Despite the comprehensive nature of these studies, a number of important questions remain. For example, although this study reveals that increased expression of snoRNAs results in augmented rRNA methylation and protein translation, it is unclear whether C/D box snoRNA leukaemogenic activity depends on increased global translation or is mediated by other consequences of rRNA modification, such as alterations in rRNA binding to non-ribosomal protein components that regulate other cellular processes. It will be important to test whether leukaemogenesis requires alterations in global protein translation or in specific proteins. Related to this issue, since rRNA methylation affects the fidelity of translation as well as usage of promoters and internal ribosome entry sites[13,14], it would be informative to measure such alterations directly following AE9a overexpression. Finally, while the authors identified increased translation as a direct consequence of oncogene expression, the importance of this finding for LSCs is unclear since studies in both AML[15] and myelodysplastic syndrome[16] have shown that disease stem cells exhibit reduced levels of translation. Thus, one might speculate that increased translation might not be required for LSC function or that it may be necessary at distinct steps during leukaemia development.

One additional intriguing finding of the study by Müller-Tidow and colleagues is that snoRNAs are induced not only by AML1-ETO but other leukaemogenic oncogenes such as c-MYC and MLL-AF9, and by the latter in an AES-independent manner. This raises the possibility that snoRNA dysregulation may be a generalizable phenomenon in AML. If this is the case, methods to inhibit the expression or activity of snoRNAs would represent an important therapeutic strategy. Another open question is whether other AML genetic drivers (for example, point and missense mutations) similarly induce increased snoRNA levels to regulate AML transformation and/or self-renewal. Ideally, such studies would be performed using primary AML blasts, using both syngeneic and xenograft models, where LSC activity can be functionally assessed.

Although these data strongly support a model in which snoRNAs are critical to AML1-ETO leukaemogenesis, it remains unclear whether other downstream targets, aside from AES, mediate the effects of AML1-ETO. While the studies were performed using a more aggressive splice variant, AML1-ETO9a, it is possible that the more common variant, AML1-ETO, mediates its function through additional downstream mechanisms. Furthermore, given the requirement for additional mutated genes in AML1-ETO$^+$ AML (such as FLT3)[7], it would be interesting to investigate whether they act to modify snoRNA activity, as well as determine whether increased snoRNA expression itself may be required to induce the appearance of these secondary mutations. Such studies may stimulate the development of therapeutic strategies that prevent the development of these later steps in AML1-ETO-driven pathogenesis.

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OPA1 and cardiolipin team up for mitochondrial fusion

Raymond Liu and David C. Chan

Fusion between the inner membranes of two mitochondria requires the GTPase optic atrophy 1 (OPA1), but the molecular mechanism is poorly understood. A study now shows that fusion of two liposomes can be performed by OPA1 tethered to just one liposome, through an interaction with the phospholipid cardiolipin on the opposing liposome.

As dynamic organelles, mitochondria require a balance between fusion and fission events for their proper function[1]. Mitochondria have double membranes, and the merger of two mitochondria involves outer membrane (OM) fusion followed by inner membrane (IM) fusion. Mechanoenzymes from the dynamin superfamily of large GTPases act sequentially to mediate these membrane remodelling reactions[1]. In mammals, fusion of the OM is carried out by mitofusins (MFN1 and MFN2), whereas subsequent fusion of the IM is carried out by optic atrophy 1 (OPA1). These reactions are critical for cell function. Mutations in *MFN2* cause peripheral neuropathy, and mutations in *OPA1* cause dominant optic atrophy, the most common inherited optic neuropathy. The biochemical basis of OPA1 action is poorly understood. In this issue[2], Ban *et al.* achieve a breakthrough in this area by developing a liposome-based assay to measure the membrane fusion activity of recombinant OPA1, leading to key biochemical insights.

Mitochondrial fusion is a homotypic membrane fusion event. This aspect is mirrored in the mechanism of OM fusion, which requires OM-bound mitofusin molecules to be present on both of the opposing membranes. Structural studies have provided models of how mitofusin dimers might form between opposing OMs to tether mitochondria together[3–5]. Analogously, homotypic fusion

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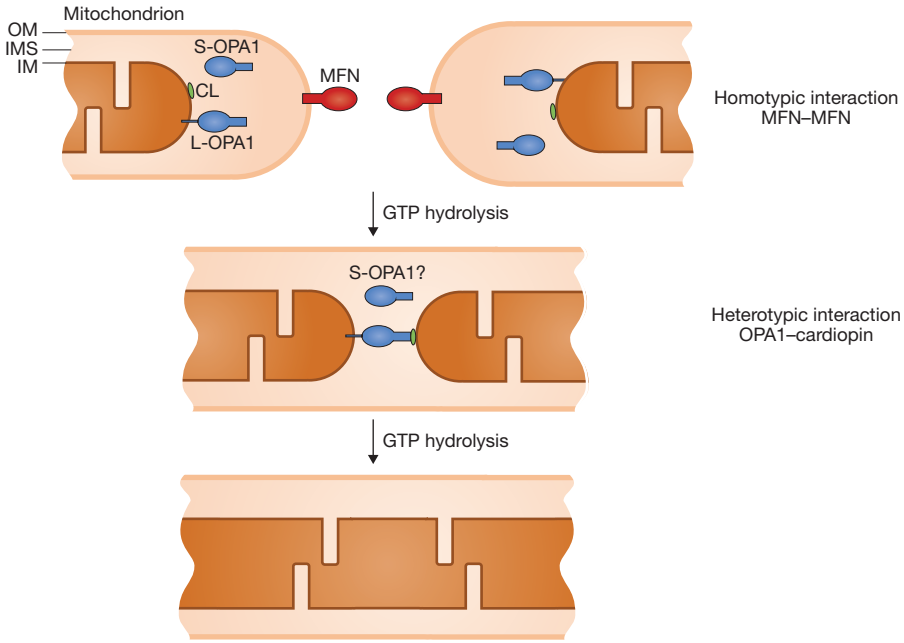

Figure 1 L-OPA1 and cardiolipin interactions mediate membrane fusion. Whereas homotypic interactions between MFN molecules in *trans* are required for OM fusion, OPA1 is required in only one of the organelles for IM fusion. Ban *et al.* show that L-OPA1 partners with cardiolipin (CL) on the opposing IM; this heterotypic interaction is sufficient for IM fusion. IMS, intermembrane space.

of the endoplasmic reticulum (ER) requires *trans*-complexes of atlastin[6], a large GTPase embedded in the ER membrane. This molecular symmetry, however, does not apply to mitochondrial IM fusion (Fig. 1). In contrast to the requirement for mitofusins, mitochondrial fusion assays involving cells or isolated mitochondria indicate that mitochondria from wild-type cells are perfectly capable of fusing with mitochondria from *OPA1*-null cells[7,8]. Complicating things further, OPA1 exists in the mitochondrial intermembrane space as both an IM-bound 'long form' (L-OPA1), and a soluble 'short form' (S-OPA1) produced from proteolytic cleavage of L-OPA1. It is clear that optimal fusion of mitochondria requires a combination of L-OPA1 and S-OPA1, and that S-OPA1 alone is insufficient[9]. L-OPA1 alone does not have significant fusion activity under normal circumstances[9], but appears sufficient for fusion when cells are placed under specific stress conditions[10]. In addition, cells lacking the proteases responsible for generating S-OPA1 are left with only L-OPA1, but nevertheless display some level of fusion activity[11]. Therefore, OPA1 fuses the IM in an asymmetric manner, and some of the evidence describing the relative contribution of L- versus S-OPA1 is difficult to reconcile.

In order to gain insight into the mechanism of OPA1-mediated membrane fusion, Ban *et al.* reconstituted OPA1-mediated fusion *in vitro* to allow dissection of the molecular requirements. By expressing, purifying, and incorporating recombinant L-OPA1 into liposomes, the authors successfully developed a fluorescence resonance energy transfer (FRET)-based membrane fusion assay to detect fusion between liposomes containing L-OPA1 and cardiolipin. This fusion activity increased with increased L-OPA1, required GTP, and was absent in liposomes containing an OPA1 mutant lacking GTP hydrolysis activity. These experiments generated two key insights. First, they demonstrate that L-OPA1 alone is clearly sufficient to mediate membrane fusion. Second, they satisfyingly explain why OPA1 is required on only one mitochondrion to mediate IM fusion. Liposomes containing L-OPA1 can fuse with liposomes devoid of L-OPA1, as long as the latter liposomes contain sufficient amounts of the phospholipid cardiolipin. Cardiolipin is enriched in the mitochondrial IM, where it constitutes about 20% of the phospholipid content, compared with the OM, where the value is about 4%. This striking result suggests that mitochondrial IM fusion involves a heterotypic interaction between OPA1 on one side and cardiolipin on the other (Fig. 1). This protein–phospholipid interaction may be sufficient to ensure specificity, because the mitochondrial IM is not exposed to other membranes until OM fusion by mitofusins.

In addition to mitochondrial fusion, OPA1 is important for maintaining the structure of cristae membranes, infoldings of the IM that have high membrane curvature. Interactions between OPA1 molecules on the IM have been proposed to maintain proper cristae shape[12]. Ban *et al.* modified the liposome assay to evaluate the ability of OPA1 to mediate liposome tethering. When L-OPA1 was incorporated into two sets of liposomes containing low cardiolipin, the liposomes could not fuse, but were tethered together. The authors propose that the two types of L-OPA1 interactions may lead to different functional outcomes. In one scenario, non-fusogenic *trans*-complexes of L-OPA within a single mitochondrion may help to maintain cristae structural integrity. In the other, L-OPA1 becomes fusogenic when it interacts with cardiolipin-rich domains from an opposing IM.

Although L-OPA1 alone is sufficient for fusion *in vitro*, fusion events *in vivo* appear more complex, because they are clearly affected by S-OPA1. To illuminate the function of S-OPA1, the authors examined its effect on liposome fusion under two different conditions — one in which L-OPA1 levels are held constant, and one in which total OPA1 levels are held constant. Addition of S-OPA1 to a constant concentration of L-OPA1 enhanced both liposome binding and fusion activity, consistent with a model where L-OPA1 cleavage into S-OPA1 promotes fusion[8]. However, when total OPA1 levels were held constant, and S-OPA1 levels were increased at the expense of L-OPA1, the authors observed a decrease in fusion activity. The interpretation of these results is complicated, because it is not clear whether addition of soluble S-OPA1 faithfully mimics physiological proteolytic cleavage of L-OPA1 to S-OPA1. To address these issues, it will be important to reconstitute liposomes with engineered versions of L-OPA1 that can be artificially cleaved. Consistent with the critical role of S-OPA1 for mitochondrial fusion in mammalian cells[9], the yeast orthologue of OPA1, Mgm1, requires proteolytic processing for activity. Together, L-Mgm1 and S-Mgm1 act as a heterodimer to mediate fusion. In a division of labour, L-Mgm1 functions as an IM anchor, while S-Mgm1 drives fusion via GTP hydrolysis[13,14]. In fact, a heterodimer containing GTPase-defective L-Mgm1 is

functional for fusion, as long as the associated S-Mgm1 has GTPase activity. Such results underscore the need to further examine the collaboration between L- and S-OPA1.

This study raises additional interesting questions. The demonstration that L-OPA1 is sufficient for fusion raises the issue of why L-OPA1 has little activity when expressed in *OPA1*-null cells. In contrast, L-OPA1 appears to be fusogenic when cells are treated with cycloheximide or other stressors, in a phenomenon termed stress-induced mitochondrial hyperfusion[10]. These observations suggest that unknown regulatory mechanisms exist to control the activity of L-OPA1 *in vivo*. In addition, it will be important to address how the heterotypic interaction between L-OPA1 and cardiolipin leads to activation of membrane fusion. Conversely, given that cardiolipin is present in *cis*, how is the fusogenic activity of OPA1 suppressed until OM fusion brings it face to face with the opposing IM? The GTP hydrolysis activity of OPA1 is increased by cardiolipin binding, and a disease allele of OPA1 has been shown to abrogate this effect and impair fusion[15]. Moreover, like other dynamins, OPA1 is known to self-assemble, and S-OPA1 is capable of forming large assembles that can tubulate membranes *in vitro*[15]. These biochemical properties are likely to be relevant to the fusion process, and it will be interesting to determine whether cardiolipin can regulate OPA1 conformation and self-assembly.

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