*Review*

**The Necessity of NEDD8/Rub1 for Vitality and its Association with Mitochondria-Derived Oxidative Stress**

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**Highlights:**

* NEDD8/Rub1 is a key regulator of cellular redox homeostasis.
* Ascomycota species that produce mitochondria-derived reactive oxygen species during glycolysis require NEDD8/Rub1for viability.
* NEDD8/Rub1 essentiality correlates with the existence of NEDP1 in the organism genome.

**Introduction**

The eukaryotic family of ubiquitin-like (Ubl) polypeptides is a class of evolutionary conserved reversible protein modifiers that regulate a variety of fundamental cellular processes [1,2]. The first discovered and most widely studied member of this family is ubiquitin (Ub) [3]. Ub is a most conserved polypeptide, showing only 3:76 amino acid difference between the *Saccharomyces cerevisiae* and human orthologs (Fig 1). Ub modifies thousands of targets in processes that require hundreds of enzymes, involving the recognition, covalent modification and release of Ub to and from substrates [4]. The Ubl family comprises additional members, among which, small ubiquitin-like modifiers (SUMOs) and neural precursor cell expressed developmentally downregulated gene 8 (NEDD8) are the most widely studied to date. Each of the Ubl modifiers requires a cognate cascade of enzymes for the covalent attachment to substrates (Fig 2). NEDD8 is the closest paralog of Ub; however, it shows a higher variety in sequence then Ub, with a diversity of as many as 32 out of its 76 amino acids between the human NEDD8 and its *S. cerevisiae* ortholog, related Ub 1 (Rub1) (Fig 1). Unlike the expended Ub pathway, the NEDD8/Rub1 modification pathway includes numerous enzymes and the list of substrates is much shorter, with extensive reports on a single family of conserved protein targets, namely “cullins” (in *S. cerevisiae* Cdc53/yCul1, yCul3 and Rtt101/yCul4; in humans the typical Cul1-5, and atypical Cul7 and Cul9/PARC) [5–7]. Each cullin serves as a platform for the construction of modular multi-subunit Ub ligases, belonging to the family of cullin-RING E3 ligases (CRLs). Similar to ubiquitination, the conjugation of NEDD8/Rub1 to cullin substrates (known as “NEDDylation”) requires a cascade of enzymes (Fig 3). Both NEDD8 and Rub1 orthologs are synthesized as precursors that first need to be trimmed by C-terminal hydrolases [8,9]. The subsequent conjugation of NEDD8/Rub1 to a precise Lys residue of each cullin is mediated by the NEDDylation cascade of enzymes, consisting of a dimeric NEDD8 E1 activating enzyme (NAE1), consisting of Ula1/Uba3 in *S. cerevisiae* and APPBP1/UBA3 in humans, an E2 conjugating enzyme (Ubc12 in *S. cerevisiae*; UbE2M or UbE2F in humans) [10–12] and the RING E3 subunit of CRLs (Hrt1 in *S. cerevisiae*; RBX1 and RBX2 in humans) together with the co-E3, DCN1 that stimulates the NEDDylation reaction [13,14]. Cullin NEDDylation enhances CRL activity *in vitro* probably by facilitating the recruitment of the Ub-charged E2 enzyme [15]. The NEDDylation site is located at the C-terminal “winged-helix B” (WHB) inhibitory domain of the cullin [16]. Recent structural studies of CRL1 (a.k.a. the SCF) through cryo-electron microscopy have revealed that covalent attachment of NEDD8 to the WHB domain of Cul1 leads to extensive rearrangements of the CRL components, which could explain the enhanced ubiquitination activity [17]. Cullin modification is reversed by the COP9 signalosome (CSN), a multi-subunit cullin-NEDD8/Rub1-specific deNEDDylase [18,19] (Fig 3). Over time, studies undertaken by multiple laboratories have identified additional substrates for NEDD8 [20–30]. These non-cullin substrates are conjugated to NEDD8 monomers either directly, or indirectly through poly-NEDD8 or mixed Ub–NEDD8 chains. These studies have led to the finding of an additional deNEDDylase, namely NEDP1 (Ub-like protease (ULP)8, SENP8, DEN1) that detaches NEDD8 from non-cullin substrates [31–33] (Fig 4). While the NEDDylation cascade of enzymes is highly conserved across phyla, deNEDDylases are more diverse in sequence, complexity and even in their existence. This review analyzes the knowledge on Ascomycota lifestyle instrumentally to present a link between NEDD8/Rub1 functionality, vitality and cell metabolic programming (Fig 5).

1. **Crossroads between ubiquitin and NEDD8 pathways**

The NEDDylation pathway intersects with ubiquitination at several junctions. The first intersecting enzyme is the ubiquitin C-terminal hydrolase 3 ([UCHL3] Yuh1 in *S. cerevisiae*), which exposes the conjugating residue, Gly76 of both, Ub and NEDD8 (Fig 2). Similarly, all documented NEDD8/Rub1 E3s are also Ub ligases [34]. Apparently, the Ub and NEDD8/Rub1 pathways split into distinct cascades for the E1 and E2 activities. Notably, residue 72 within each modifier is the key recognition site for each the E1 enzymes: ubiquitin activating enzyme (UAE) and NAE [35–37]. NAE exhibits specificity to NEDD8/Rub1 due to an arginine residue near the catalytic site of UBA3/Uba3 that could possibly clash with the highly conserved Arg72 residue of Ub [35–38]. In contrast, UAE prefers Ub over Rub1 [39]. Notably, in certain circumstances, NEDD8/Rub1 approaches UAE to enter the ubiquitinome [39–42]. This can be achieved if most of the Ub bulk is incorporated into chains due to a pharmacological treatment with proteasome inhibitors, or in response to stress [40–44]. In such circumstances, NEDD8/Rub1 is activated by UAE, transferred to Ub-E2s and eventually incorporated into Ub chains. It had been suggested that introducing Rub1 into the ubiquitinome decreases the average length of heterologous mixed Rub1-Ub chains relative to homogenous poly-Ub chains [45]. NEDD8 has been reported to form *in vivo* homogenous polymers and tripeptides in cellular stresses, including DNA damage and oxidative stress, respectively [46–48]. The spillover of NEDD8/Rub1 into the Ub pathway and the ability to form homogenous chains could indicate other function(s) of NEDD8/Rub1 apart from activating CRLs.

**2. The NEDD8/Rub1 deconjugating enzymes**

2.1 The CSN complex

*2.1.1 Evolutionary conservation of the CSN*

The CSN is a multi-subunit cullin-deNEDDylating enzyme harboring a JAMM/MPN+ metalloprotease motif within the catalytic subunit Csn5. The CSN shows high paralogy to the 19S proteasome lid, which contributes deubiquitination activity to the 26S holocomplex through the metalloprotease subunit Rpn11 [49,50]. The proteasome lid and the CSN complexes are required for viability of multicellular organisms. Yet, unlike the high conservation of the proteasome lid across all eukaryotic phyla, the CSN shows more diversity, especially in fungal species [51–53]. For example, *S. cerevisiae* proteasome lid subunits can be substituted with *Arabidopsis thaliana* orthologs. In contrast, *S. cerevisiae* CSN subunits are not interchangeable with orthologs of higher organisms [54,55]. The CSN had been studied in model organism species belonging to the three monophyletic subphyla of the fungal phylum Ascomycota: Saccharomycotina (including *S. cerevisiae* and *Candida albicans*), Pezizomycotina (including *Aspergillus nidulans* and *Neurospora crassa*), and Taphrinomycotina (including *Schizosaccharomyces pombe*). In these species, the CSN is not vital, although mutants sometimes lead to physiological and morphological alterations, among them, defects in cell-cycle progression, vacuole morphology, circadian clock or fruit body formation [56–61].

*2.1.2 Conserved and diverged CSN functions*

The specificity of the CSN complex to cullin-NEDD8 conjugates stems from the JAMM/MPN+ motif in Csn5/Jab1, which becomes functional only upon interaction of the holocomplex with a CRL. Indeed, the complex-incorporated CSN5 is autoinhibited at the Glu 104 residue in the autoinhibitory loop (Ins-1). Interaction of the complex with a NEDDylated CRL leads to a series of conformational change events in Csn2, Csn4 and Csn7, triggering rearrangements in the Csn5–Csn6 dimer, resulting in Csn5 activation by priming the Csn5 MPN+/JAMM motif for deNEDDylation [19,62]. In addition to intrinsic deNEDDylation activity, the CSN also possesses two kinds of deubiquitination activity: one that deconjugates ubiquitin from mono-ubiquitinated substrates and is mediated by Csn5 [42,63]; and the other that depolymerizes polyubiquitin chains through CSN-associated deubiquitinating enzymes: Usp48 in humans, Ubp12/Usp15 in *S. pombe*, *A. nidulans* and Humans, and Ubp3 in *S. cerevisiae* [54,64,65]. Both deNEDDylation and deubiquitination functions of the CSN inhibit CRLs *in vitro* [65-67]. Additionally, the CSN also controls CRL activity in two non-enzymatic manners: inhibition of cullin NEDDylation through the binding of Csn2 and Csn4 to the CRL-RING subunit Rbx1 [19,62,68,69]; and hindrance of the target binding site in the substrate receptor (SR) of CRLs by the Csn3 and Csn8 subunits [19,70]. The overall negative activity of the CSN is believed to protect CRL ligases *in vivo* from auto-ubiquitination of self-components. Consequently, the CSN is considered a positive regulator of CRL-mediated physiological functions. Consistent with this, in the absence of CSN, many SRs are auto-ubiquitination , and hence are destabilized [71,72]. Indeed, a direct interaction between the CSN and CRL-SRs has been described in many organisms; in each of them, dissociation of CSN subunits results in the loss of BTB and F box protein stability [71–77]. The CRL SR interacting components, Csn3 and Csn8, are two atypical Proteasome, COP9, Initiation factor 3 (PCI)-containing subunits [50]. In canonical CSN complexes, these subunits co-interact through their N-terminal repeats, and are considered as a module [19]. Interestingly, both Csn3 and Csn8 exhibit diverged phylogenetic performance in the Ascomycota species by not always participating in CSN assemblages [54,78–80] (Fig 3a,b), even if a direct ortholog gene exists in their genome [50,81]. Indeed, orthologs of these subunits exist and participate in CSN assemblages of *A. nidulans* (Csn3, Csn8), *N. crassa* (Csn3) and *S. pombe* (Csn3), yet their mutants are distinguished from other *csn* mutants by showing a slighter CSN characteristic biochemical phenotype of accumulated cullin-NEDD8 conjugates [56,67,71,82] (Fig 3b). The minor accumulation of NEDDylated cullins could be explained if these subunits are not part of the core deNEDDylase complex, and instead are primarily involved in the regulation of substrate ubiquitination and turnover [53] (Fig 3c). Evidently, *S. cerevisiae* CSN complexes lacking the Csn3/Csn8 module harbor a highly conserved deNEDDylase activity, but do not affect the turnover of typical CRL substrates such as p27/Sic1 or other key cell-cycle regulators [83,84]. The turnover of a single CRL substrate was found to be regulated by the *S. cerevisiae* CSN complex Mth1, a negative regulator of the glucose-sensing signal transduction pathway and a substrate of SCFGrr1 [84]. Altogether, this suggests that the requirement of the CSN for vitality and/or functionality exhibits a great phylogenetic diversity.

2.2 Other deNEDDylases

Identification of the CSN as a cullin-specific deNEDDylase indicates a requirement for a more general deNEDDylase to facilitate the exposure of the conjugation residue Gly76 of NEDD8/Rub1 and to release NEDD8 from non-cullin substrates. These distinct functions could be achieved either by a difunctional enzyme similar to the deSUMOylating enzyme ULP1 [85], or by two distinct enzymes, with each one bearing one of the functions. In fact, UCHL3/Yuh1 catalyzes the initial processing of NEDD8/Rub1 by hydrolyzing the peptide bond at the carboxyl terminus of both Ub and NEDD8/Rub1 (Fig 2). UCHL3/Yuh1 is highly conserved in eukaryotes, including the three Ascomycota subphyla. Nevertheless, Ub is probably a better substrate of UCHL3/Yuh1 as the inhibitory constant (*K*i)for NEDD8/Rub1 is 20-fold higher compared to Ub [8]. Many eukaryotes harbor an additional deNEDDylase, namely NEDP1. NEDP1 is a NEDD8-specific hydrolase from the ULP family that plays a dual role by both processing the carboxyl terminus of NEDD8 and also releasing covalently attached NEDD8 molecules from multiple substrates [32,33,86–88]. NEDP1 is a direct paralog of Ulp1 (Fig 4). Orthologs of NEDP1 are included in Pezizomycotina and Taphrinomycotina, but not in Saccharomycotina species. Interestingly, the *S. pombe* genome includes two orthologs of NEDP1 known as Nep1 and Nep2 [88] (Fig. 4). NEDP1 deletion mutants of *A. nidulans* (*ΔdenA*) and *S. pombe* (Δ*nep1/Δnep2*) are viable [88,89]. Notably, the functional redundancy in *S. pombe* could be connected with the essential nature of NEDD8 conjugation in fission yeast.

**3. Essentiality of NEDD8 and the cullin-NEDDylation site in Ascomycota**

If the regulation of CRL activity was the only important function of NEDD8, it would be expected that the NEDDylation and deNEDDylation enzymes would be similarly required for organism viability. Yet, non-essentiality of the CSN in Ascomycota species does not constantly conform with the requirement of NEDD8/Rub1 for vitality. Indeed, NEDD8/Rub1 is essential for the viability of most studied Ascomycota model species, except for *S. cerevisiae* and *C. albicans* [90,91]. In correlation, *S. cerevisiae* strains, mutated in the NEDDylation modification site of either Cdc53/Cul1 (K760R) or Rtt101 (K791R) are viable and show moderated phenotypes of altered ergosterol quantity and sensitivity to a DNA single-strand break, respectively [61,92]. Similarly, the *C. albicans* mutant of Rub1 (*rub1−*/−) exhibits mild growth defects, including a pseudohypha-like phenotype associated with the pathogenic morphology of this fungus. The phenotype is shared with the non-NEDDylated point mutant of Cul1 (K699R), but not with *csn5−*/− [90]. The non-essentiality of NEDD8/Rub1 in both, *S. cerevisiae* and *C. albicans* is correlated with the absence of DENP1-encoding genes in their genomes.

Although belonging to the same phylum, the fission yeast *S. pombe* is only distantly related to *S. cerevisiae* and is believed to have separated more than 350 million years ago [93]. Indeed, *S. pombe* NEDD8/Rub1 is required for organism viability and the NEDDylation site of the only essential *S. pombe* cullin, Cul1/Pcu1 (K713) is also vital [94]. Unexpectedly, the overexpression of *pcu1Δ720–767,* a C-terminal truncated mutant lacking the autoinhibitory WHB domain of Cul1/Pcu1, rescues *pcu1* K713/R unviability [46,95,96]. These findings could be explained if increased flexibility of the WHB free mutant suppresses the necessity of NEDD8/Rub1 for CRL1/SCF activation [17]. Surprisingly, NAE is still essential in this mutant, indicating the vital NEDDylation of the non-cullin substrate(s) in *S. pombe*.

**4. Cullin-free NEDD8 and the response to mitochondrial oxidative stress**

Oxidation is the strongest stress inducer of the formation of non-cullin conjugates of NEDD8 [43]. A growing number of studies identify a direct link between mitochondrial-derived oxidation and the performance of non-cullin NEDD8 conjugates. For example, mice flavoproteins of the mitochondrial electron transfer chain (ETC) are stabilized by NEDDylation and are rapidly degraded upon deficiency in NEDDylation cascade enzymes, leading to pathologies related to fatty acid oxidation disorders [97]. The modification of hypoxia-inducible factor (HIF)-α1 protein by NEDD8 has a great impact on cell survival under circumstances where hypoxia and oxidative stress coexist [98]. Stressed mammalian cells accumulate poly-NEDD8 chains [43], and the treatment with hydrogen peroxide inactivates NEDP1, resulting in an accumulation of unanchored NEDD8 trimers [47]. These NEDD8 trimers bind and inhibit poly-ADP ribose polymerase (PARP)1 to prevent the activation of apoptosis. In correlation with these data, the proliferating cell nuclear antigen is modified by NEDD8 and stabilized upon hydrogen peroxide treatment due to inhibition of NEDP1 [99].

The link between NEDD8/Rub1 and oxidative stress is highly conserved. Indeed, the loss of NEDD8/Rub1 in *C. albicans* promotes sensitivity to oxidative stress [90]. A null mutant of the *S. cerevisiae* ortholog exhibits altered mitochondrial morphology and decreased ergosterol quantity, a well-known phenotype of cells with a complete loss of mitochondrial DNA (mtDNA ρ0) [45,61,100]. Altogether, this suggests a highly conserved link between the biology of NEDD8/Rub1 and mitochondria-derived oxidation.

**5. NEDD8/Rub1 and the respiration lifestyle of various fungal species**

The mitochondria ETC is a central source of cellular reactive oxygen species (ROS) [101–103]. Indeed, mitochondrial ROS is considered as a key pathogenic trigger of diseases such as inflammation, cancer, cardiac, neurodegeneration and other aging disorders [104–106]. Accordingly, it will not be surprising if the diverged existence or essentiality of NEDD8 and NEDP1 in Ascomycota is linked with aerobic lifestyle and essentiality of the mitochondria. The stated Ascomycota groups have the ability to ferment glucose in the presence of oxygen and to proliferate under anaerobic conditions, and thereby employ the mitochondria for respiration by different metabolic programs. Unfortunately, with the exception of the information on these popular species, reliable data concerning respiration strategies of other Ascomycota species are poorly known.

5.1 The respiration lifestyle of *S. cerevisiae*

Much of our knowledge in this area is based on research of *S. cerevisiae* due to biotechnological interest in this organism, which began in the 19th century following the identification of glucose fermentation by Louis Pasteur. *S. cerevisiae* cells approach anaerobic metabolism even under aerobic conditions in the presence of oxygen. This phenomenon is known as “aerobic glycolysis” or the “Crabtree effect.” Aerobic glycolysis in Crabtree-positive organisms such as *S. cerevisiae* enables a higher rate of ATP production at the fermentation growth phase, usually without accumulating ROS [107,108]. As a Crabtree-positive organism, *S. cerevisiae* cells convert glucose to ethanol and carbon dioxide through alcoholic fermentation, as long as glucose is available (logarithmic phase) and regardless of the presence of oxygen. During this phase, the oxidation of carbohydrates in the mitochondrial ETC is suppressed [109]. Towards the end of the fermentative phase, under a critical dilution rate of glucose, *S. cerevisiae* cells undergo a physiological transition from anaerobic glycolysis to mitochondrial respiration, which is accompanied by a high production of ROS. This critical point is known as the “diauxic shift,” and is characterized by *de novo* transcription of metabolic and antioxidant genes [110–112]. In fact, *S. cerevisiae* cells can survive without mtDNA (ρ0) as long as they are grown on a fermentative carbon source [113]. Moreover, ρ0 yeast cells, which do not have respiratory capacity, form a characteristically reduced colony size, termed “petite” [113].

5.2 *S. cerevisiae* mitochondria and NEDD8/Rub1

We asked how the respiration lifestyle of *S. cerevisiae* might be linked with NEDD8/Rub1 functionality. Recent studies present a dramatic loss in the *S*. *cerevisiae* yCul1/Cdc53 NEDDylation status at the diauxic shift [84,108]. Indeed, the *S*. *cerevisiae* NEDDylation cascade of enzymes is sensitive to a natural increase in metabolic ROS during mitochondrial respiration, leading to the loss of Ubc12~NEDD8 thioester forms and eventually in Cdc53/yCul1 NEDDylation status. Correspondingly, elevation of ROS in mutants lacking a proper antioxidant machinery, or in wild-type cells treated by uncouplers of the mitochondria ETC, leads to a decrease in Cdc53/yCul1 NEDDylation status. Interestingly, this phenomenon is evolutionarily conserved, as induction of ROS in human cells by low concentrations of hydrogen peroxide blocks the NEDDylation enzymes and the transfer of NEDD8 to cullins [114,115]. Conversely, *rpn11-m1*, a proteasome mutant strain that does not accumulate ROS due to a high capacity of antioxidants, exhibits continuous Ubc12~NEDD8 thioester formation in all growth phases [108,116]. On the other hand, CSN metalloprotease activity is stable during oxidation (diauxic and post-diauxic phases) [84,108,117]. Inhibition of the *S*. *cerevisiae* NEDDylation cascade by cumulative mitochondrial and exogenous ROS, supplemented by continuous CSN activity results in the loss of cullin NEDDylation and the appearance of cullin-free NEDD8/Rub1molecules.

5.3 *C. albicans* mitochondria and NEDD8/Rub1

*C. albicans* is an mtDNA ρ+ Crabtree-positive organism, harboring effective mitochondria during the pre-diauxic anaerobic growth phase [118]. This attribute might appear to conflict with the suggestion that NEDD8/Rub1 essentiality correlates with mitochondrial respiratory activity. However, the lifestyles of *C. albicans* and *S. cerevisiae* are quite dissimilar. The mitochondria of *C. albicans* have a central role in fungal virulence, even at the fermentation phase [119]. *C. albicans* is a pleomorphic pathogenic fungus with two distinct morphologies: yeast-like and filamentous-like. The filamentous morphology could be further dissected into a pseudo-hyphae form and a pathogenic invasive-hyphae form [120]. The most potent inducers of the invasive form are amino acids such as arginine and proline, which are catabolized to glutamate [121]. These amino acids enter the tricarboxylic acid (TCA) cycle in the mitochondria through α-ketoglutarate. The catabolism of proline in the mitochondria leads to increased levels of intracellular ATP, which subsequently promotes the switch between the yeast and hyphal morphologies, a key strategy to escape from degradation in macrophages [122]. Interestingly, similar to *S. cerevisiae* cells, when *C. albicans* is grown aerobically in a high-glucose medium complemented with proline, mitochondria are no longer required [122]. Moreover, even without the addition of proline, cells at the pre-diauxic phase exhibit a rapid induction of oxidative stress-resistant genes and are less susceptible to oxidative stress [123]. This could be explained as survival tactics of pathogens, which are attracted by neutrophils that try to kill them by oxidative burst [124]. Accordingly, the non-essentiality of NEDD8/Rub1 in *C. albicans* can be explained by the function of mitochondria in the aerobic fermentation phase mainly for the metabolism of amino acids and not for the purpose of respiration.

5.4 *S. pombe* mitochondria and NEDD8/Rub1

Similar to *C. albicans*, the fission yeast *S. pombe* is also an mtDNA ρ+ Crabtree-positive organism with partial active mitochondria at the fermentation growth phase. However, unlike species of the Saccharomycotina sub-phylum, *S. pombe* cells respire some oxygen during the fermentation growth phase [125]. *S. pombe* cells treated at the fermentation phase with antimycin A, a potent ETC inhibitor, have a lower biomass, consume more glucose and produce more ethanol than control cells, indicating that the mitochondrial respiratory loss is compensated for by increased alcoholic fermentation. The above suggests that the oxidative phosphorylation of ETC is active during fermentation and that energy gained in the respiration process is required for yeast proliferation and increase of biomass [126]. Indeed, Malecki *et al.* [126] identified 154 genes in *S. pombe* that were found to be important for respiration but did not have orthologs in *S. cerevisiae*, suggesting that the genetic basis for respiratory growth is remarkably distinct between these yeast species. The increased respiration at the fermentation phase in *S. pombe* is associated with low activity of a sole enzyme, pyruvate kinase 1 (Pyk1), a protein that forms a homo-tetramer during glycolysis to convert phosphoenolpyruvate to pyruvate, the input for aerobic respiration (TCA cycle). Accelerated Pyk1 activity restricts the respiration at the fermentation phase and leads to changes in cellular metabolism and physiology, most notably sensitivity to oxidative stress [127]. Mitochondrial respiration accompanied by the formation of ROS in the fermentation phase of *S. pombe*, correlates with the requirement of NEDD8/Rub1 for viability.

5.5 Mitochondria of the *Aspergillus* and *Neurospora* species

*A. nidulans* and *N. crassa* are Crabtree-negative filamentous fungal species. They predominantly oxidize pyruvate to carbon dioxide through the TCA cycle, and show little or no repression of the TCA cycle in glucose-rich growth conditions. Consequently, thus, the mitochondria are essential all long [128]. While the CSN and CRLs have been extensively studied in these species, little is known about NEDD8/Rub1 *per se* [60,71,80,82,129,130]. NEDD8 and the NEDDylation cascade enzymes are required for the viability of *A. nidulans* [89]. To date, experimental data on NEDD8/Rub1 in *N. crassa* are not available. However, considering the biology of the CSN/NEDD8 axis in this organism and its aerobic lifestyle, NEDD8 is probably essential for vitality [80].

**Concluding remarks and perspectives**

Access of molecular oxygen to the mitochondria ETC leads to the generation of ROS [101–103]. ROS induces progressive damage to macromolecules, cells and tissues. The cumulative damage could trigger diseases such as inflammation, cancer, cardiac, neurodegeneration and other aging disorders [104–106]. Ascomycota model species offer an opportunity to identify a highly conserved link between metabolic oxidative stress and NEDD8/Rub1essentiality. These species are facultative anaerobic organisms that utilize both anaerobic and aerobic lifestyles for optimal growth and have fundamental adaptations to ecological niches and environmental pressures to which they may be exposed. The vitality of NEDD8/Rub1 in Ascomycota species is consistent with the presence of functional mitochondrial respiration that accompanies ROS production along all stages of growth (Fig. 5).

The NEDDylation cascade mediates the conjugation of NEDD8/Rub1 to cullin and non-cullin substrates. As expected, vitality of the conjugation pathway enzymes correlates with that of NEDD8/Rub1. Interestingly, orthologs of the ROS-sensitive deNEDDylase NEDP1 appear only in genome of organisms with vital mtDNA and NEDD8/Rub1. Conversely, subunits of the CSN, the only cullin deNEDDylase eraser, are not essential in all three Ascomycota sub-phyla, regardless of the species’ respiration lifestyle. Hence, the seemingly strict function of CSN in regulation of CRL substrate turnover is less conserved than imagined. Why the CSN exists in species of the three sub-phyla if it is not vital and does not significantly regulate CRL activity is unknown. It is possible that the most conserved function of the CSN is to release NEDD8/Rub1 from the cullin “reservoirs” to support the non-cullin function of this modifier. Indeed, CSN activity is constitutive and not sensitive to ROS [117]. Furthermore, the accumulation of cullin-free NEDD8/Rub1 molecules during oxidation is conserved from *S. cerevisiae* to humans [47,108]; therefore, it could be considered as a most conserved character of NEDD8/Rub1. In fact, the anaerobic flagellated protozoan parasite *Giardia* has a relic mitochondrion (mitosome) that lacks mtDNA and has a genome lacking CSN subunits and a clear ortholog for NEDD8/Rub1 [54,131,132]. Most interestingly, reports of recent genome sequencing of an anaerobic microbe *Monocercomonoides* sp. PA203 (now termed Monocercomonoides exilis), present a compelling case for the complete lack of mitochondrial organelles [133]. The genome of this organism includes a single gene of tri-Ub (AAW22168.1a), but lacks an ortholog for NEDD8/Rub1. This is a first clear case of an organism that has Ub but lacks NEDD8/Rub1.

Despite the considerable research in the field of NEDD8/Rub1, many questions remain unanswered. For example, there is not yet have a clear understanding of how the different biochemical activities attributed to NEDD8/Rub1 translate into its physiological functions in different organisms, particularly mammals. One interesting point is the increased production of ROS in many cancers, which originate from mitochondrial dysfunction [134]. It has been shown that various cancer cells deal with ROS through a metabolic switch between glycolytic and oxidative metabolism in a reversible fashion, analogous to the Crabtree effect, known as the “Warburg effect” [135]. Indeed, information on [glucose uptake](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/glucose-transport), glycolytic activity and downregulation of [mitochondrial metabolism](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/mitochondrial-respiration) in Crabtree-positive species corresponds to the analogous Warburg effect in cancer cells. Given the growing interest in the use of NEDD/Rub1 as a therapeutic target for cancer, and the development of MLN-4924 (pevonedistat), a potent inhibitor of NAE [115,136,137], understanding the role of NEDD8/Rub1 in mitochondria metabolism could potentially lead to a better prediction of cancer types, developmental stages, and tumor progression that could potentially be targeted by this inhibitor.

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