# SUMMARY

Maturation of the immune system and the establishment of protective immunity relies on programmed induction of transcription-coupled, site-directed DNA breaks with the subsequent repair in germinal center (GC) B cells, leading to immunoglobulin (Ig) diversification and affinity maturation. GANP is a protein selectively upregulated in GC B cells. The purpose of this study is to elucidate the molecular mechanism by which GANP would regulate the diversification of the IgV-region gene.

To address this issue, we implemented the well characterized system of chicken DT40 B cell. Using different mutants lacking proteins involved in SHM (AID and UNG) or DNA repair (Rad54 and Ku70) and implementing classical tools for measuring gene conversion (GCV) and SHM we elucidated the role of GANP played in Ig diversification. We also implemented survival assays using DNA damaging drugs, to assess cell survival upon GANP overexpression or GANP deficiency. Immunoprecipitation and western blot analyses were used to elucidate GANP interaction with DNA repair proteins in human Ramos B cells. GANP haplo-sufficiency caused reduction in the rates of IgV GCV, and conversely, the introduction of ganp cDNA enhanced the GCV in DT40 cells. The results indicated that GANP is an integral molecule in GCV process. Also, GANP increased the SHM rate in the IgVL in an AID-dependent manner. Using reporter constructs to measure DNA repair activity, GANP clearly promotes homologous recombination (HR) repair, while it suppresses the activity of non-homologous end-joining (NHEJ) repair. Moreover, GANP could efficiently

restore compromised GCV/HR in HR-defective Rad54-/- cells, but suppressed it in Ku70-/- mutant cells lacking the NHEJ pathway, suggesting that GANP regulates the choice of DNA repair pathways. In cell survival assay, GANP sensitizes the cells to etoposide, while it protects cells against camptothecin-induced damage, confirming its suppressive effect on NHEJ repair and positive regulation of HR repair. GANP interacts with DNA-PKcs, a core NHEJ protein, in Ramos B cells. This interaction of GANP with DNA-PKcs undergoes dissociation upon etoposide treatment or AID overexpression, however their association is not altered in AID deficient cells, denoting that this interaction is DNA damage dependent.

GANP protein regulates the choice among DNA repair pathways provoked post AID-induced lesions at the transcriptionally active IgV-region, directing the repair to HR repair by abrogating NHEJ repair, thus positively impacting both B cell survival and IgV functional integrity.