Summary

Antibodies are produced by B cells before antigen contact. A given B cell synthesizes antibodies of one specificity only. However, the large number of B lymphocytes allows for a large diversity, which stands ready to meet the antigenic universe. Upon contact with the cognate antigen, the B cell expands into a clone and eventually secretes the antibody in large amounts. The diversity of antibodies is generated in two steps, first before antigen contact by random and imprecise joining of gene segments. Second, upon encounter with the antigen, three types of genetic changes in the antibody encoding loci may occur: somatic hypermutation (SHM) and gene conversion in the antigen binding part; and class switch recombination (CSR) in the non-antigen binding part of the antibody. All three phenomena are mediated by the enzyme activation-induced cytidine deaminase (AID), which is specifically expressed in activated B cells.

WEHI-231 is known as an immature B cell line with no evidence for SHM or CSR. Indeed, in early freezings of the WEHI-231 line the canonical AID is absent. However, we found some variant lines that express AID. But despite the presence of AID and other known factors required for SHM and CSR, cells of the WEHI-231 variants do not switch the Ig class and hypermutate only at low level. This level, though, was enough in a subclone to cause decreased surface IgM via accumulation of mutations in the κ light chain, which decreased κ expression, and, in turn, decreased the amount of complete IgM on the cell surface. And instead of CSR, we found in a WEHI-231 variant recombination between alleles. In early WEHI-231 freezings, instead of the canonical AID, an 18-kD protein is expressed. The protein reacts with the antibody to AID, and therefore may represent a temporal precursor to functional AID. Based on the presence the 18-kD protein and expression of the mature B cell marker IgD, we re-classified WEHI-231 as a mature B cell line, and the WEHI-231 variants as activated B cell lines.

We used the WEHI-231 lines to search for additional unknown factors required for SHM and CSR. By retroviral integration mutagenesis we found the chaperone Tid-1 as a potential missing factor. As a second candidate, we studied the chromatin-remodeling factor Supt6h, which is thought to help transcription by displacing histones for the RNA polymerase II to access the DNA. The rate of mutation introduced by AID depends on the rate of transcription, and so a transcription factor seemed to be a good choice after we had found previously that Supt6h binds AID in yeast two-hybrid screens.