a chaperonin-like protein may be required for the assembly of an snRNP core domain in vivo. In this connection, the observation by Fury and Zieve (1993) of a 70-kD protein that appears to interact with other Sm proteins exclusively in the cytoplasm is of interest.

In addition to a possible function in the assembly pathway of the Sm core domain, the 69-kD protein could also contribute to the cytoplasmo-nuclear transport of an snRNP particle in a more direct way, e.g., by facilitating the interaction of the snRNP-transport factors with the Sm core domain. With in vitro snRNP transport systems in sight, such possibilities could soon be examined.

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