COMMENTARY

Faulty ribosome biogenesis underlies the ribosomopathy alopecia, neurological defects, endocrinopathy (ANE) syndrome

Jennifer L. Gerton[a,b,1]

Most cells spend the vast majority of their energy making ribosomes, the machines that translate messenger RNA into protein. The ribosome is a tremendously elaborate machine comprising over 80 individual ribosomal proteins and four ribosomal RNAs. The biogenesis of these machines requires an additional 200 different factors. This amazing assembly feat is accomplished 30 times per second in a growing yeast cell (1) and 125 times per second in a growing human tissue culture cell (2). Given the complexity, frequency, and significance of the task, it is no wonder that human syndromes exist in which ribosome biogenesis is disrupted by a genetic mutation, such as the one described in PNAS (3).

A Brief Introduction to Ribosome Biogenesis and Ribosomopathies

Ribosome biogenesis is a spatially organized process in cells. The genes encoding three of the ribosomal RNAs (18S, 5.8S, and 28S) are located in a specialized part of the nucleus known as the nucleolus. The nucleolus is where these RNAs are transcribed as a single transcript, which is then further processed and modified by a host of nucleolar factors that include exonucleases and endonucleases. Due to the demand for ribosomes, genes encoding the ribosomal RNAs are present in many copies that are simultaneously transcribed. Transcription of the ribosomal RNAs takes place via the specialized RNA polymerase I and RNA polymerase III, whereas genes encoding the ribosomal proteins are transcribed by RNA polymerase II, making ribosome biogenesis a process that requires the action of all three major nuclear RNA polymerases. Ribosomal proteins are translated in the cytoplasm and then imported into the nucleus to form ribosome subassemblies that develop and mature in the nucleolus, initially forming in a single large assembly and then splitting into the 40S small subunit assembly and the 60S large subunit assembly. These subassemblies are exported to the cytoplasm, where they fully mature into complete functional ribosomes, active for translation.

Ribosomopathy is the term used to describe human syndromes in which ribosome biogenesis is disrupted. Ribosomopathies come in several varieties. While each ribosomopathy on its own is rare, the large and growing collection of syndromes associated with aberrant ribosome biogenesis demands that the molecular mechanisms of ribosome assembly be understood. Spontaneously occurring mutations have been identified in the human genome that affect various steps in the biogenesis process, resulting in disease. One class of mutations affects transcription of the ribosomal RNA components of the ribosomes, a second class impacts processing and modification of the ribosomal RNAs, a third class impacts ribosome assembly factors, and a fourth class occurs in the ribosomal protein genes themselves. Farley-Barnes et al. (4) provide an excellent recent broad review. One of the most fascinating aspects of this group of human diseases is how mutations impacting a general housekeeping process give rise to specific disease phenotypes. In PNAS, Bryant et al. (3) describe and characterize mutations in *RBM28* that impact ribosomal RNA processing and cause a rare ribosomopathy known as alopecia, neurologic defects, and endocrinopathy (ANE) syndrome.

The ANE Syndrome Ribosomopathy

ANE syndrome is a rare inherited ribosomopathy disorder caused by mutations in *RBM28* (Fig. 1). In an initial report of ANE syndrome, five affected male siblings were identified, and all had identical homozygous loss of function mutations in *RBM28*, which result in Leucine 351 being changed to Proline (5). These patients lacked puberty at 20 y to 39 y of age, and displayed hypogonadism, short stature, and alopecia. In the PNAS study (3), a new case of ANE syndrome was

[a]Stowers Institute for Medical Research, Kansas City, MO 64110; and [b]Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS 66160

Author contributions: J.L.G. wrote the paper.

The author declares no competing interest.

See companion article, "Biallelic splicing variants in the nucleolar 60S assembly factor RBM28 cause the ribosomopathy ANE syndrome," 10.1073/pnas.2017777118.

[1]Email: jeg@stowers.org.

Published May 25, 2021.

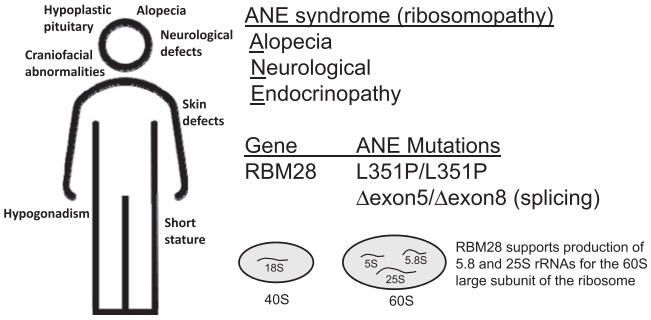

Fig. 1. ANE syndrome is a ribosomopathy characterized by alopecia and neurological and endocrine defects. The syndrome is caused by biallelic mutations in RBM28 that create greater than 50% loss of function. RBM28 encodes a nucleolar protein that supports the processing of ribosomal RNAs for inclusion in the 60S large ribosomal subunit.

discovered in a female pediatric patient, who shared many clinical characteristics with the first patient cohort. However, the new case is caused by biallelic mutations predicted to affect the splicing of *RBM28*. One copy of *RBM28* contains a mutation that is predicted to affect the canonical 5′ splice site of exon 5, and the second copy of the *RBM28* gene contains a mutation predicted to affect the 5′ splice site of exon 8. This patient presented with alopecia, craniofacial malformations, hypoplastic pituitary, and hair and skin abnormalities. The young age of the patient precludes any information about delayed or absent puberty. Because the number of individuals reported with ANE syndrome is so low, it remains to be seen which features will be typical, which may be more variable, and whether genotype–phenotype correlations will exist. Furthermore, there may be additional gene mutations discovered to cause ANE syndrome, either in *RBM28* or in genes with related function.

Mutations that disrupt ribosome biogenesis often affect tissues that rely on cell division for their function. Many ribosomopathies have an anemia component, as blood cell production from the bone marrow relies heavily on cell division. Many ribosomopathies also have a neurological component. While neurons themselves are not highly proliferative, the neural crest cells, progenitors of many cell types, including melanocytes, craniofacial cartilage and bone, smooth muscle, peripheral and enteric neurons, and glia, are highly proliferative during embryogenesis, and these cells may be particularly sensitive to defects in ribosome biogenesis. Consistent with this proposal, ANE syndrome is associated with neurological defects. ANE syndrome is somewhat unique among ribosomopathies in its association with hair loss (alopecia). Following the first reports of ANE syndrome, organoid culture was used to demonstrate that hair follicle growth depends on *RBM28* (6). Finally, endocrine dysfunction is associated with ANE syndrome in the previous report; specifically, the lack of secretion of several hormones suggested combined anterior pituitary hormone deficiency (7). In the current report (3), the individual has yet to display endocrine dysfunction. It is not clear whether this phenotype may manifest later in the individual's life, whether this a variable phenotype in ANE syndrome, or whether endocrine dysfunction may covary with the specific causative mutations.

Ribosome Biogenesis Functions of RBM28

Much of what is known about the molecular function of *RBM28* is derived from work on the budding yeast ortholog *NOP4*. In fact, the evolutionary conservation of ribosome biogenesis in eukaryotes has made research in budding yeast a rich resource for understanding the molecular mechanisms of ribosome assembly and has often served as a starting point for illuminating the molecular processes disrupted in ribosomopathies. Nop4 is so named because it is a nucleolar protein, critical for the processing and maturation of the initial single large transcript that encodes 18S, 5.8S, and 25S ribosomal RNAs.

NOP4 is an essential gene in budding yeast, and the Nop4 protein has many interacting partners in the large subunit processome, including helicases and GTPase, acting as a hub to coordinate multiple aspects of ribosome biogenesis (8, 9). RBM28 and Nop4 both contain four RNA recognition motifs (RRMs) and are 26% identical (10). The specific processing defect without *NOP4* is stalled production of the 25S and 5.8S rRNAs from the precursor ribosomal RNA (rRNA) (9). The rRNA processing defects are similarly observed in human cells depleted for RBM28 (11). This rRNA processing defect will slow biogenesis of the 60S subassembly and ultimately has the effect of reducing ribosome number, as has been shown in ANE patient cells (5). Fewer ribosomes will hinder translation, cell growth, and proliferation. RBM28 is part of the spliceosomal small nuclear ribonucleoprotein complex that associates with small nuclear RNAs (12). The first ANE mutation identified, Leu-351 Pro, when modeled in budding yeast Nop4, disrupts its hub function by abrogating protein–protein interactions (13).

Given the limitations associated with patient samples, the biallelic mutations identified in Bryant et al. (3) were modeled in laboratory experiments for their effects on splicing of *RBM28* and impact on production of rRNA cleavage products. The mutations predicted to affect splicing were modeled in a minigene assay. The mutation in the 5′ splice site of exon 5 created a spliced product without exon 5, resulting in an in-frame loss of 31 amino acids that included partial loss of the second RRM domain. The mutation in the 5′ splice site of exon 8 resulted in a spliced product without exon 8, and premature termination codons. The resulting protein detected was majorly truncated. Coupled with lethality and poor rRNA processing in yeast bearing this allele, Bryant et al. suggest this mutation acts as a null allele. The authors find that the exon 5 mutant gene makes nearly full-length protein and reduces overall growth and rRNA processing, similar to the L351P mutation. The authors conclude that the exon 5 allele enables survival but precludes normal development. Furthermore, because the exon 8 allele was inherited from an unaffected parent, ANE syndrome is not a haploinsufficiency and only occurs when RBM28 function is less than 50% of normal levels, unlike some other ribosomopathies that occur as dominant haploinsufficiencies (e.g., 5q– syndrome).

Future Directions

Human genome sequencing projects are expanding at an enormous rate as the cost of sequencing continues to decline and sequencing and related computational technologies continue to improve. Such projects will almost certainly reveal the genetic origins of many rare diseases, including new ribosomopathies, given the number of factors involved in ribosome biogenesis. In the new case of ANE syndrome described, whole exome sequencing was key to identifying mutations predicted to impact splicing. In the future, whole genome sequencing may reveal an even broader range of mutations that impact gene function, such as promoter and enhancer mutations. However, limitations in patient material will continue to make model organism research indispensable for understanding the consequences of the mutations at the molecular level. In fact, as more candidate rare disease

mutations of potential significance are discovered, efforts to systematically model rare disease pathogenesis will need to be expanded and improved, and working at this interface will be an exciting endeavor. Wangler et al. (14) have argued that "merging human genetics with model organism research guides experimental studies to solve these medical mysteries, gain new insights into disease pathogenesis, and uncover new therapeutic strategies." In the case of ANE syndrome in particular, budding yeast has been incredibly useful to understand the consequences of loss of function of the yeast ortholog of *RBM28*, *NOP4*. In the future, animal models, utilizing mouse and zebrafish, for example, will help to further elucidate why specific tissues and cell types are impacted. Ribosome biogenesis is critical for normal development, but defective ribosome biogenesis, nucleolar dysmorphology, and altered translation also accompany cancer and aging. Therefore, it is imperative to continue investigating fundamental molecular mechanisms of ribosome biogenesis in order to ultimately harness this information to improve human health.

1 J. R. Warner, The economics of ribosome biosynthesis in yeast. *Trends Biochem. Sci.* **24**, 437–440 (1999).
2 J. D. Lewis, D. Tollervey, Like attracts like: Getting RNA processing together in the nucleus. *Science* **288**, 1385–1389 (2000).
3 C. J. Bryant *et al.*, Biallelic splicing variants in the nucleolar 60S assembly factor RBM28 cause the ribosomopathy ANE syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2017777118 (2021).
4 K. I. Farley-Barnes, L. M. Ogawa, S. J. Baserga, Ribosomopathies: Old concepts, new controversies. *Trends Genet.* **35**, 754–767 (2019).
5 J. Nousbeck *et al.*, Alopecia, neurological defects, and endocrinopathy syndrome caused by decreased expression of RBM28, a nucleolar protein associated with ribosome biogenesis. *Am. J. Hum. Genet.* **82**, 1114–1121 (2008).
6 E. Warshauer *et al.*, RBM28, a protein deficient in ANE syndrome, regulates hair follicle growth via miR-203 and p63. *Exp. Dermatol.* **24**, 618–622 (2015).
7 R. Spiegel, S. A. Shalev, A. Adawi, E. Sprecher, Y. Tenenbaum-Rakover, ANE syndrome caused by mutated RBM28 gene: A novel etiology of combined pituitary hormone deficiency. *Eur. J. Endocrinol.* **162**, 1021–1025 (2010).
8 K. L. McCann, J. M. Charette, N. G. Vincent, S. J. Baserga, A protein interaction map of the LSU processome. *Genes Dev.* **29**, 862–875 (2015).
9 C. Sun, J. L. Woolford Jr, The yeast NOP4 gene product is an essential nucleolar protein required for pre-rRNA processing and accumulation of 60S ribosomal subunits. *EMBO J.* **13**, 3127–3135 (1994).
10 C. Sun, J. L. Woolford Jr, The yeast nucleolar protein Nop4p contains four RNA recognition motifs necessary for ribosome biogenesis. *J. Biol. Chem.* **272**, 25345–25352 (1997).
11 K. E. Sloan *et al.*, Both endonucleolytic and exonucleolytic cleavage mediate ITS1 removal during human ribosomal RNA processing. *J. Cell Biol.* **200**, 577–588 (2013).
12 A. Damianov, M. Kann, W. S. Lane, A. Bindereif, Human RBM28 protein is a specific nucleolar component of the spliceosomal snRNPs. *Biol. Chem.* **387**, 1455–1460 (2006).
13 K. L. McCann, T. Teramoto, J. Zhang, T. M. Tanaka Hall, S. J. Baserga, The molecular basis for ANE syndrome revealed by the large ribosomal subunit processome interactome. *eLife* **5**, e16381 (2016).
14 M. F. Wangler *et al.*; Members of the Undiagnosed Diseases Network (UDN), Model organisms facilitate rare disease diagnosis and therapeutic research. *Genetics* **207**, 9–27 (2017).

https://doi.org/10.1073/pnas.2107030118