Biochimica et Biophysica Acta 1828 (2013) 2801–2807

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

The site-2 protease ☆

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ARTICLE INFO

Article history:
Received 4 January 2013
Received in revised form 6 March 2013
Accepted 25 March 2013
Available online 6 April 2013

Keywords:
ER stress
Regulated intramembrane proteolysis
Rip
Site-2 protease
S2P
SREBP

ABSTRACT

The site-2 protease (S2P) is an unusually-hydrophobic integral membrane protease. It cleaves its substrates, which are membrane-bound transcription factors, within membrane-spanning helices. Although structural information for S2P from animals is lacking, the available data suggest that cleavage may occur at or within the lipid bilayer. In mammalian cells, S2P is essential owing to its activation of the sterol regulatory element binding proteins (SREBPs); in the absence of exogenous lipid, cells lacking S2P cannot survive. S2P is also important in the endoplasmic reticulum (ER) stress response, activating several different membrane-bound transcription factors. Human patients harboring reduction-of-function mutations in S2P exhibit an array of pathologies ranging from skin defects to neurological abnormalities. Surprisingly, *Drosophila melanogaster* lacking S2P are viable and fertile. This article is part of a Special Issue entitled: Intramembrane Proteases.

Contents

1. Introduction

The site-2 protease (S2P) holds a unique place in the field of intramembrane proteolysis. Prior to the identification of S2P, the notion that membrane-spanning helices might be cleaved within the plane of the membrane seemed almost heretical. Of course, if one considered the issue, mechanisms must exist to deal with helical portions of larger proteins left in the membrane by the action of sheddases or signal peptidase. Nevertheless, it seemed unnecessary to postulate proteases with active sites at or in the bilayer. When the S2P gene was cloned in 1997, new discoveries from the fields of Alzheimer's disease and cholesterol metabolism led some scientists to entertain this rather unorthodox hypothesis. Since the discovery of S2P, intramembrane proteolysis moved from being what was seemingly wild speculation to become a well-established mechanism for cellular signal transduction. This phenomenon is found in all kingdoms of life [1].

2. S2P

S2P was the first of the intramembrane-cleaving proteases (I-CLiPs; [2]) to be isolated [3]. In humans, S2P is a 519 amino acid protein encoded on the X chromosome. The sterol regulatory element

☆ This article is part of a Special Issue entitled: Intramembrane Proteases.

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http://dx.doi.org/10.1016/j.bbamem.2013.03.031

binding proteins (SREBPs) are membrane-bound transcription factors responsible for feedback regulation of transcription of genes required for fatty acid and cholesterol synthesis and uptake. In mammals there are two related SREBP genes, encoding SREBP-1 and SREBP-2. They are synthesized as integral membrane proteins of the endoplasmic reticulum (ER). The amino-terminal portion of SREBP contains the basic helix-loop-helix transcription factor domain while the carboxy terminal portion interacts with SREBP cleavage-activating protein (Scap), an escort factor. When cellular demand for lipid rises, SREBP:Scap complexes exit the ER *via* COP-II-coated vesicles and transit to the Golgi apparatus. In the Golgi, SREBPs are substrates for two different proteases, site-1 protease (S1P) and S2P. S1P is a membrane-bound serine protease with a luminally-disposed active site. It is related to other secretory pathway processing proteases such as furin and kexin. S1P cleaves SREBP within the short luminal loop between the first and second membrane-spanning helices. This cleavage creates a new carboxy-terminus for the transcription factor domain of SREBP. This is the substrate for S2P [4].

The isolation of S2P has been described in detail elsewhere [5]. Briefly, mutant M19 Chinese hamster ovary (CHO) cells are auxotrophic for cholesterol owing to inability to cleave SREBPs. In these cells, the membrane-bound intermediate form of SREBP, which is the product of S1P cleavage, accumulates [3]. This is because they harbor loss-of-function mutations in the gene encoding S2P. Complementation of the defect in these cells using human genomic DNA enabled isolation of the gene [3,6].

Certain families of metalloproteases are characterized by the presence of an His-Glu-(any amino acid)$_2$-His (HEXXH) motif that is involved in coordinating the active-site metal atom [7]. Although it exhibited no significant sequence similarity with any previously-reported protein, the presence of a His-Glu-Ile-Gly-His motif suggested that S2P might indeed be a protease.

Without an *in vitro* biochemical assay, data about the mechanism of intramembrane proteolysis by S2P remain indirect. In metalloproteases such as thermolysin, the histidines of the HEXXH motif help to coordinate the active-site zinc atom. The glutamate residue activates a water molecule that then makes the nucleophilic attack on the scissile bond. Other metalloproteases also contain an additional coordinating residue (such as Asp, Glu, His, or Tyr) at some distance in the linear sequence of the protein from the HEXXH motif. The Asp residue within the sequence L_{466}DG in human S2P lies 291 residues after the H_{171}EIGH motif. This residue is required for S2P function and is likely to provide the additional coordinating bond for the active site metal atom [8].

Mutation of either His in the HEIGH sequence or of the aspartate of the LDG sequence eliminates the ability of an S2P cDNA to restore SREBP cleavage in S2P$^-$ M19 cells [3,8]. Similarly, mutation of Glu_{172} to Ala abolished cleavage of SREBPs in mutant cells. However, unlike other metalloproteases, S2P retains significant complementation activity when Glu_{172} is replaced by Asp. The *Bacillus subtilis* SpoIVFB protein is an S2P-like metalloprotease. It cleaves membrane-localized Pro-σK precursor protein to produce the mature and active transcription factor σ(K), by removing an amino-terminal extension of 20 amino acids [9]. SpoIVFB likewise retains the ability to rescue mutants when an Asp residue is substituted for its active-site Glu [9]. These results from complementation assays, while indirect, provide the evidence that S2P is a protease. Accordingly, owing to its highly unusual sequence, S2P was placed in its own family of metalloproteases (MEROPS classification: clan MM, family M5A, peptidase M50.001).

Proteins related to S2P have been successfully assayed for proteolytic activity. Feng et al., [10] demonstrated proteolytic activity for an S2P homolog from *Methanocaldococcus jannaschii.* In *B. subtilis*, cleavage of Pro-σK by SpoIVFB releases σK to mediate the transcription of genes required during spore formation. Cleavage of Pro-σK *in vitro* by purified SpoIVFB requires zinc and, perhaps more interestingly, ATP [11]. While these prokaryotic homologs of S2P clearly are proteases, an *in vitro* assay for S2P from animals has not yet been reported.

3. Structural features of S2P

S2P is a hydrophobic protein with several putative membrane-associated or -embedded domains that are separated by distinct hydrophilic domains. These latter are accessible to the glycosylation machinery of the ER and Golgi [8]. Kinch et al., offer a model of S2P core domain topology (their Fig. 3) based on biochemical data and sequence comparisons [12].

In order for S2P to act, its substrate must first be cleaved by another protease [1]. Golgi localization of S2P was inferred from studies that demonstrated that initial cleavage of the substrate occurs in the Golgi apparatus [13,14]. Since S2P apparently cleaves its substrates immediately following this initial cleavage, the parsimonious hypothesis was that S2P is also in the Golgi. Studies of over-expressed, epitope-tagged S2P supported this notion [15]. We conducted immunolocalization studies in Chinese hamster ovary cells. These experiments provided direct evidence that the Site-1 protease (S1P) and S2P colocalize to the Golgi apparatus in human and hamster cells [5].

S2P is distinguished from soluble proteases by its notable hydrophobicity, especially of the residues around the HEXXH motif. The hydrophobic nature of its active-site region is consistent with the character of S2P substrates, which are hydrophobic membrane-spanning helices.

Conservation of sequence motifs among S2P homologs from widely-divergent organisms suggests that these motifs are important for S2P function [12]. In addition to the metal–atom coordinating residues, S2P contains other intriguing features in its primary structure. For example, in humans, starting at residue 108, S2P contains a run of 29 residues of which 26 are Ser. In hamster, the corresponding sequence is shorter, having Ser at 18/19 positions and the hamster protein is only 510 amino acids long [3]. The variability of this motif is evident even among primates. Gorilla and chimpanzee S2Ps differ from the human sequence by the addition to this motif of 1 and 2 serines respectively. Thus, the precise length of this motif is unlikely to be important in S2P function. However, even in fruit flies, there is a run of 6/7 Ser at this position [16]. What function these residues serve, if any, is unknown.

Residues 258–446 include 12 conserved Cys residues and display sequence similarity to PDZ domains. Analysis of S2P-family sequences suggests a functional role for this feature [12]. The putative PDZ domain of S2P may be involved in the recognition and/or binding of the newly-generated carboxy-terminus of the substrate. This is created by cleavage of the precursor by S1P. Studies of mutant *Escherichia coli* indicate that the PDZ domains of the S2P homologue RseP are needed for its activity [17]. As with other hypotheses regarding the mechanism of S2P cleavage, direct biochemical data for the function of the putative PDZ domain in mammalian S2P are not available. Fig. 1 shows models for the predicted membrane topology of S2P and its SREBP substrate. The positions of the serine-rich region and the putative PDZ domain are also indicated [8,12]. Compare the S2P model to that of a rhomboid protease, which cleaves type 1 membrane proteins.

Many soluble or membrane-associated proteases, such as trypsin, caspases, or even S1P, are made as proenzymes or preproenzymes. These require the cleavage of an inhibitory portion of the protein prior to becoming active enzymes. No evidence suggests that S2P requires any posttranslational modification(s) for activation. S2P substrates share the unusual characteristics of being membrane-spanning helices with short carboxy termini projecting into an extra-cytosolic compartment. As noted above, these arise only once a precursor has been cleaved by another, more conventional protease such as S1P. This fact may serve to limit S2P activity sufficiently to prevent it from cleaving inappropriate substrates.

4. Substrate features

A major difficulty in understanding the mechanism of intramembrane proteolysis is the likelihood that hydrolysis occurs within the plane of the lipid bilayer, possibly near the surface [10]. The substrates known for S2P

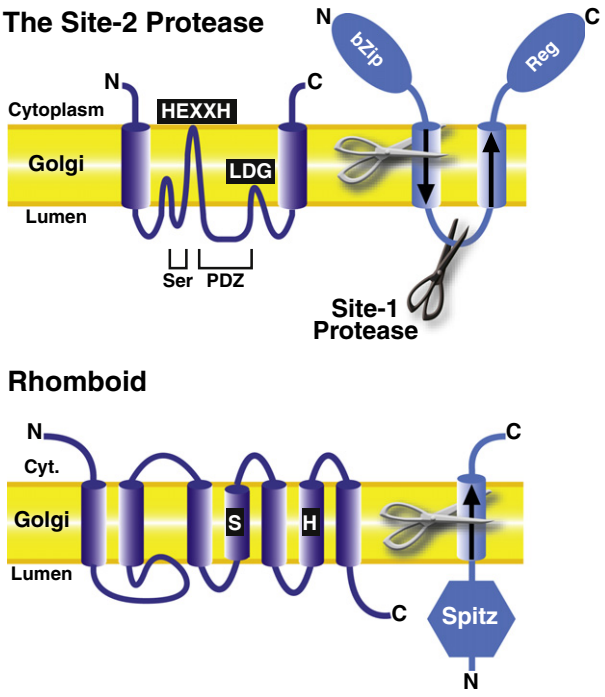

Fig. 1. Intramembrane-cleaving proteases and their substrates. The diagram shows membrane topology of S2P, Rhomboid, and representative substrates (SREBP and Spitz, respectively). The topologies shown are based on [8,12] (S2P) and [64,65] (rhomboid) and references cited therein. The rectangular bar represents the lipid bilayer of the Golgi apparatus with the cytosolic and luminal faces indicated. White lettering indicates the relative positions of crucial catalytic residues in these enzymes. Membrane-spanning helices are depicted as shaded cylinders. For the substrates, arrows indicate the membrane orientation of the substrate helices (Type 1, up-arrow; type 2, down-arrow). Cleavage sites are shown by scissors. The S2P substrate, which is a type 2 membrane protein, is produced by an initial cleavage of the target protein by S1P. In SREBP, this occurs in the short luminal loop (scissors). By contrast with other families of I-CLiPs, rhomboid proteases do not require initial cleavage of their substrates by separate proteases [64]. Brackets show the positions of the serine-rich region (Ser) and putative PDZ domain (PDZ) in S2P. The transcription factor domain of SREBP is indicated by bZip. The regulatory domain is labeled Reg. N and C designate the amino- and carboxy-termini of the proteins shown.

are all membrane-bound transcription factors of the ER that require two-step cleavage for activation (Table 1). They each have at least one type 2 membrane-spanning helix (amino-terminus cytoplasmic, carboxy-terminus extra-cytoplasmic;) within which cleavage by S2P occurs (Fig. 1).

Ye et al., showed that an Asp-Pro motif in the middle of the first membrane-spanning helix of SREBP is important for its cleavage by S2P [18]. Replacement of either the Asn or Pro residue by itself does not abrogate cleavage. Replacement of the two residues together almost completely blocks cleavage by S2P. These substitutions do not interfere with the anchoring function of the membrane-spanning helix; they merely make it resistant to cleavage by S2P [18]. Ye et al., proposed that this motif serves as an amino-terminal cap for a portion of the membrane-spanning helix. Such capping enables the remainder of the helix to partially unwind, permitting S2P access to the scissile bond. Similar helix-destabilizing motifs are features of the substrates of other I-CLiPs such as the signal peptide peptidase aspartyl protease and rhomboid intramembrane serine proteases [19,20].

Extracting a hydrophobic membrane-spanning helix from the bilayer into the aqueous environment entails substantial thermodynamic costs. When only a portion of a helix is extracted, these costs are reduced. Polypeptide chains within the bilayer are predisposed to adopt alpha helical structures. Moreover, peptide bonds in an alpha helix are resistant to hydrolysis compared to those in a more extended conformation [21]. It seems likely, then, that partial unwinding of substrate helices into a more extended conformation may be a typical feature of intramembrane proteolysis.

Like other I-CLiPs, S2P, is a large protease (519 amino acids in human). While the 'core' structure of S2P-like proteases consists of four membrane-spanning helices [10], S2P itself includes additional membrane-associated helices whose function is unclear. These extra sequences may help S2P create a local environment within the bilayer, perhaps near one surface, where both helix unwinding and hydrolysis are thermodynamically-favorable. The crystal structure of the rhomboid family protease GlpG, suggests just such a situation [22,23]. This may be a general strategy for cleaving membrane-spanning helices [24]. As with other aspects of S2P mechanism of action, structural and enzymological data will be necessary to test this idea.

5. Biological roles for S2P

S2P-like proteins are found in all kingdoms of life, showing that they arose very early in evolution. The genomes of archea, and those of gram-positive and gram-negative eubacteria encode clearly homologous proteins [3,9]. Homologs are also found in green plants, where they are likewise involved in the activation of membrane-bound transcription factors [25,26]. In the human fungal pathogen *Cryptococcus neoformans*, Stp1 is a functionally conserved ortholog of the mammalian site-2 protease. It cleaves the *C. neoformans* SREBP ortholog Sre1 within its predicted first membrane-spanning helix [27].

In metazoans, S2Ps play crucial roles in signaling pathways involved in cellular processes such as lipid metabolism and the unfolded protein response. These processes are necessary if the organism is to survive in changing environments. Because they entirely lack S2P, CHO M19 cells are suitable for testing the ability of transfected S2P cDNAs to restore the second-site cleavage of substrates such as SREBP, ATF6α, and CREBH. Such transfection–complementation experiments demonstrate that S2P is required for activation of these

Table 1
S2P substrates. Listed are mammalian proteins known or thought to be substrates for S2P because they are membrane-bound transcription factors that are substrates for S1P. Homologs of S1P, S2P and SREBP are also found in insects and other invertebrates.

Protein	Synonym	Membrane orientation	Function of released fragment	Process	Ref
SREBP-1a, c	ADD1	Type 2	Transcription factor	Lipid metabolism	[1]
SREBP-2		Type 2	Transcription factor	Lipid metabolism	[1]
ATF6α		Type 2	Transcription factor	ER stress response	[1,28]
ATF6β	CREB-RP	Type 2	Transcription factor	ER stress response	
Luman	CREB3, LZIP	Type 2	Transcription factor	Dendritic cell activation	[49,60]
OASIS	CREB3L1	Type 2	Transcription factor	ER stress response	[50]
BBF2H7	CREB3L2	Type 2	Transcription factor		[45,46,61]
CREBH	CREB3L3	Type 2	Transcription factor	ER stress response Gluconeogenesis	[29]
CREB4	AlbZIP, Tisp40, CREB3L4	Type 2	Transcription factor	ER stress response?	[62,63]

transcription factors [3,8,28,29]. Table 1 lists known and suspected S2P substrates and the pathways in which they participate.

5.1. Lipid metabolism

SREBPs are membrane-bound transcription factors that target genes needed for cholesterol uptake and synthesis as well as genes involved with fatty acid metabolism [30]. Vertebrates have two loci encoding SREBPs while other metazoans have a single SREBP gene. The role of S2P in activating SREBP is the same in all systems studied.

The SREBP precursor resides in the membranes of the endoplasmic reticulum. In mammals, increased cellular demand for cholesterol is signaled by alteration of the lipid composition of the ER membrane [31]. In response, the ~1150 amino acid precursor form of SREBP exits the ER *via* its inclusion in COPII vesicles and travels to the Golgi apparatus. There, it serves as the substrate for S1P (also termed SKI) [32,33]. A serine protease related to other secretases such as subtilisin and kexin, S1P is anchored to the Golgi membrane with its active site in the lumen.

S1P cleaves SREBP at a site within its luminal loop, which separates the two membrane spanning helices of the precursor. This produces two fragments which remain membrane-bound [34]. The amino-terminal fragment contains the SREBP transcription factor domain. This fragment is the substrate for S2P, which cleaves at a Leu–Cys bond within the membrane-spanning helix, near the cytoplasmic face of the bilayer [35]. This releases the transcription factor, enabling it to travel to the nucleus and increase the transcription of genes required for lipid synthesis. In the nucleus, SREBP is targeted for degradation by the ubiquitin–proteasome system. The half-life of nuclear SREBP is about 30 min [36]. As lipid accumulates, ER-to-Golgi transport of SREBP is blocked, thus halting production of the active transcription factor. This mechanism is the basis for the long-ago observed effect of cholesterol-feeding on the suppression of its own synthesis [37].

In cultured mammalian cells, S2P is an essential gene. In the absence of S2P, Chinese hamster ovary (CHO) cells require exogenous cholesterol to survive [3]. They also require mevalonate, and oleate [38] and exhibit reduced content of unsaturated fatty acids [39]. The original isolation of SRD-6 mutants, which lack S2P, did not determine a requirement for oleate [40]. This is because serum centrifugally-depleted of lipoproteins retains sufficient fatty acid to support the growth of these auxotrophic mutants. By contrast, lipid extraction of serum with organic solvents removes fatty acids in addition to those in lipoproteins and reveals fatty acid auxotrophy [41].

We tested whether the requirement of S2P$^-$ cells for oleate was absolute or if other unsaturated fatty acids would support their survival. Fig. 2 shows the growth of wild-type and S2P$^-$ cells in medium supplemented with cholesterol, mevalonate, and various fatty acids as indicated. Growth in the standard culture medium for cholesterol auxotrophic cells is shown on the left. The wild-type cells grow in all conditions tested. In medium containing delipidated serum, cells lacking S2P grow only in medium supplemented with an unsaturated fatty acid (oleate, linoleate, or arachidonate). Thus, S2P is essential in lipid metabolism for synthesis of cholesterol and unsaturated fatty acids owing to its role in SREBP activation.

5.2. The ER stress response

After SREBPs, the next S2P substrate identified was activating transcription factor 6 (ATF6). ATF6 is synthesized as a single-pass type 2 membrane-spanning protein embedded in the ER membrane [42]. The amino-terminal domain encodes a member of the basic-leucine zipper (bZip) transcription factor family and is disposed to the cytoplasm. Its carboxy-terminal domain of about 270 amino acids is luminal.

ATF6 is an ER stress sensor/transducer involved in all steps of the UPR, from initially sensing stress in the ER to the transcriptional activation of stress-responsive genes. It is one of a trio of proteins that are master regulators of the ER stress response. The other branches of the ER stress response are mediated by IRE1 (inositol requiring 1) and PERK (PKR-like endoplasmic reticulum kinase) [43]. Each of these ultimately induces the transcription of stress-response genes when, for example, unfolded proteins accumulate in the ER.

The luminal domain of ATF6 interacts with immunoglobulin binding protein BiP (also known as glucose-regulated protein 78, Grp78), an endoplasmic reticulum chaperone [43]. In cells undergoing ER stress, BiP binds to unfolded proteins. When, unfolded proteins accumulate in the lumen of the ER, BiP dissociates from the luminal domain of ATF6 *via* an active mechanism [44] and ATF6 moves to the Golgi apparatus. There, it is cleaved first by S1P and then by S2P. Like SREBP, ATF6 also contains helix-breaking residues in its membrane-spanning domain. In this case, the Asn and Pro residues are separated by two amino acids. Again, substituting either residue alone has no discernible effect on cleavage. Simultaneously mutating the Asn to Phe and the Pro to Leu abolishes cleavage by S2P [18]. In cells lacking S2P, ATF6 is not activated and the ER stress response is attenuated [28].

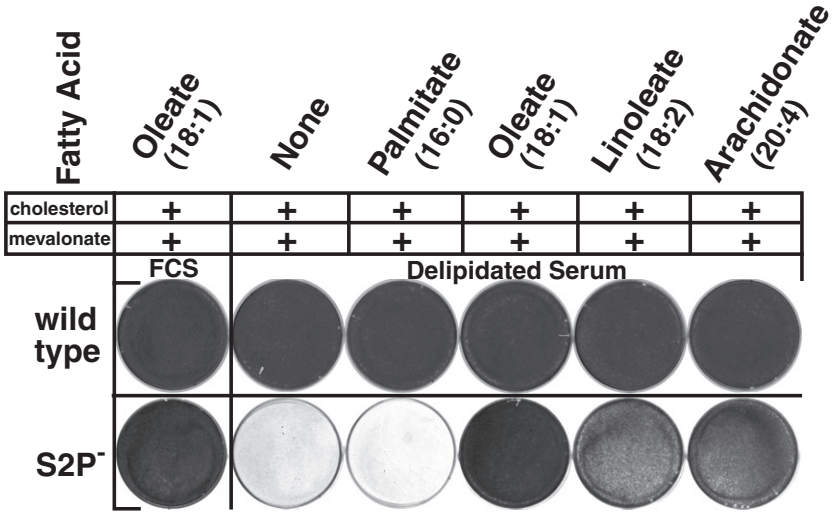

Fig. 2. Unsaturated fatty acids other than oleate can support the growth of cells lacking S2P. Wild-type CHO-7 and S2P$^-$ M19 cells were set up on day 0 at 3.5×10^5 cells/60 mm dish in a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium containing 100 U/ml of penicillin, 100 μg/ml streptomycin sulfate, 5 μg/ml cholesterol, 1 mM mevalonate, and 5% (v/v) fetal calf serum or delipidated serum [41] as indicated, along with 20 μM of the indicated fatty acid complexed to BSA [66]. On day 1, and every 1–2 days thereafter, the cells were refed with fresh medium. On day 14, the cells were washed with PBS, fixed in 95% ethanol, and stained with crystal violet.

In addition to ATF6, S2P is implicated in the activation of other transcription factors involved in the ER stress response (Table 1). These include BBF2 human homolog on chromosome 7 (BBF2H7), CREBH, Luman, and old astrocyte specifically induced substance (OASIS). These proteins each harbor the distinctive features of Rip substrates: ER localization, a cytoplasmic bZip domain, a predicted type 2 membrane-spanning helix, and a luminal cleavage site for S1P.

BBF2H7 is cleaved in response to endoplasmic reticulum stress [45,46] and may function in what the authors term the late phase of the response [45]. CREBH is a liver-specific, membrane-bound transcription factor related to ATF6. It undergoes intramembrane proteolysis in response to endoplasmic reticulum stress [29]. It also regulates gluconeogenesis [47]. Like SREBP, the nuclear form of CREBH is short-lived [48]. In addition to roles in the ER stress response in various tissues, Luman is also involved in dendritic cell activation [49]. OASIS, is another membrane-bound transcription factor believed to play a role in the endoplasmic reticulum stress response [50]. It is expressed at high levels in osteoblasts and plays a crucial role in the formation of bone [51].

5.3. S2P in disease

Mutations in human S2P (designated MBTPS2 for 'membrane-bound transcription factor peptidase, site 2' by the HUGO Gene Nomenclature Committee) are associated with disease. Oeffner et al., identified hypomorphic (reduction-of-function) mutations in the human S2P gene as the cause of ichthyosis follicularis, alopecia, and photophobia (IFAP) syndrome. This is a rare X-linked genetic disorder [52]. Accordingly, males are more frequently affected than females. The hypomorphic mutant allele is typically recessive to the wild-type allele in females. However, some female carriers are symptomatic owing to skewed X chromosome-inactivation of the wild-type allele [52,53]. Afflicted individuals are typically hairless from birth and frequently present with neurological defects along with the light-sensitivity and follicular abnormalities from which its name derives. Hypomorphic mutations in S2P are also associated with a similar syndrome, keratosis follicularis spinulosa decalvans (KFSD) [53]. These somewhat distinct clinical presentations share the same underlying biochemical deficit: insufficient S2P activity. It remains unclear whether the observed clinical phenotypes result from misregulation of lipid metabolism or of the unfolded protein response. Possibly, they arise from some as-yet unidentified role(s) for S2P in humans. It is interesting that in the human mutations identified, at least some S2P function is retained [52,53]. No null mutations of S2P have been reported in vertebrates. This is consistent with the prediction that S2P is an essential gene, as in cultured mammalian cells. An essential role for S2P may be specifically owing to its role in cholesterol homeostasis, since in cultured cells, exogenous lipid fully restores normal growth [40,54].

5.4. When S2P is dispensable

The situation is different for invertebrates. In *Drosophila melanogaster*, which, like the majority of metazoan species, cannot synthesize cholesterol *de novo*, the SREBP gene is essential. In its absence, flies require exogenous fatty acids to survive [55]. By contrast, the *Drosophila* S2P gene is dispensable under laboratory culture conditions [56]. This is true even though S2P cleaves SREBP in flies. Flies homozygous for deletion of the S2P gene are readily recovered as adults and may be maintained as stable populations. These S2P-null mutants exhibit somewhat delayed growth in comparison to their heterozygous siblings but are otherwise similar to them. They do not display severe phenotypes as seen in human patients [56].

Fig. 3 shows immunoblots of proteins extracted from adult flies that have been deprived of food to induce cleavage of SREBP (Fig. 3, lane 1, middle panel). Unlike heterozygotes (lane 3, middle panel), flies homozygous for loss of S2P show a profound deficit in nuclear accumulation of dSREBP (lane 4, middle panel). Instead, the intermediate form (I), which is the product of S1P cleavage, accumulates in their membranes (lane 4, upper panel). This is just what is observed in S2P^{-} mammalian cells [34]. Thus, while exhibiting the same defect in SREBP processing as mammalian cells, flies lacking S2P are viable and fertile. Flies lacking S2P survive because SREBP is also a substrate of the caspase Drice [57]. This alternative processing of SREBP is sufficient to compensate significantly for the loss of S2P. Flies harboring null mutations in both S2P and Drice exhibit the same phenotypes as flies lacking dSREBP. These animals survive on medium supplemented with fatty acids [57]. In mammals, both SREBP-1 and -2 are substrates for caspases-3 and -7 [58,59]. The normal physiological role of caspase cleavage of SREBP is known neither for vertebrates nor invertebrates.

6. Conclusions

All substrates known for S2P are membrane-bound transcription factors with type 2 membrane-spanning helices. These factors only become substrates for S2P once another, more conventional, protease cleaves the precursor form. Cleavage by S2P then releases a cytoplasmic domain that travels to the nucleus to mediate the increased transcription of target genes.

S2P is essential in mammalian cells owing to its role in the production and uptake of unsaturated fatty acids and cholesterol. It is almost certainly essential in humans as well. Strikingly, it is dispensable in fruit flies under laboratory conditions. This latter finding suggests that any role for S2P in activating transcription factors in other signaling pathways of *Drosophila* is not essential.

S2P is an exceptionally hydrophobic metalloprotease of the Golgi apparatus. Although it was the first such enzyme discovered, more is known about the mechanism of action of rhomboid family of intramembrane serine proteases and S2P homologs from prokaryotes. This may be due to the difficulty of simultaneously solubilizing a membrane-embedded protease and its membrane-embedded substrate,

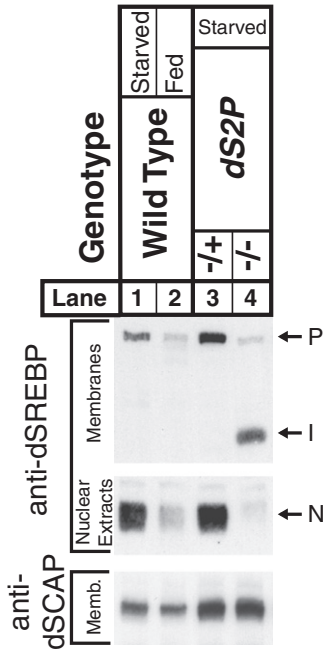

Fig. 3. *Drosophila melanogaster* mutants harboring a deletion of the S2P gene are viable and exhibit the same defect in SREBP processing as mutant mammalian cells. Flies were raised, proteins extracted, and immunoblots analysis performed as described [56,67], using monoclonal antibody 3B2 against the amino terminal domain of dSREBP [16]. P, the ~120 kDa precursor form of SREBP. I, the membrane-bound intermediate form. N, the ~70 kDa nuclear form. Anti-dScap [67] is shown as a loading control for membrane fractions.

although this challenge has been overcome for other I-CLiPs. It will be interesting to see if this can be done for S2P from animals.

Acknowledgements

The work described here was done while the author was a member of the Department of Molecular Genetics at the University of Texas Southwestern Medical Center at Dallas. It was supported by grants from the National Institutes of Health (HL20948 and R01 GM07145701A1) and the Perot Family Foundation. The author wishes to thank his former colleagues in the Rawson lab and the Department of Molecular Genetics for many exciting years of discovery.

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