The Nuclear Tyrosine Kinase BRK/Sik Phosphorylates and Inhibits the RNA-binding Activities of the Sam68-like Mammalian Proteins SLM-1 and SLM-2*

Expression of the intracellular tyrosine kinase BRK/Sik is epithelial-specific and regulated during differentiation. Only a few substrates have been identified for BRK/Sik, including the KH domain containing RNA-binding protein Sam68 and the novel adaptor protein BKS. Although the physiological role of Sam68 is unknown, it has been shown to regulate mRNA transport, pre-mRNA splicing, and polyadenylation. Here we demonstrate that the Sam68-like mammalian proteins SLM-1 and SLM-2 but not the related KH domain containing heterogeneous nuclear ribonucleoprotein K are novel substrates of BRK/Sik. The expression of active BRK/Sik results in increased SLM-1 and SLM-2 phosphorylation and increased retention of BRK/Sik within the nucleus. The phosphorylation of SLM-1 and SLM-2 has functional relevance and leads to inhibition of their RNA-binding abilities. We show that SLM-1, SLM-2, and BRK/Sik have restricted patterns of expression unlike the ubiquitously expressed Sam68. Moreover, BRK/Sik, SLM-1, and Sam68 transcripts were coexpressed in the mouse gastrointestinal tract and skin, suggesting that SLM-1 and Sam68 could be physiologically relevant BRK/Sik targets in vivo. The ability of BRK/Sik to negatively regulate the RNA-binding activities of the KH domain RNA binding proteins SLM-1 and Sam68 may have an impact on the posttranscriptional regulation of epithelial cell gene expression.

The intracellular tyrosine kinase BRK/Sik (also named PTK6) was identified in a screen for protein tyrosine kinases involved in breast cancer (1), from the mouse small intestine in a screen for factors that regulate epithelial cell differentiation (2), and from cultured human melanocytes (3). Highest levels are expressed in differentiating epithelial linings of the gastrointestinal tract and skin (4, 5) and in prostate epithelial cells (6). Activation of BRK/Sik has been correlated with the differentiation of cultured keratinocytes (7). Although BRK/Sik is expressed in many breast carcinoma cell lines and primary breast tumors, it has not been detected in normal human breast tissue (1, 6) or at any stage of mammary gland differentiation in the mouse (5). Modest increases in BRK/Sik levels have been detected in colon tumors relative to normal colonic tissue (5). BRK/Sik expression has also been detected in normal human oral epithelium and oral squamous cell carcinomas (9).

Although related to Src, BRK/Sik belongs to a distinct family of intracellular tyrosine kinases (10). It lacks an amino-terminal myristoylation signal, and it is not specifically targeted to the membrane. Like the Src family kinases, mutation of the carboxyl-terminal tyrosine of BRK/Sik (Y447) results in increased enzyme activity, supporting a role for this residue in autoinhibition (11–13). However Csk, the kinase that phosphorylates the carboxyl-terminal tyrosine in Src kinases, does not appear to be the enzyme that regulates carboxyl-terminal phosphorylation of BRK/Sik (12). Mutation of the BRK/Sik regulatory tyrosine did not enhance its ability to induce anchorage-independent growth of NIH 3T3 cells (14).

Thus far two substrates have been identified for BRK/Sik, the novel adaptor-like protein BKS (15) and the nuclear RNA-binding protein Sam68 (11). Sam68 is a member of the STAR (signal transducers and activators of RNA) family of KH (heteronuclear ribonucleoprotein K homology) domain containing RNA binding proteins that regulate different aspects of RNA metabolism, including transport, stability, translation, and processing (16–18). The Sam68 KH domain is required for RNA binding, and it is embedded in a larger conserved domain called the GSG (GRP33, Sam68, GLD1) domain that is found in several RNA-binding proteins that regulate developmental processes (19, 20).

Several studies support roles for Sam68 in the regulation of RNA metabolism. It may act as a functional homologue of the human immunodeficiency virus (HIV)1 type 1 Rev protein, which transports RNA from the nucleus to the cytoplasm (21–24). Sam68 may also regulate polyadenylation and has been found to enhance the 3′-end processing of HIV-1 RNA (25). In addition, Sam68 colocalizes with and associates with RNA splicing factors (26, 27) and was shown to be a regulator of alternative splicing (28). Because of its interactions with a number of signaling proteins, including Src, Fyn, Grb2, and

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1 The abbreviations used are: HIV, human immunodeficiency virus; GFP, green fluorescent protein; hnRNP, heterogeneous nuclear ribonucleoprotein; GST, glutathione S-transferase.
phospholipase C-γ has been proposed that Sam68 may also act as an adaptor protein (29).

We showed previously that BRK/Sik phosphorylation of Sam68 inhibits its ability to bind RNA and function as a cellular HIV-1 Rev homologue (11). Although Sam68 can be phosphorylated by other intracellular tyrosine kinases, only BRK/Sik has been shown to colocalize with Sam68 in the nucleus (11). BRK/Sik also regulates the ability of Sam68 to regulate utilization of specific RNAs in the cytoplasm (30).

Two Sam68-like mammalian proteins, SLM-1 and SLM-2, were identified based on their high degree of sequence similarity with Sam68 within the GSG domains (31, 32). SLM-1 shares many similarities with Sam68; it interacts with many of the same proteins and is also tyrosine-phosphorylated by Src during mitosis (31). SLM-2 was also identified by its ability to interact with RNA-binding motif in spermagenes and was also named T-STAR or ETOILE (33). Because of the similarities that SLM-1 and SLM-2 share with Sam68, we asked if SLM-1 and SLM-2 are BRK/Sik substrates. We then examined the possible biological significance of BRK/Sik tyrosine phosphorylation of SLM-1 and SLM-2 by examining their RNA-binding activities after tyrosine phosphorylation and their coexpression with BRK/Sik in mouse tissues.

EXPERIMENTAL PROCEDURES

Expression Constructs—Myc-tagged wild-type BRK/Sik, BRK/Sik YF, and BRK/Sik KM coding sequences were cloned into the vector pCDNA3 (7). BRK/Sik YF has a substitution of the regulatory tyrosine at position 447 of wild-type mouse BRK/Sik to phenylalanine, while BRK/Sik KM is kinase defective and has a substitution of the lysine at position 219 to methionine. SLM-1 and SLM-2-GFP fusion proteins were prepared as described previously (34). Myc epitope-tagged Sam68, SLM-1, and SLM-2 cDNAs in pBluescript KS have been described (31) and were also inserted into the expression vector pCDNA for studies described here.

Cell Lines and Protein Expression—Cell lines were obtained from the American Type Culture Collection. NMuMG cells were transfected using the Lipofectamine reagent (Invitrogen Corp.). HeLa cells were maintained in Dulbecco’s modified Eagle’s medium with 1.0 mM sodium pyruvate and 10% bovine calf serum and transfected with the vaccinia virus T7 expression system (29).

Antibodies and Immunoblotting—Anti-Sik C17, BRK, GFP, and Sp1 antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-Myc (1:5000) antibody was obtained from Invitrogen. Anti-Sam68 AD1 antibodies were described previously (35). Anti-phosphotyrosine antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-p38 antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Immunoprecipitations and in Vitro Kinase Assays—Bacterially expressed His6-tagged myc epitope-Tagged Sam68, SLM-1, or SLM-2 cDNAs in pBluescript KS have been described (31) and were also inserted into the expression vector pCDNA for studies described here.

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Subcellular Fractionation—Transfected NMuMG cells were fractionated as described previously (11). Cells were treated with 2 ml of hypotonic lysis buffer (HLB, 20 mM Tris-HCl (pH 7.5), 1 mM MnCl2, 2 mM EGTA, 2 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride) and shaken for 20 min on ice. Cells were then lysed, and the supernatant was kept as cytosolic and membrane fractions. The pellet was washed in 1 ml of HLB and resuspended in 1 ml of Dignam buffer (20 mM HEPES (pH 7.9), 420 mM NaCl, 1.5 mM MgCl2, 0.1 mM EDTA, 25% glycerol, 0.5 mM diethiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin/ml, 2 μg/ml aprotinin/ml, 1 mM Na3VO4).

RNA-binding assays—Myc-tagged Sam68, SLM-1, or SLM-2 and pCDNA or BRK/Sik YF were introduced into NMuMG cells, and cell lysates were prepared. These cell lysates were used for homopolymeric RNA-binding assays using poly(A), poly(G), and poly(U) (Sigma) covalently coupled to beads in lysis buffer supplemented with 2 mg/ml heparin as described (31, 36, 37). Controls were performed by incubating lysates with Sepharose 4B beads (Sigma) alone. Beads were washed twice with lysis buffer and once with phosphate-buffered saline, and proteins were eluted in Laemmli sample buffer. Samples were analyzed on 9% SDS-PAGE.

RNAse Protection Assays—Total RNA was isolated from various tissues of CD1 mice using TRIzol reagent (Invitrogen). A 350-bp HindIII/XhoI fragment of the SLM-1 cDNA, a 500-bp HindIII/XhoI fragment of the SLM-2 cDNA, and a 220-bp Apal/HindIII fragment of the BRK/Sik cDNA were subcloned into the vector pBluescript KS, and 32P-labeled antisense cRNA probes were generated. RNAse protection assays were performed as described previously (2, 38).

RESULTS

BRK/Sik Phosphorylates SLM-1 and SLM-2 in Vitro and in Vivo—To determine whether SLM-1 and/or SLM-2 are substrates of BRK/Sik, the normal murine mammary gland cell line NMuMG was transiently cotransfected with either wild-type BRK/Sik WT or activated BRK/Sik YF, and GFP-tagged GFP-SLM-1 and GFP-SLM-2. The NMuMG cell line does not express endogenous BRK/Sik and is an excellent epithelial cell line model for evaluating BRK/Sik activities (5). Tyrosine-phosphorylated GFP-SLM-1 and GFP-SLM-2 were detected by immunoblotting with anti-phosphotyrosine in total cell lysates from NMuMG cells cotransfected with either wild-type BRK/Sik or activated BRK/Sik YF but not in cells cotransfected with vector alone (Fig. 1). BRK/Sik YF, which contains a substitution of the carboxyl-terminal negative regulatory tyrosine, was most effective in phosphorylating the SLM proteins because this mutation results in increased BRK/Sik activation (11). Immunoblotting with BRK/Sik, β-actin, and GFP antibodies confirmed the equivalent expression of the transfected proteins (Fig. 1). These data demonstrate that SLM-1 and SLM-2 are substrates for BRK/Sik.

BRK/Sik phosphorylation of SLM-1 and SLM-2 was also demonstrated in vitro using recombinant GST-BRK/Sik. Myc-tagged Sam68 (Myc-Sam68), Myc-SLM-1, and Myc-SLM-2 were immunoprecipitated from HeLa cells and incubated with GST-BRK/Sik and [γ−32P]ATP. The GST-BRK/Sik phosphorylated Myc-tagged Sam68, SLM-1, and SLM-2 as visualized in duplicate Myc immunoprecipitations (Fig. 2A), providing further evidence that Sam68, SLM-1, and SLM-2 are substrates of BRK/Sik.

To examine the specificity of phosphorylation, HeLa cells were cotransfected with BRK/Sik YF and Myc-Sam68, Myc-SLM-1, Myc-SLM-2, Myc-hnRNP K, or Myc-Sam68 (68–347). Sam68 (68–347) includes amino acids 68–347 of Sam68 with its four proline rich motifs, but it lacks the tyrosine-rich carboxy terminus (29). Cells were lysed, and the proteins were analyzed by immunoblotting with anti-BRK/Sik, anti-Myc, or anti-phosphotyrosine antibodies. Tyrosine phosphorylation of Sam68, Myc-SLM-1, and Myc-SLM-2 increased in these cotransfection experiments with BRK/Sik YF (Fig. 2B). The related STARP protein hnRNP K as well as the truncated form of Sam68 (68–347) were not phosphorylated, even though immunoblotting with anti-Myc-antibodies confirmed protein expression.

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BRK/Sik phosphorylates SLM-1 and SLM-2 in NMuMG cells. GFP-tagged SLM-1 (A) or SLM-2 (B) was cotransfected with wild-type BRK/Sik WT, BRK/Sik YF (with a mutation of the carboxyl-terminal regulatory tyrosine), or empty expression vector. Immunoblotting experiments were performed with total cell lysates and the indicated antibodies. Anti-phosphotyrosine (PY) antibodies were used to detect tyrosine phosphorylation. NMuMG cells do not express endogenous BRK/Sik. Controls examining expression of the transfected proteins are shown at the right of each panel. Expression of β-actin was examined as a control for protein loading.

**Fig. 1.** BRK/Sik phosphorylates SLM-1 and SLM-2 in NMuMG cells. GFP-tagged SLM-1 (A) or SLM-2 (B) was cotransfected with wild-type BRK/Sik WT, BRK/Sik YF (with a mutation of the carboxyl-terminal regulatory tyrosine), or empty expression vector. Immunoblotting experiments were performed with total cell lysates and the indicated antibodies. Anti-phosphotyrosine (PY) antibodies were used to detect tyrosine phosphorylation. NMuMG cells do not express endogenous BRK/Sik. Controls examining expression of the transfected proteins are shown at the right of each panel. Expression of β-actin was examined as a control for protein loading.

**Fig. 2.** Specificity of STAR protein phosphorylation by BRK/Sik. A, GST-BRK/Sik was incubated with immunoprecipitated Myc-tagged Sam68, SLM-1, and SLM-2 and [γ-32P]ATP. Immunoprecipitated Myc-tagged Sam68, SLM-1, and SLM-2 were phosphorylated following the addition of the GST-BRK/Sik fusion protein. B, BRK/Sik YF was introduced into HeLa cells expressing Myc-tagged Sam68, SLM-1, SLM-2, hrRNP K, and the deletion mutant Sam68 (68–347). Immunoblotting was performed with antibodies against BRK/Sik, Myc, and phosphotyrosine. BRK/Sik YF expression led to tyrosine phosphorylation of Myc-Sam68, Myc-SLM-1, and Myc-SLM-2 but not the related protein hrRNP K or Sam68 (68–347) lacking the tyrosine-rich carboxyl terminus. Wild-type BRK/Sik protein, visualized by rhodamine-avidin (red), was distributed throughout the cell (Fig. 3, panels F and G). Colocalization of GFP-SLM-1 and wild-type BRK/Sik in the nucleus is shown in Fig. 3G (yellow). In contrast to the wild-type protein, constitutively active BRK/Sik YF was present primarily in nuclei of transfected cells where it colocalized with GFP-SLM-1 (Fig. 3A, panels J and K). A rare instance in which both expression constructs were not taken up by the same cell is shown in Fig. 3B. BRK/Sik YF is localized throughout the cell in the cell lacking expression of GFP-SLM-1, but it is nuclear in the cell expressing GFP-SLM-1 (Fig. 3B, panels B and C). These data suggest that activated BRK/Sik YF is more efficiently retained in the nuclei of cells expressing high levels of substrate. Similar patterns of colocalization of GFP-SLM-2 with wild-type BRK/Sik and BRK/Sik YF were detected (data not shown).

Following transfection of wild-type BRK/Sik and BRK/Sik YF differences in the intracellular localization of phosphotyrosine specific immunoreactivity were also observed. Differences in the localization of phosphotyrosine in cells transfected with wild-type BRK/Sik and BRK/Sik YF and GFP-tagged SLM-2 are shown in Fig. 4. Expression of wild-type BRK/Sik resulted in the tyrosine phosphorylation of cytoplasmic and nuclear proteins (Fig. 4, B and C), whereas expression of activated BRK/Sik YF led to enhanced phosphotyrosine immunoreactivity within the nucleus (Fig. 4, F and G). Phosphotyrosine activity was dependent on BRK/Sik activity and not detected in cells expressing kinase-defective BRK/Sik KM with a mutation in its ATP binding site (Fig. 4, F and G). Most of the BRK/Sik tyrosine phosphorylated proteins colocalized with SLM-2 (Fig. 4, C and G). Cotransfection of the GFP expression vector pEGFP-C1 and the empty BRK/Sik expression vector pcDNA3 resulted in diffuse GFP fluorescence throughout the cell and no detectable anti-phosphotyrosine staining (Fig. 4, M and N). In additional control experiments with IgG, no specific fluorescent signal was detected (data not shown). Increased nuclear localization of activated BRK/Sik YF (Fig. 3) and increased phosphotyrosine immunoreactivity in cells expressing BRK/Sik YF suggest that the carboxyl-terminal tyrosine of BRK/Sik functions to regulate both activity and subsequent localization of the kinase.

The intracellular localization of wild-type and activated BRK/Sik YF was also examined following fractionation of transfected NMuMG cells (Fig. 5A). Higher levels of BRK/Sik YF were present in the nuclear fractions than wild-type BRK/Sik YF. Immunoblotting with antibodies against Sp1 and α-tubulin served as a control to demonstrate the enrichment of nuclear and cytoplasmic/membrane proteins in the respective fractions. Confocal microscopy also indicated that higher levels of BRK/Sik YF were present in nuclei of transfected NMuMG...
BRK/Sik is transfected into the cells but only in the nucleus following transfection, and localization of GFP-SLM-1 and BRK/Sik was examined. BRK/Sik immunolocalization was detected using rhodamine (red), and 4',6-diamidino-2-phenylindole (DAPI) was used to stain the nuclei. BRK/Sik is not expressed in NMuMG cells (A–D). Transfected wild-type BRK/Sik showed both cytoplasmic and nuclear localization. Some nuclear colocalization of GFP-SLM-1 and wild-type BRK/Sik was detected (G, yellow); BRK/Sik YF was primarily nuclear in cells expressing SLM-1 (J and K). B, enhanced nuclear localization of BRK/Sik YF in a cell expressing GFP-SLM-1. GFP-SLM-1 expression (A, green) increases BRK/Sik YF nuclear localization (B, red) and BRK/Sik YF colocalization with SLM-1 (C, yellow). Bars represent 10 μm.

Fig. 4. Increased nuclear phosphotyrosine in cells expressing BRK/Sik and SLM-2. NMuMG cells were transfected with GFP-SLM-2 and wild-type BRK/Sik (A–D), GFP-SLM-2 and BRK/Sik YF (E–H), GFP-SLM-2 and kinase defective BRK/Sik KM (I–L), or the GFP expression vector pEGFP-C1 and pcDNA3 (M–P). Cells were fixed 24 h after transfection, and tyrosine-phosphorylated proteins were localized using anti-phosphotyrosine antibodies (B, F, J, and N). 4',6-Diamidino-2-phenylindole (DAPI) was used to stain the nuclei (D, H, L, and P). In NMuMG cells, SLM-2 displays diffuse, nuclear localization visible by green fluorescence (A, E, and I). Cells cotransfected with GFP-SLM-2 and wild-type BRK/Sik or BRK/Sik YF also stain strongly with the anti-phosphotyrosine antibody visualized using rhodamine (B and F), whereas no phosphotyrosine is detected in cells expressing kinase-defective BRK/Sik KM (J). Interestingly, phosphotyrosine immunoreactivity is seen in the cytoplasm and at the membrane when wild-type BRK/Sik is transfected into the cells but only in the nucleus following transfection of BRK/Sik YF. C, G, K, and O are composites demonstrating colocalization that appears yellow. GFP alone is expressed throughout the cell (M) and is negative for anti-phosphotyrosine staining (N). Bar represents 5 μm.

Fig. 5. Intracellular localization of wild-type and activated BRK/Sik YF in transfected NMuMG cells. A, NMuMG cells were transfected with empty vector (V), wild-type (WT), or activated BRK/Sik YF expression constructs. Cells were fractionated into nuclear and membrane/cytoplasmic fractions, and localization of transfected BRK/Sik protein was examined by immunoblotting. A greater proportion of activated BRK/Sik YF is found in the nucleus. B, localization of transfected BRK/Sik protein was examined using immunohistochemistry and confocal microscopy. BRK/Sik YF exhibits increased nuclear localization in NMuMG cells (panel C). Bar represents 10 μm.
of BRK/Sik, SLM-1, SLM-2, and Sam68 RNAs by performing RNase protection assays using total RNA from multiple different mouse tissues of male and female mice. Because BRK/Sik plays a role in breast cancer, we examined expression of its substrates in mammary glands isolated from nulliparous virgin female mice and multiparous breeder females that had raised litters (Fig. 7).

As expected, BRK/Sik was expressed at highest levels in the gastrointestinal tract and skin. No BRK/Sik expression was detected in normal mammary gland at any stage. SLM-1 and SLM-2 exhibited restricted patterns of expression, whereas Sam68 was ubiquitously expressed. Notable levels of SLM-1 were coexpressed with BRK/Sik in skin, colon, and cervix, whereas SLM-2 exhibited testes-specific expression. No expression of BRK/Sik protein has been detected in the mouse testes (data not shown). These findings indicate that SLM-1 and Sam68 may be relevant substrates of BRK/Sik in vivo.

BRK/Sik negatively regulates the ability of SLM-1 and SLM-2 to bind RNA. NMuMG cells were cotransfected with Myc epitope-tagged SLM-1 (A), SLM-2 (B), or Sam68 (C) and the expression vector pcDNA3 alone or activated BRK/Sik YF. Total cell lysates were divided equally and precipitated with Sepharose 4B as a control (C) or poly(U)-agarose (pU), poly(A)-agarose (pA) and poly(G)-agarose (pG) followed by anti-Myc immunoblotting (right panels). Aliquots of total cell lysates were probed for Myc-SLM-1, SLM-2, or Sam68 and BRK/Sik YF as well as tyrosine phosphorylation (PY) of SLM-1, SLM-2, or Sam68 (left panels). The ability of Myc-SLM-1 to bind poly(A)-agarose and Myc-SLM-2 to bind poly(G)-agarose was inhibited in cells transfected with BRK/Sik YF.

FIG. 7. Expression of BRK/Sik RNA-binding protein substrates in mouse tissues. RNase protection assays were performed with total RNAs from a variety of tissues from the mouse (male mice and multiparous and virgin females as indicated) and 32P-labeled antisense probes specific for BRK/Sik, Sam68, SLM-1, and SLM-2. Cyclophilin expression was examined as a control. Sam68 is ubiquitously expressed, whereas SLM-1 and SLM-2 have a much more restricted pattern of expression. Although BRK/Sik is induced in a high percentage of breast tumors, mouse BRK/Sik and its SLM substrates are not expressed in adult female mammary gland.

FIG. 8. Functions of BRK/Sik in normal tissues and tumors may be dependent on its intracellular localization and access to different substrates. In breast cancer cells, BRK/Sik appears to promote growth by enhancing epidermal growth factor receptor and ErbB3 receptor signaling (14, 53). However in normal tissues, BRK/Sik expression is associated with cell cycle exit and differentiation of epithelial linings (4–7, 9). In normal prostate epithelial cells BRK/Sik is enriched in the nucleus where it associates with Sam68, whereas BRK/Sik is relocalized to the cytoplasm in prostate tumors (6). Differential localization of BRK/Sik in normal tissues and cancer cells may lead to activation of divergent signaling pathways.
**DISCUSSION**

Previously we showed that BRK/Sik phosphorylates the nuclear protein Sam68 and inhibits its RNA-binding functions (11). Here we demonstrated that SLM-1 and SLM-2 are substrates of BRK/Sik and that their RNA-binding abilities are inhibited by tyrosine phosphorylation. The RNA-binding functions of these STAR proteins have been implicated in the post-transcriptional regulation of gene expression. Both SLM-1 and SLM-2 have been shown to stimulate HIV-1 Rev activity (22, 39) and to regulate the selection of alternative splice sites in transcripts encoded by a CD44 minigene (40, 41). However, the cellular functions of these two RNA-binding proteins and their DNA targets are still poorly understood.

To begin to understand the significance of SLM-1 and SLM-2 phosphorylation in vivo, we examined the expression of these two proteins in mouse tissues. SLM-1 is coexpressed with BRK/Sik in some epithelial tissues, including colon and skin. However our data and that of others (33, 42) indicate that SLM-2 is predominantly expressed in testis, a tissue lacking significant BRK/Sik expression. Although SLM-1 and Sam68 may be phosphorylated by other kinases such as Src and Fyn (31), BRK/Sik colocalizes with SLM-1 and Sam68 in the nucleus and may regulate nuclear functions.

In previous studies, SLM-2 was not demonstrated to be a Src or Fyn substrate (31), so it is interesting that this RNA-binding protein is a BRK/Sik substrate. However lack of coexpression of SLM-1 and BRK/Sik in epithelial tissues makes it unlikely that this STAR substrate is physiologically relevant. Further studies will be required to determine whether other nonmyristoylated members of the BRK/Sik kinase family such as Srms or FRK/RAk (reviewed in Ref. 10) are coexpressed with SLM-2 in the testis and able to regulate its nuclear functions.

Although BRK/Sik phosphorylates SLM-1, SLM-2, and Sam68 in the nucleus, it did not phosphorylate the KH domain-containing protein hnRNP K (Fig. 2). In addition BRK/Sik does not phosphorylate the ubiquitous nuclear protein YT521-B that regulates alternative splicing and associates with Sam68 (43). YT521-B is a substrate of Src and Fyn in the cytoplasm and c-Abl in the nucleus (43). In the nucleus BRK/Sik has substrate specificity and appears to regulate specific STAR family signaling pathways.

Sam68 is one of the most well characterized members of the STAR family of RNA-binding proteins (reviewed in Ref. 16), but its physiological functions are still not well understood. For example, both growth inhibiting and growth promoting functions have been reported for Sam68. A retroviral-based antisense strategy revealed that Sam68 deficiency led to transformation of murine NIH 3T3 fibroblasts (44). A recent study has implicated members of the BRK/Sik kinase family such as Srms or FRK/Rak (reviewed in Ref. 10) are coexpressed with SLM-2 in normal tissues and in cancer cells may lead to activation of divergent signaling pathways (Fig. 8). In some epithelial tissues, BRK/Sik appears to be positioned to inhibit RNA-binding activities of its nuclear STAR protein family substrates during differentiation. Further studies will be required to determine the significance of BRK/Sik and STAR family proteins in epithelial cell signal transduction.

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The Nuclear Tyrosine Kinase BRK/Sik Phosphorylates and Inhibits the RNA-binding Activities of the Sam68-like Mammalian Proteins SLM-1 and SLM-2
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