can have a large effect on the assays used in these studies. However, aside from distinct purification approaches, there are several other differences that could impact the observed biology. First, although both groups utilized group I autocatalytic introns, the exact sources were disparate, as were the coding exons. There were several additional differences as well, including the cell types used (HeLa by Chen et al. versus A549 by Wesselhoeft et al.), transfection reagents, the amount of circRNA transfected, and experimental format. Lastly, the amount (25 μg versus 350 ng) and route of inoculation (subcutaneous versus visceral fat) could contribute to differential activation of innate pathways *in vivo*. In sum, it is difficult to make definitive conclusions about circRNA immunogenicity from these studies. Perhaps instead it is best to take the data at face value. These studies likely suggest that only certain exogenous circRNAs are immunogenic in a context-, cell-, and time-specific manner. This is supported by recent work suggesting that some circRNAs are bound by the dsRNA sensor PKR in a manner that impacts downstream innate immune responses (Liu et al., 2019). Which circRNAs are immunogenic, why, and how they are detected will be an important focus moving forward, as these species may represent powerful tools for therapeutic interventions. How self-tolerance is achieved against diverse endogenous circRNAs and whether circRNAs contribute to inflammation either from endogenous or pathogenic sources are also important questions to be addressed in the future (Huang et al., 2019).

REFERENCES

Chen, Y.G., Kim, M.V., Chen, X., Batista, P.J., Aoyama, S., Wilusz, J.E., Iwasaki, A., and Chang, H.Y. (2017). Sensing Self and Foreign Circular RNAs by Intron Identity. Mol. Cell *67*, 228–238e5.

Chen, Y.G., Chen, R., Ahmad, S., Verma, R., Kasturi, S., Amaya, L., Broughton, J.P., Kim, J., Cadena, C., Pulendran, B., Hur, S., and Chang, H.Y. (2019). N6-methyladenosine modification controls circular RNA immunity. Mol. Cell *76*, 96–109.

Harrow, J., Frankish, A., Gonzalez, J.M., Tapanari, E., Diekhans, M., Kokocinski, F., Aken, B.L., Barrell, D., Zadissa, A., Searle, S., et al. (2012). GENCODE: the reference human genome annotation for The ENCODE Project. Genome Res. *22*, 1760–1774.

Huang, J.T., Chen, J.N., Gong, L.P., Bi, Y.H., Liang, J., Zhou, L., He, D., and Shao, C.K. (2019). Identification of virus-encoded circular RNA. Virology *529*, 144–151.

Liu, C.X., Li, X., Nan, F., Jiang, S., Gao, X., Guo, S.K., Xue, W., Cui, Y., Dong, K., Ding, H., et al. (2019). Structure and Degradation of Circular RNAs Regulate PKR Activation in Innate Immunity. Cell *177*, 865–880e21.

Mu, X., Ahmad, S., and Hur, S. (2016). Endogenous Retroelements and the Host Innate Immune Sensors. In Advances in Immunology, F.W. Alt, ed. (Academic Press), pp. 47–69.

Nabet, B.Y., Qiu, Y., Shabason, J.E., Wu, T.J., Yoon, T., Kim, B.C., Benci, J.L., DeMichele, A.M., Tchou, J., Marcotrigiano, J., et al. (2017). Exosome RNA Unshielding Couples Stromal Activation to Pattern Recognition Receptor Signaling in Cancer. Cell *170*, 352–366e13.

Roers, A., Hiller, B., and Hornung, V. (2016). Recognition of Endogenous Nucleic Acids by the Innate Immune System. Immunity *44*, 739–754.

Wesselhoeft, R.A., Kowalski, P.S., Parker-Hale, F.C., Huang, Y., Bisaria, N., and Anderson, D.G. (2019). RNA Circularization Diminishes Immunogenicity and Can Extend Translation Duration In Vivo. Mol. Cell *74*, 508–520e4.

Wilusz, J.E. (2018). A 360° view of circular RNAs: From biogenesis to functions. Wiley Interdiscip. Rev. RNA *9*, e1478.

Wu, J., and Chen, Z.J. (2014). Innate immune sensing and signaling of cytosolic nucleic acids. Annu. Rev. Immunol. *32*, 461–488.

Polyamines and Aging: A CLEAR Connection?

Douglas R. Green[1,*]
[1]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA
*Correspondence: douglas.green@stjude.org
https://doi.org/10.1016/j.molcel.2019.09.003

A decline in polyamine levels with age has been implicated in the pathophysiology of aging, and nutritional supplementation of spermidine can reduce age-related pathology and increase lifespan in a number of different organisms. In this issue of *Molecular Cell*, Zhang and colleagues provide a mechanistic link between polyamines, autophagy, and aging.

The wonderful children's book author, Dr. Seuss (Theodor Seuss Geisel), wrote the iconic *On Beyond Zebra* about a boy who explores the strange alphabet that follows the letter Z. So too can we travel beyond the conventional amino acid "alphabet" (On Beyond Tyrosine?) to the lesser-known amino acids that can be found in proteins, amino acids such as selenocysteine and pyrrolysine. Among these is hypusine, an amino acid that appears in only a single protein, eiF5A. Hypusine is generated by a two-step enzymatic process that transfers an *n*-butylamine moiety from spermidine to the ε-amino group of a specific lysine side chain in the eiF5A polypeptide. Without this hypusination, eiF5A does not function.

It has been suggested for decades that levels of polyamines, short molecules composed of two or more amine groups (as the name implies), decline with age and that this contributes to the

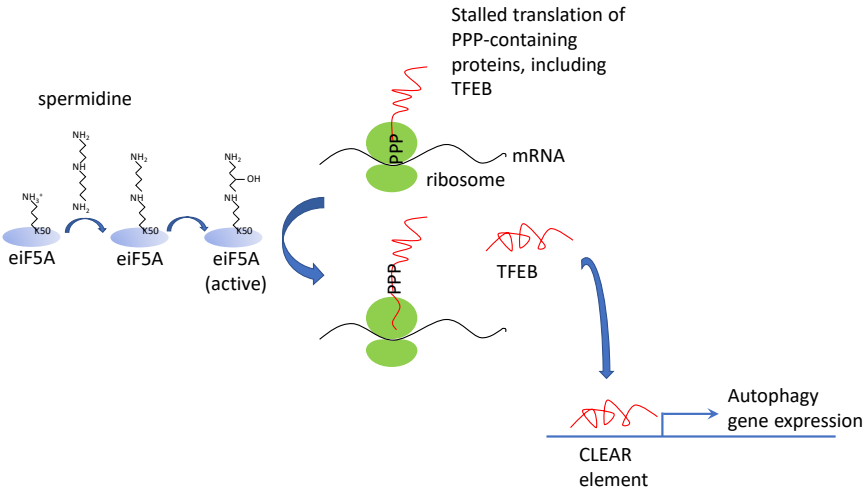

Figure 1. A Model for the Role of Polyamines in Autophagy and Aging

Spermidine, synthesized *de novo* or derived from bacterial commensals or diet, is converted to hypusine on eiF5A (in the human protein, this is at K50), the only protein containing this amino acid. Functional, hypusinated eiF5A is required for the efficient translation of several proteins, including proteins containing poly-proline repeats. These include TFEB, a transcription factor that binds to CLEAR elements in the genome, leading to the production of lysosomal and autophagy proteins. Age-related decline in polyamines is associated with decreased TFEB and autophagy, which is corrected by administration of spermidine. This model may account for the beneficial effects of dietary supplementation of spermidine in aged individuals.

pathophysiology of aging (Pucciarelli et al., 2012). The reason for this decline is obscure; animals derive polyamines from diet and microbial commensals or synthesize them from ornithine via ornithine decarboxylase. There is compelling evidence that nutritional supplementation of polyamines in the form of spermidine (the polyamines are inter-converted among the three forms, putresine, spermidine, and spermine) extend lifespan in yeast, nematodes, and *Drosophila* and prevent some aging effects in murine and human cells (Eisenberg et al., 2009), as well as providing cardioprotection and extended lifespan in humans (Eisenberg et al., 2016; Kiechl et al., 2018). These "anti-aging" effects have been ascribed to the induction of autophagy by spermidine, as genetic disruption of autophagy in yeast, worms, and flies counteracts these beneficial effects (Eisenberg et al., 2009). Now, Zhang et al. provide a mechanism that links the role of polyamines in the process of eiF5A hypusination to autophagy and the function of B lymphocytes from aged individuals (Zhang et al., 2019).

Miscalled as a protein translation initiation factor, eiF5A is actually an elongation factor required for optimal translation of proteins that carry poly-proline repeats (three or more sequential prolines) (Gutierrez et al., 2013). Zhang et al. found that among the proteins requiring eiF5A for translation are the transcription factors TFEB and its relative, TFE3. TFEB (and TFE3) drive the transcription of the coordinated lysosome expression and regulation (CLEAR) gene set to engage lysosomal biogenesis. Included in the CLEAR gene set induced by TFEB are many of the genes encoding proteins required for autophagy (Settembre et al., 2011). Zhang et al. found that defective B cells from aged ($\geq$68 years) humans displayed reduced levels of hypusinated eiF5A, TFEB, and autophagic flux, all of which were restored to levels seen in "young" B cells by providing spermidine to the cells. Further, the ability of the B cells to produce antibodies upon activation was also restored. Inhibition of hypusination with a spermidine analog (GC7) prevented these beneficial effects of polyamine supplementation *in vitro*.

Together, these results create a compelling scenario (Figure 1): as we age, declining levels of polyamines result in a loss of functional, hypusinated eiF5A, as a consequence of which TFEB is not efficiently translated and autophagy genes are therefore not efficiently transcribed. Loss of autophagy compromises optimal quality control mechanisms in cells. Restoring polyamine levels reverses this sequence, and cell function (in this case, B cell function) returns.

Does a polyamine-eiF5A-TFEB-autophagy axis explain aging and the putative beneficial effects of spermidine? Although early studies in rats showed reduced polyamine levels with aging, studies in humans were equivocal, although more recent studies suggest that such decline does, indeed, occur (Pucciarelli et al., 2012). Further, many functions have been ascribed to polyamines, including the regulation of ion channels, DNA and RNA stability, inhibition of inflammation, regulation of DNA methylases, and protein acetylation. Indeed, several studies on the beneficial effects of spermidine have attributed the effects to changes in DNA methylation and histone acetylation. Finally, eiF5A hypusination has also been convincingly shown to regulate the translation of nuclear-encoded mitochondrial proteins that contain mitochondrial localizing sequences (Puleston et al., 2019), and how this would impact the possible roles of mitochondrial defects in aging is certainly worth considering.

As mice age, their ability to generate B cells from precursors declines, and ablation of autophagy in the B cell lineage causes a similar decline in young animals (Ma et al., 2019). Nevertheless, numbers of mature B cells in aged individuals (mouse and human) and in young B cell-specific, autophagy-deficient mice are observed at normal levels, although both show defects in the long-term maintenance of memory B cells. That said, autophagy is dispensable for B cell activation and antibody production (Miller et al., 2008). On the other hand, TFEB was originally identified as a transcription factor binding to the enhancer of the IgM heavy chain. It remains possible that the effects observed by Zhang et al. regarding activation of aged B cells might therefore relate to TFEB but not necessarily to autophagy.

Nevertheless, an interesting opportunity arises from these studies. Autophagy in mature B cells appears to be required for autoimmunity in murine models of lupus (Arnold et al., 2016; Weindel et al., 2015). The studies of Zhang et al. point to the importance of eiF5A hypusination in TFEB translation and autophagy in B cells. Might the hypusine pathway be a target for modulation of autophagy and

function of autoimmune B cells? Clinical trials to explore hypusine inhibition in cancer treatment have been proposed, and pre-clinical experiments suggest that such inhibition may be feasible *in vivo*. While we may consider spermidine supplementation to combat aging (e.g., some aged cheese, rich in spermidine, to go with our red wine), there may also be opportunities to exploit these new findings in inhibiting this pathway toward clinical benefit in some settings.

ACKNOWLEDGMENTS

D.R.G. is supported by grants from the National Institutes of Health and ALSAC.

REFERENCES

Arnold, J., Murera, D., Arbogast, F., Fauny, J.D., Muller, S., and Gros, F. (2016). Autophagy is dispensable for B-cell development but essential for humoral autoimmune responses. Cell Death Differ. *23*, 853–864.

Eisenberg, T., Knauer, H., Schauer, A., Büttner, S., Ruckenstuhl, C., Carmona-Gutierrez, D., Ring, J., Schroeder, S., Magnes, C., Antonacci, L., et al. (2009). Induction of autophagy by spermidine promotes longevity. Nat. Cell Biol. *11*, 1305–1314.

Eisenberg, T., Abdellatif, M., Schroeder, S., Primessnig, U., Stekovic, S., Pendl, T., Harger, A., Schipke, J., Zimmermann, A., Schmidt, A., et al. (2016). Cardioprotection and lifespan extension by the natural polyamine spermidine. Nat. Med. *22*, 1428–1438.

Gutierrez, E., Shin, B.S., Woolstenhulme, C.J., Kim, J.R., Saini, P., Buskirk, A.R., and Dever, T.E. (2013). eIF5A promotes translation of polyproline motifs. Mol. Cell *51*, 35–45.

Kiechl, S., Pechlaner, R., Willeit, P., Notdurfter, M., Paulweber, B., Willeit, K., Werner, P., Ruckenstuhl, C., Iglseder, B., Weger, S., et al. (2018). Higher spermidine intake is linked to lower mortality: a prospective population-based study. Am. J. Clin. Nutr. *108*, 371–380.

Ma, S., Wang, C., Mao, X., and Hao, Y. (2019). B Cell Dysfunction Associated With Aging and Autoimmune Diseases. Front. Immunol. *10*, 318.

Miller, B.C., Zhao, Z., Stephenson, L.M., Cadwell, K., Pua, H.H., Lee, H.K., Mizushima, N.N., Iwasaki, A., He, Y.W., Swat, W., and Virgin, H.W., 4th (2008). The autophagy gene ATG5 plays an essential role in B lymphocyte development. Autophagy *4*, 309–314.

Pucciarelli, S., Moreschini, B., Micozzi, D., De Fronzo, G.S., Carpi, F.M., Polzonetti, V., Vincenzetti, S., Mignini, F., and Napolioni, V. (2012). Spermidine and spermine are enriched in whole blood of nona/centenarians. Rejuvenation Res. *15*, 590–595.

Puleston, D.J., Buck, M.D., Klein Geltink, R.I., Kyle, R.L., Caputa, G., O'Sullivan, D., Cameron, A.M., Castoldi, A., Musa, Y., Kabat, A.M., et al. (2019). Polyamines and eIF5A Hypusination Modulate Mitochondrial Respiration and Macrophage Activation. Cell Metab. *30*, 352–363.e8.

Settembre, C., Di Malta, C., Polito, V.A., Garcia Arencibia, M., Vetrini, F., Erdin, S., Erdin, S.U., Huynh, T., Medina, D., Colella, P., et al. (2011). TFEB links autophagy to lysosomal biogenesis. Science *332*, 1429–1433.

Weindel, C.G., Richey, L.J., Bolland, S., Mehta, A.J., Kearney, J.F., and Huber, B.T. (2015). B cell autophagy mediates TLR7-dependent autoimmunity and inflammation. Autophagy *11*, 1010–1024.

Zhang, H., Alsaleh, G., Feltham, J., Sun, Y., Napolitano, G., Riffelmacher, T., Charles, P., Frau, L., Hublitz, P., Yu, Z., et al. (2019). Polyamines Control eIF5A Hypusination, TFEB Translation, and Autophagy to Reverse B Cell Senescence. Mol. Cell *76*, 110–125.