

Nuclear Organisation of immunoglobulin genes

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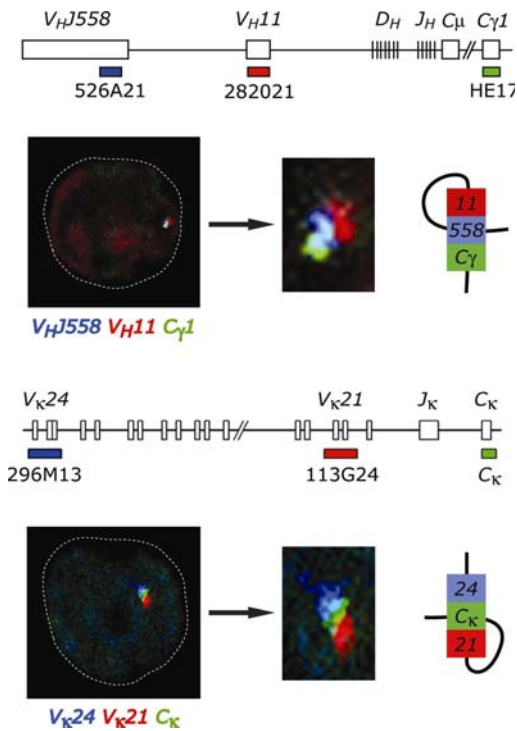
Introduction

The function of a B cell is to produce antibodies which can respond to the numerous foreign antigens which we encounter. Since there are only a limited number of *immunoglobulin genes (Ig)* this is achieved through a process whereby variable (*V*), diversity (*D*) and joining (*J*) gene segments of *immunoglobulin genes* are rearranged in different orders to achieve antibodies with a vast range of different specificities. *V(D)J* recombination occurs in a lineage specific and developmentally regulated manner in the bone marrow. Since rearrangement takes place on both alleles at the same time during B cell development, as soon as a successful rearrangement is achieved on one of these alleles the other allele has to be prevented from continuing to rearrange. Allelic exclusion thus simplifies the problem of immune recognition by ensuring that only one of the two alleles is functionally rearranged so that each B cell makes antibodies that recognise a single target.

We know that the temporal order of rearrangement is largely regulated by the accessibility of the *immunoglobulin genes* to the recombinase machinery. The way in which the *immunoglobulin genes* are packaged within chromatin is therefore important and efficient recombination occurs when these genes are packaged within active chromatin as judged by germline transcription, histone acetylation, nucleosome remodelling and DNA demethylation. The approach that we have taken is to examine the changes in configuration and nuclear positioning of the *immunoglobulin genes* at each stage of development to determine the impact this has on ordered rearrangement and allelic exclusion. For this we have used 3D-DNA fluorescent in situ hybridisation (FISH), a technique which is carried out under conditions that are designed to preserve the integrity of the nucleus.

Regulation of lineage specificity

In collaboration with Harinder Singh and Steven Kosak we demonstrated that perinuclear localisation of *IgH* and *Igκ* loci may be involved in lineage specific regulation of rearrangement, since relocation of *Ig* loci to a more central location in the nucleus is developmentally regulated and occurs just prior to the onset of rearrangement. We are currently examining the factors that may be responsible for this relocation.



Ordered rearrangement: two stage activation of the IgH locus

Activation of the *IgH* locus occurs in two stages. Through a collaboration with Meinrad Busslinger we have shown that relocation to the centre of the nucleus results in upregulation of *DJ_H* rearrangements and enables proximal *V_H-DJ_H* rearrangements. The second stage of activation is locus contraction, which enables recombination of mid and distal *V_H* gene segments, and this stage is regulated by Pax5 and an unknown factor X. We are currently trying to identify factor X and the other components involved in locus contraction in order to try and understand the mechanism underlying this process.

Using 3D FISH we have demonstrated that the long range interactions which facilitate *V_H-DJ_H* rearrangements in pro-B cells are mediated by looping of individual *IgH* subdomains (see above). The *Igκ* locus also undergoes contraction by looping in small pre-B and immature B cells, demonstrating that *Ig* loci are in a contracted state in rearranging cells.

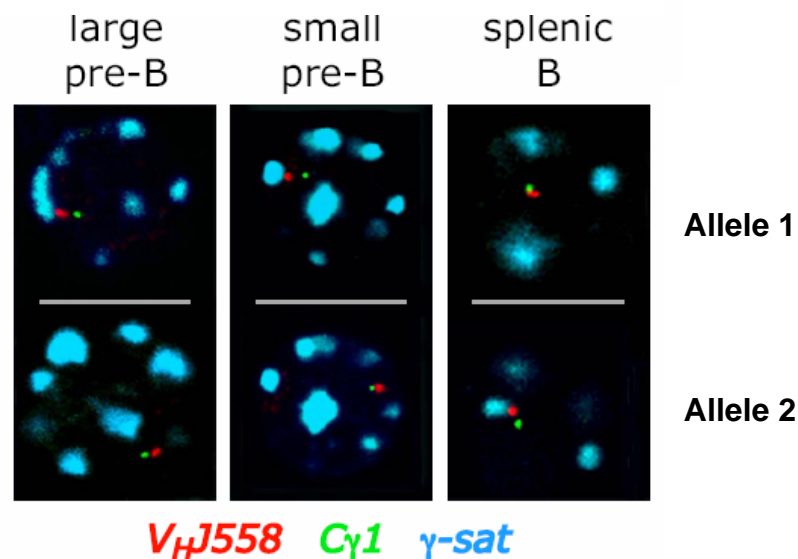
Allelic exclusion

Allelic exclusion of *immunoglobulin (Ig)* genes ensures the expression of a single antibody molecule in B cells but the mechanisms

responsible for establishing monoallelic expression are largely unknown. We have found that successful *IgH* recombination induces the rapid reversal of locus contraction in response to pre-BCR signalling which physically separates the distal *V_H* genes from the proximal *IgH* domain, thus preventing further rearrangements involving mid and distal *V_H* gene families from occurring on the second *DJ_H* rearranged allele. In collaboration with Meinrad Busslinger we have shown that in the absence of locus contraction, only the four most proximal *V_H* genes escape allelic exclusion in immature *Igμ* transgenic B-lymphocytes.

Our earlier studies have demonstrated that in mature activated splenic B cells, following allelic exclusion, endogenous *IgH*, *κ* and *λ* alleles localise to different subnuclear environments; one allele associates with centromeric heterochromatin where transcription diminishes and the second allele is positioned away from these domains and transcription from this locus predominates. These observations suggest that the differential recruitment of *Ig* alleles has a role in maintaining and favouring expression from a single rearranged *IgH* and *Igκ* allele. This led us to investigate centromeric repositioning of *Ig* alleles during B cell development to determine whether recruitment of one allele contributes to the mechanisms that establish allelic exclusion. We have now established that pre-BCR signaling leads to rapid repositioning of one *IgH* allele to repressive centromeric domains in response to down-regulation of IL-7 signaling. Centromeric recruitment of one *IgH* allele is initiated together with allelic exclusion at the onset of pre-B cell development and is transiently maintained in B-lymphocytes undergoing *IgL* gene rearrangements.

Our data suggest that it is the non-productively rearranged *IgH* allele which is recruited to the



centromere. As shown by 3D FISH analysis, the recruited *IgH* locus is oriented at the centromere in pre-B and activated B cells in such a way that the distal *V_HJ558* gene family is positioned closer to the *γ-satellite* cluster than the proximal *V_H7183* and *C_γ1* genes of recruited *IgH* alleles, respectively (see above). Centromeric recruitment of the *V_HJ558* gene family coincides with histone deacetylation of the distal *IgH* domain in pre-B cells suggesting a link between these two processes. Taken together these results suggest that repositioning may function to protect the second *DJ_H* rearranged allele from recombinases during *IgL* rearrangement by reducing accessibility. Our data implicate both locus decontraction and centromeric recruitment in the establishment of allelic exclusion at the *IgH* locus.

Future goals:

The work we have done to date has provided some insight into how recombination and allelic exclusion are regulated. Using normal and mutant mice we have begun to identify the transcription factors and signalling pathways that are involved in initiating and propagating the changes in nuclear organisation that we have observed in developing and mature B cells. Alongside this we are using DNA footprinting to look at DNA remodelling and DNA/protein interactions. In addition we are examining alterations in histone modifications using chromatin immunoprecipitation (ChIP) assays. With these combined techniques we aim to build up a comprehensive picture of what is happening to the immunoglobulin genes concurrent with positional changes at each stage of B cell development in order to unravel the molecular mechanisms underlying their epigenetic regulation.

There is increasing evidence that epigenetic mechanisms contribute to disease states including cancer. By examining the molecular and organisational status of *Ig* genes in mutant mice blocked at different stages of B cell development we hope to identify epigenetic factors that contribute to immunodeficiency. A thorough understanding of the epigenetic mechanisms regulating *Ig* genes will also enable us to determine the contribution made by alterations in regulation of these genes accompanying chromosomal translocations which cause leukaemias and lymphomas.