Fungal Ice2p has remote homology to SERINCs, restriction factors for HIV and other viruses.

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Abstract

Ice2p is an integral endoplasmic reticulum (ER) membrane protein in budding yeast *S. cerevisiae* named “ICE” because its deletion reduces inheritance of cortical ER. Ice2p has also been reported to be involved in mobilising neutral lipids from lipid droplets to the ER for phospholipid metabolism. In addition, it has been proposed that Ice2 acts as a tether that links the ER both to lipid droplets and to the plasma membrane, via a long cytoplasmic loop that contains multiple predicted amphipathic helices. We used bioinformatics to study Ice2p. First, regarding its topology, we found that members of the Ice2 family in diverse fungi are predominantly predicted to have ten transmembrane helices, casting doubt on the prediction of eight transmembrane helices for the yeast protein. Applying the dominant topology to Ice2p puts the loop with amphipathic helices is in the lumen of the ER, not the cytoplasm, so unable to tether. Secondly, we looked for homologues of Ice2p using the profile-profile search tool HHpred and supporting results with bespoke PSI-BLAST searches. This identified a strong homology to SERINCs (serine incorporators), ten transmembrane helix proteins found universally in eukaryotes. Since SERINCs are potent restriction factors for HIV and other viruses, study of Ice2p may reveal functions shared with SERINCs, including antiviral (including anti-HIV) restriction mechanisms.

Key words: Ice2p, membrane contact site, tether, SERINC, HIV, HHsearch, HHpred, virus restriction factor, cortical endoplasmic reticulum

Introduction

Ice2p is a multi-spanning ER localised yeast protein of 491 residues that was first named for its role in inheritance of cortical ER from mother to daughter cells (Estrada de Martin et al., 2005). Cortical ER is reduced to some extent upon deletion of *ICE2* alone (Estrada de Martin et al., 2005). Cortical ER in both mother and daughter cells is markedly reduced by adding *ice2*Δ to other mutations that reduce cortical ER. This was first demonstrated in yeast lacking Scs2p, the major yeast VAP - standing for VAMP-associated protein (Murphy and Levine, 2016): *ice2*Δ*scs2*Δ mutant cells have less cortical ER than *scs2*Δ cells (Loewen et al., 2007). A more extreme effect of *ice2*Δ on cortical ER is seen in yeast missing not only both VAPs, but also four other integral ER proteins that communicate with plasma membrane (three extended-synaptotagmins (Tcb1/2/3p) and the anoctamin-8 homologue Ist2p) (Quon et al., 2018).

While the molecular function of Ice2p is not known, observed genetic interactions involve a wide range of ER-related activities. *ice2*Δ prevents targeting of tRNA methyltransferase to the inner nuclear envelope (Murthi and Hopper, 2005), though this interaction is thought to be indirect and not through Ice2p acting as a tether (Diaz-Munoz et al., 2014). *ice2*Δ also prevents ER-associated degradation (ERAD) of misfolded lumenal proteins (Schuldiner et al., 2005). Ice2p levels also correlate closely with the stability of exogenously expressed ER membrane-anchored cytochrome P450 reductases (Emmerstorfer et al., 2015; Zhang et al., 2018), and Ice2p is required for normal function of the ER zinc transporter (North et al., 2012). Finally, Ice2p function has been linked to mobilisation of neutral lipid reserves upon resumption of growth after stationary phase: *ice2*Δ cells are slower to re-enter log-phase growth, because Ice2p is needed to allow phospholipid synthesis from diacylglycerol, itself made from triacylglycerol stored in lipid droplets (Markgraf et al., 2014). Decreased mobilisation of neutral lipids has also been suggested to underlie *ice2*Δ cells being slow to induce macroautophagy (Shpilka et al., 2015). To match this role in lipid metabolism, in cells in stationary phase GFP-tagged Ice2p localises to regions of the ER in contact with lipid droplets, without translocating onto that compartment, and it dissociates rapidly into general ER after initiation of growth with fresh medium (Markgraf et al., 2014).

The localisation of Ice2p at ER-lipid droplet contact sites was attributed to direct bridging. While originally predicted to have seven transmembrane helices (TMHs) (Estrada de Martin et al., 2005), subsequently the prediction was revised to eight TMHs, with a long loop predicted between TMH6 and TMH7. That loop expressed on its own targets lipid droplets (both in yeast and heterologously expressed in COS7 cells), and within that loop four regions were identified as forming amphipathic helices (Markgraf et al., 2014), making them

candidates for binding the lipid droplet outer surface (Gimenez-Andres et al., 2018; Hickenbottom et al., 2004). The proposed bridging to lipid droplets was subsequently extended to explain the role of Ice2p on forming cortical ER, by suggesting direct bridging (Quon et al., 2018).

Given that the molecular function of Ice2p is unknown, useful information might be obtained by identifying remote homologies (Soding and Remmert, 2011). One of the most sensitive approaches is profile-profile alignment, which can identify homology with as little as 10% sequence identity (Rost, 1999). An easily accessible profile-profile alignment tool is HHsearch (Soding, 2005), which is implemented online as HHpred (Soding et al., 2005), and is highly successful at identifying true homologies (Mariani et al., 2011). Here we found that Ice2p is a distant homologue of SERINCs, a ubiquitous eukaryotic family of proteins with ten TMHs (Gonzalez-Enriquez et al., 2017), also called TMS proteins, with Tms1p the sole SERINC in yeast (Grossman et al., 2000; Xu et al., 2003), named for their possible role in serine incorporation into lipids (Inuzuka et al., 2005). Analysis of the topology of Ice2, together with a structural model of Ice2p based on the cryo-EM structure of SERINC (Pye et al., 2020), show that it is unlikely that Ice2p tethers the ER either to lipid droplets or to the plasma membrane.

Methods

Alignment of yeast Ice2 sequences

A small alignment of Ice2 homologues was made from 18 sequences from experimentally relevant and diverse Ice2-positive fungi. The list shown in Figure S1 was obtained after aligning by Clustal Omega (Zimmermann et al., 2018), and creating a tree with PHYML 3.0 (Guindon et al., 2010). A medium-sized group of 95 Ice2 sequences was obtained from UNIPROT by filtering (i) to remove fragments; (ii) to include only sequences between 200 and 800 residues; (iii) using the UniRef clusters with maximum identify 90% (nr90), so that only one sequence represents any cluster of sequences sharing 90% or more sequence identity.

Amphipathic helix prediction

Sequences of amphipathic helices previously identified in Ice2p (Markgraf et al., 2014) were plotted as cartwheels using the Heliquest server (Gautier et al., 2008). Heliquest was also used to calculate the hydrophobicity and hydrophobic moment of each predicted amphipathic helix, as well as TMHs and additional hydrophobic regions in Ice2p.

Topology predictions

Transmembrane helix prediction tools were used via online servers: TOPCONS (Tsirigos et al., 2015) and TMHMM2.0 (Krogh et al., 2001) using default parameters in both cases. The TOPCONS data was considered not only on the basis of the consensus, but also the five component programmes: OCTOPUS, Philius, Polyphobius, SCAMPI, and SPOCTOPUS.

Remote homology prediction and homology modelling

HHpred, an online enactment of HHsearch (Soding, 2005; Soding and Remmert, 2011) at the Tuebingen toolkit (Zimmermann et al., 2018) was used to search for remote homologues of Ice2p in PFAM, in PDB and in model eukaryotes. Settings were standard, except using eight iterations of HHblits. For modelling a structure for Ice2p based on the structure of *D. melanogaster* SERINC, the HHpred alignment was extended beyond the regions of highest homology by setting alignment mode to local realignment and setting the MAC realignment threshold to 0.01. This alignment was forwarded to Modeller (Webb and Sali, 2016).

Phylogeny Tree and Cluster analysis

223 sequences were accumulated from three sources: (i) 184 sequences including diverse SERINCs, which was prepared by seeding the NCBI protein BLAST server with *Thecomonas trahens* hypothetical protein AMSG_07160 (XP_013756618.1; 338 aa) with results restricted by 34 taxonomic terms (Table S2), otherwise with standard settings (*e.g.* threshold p=0.005). Significant hits were re-submitted until convergence after iteration 9 (469 sequences). After aligning sequences by Clustal Omega, the alignment was edited by hand to remove long, unique insertions, fragments and highly similar repeats. The resulting 184 sequences included 167 SERINCs, nine fungal Ice2s, and eight others with no known domain, including the seed. (ii) 34 further sequences, either Ice2 or with no known domain, obtained from the second round of PSI-BLAST (no limitation on the taxonomy of hits) with both the *T. trahens* protein (above) and *Nematostella vectensis* predicted protein EDO39878.1: XP_001631941.1. (iii) Five further sequences obtained by hand curating incomplete sequences in (i) and (ii). All sequences are listed in Table S2.

To visualise relationships between sequences as a phylogeny tree, they were aligned with Kalign (Lassmann and Sonnhammer, 2005) and then relationships were identified by PHYML3.0 with branch support by aBayes (Guindon et al., 2010). To identify relationships in an all-*vs.*-all cluster map, sequences were submitted CLANS with standard settings (threshold e-values: $1e^{-4}$) (Frickey and Lupas, 2004).

Results

The Ice2p region identified as a loop may contain at least one TMH

S. cerevisiae Ice2p was initially predicted to span the ER membrane with seven TMHs (Estrada de Martin et al., 2005). In a subsequent study, Ice2p was predicted to comprise eight TMHs (Figure 1A) (Markgraf et al., 2014). To determine the most likely topology of Ice2p, we considered that the topology was highly likely to be conserved across the protein family related to Ice2, which is documented by Pfam as PF08426, and contains single Ice2 proteins per fungal species in all dikarya (Ascomycota and Basidiomycota) but missing from most Mucormycota and all Chytridiomycota, Zoopagomycota and Microsporidia (not shown). Initially we compared Ice2p with a small number of representative homologues across fungal evolution (Figure S1), finding a high degree of conservation across the full length of these proteins (Figure S2). This comparative alignment showed that *S. cerevisiae* Ice2p has a non-conserved insertion spanning residues 292-343, which is entirely within the region 241-357 previously described as a cytoplasmic loop that facilitates binding to lipid droplets *in trans* (Figure 1B) (Markgraf et al., 2014). This ER-lipid droplet bridging feature has multiple regions predicted to be helical (Figure 1A) and was proposed to contain four predicted amphipathic helices, AH1-4, using Heliquest (Figure 1B) (Gautier et al., 2008).

We re-examined these predictions for AH1-4. One possible issue is assigning a region as an AH when it is more likely to form a TMH. Heliquest will typically identify a hydrophobic face at every position in or near a TMH, but an TMH can be distinguished from an AH by its hydrophobicity being higher than an AH, and that both its hydrophobic moment and proportion of polar residues being lower than an AH. This is the case for AH1, a conserved region (residues 245-262) for which all three parameters are characteristic of a TMH (Table S1, Figure 1C) (Eisenberg et al., 1982; Wallace et al., 2004). While AH2 (residues 266-283) meets all the criteria for an amphipathic helix, AH3 (residues 293-310) has none of the parameters characteristic of an AH (Table S1). Finally, AH4 (residues 333-350) has intermediate parameters, but it contains a proline (Pro344) at position 12, and a serine residue (S342) in the middle of the proposed hydrophobic face, making it unlikely to form an AH (Figure 1C). In addition, AH3 and AH4 are both in the portion of Ice2p that has no counterparts in most fungi (Figure 1B), which indicates that any fundamental conserved function of Ice2p is unlikely to involve these regions. This analysis indicates that the long loop comprises two functionally important helices: one likely AH (AH2), and a TMH that has so far not been predicted as such (AH1).

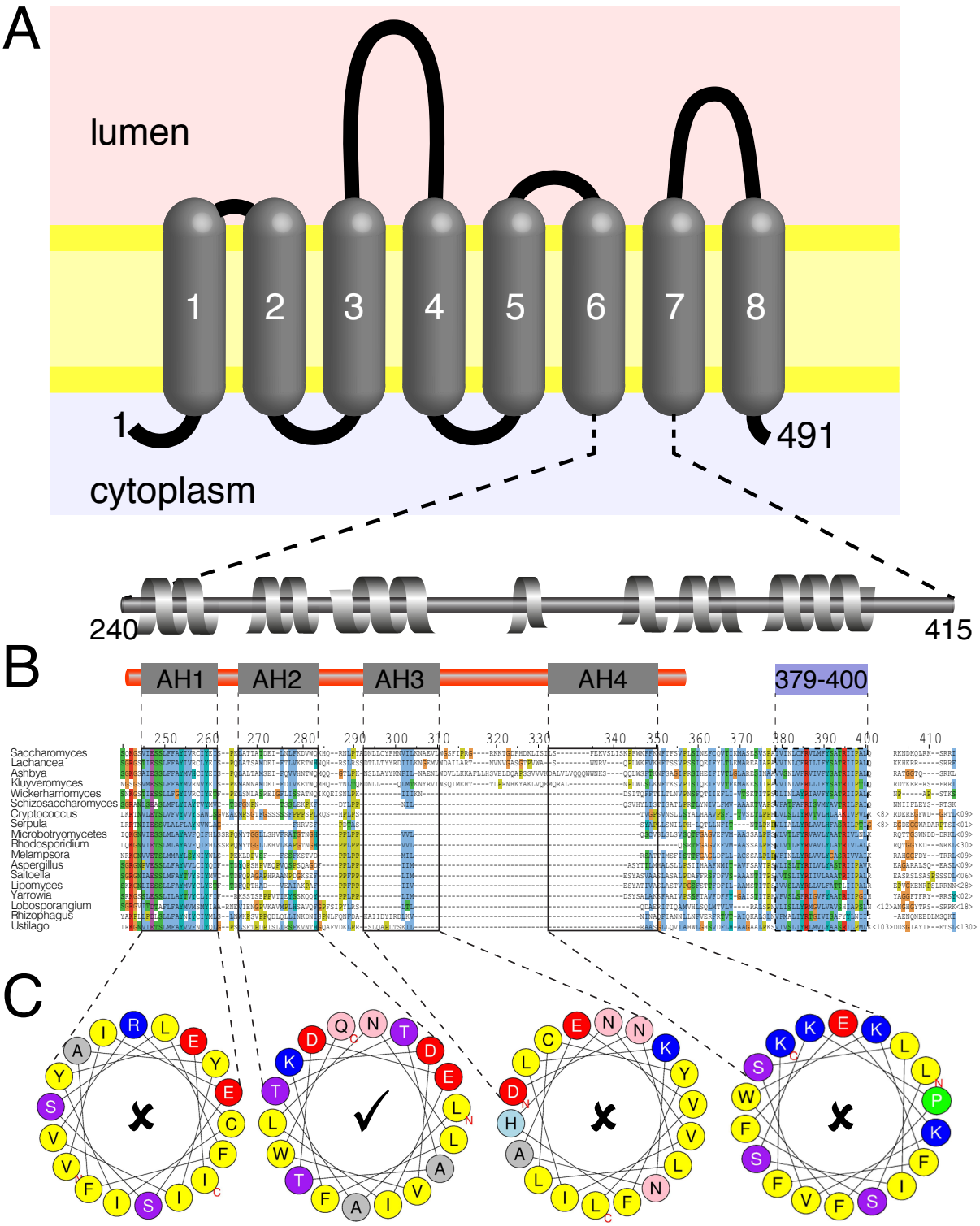

Figure 1. Analysis of Ice2p's previously predicted 176 aa cytoplasmic loop.

A. Previously predicted topology of Ice2p: eight transmembrane helices (TMH) with residues 240-415 forming a cytoplasmic loop between TMH6 and TMH7 (Markgraf et al., 2014). Also shown are predicted α-helical regions in that loop. B. Alignment of residues 240-414 in Ice2p with homologues from 17 other fungal species (see also Figures S1 and S2). Residues 241-357, previously shown to target lipid droplets (Markgraf et al., 2014), are indicated by red bar (top). Boxes indicate 4 regions previously described as amphipathic helices (AH1-4) (Markgraf et al., 2014). Box with dashed outline indicates conserved hydrophobic residues 379-400. Alignment coloured according to CLUSTALX. C. Helical wheel representations of four predicted amphipathic helices within Ice2p's longest cytoplasmic loop (depicted in helical wheel projections and labelled AH1-4) were generated in Heliquest. Tick ( ) on helical wheel indicates suitability to fold into an AH, while crosses (x) indicate hinderance to AH formation due to presence of proline, or a polar residue within the predicted hydrophobic interface or physico-chemical parameters more characteristic of a TMH (high hydrophobicity, low hydrophobic moment, low incidence of non-polar residues, see also Table S1)..

Ice2p spans the ER membrane with 10 transmembrane helices

Different topologies have already been predicted for Ice2p, and the potential TMH we identified in AH1 (residues 245-262) may suggest further revision of Ice2p topology is needed. In addition, residues 379-400 form another conserved hydrophobic region with properties of a transmembrane helix (TMH, Figure 1B; Table S1). The transmembrane hidden Markov model (TMHMM) membrane prediction method which predicted eight TMHs (Markgraf et al., 2014) also shows the region of 379-400 as having intermediate probability of forming a TMH (Figure 2A). To investigate this further, we applied another prediction tool, TOPCONS, which combines five prediction methods to generate a consensus topology (Tsirigos et al., 2015). This produced a consensus topology of eight TMHs identical to TMHMM2.0. However, two of its tools predict residues 246-266 form a TMH coinciding with AH1, and three tools predicted residues 375-395 form a TMH (Figure S3A). This uncertainty is indicated by TOPCONS reporting a low reliability for its prediction for residues 240-400 (Figure S3B). Thus, *in silico* predictions are not reliable for determining the TMH status of the two regions 246-266 and 379-400 using the sequence of *S. cerevisiae* Ice2p.

To make progress on predicting topology of Ice2p, we wondered if the analysis could be optimised by looking more widely than the sole family member in *S. cerevisiae*. Given that Ice2 proteins form a single region of homology across their entire length (Figure S2), we assumed that all Ice2 homologues might share the same topology. On this basis, estimates of the topology of a wide range of Ice2 homologues would be informative about Ice2p. We analysed the topologies of a medium-sized group of 95 diverse Ice2 family members in TOPCONS. Among these, 10 TMHs were predicted in 77% of fungal species (Figure 2B). The additional two TMHs coincided with the two regions possible TMHs we had already identified in Ice2p. This is illustrated by plotting average hydropathy across the Ice2 multiple sequence alignment (Figure 2C, black line). The 10 hydrophobic regions coincided with the regions of the alignment that were ungapped (Figure 2C, green dashed line), indicating that the TMHs are the most conserved elements. These results strongly indicate that Ice2p in *S. cerevisiae* spans the ER membrane with 10 TMHs, which place the loop with the sole conserved amphipathic helix in the ER lumen, not the cytoplasm (Figure 2C, top).

Ice2p is a remote homologue of SERINCs

Although the Ice2 family includes only fungal proteins, we wondered if Ice2 has previously unrecognised remote homologues. We used the profile-profile search tool HHsearch, which creates Hidden Markov Models of both query and targets, and is implemented online as

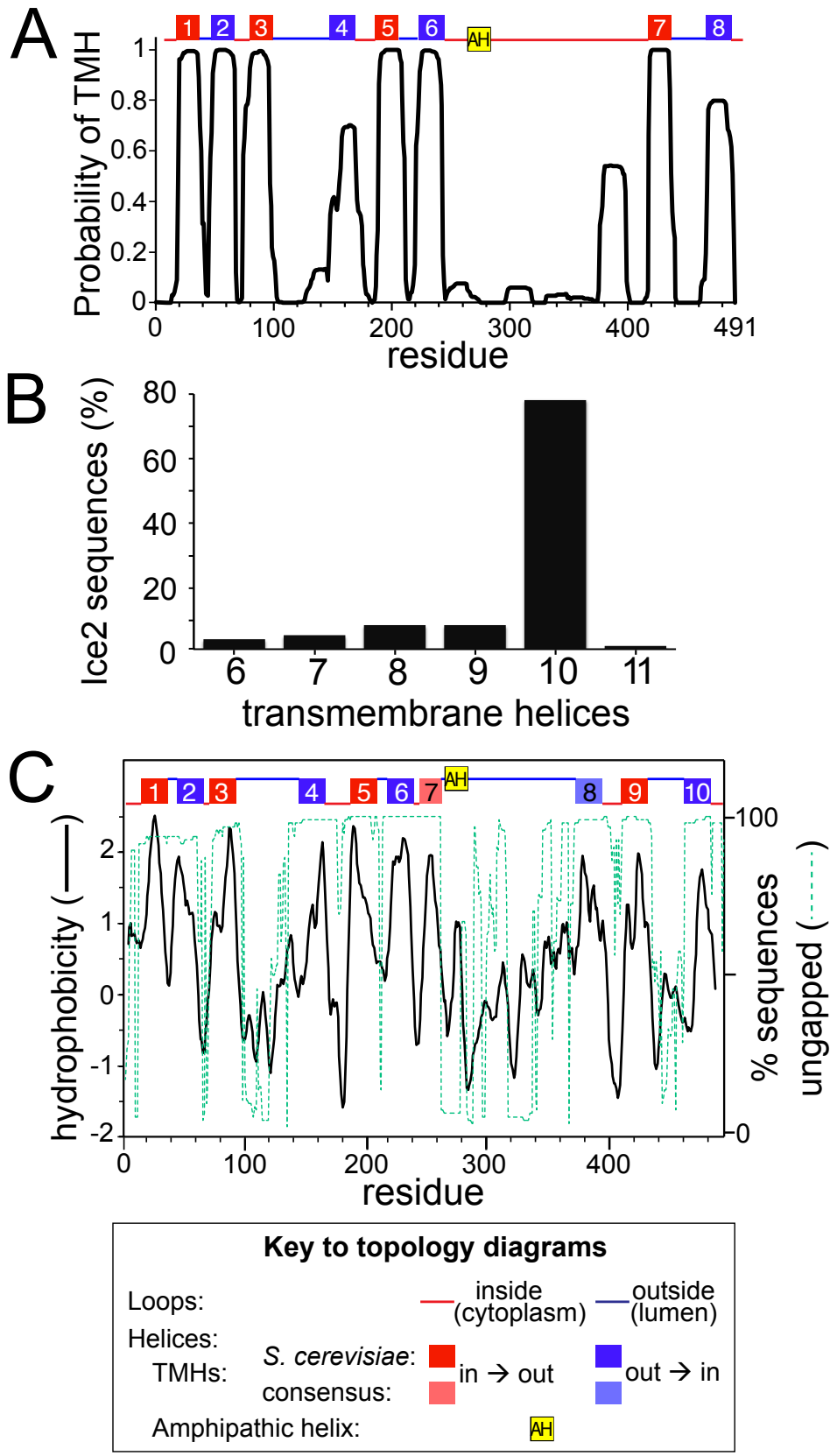

Figure 2. Consensus topology across the Ice2 family is presence of 10 TMHs.

A. Topology analysis of S. cerevisiae Ice2p by TMHMM2.0 (Krogh et al., 2001). B. Range of TMHs predicted among 95 fungal Ice2 sequences by TOPCONS (see also Figure S3 for report on S. cerevisiae Ice2p only). C. Hydrophobicity in aligned Ice2 sequences (black line) using an average sliding window of 11 residues (Kyte and Doolittle, 1982). Also shown are the proportions of ungapped sequences across the alignment (green dashed line). Key below indicates how topology is indicated in A and C.

HHpred (Soding, 2005; Soding and Remmert, 2011). This aligned Ice2p with proteins in the family of SERINCs,10 TMH proteins found in most eukaryotes. The tool's estimated probability that the match is a true positive was 95% for human homologues and 93% for yeast Tms1p (Figure 3A), and similarly high for other eukaryotes (data not shown). The region of greatest homology was TMHs 1–7 of both proteins, covering >200 residues (Figure 3B). This homology is bolstered by the overall topological similarity. While the alignment is not strong immediately beyond TMH7, when the alignment was extended to full length, sequence similarity returned from the end of TMH9 and was considerable in TMH10 (Figure S4). Overall, these results indicate that Ice2p is a variant of SERINC.

To examine the evolutionary relationship between Ice2p and SERINCs, we attempted to link the two families using PSI-BLAST alone. Searches seeded with Ice2p produced distant hits in a family of proteins with no known domain. Searches seeded in turn with one of these sequences included Ice2s from the second iteration onwards, and SERINCs from the third iteration (data not shown). We visualised the relationships between these proteins, SERINCs and Ice2 as both a phylogeny tree and a cluster map (Figure 4). Both visualisations showed that the unknown family is closely related to Ice2. Both this new family and Ice2 share a common branch from SERINC (Figure 4A). In addition, the new family has more sequence similarities to SERINCs than it does to Ice2, indicating that it is intermediate between SERINC and Ice2 (Figure 4B). The ability to link Ice2 and SERINCs by PSI-BLAST confirms the HHpred result. The members of the intermediate family are mostly in animals (invertebrates, including chordates and some insects) and also in other dispersed unikonts, including one fungal sequence and several amoebae. This indicates that the ancestor of the new intermediate family diverged from the more ubiquitous SERINCs at an early stage of unikont evolution, maybe even in LECA, with fixation of a Ice2 in many fungal lineages.

Homology modelling of Ice2 reveal a conserved structural fold

Based on the remote homology between Ice2p and SERINCs, we used the cryo-EM structure of full-length *D. melanogaster* SERINC to model the full-length structure of Ice2p. This indicates that Ice2p, like SERINC, is arranged in two subdomains, each with four TMHs (TMH 1,2,3 and 9 in subdomain A and TMHs 5, 6,7 and 10 in subdomain B), with the subdomains linked by TMH4 lying diagonally at the centre and TMH8 crossing back to subdomain A (Figure 5A).

The Ice2p model includes both a pocket and a cleft identified as potential functional sites in SERINC. The pocket (on the cytoplasmic face of subdomain A) is lined by W140 and K143

A

Target	p(Hom %)	Columns
Serinc 1	95	183
Serinc 2	94	183
Serinc 3	97	208
Serinc 4	95	183
Serinc 5	95	181
Tms1p	93	209

B

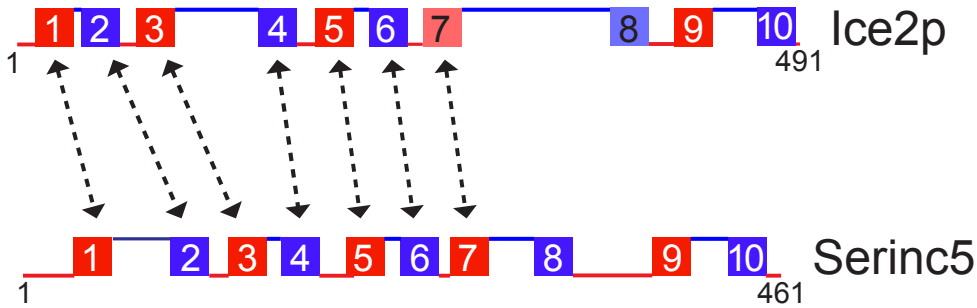

Figure 3. Ice2p is homologous to SERINCs.

A. Probabilities of SERINCs in human and yeast to be homologous to Ice2p (pHom, %), and number of residues aligned (Columns), as determined by HHpred. B. Regions conserved between Ice2p (with topology from Figure 2) and SERINC5

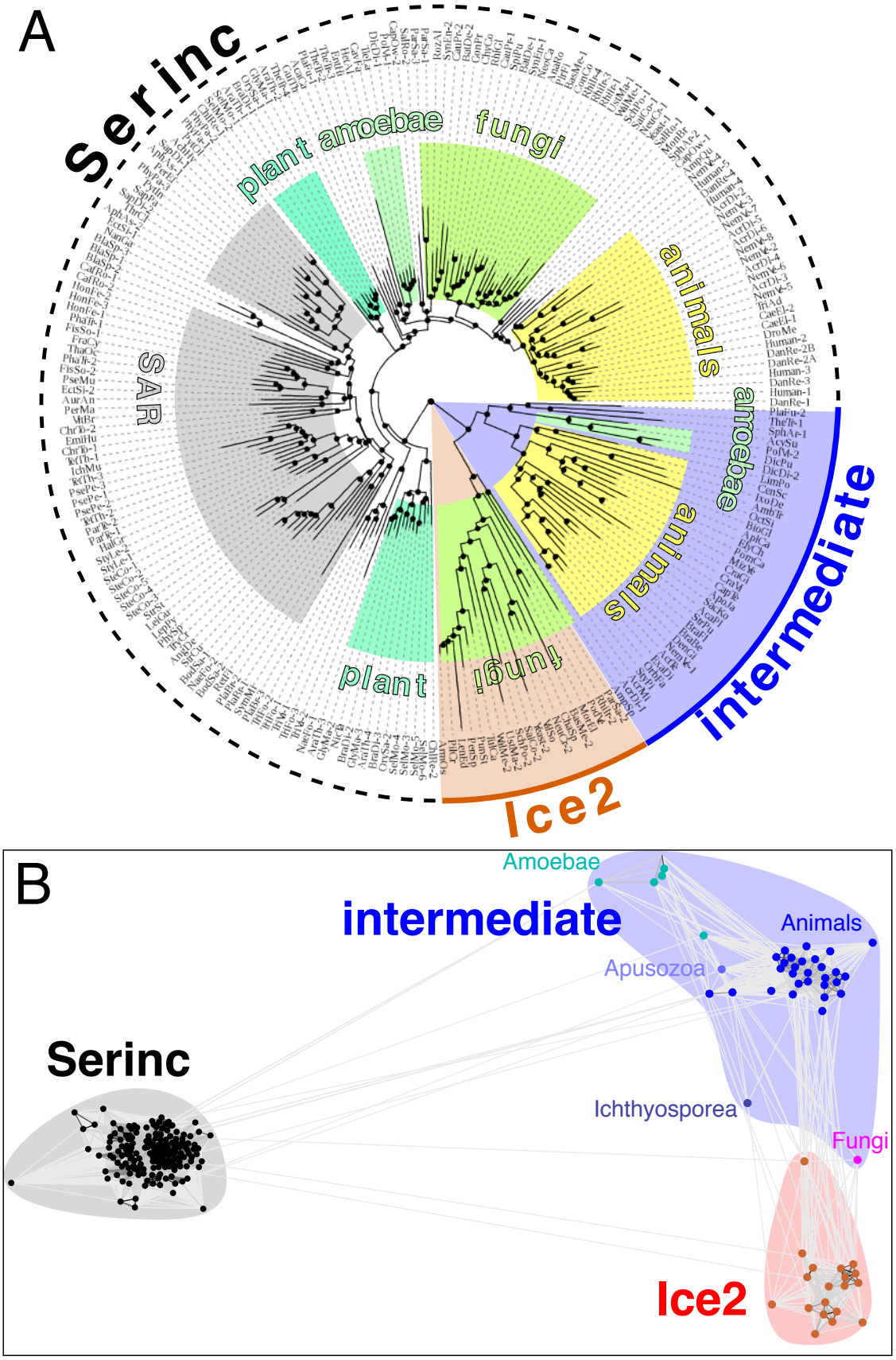

Figure 4. Relationships between SERINC, Ice2 and a previously unknown intermediate family.

A. Phylogeny tree of three families of related proteins: SERINC, Ice2, and intermediate family. B. Cluster map of same proteins. Both diagrams show relationships for 223 sequences consisting of 171 divergent SERINCs, 19 Ice2s and 33 sequences not previously identified with any domain (see Table S2). Protein families are coloured: SERINC: black, Ice2: red, intermediate family: blue. Additional colours indicate in the tree (A) major taxonomic groupings; in the cluster map (B) 5 clades within the intermediate family.

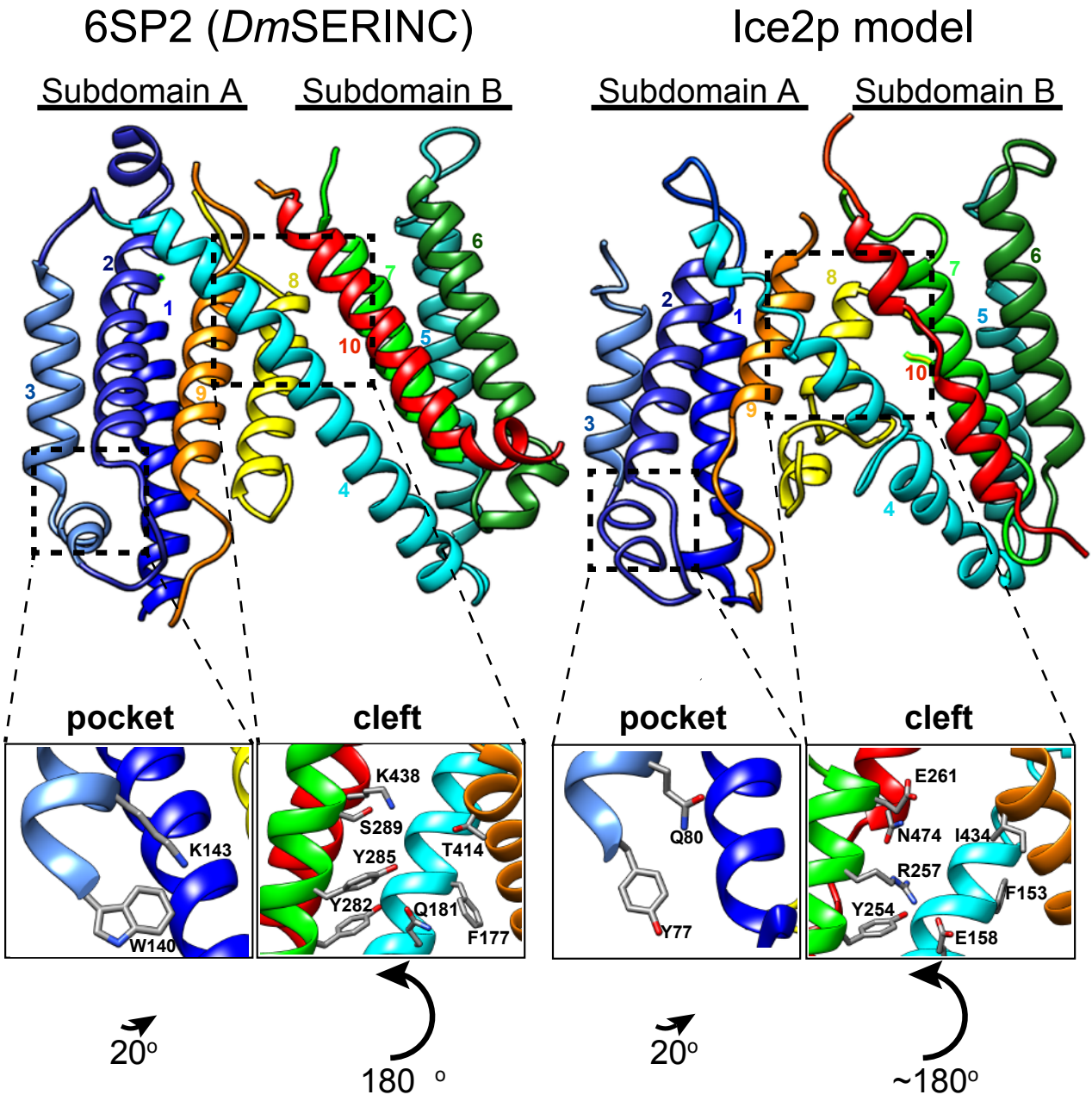

Figure 5. Structural model of Ice2p.

A. The Cryo-EM structure of DmSERINC (6SP2, chain C) in comparison with the structural model of Ice2p. Transmembrane helices are numbered and coloured in a spectrum from blue (N-terminus) to red (C-terminus). The Ice2p model was generated by Modeller, based on an HHpred alignment that maximally extends the homology between Ice2p and 6SP2 (see also Figure S4). B. Zoomed in view of two structural elements in DmSERINC and corresponding regions in the Ice2p model: (i) pocket on the cytoplasmic face of subdomain A, showing TMH1/3, with 2 conserved residues; (ii) cleft on the lumenal side where Subdomains A and B meet, made up of TMH4/7/9/10 and TMH8 (removed), with a hydrophobic phenylalanine plug at the base and lined by 6 hydrophilic residues.

in *Dm*SERINC, which align with Y77 and Q80 in Ice2p respectively (Figure 5B). The hydrophilic cleft (between SERINC's two subdomains) lies near a lipid binding groove that fills with lipids during simulations and contains an acyl chain by cryo-EM (Pye et al., 2020). The base of the cleft in SERINC is plugged by F177 in TMH4 (Pye et al., 2020), which aligns with F154 in Ice2p (Figure 5B). In addition, polar side chains lining the cleft in SERINC (for example, Q181/ Y282) align with residues in Ice2p (E158/Y254). Conservation of residues that are indicated to play key roles from the SERINC structure suggest that Ice2p has a similar activity to SERINC.

DISCUSSION

Ice2p is a member of the SERINC Superfamily

Our aim was to use structural bioinformatics to identify a function for Ice2p, a member of a family of integral ER membrane proteins of unknown function, with single members in many fungal species. We found that Ice2p is a distant homologue of SERINCs, another family of integral membrane proteins of unknown function, which are distributed across all eukaryotes, often with multiple members per species (for example, five in humans). The Ice2p-SERINC homology is stronger than many homologies identified by HHpred that subsequently have been confirmed by structural studies (Mariani et al., 2011; Schauder et al., 2014; Su et al., 2020). Additional factors cement the Ice2/SERINC homology: overall topologies are identical (Figure 2); sequence similarities stretch the whole length (Figure S4); key residues for SERINC function are among those conserved in Ice2p (Figure 5B). Together, this evidence makes a very strong prediction that adds the Ice2 family to a newly appreciated SERINC superfamily. In addition, a previously unknown protein family intermediate between Ice2 and SERINC is present from amoebae to opisthokonts (Figure 4), suggesting that this family and Ice2 branched from SERINC in the last unikont common ancestor or earlier (Figure 5).

Ice2p is predicted to interact with specific lipids

Structural (Pye et al., 2020) and mechanistic (Chen et al., 2020) studies of SERINC allow useful predictions about Ice2p based on its membership of the SERINC superfamily. Initially, SERINCs were proposed to modulate membrane lipid composition (Inuzuka et al., 2005). However, lipidomic studies have failed to detect the impacts of SERINC1 or SERINC5 on membrane PS and sphingolipid composition (Chu et al., 2017; Trautz et al., 2017). This suggests that SERINCs exert their antiviral actions through other mechanisms. The structure of *Drosophila* SERINC shows a cleft in the lumenal leaflet that is accessible to solvent molecules in Molecular Dynamics simulations, with a nearby hydrophobic groove occupied

by an acyl chain in the structure (Pye et al., 2020). In addition, specific lipids (cholesterol, PS and sulphatide) stabilised *Drosophila* SERINC against breakdown during heating, suggesting that these lipids might specifically bind the protein.

The structural homology between Ice2p and SERINC5 is focussed on TMH 1-7 (Figure 3) but extends across the whole protein (Figure S4), and includes surface features such as the cleft and groove (Figure 5B). This suggests that like SERINC, Ice2p has an intramembrane lipid binding site, which might explain the repeated finding that Ice2p is linked to phospholipid synthesis (Chen et al., 2020; Markgraf et al., 2014), though the specific lipid involved is not yet known.

Ice2p is unlikely to be a conventional tether

The link to SERINC calls into question some of the previous ideas about Ice2p. Ice2p reversibly localises during stationary phase to parts of the ER forming contact with lipid droplets (Markgraf et al., 2014). This was attributed to direct bridging, also called “tethering”, of Ice2p to lipid droplets by four predicted amphipathic helices in a long loop that was predicted to be in the cytoplasm and that, when expressed on its own, targeted lipid droplets (Markgraf et al., 2014). We show that the sole conserved amphipathic helix in this region (AH2, Figure 1) is most likely to lie on the lumenal side of the membrane, based on both the consensus predicted topology of Ice2s (Figure 2) and the homology to SERINC (Figures 3 and 5). Therefore, the loop can only access lipid droplets *in vivo* if two or more TMHs change topology reversibly. Such changes have been reported for bacterial permeases and transporters, and they have been predicted in eukaryotes (Dowhan et al., 2019), with a few examples reported, though not yet studied in detail (Herate et al., 2016; Zhang et al., 2014). This makes it is worth considering whether Ice2p, and therefore also SERINC, might have reversible topology.

Along with multiple ER-related functions attributed to Ice2p (Emmerstorfer et al., 2015; Markgraf et al., 2014; Murthi and Hopper, 2005; North et al., 2012; Schuldiner et al., 2005), it is required for normal formation of cortical ER in multiple different genetic backgrounds, including wild-type (only buds affected) (Estrada de Martin et al., 2005), cells with excess cortical ER because of unregulated phospholipid synthesis (*opi1*Δ) (Bircham and al., 2020), and cells lacking cortical ER, either 50% decreased after loss of Scs2p (Loewen et al., 2007), or 90% decreased after additional loss of five other ER-plasma membrane bridging proteins (Scs22p, Tcb1/2/3p and Ist2p) (Quon et al., 2018). Our results call into question the idea that Ice2p supports the formation of cortical ER through forming a physical bridge, or “tether”, to the plasma membrane (Quon et al., 2018). Even if AH2 flips into the cytoplasm, it does not have the polybasic nature of helices such as that in Ist2p to bind the plasma

membrane (Ercan et al., 2009). An alternative means of bridging for Ice2p is if it has a plasma membrane protein binding partner, but none was found in a proximity screen (Bircham and al., 2020). A more parsimonious hypothesis is that Ice2p acts to modulate a fundamental aspect of ER function that regulates other proteins in the same compartment, for example the Nem1/Spo7 phosphatase complex (Bircham and al., 2020). This would parallel the ability of SERINCs in viral envelopes to indirectly modulate the conformation and clustering of other integral proteins including HIV Env (Chen et al., 2020).

Yeast as a future model for SERINC research

Although more is known at the molecular level about SERINCs than Ice2p (Chen et al., 2020; Pye et al., 2020), identifying the link to Ice2p may aid research into SERINCs. *S. cerevisiae* is typical of fungi that express an Ice2 family member, in that it also expresses a SERINC: Tms1p. Little is known about this protein, except that it localises to the degradative vacuole (equivalent of lysosome) (Tkach et al., 2012), and that it can binds a limited range of lipids (Gallego et al., 2010). Targeting of multiple subcellular compartments by a pair of yeast SERINC superfamily proteins resembles the wide intracellular distribution of human SERINCs to plasma membrane (Gonzalez-Enriquez et al., 2017), ER (Inuzuka et al., 2005), and pre-autophagic vesicles (Davies et al., 2018). To date, attempts to produce a SERINC-null organism have been limited to a high-throughput study of *C. elegans*, where double RNAi of its two recently duplicated SERINCs produced no synthetic negative interaction (Tischler et al., 2006). Future yeast studies with individual and double *ice2/tms1* deletion mutants may lead to improved understanding of the whole superfamily.

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Contributions

G.O.A-B. and T.P.L. were both responsible for Conceptualisation, Formal Analysis, Writing (Original Drafted Review/Editing) and Visualisation.

Supplemental Information

4 Supplemental Figures and 2 Supplemental Tables.

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