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Cell Cycle News & Views

Interchanging heads: p53 re-composes the DREAM/MMB complex to repress transcription

Comment on: Quaas M, et al. Cell Cycle 2012; 11:4661–72;
PMID:23187802; http://dx.doi.org/10.4161/cc.22917

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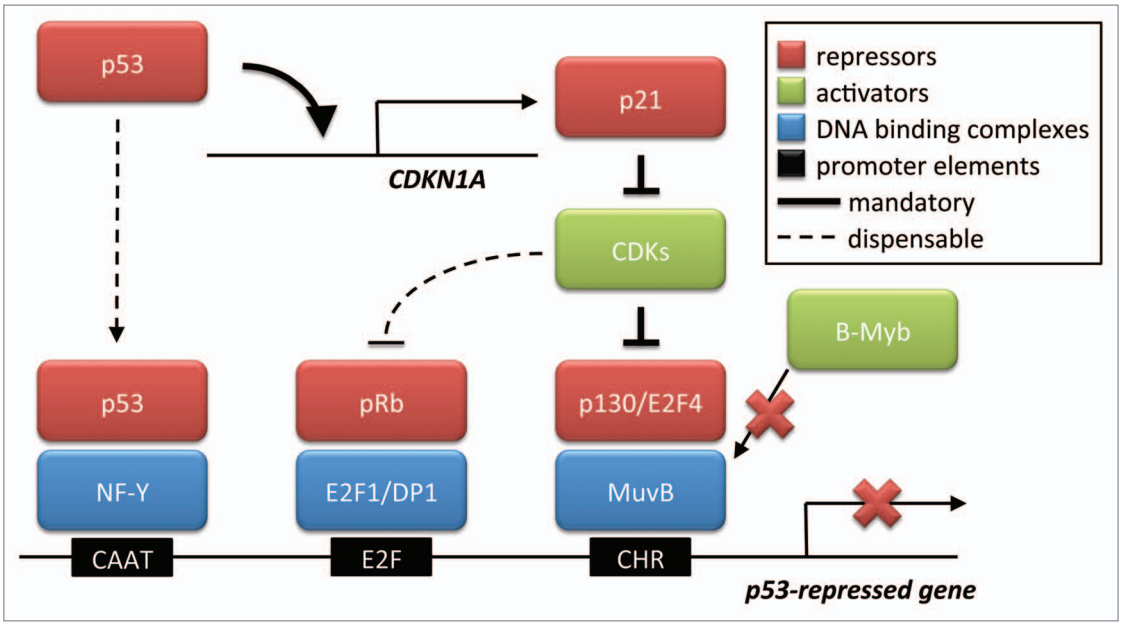

Figure 1. p53-mediated gene repression. p53 reportedly can act as a repressor of genes by at least three mechanisms. The first one involves the association of p53 with the transcription factor complex NF-Y, which, in turn, binds the CAAT box of promoter DNA. The two other mechanisms each depend on the transactivation of p21 by p53. p21 blocks cyclin-dependent kinases (CDKs), leading to the hypophosphorylation of retinoblastoma (Rb) family members. These then associate with E2F proteins. E2Fs can bind their cognate DNA elements, in cooperation with DP1, and the associated Rb proteins then mediate repression. However, p130, while binding E2F4 but independent of an E2F-binding DNA element, associates with the MuvB-complex, replacing B-Myb. MuvB binds the CHR element of DNA. As a consequence of E2F4 and p130 being tethered to the CHR, the promoter is repressed. The first two mechanisms were reported earlier, but the article in this issue of *Cell Cycle*[1] argues against a direct association of p53 with the cyclin B promoter; nor did the authors observe a need for E2F binding sites in repression. Only the last mechanism, driven by MuvB and the CHR element, appears indispensable for gene repression in these experiments. This mandatory mechanism is therefore indicated by bold arrows in the scheme, whereas the other two seem dispensable, reflected by dashed lines.

In the popular tale "Ozma of Oz," Lyman Frank Baum describes the princess Langwidere, who is capable of interchanging her currently worn head with another one, using a collection of heads stored in a cabinet. Remarkably, she changes her character as well when putting a new head on her neck.

In the December 15, 2012 issue of *Cell Cycle*, Kurt Engeland and colleagues report a similar phenomenon[1] with regard to a DNA-associated, transcription-regulatory complex of proteins, the DREAM/MMB complex. A central core of this complex, MuvB, binds to a DNA motif called CHR for cell cycle genes homology region.[2] In addition, the complex has facultative members. Either the B-Myb oncoprotein joins to activate transcription from the adjacent gene—this composition is termed MMB for Myb-MuvB. Alternatively, the retinoblastoma protein homolog p130, along with the prototype-repressive member of the E2F family, E2F4 and the auxiliary DNA-binding partner protein DP1, associate with MuvB to form an entity called DREAM for DP, RB-like, E2F and MuvB complex. This complex was first purified from Drosophila embryos[3] and then characterized in mammalian cells.[4] Exchanging the "head" of the complex in this way also changes its "character," converting the transactivator MMB to the repressive DREAM complex.

Strikingly, the new report shows how this re-association of the MMB/DREAM complex can be induced. Activating the tumor suppressor p53 drives the expression of the cyclin-dependent kinase p21/Cip1/Waf1/CDKN1A. This, in turn, leads to the hypophosphorylation of p130 and thereby enables its association with MuvB, replacing B-Myb and mediating

the repression of at least one cell cycle regulatory gene, i.e., CCNB2 encoding cyclin B2. Thus, by interchanging the association partners of MuvB, p53 indirectly represses a cell cycle regulatory gene to mediate G_2 arrest.

p53 carries out most (though not all) of its tumor suppressive functions by regulating transcription; it activates target genes by DNA binding. However, p53 also represses genes, the underlying mechanisms being much less obvious.

It was previously reported that p53 binds to the promoters of repressed genes. However, unlike for transactivation, p53 did not seem to directly bind the DNA of repressed promoters, but rather associate with the DNA-bound transcription factor complex NF-Y.[5] In the current report, however, the authors did not observe an association of p53 with repressed promoters,[1] arguing against a general need for this interactions in repression.

A second possibility is that p53 may induce genes that encode repressors. Accordingly, preventing protein synthesis by cycloheximide abolishes p53-mediated repression but not activation.[6] Strikingly, cells that lack p21 no longer show gene repression by p53.[7,8] p21 is an inhibitor of cyclin-dependent kinases, thus contributing to a hypophosphorylated state of the retinoblastoma protein family members pRb, p107 and p130. In turn, hypophosphorylated Rb proteins bind to members of the E2F family of transcription factors, often turning transactivators into repressors. Many p53-repressible genes contain promoter elements that bind E2F. Thus, it appeared conceivable that gene repression by p53 is largely performed through E2F-binding DNA.

The new report shows that this scenario is unlikely to reflect the full truth. The authors show that it is a CHR site, rather than E2F-responsive elements, that confers p53-mediated repression. Hence, while still involving p21 and members of the Rb and E2F families, the new model (**Fig. 1**) suggests that the association of a repressive DREAM complex with the CHR site is the major route of negative gene regulation by p53. This does not exclude that the other two mechanisms (p53-NF-Y and pRb-E2F) and their respective promoter DNA motifs still contribute to the repression of a different set of promoters; however, the work by Quaas et al. clarifies a necessary role of converting MMB to DREAM by p53 and p21 for the repression of CCNB2 and for G_2 arrest.

If this concept can be generalized to other p53-repressed promoters, the outlined mechanism may explain how p53 and p21 prevent premature mitosis, especially upon DNA damage. In the context of cancer chemotherapy, it may prove helpful to deliberately interfere with this route to G_2 arrest, thus sensitizing tumor cells by promoting mitotic failure. Putting a new head on MuvB may thereby determine the sensitive or resistant character of a cell.

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The Golgi complex: A common platform for canonical and non-canonical autophagy?

Comment on: Naydenov NG, et al. Cell Cycle 2012; 11:4613–25; PMID:23187805; http://dx.doi.org/10.4161/cc.22885

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Naydenov et al. found that siRNA-mediated downregulation of αSNAP, a component of the SNARE system, triggers autophagy as measured by the lipidation of LC3 and flux analysis.[1] The SNARE system participates in vesicular fusion. Its key components include N-ethylmaleimide sensitive factor (NSF) and its adaptor soluble NSF-attachment protein α (αSNAP). Notably, downregulation of NSF had no effects on LC3 lipidation. This suggests that in mammalian cells, the autophagy regulatory function of αSNAP is separated from its other functions that involve its partner protein NSF.

The SNARE system is involved in the ER-Golgi trafficking. It is thus possible that disruption of the normal ER-Golgi interaction, ER function or the Golgi complex could be an autophagy signal. Indeed, knockdown of αSNAP caused Golgi fragmentation. Pharmacological agents that cause Golgi fragmentation and inhibit ER to Golgi trafficking, such as Brefeldin A (BFA) and Golgicide A (GA), are known to cause autophagy.[2] BFA actually inhibit three Golgi-resident guanine nucleotide exchange factors (GEFs) for the Arf small GTPases, GBF1, BIG1 and BIG2, whereas GA only inhibits GBF1. Consistently, knockdown of GBF1, but not BIG1 and BIG2, induced LC3 lipidation. These observations indicate that an important autophagy trigger could be the disruption of Golgi function and/or structure.

Interestingly, the Golgi complex has been closely linked to autophagy in several ways. The Golgi complex has been considered to be one of the potential membrane sources of autophagosomes.[3] Several well-defined autophagy molecules, such as Beclin-1, Atg9, Rab32 and Rab33B, can be located at the Golgi complex.[4-7] Moreover, as strengthened by the present work, disruption of Golgi function and/or structure can trigger an autophagic process. Fission of the Golgi membranes had been observed during starvation-induced autophagy, which is thought to be related to the re-distribution of Atg9 from the Golgi complex to vesicles that may be related to the biogenesis of autophagosomes.[8] Could Golgi fragmentation, caused by the knockdown of αSNAP and GBF1, or by BFA and GA, be related to autophagosome generation in these cases?

While this possibility exists, there is at least one important distinction between the two cases. In the starvation-induced autophagy, both Bif-1 and Beclin-1 are required for the Atg9 redistribution and fission of Golgi complex. In the study by Naydenov et al., Bif-1 but not Beclin-1 was required for the LC3 lipidation. It is not clear whether these molecules are required for Golgi fission and for Atg9 redistribution, if it ever occurs, in the scenario involving αSNAP or GBF1 knockdown. Likewise, if BFA and GA trigger autophagy in the same way as the downregulation of αSNAP, the role of Bif-1, Atg9 and Beclin-1 would have to be examined as well.

Nevertheless the Beclin-1-independent nature suggests a different type of autophagy, known as non-canonical autophagy (NCA), which has been reported in many other cases.[9] It seems that NCA could be triggered by many different factors, and all could be independent on one or more components of the Beclin-1-PI3KC3 complex or other autophagy molecules. However, it is not clear at all, how NCA could occur in the absence of the initiation/nucleation complexes. One caveat of NCA is that it is measured in many studies by LC3 lipidation only without further functional assessment and/or strong electron microscopic morphological evidence. Thus, whether NCA is an autophagy process in the normal sense, i.e., as functional and productive as the canonical autophagy, has yet to be fully determined. The present study by Naydenov et al., however, did provide supportive evidence based on flux analysis that NCA triggered by αSNAP knockdown could be productive. Future studies would need to be directed further on its functional significance.

If fission of Golgi membrane is a key step toward autophagosome biogenesis, then molecules like αSNAP and GBF1 would be important gate keepers to restrict the fission and, thus, the membrane supply for autophagosome biogenesis. It also seems that just removing the gate keepers without additional signals could be sufficient to trigger an autophagy process that can bypass some of the key mechanisms. It could be speculated that the bypassed mechanisms, such as the one controlled by the Beclin-1-PI3KC3 complex, may function in fragmenting the Golgi complex, in addition to other possible capacities, during canonical autophagy.

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Genomic instability: Ada3 and HPV E6-acetyltransferase connections?

Comment on: Mirza S, et al. Cell Cycle 2012; 11:4266–74; PMID:23095635; http://dx.doi.org/10.4161/cc.22613

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DNA damage induced by chemicals or radiation must be detected, checkpoints activated, repair enzymes induced and recruited to the lesion and DNA crosslinks and breaks resolved. Because genotoxic damage can eventuate in cell cycle arrest, senescence or death, a tightly organized cascade of complex events must ensue. How DNA damage signals induce these very different outcomes remains to be precisely determined. Two mechanisms, non-homologous end-joining and homologous recombination, are available for restoration of DNA integrity. We know many of the players, yet how these proteins are integrated in the cellular response is a tangle of protein-protein interactions.

Two manuscripts[1,2] from Vimla Band's group report the consequences of disrupting the murine Ada3 gene. Loss of Ada3 increased basal phosphorylation of several key DNA damage pathway factors, including ATM and γH2AX. Ada3-null cells exhibited a prolonged response to irradiation and a variety of chromosomal alterations indicative of ineffective DNA repair.

What is the operative mechanism? The 432-amino acid Ada3 protein has attributes of a signal integrator. The C-terminal half of Ada3 binds to lysine acetyltransferases (KATs) that acetylate histones, also referred to as HATs, which influence local chromatin structure. KATs also interact with a host of key regulatory factors, including p53, the tumor suppressor that controls cell fate decisions following genotoxic stress. The N-terminal domain of Ada3 binds p53. Ada3 modulates acetylation of C-terminal lysines in p53 that are necessary for p14ARF signaling to p53 for induction cell senescence.[3] The activation status of p53, including specific acetylation and phosphorylation sites, following homozygous Ada3 deletion in mouse tissues was not reported. Inability to activate p53 and induce its effector functions might lead to the observed accumulation of DNA damage and genetic instability. Nonetheless, p53 is not the sole determinant of Ada3 function. Homozygous deletion of Ada3 is embryonic lethal,[2] while p53 nullizygous mice are viable. Ada3 haploinsufficent mice were not reported to have increased tumor development.

Several KAT proteins are involved in control of DNA repair. The Band laboratory reported the KAT family members p300, PCAF and GCN5 co-immunoprecipitate with Ada3.[4] GCN5 in conjunction with E2F1 was found to stimulate recruitment of excision repair factors following UV radiation.[5] Although not reported to associate with Ada3, the KAT protein Tip60 is an intriguing possibility, as its acetyltransferase activity is rapidly stimulated upon irradiation and, together with the mre11-rad50-nbs (MRN) complex, increased ATM kinase activity in response to double-stranded DNA breaks.[6] Another consequence of Ada3 absence is stalling of cell cycle progression from G_1 and

accumulation of the cdk inhibitor $p27^{Kip1}$.[2] There was concomitant reduction in the levels of p300 and PCAF. PCAF, but not p300/CBP, regulates expression of the cell cycle regulator $p21^{cip1}$. Because Ada3-deficient cells accumulate at G1/S, might Ada3 and its associated histone acetyltransferases be required for modifying chromatin structure at origins of DNA replication or for resolution of DNA damage that occurs during strand separation and synthesis? Clearly the interaction of Ada3 and KAT proteins in the DNA damage response deserves further investigation.

The relationship between genetic abnormalities accompanying Ada3 deficiency to the viral oncoprotein HPV E6 are provocative. The cervical cancer-associated HPV types 16 and 18 E6 proteins bind to and inactivate Ada3, which correlates with the ability to immortalize human epithelial cells and HPV 16 E6 mutants that retain binding to Ada3 inhibit p53 acetylation.[7] Mechanistically, E6 could interfere with binding of p53 or a KAT to Ada3, although this remains to be shown. HPV 18 E6 also induces degradation of Tip60, which may result in inability to induce DNA repair mechanisms.[8] Interestingly, E6 proteins from HPV types associated with cutaneous cancers were found to stimulate p300 degradation, inhibit expression of the DNA repair factor ATR and leading to persistence of UV-induced thymine dimers.[9] Nonetheless, cell lines expressing HPV are remarkably stable and do not accumulate chromosomal damage observed in Ada3-knockout cells.

Does Ada3 complex with specific DNA damage pathway proteins? Sorting out the functions of Ada3, HPV E6, the relevant HAT proteins and their targets will be most informative.

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NCF2/p67phox: A novel player in the anti-apoptotic functions of p53

Comment on: Italiano D, et al. Cell Cycle 2012; 11:4589–96; PMID:23187810; http://dx.doi.org/10.4161/cc.22853

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p53 is best known for its role as a potent tumor suppressor and transcription factor that induces processes such as apoptosis or cell cycle arrest in response to a variety of stresses. The decision to survive or to die is most likely dependent on the stimulus and the duration or severity of that stimulus (reviewed in ref. 1). Low amounts of stresses are thought to favor the induction of p53-dependent cell cycle arrest via p21 or 14-3-3 proteins to allow for DNA repair. Interestingly, such proteins have also been shown to actively inhibit apoptosis and favor survival. In fact, in recent years it has become apparent that p53 can employ multiple strategies to promote survival, including inducing a multitude of anti-apoptotic genes.[2] A small number of these genes can function by decreasing ROS levels, including MnSOD (Manganese superoxide dismutase), ALDH4 (aldehyde dehydrogenase 4), GPX (glutathione peroxidase), sestrins, which have antioxidant activities themselves, or TIGAR (p53-induced glycolysis and apoptosis inhibitor), which inhibits glycolysis and, thus, lowers ROS (**Fig. 1**).

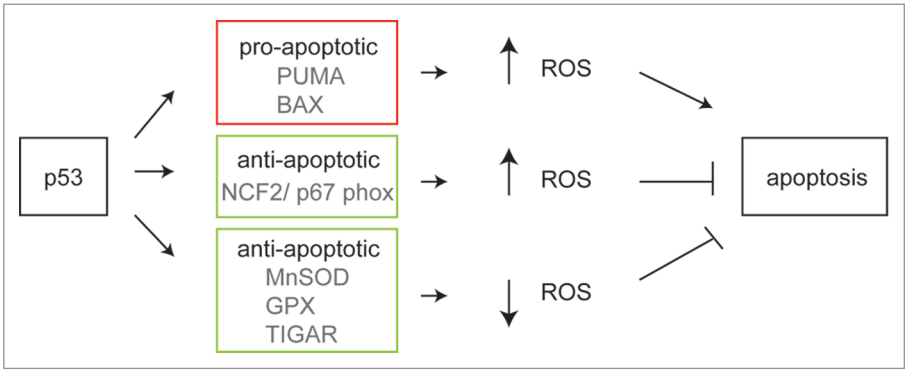

Figure 1. p53 can both induce and inhibit apoptosis by regulating ROS levels. Some of the pro- (red box) and anti- (green box) apoptotic genes regulated by p53 are depicted in gray.

In the December 15, 2012 issue of *Cell Cycle*, Italiano et al. explored the possibility that NCF2/p67phox is a novel p53 target gene that could potentially be added to the list of anti-apoptotic p53 target genes (**Fig. 1**).[3] Overexpression of p53 or doxorubicin-mediated stabilization of p53 induced the expression of NCF2/ p67 phox. Furthermore, using luciferase assays and chromatin IPs, they demonstrate the presence of a p53 response element in the promotor region of NCF2/p67 phox. Although p53 shares a number of target genes with its family members p63 and p73, and all family members have a similar consensus response element, the authors show that neither p63 or p73 could induce NCF2/p67 phox expression. NCF2/p67 phox is the activating unit of the NAD(P)H oxidase enzyme complex 2 (Nox2) localized at the plasma membrane and endosomes, involved in generating NADP+ or NAD+ and, thus, increasing ROS. Not surprisingly, siRNA-mediated loss of NCF2/p67 phox expression resulted in a decrease in ROS levels. However, and contrary to ROS generated by the p53 target genes Puma or Bax via the mitochondrial-dependent apoptotic pathway, a decrease in ROS by loss

of NCF2/p67 phox expression coincided with increased apoptosis. These results suggest that ROS generated from different sources or possibly at different cellular localizations have differential effects on apoptosis. Although Nox2 has been shown to drive ROS-dependent cell death, others have demonstrated pro-survival and anti-apoptotic effects of ROS production by Nox2[4] or the Nox family member Nox4[5] that inhibited apoptosis by enhancing the AKT-mediated phosphorylation of apoptosis signal kinase 1 (ASK1). The data by Italiano et al. add to the complexity of p53 signaling toward ROS, and it will be interesting to further characterize the orchestration of all the p53 signaling routes leading to ROS and apoptosis during various stresses.

As NOXes, including Nox2, have also been shown to induce signaling cascades that enhance cell migration and proliferation,[6] and Nox activity seems required for H-Ras transformation,[7] the work of Italiano et al. gives room for some further speculations. Frequently, mutations in p53 give rise to a mutant p53 protein that drives cell migration and proliferation. Although these mutant p53 proteins have often lost the ability to regulate the pro-apoptotic genes, some mutants still retain the ability to regulate some target genes.[8,9] It will be interesting to further explore whether some mutant p53 proteins can still upregulate NCF2/p67 phox, and whether the transforming abilities of mutant p53, like H-Ras, require Nox activity.

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SNAPping off Golgi membranes for autophagosome formation

Comment on: Naydenov NG, et al. Cell Cycle 2012; 11:4613–25; PMID:23187805; http://dx.doi.org/10.4161/cc.22885

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Macroautophagy, hereafter referred as autophagy, is a lysosomal degradation process that is initiated from phagophore assembly sites (PAS), which expand to recruit Atg8/LC3 for the formation of isolation membranes/phagophores. The processing of Atg8/LC3 is required for the elongation and sealing of the phagophore to generate the completed autophagosome. Recent studies have identified SNARE proteins, evolutionarily conserved mediators of intracellular membrane fusion, as key regulators for the expansion of PAS and recruitment of Atg8/LC3 in both yeast[1] and mammalian cells.[2] Loss of Sec18 and Sec17, the yeast orthologs of the mammalian AAA-ATPase NSF and its cofactor αSNAP, respectively, which regulate the activation of SNARE complexes,[3] results in a defect in autophagy,[1] demonstrating that SNARE-mediated membrane fusion plays a key role in autophagosome biogenesis.

In stark contrast with these findings, Naydenov et al. report within the December 15, 2012 issue of *Cell Cycle* that knockdown of α*SNAP* promotes autophagic flux in cultured human epithelial cells under nutrient-rich culture conditions (**Fig. 1**).[4] They found that α*SNAP* siRNA (siα*SNAP*)-induced autophagy is accompanied by Golgi fragmentation, and that the fragmented Golgi membranes (fGMs) co-localize with an autophagosomal marker, GFP-LC3. Moreover, loss of the membrane curvature-inducer *Bif-1* attenuates siα*SNAP*-induced autophagy. Notably, Bif-1 promotes the fission of Golgi membranes that contain the autophagy-essential transmembrane protein Atg9 to promote autophagosome formation in mammalian cells during starvation,[5] suggesting that Bif-1 may serve as a key regulator for the PAS formation and expansion during the suppression of α*SNAP*. Intriguingly, deletion of a secretory SNARE gene, *Sec22*, in yeast suppresses the PAS translocation of Atg9 and abrogates autophagosome biogenesis,[1] whereas knockdown of *Sec22B* in HeLa cells has minimal effects on the formation and expansion of PAS,[2] suggesting that the membrane supply process for autophagosome biogenesis in mammalian cells may differ from that in yeast.

As siα*SNAP*-induced LC3 lipidation and GFP-LC3 foci formation are observed in the absence of Beclin 1, the authors propose that siα*SNAP*-induced autophagy occurs through a non-canonical pathway that bypasses the Beclin 1-dependent phagophore nucleation step.[4] However, it is worth noting that LC3 processing can be induced in several autophagy-defective cells.[6] Moreover, while siα*SNAP*-induced autophagy requires *Bif-1*, Bif-1-mediated Golgi fission and autophagosome formation during nutrient starvation require the Beclin 1-UVRAG complex.[5] Further analyses are warranted to clarify whether and how the autophagy induced in response to α*SNAP* knockdown occurs in a Beclin 1-independent manner.

Although it remains unclear if the dispersion of fGMs is in itself sufficient for the induction of autophagy, the authors demonstrate that siα*SNAP* diminishes the expression of several Golgi-associated proteins, including the guanine nucleotide exchange factor GBF[1,4] which acts upstream of Arf to regulate COPI-mediated vesicle trafficking. Dysregulation of Arf1 has been shown to inhibit mTORC1,[7] a negative regulator of the autophagy-essential Atg1/ULK kinase complex.[6] Consistently, the mTOR signaling pathway is suppressed by α*SNAP* knockdown. As the dispersion of fGMs by the knockdown of *Rab1* is not sufficient for inducing autophagosome formation,[8] the activation of Atg1/ULK may be a critical factor for the induction of Golgi fragmentation-induced autophagy.

SNARE-mediated membrane fusion is indispensable not only for the expansion of PAS but also for the formation and maturation of completed autophagosomes. This raises a question, how can fGMs form degradative autophagosomes in the absence of α*SNAP*?

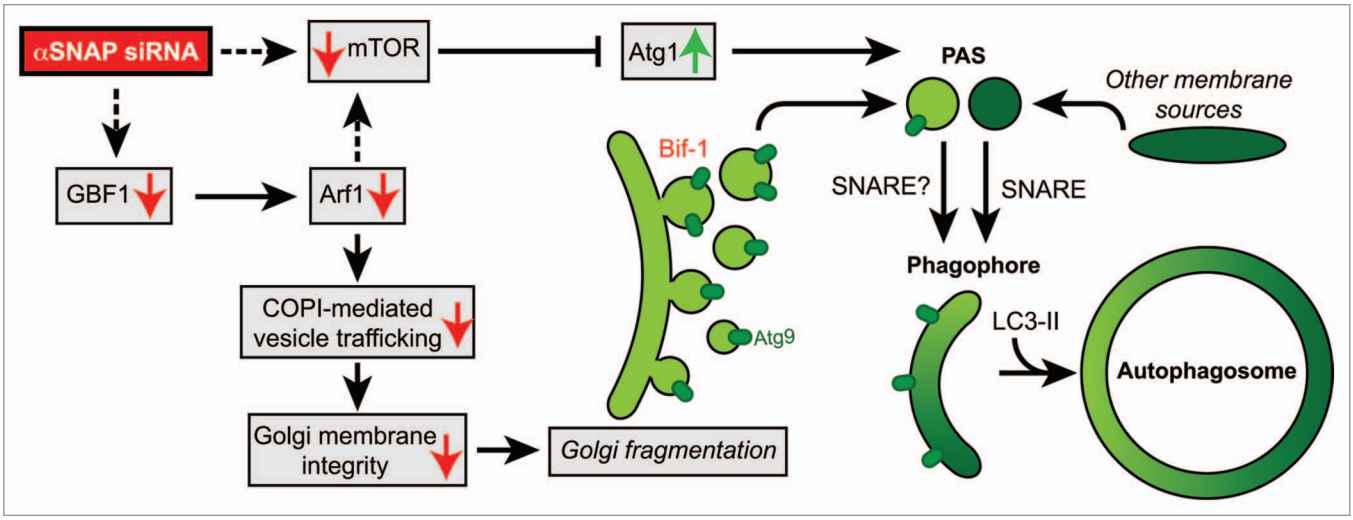

Figure 1. Knockdown of αSNAP promotes autophagic flux through the inhibition of mTOR-related signaling and the fragmentation of Golgi apparatus to provide Atg9-containing membranes for the formation and expansion of PAS during autophagosome biogenesis.

Unlike yeast *Sec17/Sec18* deletion mutants, the siRNA-mediated gene silencing system used by Naydenov et al. did not completely deplete α*SNAP*.[4] Thus, it is possible to speculate that a trace amount of αSNAP in the siRNA-treated cells is sufficient for activating SNAREs to mediate autophagic membrane fusion events. In addition, as the SNAP family is composed of two ubiquitously expressed α- and γ-SNAPs and neuronal cell-specific βSNAP,[3] the expression of β-/γ-SNAPs may compensate for the loss of αSNAP. In this scenario, depletion of β-/γ-*SNAPs* or *NSF* would abrogate siα*SNAP*-induced GFP-LC3 foci formation and cause the accumulation of LC3-negative nascent autophagosomal membranes, as SNAREs are required for the expansion of PAS prior to the recruitment of Atg8/LC3.[1,2] Indeed, unlike α*SNAP* depletion, knockdown of *NSF* fails to induce the autophagic processing of LC3.[4] Further investigation of the mechanism behind the regulation of autophagic membrane fusion in siα*SNAP*-treated cells is anticipated in the future.

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