Jane Skok

Nuclear organisation of *immunoglobulin* genes

Introduction

B cell development takes place in the bone marrow where immunoglobulin (Ig) loci undergo a series of genetic modifications resulting in phenotypic and functional changes. Recombination of variable (V), diversity (D) and joining (J) regions occurs in a lineage specific and developmentally regulated manner giving rise to a diverse repertoire of functional B cell receptors. Allelic exclusion subsequently ensures monospecificity in terms of antigen recognition and, at a later stage, antibody secretion. Current research in my group is focused on identifying the factors involved in these processes and investigating their relationship with the initiation and propagation of changes that determine B cell identity.

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.

Two stage activation of the *IgH* locus

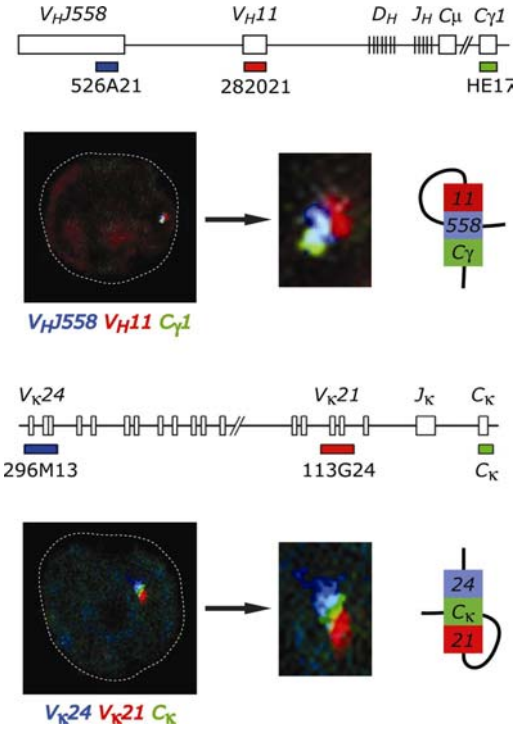
Through a collaboration with Meinrad Busslinger we have shown that there are two stages of activation of the IgH locus and each is regulated by distinct factors. The first stage is relocation to the centre of the nucleus which results in upregulation of DJ_H rearrangements and enables proximal V_H-DJ_H rearrangements. The second stage is locus contraction, which enables recombination of distal V_H gene segments, and this stage is regulated by Pax5 and an unknown factor X.

Lab members:

Jane Skok
Senior Lecturer
Department of Immunology and Molecular Pathology
University College London
46 Cleveland Street
London W1T 4JF, UK

Tel: +44 20 7679 9604,
Fax: +44 20 7679 9652
Email: j.skok@ucl.ac.uk

Winnie Chong (Post Doc)
Esther Roldan (former Post Doc)



Using 3D FISH we have shown 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 through largely unknown mechanisms. 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. 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.

We have previously demonstrated that following allelic exclusion, endogenous *IgH,* κ and λ alleles localise to different subnuclear environments in activated cells; 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. We now know 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 $C_\gamma1$ genes of recruited *IgH* alleles, respectively (see below). 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. Our data implicate both locus decontraction and centromeric recruitment in the establishment of allelic exclusion at the *IgH* locus.