Biochemical and Biophysical Research Communications 370 (2008) 62–66

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Subcellular localization of the Schlafen protein family

Brent Neumann, Liang Zhao, Kathleen Murphy [1], Thomas J. Gonda *

University of Queensland Diamantina Institute for Cancer, Immunology and Metabolic Medicine, Level 4, R-Wing, Building 1, Princess Alexandra Hospital, Brisbane, Qld. 4102, Australia

ARTICLE INFO

Article history:
Received 4 March 2008
Available online 18 March 2008

Keywords:
Murine
Schlafen
Subcellular localization
Cytoplasm
Nucleus
Differentiation

ABSTRACT

Although the first members of the *Schlafen* gene family were first described almost 10 years ago, the precise molecular/biochemical functions of the proteins they encode still remain largely unknown. Roles in cell growth, haematopoietic cell differentiation, and T cell development/maturation have, with some experimental support, been postulated, but none have been conclusively verified. Here, we have determined the subcellular localization of Schlafens 1, 2, 4, 5, 8, and 9, representing all three of the murine subgroups. We show that the proteins from subgroups I and II localize to the cytoplasm, while the longer forms in subgroup III localize exclusively to the nuclear compartment. We also demonstrate upregulation of Schlafen2 upon differentiation of haematopoietic cells and show this endogenous protein localizes to the cytoplasm. Thus, we propose the different subgroups of Schlafen proteins are likely to have functionally distinct roles, reflecting their differing localizations within the cell.

The first *Schlafen* (*Slfn*) genes were identified by Schwarz et al. as a multigene family in mice whose members were differentially expressed during thymocyte maturation and T cell activation [1]. Subsequent studies revealed three subgroups based on overall sequence homology and size of the encoded proteins [2]: subgroup I is composed of the shortest three proteins (Slfn1, Slfn2, and Slfn Like 1 [3]); Slfn3 and Slfn4 form subgroup II; while subgroup III comprises five proteins, Slfn5, Slfn8–10, and Slfn14, all of which possess large C-terminal extensions when compared to subgroups I and II [2]. Thus, 10 murine Slfn proteins exist, all which contain several highly conserved domains/motifs (Fig. 1). Within all Slfn proteins is a domain not found in any other characterized proteins, a so-called "Slfn box", which lies adjacent to a divergent AAA domain [2,4], a motif thought, based on homology, to function similarly to the classical AAA domains in GTP/ATP binding. The classical AAA domain-containing proteins perform a vast range of roles including protein degradation and folding, vesicle transport/fusion, and transcription [5–7]. The highly conserved "SWADL" domain, defined by a five amino acid (Ser-Trp-Ala-Asp-Leu) signature, also appears to be Slfn protein-specific and is confined to subgroups II and III. Additionally, within the C-terminal extensions found in the subgroup III Slfns are sequence motifs homologous to the superfamily I of DNA/RNA helicases [2], domains known to mediate many different aspects of DNA and RNA metabolism, ranging from transcription, splicing and translation to RNA degradation [8,9]. Thus, the identification of these potential functional domains within the Slfn protein family provides few clues to their activities and roles.

Slfns 1–3 have been reported to possess potent growth inhibitory properties *in vitro* [1,2,4], presumably through the inhibition of cyclin D1 expression [4]. However, these observations cannot be replicated in our laboratory [10]. Mice lacking *Slfn1* appear to exhibit a normal phenotype [1], while transgenic mice expressing *Slfn1* or *Slfn8* within the T-cell lineages displayed an overall decrease in thymocyte number [1,2]. *Slfn* gene expression has been reported to increase after viral and bacterial infection [2,11–19], and upon stimulation of macrophages [2,20,21]. Furthermore, AP-1 and NF-κβ are reported to regulate *Slfn2* expression [11]. These data hint at a role for the Slfn proteins in the immune response.

There is evidence that *Slfn* expression increases during cellular differentiation. Geserick et al. showed that the majority of *Slfns* are upregulated upon IL6- or LIF-driven differentiation of the M1 monocytic leukemia cell line into macrophage-like cells [2]. While other studies have demonstrated similar findings in other, predominantly haematopoietic cell lines [22–25]. Our own studies revealed that *Slfn1*, *2*, *3*, and *4* were upregulated after differentiation was induced in ERMYB [26] and FDB1 [27] myeloid cells [10,28].

Given our lack of knowledge on the function of Slfn proteins, we have determined the subcellular localization of the murine Slfn proteins, as a step towards elucidation of their molecular/biochemical activities. Using mammalian cell lines and epitope tagged versions of the proteins, we have found clear differences in localization between the subgroups I and II Slfns compared with those from subgroup III. In addition, we have analysed the localization of endogenous Slfn2 following its upregulation in differentiated myeloid haematopoeitic cells.

* Corresponding author. Fax: +61 7 3240 5946.
E-mail address: t.gonda@uq.edu.au (T.J. Gonda).
[1] Present address: Corbett Robotics, Brisbane, Qld., Australia.

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.03.032

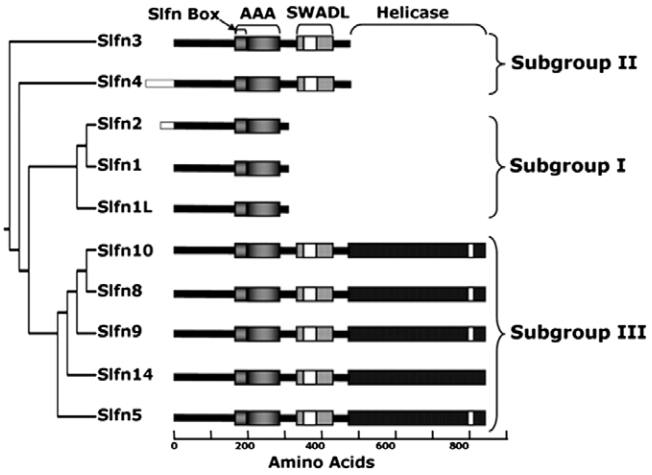

Fig. 1. The murine Schlafen protein family. The 10 murine Slfn proteins are aligned in a phylogenetic tree to the left, which evidently divides the family into three distinct subgroups. Shaded boxes represent regions of high conservation within the Slfn proteins. The "Slfn box" and the divergent AAA domain characterize all of the Slfn proteins, while the "SWADL" and DNA/RNA helicase domains are exclusive to subgroups II and/or III. Figure is modified from [2] and the phylogenetic tree prepared using the BioManager program (http://www.angis.org.au) [37,38].

Materials and methods

Slfn constructs. Slfn1, *Slfn4*, and *Slfn8* clones were obtained from Dr. Jens Zerrahn (Max-Planck Institute for Infection Biology, Germany), *Slfn2* was amplified from ER-MYB cells, *Slfn5* and *Slfn9* were purchased from the Fantom clone repository (Institute for Molecular Bioscience, Brisbane, Australia). Full length 3′FLAG-tagged *Slfn* coding regions were ligated into the pcDNA3 plasmid. *Slfn9* coding region was ligated into pCM5-KHA$_2$M1 [29], downstream of two HA epitope sequences.

Cell culture. HEK 293T cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. HEK 293 T cells (1×10^5 in six-well plates) were transfected with 4 μg DNA using Lipofectamine 2000 reagent (Invitrogen). ER-MYB cells [25] were growth in Iscove's Modified Dulbecco's Medium (IMDM) containing 10% FCS, 80 U/mL GM-CSF and 1 μM β-estradiol (Sigma–Aldrich). To induce differentiation, β-estradiol was removed and cells cultured for 3 days with 1 μM estrogen antagonist ICI,182,780 (Tocris Bioscience).

Immunofluorescence. HEK 293T cells were grown on coverslips and 48 h post-transfection, were washed with PBS and fixed with methanol:acetone:formaldehyde (47.5:47.5:5) for 1 min at −20 °C. Cells were blocked with PBS/BSA (3%)/Tween-20 (1%) for 1 h on ice, then incubated for 1.5 h on ice with the following primary antibody/s: anti-FLAG M2 (Sigma–Aldrich) (1:150 dilution), anti-HA (Abcam) (1:2000 dilution), anti-SC-35 (Sigma–Aldrich) (1:1000 dilution), anti-RNA Polymerase II (Covance) (1:50 dilution), anti-Slfn2 (Santa Cruz) (1:25 dilution). Cells were washed with PBS and incubated for 45 min on ice in DAPI (Invitrogen) (1:1000 dilution) and the following secondary antibody/s: anti-mouse IgG-FITC (Sigma–Aldrich) (1:64 dilution), anti-Rabbit IgG-Texas Red (Invitrogen) (1:750 dilution), anti-Goat IgG-FITC (Santa Cruz) (1:100 dilution). Cells were washed with PBS, dried, mounted using ProLong® Gold anti-fade reagent (Invitrogen) and visualized with a Zeiss Imager Z1 microscope fitted with an ApoTome imaging system.

Whole cell extracts. Cells were collected from 10 cm dishes, washed with PBS and resuspended in 500 μL lysis buffer (100 mM NaCl, 30 mM Tris–HCl (pH 8.0), 10 mM EDTA, 0.1% NP-40 and protease inhibitors). Cells were sonicated twice at 50% output for 10 s and centrifuged for 40 min at 4 °C.

Immunoblotting. Total protein (30 μg) was suspended in 1× SDS-buffer (50 mM Tris–HCl (pH 6.8), 2% SDS, 0.1% bromophenol blue, 10% glycerol, and 100 mM β-mercaptoethanol) and fractionated by SDS–PAGE. Proteins were transferred to polyvinylidene difluoride membranes, and incubated in 5% nonfat milk powder in TBS-T buffer (Tris-buffered saline, pH 7.2, 0.1% Tween 20) for 1 h before O/N incubation with primary antibody (anti-FLAG (1:1000), anti-Slfn2 (1:50), anti-β-actin (1:4000)). Membranes were washed thrice, before incubation with secondary, horseradish peroxidase conjugated antibodies for 1 h. Membranes were washed thrice and developed with ECL reagent (Amersham Biosciences).

Results

Subcellular localization of the Slfn proteins

To date, nothing has been reported in regard to the subcellular localization of the Slfn proteins, which may in part be due to the lack of specific commercial antibodies to the majority of the proteins. Thus, to determine the subcellular localization of the murine Slfn proteins, FLAG-tagged versions were expressed in HEK 293T cells and immunofluorescence analyses were performed. As shown in Fig. 2A, the majority of Slfn1 is present in the cytoplasm, with a minor proportion also evident in the nucleus. Slfn2 and Slfn4 show similar patterns of expression. Both of these proteins are clearly and exclusively cytoplasmic (Fig. 2B and C). Hence, these three proteins, from subgroups I and II of the family, predominantly or exclusively localize to the cytoplasm in mammalian cells. Similar results were found with other cell lines, including murine NIH/3T3 cells (data not shown). To confirm the correct proteins were expressed in each instance, immunoblotting was carried out and the expected sized proteins for Slfn1 (∼40 kDa), Slfn2 (∼42 kDa), and Slfn4 (∼69 kDa) were observed (Fig. 2D).

Next, several Slfn proteins from subgroup III were analysed. Within the C-terminal extensions of these proteins is a putative nuclear localization signal, indicating that these may in fact be nuclear proteins. A RKRRR motif is found at amino acid position 832–836 in Slfn8 and Slfn9, and is partially conserved in Slfn5 (aa805–807) and Slfn10 (aa830–834). FLAG-tagged versions of Slfn5, Slfn8, and Slfn9 expressed in HEK 293T cells revealed that, in contrast to the Slfn proteins in subgroups I and II, Slfns 5, 8, and 9 are exclusively localized to the nucleus (Fig. 3). The proteins are all excluded from the nucleolus and display a "speckled" staining pattern across the remainder of the nucleus, which is especially evident for Slfn9 (Fig. 3C). Again, expression of each specific Slfn protein was verified by immunoblotting (Slfn5 ∼101 kDa, Slfn8 and Slfn9 ∼105 kDa; Fig. 3D).

Co-localization of subgroup III Schlafen proteins with nuclear factors

Slfns 5, 8, and 9 were found to be expressed in distinctive speckled patterns across the nucleus. This suggested that these proteins may localize to the splicing machinery or to active sites of transcription, which often show similar patterns [30,31]. Thus, we conducted co-localization studies using HA-tagged Slfn9 and

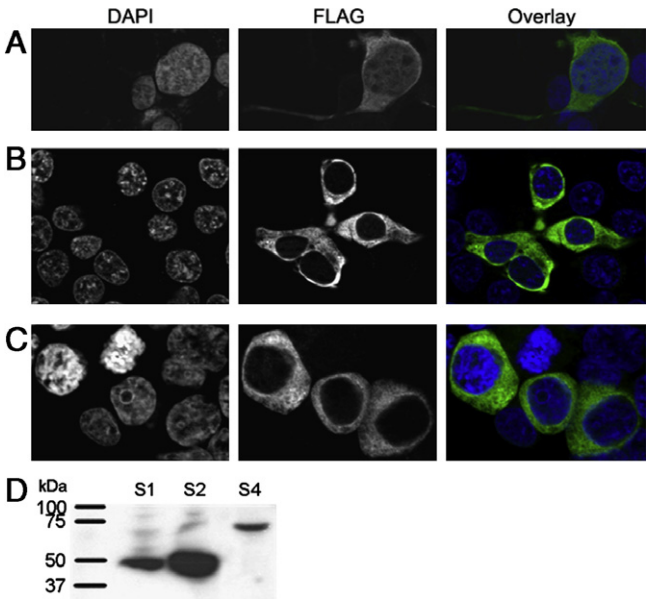

Fig. 2. Immunofluorescence analysis of subgroups I and II Schlafen proteins. FLAG-tagged Schlafen1 (A), Schlafen2 (B), and Schlafen4 (C) proteins were expressed by transient transfection in HEK 293T cells. From left to right is DAPI staining of the nuclei, the Schlafen proteins and the overlays of these two images (nuclei in blue, Schlafen proteins in green). Immunoblotting confirmed expression of each specific protein (D).

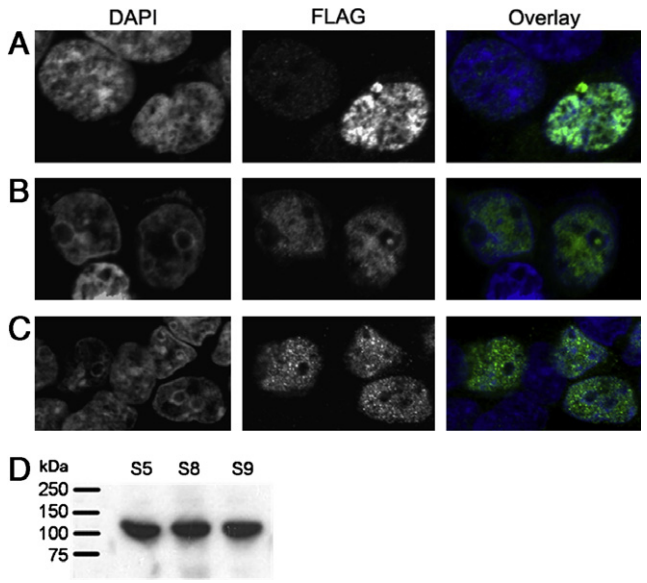

Fig. 3. Localization of subgroup III Schlafen proteins. Expression of FLAG tagged Schlafen5 (A), Schlafen8 (B), and Schlafen9 (C) in HEK 293T cells is shown. In each case, the nuclei are shown stained with DAPI on the left, Schlafen proteins in the middle panels and the overlays are shown on the right (nuclei in blue, Slfn proteins in green). Expression of each protein was confirmed by immunoblotting (D).

antibodies against the SC-35 protein, which forms part of the splicing machinery [32], and against the active, phosphorylated form of RNA polymerase II (pRNA Pol II). Co-localization of Slfn9 with pRNA Pol II (A) and SC-35 (B) is shown in Supplementary Fig. 1. While expression patterns are not exactly the same, it is clear that there is overlap of staining between Slfn9 and pRNA Pol II (Supplementary Fig. 1A Overlay 1). However, co-immunoprecipitation experiments failed to detect binding of any Slfn protein to either of these nuclear factors (data not shown). Thus, we have observed co-localization of subgroup III Slfn proteins with regions of active transcription, which is independent of detectable direct association with pRNA Pol II.

Expression of Schlafen2 in differentiated myeloid cells

To confirm upregulated expression upon differentiation and to analyse the subcellular localization of the endogenous Slfn2 protein, the ERMYB cell line, which proliferates with an immature myeloid phenotype and can be induced to differentiate into macrophages [26], was used. Q-PCR confirmed that *Slfn2* is upregulated at the mRNA level under these conditions [10]. Proliferating and differentiated cells were analysed by immunoblotting and immunofluorescence using the commercially available Slfn2 antibody (Santa Cruz). No Slfn2 expression was detected in proliferating cells (Fig. 4A and B), but a clear upregulation was seen in those cells induced to differentiate (Fig. 4A and C). Furthermore, it is clear that the endogenous Slfn2 protein localizes exclusively to cytoplasm (Fig. 4C). These results agree with those of Fig. 2B, demonstrating that endogenous Slfn2 localizes to the cytoplasm.

Discussion

Here we have determined the subcellular localization of several murine Slfn proteins. Our analyses have established distinct localization patterns among the three Slfn subgroups, with the proteins from subgroups I and II displaying cytoplasmic staining and the members from subgroup III exhibiting exclusively nuclear staining. Our results also demonstrate possible co-localization of subgroup III Slfn proteins with the active form of RNA polymerase II. Furthermore, we have confirmed upregulation of Slfn2 upon differentiation of haematopoeitic cells and established that upregulated, and presumably active, Slfn2 shows an exclusive and ubiquitous cytoplasmic localization.

The lack of commercial antibodies against the majority of Slfn family members necessitated the use of ectopically expressed, epitope-tagged versions of each protein for this study. However, the use of such proteins is not without its limitations. One potential problem is that addition of a tag may alter the way in which the native protein folds, interfering with the secondary structure of the tagged protein and thus, possibly altering its function and localization. However, confirmation of the cytoplasmic localization of endogenous Slfn2 (Fig. 4C) and that fact that similar localization patterns were observed using amino terminal FLAG tagged Slfn1, Slfn2, Slfn5, and Slfn8 constructs and carboxyl terminal HA tagged Slfn1 and Slfn9 (Supplementary Fig. 1 and data not shown) indicate the results provided here are likely to be an accurate representation of the localization of the Slfn proteins. Furthermore, Gubser et al. have shown the camelpox viral Slfn homolog (v-Slfn), which is most similar to Slfns 1 and 2, localizes predominantly to the cytoplasm in mammalian cells [33], agreeing with our findings.

Protein localization goes hand in hand with protein function, with correct localization both essential for and a major determinant of function. Thus, the different subcellular localizations found between the Slfn proteins, implies functional diversity. This notion is supported by previously published data. For example, subgroups I and II Slfn proteins, which are cytoplasmically localized (Fig. 2) are reported to inhibit cellular proliferation [1,2,4], while those found in the nucleus (Fig. 3) do not [2]. However, the cytoplasmic localization of Slfn1 creates something of a conundrum, as the reported growth suppression by Slfn1 occurred through inhibition of Cyclin D1 expression, at the level of promoter activity [4]. How the cytoplasmic Slfn1 could cause such an effect without being present in the nucleus is unclear. Indeed, our recent data do not support a role for either Slfn1 or Slfn2 in growth suppression [10]. A further possible disparity arising from the data presented here relates to the similar effects of transgenically expressed Slfn1 [1] and Slfn8 [2] on thymocyte numbers. The opposing subcellular localizations of these two proteins combined with their reported differential effects on cell growth [1,2] are somewhat difficult to reconcile with similar suppressive effects on thymocyte proliferation.

Further evidence for functional differences between the Slfn proteins is provided through analysis of the predicted proteins themselves. As shown in Fig. 1, the subgroup III proteins all contain large carboxyl terminal extensions, not found within the proteins from other subgroups. Highly homologous regions with the superfamily I RNA/DNA helicases are found in these extensions and indicate potential functions in DNA/RNA metabolism [2]. Indeed our results showing possible co-localization with the active form of RNA Polymerase II, indicates that Slfn5, 8 and 9 may be involved in the process of transcription.

Using the ERMYB cell line, we have found upregulated expression of Slfn2 during myeloid differentiation (Fig. 4). Various other studies have also linked the expression of Slfn2 with cellular, and in particular haematopoeitic cell, differentiation [2,23,34–36]. Thus, while little is known regarding the precise function of Slfn2, current evidence suggests a role in haematopoeitic differentiation. However, it should also be noted that a function within mature, fully differentiated cells is equally plausible. In addition to enhanced expression upon differentiation, this view is further supported by the upregulation of the *Slfns* in response to various inflammatory stimuli, including infection with bacteria and viruses. Response to such stimuli requires mature cells, such as macrophages and T cells. Hence, while Slfn2 could be involved in

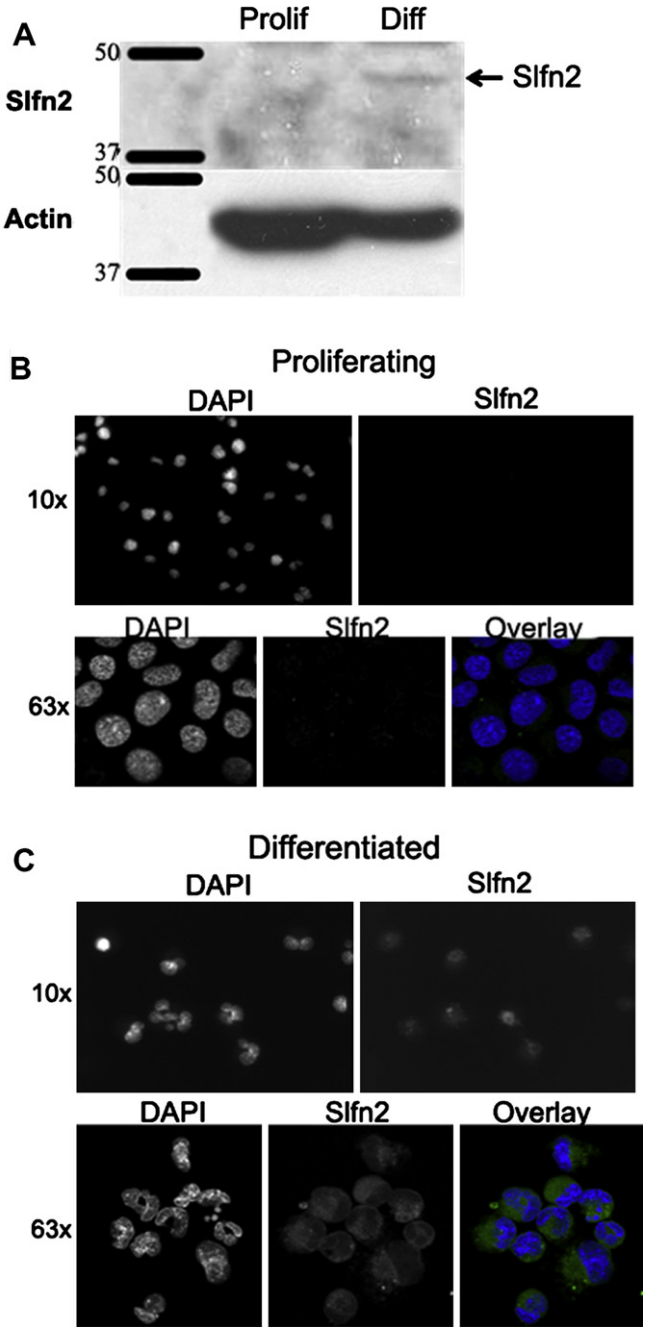

Fig. 4. Schlafen2 expression in proliferating and differentiated myeloid cells. ER-MYB cells were cultured in the presence or absence of β-estradiol for 72 h to induce proliferation or differentiation, respectively. (A) Whole cell extracts were analysed by immunoblotting using anti-Schlafen2 antibody (top panel), with β-actin as a loading control. Subcellular localization of the Schlafen2 protein was analysed in proliferating (B) or differentiated (C) ERMYB cells. In each case, the top panels display cells magnified 10 times (10×) and the bottom panels show cells visualized with 63× magnification. Overlay images show expression of Slfn2 in green and DAPI stained nuclei in blue.

the process of differentiation, it may also be involved in the functioning of mature cells.

While we believe we have gained significant insights into the cellular characteristics of the Slfn proteins, the identification of Slfn binding partners will be a critical step in confirming the putative functional roles suggested by the limited functional data currently available. In parallel, it will be essential to examine in detail the consequences of loss of function on relevant cell types.

Acknowledgments

We are grateful to Jens Zerrahn (Max Planck Institute for Infection Biology, Berlin) for providing several *Schlafen* clones. This work was supported in part by a Grant (to T.J.G.) from the Australian Research Council and an ANZ Trustees PhD scholarship (to B.N.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.03.032.

References

[1] D.A. Schwarz, C.D. Katayama, S.M. Hedrick, Schlafen, a new family of growth regulatory genes that affect thymocyte development, Immunity 9 (1998) 657–668.
[2] P. Geserick, F. Kaiser, U. Klemm, S.H. Kaufmann, J. Zerrahn, Modulation of T cell development and activation by novel members of the *Schlafen* (*slfn*) gene family harbouring an RNA helicase-like motif, Int. Immunol. 16 (2004) 1535–1548.
[3] D.A. Ferguson, J.T. Chiang, J.A. Richardson, J. Graff, eXPRESSION: an in silico tool to predict patterns of gene expression, Gene Expr. Patterns 5 (2005) 619–628.
[4] G. Brady, L. Boggan, A. Bowie, L.A. O'Neill, Schlafen-1 causes a cell cycle arrest by inhibiting induction of cyclin D1, J. Biol. Chem. 280 (2005) 30723–30734.
[5] P.I. Hanson, S.W. Whiteheart, AAA+ proteins: have engine, will work, Nat. Rev. Mol. Cell Biol. 6 (2005) 519–529.
[6] A.N. Lupas, J. Martin, AAA proteins, Curr. Opin. Struct. Biol. 12 (2002) 746–753.
[7] R.D. Vale, AAA proteins. Lords of the ring, J. Cell Biol. 150 (2000) F13–F19.
[8] S.G. Mackintosh, K.D. Raney, DNA unwinding and protein displacement by superfamily 1 and superfamily 2 helicases, Nucleic Acids Res. 34 (2006) 4154–4159.
[9] F.V. Fuller-Pace, RNA helicases: modulators of RNA structure, Trends Cell Biol. 4 (1994) 271–274.
[10] L. Zhao, B. Neumann, K. Murphy, J. Silke, T.J. Gonda, Lack of reproducible growth inhibition by *Schlafen1* and *Schlafen2 in vitro*, Blood Cells Mol. Dis., in press (2008).
[11] W.J. Sohn, D. Kim, K.W. Lee, M.S. Kim, S. Kwon, Y. Lee, D.S. Kim, H.J. Kwon, Novel transcriptional regulation of the *schlafen-2* gene in macrophages in response to TLR-triggered stimulation, Mol. Immunol. 44 (2007) 3273–3282.
[12] J. Caron, L. Lariviere, M. Nacache, M. Tam, M.M. Stevenson, C. McKerly, P. Gros, D. Malo, Influence of Slc11a1 on the outcome of *Salmonella enterica* serovar Enteritidis infection in mice is associated with Th polarization, Infect. Immun. 74 (2006) 2787–2802.
[13] A. Engler, S. Roy, C.K. Sen, D.A. Padgett, J.F. Sheridan, Restraint stress alters lung gene expression in an experimental influenza A viral infection, J. Neuroimmunol. 162 (2005) 103–111.
[14] L. Eskra, A. Mathison, G. Splitter, Microarray analysis of mRNA levels from RAW264.7 macrophages infected with *Brucella abortus*, Infect. Immun. 71 (2003) 1125–1133.
[15] C. Keller, R. Hoffmann, R. Lang, S. Brandau, C. Hermann, S. Ehlers, Genetically determined susceptibility to tuberculosis in mice causally involves accelerated and enhanced recruitment of granulocytes, Infect. Immun. 74 (2006) 4295–4309.
[16] S. Lund, K.V. Christensen, M. Hedtjarn, A.L. Mortensen, H. Hagberg, J. Falsig, H. Hasseldam, A. Schrattenholz, P. Porzgen, M. Leist, The dynamics of the LPS triggered inflammatory response of murine microglia under different culture and in vivo conditions, J. Neuroimmunol. 180 (2006) 71–87.
[17] W. Rahn, R.W. Redline, T.G. Blanchard, Molecular analysis of *Helicobacter pylori*-associated gastric inflammation in naive versus previously immunized mice, Vaccine 23 (2004) 807–818.
[18] J.R. Schurr, E. Young, P. Byrne, C. Steele, J.E. Shellito, J.K. Kolls, Central role of toll-like receptor 4 signaling and host defense in experimental pneumonia caused by Gram-negative bacteria, Infect. Immun. 73 (2005) 532–545.
[19] J.A. Smith, S.C. Schmechel, A. Raghavan, M. Abelson, C. Reilly, M.G. Katze, R.J. Kaufman, P.R. Bohjanen, L.A. Schiff, Reovirus induces and benefits from an integrated cellular stress response, J. Virol. 80 (2006) 2019–2033.
[20] R.S. Kota, J.C. Rutledge, K. Gohil, A. Kumar, R.I. Enelow, C.V. Ramana, Regulation of gene expression in RAW 264.7 macrophage cell line by interferon-gamma, Biochem. Biophys. Res. Commun. 342 (2006) 1137–1146.
[21] J.S. Welch, M. Ricote, T.E. Akiyama, F.J. Gonzalez, C.K. Glass, PPARgamma and PPARdelta negatively regulate specific subsets of lipopolysaccharide and IFN-gamma target genes in macrophages, Proc. Natl. Acad. Sci. USA 100 (2003) 6712–6717.
[22] Y.H. Huang, D. Li, A. Winoto, E.A. Robey, Distinct transcriptional programs in thymocytes responding to T cell receptor, notch, and positive selection signals, Proc. Natl. Acad. Sci. USA 101 (2004) 4936–4941.
[23] L. Bruno, R. Hoffmann, F. McBlane, J. Brown, R. Gupta, C. Joshi, S. Pearson, T. Seidl, C. Heyworth, T. Enver, Molecular signatures of self-renewal,

differentiation, and lineage choice in multipotential hemopoietic progenitor cells in vitro, Mol. Cell. Biol. 24 (2004) 741–756.
[24] S. Rossetti, A.T. Hoogeveen, P. Liang, C. Stanciu, P. van der Spek, N. Sacchi, A distinct epigenetic signature at targets of a leukemia protein, BMC Genomics 8 (2007) 38.
[25] C.G. James, C.T. Appleton, V. Ulici, T.M. Underhill, F. Beier, Microarray analyses of gene expression during chondrocyte differentiation identifies novel regulators of hypertrophy, Mol. Biol. Cell 16 (2005) 5316–5333.
[26] A. Hogg, S. Schirm, H. Nakagoshi, P. Bartley, S. Ishii, J.M. Bishop, T.J. Gonda, Inactivation of a c-Myb/estrogen receptor fusion protein in transformed primary cells leads to granulocyte/macrophage differentiation and down regulation of c-kit but not c-myc or cdc2, Oncogene 15 (1997) 2885–2898.
[27] M.P. McCormack, T.J. Gonda, Novel murine myeloid cell lines that exhibit a differentiation switch in response to IL-3 or GM-CSF, or to different constitutively active mutants of the GM-CSF receptor beta subunit, Blood 95 (2000) 120–127.
[28] A.L. Brown, C.R. Wilkinson, S.R. Waterman, C.H. Kok, D.G. Salerno, S.M. Diakiw, B. Reynolds, H.S. Scott, A. Tsykin, G.F. Glonek, G.J. Goodall, P.J. Solomon, T.J. Gonda, R.J. D'Andrea, Genetic regulators of myelopoiesis and leukemic signaling identified by gene profiling and linear modeling, J. Leukoc. Biol. 80 (2006) 433–447.
[29] D.H. Dowhan, E.P. Hong, D. Auboeuf, A.P. Dennis, M.M. Wilson, S.M. Berget, B.W. O'Malley, Steroid hormone receptor coactivation and alternative RNA splicing by U2AF65-related proteins CAPERalpha and CAPERbeta, Mol. Cell 17 (2005) 429–439.
[30] C.S. Osborne, L. Chakalova, K.E. Brown, D. Carter, A. Horton, E. Debrand, B. Goyenechea, J.A. Mitchell, S. Lopes, W. Reik, P. Fraser, Active genes dynamically colocalize to shared sites of ongoing transcription, Nat. Genet. 36 (2004) 1065–1071.
[31] D.L. Spector, X.D. Fu, T. Maniatis, Associations between distinct pre-mRNA splicing components and the cell nucleus, EMBO J. 10 (1991) 3467–3481.
[32] X.D. Fu, T. Maniatis, Factor required for mammalian spliceosome assembly is localized to discrete regions in the nucleus, Nature 343 (1990) 437–441.
[33] C. Gubser, R. Goodbody, A. Ecker, G. Brady, L.A. O'Neill, N. Jacobs, G.L. Smith, Camelpox virus encodes a schlafen-like protein that affects orthopoxvirus virulence, J. Gen. Virol. 88 (2007) 1667–1676.
[34] G. Despars, K. Ni, A. Bouchard, T.J. O'Neill, H.C. O'Neill, Molecular definition of an in vitro niche for dendritic cell development, Exp. Hematol. 32 (2004) 1182–1193.
[35] S. Greenbaum, A.S. Lazorchak, Y. Zhuang, Differential functions for the transcription factor E2A in positive and negative gene regulation in pre-B lymphocytes, J. Biol. Chem. 279 (2004) 45028–45035.
[36] N. McCarty, M.L. Shinohara, L. Lu, H. Cantor, Detailed analysis of gene expression during development of T cell lineages in the thymus, Proc. Natl. Acad. Sci. USA 101 (2004) 9339–9344.
[37] J.D. Thompson, D.G. Higgins, T.J. Gibson, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Res. 22 (1994) 4673–4680.
[38] J. Felsenstein, PHYLIP—Phylogeny Inference Package (Version 3.2), Cladistics 5 (1989) 164–166.