

Chromatin, transcription and somatic hypermutation of immunoglobulin genes

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Abstract

The humoral arm of adaptive immunity is characterized by a large and diverse population of antibodies generated by V(D)J recombination, followed by random mutations in the antigen binding region of the antibody gene by a process of somatic hypermutation (SHM), which creates further diversity as well as enhances antibody affinity more than 1000-fold. The somatic hypermutation of antibody genes is initiated by the cytidine deaminase AID creating cytidine (C) to uridine (U) mutations, starting after ~100-200 bp from the promoter and extending for about 2 Kbp. During SHM these 'U's are repaired in error-prone fashion, using translesion DNA polymerases leading to mutations at and near the 'U'. The process of SHM is linked to transcription initiation. We have postulated that AID, which initiates SHM, gets associated with the transcription complex at or near the promoter and deaminates 'C's, while it travels with the RNA polymerase. However, the influence of chromatin on SHM remains enigmatic. Transcription occurs in the context of chromatin which likely modifies the targeting of AID. Our previous cell-free studies indicate that AID cannot access nucleosomal DNA in the absence of transcription. We have now investigated the influence of nucleosome stability on mutability *in vivo*. We introduced two copies of a high affinity nucleosome positioning sequences (MP2) into a variable Ig gene region to assess its impact on SHM *in vivo*. The MP2 sequence significantly reduces the mutation frequency throughout the nucleosome and especially near its center, despite similar proportions of AID hotspots as in Ig genes. A weak positioning sequence (M5) was designed based on rules deduced from published whole genome analyses. Replacement of MP2 with M5 resulted in much higher mutations throughout the nucleosome. This indicates that both nucleosome stability and positioning significantly influence the SHM pattern. We postulate that, unlike RNA polymerase, AID has reduced access to stable nucleosomes. This study defines the limits of nucleosome positioning for SHM of Ig genes and suggests that stable nucleosomes need to be disassembled for access of AID. Possibly the variable regions of Ig genes have evolved for low nucleosome stability to enhance access to AID, DNA repair factors and error-prone polymerases and hence, maximize variability. Our experiments are important for determining how the varied repertoire of antibody genes is created with the potential to react against any foreign antigenic substance, including tumor cell antigens.