Current Genetics
https://doi.org/10.1007/s00294-020-01133-4

MINI-REVIEW

Forever young: the key to rejuvenation during gametogenesis

Bailey A. Koch-Bojalad[1,2] · Lauren Carson[1] · Hong-Guo Yu[1]

Received: 23 October 2020 / Revised: 9 November 2020 / Accepted: 12 November 2020

Abstract
Cell aging is the result of deteriorating competence in maintaining cellular homeostasis and quality control. Certain cell types are able to rejuvenate through asymmetric cell division by excluding aging factors, including damaged cellular compartments and extrachromosomal rDNA circles, from entering the daughter cell. Recent findings from the budding yeast *S. cerevisiae* have shown that gametogenesis represents another type of cellular rejuvenation. Gametes, whether produced by an old or a young mother cell, are granted a renewed replicative lifespan through the formation of a fifth nuclear compartment that sequesters the harmful senescence factors accumulated by the mother. Here, we describe the importance and mechanism of cellular remodeling at the nuclear envelope mediated by ESCRT-III and the LEM-domain proteins, with a focus on nuclear pore biogenesis and chromatin interaction during gamete rejuvenation.

Keywords ESCRT-III · LEM-domain · Nuclear pore complex · Replicative lifespan · Meiosis

Introduction

A hallmark of aging is the progressive accumulation of cellular damage. Age-induced damage is caused by a decrease in cellular homeostasis and protein quality control, affecting both the chronological age of the cell and its replicative lifespan (Longo et al. 2012). While the occurrence of self-renewal and rejuvenation has been well documented for a variety of cell types, the cellular mechanisms utilized to mitigate the effects of aging are not fully understood. Recent work in the budding yeast *S. cerevisiae* has begun to shine a light on these fundamental processes.

A crucial determinant in reducing the effects of cellular aging is proper trafficking across the nuclear envelope which forms a selective barrier between the cytoplasm and nucleoplasm (Ungricht and Kutay 2015). The semi-permeable barrier provided by the nuclear pore complexes (NPCs), which are embedded within the nuclear envelope, aids in maintaining nuclear integrity and nuclear envelope homeostasis (Beck and Hurt 2017; Kim et al. 2018). In budding yeast, malformed NPCs accumulate in the Storage of Improperly Assembled Nuclear pore Complexes compartment, called the SINC (Webster et al. 2014). The SINC is not passed on to daughter cells but instead remains with the mother during mitosis, which is asymmetric in budding yeast (Colombi et al. 2013; Makio et al. 2013; Webster et al. 2014). Consequently, the daughter cell is born with a renewed replicative lifespan while the mother ages, accumulating damaged cellular compartments and protein aggregates, with each new bud until death (Sinclair et al. 1998).

In contrast to mitosis, meiosis is morphologically symmetrical and produces gametes, also referred to as spores in budding yeast (Neiman 2011). During gametogenesis, meiotic cells reset aging such that all gametes have a renewed replicative lifespan (Unal and Amon 2011; Unal et al. 2011). Senescence factors such as extrachromosomal rDNA circles and protein aggregates originally observed in the mother cell are not present in the newly formed gametes (Fuchs and Loidl 2004; Unal and Amon 2011; Unal et al. 2011; King et al. 2019; Koch et al. 2020). Recent studies of the formation of the Gametogenesis Uninherited Nuclear Compartment (GUNC) during budding yeast meiosis offer a mechanistic explanation for how aged mother cells can produce four fully renewed gametes (King et al. 2019; King and Unal 2020; Koch et al. 2020) and the ramifications on gamete

Communicated by M.Kupiec.

✉ Hong-Guo Yu
hyu@fsu.edu

[1] Department of Biological Science, Florida State University, Tallahassee, FL 32306, USA

[2] Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Published online: 27 November 2020

lifespan of hindering this sequestration (Koch et al. 2020). This knowledge indicates a role for nuclear envelope remodeling and nuclear pore biogenesis in the rejuvenation of gametes in budding yeast, which may have implications for understanding self-renewal during animal gametogenesis.

GUNC formation during budding yeast gametogenesis

During gametogenesis in budding yeast, age-induced factors, including extrachromosomal rDNA circles, heat shock proteins, and NPCs are selectively sequestered to the GUNC (King et al. 2019; Koch et al. 2020). The wide-scale nucleoporin sequestration observed in meiosis indicates that the formation of the GUNC involves both the nuclear envelope and nucleoplasm in a coordinated fashion. GUNC initially forms via the biogenesis of the prospore membrane, projecting from the spindle pole body (King et al. 2019; King and Unal 2020). There are two potential mechanisms by which the prospore membrane biogenesis may affect GUNC formation. First, as the prospore membrane expands, it actively pushes NPCs into the GUNC. Alternatively, the rapid growth of the nuclear membranes during meiosis II, corresponding to the formation of the prospore membrane, may passively leave behind NPCs in the GUNC. In either scenario, we envision that the prospore membrane functions as a selective filter facilitating the initial constriction of NPCs, with the Endosomal Sorting Complex Required for Transport (ESCRT)-III complex finalizing the sequestration of nucleoporins to GUNC (Koch et al. 2020).

ESCRT-III constriction of NPCs to GUNC during gametogenesis

ESCRT-III and its associated factors are known to function in NPC biogenesis (Webster et al. 2014; Thaller et al. 2019), and we propose many of these interactions are required for sequestration of NPCs to the GUNC. The LEM (Lap2-emerin-MAN1) family of integral inner nuclear membrane proteins, Heh1 (which is also called Src1) and Heh2 have been shown to mediate the surveillance of NPC biogenesis in conjunction with ESCRT-III components, Chm7, Snf7, and Vps4 (Webster et al. 2014, 2016). This surveillance mechanism involves Heh1 as the recruiting factor responsible for the focal accumulation of Chm7 at specific sites along with the nuclear envelope, in turn activating ESCRT-III likely through its direct interaction between Snf7 and Chm7 (Webster et al. 2016; Thaller et al. 2019).

During meiosis, Heh1 and ESCRT-III are required for the constriction of nucleoporins to the GUNC (Koch et al. 2020). We propose that during late anaphase II, Heh1 recruits ESCRT-III and Chm7 to the junction between the expanding gamete nuclei and the GUNC. The ESCRT-III complex then likely binds Vps4 at these sites and constricts the nuclear envelope, leading to the eventual scission of the membrane leaving four gamete nuclei separated from the GUNC (Koch et al. 2020).

The formation of GUNC is analogous to nuclear division in budding yeast mitosis, in which the mother cell retains all of the nuclear senescence factors (Kaeberlein 2010; Longo et al. 2012). Functionally, the GUNC, therefore, resembles the mitotic mother cell retaining the old or damaged NPCs and other aging factors. The meiotic constriction of NPCs to the GUNC is facilitated by the actions of Heh1 and ESCRT-III (Koch et al. 2020), and given that Heh1 directs ESCRT-III during mitotic NPC biogenesis through binding Chm7 following cytoplasmic exposure (Webster et al. 2016), Heh1 may be the determining factor of where and how the GUNC constriction sites are located. In support of a Heh1 dependent mechanism, recent data suggests the LEM-domain protein, LEM2, in human cells is capable of condensing on microtubules in a liquid-like phase and co-assembles with the Chm7 homolog, CHMP7, to activate ESCRT and function as an O-ring seal at the confluence between membranes (von Appen et al. 2020). The idea that budding yeast gametogenesis may utilize a similar phase separation to constrict aging factors from nascent gametes is an attractive model that requires further analysis.

Is NPC remodeling related to nuclear envelope breakdown?

We propose that ESCRT-III/Vps4 in conjunction with Heh1 and Chm7 is responsible for the membrane cleavage separating the GUNC from the newly formed gametic nuclei. One question yet to be answered is: what is the mechanism by which NPCs are remodeled at the nuclear envelope during the process of GUNC formation? Looking to other single-celled eukaryotes, the fission yeast *S. pombe* may provide insight into nuclear envelope dynamics and NPC remodeling. In fission yeast, the nuclear envelope is intact during meiosis I, but during anaphase II there is a virtual Nuclear Envelope Breakdown (vNEB), creating an increase in permeability which allows for diffusion of nuclear proteins into the cytoplasm (Arai et al. 2010; Asakawa et al. 2010). A localized NEB, in which the nuclear envelope breaks down at specific regions, has also been observed in fission yeast mitosis, at the microtubule bridge, giving the dividing nuclei an intermediate dumbbell shape (Dey et al. 2020) and at the centrosome (Fernandez-Alvarez et al. 2016). NPCs aggregate in the middle of the bridge in a manner dependent upon Les1 (Dey et al. 2020). The Les1 protein localizes exclusively to the inner nuclear membrane to form stalks around

the mitotic spindle, constricting movement along the microtubule bridge and orienting the location of NEB (Dey et al. 2020). It has been shown that Les1 interacts with ESCRT-III proteins to ensure proper nuclear envelope sealing, and upon deletion of *LES1,* NPCs are no longer sequestered to the microtubule bridge (Dey et al. 2020). This new information on Les1 in fission yeast mitosis provides insight into the budding yeast homolog Msc1, which may have a similar function in the sequestration of NPCs in meiosis. Future study of Msc1 may provide insight into the connection of NEB to NPC remodeling in budding yeast meiosis.

Nuclear envelope remodeling and chromosome segregation

During GUNC formation, the core subunits of the NPC are sequestered, whereas basket nucleoporins appear distributed along the periphery of the gamete nuclei (King et al. 2019; Koch et al. 2020). The significance of this partial disassembly of the NPC remains unclear, but it is consistent with the idea of dynamic NPC modularity, which is tightly regulated (Knockenhauer and Schwartz 2016). The dissociation of basket nucleoporins from the core and their subsequent localization to the gamete nuclei late in meiosis may allow the amphipathic helices of basket nucleoporins to dock onto and curve the nuclear membrane (Meszaros et al. 2015). Also in meiosis II, chromosomes initially localize to the midzone, then migrate to the nascent gamete nuclei upon GUNC formation (King et al. 2019; Koch et al. 2020). Chromosome movement during this process is concomitant with that of the basket nucleoporins (King et al. 2019; Koch et al. 2020). Given that basket nucleoporins associate with chromatin during cell divisions (Dultz et al. 2008; Markossian et al. 2015; Suresh et al. 2017), we speculate that separation of basket nucleoporins from the core may be crucial for disrupting the interaction between the NPC and chromatin that would otherwise inhibit NPC constriction to the GUNC. LEM proteins tether chromatin to the nuclear periphery (Grund et al. 2008; Mekhail et al. 2008), we, therefore, hypothesize that the interaction between NPCs and chromatin is perturbed in the absence of Heh1, Heh2 or Vps4. Along with the lack of sequestration to the GUNC in the absence of these proteins, we observed a mutant phenotype in which the NPCs coalesced around a single gamete nucleus (Fig. 1a; Koch et al. 2020), indicating that the NPC and chromatin interaction somehow remains intact. This phenotype was observed in approximately one quarter of *heh1* and *vps4* mutants but in over two-thirds of *heh2* mutants (Koch et al. 2020). Another chromosomal defect we observed was NPCs encapsulating a fifth chromatin mass in the GUNC-like compartment in a little under one quarter of *heh1* and *vps4* mutants (Fig. 1a; Koch et al. 2020). The five nuclear bodies produced from these meiotic cycles were inviable, indicating a severe chromosome segregation defect (our unpublished data). We hypothesize that ESCRT-III and the LEM-domain proteins play dual roles during yeast gametogenesis (Fig. 1b). First, they participate in NPC constriction and GUNC formation (Koch et al. 2020). Second, they function in meiotic chromosome segregation, by linking chromosomes to NPCs either directly or indirectly. The LEM-domain protein to chromatin interaction at the NPC potentially can be severed by ESCRT-III/Vps4, as shown in fission yeast (Pieper et al. 2020). In contrast to Heh1, which is highly abundant in late meiosis II, the expression level of *HEH2* is dramatically downregulated (Chu et al. 1998), suggesting another layer of regulation of LEM protein and chromatin interaction. Disconnecting chromatin from the NPC may trigger the release of the chromosomes and basket nucleoporins into the daughter nuclei, while the NPC core subunits remain constricted to GUNC.

The idea of a dual action of ESCRT-III/Vps4 functioning with either Heh1/Chm7 or Heh2 to ensure gamete rejuvenation via restriction of senescence factors to GUNC and faithful chromosome segregation provides a testable hypothesis. Determining the role of LEM-domain proteins' interaction with the NPC and chromatin, and how ESCRT-III helps mediate the process of NPC remodeling may be the key to understanding GUNC formation and gamete rejuvenation.

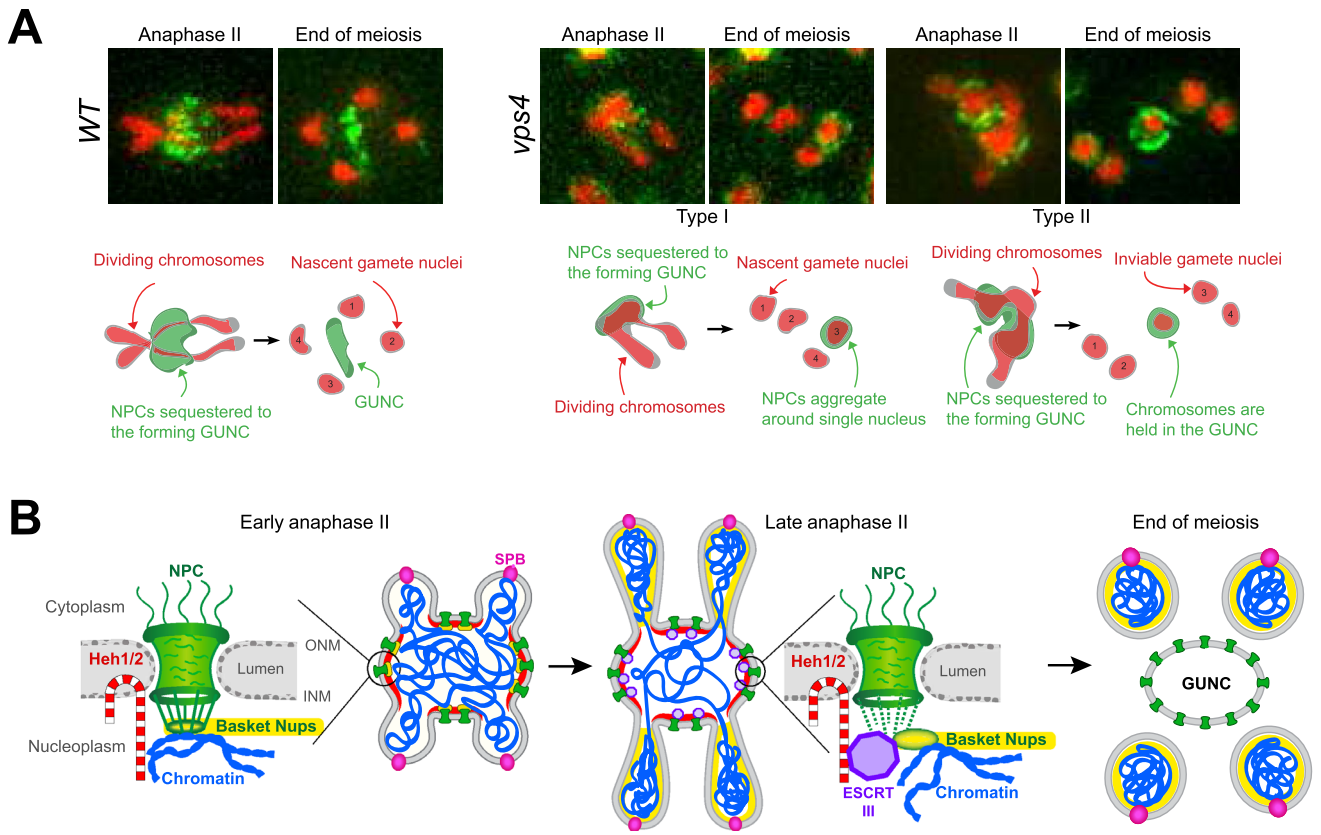

Fig. 1 LEM-domain proteins and ESCRT-III regulate NPC sequestration and chromosome segregation during gametogenesis in budding yeast. **a** Selected images from time-lapse microscopy showing the nucleoporin Pom34-GFP (green) localization in wild-type (*WT*) and *vps4* mutant cells. Hta1-mApple (red) marks the chromosomes. The corresponding diagrams detail chromosome movement and GUNC (gametogenesis uninherited nuclear compartment) formation in *WT* and *vps4* mutants during meiosis II. **b** Hypothetical model for LEM protein and ESCRT-III action at the nuclear envelope during gametogenesis in budding yeast. Recruitment of ESCRT-III/Vps4 (shown in purple) by the LEM-domain proteins (red) to the nuclear envelope promotes the disassociation of basket nucleoporins (yellow) from the core. Nuclear pore complexes (NPCs) are shown in green, chromosomes in blue, and the spindle pole body (SPB) in pink

Acknowledgements We thank Charles Badland and Jen Kennedy for their assistance. The work in authors' lab is supported by the National Institutes of Health GM138838 and the National Science Foundation MCB1951313.

References

Arai K, Sato M, Tanaka K, Yamamoto M (2010) Nuclear compartmentalization is abolished during fission yeast meiosis. Curr Biol 20:1913–1918

Asakawa H, Kojidani T, Mori C, Osakada H, Sato M, Ding DQ, Hiraoka Y, Haraguchi T (2010) Virtual breakdown of the nuclear envelope in fission yeast meiosis. Curr Biol 20:1919–1925

Beck M, Hurt E (2017) The nuclear pore complex: understanding its function through structural insight. Nat Rev Mol Cell Biol 18:73–89

Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, Brown PO, Herskowitz I (1998) The transcriptional program of sporulation in budding yeast. Science 282:699–705

Colombi P, Webster BM, Frohlich F, Lusk CP (2013) The transmission of nuclear pore complexes to daughter cells requires a cytoplasmic pool of Nsp1. J Cell Biol 203:215–232

Dey G, Culley S, Curran S, Schmidt U, Henriques R, Kukulski W, Baum B (2020) Closed mitosis requires local disassembly of the nuclear envelope. Nature 585:119–123

Dultz E, Zanin E, Wurzenberger C, Braun M, Rabut G, Sironi L, Ellenberg J (2008) Systematic kinetic analysis of mitotic dis- and reassembly of the nuclear pore in living cells. J Cell Biol 180:857–865

Fernandez-Alvarez A, Bez C, O'Toole ET, Morphew M, Cooper JP (2016) Mitotic nuclear envelope breakdown and spindle nucleation are controlled by interphase contacts between centromeres and the nuclear envelope. Dev Cell 39:544–559

Fuchs J, Loidl J (2004) Behaviour of nucleolus organizing regions (NORs) and nucleoli during mitotic and meiotic divisions in budding yeast. Chromosome Res 12:427–438

Grund SE, Fischer T, Cabal GG, Antunez O, Perez-Ortin JE, Hurt E (2008) The inner nuclear membrane protein Src1 associates with subtelomeric genes and alters their regulated gene expression. J Cell Biol 182:897–910

Kaeberlein M (2010) Lessons on longevity from budding yeast. Nature 464:513–519

Kim SJ, Fernandez-Martinez J, Nudelman I, Shi Y, Zhang W, Raveh B, Herricks T, Slaughter BD, Hogan JA, Upla P, Chemmama IE, Pellarin R, Echeverria I, Shivaraju M, Chaudhury AS, Wang J, Williams R, Unruh JR, Greenberg CH, Jacobs EY, Yu Z, de la Cruz MJ, Mironska R, Stokes DL, Aitchison JD, Jarrold MF, Gerton JL,

Ludtke SJ, Akey CW, Chait BT, Sali A, Rout MP (2018) Integrative structure and functional anatomy of a nuclear pore complex. Nature 555:475–482

King GA, Unal E (2020) The dynamic nuclear periphery as a facilitator of gamete health and rejuvenation. Curr Genet 8:1–7

King GA, Goodman JS, Schick JG, Chetlapalli K, Jorgens DM, McDonald KL, Unal E (2019) Meiotic cellular rejuvenation is coupled to nuclear remodeling in budding yeast. Elife 8:e47156

Knockenhauer KE, Schwartz TU (2016) The nuclear pore complex as a flexible and dynamic gate. Cell 164:1162–1171

Koch BA, Staley E, Jin H, Yu HG (2020) The ESCRT-III complex is required for nuclear pore complex sequestration and regulates gamete replicative lifespan in budding yeast meiosis. Nucleus 11:219–236

Longo VD, Shadel GS, Kaeberlein M, Kennedy B (2012) Replicative and chronological aging in Saccharomyces cerevisiae. Cell Metab 16:18–31

Makio T, Lapetina DL, Wozniak RW (2013) Inheritance of yeast nuclear pore complexes requires the Nsp1p subcomplex. J Cell Biol 203:187–196

Markossian S, Suresh S, Osmani AH, Osmani SA (2015) Nup2 requires a highly divergent partner, NupA, to fulfill functions at nuclear pore complexes and the mitotic chromatin region. Mol Biol Cell 26:605–621

Mekhail K, Seebacher J, Gygi SP, Moazed D (2008) Role for perinuclear chromosome tethering in maintenance of genome stability. Nature 456:667–670

Meszaros N, Cibulka J, Mendiburo MJ, Romanauska A, Schneider M, Kohler A (2015) Nuclear pore basket proteins are tethered to the nuclear envelope and can regulate membrane curvature. Dev Cell 33:285–298

Neiman AM (2011) Sporulation in the budding yeast *Saccharomyces* cerevisiae. Genetics 189:737–765

Pieper GH, Sprenger S, Teis D, Oliferenko S (2020) ESCRT-III/Vps4 controls heterochromatin-nuclear envelope attachments. Dev Cell 53(27–41):e26

Sinclair D, Mills K, Guarente L (1998) Aging in *Saccharomyces* cerevisiae. Annu Rev Microbiol 52:533–560

Suresh S, Markossian S, Osmani AH, Osmani SA (2017) Mitotic nuclear pore complex segregation involves Nup2 in *Aspergillus nidulans*. J Cell Biol 216:2813–2826

Thaller DJ, Allegretti M, Borah S, Ronchi P, Beck M, Lusk CP (2019) An ESCRT-LEM protein surveillance system is poised to directly monitor the nuclear envelope and nuclear transport system. Elife. 8:e45284

Unal E, Amon A (2011) Gamete formation resets the aging clock in yeast. Cold Spring Harb Symp Quant Biol 76:73–80

Unal E, Kinde B, Amon A (2011) Gametogenesis eliminates age-induced cellular damage and resets life span in yeast. Science 332:1554–1557

Ungricht R, Kutay U (2015) Establishment of NE asymmetry-targeting of membrane proteins to the inner nuclear membrane. Curr Opin Cell Biol 34:135–141

von Appen A, LaJoie D, Johnson IE, Trnka MJ, Pick SM, Burlingame AL, Ullman KS, Frost A (2020) LEM2 phase separation promotes ESCRT-mediated nuclear envelope reformation. Nature 582:115–118

Webster BM, Colombi P, Jager J, Lusk CP (2014) Surveillance of nuclear pore complex assembly by ESCRT-III/Vps4. Cell 159:388–401

Webster BM, Thaller DJ, Jager J, Ochmann SE, Borah S, Lusk CP (2016) Chm7 and Heh1 collaborate to link nuclear pore complex quality control with nuclear envelope sealing. EMBO J 35:2447–2467