Symplekin, a Constitutive Protein of Karyo- and Cytoplasmic Particles Involved in mRNA Biogenesis in *Xenopus laevis* Oocytes

Ilse Hofmann,^{*†} Martina Schnölzer,[§] Isabelle Kaufmann,[‡] and Werner W. Franke^{*}

^{*}Division of Cell Biology and [§]Protein Analysis Facility, German Cancer Research Center, D-69120 Heidelberg, Germany; and [‡]Department of Cell Biology, Biozentrum, University of Basel, CH-4056 Basel, Switzerland

Submitted December 4, 2001; Revised January 30, 2002; Accepted February 7, 2002
Monitoring Editor: Marvin P. Wickens

Symplekin is a dual location protein that has been localized to the cytoplasmic plaques of tight junctions but also occurs in the form of interchromatin particles in the karyoplasm. Here we report the identification of two novel and major symplekin-containing protein complexes in both the karyo- and the cytoplasm of *Xenopus laevis* oocytes. Buffer-extractable fractions from the karyoplasm of stage IV–VI oocytes contain an 11S particle, prepared by immunoselection and sucrose gradient centrifugation, in which symplekin is associated with the subunits of the cleavage and polyadenylation specificity factor (CPSF). Moreover, in immunofluorescence microscopy nuclear symplekin colocalizes with protein CPSF-100 in the “Cajal bodies.” However, symplekin is also found in cytoplasmic extracts of enucleated oocytes and egg extracts, where it occurs in 11S as well as in ca. 65S particles, again in association with CPSF-100. This suggests that, in *X. laevis* oocytes, symplekin is possibly involved in both processes, 3'-end processing of pre-mRNA in the nucleus and regulated polyadenylation in the cytoplasm. We discuss the possible occurrence of similar symplekin-containing particles involved in mRNA metabolism in the nucleus and cytoplasm of other kinds of cells, also in comparison with the nuclear forms of other dual location proteins in nuclei and cell junctions.

INTRODUCTION

Cell biologists had recently to recognize, much to their surprise, that certain proteins appear, often in the same cells, as “dual location proteins,” i.e., as general constituents of two rather distant and different structures: On the one hand, they occur as components of cytoskeletal plaques of a specific kind of intercellular junction, and on the other hand, they are located in karyoplasmic, interchromatinic granules, even in cells devoid of any junctions. Examples include the plakophilins PKP 1–3, typical of desmosomal plaques (Mertens *et al.*, 1996; Schmidt *et al.*, 1997, 1999; Bonnè *et al.*, 1999), the adherens junction proteins, ARVCF (Borrmann, 2000; Bor-

rmann *et al.*, 2000; for cDNA transfection experiments see also Mariner *et al.*, 2000), afadin (Mandai *et al.*, 1997) and protein 4.1 (Krauss *et al.*, 1997; Lallena *et al.*, 1998), and the tight junction plaque proteins ZO-1 (Gottardi *et al.*, 1996), symplekin (Keon *et al.*, 1996), Ash-1 (Nakamura *et al.*, 2000), and ZONAB (Balda and Matter, 2000). Obviously, this constitutively dual location at junctions and in nuclei has to be distinguished from observations of transient nuclear accumulations of certain other junctional plaque proteins in special stages of the cell cycle or differentiation or upon expression of certain transfected cDNAs or genes (for examples see, e.g., Funayama *et al.*, 1995; Karnovsky and Klymkowsky, 1995; Behrens *et al.*, 1996; Huber *et al.*, 1996; Molenaar *et al.*, 1996; Schneider *et al.*, 1996; Yost *et al.*, 1996; Daniel and Reynolds, 1999; for reviews see Behrens, 2000; Hübner *et al.*, 2001).

Such a constitutively dual localization has also been reported in many diverse cultured cells and tissues for the tight junction-associated M_r 150,000 protein, symplekin, which occurs in mostly granular-looking karyoplasmic structures, and on mitotic telophase, rapidly reaccumulates, in the nucleus, like a typical nuclear protein (Keon *et al.*,

Article published online ahead of print. Mol. Biol. Cell 10.1091/mbc.01-12-0567. Article and publication date are at www.molbiol-cell.org/cgi/doi/10.1091/mbc.01-12-0567.

[†] Corresponding author. E-mail address: i.hofmann@dkfz.de.

Abbreviations used: CPSF, cleavage and polyadenylation specificity factor; CstF, cleavage stimulation factor; DAPI, 4',6-diamidino-2-phenylindole; HSP, high-speed pellet; HSS, high-speed supernatant; LSP, low-speed pellet; PSD, post-source decay.

1996; for review see Stevenson and Keon, 1998). Analysis of the amino acid sequence of this protein, however, has not revealed homologies—or at least similarities—to any known nuclear protein (cf. reviews of Cáceres and Krainer, 1997; de la Cruz *et al.*, 1999).

To elucidate the nuclear function(s) of symplekin we have applied biochemical methods for isolating and characterizing the protein, using methods that recently have been successful in studies of the nuclear forms of plakophilin PKP2, which has been shown to be part of RNA polymerase III complexes (Mertens *et al.*, 2001). For the sake of clarity we have further decided to start with the exceptionally large nuclei of *Xenopus laevis* oocytes (“germinal vesicles”) allowing both mass and manual isolations with minimal cytoplasmic contamination as well as the preparation of enucleated “ooplasms,” and thus the parallel analysis of both karyoplasm and cytoplasm (cf. e.g., Bonner, 1975a, 1975b; De Robertis *et al.*, 1978; Krohne and Franke, 1980a, 1980b, 1983; Kleinschmidt and Franke, 1982; Kleinschmidt *et al.*, 1983; Peters *et al.*, 1990, 1994). Here we report the identification of the nuclear form of *Xenopus* oocyte symplekin as a distinct particle of about 11S in association with subunits of the CPSF complex known to be part of the 3′-end pre-mRNA processing machinery. In addition, symplekin occurs, together with protein CPSF-100, coilin and many other proteins known to function in RNA synthesis and processing, in the so-called “Cajal bodies,” which are also very large in these oocytes (cf. Gall *et al.*, 1999; Gall, 2000; Morgan *et al.*, 2000). Surprisingly, however, we have also found that symplekin-containing particles are not restricted to the nucleus but can also be detected in enucleated oocytes and in eggs.

MATERIALS AND METHODS

Biological Materials

Clawed toads (*Xenopus laevis*) were purchased from the African *Xenopus* Facility C.C. (Knysna, Republic of South Africa). Tissue samples from *X. laevis* (skin, heart, ovaries) were snap-frozen in isopentane cooled by liquid nitrogen to about -140°C and stored at -80°C . For *X. laevis* blood smear preparations, blood was obtained from larger vessels of decapitated toads. The blood was directly smeared on glass slides and air-dried for 3 h. Subsequently the smears were fixed for 10 min with freshly prepared 2% formaldehyde in PBS with 1 mM MgCl_2 and permeabilized for 3 min with 0.3% Triton X-100 in PBS with 1 mM MgCl_2 .

Cell culture cell lines used included *X. laevis* kidney epithelial (XLKE) line A6, human colon carcinoma line CaCo2, and human SV-40 transformed fibroblasts line SV80 (for sources see American Tissue Culture Collection, Manassas, VA, and Cordes *et al.*, 1996).

Antibodies and Reagents

The monoclonal antibodies (mAbs) specific for symplekin used were mAb Sym-TJ-E150 (Keon *et al.*, 1996) and mAb Sym-Nu (Becton Dickinson, Heidelberg, Germany). Guinea pig antibodies specific for symplekin (sym-CT) were obtained by immunization with a synthetic peptide representing the C-terminal sequence (AMKTP-SPAAEDAREPEAKGNS, aa 1122–1144; cf. Keon *et al.*, 1996; Ueki *et al.*, 1997), coupled to keyhole limpet hemocyanin (Peptide Specialty Laboratories, Heidelberg, Germany). Rabbit sera specific for CPSF-100 (Jenny *et al.*, 1994) or CPSF-73 (Jenny *et al.*, 1996) have been described before. Rabbit antibodies specific for coilin (Bohmann *et al.*, 1995) were generously provided by Dr. A. Lamond (University of Dundee, Scotland, United Kingdom). Mouse mAb H1 specific for

coilin (Tuma *et al.*, 1993) was obtained from Zytomed (Berlin, Germany), and mAb No-185 against a nucleolar protein (Schmidt-Zachmann *et al.*, 1987) was kindly provided by M. Schmidt-Zachmann (German Cancer Research Center).

Secondary antibodies used for immunofluorescence microscopy were Texas Red-, Alexa 488-, Cy3-, Cy2-conjugated antibodies to immunoglobulins of mouse, guinea pig, or rabbit, respectively (Dianova, Hamburg, Germany).

Gel Electrophoresis and Immunoblotting

Proteins of total cells and cell fractions were separated by SDS-PAGE (Laemmli, 1970). After electroblotting of the proteins to PVDF membranes, the filters were blocked for 1 h in Tris-HCl-buffered saline (TBS) containing 0.05% Tween (TBST) and 5% nonfat dry milk. The specific antibodies were incubated with the membranes for 1 h in TBST. Bound antibodies were detected by chemiluminescence using the ECL-system (NEN, Cologne, Germany) after incubation with horseradish peroxidase-coupled secondary antibodies.

Isolation and Fractionation of *Xenopus* Oocytes and Egg Extract

Ovaries were surgically removed, and oocytes were defolliculated in 2 mg/ml collagenase (Sigma, Munich, Germany) in 87 mM NaCl, 2.5 mM KCl, 1 mM MgCl_2 , 1 mM Na_2HPO_4 , 5 mM HEPES (pH 7.8) with constant agitation at 28°C for 2–3 h. For separation of nuclear content and nuclear envelope, nuclei were isolated in “5:1 buffer” supplemented with 10 mM MgCl_2 (for review see Krohne and Franke, 1983). This allowed stripping of the nuclear envelope and yielded a fraction containing only the somewhat gelified nuclear content. Manual isolation of nuclei from stage VI oocytes (Dumont, 1972) was in “5:1 buffer” as described (Krohne and Franke, 1983), followed by fixation in ethanol. Total oocytes or enucleated ooplasm were transferred into a microcentrifuge tube and excess buffer was removed. The oocytes were resuspended in a small volume of “5:1 buffer” supplemented with $1 \times$ “complete protease inhibitors” (Roche Diagnostics, Mannheim, Germany) and homogenized by pipetting up and down in a narrow bore pipette. Homogenates were centrifuged at $13,000 \times g$ for 10 min at 4°C . To examine the efficiency of the removal of follicle epithelial cells from the oocytes, the total cytoskeletal material of collagenase-treated and untreated oocytes was separated by SDS-PAGE and probed in Western blots with the mAb, Vim 3B4, which specifically recognized vimentin, a marker protein for follicle cells (Herrmann *et al.*, 1989). Large-scale isolation of nuclei from mature (stages IV–VI) *X. laevis* oocytes was carried out as described by Scalenghe *et al.* (1978) with the modifications of Kleinschmidt and Franke (1982). Subsequent fractionation of nuclear contents into low-speed pellet (LSP), high-speed pellet (HSP), and high-speed supernatant (HSS) was as described by Hügle *et al.* (1985). LSP fractions were cleared from yolk proteins by Freon extraction (Evans and Kay, 1991). Egg extracts were prepared as described by Cordes *et al.* (1993), and small scale preparations of nuclear extracts from A6 cells were done according to the method of Lee and Green (1990).

Sucrose Gradient Centrifugation

The supernatant fractions were directly loaded on top of a 5–30% linear sucrose gradient buffered with “extraction buffer” (80 mM KCl, 20 mM NaCl, 15 mM HEPES, pH 7.5, 5 mM EDTA, 1.0 mM DTT, 250 mM sucrose). Centrifugation was performed in a SW40 rotor (Beckman Instruments, Munich, Germany) at 35,000 rpm for 19 h at 4°C . Sixteen fractions of 0.8 ml each were collected from top to bottom of the gradient and tested by immunoblotting. Extracts were also layered on linear 10–40% sucrose gradients in 5:1 buffer (80 mM KCl, 20 mM NaCl, 10 mM Tris-HCl, pH 7.4, $1 \times$ Complete protease inhibitors). After centrifugation, fractions of 0.4 ml were

collected from top to bottom. Marker proteins (bovine serum albumin [BSA], catalase, thyroglobulin; all from Sigma) or ribosomal subunits from *X. laevis* ovaries were used in parallel.

Immunoselection Experiments

Immunoselection was performed with Dynabeads (Dyna, Hamburg, Germany) coated with antibodies specific to mouse IgG. Samples were cleared by addition of Dynabeads for 2 h on a rotating wheel at 4°C. The beads were then separated, and the supernatants were transferred to a tube containing Dynabeads preloaded with symplekin-specific mAb Sym-Nu. After incubation overnight at 4°C on a rotating wheel, the Dynabeads were washed four times in ice-cold buffer (140 mM NaCl, 5 mM EDTA, 20 mM HEPES, pH 7.5, 1% Nonidet-P40), then boiled in sample buffer, processed by SDS-PAGE, and either stained with Coomassie Brilliant Blue or blotted to PVDF membranes. As a control, the Dynabeads used for preclearing were processed in parallel. Protein bands were excised from the gel and cut into 1 × 1-mm pieces that were washed twice with deionized water, 50% acetonitrile/water 1:1, and acetonitrile. Proteins were digested with sequencing grade modified trypsin (Promega, Mannheim, Germany) in 40 mM ammonium bicarbonate at 37°C overnight. The reaction was stopped by freezing.

MALDI Mass Spectrometry

MALDI mass spectra were recorded in the positive ion reflector mode with delayed extraction on a Reflex II time-of-flight instrument (Bruker-Daltonik GmbH, Bremen, Germany) equipped with a SCOUT multiprobe inlet and a 337-nm nitrogen laser. Ion acceleration voltage was set to 20.0 kV, the reflector voltage was set to 21.5 kV, and the first extraction plate was set to 15.4 kV. Mass spectra were obtained by averaging 50 to 200 individual laser shots. Calibration of the spectra was performed internally by a two-point linear fit using the autolysis products of trypsin at *m/z* 842.50 and *m/z* 2211.10. For the mass spectrometric analysis of tryptic digests MALDI samples were prepared on thin film spots (Jensen *et al.*, 1996).

Post-Source Decay Analysis

Post-source decay (PSD) analysis was performed in the positive ion reflector mode with delayed extraction by setting an ion gate width of 40 Da around the ion of interest. Data were acquired in 14 segments by decreasing the reflector voltage in a stepwise manner. For each segment 100–200 individual laser shots were accumulated. The fragment ion spectrum was obtained by pasting together all segments to a single spectrum using the FAST software provided by Bruker. Fragment ion calibration was performed externally with the fragment masses of the adrenocorticotrophic hormone (ACTH) 18–39 clip. Sample preparation for PSD analysis was achieved by cocrystallization of matrix with ZipTip C18 (Millipore, Bedford, MA) concentrated samples (Regula *et al.*, 2000).

Database Search

Singly charged monoisotopic peptide masses were used as inputs for database searching. Searches were performed against the NCBIInr database using the ProFound search algorithm (<http://129.85.19.192/prowl/cgi/ProFound.exe>) and the Protein prospector software developed at the University of California, San Francisco, (<http://prospector.ucsf.edu>). Isoelectric points were allowed to range from 0 to 14, and the oxidation of methionine was included as possible modification. Up to one missed tryptic cleavage was considered, and the mass tolerance for the monoisotopic peptide masses was set to ± 100 ppm or ± 0.1 Da.

Searches with fragment masses from PSD experiments were performed against the NCBIInr database using the MS-Tag search algorithm provided by the Protein prospector software package. Parent

mass tolerance was set to ± 0.1 Da and fragment ion tolerance was set to ± 0.7 Da.

Immunofluorescence Microscopy

For immunofluorescence microscopy studies of cultured cells, cells grown on coverslips were fixed in methanol (5 min, -20°C), followed by acetone (30 s, -20°C), washed twice in PBS, and incubated with antibodies for 20 min at room temperature. After several PBS washes, cells were incubated for 20 min with the appropriate secondary antibodies, washed in PBS, dehydrated in ethanol, air-dried, and mounted in Fluoromount (Biozol, Eching, Germany).

Cryosections (5 μm) of frozen tissues were fixed either in acetone (-20°C) for 10 min or in PBS with 1 mM MgCl_2 (PBS- MgCl_2) containing 2% formaldehyde for 10 min at room temperature. Formaldehyde-fixed samples were washed in PBS- MgCl_2 containing 50 mM NH_4Cl for 5–10 min and twice for 5 min in PBS- MgCl_2 before incubation with antibodies. In some experiments cells were stained with 4',6-diaminidino-2-phenylindole (DAPI, 0.1 $\mu\text{g}/\text{ml}$; Serva, Heidelberg, Germany) for 5 min during incubation with the secondary antibodies. Micrographs were taken with an Axiophot microscope (Zeiss, Jena, Germany).

Confocal laser scanning immunofluorescence microscopy was done on a Zeiss LSM 410 UV instrument (Zeiss). For simultaneous double-label fluorescence, an argon ion laser operating at 488 nm and a helium-neon laser operating at 543 nm were used together with a band-pass filter combination of 510–525 nm and 590–610 nm for visualization of Cy-2 and Cy-3 fluorescence.

RESULTS

Immunofluorescence Microscopy

Symplekin antibody mAb Sym-TJ-E150 reacted with cell-cell junctions of cultures of human CaCo2 cells, corresponding to tight junction markers such as occludin and protein ZO-1 (Keon *et al.*, 1996) as well as throughout the karyoplasm (Figure 1A), whereas mAb Sym-Nu specifically recognized only the nuclear form of symplekin (Figure 1B). Gradual focusing through such nuclei allowed the resolution of individual granular structures, leaving the nucleoli negative. In dividing cells, symplekin staining with mAb Sym-TJ-E150 was still positive at the tight junction plaques (Figure 1A, inset), whereas the nuclear form was dispersed throughout the cytoplasm.

A similarly intense karyoplasmic immunofluorescence was observed in cultured *Xenopus* kidney epithelial cells (Figure 1, C and C'). On frozen sections of *Xenopus* tissues, intense immunofluorescence was seen in nuclei of all the different cell types examined, including epidermal keratinocytes of all layers of the skin (Figure 1, D–D'), epithelial cells of glands and ducts, fibroblasts, and other dermal cells in the skin (our unpublished results), cardiomyocytes, endothelial cells, and erythrocytes of heart tissue (Figure 1, E and E'). Because of the unexpected reaction in *Xenopus* erythrocytes, in which transcriptional and replicational activities are notoriously low or absent, we further examined whole mount preparations of erythrocytes in blood smears. As shown in Figure 1F, the nuclei of erythrocytes were clearly positive for symplekin, indicating that this protein is a general nuclear constituent and that its presence is not directly depending on ongoing nuclear RNA synthesis activities.

Figure 1. Immunofluorescence microscopy of symplekin on cultured human and *Xenopus laevis* cell lines, frozen tissue sections, and blood smear preparations of *Xenopus*, demonstrating dual localization in nuclear and tight junctional forms. (A and B) Human colon carcinoma CaCo2 cells reacted with mAb Sym-TJ-E150 (A) or mAb Sym-Nu (B), showing dual (A) or exclusively nuclear (B) localization. The inset in A shows the distribution of symplekin during mitosis. (C and C') Cultured *X. laevis* kidney epithelium XLKE cells, line A6 (C, epifluorescence with mAb Sym-Nu; C', phase contrast). (D–D'') *Xenopus* skin tissue, showing nuclear immunofluorescence in epidermal keratinocytes (D, epifluorescence with mAb Sym-Nu; D', nuclear DAPI staining; D'', phase contrast). (E and E') *Xenopus* heart tissue, including numerous cardiomyocyte nuclei and a blood vessel with positive erythrocyte nuclei (E, epifluorescence with mAb Sym-Nu; E', DAPI staining). (F) Blood smear preparation, showing the nuclear immunoreaction in the transcriptionally almost fully inactive erythrocyte nuclei. Bars, 20 μm ; Inset, 10 μm .

Figure 2. Immunoblot detection of symplekin in *Xenopus* (*X.l.*) oocyte extracts. Total cell proteins of human CaCo2 cells (CaCo, lane 1) are compared with proteins of total *X.l.* oocyte extracts (T), cytoplasmic extracts from enucleated oocytes ("ooplasm," C, equivalent to 0.5 ooplasm), total proteins from hand-isolated *Xenopus* oocyte nuclei (NU₁, equivalent to one nucleus), total cell proteins from *Xenopus* XLKE-A6 cell culture (A6, total proteins), total proteins from five hand-isolated *X.l.* oocyte nuclei (NU₅) side by side with the nuclear contents of five nuclei (nuclei devoid of nuclear envelopes, NC, last lane). Extracts were separated by SDS-PAGE and analyzed by immunoblotting with mAb Sym-Nu. Note the reaction of a single band at ca. 140 kDa. In lanes 2 and 6 no sample was loaded. The bars correspond to reference proteins with 212, 158, 116, 97, 66, 56, and 42 kDa (from top to bottom).

Symplekin in *Xenopus* Oocytes

To study the nuclear structures containing symplekin, we decided to examine *Xenopus* oocytes as its large nucleus

("germinal vesicle") can be isolated with minimal cytoplasmic contamination. We prepared extracts of total *Xenopus* oocytes, manually isolated their nuclei or ooplasmic material from enucleated oocytes, and analyzed the fractions obtained side-by-side by SDS-PAGE, followed by immunoblotting with symplekin-specific mAbs (Figure 2). A protein band of the typical size (M_r 150,000 as introduced for mammalian cells; cf. Keon *et al.*, 1996, or slightly lower corresponding to ca. 140,000) was selectively reactive in total cell lysates from human CaCo2 and *Xenopus* A6 cells and in extracts from total *Xenopus* oocytes (Figure 2, compare CaCo, T, and A6), indicating that this band contains the *Xenopus* homologue to human symplekin. This band was also detected in samples from manually isolated nuclei and in extracts of enucleated oocytes. The protein reacting with the symplekin-specific antibodies seemed to be present in rather large amounts in *Xenopus* oocytes, as the material from one or two hand-isolated nuclei and one ooplasm was sufficient to yield a strong signal. Moreover, this protein was enriched in the nuclear content, as comparable amounts were detected in hand-isolated nuclei and in a fraction of nuclear contents from which the nuclear envelopes had been removed (compare Figure 2, NU₅ and NC).

The fractionation of particles from isolated *Xenopus* oocyte nuclei by differential centrifugation leads to well-defined subnuclear fractions (Figure 3, left part; cf. Hügler *et al.*, 1985; Schmidt-Zachmann *et al.*, 1998). When the distribution of symplekin in such fractions from oocyte nuclei, egg extracts and somatic cells was analyzed by immunoblotting (Figure 3, A and B), symplekin was detected in total oocyte nuclei, in the HSP and HSS fractions and in egg extracts. For SDS-PAGE equal volumes of LSP, HSP, and HSS fractions were loaded. As the two pellets (LSP and HSP; Figure 3) were solubilized in small volumes their material was consequently concentrated, with respect to the HSS fraction. Thus, we concluded that the major proportion of symplekin was

Figure 4. Immunoselected proteins of symplekin-containing particles present in the HSS fraction from *Xenopus* oocyte nuclei (A) and from egg extracts (B). The precipitates were solubilized in sample buffer, separated by SDS-PAGE and stained with Coomassie Brilliant Blue. The prominent protein bands (lane 2) were analyzed by peptide mass fingerprinting. In addition to symplekin, 3 out of 4 proteins of the CPSF complex were identified as CPSF-160, CPSF-100, and CPSF-73. Lane 1, reference proteins (molecular mass values are indicated on the left).

recovered in the HSS fraction containing nuclear proteins in soluble form or in small particles. In addition, some symplekin was bound to larger nucleoplasmic particles, as indicated by the reaction in the HSP fraction. The fractions were also characterized by immunoblotting with mAb No-185 against nucleolar protein NO38, known to be enriched in the LSP and HSP but absent from the HSS fraction (Schmidt-Zachmann *et al.*, 1998), and by immunoblotting with mAb H1 against coilin. The protein coilin was enriched in the HSP fraction, minor amounts were also detected in the LSP and HSS fractions (our unpublished results).

Association of Symplekin with Cleavage and Polyadenylation Specificity Factors

The HSS fraction obtained from *Xenopus* oocyte nuclei was used for immunoselection to obtain symplekin-containing protein complexes. Material bound to Dynabeads loaded with symplekin antibodies was solubilized with sample buffer and separated by 10% SDS-PAGE. After staining with Coomassie Brilliant Blue, five major protein bands, ranging from 70 to 160 kDa, were visible among the immunoselected proteins (Figure 4A), whereas no such enrichment was seen after preclearing of the lysate (unpublished results). By mass spectrometric sequencing using PSD analysis, two amino acid sequences were identified in the protein band of ca. 140 kDa: RRPEIIPVTQGR and DPLLAHVLR. From database search these sequences were identified as homologous to the tryptic peptides 521–533 and 1073–1080 of human symplekin (Keon *et al.*, 1996; Ueki *et al.*, 1997), with exchanges of amino acid P532 to G and I1073 to D, respectively. The other proteins present in these immunoprecipitates were identified as the *Xenopus* proteins CPSF-160, CPSF-100, and CPSF-

73, respectively, and these identifications were verified by immunoblotting with specific antibodies. Interestingly, the two protein bands of ~95 and 100 kDa were both identified as CPSF-100, corresponding to a recent report showing the existence of two forms of CPSF-100 in *X. laevis* oocytes (Dickson *et al.*, 1999).

Because all three proteins were known as constituents of the protein complex named CPSF (Bienroth *et al.*, 1991; Murthy and Manley, 1992; for review see Wahle and Rügsegger, 1999), we concluded that symplekin is also a component of this complex. A fourth polypeptide of 30 kDa that had also been described for this complex (Bienroth *et al.*, 1991) was not identified in these immunoprecipitates, perhaps because it was obscured by the large amount of immunoglobulin light chains.

We also wondered to which proteins symplekin was associated in eggs, i.e., after the breakdown of the nuclear envelope, and examined egg extracts obtained by immunoselection with symplekin antibodies. After staining with Coomassie Brilliant Blue, four major polypeptide bands, ranging from 70–160 kDa, were visible (Figure 4B). By mass spectrometric analysis the proteins of 160, 100, and 70 kDa were identified as CPSF-160, CPSF-100, and CPSF-73, respectively. The protein band of ~140 kDa showed a spectrometric pattern of fragments similar to that of the protein identified as symplekin. Moreover, by mass spectrometric sequencing using PSD analysis, the same amino acid sequence RRPEIIPVTQGR was determined as mentioned for the tryptic fragment of nuclear symplekin (peptide 521–533; see above). The 160-kDa protein band showed an unexpectedly intense dye staining, indicative of either supraproportional amounts of the CPSF-160 subunit or the coincidence of an additional yet unidentified protein.

To analyze the physical state of symplekin-containing material in the HSS fraction of fractionated *Xenopus* oocyte nuclei, this fraction was further subjected to centrifugation in 5–30% sucrose gradients, and the resulting fractions were analyzed by SDS-PAGE and immunoblotting using PVDF membranes (Figure 5A). Symplekin was recovered in fractions 4–8, with a maximum in fractions 6 and 7, corresponding to ~11S. When the PVDF membranes were reprobed with antibodies against protein CPSF-100, two protein bands of ~100 and 95 kDa were decorated. The 100-kDa form of CPSF-100 was found in fractions 6–8, with a maximum between fractions 6 and 7, corresponding to the distribution of symplekin. The 95-kDa version of CPSF-100 was detected in fractions 7–9, with a maximum in fractions 7 and 8 (Figure 5A'). An identical distribution was found with CPSF-73 antibodies (unpublished results).

As a smaller proportion of the nuclear symplekin was recovered in the HSP fraction, the protein material of the pellet was resuspended and separated by sucrose gradient centrifugation. Some symplekin was again recovered in fractions corresponding to 10–14S, whereas the main portion was detected in the pellet, indicative of its association with relatively large structures (for Cajal bodies see below).

As the *Xenopus* oocyte is a highly specialized cell, we also used nuclear extracts from cultured *Xenopus* cells of line A6 (cf. Figure 1C) for sucrose gradient centrifugation. Here most of the symplekin present could also be detected in fractions corresponding to 10–11S (unpublished results).

Concentration of Symplekin in Cajal Bodies

To examine the intranuclear localization of symplekin, we performed immunocytochemistry on cryosections through *Xenopus laevis* ovaries. Using symplekin-specific antibodies, distinct intranuclear, nonnucleolar bodies of diameters of 5–10 μm were intensely stained (Figure 6A). Double-label experiments with CPSF-100 antibodies revealed a clear colocalization of both proteins (Figure 6C). Moreover, double-labeling with coilin-specific antibodies also showed colocalization, thus indicating that symplekin is concentrated in Cajal bodies, as has also been described for CPSF-100 and other factors of the 3'-end pre-mRNA processing complex by Gall and coworkers (Gall *et al.*, 1999; Gall, 2000; Morgan *et al.*, 2000). Using standard protocols, a weak immunofluorescence with symplekin antibodies was also seen throughout the karyoplasm. However, the intense immunolocalization of symplekin in the Cajal bodies seems to represent only a minor portion of the total nuclear symplekin, as indicated by our estimations from recovery experiments that only a small proportion of the nuclear symplekin is contained in HS pelletable structures (for general difficulties of demonstrating even most abundant extractable karyoplasmic proteins and particles such as actin, histones, nucleoplasm, and their complexes see, e.g., Krohne and Franke, 1980b; Ankenbauer *et al.*, 1989; Hofmann *et al.*, 2001).

Symplekin Complexes in Cytoplasmic Extracts of Enucleated Oocytes

Unexpectedly, a large proportion of the oocyte's symplekin was found in extracts from "ooplasm," i.e., enucleated oocytes (see Figure 2). To prove that hand-isolated, enucleated

Figure 5. Analysis of the native state of the particles containing proteins symplekin (A) and CPSF-100 (A') by sucrose gradient centrifugation. The HSS fraction from *Xenopus* oocyte nuclei was analyzed by sucrose gradient centrifugation, fractions were collected from top to bottom, and the portions analyzed by immunoblotting either with mAb Sym-Nu (A) or CPSF-100-specific antibodies (A'). Reference proteins were BSA (4.3S, fraction 3), catalase (11.3S, fraction 6) and thyroglobulin (16.5S, fraction 9). The points correspond to SDS-PAGE reference proteins with 212, 158, 116, 97, 66, and 56 kDa (from top to bottom).

oocytes were not contaminated with follicle cells or remnants thereof, we performed immunoblotting with antibodies specific for vimentin as a marker of follicle cells (cf. Herrmann *et al.*, 1989). These controls revealed that cytoskeletal preparations of collagenase-treated oocytes contained only traces if any vimentin (our unpublished results). Cytoplasmic extracts from enucleated oocytes were used for immunoselection (Figure 7). When the material bound to Dynabeads loaded with antibodies to symplekin was solubilized and separated by SDS-PAGE, enrichment of symplekin (Figure 7A) and protein CPSF-100 (Figure 7B) was detected by immunoblotting. This again showed that the immunoselected symplekin complexes also contained protein CPSF-100 (compare lane 3 in Figure 7, A and B).

To determine the physical state of this symplekin-containing cytoplasmic material, sucrose gradient centrifugation was applied, and the resulting fractions were analyzed by SDS-PAGE, followed by immunoblotting for symplekin (Figure 8). Symplekin was distributed in three classes, one in fractions 5–8 with a peak in fraction 6, corresponding to ca. 11S, and the other in fractions 24 and 25, corresponding to particles larger than 65S, and some material accumulated in the last gradient fraction and the pellet.

Figure 6. Laser scanning confocal microscopy showing the result of a double-labeling experiment, comparing here the localization of symplekin (A) with that of protein CPSF-100 (B) in a cryosection through a *Xenopus laevis* ovary. The corresponding merge picture is shown in (C), the corresponding phase contrast in (D). Note that the Cajal bodies in the nuclei are strongly positive for both symplekin (A) and CPSF-100 (B), as indicated by the yellow color in (C), whereas under the immunolocalization conditions applied here only a weak and diffuse reaction is seen in the karyo- and the cytoplasm. Bar, 100 μ m.

DISCUSSION

In oocytes and eggs of *X. laevis* we have discovered and characterized distinct karyo- and cytoplasmic particles containing symplekin, a protein also described as a component of the tight junction plaque. A major result of our study is that a large part of the nuclear symplekin occurs in particles with an approximate mean peak value of 11S ("11S particles"), where it is complexed with proteins involved in mRNA biogenesis, notably 3'-end processing. Symplekin particles have also been found in egg extracts and, most surprisingly, in cytoplasmic particles of enucleated oocytes. In addition, a notable proportion of the nuclear symplekin is associated with much larger, i.e., readily pelletable structures, and this seems to include the Cajal body symplekin.

Clearly, the major part of the symplekin-containing nuclear particles can be precipitated together with CPSF subunits such as proteins CPSF-160, CPSF-100, and CPSF-73. Here, the identification of CPSF-73 presents further evidence that we have identified a nuclear protein complex, because this subunit has not been found in cytoplasmic CPSF complexes involved in regulated polyadenylation of mRNAs in *Xenopus* oocytes (Dickson *et al.*, 1999, 2001). Furthermore, the relative staining intensities of the separated polypeptides of

the immunoprecipitated particles indicate that symplekin is an iso-stoichiometric component with respect to the other CPSF proteins. The relative amount of total nuclear CPSF particle-bound symplekin may actually be even higher because we cannot exclude that pelleted material, including Cajal bodies, contains similar ~11S particles, although in a state associated with—or integrated into—larger structures (Gall *et al.*, 1999; Gall, 2000).

The finding of symplekin as a constituent of CPSF particles is not restricted to *X. laevis* oocytes and cultured A6 kidney-derived cells, as shown in the present study. In similar experiments using cell culture lines from various tissues and species, including human HeLa cells, we have also identified symplekin in association with such particles. Moreover, by immunodepletion the functional importance of symplekin for 3'-end cleavage and polyadenylation has recently been demonstrated in *in vitro* assays using extracts from HeLa cell nuclei (I. Hofmann, I. Kaufmann, W. Keller, and W.W. Franke, unpublished results). Therefore, we think it is a sensible working hypothesis that symplekin is a widespread, if not ubiquitous CPSF protein.

It is perhaps somewhat astonishing that in the numerous previous studies symplekin has been overlooked as a com-

Figure 7. Immunoselection of symplekin-containing complexes present in cytoplasmic (ooplasmic) extracts of enucleated *Xenopus* oocyte. The precipitate has been solubilized in sample buffer, separated on SDS-PAGE and transferred to a PVDF-membrane. Lane 1, sample of cytoplasmic extract from five enucleated oocytes before immunoselection; lane 2, sample of cytoplasmic extract from five enucleated oocytes after immunoselection; lane 3, precipitate obtained with mAb Sym-Nu; lane 4, control reaction obtained with the extract using beads alone. Symplekin has been detected by Western blotting using mAb Sym-Nu (A), reaction with CPSF-100 specific antibodies is shown in (B). The dots correspond to reference proteins with 158, 116, 97 kDa (from top to bottom).

ponent involved in 3'-end processing of pre-mRNA. So far six transacting factors have been listed that are required for the *in vitro* reconstitution of mammalian 3'-end processing (reviewed by Wahle and Rügsegger, 1999). Besides the symplekin-containing factor CPSF, composed of the five subunits CPSF-160, CPSF-100, CPSF-73, CPSF-30, and symplekin (cf. Bienroth *et al.*, 1991; Murthy and Manley, 1992; this study), the trimeric cleavage stimulation factor (CstF), with the subunits CstF-77, CstF-64, and CstF-50, rec-

ognizes sequence elements on the pre-mRNA (for references see Wahle and Rügsegger, 1999). In addition, cleavage factors I (CFI) and II (CFII), poly(A)-binding protein II and poly(A) polymerase are needed for 3'-end processing in vertebrates (Raabe *et al.*, 1991; Wahle, 1991; Wahle *et al.*, 1991; Bienroth *et al.*, 1993; Rügsegger *et al.*, 1996; de Vries *et al.*, 2000; Kim *et al.*, 2001).

The concept of symplekin as a constituent of the larger 3'-end cleavage and polyadenylation complex is also supported by the observation of Takagaki and Manley (2000) who, in "far Western" screens with the protein CstF-64, have identified HeLa cell symplekin as a strongly interacting protein that in solid phase and pull down assays competes for binding to CstF-64. Moreover, using immuno-affinity column chromatography on CstF-64, these authors have co-purified subunits of CstF, CPSF, and symplekin from nuclear extracts, indicating that CstF, CPSF, and symplekin are part of a large complex. That symplekin serves an important role in fundamental cellular processes is also suggested from the existence of homologues in the genomes of very distantly related species such as *Caenorhabditis elegans* (AF022973), *Drosophila melanogaster* (AE003601) and *Arabidopsis thaliana* (AL161746; these authors, unpublished results). The involvement of symplekin in 3'-end processing of premRNA is also supported by the notion of a homologue in *Saccharomyces cerevisiae*, protein PTA1p, described to be associated with the proteins of the yeast equivalent of CPSF (YHH1, YDH1, YSH1, YTH1) and to be important for cleavage and polyadenylation of pre-mRNA (Preker *et al.*, 1997; Zhao *et al.*, 1999).

Certainly, the most unexpected finding of our study is the discovery of ca. 11S complexes containing symplekin together with CPSF proteins, notably CPSF-100, in the cytoplasm. As these particles have also been obtained from manually enucleated ooplasm, a contamination by nuclear particles appears to be excluded. This finding indicates that reactions known to be involved in cytoplasmic polyadenylation (Hake and Richter, 1994; Dickson *et al.*, 1999, 2001; for review see also Wickens *et al.*, 2000) and in the regulation of translation (Stebbins-Boaz *et al.*, 1999; Mendez *et al.*, 2000; for reviews see Macdonald, 2001; Mendez and Richter, 2001) are located in symplekin-containing complexes.

Figure 8. Characterization of symplekin-containing particles in cytoplasmic extracts of enucleated *Xenopus* oocytes by sucrose gradient centrifugation. Extracts from 40 ooplasm have been subjected to sucrose gradient centrifugation, as described in Figure 5, using catalase (11.3S, fraction 6), thyroglobulin (16.5S, fraction 9) and the 40S and 60S X.l. ribosomal subunits as reference. Dots on the right margin correspond to reference proteins with 158, 116, 97, 66, and 56 kDa (from top to bottom). Note that most of the symplekin is recovered in particles with a peak value of ~11S, whereas minor portions appear with a mean peak corresponding to ~65S or in the pellet.

Interestingly, all so far characterized nuclear forms of junctional plaque proteins have in common that they are somehow involved in processes of transcription, splicing, or 3'-end processing: Plakophilin 2 has been detected in RNA polymerase III complexes, p120^{ctn} and β -catenin are involved in regulations of RNA polymerase II transcription, and protein 4.1 has been found in splicing factors (Behrens *et al.*, 1996; Huber *et al.*, 1996; Molenaar *et al.*, 1996; Krauss *et al.*, 1997; Lallena *et al.*, 1998; Daniel and Reynolds, 1999; Mertens *et al.*, 2001). Symplekin is the first protein associated with factors involved in 3'-end processing of premRNA in the nucleus as well as in cytoplasmic translational control. Future studies will help understanding the biological significance of the interactions between CPSF and their regulation and determining the functions of symplekin in oocytes as well as in somatic cells. In addition, it will be important to clarify whether the nuclear and the plaque-bound forms exist in a regulated exchange equilibrium.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Walter Keller (Biozentrum, University of Basel, Switzerland) for continuous support and advice as well as to Drs. Reimer Stick (University of Bremen, Germany) and Marion Schmidt-Zachmann (German Cancer Research Center, Heidelberg, Germany) for stimulating discussions. They also thank Jutta Osterholt for preparing the photographs and Eva Ouis for arranging the manuscript. The technical assistance of Sonja Reidenbach and the expert help of Dr. Herbert Spring with the laser scanning confocal microscopy is also gratefully acknowledged. This study has been supported in part by the Deutsche Forschungsgemeinschaft.

REFERENCES

- Ankenbauer, T., Kleinschmidt, J.A., Walsh, M.J., Weiner, O.H., and Franke, W.W. (1989). Identification of a widespread nuclear actin binding protein. *Nature* 342, 822–825.
- Balda, M.S., and Matter, K. (2000). The tight junction protein ZO-1 and an interacting transcription factor regulate ErbB-2 expression. *EMBO J.* 19, 2024–2033.
- Behrens, J. (2000). Control of β -catenin signaling in tumor development. *Ann. NY Acad. Sci.* 910, 21–33.
- Behrens, J., von Kries, J.P., Kühl, M., Bruhn, L., Wedlich, D., Grosschedl, R., and Birchmeier, W. (1996). Functional interaction of β -catenin with the transcription factor LEF-1. *Nature* 382, 638–642.
- Bienroth, S., Wahle, E., Suter-Crazzolara, C., and Keller, W. (1991). Purification of the cleavage and polyadenylation factor involved in the 3'-processing of messenger RNA precursors. *J. Biol. Chem.* 266, 19768–19776.
- Bienroth, S., Keller, W., and Wahle, E. (1993). Assembly of a processive messenger RNA polyadenylation complex. *EMBO J.* 12, 585–594.
- Bohmann, K., Ferreira, J.A., and Lamond, A.I. (1995). Mutational analysis of p80 coilin indicates a functional interaction between coiled bodies and the nucleolus. *J. Cell Biol.* 131, 817–831.
- Bonné, S., van Hengel, J., Nollet, F., Kools, P., and van Roy, F. (1999). Plakophilin-3, a novel armadillo-like protein present in nuclei and desmosomes of epithelial cells. *J. Cell Sci.* 112, 2265–2276.
- Bonner, W.M. (1975a). Protein migration into nuclei. I. Frog oocyte nuclei in vivo accumulate microinjected histones, allow entry to small proteins, and exclude large proteins. *J. Cell Biol.* 64, 421–30.

- Bonner, W.M. (1975b). Protein migration into nuclei. II. Frog oocyte nuclei accumulate a class of microinjected oocyte nuclear proteins and exclude a class of microinjected oocyte cytoplasmic proteins. *J. Cell Biol.* 64, 431–437.
- Borrmann, C. (2000). Molekulare Charakterisierung der Adhärens-Zellverbindungen des Herzens. Identifizierung einer neuen Art, der Area composita. Ph.D. Thesis. Faculty of Biology, University of Heidelberg, Germany.
- Borrmann, C.M., Mertens, C., Schmidt, A., Langbein, L., Kuhn, C., and Franke, W.W. (2000). Molecular diversity of plaques of epithelial adhering junctions. *Ann. NY Acad. Sci.* 915, 144–150.
- Cáceres, J.F., and Krainer, A.R. (1997). Mammalian pre-mRNA splicing factors. In: *Eukaryotic mRNA Processing*, ed. A.R. Krainer, Oxford, UK: IRL Press at Oxford University Press, 174–212.
- Cordes, V.C., Reidenbach, S., Köhler, A., Stuurman, N., van Driel, R., and Franke, W.W. (1993). Intranuclear filaments containing a nuclear pore complex protein. *J. Cell Biol.* 123, 1333–1344.
- Cordes, V.C., Reidenbach, S., and Franke, W.W. (1996). Cytoplasmic annulate lamellae in cultured cells: composition, distribution, and mitotic behavior. *Cell Tissue Res.* 284, 177–191.
- Daniel, J.M., and Reynolds, A.B. (1999). The catenin p120(ctn) interacts with Kaiso, a novel BTB/POZ domain zinc finger transcription factor. *Mol. Cell. Biol.* 19, 3614–3623.
- de la Cruz, J., Kressler, D., and Linder, P. (1999). Unwinding RNA in *Saccharomyces cerevisiae*: DEAD-box proteins and related families. *Trends Biochem. Sci.* 24, 192–198.
- De Robertis, E.M., Longthorne, R.F., and Gurdon, J.B. (1978). Intracellular migration of nuclear proteins in *Xenopus* oocytes. *Nature* 272, 254–256.
- de Vries, H., Rügsegger, U., Hübner, W., Friedlein, A., Langen, H., and Keller, W. (2000). Human pre-mRNA cleavage factor II_m contains homologs of yeast proteins and bridges two other cleavage factors. *EMBO J.* 19, 5895–5904.
- Dickson, K.S., Bilger, A., Ballantyne, S., and Wickens, M.P. (1999). The cleavage and polyadenylation specificity factor in *Xenopus laevis* oocytes is a cytoplasmic factor involved in regulated polyadenylation. *Mol. Cell. Biol.* 19, 5707–5717.
- Dickson, K.S., Thompson, S.R., Gray, N.K., and Wickens, M. (2001). Poly(A) polymerase and the regulation of cytoplasmic polyadenylation. *J. Biol. Chem.* 276, 41810–41816.
- Dumont, J.N. (1972). Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory-maintained animals. *J. Morphol.* 136, 153–164.
- Evans, J.P., and Kay, B.K. (1991). Biochemical fractionation of oocytes. *Methods Cell Biol.* 26, 133–146.
- Funayama, N., Fagotto, F., McCrea, P., and Gumbiner, B.M. (1995). Embryonic axis induction by the *armadillo* repeat domain of β -catenin: evidence for intracellular signaling. *J. Cell Biol.* 128, 959–968.
- Gall, J.G. (2000). Cajal bodies: the first 100 years. *Annu. Rev. Cell Dev. Biol.* 16, 273–300.
- Gall, J.G., Bellini, M., Wu, Z., and Murphy, C. (1999). Assembly of the nuclear transcription and processing machinery: Cajal bodies (coiled bodies) and transcriptosomes. *Mol. Biol. Cell* 10, 4385–4402.
- Gottardi, C.J., Arpin, M., Fanning, A.S., and Louvard, D. (1996). The junction-associated protein, zonula occludens-1, localizes to the nucleus before the maturation and during the remodeling of cell-cell contacts. *Proc. Natl. Acad. Sci. USA* 93, 10779–10784.
- Hake, L.E., and Richter, J.D. (1994). CPEB is a specificity factor that mediates cytoplasmic polyadenylation during *Xenopus* oocyte maturation. *Cell* 79, 617–627.

- Herrmann, H., Fouquet, B., and Franke, W.W. (1989). Expression of intermediate filament proteins during development of *Xenopus laevis*. I. cDNA clones encoding different forms of vimentin. *Development* 105, 279–298.
- Hofmann, W., Reichart, B., Ewald, A., Müller, E., Schmitt, I., Stauber, R.H., Lottspeich, F., Jockusch, B.M., Scheer, U., Hauber, J., and Dabauvalle, M.-C. (2001). Cofactor requirements for nuclear export of Rev response element (RRE)- and constitutive transport element (CTE)-containing retroviral RNAs: an unexpected role for actin. *J. Cell Biol.* 152, 895–910.
- Huber, O., Korn, R., McLaughlin, J., Ohsugi, M., Herrmann, B.G., and Kemler, R. (1996). Nuclear localization of β -catenin by interaction with transcription factor LEF-1. *Mech. Dev.* 59, 3–10.
- Hübner, S., Jans, D.A., and Drenckhahn, D. (2001). Roles of cytoskeletal and junctional plaque proteins in nuclear signaling. *Int. Rev. Cytol.* 208, 207–265.
- Hügler, B., Scheer, U., and Franke, W.W. (1985). Ribocharin: a nuclear Mr 40,000 protein specific to precursor particles of the large ribosomal subunit. *Cell* 41, 615–627.
- Jenny, A., Hauri, H.-P., and Keller, W. (1994). Characterization of cleavage and polyadenylation specificity factor and cloning of its 100-kilodalton subunit. *Mol. Cell. Biol.* 14, 8183–8190.
- Jenny, A., Minvielle-Sebastia, L., Preker, P.J., and Keller, W. (1996). Sequence similarity between the 73-kilodalton protein of mammalian CPSF and a subunit of yeast polyadenylation factor I. *Science* 274, 1514–1517.
- Jensen, O.N., Podtelejnikov, A., and Mann, M. (1996). Delayed extraction improves specificity in database searches by matrix-assisted laser desorption/ionization peptide maps. *Rapid Commun. Mass Spectrom.* 10, 1371–1378.
- Karnovsky, A., and Klymkowsky, M.W. (1995). Anterior axis duplication in *Xenopus* induced by the over-expression of the cadherin-binding protein plakoglobin. *Proc. Natl. Acad. Sci. USA* 92, 4522–4526.
- Keon, B.H., Schäfer, S., Kuhn, C., Grund, C., and Franke, W.W. (1996). Symplekin, a novel type of tight junction plaque protein. *J. Cell Biol.* 134, 1003–1018.
- Kim, J., Choi, S.-C., Chang, J.-Y., and Han, J.-K. (2001). Poly(A) binding protein II in *Xenopus laevis* is expressed in developing brain and pancreas. *Mech. Dev.* 109, 111–114.
- Kleinschmidt, J.A., and Franke, W.W. (1982). Soluble acidic complexes containing histones H3 and H4 in nuclei of *Xenopus laevis* oocytes. *Cell* 29, 799–809.
- Kleinschmidt, J.A., Scheer, U., Dabauvalle, M.-C., Bustin, M., and Franke, W.W. (1983). High mobility group proteins of amphibian oocytes: a large storage pool of a soluble high mobility group-1-like protein and involvement in transcriptional events. *J. Cell Biol.* 97, 838–848.
- Krauss, S.W., Larabell, C.A., Lockett, S., Gascard, P., Penman, S., Mohandas, N., and Chasis, J.A. (1997). Structural protein 4.1 in the nucleus of human cells: dynamic rearrangements during cell division. *J. Cell Biol.* 137, 275–289.
- Krohne, G., and Franke, W.W. (1980a). A major soluble acidic protein located in nuclei of diverse vertebrate species. *Exp. Cell Res.* 129, 167–189.
- Krohne, G., and Franke, W.W. (1980b). Immunological identification and localization of the predominant nuclear protein of the amphibian oocyte nucleus. *Proc. Natl. Acad. Sci. USA* 77, 1034–1038.
- Krohne, G., and Franke, W.W. (1983). Proteins of pore complex-lamina structures from nuclei and nuclear membranes. *Methods Enzymol.* 96, 597–608.
- Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–685.
- Lallena, M.J., Martinez, C., Valcarcel, J., and Correas, I. (1998). Functional association of nuclear protein 4.1 with pre-mRNA splicing factor. *J. Cell Sci.* 111, 1963–1971.
- Lee, K.A.W., and Green, M.R. (1990). Small-scale preparation of extracts from radiolabeled cells efficient in pre-mRNA splicing. *Methods Enzymol.* 181, 20–30.
- Macdonald, P. (2001). Diversity in translational regulation. *Curr. Opin. Cell Biol.* 13, 326–331.
- Mandai, K., Nakanishi, H., Satoh, A., Obaishi, H., Wada, M., Nishioka, H., Itoh, M., Mizoguchi, A., Aoki, T., Fujimoto, T., Matsuda, Y., Tsukita, S., and Takai, Y. (1997). Afadin: a novel actin filament-binding protein with one PDZ domain localized at cadherin-based cell-to-cell adherens junction. *J. Cell Biol.* 139, 517–528.
- Mariner, D.J., Wang, J., and Reynolds, A.B. (2000). ARVCF localizes to the nucleus and adherens junction and is mutually exclusive with p120^{cas} in E-cadherin complexes. *J. Cell Sci.* 113, 1481–1490.
- Mendez, R., and Richter, J.D. (2001). Translational control by CPEB: a means to the end. *Mol. Cell. Biol.* 2, 521–529.
- Mendez, R., Murthy, K.G.K., Ryan, K., Manley, J.L., and Richter, J.D. (2000). Phosphorylation of CPEB by Eg2 mediates the recruitment of CPSF into an active cytoplasmic polyadenylation complex. *Mol. Cell* 6, 1253–1259.
- Mertens, C., Kuhn, C., and Franke, W.W. (1996). Plakophilins 2a and 2b: constitutive proteins of dual location in the karyoplasm and the desmosomal plaque. *J. Cell Biol.* 135, 1009–1025.
- Mertens, C., Hofmann, I., Wang, Z., Teichmann, M., Sepehri Chong, S., Schnölzer, M., and Franke, W.W. (2001). Nuclear particles containing RNA polymerase III complexes associated with the junctional plaque protein plakophilin 2. *Proc. Natl. Acad. Sci. USA* 98, 7795–7800.
- Molenaar, M., van de Wetering, M., Oosterwegel, M., Peterson-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destree, O., and Clevers, H. (1996). XTcf-3 transcription factor mediates β -catenin-induced axis formation in *Xenopus* embryos. *Cell* 86, 391–399.
- Morgan, G.T., Doyle, O., Murphy, C., and Gall, J.G. (2000). RNA polymerase II in Cajal bodies of amphibian oocytes. *J. Struct. Biol.* 129, 258–268.
- Murthy, K.G.K., and Manley, J.L. (1992). Characterization of the multisubunit cleavage-polyadenylation specificity factor from calf thymus. *J. Biol. Chem.* 267, 14804–14811.
- Nakamura, T., Blechman, J., Tada, S., Rozovskaia, T., Itoyama, T., Bullrich, F., Mazo, A., Croce, C.M., Geiger, B., and Canaani, E. (2000). huASH1 protein, a putative transcription factor encoded by a human homologue of the *Drosophila* ash1 gene, localizes to both nuclei, and cell-cell tight junctions. *Proc. Natl. Acad. Sci. USA* 97, 7284–7289.
- Peters, J.-M., Walsh, M.J., and Franke, W.W. (1990). An abundant and ubiquitous homo-oligomeric ring-shaped ATPase particle related to the putative vesicle fusion proteins Sec18p and NSF. *EMBO J.* 9, 1757–1767.
- Peters, J.-M., Franke, W.W., and Kleinschmidt, J.A. (1994). Distinct 19 S and 20 S subcomplexes of the 26 S proteasome and their distribution in the nucleus and the cytoplasm. *J. Biol. Chem.* 269, 7709–7718.
- Preker, P.J., Ohnacker, M., Minvielle-Sebastia, L., and Keller, W. (1997). A multisubunit 3' end processing factor from yeast containing poly(A) polymerase and homologues of the subunits of mammalian cleavage and polyadenylation specificity factor. *EMBO J.* 16, 4727–4737.

- Raabe, T., Bollum, F.J., and Manley, J.L. (1991). Primary structure and expression of bovine poly(A) polymerase. *Nature* 353, 229–234.
- Regula, T., Ueberle, B., Boguth, G., Görg, A., Schnölzer, M., Herrmann, R., and Frank, R. (2000). Towards a two-dimensional proteome map of *Mycoplasma pneumoniae*. *Electrophoresis* 21, 3765–3780.
- Rüegsegger, U., Beyer, K., and Keller, W. (1996). Purification and characterization of human cleavage factor I_m involved in the 3' end processing of messenger RNA precursors. *J. Biol. Chem.* 271, 6107–6113.
- Scalenghe, F., Buscaglia, M., Steinheil, C., and Crippa, M. (1978). Large scale isolation of nuclei and nucleoli from vitellogenic oocytes in *Xenopus laevis*. *Chromosoma* 66, 299–308.
- Schmidt, A., Langbein, L., Rode, M., Prätzel, S., Zimbelmann, R., and Franke, W.W. (1997). Plakophilins 1a and 1b: widespread nuclear proteins recruited in specific epithelial cells as desmosomal plaque components. *Cell Tissue Res.* 290, 481–499.
- Schmidt, A., Langbein, L., Prätzel, S., Rode, M., Rackwitz, H.-R., and Franke, W.W. (1999). Plakophilin 3—a novel cell-type-specific desmosomal plaque protein. *Differentiation* 64, 291–306.
- Schmidt-Zachmann, M.S., Hügler-Dörr, B., and Franke, W.W. (1987). A constitutive nucleolar protein identified as a member of the nucleoplasmin family. *EMBO J.* 6, 1881–1890.
- Schmidt-Zachmann, M.S., Knecht, S., and Krämer, A. (1998). Molecular characterization of a novel, widespread nuclear protein that colocalizes with spliceosome components. *Mol. Biol. Cell* 9, 143–160.
- Schneider, S., Steinbeisser, H., Warg, R.M., and Hausen, P. (1996). β -Catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. *Mech. Dev.* 57, 191–198.
- Stebbins-Boaz, B., Cao, Q., de Moor, C.H., Mendez, R., and Richter, J.D. (1999). Maskin is a CPEB-associated factor that transiently interacts with eIF-4E. *Mol. Cell* 4, 1017–1027.
- Stevenson, B.R., and Keon, B.H. (1998). The tight junction: morphology to molecules. *Annu. Rev. Cell Dev. Biol.* 14, 89–109.
- Takagaki, Y., and Manley, J.L. (2000). Complex protein interactions within the human polyadenylation machinery identify a novel component. *Mol. Cell. Biol.* 20, 1515–1525.
- Tuma, R.S., Stolk, J.A., and Roth, M.B. (1993). Identification and characterization of a sphere organelle protein. *J. Cell Biol.* 122, 767–773.
- Ueki, K., Ramaswamy, S., Billings, S.J., Mohrenweiser, H.W., and Louis, D.N. (1997). Chromosomal localization to 19q13.3., partial genomic structure and 5' cDNA sequence of the human symplekin gene. *Somatic Cell Mol. Gen.* 23, 229–231.
- Wahle, E. (1991). A novel poly(A)-binding protein acts as a specificity factor in the second phase of messenger RNA polyadenylation. *Cell* 66, 759–768.
- Wahle, E., and Rüegsegger, U. (1999). 3'-End processing of pre-mRNA in eukaryotes. *FEMS Microbiol. Rev.* 23, 277–295.
- Wahle, E., Martin, G., Schiltz, E., and Keller, W. (1991). Isolation and expression of cDNA clones encoding mammalian poly(A) polymerase. *EMBO J.* 10, 4251–4257.
- Wickens, M., Goodwin, E.B., Kimble, J., Strickland, S., and Hentze, M. (2000). Translational control of developmental decisions. In: *Translational Control of Gene Expression*, eds. N. Sonenberg, J.W.B. Hershey, and M.B. Mathews, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 295–370.
- Yost, C., Torres, M., Miller, J.R., Huang, E., Kimelman, D., and Moon, R.T. (1996). The axis-inducing activity, stability, and subcellular distribution of β -catenin is regulated in *Xenopus* embryos by glycogen synthase kinase 3. *Genes Dev.* 10, 1443–1454.
- Zhao, J., Kessler, M., Helmling, S., O'Connor, J.P., and Moore, C. (1999). Pta1, a component of yeast CF II, is required for both cleavage and poly(A) addition of mRNA precursor. *Mol. Cell. Biol.* 19, 7733–7740.