DNA DAMAGE TOLERANCE, PROGRAMMED MUTAGENESIS AND LYMPHOMAGENESIS

B cells have the unique capacity to mutate their immunoglobulin genes by programmed mutagenesis. Our research is focused on the following aspects: 1) To unravel the molecular mechanism of programmed mutagenesis, 2) To determine the targeting specificity of the mutation process and its contribution to lymphoma development, and 3) To understand the decision making between repair and mutagenesis.

In vivo binding preferences of the mutator protein AID in the genome
To improve adaptive immunity, the activation induced cytidine deaminase (AID) initiates somatic hypermutation (SHM) and class switch recombination (CSR) in immunoglobulin (Ig) genes. As shown by selective mutation analysis, non-Ig genes are targeted by AID, although the genome-wide impact of aberrant targeting remains to be determined. The aim of this study was to establish a genome-wide AID binding profile and identify molecular parameters controlling the targeting of AID. To accomplish these goals, we apply the DamID technique for AID in a hypermutation-competent Burkitt lymphoma cell line. This unbiased approach revealed that AID is targeted throughout the genome and preferentially to actively transcribed genes. Moreover, AID binding is favored in regions enriched in their G/C content and enriched for G-stretches in the coding strand. Furthermore, AID does not exhibit an intrinsic preference for binding to RGYW motifs, suggesting that these hot spots of SHM are deamination- rather than binding hot spots of AID. Future studies should allow us to provide a detailed, genome-wide binding profile of AID. This profile will be critical in identifying aberrant AID target sites (ATS), which will help to identify cancer-associated ATS causal to the development of GC- and post-GC derived B cell lymphomas. ATS are presently used to identify molecular predictors that favor AID binding.

The Fanconi Anemia Core Complex is dispensable during Somatic Hypermutation To generate high affinity antibodies during an immune response, B cells undergo somatic hypermutation (SHM) of their Ig genes. Error-prone translesion synthesis (TLS) DNA polymerases have been reported to be responsible for all mutations at template A/T and at least a fraction of G/C transversions. In contrast to A/T mutations, which depend on PCNA ubiquitination, it remains unclear how G/C transversions are regulated during SHM. Several lines of evidence indicate a mechanistic link between the Fanconi Anemia (FA) pathway and TLS. To investigate the contribution of the FA pathway in SHM we analyzed B cells deficient for FancG, an essential member of the FA core complex. These B cells were hypersensitive to treatment with cross-linking agents, but the frequencies and nucleotide exchange spectra of SHM remained comparable between wild-type and FancG-deficient B cells. These data indicate that the FA pathway is not involved in regulating the outcome of SHM in mammals. In addition, the FA pathway appears dispensable for class switch recombination.

Allelic exclusion at the immunoglobulin heavy chain locus initiates at the level of transcription Developing lymphocytes generate their antigen receptor genes by DNA recombination. Once a gene has been productively assembled, the receptor is expressed and terminates rearrangement of the second allele to ensure mono-specificity. Using specific, non-functional IgH knock-in and transgenic mice, we showed in collaboration with Dr J Lutz and Dr H-M Jäck (University of Erlangen, Germany) that stable expression of a non-coding µ heavy chain (HC) mRNA in B cells reduces DNA recombination at the IgH locus and impairs early B cell development. Functional µHC mRNA serves therefore not only as a classical messenger, but also as a sensor for productive IgH rearrangements and as a regulator of allelic exclusion during B cell development.

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Publications

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