論文内容の要旨

論文題目　Mechanism and regulation of the antigen-receptor gene rearrangement in the jawless vertebrates
(無顎類における抗原受容体の遺伝子再編成機構と制御)

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Immune system is a very important and highly organized host defense system and throughout evolution, it has been developed in various ways to protect from countless pathogens in the environment. Most species in the animal kingdom rely on innate immunity, which constitutes the first line of defense against infection and attacks pathogens in non-antigen-specific manner. Recent studies have shown that innate immune systems are highly diverse among species and have uniquely developed in a species-specific manner. On the other hand, highly sophisticated adaptive immunity, which shows antigen-specific response and create immune memory, is only observed in the vertebrates; for a long time, only one type of the adaptive immune system was known in higher vertebrates, commonly referred to as jawed vertebrates (sharks to mammals). In these animals, antigen receptor genes are somatically diversified under strict regulations; genes encoding T cell receptors and immunoglobulins (Ig) are rearranged and expressed in T cells and B cells, respectively. T cells and B cells functionally interact with each other, providing the basis for highly specific and effective immune responses and the development of immune memory.

Although the most basal vertebrates, jawless vertebrates, show adaptive immune responses, they do not possess conventional antigen receptor genes. The paradox causes a question what is responsible for adaptive immune responses and whether or not they have a system to execute a highly complex immune response as jawed vertebrates. Several years ago, a study showed jawless

vertebrates possess an alternative antigen receptor named variable lymphocyte receptors (VLR), which consist of multiple leucine-rich repeat (LRR) modules. In hagfish and lampreys, which are the only extant orders of jawless vertebrates, two types of the *VLR* gene, VLR_A and VLR_B, are present. Recent reports showed that in the sea lamprey (*Petromyzon marinus*), VLR_A and VLR_B are expressed each by separate lymphocyte subpopulations, and that lymphocytes expressing VLR_A are T cell-like, while those expressing VLR_B are B cell-like.

Diverse *VLR* genes are somatically generated in lymphocyte-like cells in hagfish and lampreys; the intervening sequence in the incomplete germline *VLR* gene is replaced by a set of LRR-coding gene segments residing around the germline *VLR* gene (Fig. IA). A great number of LRR segments, which come in several types, lie randomly in the genome and the size of VLR_A and VLR_B loci spread over 2 mega bases. The mechanism to generate diverse *VLR* genes was, however, largely unknown. In order to understand *VLR* diversification mechanism, I examined how diverse VLR_B (VLR_A was not reported at that time) genes were generated in the Japanese lamprey (*Lethenteron japonicum*). Sequence analysis of VLR_B genes before, during and after somatic rearrangement revealed that short homologies were present between germline LRR segments and seemed to be used as an anchor site for LRR segment insertion.

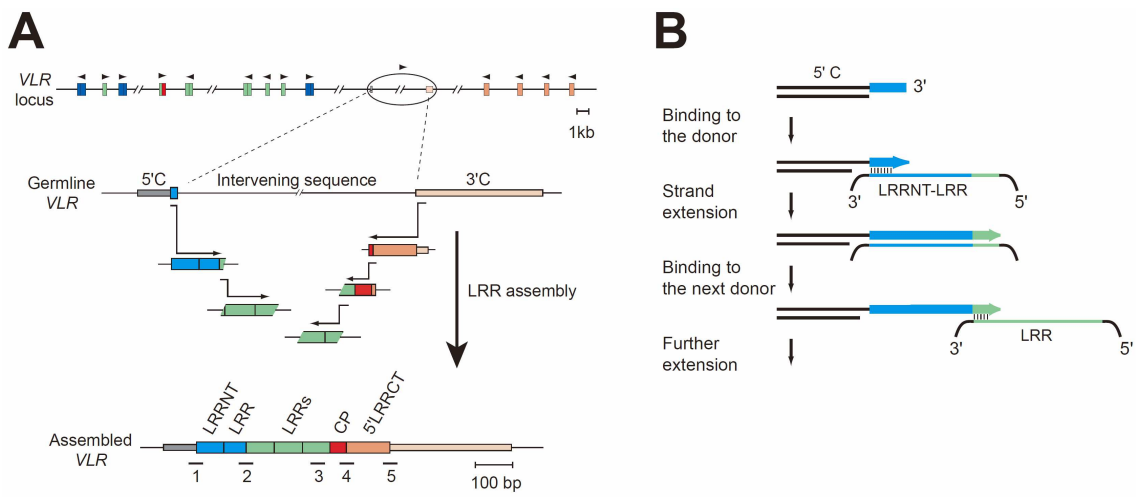

Figure I. A model of *VLR* assembly mechanism.
(A) A model of *VLR* assembly. Several LRR segments are inserted in order using short homologies. Through somatic assembly, the *VLR* gene is greatly diversified not only by inserting LRR segments in various combinations, but also by shifting combining sites between LRR segments.
(B) A model of LRR segment insertion into incomplete *VLR* gene. Only the 5'C end is illustrated. Short homologies between LRR segments are used as an anchor site for LRR segment insertion. Single-stranded extension of the 5' C (recipient) hybridizes and copies the LRRNT-LRR sequence (donor). After incorporating one LRR segment, copying continues with the next segment.

Based on close analysis of LRR junction sequences, I have proposed that *VLR* assembly is mediated by a process involving copy choice/template-switching, in which DNA polymerase dissociates from one DNA region (template) to another through repeat sequences in templates. In

VLR assembly, short homologies in LRR segments are used as template-switching sites and several LRR segments are inserted in order (Fig. I). For instance, the short homology in 5' constant region (5'C) of the germline VLR_B gene draws a particular LRR segment, an LRRNT plus LRR (Fig. IB). An LRRNT-LRR segment is inserted into the germline VLR_B gene, and an inserted sequence now contains a short homology which draws a different LRR segment, an LRR (Fig. IB). Insertion of LRR segments occurs at both 5'C and 3'C ends of the intervening sequence; several kinds of LRR segments are inserted at each end, and joined at the end to generate a *VLR* gene (Fig. IA). When a pair of LRR segments share homology at several sites, LRR segments were connected at different short homology sites, generating various hybrid LRRs from a single pair of LRR segments. Thus, in *VLR* assembly, a vast repertoire of assembled *VLR* genes could be generated, not only by inserting LRR segments in various combinations, but also by joining LRR segments at multiple sites.

The research of VLR_B gene assembly in the Japanese lamprey also demonstrated monoallelic assembly of VLR_B gene, leaving non-assembled allele intact. Since there are two types of *VLR* gene in the jawless vertebrates, important questions arise such as how VLR_A and VLR_B are assembled in a lymphocyte and how the assembly is controlled to be monoallelic. It is, in other words, whether or not jawless vertebrates also have developed a certain mechanism to ensure that a single antigen receptor is expressed in a lymphocyte, as highly strict regulations that are seen in gene rearrangement of Ig-type antigen receptors. In order to understand how *VLR* assembly is regulated, I analyzed the *VLR* gene assembly in the inshore hagfish (*Eptatretus burgeri*) at the single-cell level, using over 1,000 sorted lymphocytes. Single-cell PCR analysis showed that each lymphocyte assembled only one type of the *VLR* gene, either VLR_A or VLR_B, mostly in a monoallelic fashion. On the other hand, single-cell RT-PCR demonstrated that *VLR* transcription was detected in both alleles and not restricted to one of the two alleles. In minority of lymphocytes, *VLR* assembly occurred in both alleles; in many of such cases, only one allele contained a functional *VLR* gene, and the other allele contained a defective *VLR* gene with an in-frame stop codon or a frameshift.

Based on these results, I have proposed a model describing how *VLR* assembly proceeds, referring to feedback inhibition hypothesis that a functional VLR protein acts to prevent *VLR* assembly on the other allele (Fig. II). At first, lymphoid progenitors, if they exist, separate into two populations, each of which somehow activates either VLR_A or VLR_B locus. While transcription occurs on both alleles, *VLR* assembly occurs on either allele to generate a functional *VLR* gene. A functional VLR protein induces feedback inhibition on the other allele so that *VLR* assembly no longer occurs. If defective *VLR* is generated (or VLR protein somehow can not induce feedback inhibition), *VLR* assembly continues on the other allele, generating one defective and one functional *VLR* gene (or two apparently functional *VLR* genes) in a lymphocyte. When defective *VLR* genes are generated on both alleles, such lymphocytes might be subject to apoptosis. Only lymphocytes containing at least one functional *VLR* gene survive and come out into the peripheral blood.

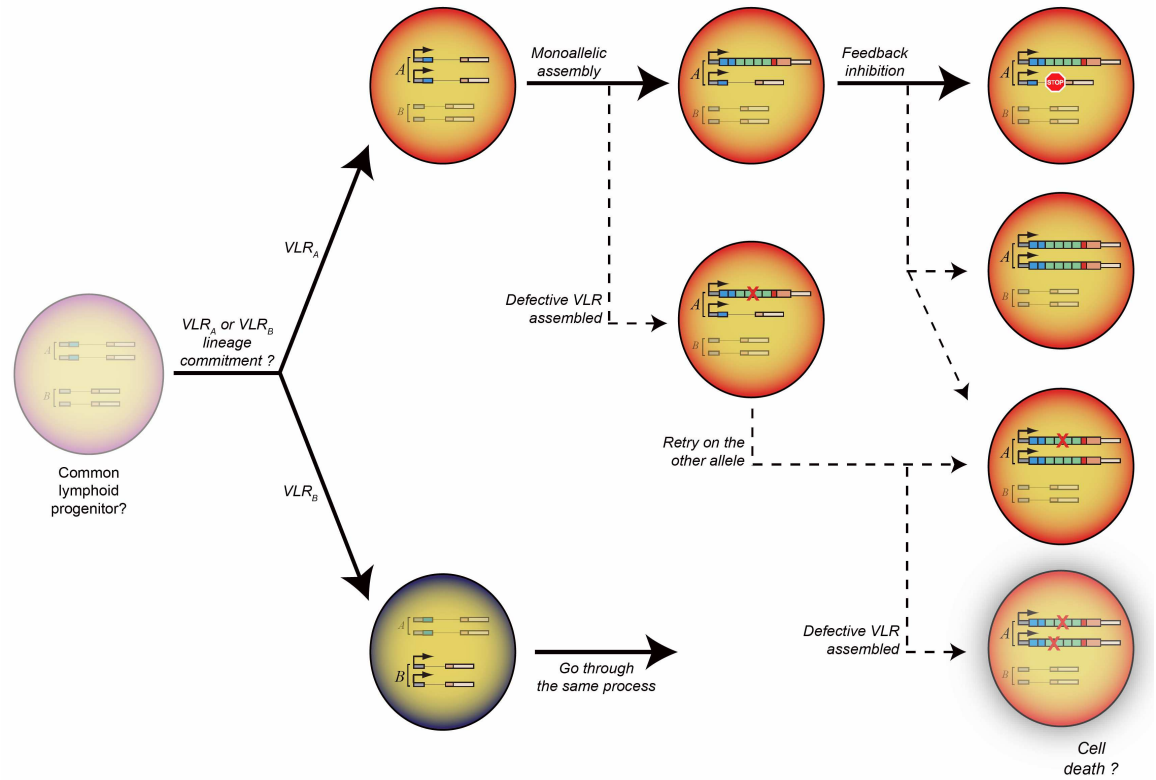

Figure II. A model of *VLR* assembly in lymphocyte development.
Before *VLR* assembly takes place, lymphocytes separate into two groups, each of which undergo VLR_A or VLR_B assembly in a mutually exclusive manner. At each VLR_A or VLR_B locus, both alleles are activated in transcription. Somehow, *VLR* assembly occurs in a single allele and a successful assembly of a functional *VLR* gene would lead to feedback inhibition on the other allele. When a defective *VLR* gene is generated, assembly might occur on the other allele, which would result in a lymphocyte with one defective and one functional VLR. In some cases, two functional *VLR* genes were found in a single lymphocyte, which might be due to a failure of feedback inhibition. If a lymphocyte contains only defective *VLR* genes, it would result in cell death.

At an early stage of vertebrate evolution, there seems to have been an overwhelming demand for adaptive immunity; two adaptive immune systems mediated by Ig-type and VLR-type antigen receptors have developed in jawed and jawless vertebrates, respectively. I have revealed that a copy-choice-involving mechanism is used for somatic assembly of the *VLR* gene in jawless vertebrates. Although VLR antigen receptors are very different from Ig-type antigen receptors, I have shown that various *VLR* genes are generated through combinatorial and junctional diversifications and that the *VLR* assembly is highly regulated to ensure that a single receptor is expressed by each lymphocyte, as in V(D)J recombination. Thus, although the two adaptive immune systems have evolved with distinct antigen receptors in parallel, they show common features that diverse antigen receptor genes are generated through similar diversification strategies, and each lymphocyte is regulated to express a single receptor.