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Gene conversion at the chicken immunoglobulin L genes

with

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What is gene conversion?

Kerstin Meyer: The key feature of antibodies (also called immunoglobulins, Ig) is their ability to recognise and bind a huge array of foreign antigen. Different organisms have evolved unique strategies to achieve this. In man and mouse the primary immune repertoire is generated by the rearrangement of a large number of variable (V), diversity (D) and joining (J) gene segments giving rise to a highly diverse variable region of the antibody. In chickens on the other hand a single functional V and J segment and highly homologous D segments are rearranged to encode, in effect, a single type of Ig molecule. Subsequently diversity is generated by gene conversion, copying stretches of 8 - 200 base pairs from an array of upstream pseudo-V genes into the expression cassette. Repeated rounds of gene conversion (GC) yield highly diverse immunoglobulins. Although distinct from the mechanisms found in man and mouse, the majority of farm animals utilise GC in the generation of their antibody repertoire and studies of GC in chicken cells have shed light on the molecular mechanisms used for Ig diversification in man.

What is the molecular mechanism of gene conversion?

Kerstin Meyer: Recent years have seen a dramatic increase in our understanding of gene conversion. In particular the genetic analysis of the chicken cell line DT40 has allowed us to tease apart the enzymes required in gene conversion (1). It is now widely accepted that the enzyme activation-induced cytidine deaminase (AID), which is essential for GC, somatic hypermutation (SHM) and class switching recombination (CSR), initiates these Ig modifications by deaminating single stranded DNA at specific target sites (hot spots), thus converting a G:C to a G:U base pair. At the chicken Ig locus the processing of this lesion determines whether this mismatch is repaired or gives rise to GC or SHM (2). It has recently been shown that UNG (uracyl DNA glycosylase) is essential for gene conversion (3). It removes the uracyl, leading to an abasic site that is recognised by an apyrimidinic endonuclease. This single stranded break could then initiate a homologous recombination reaction involving the upstream pseudo V-genes. Indeed deletion of enzymes of the homologous repair pathway, such as RAD54, NSB1 and the Rad51 paralogues (XRCC2, XRCC3 and RAD51b) leads to a reduction in GC rates (1). G:U mismatches are normally recognised by the mismatch repair pathway and it remains to be clarified how this pathways impacts on GC.

How is gene conversion regulated?

Kerstin Meyer: AID is capable of introducing permanent changes into the genome and therefore needs to be tightly regulated and targeted to its correct substrate. The expression of AID expression is restricted to activated B cells, thus protecting the majority of cell types from its effect. But how are Ig genes targeted specifically? As GC and SHM have been shown to proceed through the same intermediate, most of our insights of the targeting of AID to the V-genes have come from the analysis of SHM. While transcription is a pre-requisite for SHM and targets the mutation machinery to within 1kb downstream of the promoter, it is the enhancers that appear to confer specificity of SHM to the Ig genes. However in certain assay systems mutation rates simply correlate with rate of transcription. First candidate proteins that may contribute to AID targeting have also been identified. For example phosphorylated AID is able to interact directly with replication factor A which can stabilise single stranded DNA that has been generated by transcription (4). In addition, the mismatch repair protein Msh6 may provide a scaffold that helps to target AID to the Ig locus in mice (5). However it remains unclear how either of these proteins would be able to distinguish the Ig locus from other loci.

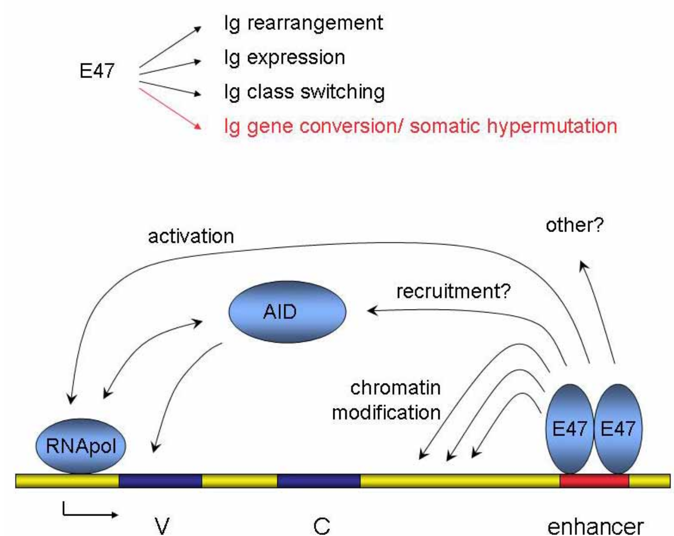

Figure 1: Digram depicting the role of E2A in Ig gene diversification.

What is the role of E2A in gene conversion?

Kerstin Meyer: While transcription appears to be a pre-requisite it is not sufficient to recruit the AID activity since a number of genes that are highly expressed in mature B cells show either no or very low levels of SHM. A potential candidate for directing the AID machinery to the Ig locus is the transcription factor E2A. E2A plays a pivotal role in B cell development and binding sites for E47 and E12, the 2 splice products of E2A, are present in all Ig enhancers and in mutating transgenes. Furthermore E2A protein levels are highest in germinal centres where SHM and CSR are thought to occur.

In recent experiments we have now shown that E2A is able to regulate GC (6). Inhibition of E2A led to a decrease in GC, which was mirrored by reduced rates of gene expression. Thus, like for SHM the level of GC broadly correlates with the levels of transcription. Significantly, overexpression of E47 greatly increased the level of GC but did not increase in the levels of transcription (6). These results argue that GC is actively regulated by E2A and not merely a result of increased transcription. E2A has now been implicated in regulating all the Ig modification processes, ranging from Ig gene rearrangement, expression and class switching to gene diversification. It will be very interesting to determine whether these processes are mediated via epigenetic changes and chromatin modification or by the direct recruitment of the enzymatic components of the AID complex to the Ig locus by the E2A proteins.

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