The development of spermatids into spermatozoa, termed spermiogenesis, is characterized by the replacement of histones by the highly basic, arginine-rich, protamines (1). As a result of this exchange, the nucleosomal-type chromatin is transformed into a smooth fiber and compacted in a volume of about 5% of that of a somatic cell nucleus (2, 3). Although the exchange of chromatin proteins during spermiogenesis has long been known, the molecular mechanisms and the signaling pathways governing the histone to protamine transition have remained obscure.

The deposition of protamines on sperm chromatin and the subsequent chromatin condensation appear to be controlled by phosphorylation-dephosphorylation events. Protamines are highly phosphorylated, shortly after their synthesis and before binding to DNA, whereas they become largely dephosphorylated during sperm maturation (4–8). Phosphorylation of P2 protamine has been shown to be essential, because deletion of the calmodulin-dependent protein kinase Camk4, which phosphorylates P2 protamine, impairs the replacement of transition protein-2 with P2 protamine, resulting in defective spermiogenesis and male sterility (9). On the other hand, all P1 protamines contain short arginine-serine-rich (RS) domains that are efficiently phosphorylated by SRPK1 (SR protein kinase 1) (10), but the physiological significance of this modification is mostly unknown.

In this respect, Biggiogera et al. (11) reported that protamines initially appear at the nuclear periphery, implying that the nuclear envelope might play a role in the replacement of transition proteins by protamines during spermiogenesis. Given that RS domains mediate protein-protein interactions (12), we sought to investigate the potential interaction of P1 protamine with the inner nuclear membrane protein lamin B receptor (LBR), which also possesses a repeat of RS dipeptides at its nucleoplasmic NH₂-terminal domain. In the present study we demonstrate a direct association of these two proteins, in a phosphorylation-dependent manner.

SRPK1 was initially purified and cloned on the basis of its ability to phosphorylate and regulate the intracellular localization of SR splicing factors that commit precursor mRNA to splicing and promote spliceosome assembly (13). A polypeptide with an apparent molecular mass of 32 kDa (p32), isolated first as a protein tightly associated with the essential SR splicing factor ASF/SF2 (14), was shown to inactivate ASF/SF2 as both a splicing repressor and splicing enhancer protein (15). p32 was also found to form an in vivo complex with LBR (16, 17). In this study we reinvestigated the significance of the p32-LBR interaction, presenting evidence that p32 prevents LBR phosphorylation and P1 protamine association with LBR. Immunofluorescence analysis suggests that dissociation of this protein precedes P1 protamine association in early spermiogenesis. Taken together our data provide some insights into the molecular machinery that spatially directs the histone to protamine transition, revealing a new role for SR protein kinases and p32.

MATERIALS AND METHODS

Plasmids, Expression of Proteins, and Antibodies—Human SRPK1, the wild-type NH₂-terminal domain of LBR (GST-wtNt; amino acids 1–205), and a mutant form lacking the RS motifs (GST-ΔRS; lacks amino acids 75–84; 75RSRSRSRSRS84) were expressed as fusion proteins with GST using the pGEX-2T vector (Amersham Biosciences) (18, 19). Full-length SRPK1 was also subcloned into the p-FLAG-CMV-2 (Eastman Kodak) vector and expressed in 293T cells with a FLAG tag fused at its NH₂ terminus (20). 293T cells were transiently transfected with 3–5 µg of plasmid DNA using the CaCl₂ method and harvested 48 h later (20).

Received for publication, October 31, 2003, and in revised form, December 22, 2003
Published, JBC Papers in Press, December 30, 2003, DOI 10.1074/jbc.M311949200
nant p32 (kindly provided by G. Akuesjarvi, Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden) was isolated as a His-tagged protein by standard nickel column chromatography (Qiagen) according to the manufacturer's instructions. The polyclonal anti-protamine 1 antibody was generated by immunizing rabbits with a keyhole limpet hemocyanin-coupled peptide (MARYRCCRSKSSRSGCR) corresponding to the NH2-terminal sequence (residues 1–16) of mouse protamine 1. A rabbit anti-LBR polyclonal antibody raised against GST-wtNt was kindly provided by P. S. Singh (Division of Gene Expression and Development, The Roslin Institute, Edinburgh, UK), whereas the M5 anti-FLAG monoclonal antibody was a kind gift from G. Mosialos (Biomedical Sciences Research Center “AI. Fleming,” Vari, Attiki). The polyclonal antibodies against p32 and SRPK1 were previously characterized (18). GST-wtNt was added to the separating gel at a concentration of 0.1 mg/ml prior to polymerization. For control experiments, GST-wtNt was replaced by 0.1 mg/ml GST.

Preparation of Testis Cytosolic and Nuclear Extracts—Tests from 5-month-old Wistar rats or C57/BL/6 mice were homogenized by means of a Potter-Elvehjem tissue homogenizer in 3 volumes of an ice-cold solution containing 0.25 m sucrose, 50 mM Tris-HCl, pH 7.5, 25 mM KCl, 5 mM MgCl₂, 5 mM dithiothreitol, and 3 mM phenylmethylsulfonil fluoride. The homogenate was passed through two layers of surgical gauze and then centrifuged at 1000 × g for 10 min at 4 °C to yield a crude nuclear fraction. The supernatant was centrifuged at 100,000 × g for 2 h to obtain the cytosolic fraction (S100). The nuclei were further purified from the crude nuclear fraction by centrifugation through a cushion of 2.3 m sucrose at 100,000 × g for 90 min according to the method of Blobel and Potter (21). The nuclear pellets were resuspended in 10 mM NaH₂PO₄/NaH₂PO₃, pH 7.5, 2 mM MgCl₂, and 1 mM phenylmethylsulfonil fluoride and digested with 40 μg/ml DNase I for 30 min at room temperature. NaCl to a final concentration of 1 M was then added, and the mixture was further incubated at 4 °C for 2 h on a rotator. Nuclear extracts were collected by centrifugation at 10,000 × g for 30 min at 4 °C. Protein concentration was determined by the method of Bradford (22).

Kinase Assays—In vitro phosphorylation assays were carried out at 30 °C in a total volume of 25 μl containing 25 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 50 mM NaCl, 50 μM γ-32PATP (6000 Ci/mmol), 2–3 μg of the appropriate substrate (bacterially expressed LBR or P1 protamine), and 0.1 μg of FLAG-SRPK1 (at a concentration of 5 μg/ml). The samples were incubated for 30 min, and the reaction was stopped by adding 6 μl of 5× SDS sample buffer and heating at 95 °C for 3 min. In situ kinase assays using rat testis cytosolic and nuclear extracts were performed as previously described (18), following essentially the protocol of Kameshita et al. (23).

Mouse seminiferous tubule segments were isolated in sequential order after stages VII–VIII using the transillumination-assisted microdissection method (24). Cells from the spermatogenic stages were spread onto polylysine-coated slides and allowed to adhere for 20 min at room temperature. The slides were then fixed with 4% paraformaldehyde in PBS for 20 min at room tempera-
ture. The nuclear pellets were resuspended twice with 50 mM ammonium chloride, and permeabilized with PBS containing 0.2% Triton X-100 for 5 min. After washing twice with PBS the slides were incubated for 45 min with the primary antibodies (diluted 1:100 in PBS), washed twice with PBS, and incubated for 30 min with the Cy3-conjugated anti-rabbit secondary antibody (1:500 dilution in PBS). The immunostained samples were counterstained with 4′, 6-diamidino-2-phenylindole dihydrochloride before being subjected to microscopy. The images were collected on a DMLB Leica microscope with an HBO 100-W lamp.

RESULTS

SRPK1 Phosphorylates LBR and P1 Protamine in Testes—In a previous report, we have detected high levels of LBR kinase activity in testes (10). This activity was found primarily in the cytosol (Fig. 1A; see also Ref. 10). To demonstrate that the cytosolic and nuclear kinase activities targeting LBR, from rat testes, were associated with the same enzyme, we performed in situ kinase assays in SDS-polyacrylamide gels, to which 0.1 mg/ml GST-wtNt (the wild-type nucleoplasmic NH2-terminal domain of LBR; amino acids 1–205) had been added immediately prior to gel polymerization. Fig. 1B shows that, in both cytosolic and nuclear fractions, a protein with a relative molecular mass of ~97 kDa could modify LBR. This was specific because no labeling was detected when GST-wtNt was omitted from the gel or replaced by GST (data not shown). In a following step, GST-wtNt was incubated with rat testis cytosol and recovered by pull-down with glutathione-Sepharose beads. The beads were then used for kinase reactions in the presence of radiolabeled ATP. Phosphorylation of the GST-wtNt protein, detected by autoradiography, suggested the existence of an LBR-associated kinase in testis cytosol (Fig. 1C, left panel). Furthermore, in a similar experiment the complexes recovered with glutathione-Sepharose beads were analyzed by SDS-PAGE and Western blotsing using an anti-SRPK1 polyclonal antibody (20). As shown in Fig. 1C (right panel), the 97-kDa protein that could associate with LBR corresponded to the molecular mass of SRPK1.

SRPK1 also phosphorylated efficiently protamine 1 (Fig. 1D; see also Ref. 10). Consistent with SRPK1 being the major protamine 1 kinase, Ser⁸ and Ser¹⁰ (RSQSRSR⁰⁸) were previously identified as the phosphorylation sites of monophosphorylated and diphosphorylated human P1 protamine (6).

Phosphorylation-mediated Association of P1 Protamine with LBR—Given that RS domains are well known for their bridging properties (12), we set out to investigate the potential interaction of P1 protamine with LBR through their RS regions. To this end we tested the ability of purified P1 protamine to bind GST-wtNt and GST-ARS (the mutant form lacking the RS motifs; RSRSRSRSRS⁰⁴). The complexes were recovered by pull-down with glutathione-Sepharose beads and analyzed by SDS-PAGE and Coomassie Blue staining. As shown in Fig. 2, we were unable to detect any binding of unphosphorylated P1 protamine to the unphosphorylated NH2-terminal domain of LBR (lane 4). However, phosphorylation of P1 protamine by SRPK1 (either GST-SRPK1 or 293T cell extracts overexpressing FLAG-SRPK1) was able to promote its binding to the NH2-terminal domain of LBR (lane 5), whereas no such interaction
Temporal Association of Protamine 1 with LBR during Spermiogenesis

Fig. 1. A, subcellular distribution of LBR kinase activity in rat testes. Cytosolic (cyt) and nuclear (nuc) extracts (15 μg) were assayed for LBR kinase activity toward GST-wtNt. The samples were analyzed by SDS-PAGE and autoradiographed. The radioactive bands corresponding to GST-wtNt were excised and measured by scintillation counting. Kinase activity is expressed as units/g of tissue. One unit is the amount of enzyme required to catalyze the transfer of 0.1 nmol of phosphate to 2 μg of GST-wtNt in 30 min at 30 °C. Under the assay conditions no detectable phosphorylation was observed when GST-ΔRS was used as substrate. B, in situ kinase assay of cytosolic and nuclear extracts of rat testes (150 μg each). The samples were electrophoresed on a 10% SDS-polyacrylamide gel containing 0.1 mg/ml GST-wtNt, renatured in situ, incubated with [γ-32P]ATP, and subjected to autoradiography. C, GST or GST-wtNt immobilized on glutathione-Sepharose beads were incubated with rat testis cytosol for 1 h at room temperature. The sediments were incubated with [γ-32P]ATP, analyzed by SDS-PAGE, and autoradiographed (left panel). The full-length GST-wtNt migrates with an apparent molecular mass of 51 kDa. The lower bands represent degradation products (see also Ref. 18). Bound SRPK1 was detected by Western blotting using an anti-SRPK1 polyclonal antibody (right panel). D, phosphorylation of purified human P1 protamine by GST-SRPK1. Left panel, Coomassie Blue staining; right panel, autoradiography.

![Diagram](https://example.com/diagram.png)

**Fig. 2.** Phosphorylated P1 protamine binds to the RS domain of LBR. Pull-down assays using purified P1 protamine and GST-wtNt or GST-ΔRS. GST was included as a control. Bound P1 protamine was detected by Coomassie Blue staining. Phosphorylation of GST-wtNt was achieved by incubating the recombinant protein with GST-SRPK1 (0.3 μg) in the presence of 0.5 mM ATP for 3 h at 30 °C prior to immobilization on glutathione-Sepharose beads. P1 protamine was phosphorylated by GST-SRPK1 under the same assay conditions prior to incubation with the immobilized recombinant proteins. Phosphorylated GST-wtNt and P1 protamine are denoted by asterisks.

![Diagram](https://example.com/diagram.png)

Surprisingly, unphosphorylated P1 protamine was unable to bind to the SRPK1-phosphorylated NH₂-terminal domain of LBR (lane 6), leading to the conclusion that the phosphorylation of the two RS domains is not functionally equivalent. A minimal interaction was obtained when both RS regions were modified (lane 7).

**p32 Protein Prevents Association of both SRPK1 and Protamine 1 with LBR**—A polypeptide with an apparent mass of 32 kDa (p32) was previously shown to associate both in vitro and in vivo with the NH₂-terminal domain of LBR (16–18). Using in vitro binding assays with GST-wtNt and His-tagged p32, we confirmed the tight interaction between p32 and the RS dipeptides of LBR, only when the latter were unphosphorylated (Fig. 3A). Binding of His-p32 to GST-wtNt was saturable (Fig. 3B) and could still occur in the presence of 0.5 mM salt, revealing a high affinity interaction. In this respect, it is noteworthy that p32 was unable to bind to P1 protamine under our assay conditions (data not shown), suggesting that the RS domain of LBR, although indispensable to p32 interaction, may not be sufficient. In favor of this hypothesis, p32 was reported to interact both with the RS domain and with the RNA-binding domain of ASF/SF2, a member of the SR family of splicing factors (15, 25).

Because p32 interacts with the RS domain, it may also control the accessibility of LBR to SRPK1. To test this hypothesis GST-wtNt or GST-wtNt/p32 complexes immobilized on glutathione-Sepharose beads were incubated with 293T cell extracts overexpressing FLAG-SRPK1. The beads were then harvested, washed three times with PBST, and used for in vitro phosphorylation assays in the presence of radiolabeled ATP. Bound proteins were also analyzed by Western blotting, using the M5 anti-FLAG monoclonal antibody. As shown in Fig. 4A, binding of p32 completely inhibited binding of SRPK1 (left panel) and the subsequent phosphorylation of the NH₂-terminal domain of LBR (right panel).

In a following step, to examine whether p32 could also regulate the binding of phosphorylated protamine 1 to GST-wtNt,
we performed pull-down assays using GST-wtNt/p32 complexes immobilized on glutathione-Sepharose beads and phosphorylated P1 protamine. Analysis of bound P1 protamine on SDS-polyacrylamide gels followed by Coomassie Blue staining demonstrated that p32 protein prevented the binding of protamine 1 to LBR (Fig. 4B), suggesting that p32 actually competes with both P1 protamine and SRPK1 for the RS domain of LBR.

The NH₂-terminal Domain of LBR Pulls Down a Fraction of P1 Protamine and p32 from Testis Nuclear Extracts—To confirm the observed interactions between purified proteins, GST-wtNt immobilized on glutathione-Sepharose beads was incubated with mouse testis nuclear extracts. SDS-PAGE and immunoblotting analysis of the proteins co-sedimenting with the beads showed that a fraction of protamine 1 was able to associate with GST-wtNt (Fig. 5A). The association of p32 could also be readily documented using the same pull-down approach (Fig. 5B).

Temporal Perinuclear Localization of Protamine 1 and p32 during Mouse Spermiogenesis—To study in detail the distribution of LBR, protamine 1, and p32 during mouse spermiogenesis, several isolated spermiogenic cells during the elongation process were subjected to immunofluorescence microscopy, and a representative analysis is shown in Fig. 6. At the beginning of the elongation process LBR exhibited a peripheral, nuclear envelope distribution (Fig. 6A, a-LBR). With progression of spermiogenesis, a polarization of the signal to the posterior pole of the nucleus (Fig. 6D, a-LBR). A similar redistribution was also observed with LAPs (lamin-associated polypeptides), which are also integral proteins of the inner nuclear membrane, and probably reflects the nuclear envelope remodeling during spermiogenesis (26).

![Image](https://example.com/image.png)
tion of the elongation process nuclear labeling increased (Fig. 6C, a-P1), whereas in elongated spermatids the whole nucleus was labeled (Fig. 6D, a-P1), indicating the termination of P1 protamine deposition on sperm chromatin. The redistribution of p32 protein was impressive. p32 exhibited perinuclear localization, similarly to LBR, during the initial stages of the elongating process (Fig. 6A, a-p32). Concomitantly with the appearance of P1 protamine at the nuclear periphery, the perinuclear distribution of p32 was less apparent, and significant labeling of the central area of the nucleus could be observed (Fig. 6B, a-p32). The labeling became exclusively nuclear at a later stage of the elongation process with the first indications of acrosomal localization (Fig. 6C, a-p32), and finally p32 exhibited a clear acrosomal localization in elongated spermatids (Fig. 6D, a-p32). The acrosomal distribution of p32 has been also previously observed, during late spermiogenesis (27), however without any indication of its potential function in this organelle.

**DISCUSSION**

In the present study we demonstrated a direct association of LBR with protamine 1 only when the latter was phosphorylated by SRPK1. SRPK1 is predominantly expressed in testes, whereas it is present at low levels in almost all tissues (10). In situ hybridization experiments (10) and Western blotting analysis (data not shown) revealed that SRPK1 is highly expressed in all spermatogenic cells except spermatocytes. The reason for the high level of expression of SRPK1 during the early stages of spermiogenesis may be due to the multiple functions associated with this kinase. SRPK1 was found to regulate through the phosphorylation of SR splicing factors constitutive and alternative pre-mRNA splicing (13) as well as export and translation of mRNAs (28). Moreover, SRPK1 was able to mediate the uptake of polyamines through an as yet unidentified signaling pathway (29). Consistent with the diversity of roles displayed by SRPK1 during spermiogenesis, SPK-1 (the *Caenorhabditis elegans* homologue of SRPK1) is required for germ-line development in *C. elegans*, including proliferation, maintenance, and gametogenesis (30).

Postmeiotically, the beginning of spermiogenesis is characterized by a powerful wave of transcription, which is accompanied by high levels of splicing activity (3). Splicing proceeds through phosphorylation-dephosphorylation cycles. Phosphorylation of SR splicing factors is essential for the spliceosome assembly, whereas their dephosphorylation is critical for the catalytic step after spliceosome formation (31). The ASF/SF2 protein is one of the essential members of the SR family of splicing factors. p32 inhibits ASF/SF2 function as a splicing enhancer by preventing stable ASF/SF2 interaction with RNA and also by inhibiting ASF/SF2 phosphorylation by SR protein

---

**Fig. 6.** Immunolocalization of LBR, p32, and P1 protamine in isolated mouse germ cells. Germ cells from various elongation stages were subjected to immunofluorescence microscopy after incubation with rabbit polyclonal antibodies against LBR, p32, and P1 protamine (a-LBR, a-p32, and a-P1) (red). Nuclear staining was achieved by using 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) dye (blue). A, spermatids at the beginning of the elongation process; B, elongating spermatids; C, elongating spermatids isolated from a later stage of the elongation process; D, elongated spermatids.

**Fig. 7.** A provisional model illustrating the interactions between the NH2-terminal nucleoplasmic domain of LBR, p32, and P1 protamine. In early haploid cells, p32 is associated with the RS domain of LBR. As spermiogenesis proceeds, an as yet unidentified signal triggers its dissociation from LBR, thereby unmasking the RS domain of LBR and allowing its subsequent binding to phosphorylated protamine 1. LBR may act as a docking site for the replacement of transition proteins (TP) by P1 protamine in certain chromatin layers that come close to the nuclear periphery. Enzymes trapped in the inner nuclear membrane (INM) may also further modify the P1 protamine molecules, thereby facilitating their deposition on sperm chromatin. The detachment of P1 protamine from the nuclear envelope and its tight binding to DNA is postulated to occur through its dephosphorylation.

---

a J. F. Caceres, personal communication.
kinases (15). The sequestration of p32 at the nuclear periphery, via its association with LBR, would therefore allow splicing to proceed unimpaired in early haploid cells. Furthermore, the masking of the RS domain of LBR would prevent its phosphorylation and consequently would leave LBR ready to act as a “docking site” for the protamine 1 molecules.

As spermiogenesis proceeds, histones are sequentially replaced by transition proteins and protamines, resulting in transcriptional silencing. P1 protamine is presumably phosphorylated in the cytoplasm, shortly after its synthesis, considering the predominant cytoplasmic localization of SRPK1 (Ref. 10 and this study). The appearance of phosphorylated P1 protamine in the nucleus is well coordinated with the migration of p32 to the central area of the nucleus and the unmasking of the RS domain of LBR (for a model see Fig. 7). The nature of the event that triggers the dissociation of p32 from the RS domain of LBR still remains obscure.

The association of P1 protamine with the nuclear envelope probably represents an important intermediate step prior to its deposition on sperm chromatin. One possibility is that certain layers of the transition protein-organized chromatin come close to the nuclear membrane, and the replacement takes place. Another possibility, not mutually exclusive, is that the nuclear envelope functions as a “working platform” where additional modifications (i.e. methylation) of protamine 1 take place. Those modifications may not only increase the affinity of protamine 1 for sperm DNA but may also recruit specific molecules, such as HP1 (heterochromatin protein 1), that have been shown to be coupled to chromatin condensation and transcriptional silencing (3, 32).

The detachment of protamine 1 from the nuclear envelope and its binding to DNA are probably achieved through its dephosphorylation (Fig. 7). Consistent with this hypothesis, protamines were found mainly dephosphorylated in mature sperm chromatin (5, 6). As spermiogenesis proceeds, the nuclear envelope is sequestered mainly to the posterior pole (pre-implantation fossa) of the nucleus (Ref. 26 and this study). Only this specialized region is associated with chromatin in mature sperm. Fractions of P1 protamine that still remain phosphorylated (6) may be responsible for this type of interaction.

Our data clearly show that an integral nuclear envelope protein plays a crucial role in the well orchestrated exchange of basic nuclear proteins during spermiogenesis. Deciphering the molecular machinery that regulates this process and directs it spatially is important, not only from the standpoint of cell biological interest but also for providing valuable insights of biomedical importance.

Acknowledgments—We thank Goran Akusjarvi for the p32 cDNA clone, Philippe Chevaillier for human protamine 1, G. Mosialos for the M5 anti-FLAG monoclonal antibody, Prim Singh for the anti-LBR antiserum, and A. Tsiftsoglou for providing us with a tissue culture facility. We also thank S. D. Georgatos and J. G. Georgatos for valuable discussions.

REFERENCES

1. Hecht, N. B. (1995) Dev. Genet. 16, 95–103
2. Meistrach, M. L. (1989) in Histones and Other Basic Nuclear Proteins (Hnilica, L., Stein, G. S., and Stein, J. L., eds) pp. 165–182, CRC Press, Boca Raton, FL
3. Sassone-Corsi, P. (2002) Science 296, 2176–2178
4. Marushige, Y., and Marushige, K. (1978) Biochim. Biophys. Acta 518, 440–449
5. Oliva, R., and Dixon, G. H. (1991) Prog. Nucleic Acids Res. Mol. Biol. 40, 25–94
6. Chirat, F., Arkhis, A., Martimage, A., Jaquinet, M., Chevaillier, P., and Sauvière, P. (1993) Biochim. Biophys. Acta 1193, 109–114
7. Raukas, E., and Mikelsaar R. H. (1999) Biosci. Rep. 21, 440–448
8. Lewis, J. D., Song, Y., de Jong, M. M., Bagha, S. M., and Aasio, J. (2003) Chromosoma 111, 473–482
9. Wu, J. Y., Ribar, T. J., Cummings, D. E., Burton, K. A., McKnight, G. S., and Means, A. R. (2000) Nat. Genet. 23, 448–452
10. Papoutsopoulou, S., Nikolakaki, E., Chalepakis, G., Kruft, V., Chevaillier, P., and Giannakourou, T. (1999) Nucleic Acids Res. 27, 2972–2980
11. Biggiogera, M., Muller, S., Courtes, J. L., Fakan, S., and Romani, M. G. (1992) Microsc. Res. Tech. 20, 259–267
12. Valcarcel, J., and Green, M. R. (1996) Trends Biochem. Sci. 21, 296–301
13. Gui, J.-F., Lane, W. S., and Fu, X.-D. (1994) Nature 369, 678–682
14. Krainer, A. R., Mayeda, A., Kozak, D., and Binns, G. (1991) Cell 66, 383–394
15. Petersen-Mahrt, S. K., Estimer, C., Osterman, C., Matthews, D. A., Russel, W. C., and Akusjarvi, G. (1999) EMBO J. 18, 1014–1024
16. Simos, G., and Georgatos, S. D. (1992) EMBO J. 11, 4027–4036
17. Simos, G., and Georgatos, S. D. (1994) FEBS Lett. 346, 225–228
18. Nikolakaki, E., Simos, G., Georgatos, S. D., and Giannakourou, T. (1996) J. Biol. Chem. 271, 8365–8372
19. Papoutsopoulou, S., Nikolakaki, E., and Giannakouros, T. (1999) Biochem. Biophys. Res. Commun. 255, 602–607
20. Nikolakaki, E., Koken, R., Hartmann, A. M., Stamm, S., Georgatos, E. and Giannakourou, T. (2001) J. Biol. Chem. 276, 40175–40182
21. Blobel, G., and Potter, V. R. (1966) Science 154, 1662–1665
22. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
23. Kameshita, I., and Fujisawa, H. (1988) Anal. Biochem. 183, 139–143
24. Parvinen, M., and Yashiha-Perttilä, T. (1972) Anat. Res. 174, 435–449
25. Yu, L., Lowenstein, P. M., Zhang, Z., and Green, M. (1995) J. Virol. 69, 3017–3023
26. Alsheimer, M., Fecher, E., and Benavente, R. (1998) J. Cell Sci. 111, 2227–2234
27. Soltys, B. J., Kang, D., and Gupta, R. S. (2000) Histochim. Cell Biol. 114, 285–295
28. Capesius, J. F., Scratton, G. R., and Krainer, A. R. (1998) Genes Dev. 12, 55–66
29. Erez, O., and Kahana, C. (2001) Mol. Cell. Biol. 21, 175–184
30. Kuroyanagi, H., Kimura, T., Kazuhiro, W., Hisamoto, N., Matsumoto, K., and Hagiwara, M. (2000) Mech. Dev. 99, 51–64
31. Mermod, J. E., Cohen, P. T. W., and Lamsd, A. I. (1994) EMBO J. 13, 5679–5688
32. Chemung, P., Allis, C. D., and Sassone-Corsi, P. (2000) Cell 103, 263–271
Temporal Association of Protamine 1 with the Inner Nuclear Membrane Protein Lamin B Receptor during Spermiogenesis
Ilias Mylonis, Victoria Drosou, Stefano Brancorsini, Eleni Nikolakaki, Paolo Sassone-Corsi and Thomas Giannakouros

J. Biol. Chem. 2004, 279:11626-11631.
doi: 10.1074/jbc.M311949200 originally published online December 30, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M311949200

Alerts:
  - When this article is cited
  - When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 31 references, 9 of which can be accessed free at http://www.jbc.org/content/279/12/11626.full.html#ref-list-1