Defective Ribosome Assembly in Shwachman-Diamond Syndrome

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Altered ribosome formation or function characterizes a number of inherited bone marrow failure disorders, including Shwachman-Diamond syndrome (SDS). SDS is a rare autosomal recessive disorder caused by mutations in both copies of the Shwachman-Bodian-Diamond syndrome (*SBDS*) gene. Children with SDS have pancreatic dysfunction, leading to malabsorption of nutrients and abnormal growth. Defects in the bone marrow lead to impaired hematopoiesis that can develop into myeloid leukemia or aplastic anemia in 30% of SDS patients. SBDS protein levels are reduced, but not absent, in both hematopoietic and bone marrow stromal cells with *SBDS*gene mutations in SDS patients. To understand how the altered SBDS proteins affect hematopoiesis, Dr. Akiko Shimamura's laboratory in the Clinical Research Division directly assessed the role of SBDS in ribosome formation in patient cells.

Ribosomes are the molecular machines that translate messenger RNA into proteins. Each mature ribosome is comprised of a large and a small subunit, which together require the assembly of 4 ribosomal RNAs, 80 core ribosomal proteins, more than 150 associated proteins, and 70 small nucleolar RNAs. Previous studies demonstrated that the SBDS protein associates with the larger ribosomal subunit, and in yeast and animal models, *SBDS* orthologs affect ribosome formation. In the current study, co-first authors Scott Coats and Dr. Nicholas Burwick utilized the ability of different size protein complexes to sediment in a sucrose gradient to look at the relative quantity of the individual ribosomal subunits, having sizes of 40S and 60S, and the mature assembled ribosome, having a size of 80S (see figure). The authors then modified an assay to look at the formation of the mature ribosome *in vitro* by lysing cells at low salt concentrations to dissociate the ribosome subunits, and then adding back magnesium chloride to physiologic levels. The authors found a significant decrease in both the 60S subunit levels and the formation of the mature 80S ribosome in five SDS patient samples with different *SBDS*mutations compared to healthy controls, using cell extracts from both bone marrow stromal cells and lymphoblasts.

The authors then used this ribosomal subunit association assay to demonstrate that a decrease in SBDS protein levels and *SBDS* mutations led to the defect in mature ribosome assembly. Knockdown of SBDS protein by RNA interference in healthy stromal cells resulted in a decrease in 80S ribosome formation, while expressing wild-type SBDS rescued the defect of ribosome assembly

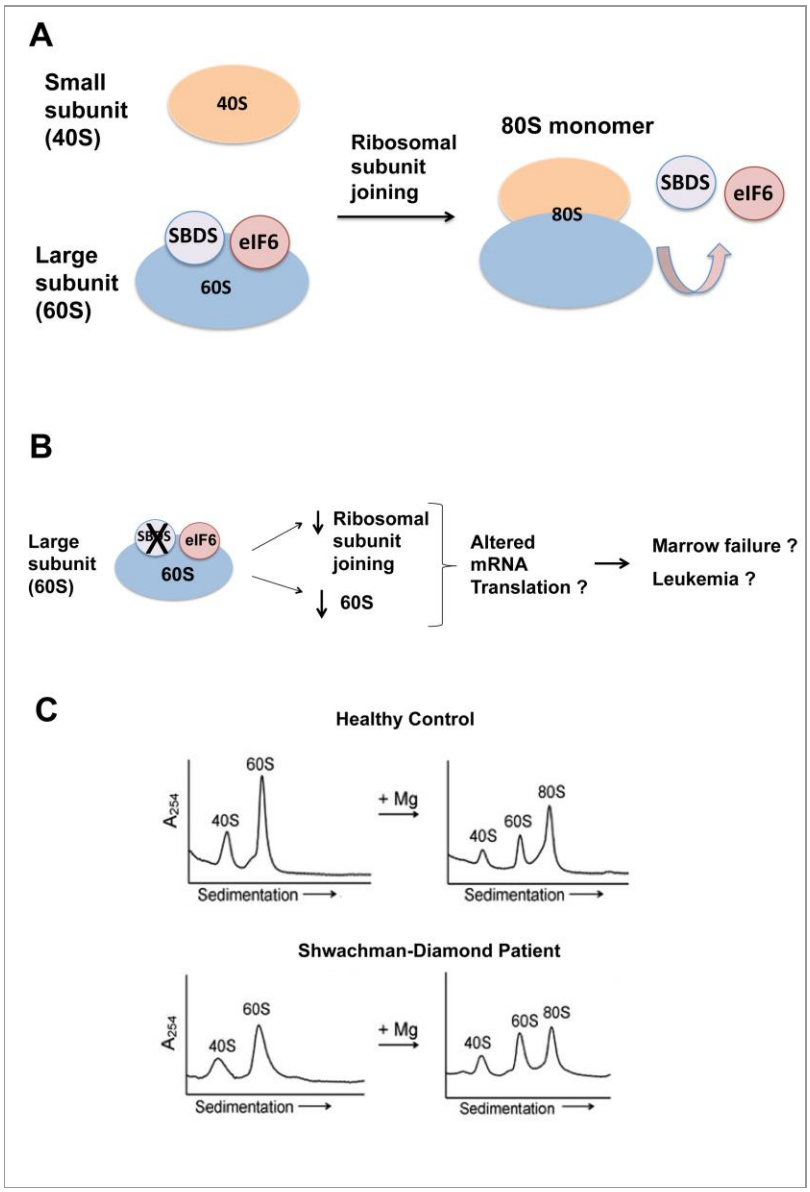
in SDS patient stromal cells. In contrast, expressing SBDS with SDS patient-specific mutations did not rescue 80S ribosome formation in SDS patient stromal cells. Progressive deletions of the amino terminus impaired the ability of SBDS to associate with the 60S subunit, which may account for decreases in ribosome assembly with *SDBS* mutations in this region. Mutations in other regions of the SBDS protein alter ribosome assembly in currently undetermined ways.

Lastly, the authors looked at the ability of 60S subunit to associate with the protein eIF6, which prevents 40S and 60S subunits from joining, and observed no change in steady-state levels of 60S-associated eIF6 in SDS patient samples. This data suggests that the SBDS protein functions after eIF6 is displaced while the individual ribosomal subunits are joining. Silencing eIF6 expression with RNA interference improved 80S formation in SDS patient cells in the in vitro assay. However, silencing eIF6 expression did not rescue the defects in hematopoietic differentiation as assessed by colony formation in methylcellulose with concurrent SBDS knockdown in hematopoietic progenitor cells.

How defective ribosome assembly affects mRNA translation, and specifically the expression of genes in SDS patients is still unknown. In a previous study, Dr. Shimamura determined that SBDS also functions to stabilize the mitotic spindle and prevent genomic instability (Austin *et al*., 2008). Additional studies are needed to tease out how the multiple functions of SBDS protein contribute to bone marrow failure and leukemogenesis in SDS patients. Other marrow failure syndromes are caused by mutations in a range of ribosomal proteins, ultimately leading to ribosome dysfunction. Notably, the authors suggest that the in vitro ribosomal formation assay may have clinical use in undiagnosed marrow failures to screen for defects in this pathway without detecting DNA mutations of specific genes.

Burwick N, Coats SA, Nakamura T, Shimamura A. 2012. Impaired ribosomal subunit association in Shwachman-Diamond syndrome. *Blood*, Epub ahead of print, doi: 10.1182/blood-2012-04-420166.

Also see: Austin KM, Gupta ML Jr, Coats SA, Tulpule A, Mostoslavsky G, Balazs AB, Mulligan RC, Daley G, Pellman D, Shimamura A. 2008. Mitotic spindle destabilization and genomic instability in Shwachman-Diamond syndrome. *Journal of Clinical Investigation*118:1511-8.



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Ribosomal subunit association is impaired in Shwachman-Diamond syndrome (SDS). (A) Schematic of normal ribosomal subunit joining. SBDS facilitates release of eIF6, allowing for subunit association to form the 80S monomer. (B) SBDS loss impairs ribosomal subunit joining and decreases 60S subunits. This may result in altered mRNA translation, marrow failure, and leukemia. (C) Healthy control or SDS patient cells were assayed for 60S:40S subunit association using an in vitro joining assay. The ribosomal profiles were analyzed by sucrose density sedimentation. In SDS patients, dramatically decreased levels of SBDS protein result in reduced 60S levels and impaired subunit association (lower panel).