immune synapse formation.[2] Recent studies have demonstrated that self-tolerance including maintenance of T-regulatory cells and B-cell tolerance checkpoints also depend on regulators of actin polymerization,[3] demonstrating the complex role that the cytoskeleton plays in cell activation and survival.

In this issue, Brigida et al describe T-cell defects in 6 patients with autosomal recessive deficiency of *ARPC1B*, a member of the ARP2/3 complex, which is an essential mediator of actin polymerization.[1] Defects in ARPC1B have been recently been associated with combined immunodeficiency and platelet abnormalities in several children.[4-6] Clinical findings were very similar to other known defects of actin organization, including infections, atopy, and autoimmunity. Brigida et al expanded on previous reports by demonstrating the degree of T-cell dysfunction in these patients, including abnormalities in proliferation, activation, and immune synapse formation. Transduction of affected T cells with wild-type *ARPC1B* corrected the T-cell proliferative defects in vitro, suggesting that condition would be amenable to hematopoietic stem cell transplantation (HSCT). Similarly, 2 of the patients had minor populations of memory $CD8^+$ T cells that expressed ARPC1B resulting from reversion mutations. V-β expression demonstrated oligoclonality in these revertant cell populations, suggesting that these populations rose from antigen-exposed T cells or from homeostatic proliferation.

ARPC1P joins a growing group of genes of actin regulatory that are proven causes of primary immunodeficiency disorders.[2] These pathways share many commonalities, including susceptibility to a wide range of infections, atopy, and immunodysregulation. Lymphocyte abnormalities are a consistent feature, whereas platelet and neutrophil abnormalities are variably present in these disorders. Presence of revertant cell populations has also been described in patients with Wiskott-Aldrich syndrome and DOCK8 deficiency,[7,8] reflecting selective pressure and the survival advantage gained by revertant lymphocytes. Identification of DOCK8 deficiency via testing of T-cell receptor excision circles in whole blood has been reported,[9] and with the described decreased in naïve T cells in these patients, it is possible that *ARPC1B*-deficient patients may similarly be detected by this method in newborn screening for severe combined immunodeficiency. Given the severity of these disorders, HSCT from a well-matched donor is considered the standard of care. Two of the patients described in this study underwent successful HSCT. Gene therapy for Wiskott-Aldrich syndrome has also been successful in previous trials,[10] and because *ARPC1B* expression is limited to hematopoietic cells, this disease should similarly be amenable to a gene therapy approach.

As next-generation sequencing is applied to more patients with undefined forms of primary immunodeficiency, it is likely that *ARPC1B* will not be the last cytoskeletal disorder to be associated with human disease. Further study of the clinical spectrum and mechanistic biology of *ARPC1B* deficiency may identify new therapeutic targets and methods for early diagnosis and definitive treatment.

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Comment on Bretones et al, page 2375

Not so lost in translation: *RPS15* mutations in CLL

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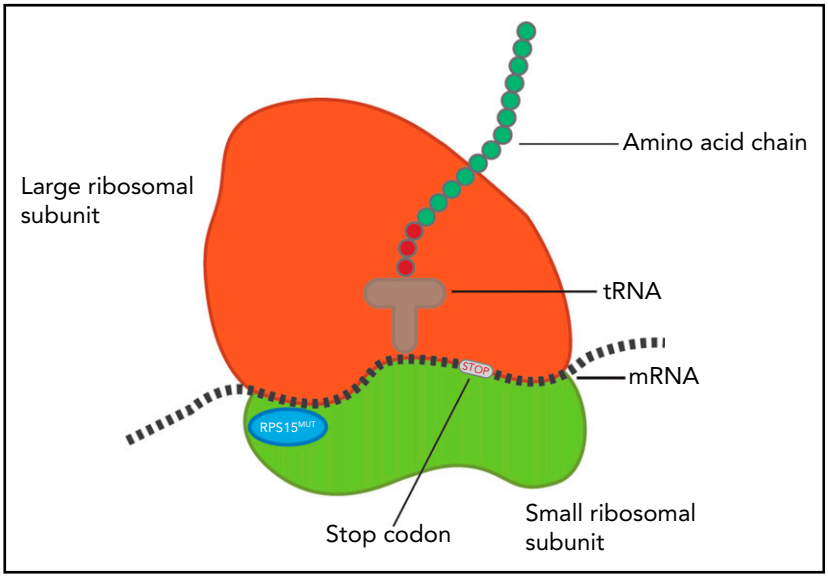
In this issue of *Blood*, Bretones et al expand knowledge of the functional consequences of recurrent mutations in *RPS15*, a gene that encodes a ribosomal protein of the 40S subunit and is enriched in patients with clinically aggressive chronic lymphocytic leukemia (CLL).[1] By transfecting *RPS15* mutants and applying different technologies to assess ribosome activity and efficiency in combination with high-throughput proteome profiling, they were able to demonstrate reduced half-life of RPS15, impaired translational fidelity, and changes in the expressed proteome in mutant vs wild-type RPS15.

The mutational landscape of CLL is heterogeneous spanning from recurrent mutations in classic tumor suppressor genes (eg, *TP53*) to mutations in genes involved in the splicing machinery (eg, *SF3B1*).[2] Recently, *RPS15* mutations were



Mutations in RPS15 may lead to altered ribosome fidelity, including a decreased ability to detect stop-codons during mRNA translation, as shown by Bretones et al. tRNA, transfer RNA.

discovered at low frequencies in newly diagnosed and untreated cohorts,[3] but they affected up to 20% of CLL patients who relapsed after chemoimmunotherapy (ie, fludarabine, cyclophosphamide, and rituximab [FCR]).[4,5] In addition, it was noted that approximately one-third of *RPS15*-mutated patients also carried a *TP53* aberration (ie, 17p deletion and/or *TP53* mutations).[5,6]

The translation of RNA into proteins is a complex and fundamental cellular process, and the recent discoveries of somatic *RPS15* mutations in CLL as well as mutations in *RPL10*, *RPL5*, and *RPL11* in T-cell acute lymphoblastic leukemia[7] have sparked interest in how these mutations may tie in to oncogenesis and disease progression in hematologic malignancies. Several ribosomal proteins, including RPS15, have a suggested alternative function besides protein translation in interaction with the MDM2-p53 axis in conditions with nucleolar stress, subsequently leading to cell cycle arrest.[8] Although preliminary functional analysis of mutant *RPS15* pointed to increased ubiquitin-mediated p53 degradation, this could not fully explain abrogation of this function as the specific oncogenic mechanism.[5] *RPS15* mutations in CLL predominantly represent heterozygous missense single nucleotide variants occurring almost exclusively in the evolutionary conserved C-terminal region between amino acids 129 and 145, indicating strong functional constraints for mutations occurring in the decoding center of the ribosome. This mutational pattern indicates an oncogenic rather than a tumor suppressor function of the mutant protein; however, the exact effects of *RPS15* mutations on ribosome function have not previously been described.

Bretones et al investigated the cellular impact of *RPS15* mutations. They started by investigating the half-life of mutant RPS15 compared with wild-type protein by stable expression of transcripts with 8 different *RPS15* mutations, and they detected a decreased half-life of mutant RPS15, most likely by increased ubiquitination. An important question concerning the effect of *RPS15* mutations is whether the mutant protein is incorporated in the ribosome or not. The authors show that mutant RPS15 is indeed incorporated in the ribosome and could therefore have an effect on the translational properties of *RPS15*-mutated cells. Next, they assessed the effects of mutant *RPS15* on ribosome function and detected increased cap-independent translation for 5 of the mutants, a decreased translation fidelity for 2 of the mutants, and a significantly decreased ability in the detection of stop-codons for 5 mutants (see figure). Taken together, these features strongly indicate that *RPS15* mutations lead to altered ribosomal function regarding both protein synthesis and translational fidelity.

Finally, the authors applied a high-throughput proteome analysis to delineate global protein alterations for 2 of the most frequently occurring *RPS15* mutations ($RPS15^{P131S}$ and $RPS15^{S138F}$) compared with wild-type RPS15 in a nonhematologic cell line (HEK293T). The proteome profiles of replicates with the same mutations clustered together, and detailed investigation of the altered protein classes showed that the $RPS15^{P131S}$ mutant led to increased amount of proteins associated with replication and DNA elongation. In turn, $RPS15^{S138F}$ led to enrichment of proteins associated with messenger RNA (mRNA) and peptide processing. CLL cell lines often prove challenging in functional studies, and a replicate study in the CLL cell line MEC-1 did not reveal mutant-specific proteome profiles. However, comparison of all mutants to the wild-type profile revealed patterns similar to those in the HEK293T experiments. These profiles also involve downregulation of pathways that could lead to metabolic reprograming toward aerobic glycolysis, which is in line with the previously described Warburg effect.

All in all, this study demonstrates that *RPS15* mutations, mainly occurring in the decoding center of the ribosome, lead to reduced RPS15 levels and an altered ribosomal efficiency that in turn causes global changes of the proteome. On the basis of data from different ribosomopathies, the authors speculate that ribosomal mutations may result in a switch from a hypo- to a hyperproliferative phenotype. Initially, ribosomal stress may select mutations that bypass the ribosome quality control, hence increasing the amount of defective ribosomes. Conversely, mutated ribosomes may cause altered protein expression patterns, which may contribute to transformation and leukemia development. Although data presented here are mainly based on experiments performed in cell lines, it will be important to further investigate whether *RPS15* mutations cause similar differences in ribosome efficiency and protein synthesis in primary CLL cells as well, particularly exploring effects in vivo (ie, in the context of the tumor microenvironment). To this end, detailed analysis of key pathways and processes affected by impaired mRNA translation and their downstream effects will be necessary to pinpoint the causative effect of ribosomal mutations on CLL pathobiology. As shown in the article by Bretones et al and by others,[3-5] *RPS15* mutations are particularly enriched in patients with aggressive disease who

have a higher propensity of disease relapse after treatment with chemo-immunotherapy. Notably, the authors observed an increased resistance to fludarabine, and to a lesser extent B-cell receptor signaling inhibitors, at least for cells carrying selected *RPS15* mutations. Hence, it will be important to study additional potential treatment targets or combinations of therapy for this poor-prognosis group of CLL patients.

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Comment on Mottok et al, page 2401

PMBCL: a molecular diagnosis?

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In this issue of *Blood*, Mottok et al[1] demonstrate the utility of a molecular assay that assesses the expression of 58 genes to distinguish primary mediastinal B-cell lymphoma (PMBCL) from diffuse large B-cell lymphoma (DLBCL) by using routinely available formalin-fixed paraffin-embedded tissue (FFPET) biopsies. The results could improve diagnostic accuracy for patients with PMBCL and may have important implications for clinical trial selection and interpretation of clinical outcomes for patients with this rare form of lymphoma.

PMBCL is a unique clinicopathologic subtype of aggressive B-cell lymphoma, the diagnosis of which is dependent on correlating clinical features and pathologic and immunophenotypic features.[2] Despite improvements in the diagnostic criteria that have been developed for many subtypes of non-Hodgkin lymphoma, distinguishing PMBCL from DLBCL and gray zone lymphoma involving the mediastinum is still a challenge when using currently available diagnostic methods.

By leveraging the wealth of gene expression profiling (GEP) data obtained from PMBCL samples,[3,4] the authors sought to establish a molecular assay that would be broadly applicable for routine clinical diagnostic testing. Important criteria were the ability to use nucleic acids obtained from routinely fixed FFPETs and to use a widely available diagnostic platform. The authors addressed this challenge by developing a quantitative gene expression assay that analyzed 58 genes using the Nanostring platform labeled Lymph3Cx.

The Mottok article describes a rigorous study that used a training set composed of 68 cases of PMBCL and DLBCL and an independent validation set composed of 158 cases. The authors used 30 genes to distinguish between PMBCL and DLBCL, 24 of which were overexpressed in PMBCL and 6 of which were overexpressed in DLBCL and represented high discriminative power. The assay also included genes that are represented in Lymph2Cx, which allowed for cell-of-origin (COO) determination in DLBCL not otherwise specified (NOS). Thus, the assay could use molecular signatures to distinguish PMBCL and also provide COO assignments to DLBCL NOS (see figure). The reproducibility of the assay performance in 2 independent laboratories was high, which supports the likelihood of this assay becoming clinically relevant.

Patients with PMBCL demonstrate selective responses to novel therapeutic approaches such as dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (EPOCH-R)[5] or anti-programmed cell death protein 1 antibody[6] vs those therapies for DLBCL. Thus, the ability to more accurately distinguish PMBCL from DLBCL has important implications for patient management.

Potential obstacles in the rapid and large-scale adoption of this assay in routine clinical settings are the availability of the technical platform and the amount of tissue required (core needle biopsies vs excisional lymph node biopsies) for the assay to perform at the levels reported in the Mottok article. Most importantly, the ability to translate the results of the Mottok study is limited by the fact that the assay was developed by using a specific technical platform, and thus the applicability to other GEP methods such as reverse transcriptase multiplex ligation-dependent probe amplification[7] is unknown.

One major diagnostic challenge that is not addressed is the distinction between PMBCL and classical Hodgkin lymphoma (CHL). Given the biologic overlap between these 2 entities, GEP assays such as those described in the Mottok article may provide a more robust and accurate molecular assay to distinguish PMBCL from CHL and provide quantitative gene expression-based cutoff values that could