## *Size Matters*

As you are all aware, Diamond Blackfan anemia is referred to as a macrocyctic anemia. The term macrocytosis, meaning large cells, relates to the increased size of red blood cells in patients with DBA. An explanation for this phenomenon may reside in a recent manuscript published by Sankaran and colleagues in the journal, *Genes and Development* [1].

Before exploring the Sankaran paper, I think it is worthwhile to provide a little background on macrocytic anemia. In this regard, it is important to point out that DBA is not the only macrocytic anemia. In fact, macrocytosis is observed in the megaloblastic anemias which are commonly encountered by the medical community. Two causes of megaloblastic anemia are deficiencies of folic acid and vitamin B12. The increased size of red blood cells in individuals with folic acid deficiency is relatively easy to envision, so I thought I would begin there.

Folic acid plays an important role in intermediary metabolism; the chemical transformations that occur within cells. Specifically, folic acid plays a role in reactions where single carbon units are used to build larger, more complex molecules. Some of these more complex molecules are the nucleotides which serve as precursors for the synthesis of DNA and RNA. Thus, individuals deficient in folic acid have a problem making copies of their DNA, a critical component of cell division. DNA needs to be copied so new cells created when cells divide will have the necessary genetic blueprint for them to fulfill their functions within the body. Another component of cell division is cell growth. If a cell were to divide without growth, the cells would get smaller and smaller until there would be nothing left. Thus, cell growth and division go hand in hand as cells multiply within the body.

Under most circumstances that is.

The process by which cells grow and divide is referred to as the cell division cycle. Some cells in the human body undergo division regularly, whereas most do not. Those cells that are not undergoing cell division still perform vital functions, they simply aren't dividing. They are referred to as being quiescent. Under the right conditions, these cells can be stimulated to divide and so enter the cell division cycle. This decision making point (also known as a cell cycle checkpoint) is called *START* and falls within a region of the cell division cycle called G1 (Gap1). In G1 a cell either decides to quit dividing and so become quiescent or pass *START* and commit to another round of cell division (see figure). Once the decision to divide has been made, the cell enters the S (synthesis) phase of the cell cycle, where DNA is copied and the cell synthesizes proteins and other basic material needed for cell growth. After S phase, the cell enters another decision making phase (G2), where the cell checks a number of critical parameters before undergoing mitosis (M) and the actual act of cell division. Once cells divide they find themselves back in G1 where they again decide whether to undergo another round of cell division or instead exit from the cell cycle and become quiescent.

So what happens to a cell where cell growth and division become uncoupled? Here we return to folic acid deficiency. Under these conditions, cells continue to grow but have a difficult time copying their DNA because of the lack of suitable building blocks. So cells grow but their division rate is reduced because of the effect on copying DNA. Consequently, the cells produced under these conditions are larger than normal. The flip side of this is a mutant form of yeast where cells divide prematurely before growth catches up with DNA replication. These mutants are referred to by the English investigators who initially discovered them as "*wee*" mutants because of their small size. Thus, factors that perturb the delicate balance between growth and division can lead to cells with altered sizes.

Normal erythrocyte development is somewhat akin to the wee mutants discussed above because cell division is occurring so quickly cells don't have time for appropriate growth and so become smaller during these cell divisions. Prior to terminal differentiation, when cells cease dividing and lose their nuclei, red blood cell precursors have already become smaller than the cells from which they are derived because of this partial uncoupling of cell growth and division.

Let's turn our attention now to the macrocytic anemia observed in DBA. As you are all aware, most cases of DBA are caused by mutations in genes encoding ribosomal proteins. This finding creates somewhat of a problem given what I've just told you regarding the megaloblastic anemias linked to folic acid deficiency (and B12 deficiency which ultimately impacts on folic acid metabolism). The reason this is somewhat problematic is that to date no one has demonstrated a specific effect of ribosomal protein haploinsufficiency on DNA replication. Instead, ribosomal protein haploinsufficiency affects the synthesis of ribosomal subunits and as a consequence has a negative effect on the protein synthetic capacity of cells. A general effect on protein synthesis would be expected to have a significant effect on cell growth since proteins/enzymes are needed to form the lipids that make cell membranes, supply the energy for cellular functions, create the nucleotides to synthesize DNA and RNA, and pretty much anything else you can think of in terms of what cells do. Since proteins have so many different functions that impact on both cell growth and division, one might predict that any affect on cell size associated with ribosomal protein haploinsufficiency might very well be a push and thus just give rise to fewer cells of normal or even smaller size. But that is not what is observed in DBA, so there must be something more going on and this is where the paper by Sankaran comes in.

Sankaran and colleagues used Genome Wide Association Studies (GWAS) to identify genes in human populations that play a role in erythrocyte size. These studies make use of single nucleotide polymorphisms (SNPs) spaced throughout the human genome as markers to identify regions of the genome that associate with whatever property is under investigation, which in this case was erythrocyte size. The gene identified in these studies was *CCND3* which encodes a protein known as cyclin D3. Cyclin D3 as the name implies is a cyclin, a member of a large protein family whose members have related structures and functions. Cyclins get their name because their level of expression was shown to rise and fall (or cycle) as cells progress through the cell cycle. We now know that cyclins regulate another family of proteins known as cell division kinases (Cdks). The Cdks regulated by cyclins tend to be on/off switches that control critical checkpoints in the cell division cycle. Without their corresponding cyclins these switches remain off thereby blocking cell cycle progression and so cell division.

What Sankaran and colleagues found was a change in sequences upstream of *CCND3* that specifically affected its expression in erythrocytes. Thus, erythrocyte precursors with this polymorphism would grow but divide less frequently and so give rise to fewer erythrocytes of increased size.

The question these studies raise is whether *CCND3* could possibly play a role in the macrocytosis observed in DBA. Cells producing suboptimal amounts of a ribosomal protein as seen in DBA patients make fewer ribosomal subunits and therefore fewer functional ribosomes. As mentioned, this reduces protein synthetic capacity which negatively impacts cell growth. But not all proteins are made equal rates in cells. The mRNAs used to make different proteins compete with one another for use by ribosomes to make their respective proteins. Intriguingly, almost 40 years ago one of the authors on the Sankaran manuscript, Harvey Lodish, published a remarkable theoretical paper arguing that as general components of the translational machinery (ribosomes) become scarcer, this competition becomes increasingly intense, and mRNAs that are inefficiently translated by ribosomes under normal conditions may be selected against under these more competitive conditions and so the synthesis of

their specific protein products may be selectively inhibited [2]. Thus, haploinsufficiency for a ribosomal protein could selectively affect the production of key proteins like cyclin D3, while leaving the synthesis of other proteins relatively unscathed. Under such circumstances effects on cell division could be more pronounced than effects on cell growth so leading to macrocytosis. Therefore, what Sankaran and colleagues have given us is a prime candidate for a protein who synthesis might be preferentially affected in erythrocyte precursors from patients with DBA.

The manuscript by Sankaran and colleagues thus provides a tantalizing clue which may help link the underlying genetic change in many patients with DBA to a specific clinical trait; a connection long sought after by those of us in the DBA field.

1. Sankaran VG, Ludwig LS, Sicinska E, et al. Cyclin D3 coordinates the cell cycle during differentiation to regulate erythrocyte size and number. *Genes Dev*. 2012;26(18):2075-2087. Prepublished on 2012/08/30 as DOI 10.1101/gad.197020.112.
2. Lodish HF. Model for the regulation of mRNA translation applied to haemoglobin synthesis. *Nature*. 1974;251(5474):385-388. Prepublished on 1974/10/04 as DOI

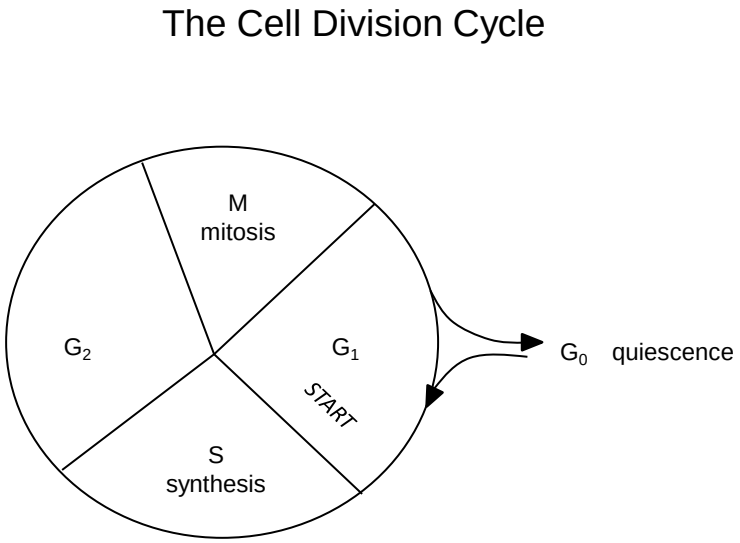


Figure 1