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Epigenetics of antigen-receptor gene assembly

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Introduction

It is established that the temporal and lineage specificity of V(D)J rearrangement is controlled at multiple levels [1,2]. These include germ-line transcription, chromatin remodeling, histone acetylation and DNA methylation. It is, however, unknown how a region spanning large genomic distances allows the assembly of antigen receptor genes. Recent data have suggested that an underlying structural order must exist that facilitates the association of DNA elements separated by large genomic distances.

B cell development and ordered immunoglobulin gene rearrangement

The developmental progression of B cells has been characterized on the basis of antigen receptor gene rearrangements and on the dynamic expression patterns of cell surface proteins, which have served as maturation markers [3]. The Igh locus is comprised of distinct DNA elements encoding the variable (V), diversity (D), joining (J), and constant (C) regions. Eight Igh constant regions encode for distinct isotypes that include C_{μ}, C_{δ}, $C_{\gamma 1}$ $C_{\gamma 2a}$, $C_{\gamma 2b}$, $C_{\gamma 3}$, C_{α} and C_{ε}. Upstream of the IgM constant region are located twelve D_H and four J_H segments. Fifteen partially dispersed V region families are present that span approximately 2 Mbp of genomic sequence. The IgK and L light chain loci are also organized into distinct DNA elements, encoding V_H, J_H and C_H segments. Ig V_H, D_H and J_H elements are flanked by recombination signal sequences that interact with the RAG1 and RAG2 gene products.

In pro-B cells, Igh D_HJ_H joining precedes that of $V_H(D_H)J_H$ gene rearrangement [4]. Once a V(D)J gene rearrangement leads to a productive joint, pre-BCR signaling acts to inhibit RAG1 and RAG2 gene expression and to promote the survival, expansion and developmental progression of large pre-B cells [5.6]. The expansion phase is followed by cell cycle arrest, during which RAG gene expression is reactivated to initiate the rearrangement of the Ig light chain genes. In the presence of self-reactivity, continued rearrangements will replace primary IgK VJ joints, generating receptors with novel specificities. Once a BCR is expressed which lacks auto-reactivity, tonic signaling mediated by the BCR will permanently suppress RAG1 and RAG2 gene expression. Tonic BCR signaling will also trigger the migration of B cells to the peripheral lymphoid organs where they, upon interacting with pathogens, will undergo class switching and somatic hypermutation.

T-lineage development and T cell receptor gene rearrangement

T-lineage cells also develop sequentially [7]. Two distinct T cell lineages develop from a common progenitor, named αβ and γδ T cells. The rearrangement of the TCRβ and γδ loci is

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initiated in a compartment that lacks the expression of the co-receptors, CD4 and CD8. This population is often referred to as the double-negative (DN) compartment. Once a productive TCRβ V(D)J gene rearrangement has been generated, a pre-TCR complex is formed and continued TCRβ rearrangement is suppressed by signals emanating from the pre-TCR. Committed αβ T cells that express a pre-TCR, will undergo extensive proliferation and ultimately will develop into cells that express both CD4 and CD8, also named double-positive (DP) cells. If developing thymocytes generate a γδ TCR prior to the expression of a pre-TCR, cells will become committed to the γδ T cell lineage. In the DP compartment, TCRα VJ rearrangement will be initiated and completed. Developmental progression at this stage depends on the ability of the αβ TCR to recognize with the appropriate affinity, peptides that are presented by the major histocompatibility complex gene products.

Regulatory factors that control B- and T-lineage specification and commitment

B cell commitment and developmental progression is regulated by the combined activities of distinct classes of transcriptional regulators, which include PU.1, E2A, EBF, and Pax5 (Figure 1A). Recent data have provided a regulatory network in which these proteins act to induce B- and T-lineage specific programs of gene expression [8,9]. In the common lymphoid compartment, PU1, E2A, EBF and Pax5 act together to promote a B cell fate. The gene encoding the IL7Rα chain is a critical target for PU.1 and E2A [8,10]. IL7Rα-mediated signaling then acts in concert with E2A and PU.1 to activate EBF transcription, which in turn induces the expression of Pax5 [11,12]. Once activated, E2A, EBF and Pax5 then act together to induce B-lineage specification and commitment (Figure 2) [8,9].

T-lineage development is controlled by the combined activities of E2A, HEB, GATA-3, TCF1 and Notch-mediated signaling (Figure 1B) [8,13]. The E-proteins, E2A and HEB, form heterodimers to induce the expression of genes involved in Notch- and pre-TCR mediated signaling [8]. Once the Notch signaling pathway is activated, T cells become committed to the T cell lineage, in part, through the induction of Hes1, GATA3 and TCF1 transcription (Figure 2) [9,13,14]. Notch signaling and the E-proteins also act to induce TCRβ V(D)J gene rearrangement [15,16]. Upon formation of a productive TCRβ V(D)J gene rearrangement, pre-TCR signaling inhibits E-protein activity to suppress continued rearrangement, to promote developmental progression and cellular expansion [17].

Developmental-specific regulation of antigen receptor gene assembly

It is established that the rearrangement of antigen receptor loci is dependent on lineage- and developmental-specific modulation of chromatin structure. For example, pro-B cells express high levels of the V(D)J recombinase, RAG1 and RAG2, but only the Igh locus will undergo V(D)J gene rearrangement. IgL VJ joining occurs in pre-B cells, but Igh V(D)J rearrangement is permanently suppressed beyond the pro-B cell stage. TCRβ V(D)J joints are generated in the DN compartment but not in the DP compartment.

How is lineage- and stage-specific antigen receptor gene assembly regulated? In vitro studies have indicated that the IgK, but not the TCRβ locus, in pre-B cells is accessible to the V(D)J recombinase, indicating that chromatin structure determines accessibility [18]. Chromatin accessibility, in part, is regulated by the activities of transcriptional regulators. Ig V(D)J gene rearrangement is controlled by STAT5, Pax5 and YY1, whereas IgL VJ gene rearrangement is regulated by the E2A proteins [10,19,20,21,22,23]. The E2A proteins interact directly with binding sites present in the IgK light chain gene enhancer to modulate IgK VJ gene rearrangement [24]. Furthermore, in the presence of self-reactivity they promote receptor editing [25]. Expression of RAG1 and RAG2 in non-lymphoid cells does not promote IgH and

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IgL chain gene rearrangement. However, upon enforced expression of E2A, EBF and Pax5, IgH and IgL chain gene rearrangements can be readily induced [22,23,26]. Collectively, these data indicate that the targeting of the V(D)J recombinase is regulated in a developmental- and lineage-specific manner by the combinatorial activities of transcriptional regulators. How is chromatin accessibility regulated by the activities of these transcriptional regulators? Various mechanisms have been demonstrated to play critical roles in modulating chromatin accessibility including, germ-line transcription, histone modification, DNA methylation, nuclear localization, chromatin compaction and looping.

The roles of histone modification, DNA methylation and germ-line transcription in antigen receptor gene assembly

More then two decades ago, it was proposed that lineage- and developmental specific regulation of antigen receptor gene assembly, is regulated by chromatin accessibility of Ig and TCR loci to the V(D)J recombinase [27]. Consistent with this proposal are in vitro studies showing that the nucleosome substantially interferes with cleavage of recombination signal sequences by the RAG proteins [28,29]. Histone modification likely plays an important role in this process. A region containing the D_HJ_H cluster initially becomes hyperacetylated in pre-pro-B cells, followed by acetylation of V_H segments once D_HJ_H joints have been generated [30]. Distinct chromatin modifiers regulate Igh accessibility. Ezh2, a H3K27 methyltransferase, normally associated with transcriptional inhibition, promotes IgH V(D)J joining of the distal V_H regions [31]. The transcription factor Pax5 modulates histone-demethylation, whereas STAT5 promotes histone acetylation of distal V_H regions [19,32]. Whereas these studies implicate epigenetic marking in the regulation of antigen receptor assembly, the precise mechanism by which they allow chromatin accessibility to the recombination machinery remains to be determined.

The most common modification of DNA involves the methylation of cytosine residues, mediated by a family of DNA methyltransferases that have the ability to promote the de novo methylation or alternatively modulate hemi-methylated DNA sequences. The role of methylation in antigen receptor assembly has been particularly well studied in the IgK loci [33]. During B-lineage development, demethylation occurs in a monoallelic fashion prior to the onset of IgK VJ gene rearrangement [34]. Interestingly, recent studies have indicated that the demethylated IgK allele is also selectively targeted by AID [35].

Both sense and anti-sense germ-line transcription have been shown to correlate well with Igh V(D)J joining [27,36]. Germ-line transcription is controlled by enhancer and promoter elements [3]. Pre-BCR signaling activates IgK germ-line transcription in large-pre-B cells, whereas TCRα germ-line transcription is induced during β-selection [3]. The presence of germ-line transcripts encoded by antigen receptor genes has raised the question whether they play a critical role in modulating antigen receptor gene assembly. Most compelling evidence for a role in germ-line transcription in antigen receptor gene assembly has been obtained from studies focused on the regulation of TCRα VJ rearrangement. Inhibition of transcriptional elongation severely perturbed TCRα VJ rearrangement as well as remodeling of Jα promoters [37].

Nuclear location, compaction, looping and antigen receptor gene assembly

During the past decade, structure preserving three-dimensional fluorescence in situ hybridization and high precision epifluorescence microscopy, have provided insight into the organization and nuclear localization of antigen receptor genes in developing lymphocytes (Figure 3). In non-lymphoid cells as well as T cells, the Igh locus is positioned in close proximity to the nuclear membrane, whereas in committed pro-B cells the Igh locus is located

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in central nuclear domains [38,39]. In peripheral B cells, the untranscribed Igh locus is associated with heterochromatic DNA domains, while the transcriptionally active Igh allele is located away from the heterochromatin [40].

The IgK locus is also repositioned during B-lineage maturation. In pre-pro-B cells, the IgK locus is associated with the nuclear membrane, whereas in committed pro-B cells, the IgK loci move to centrally located nuclear domains [41,42]. At the small pre-B cell stage, one IgK allele is localized at the centromeric regions, whereas the other allele is repositioned away from the nuclear membrane and centromeric regions. The association of the IgK locus with centromeric heterochromatin is mediated by a silencer element, termed Sis, located within the IgK locus [43].

The Igh locus undergoes substantial large-scale genomic reorganization during B cell development. The Igh locus in T-lineage cells shows an extended configuration, whereas the Igh topology is contracted in central nuclear areas of pro-B cells [38,44]. Recent observations have demonstrated that two transcriptional regulators, Pax5 and YY1, modulate Igh locus contraction (Figure 3) [19,21].

Pax5 is required to promote distal, but not proximal, V(D)J gene rearrangement [20]. Whereas the distal Igh V_H regions fail to recombine in Pax5-ablated cells, they are accessible to the recombination machinery [20]. Interestingly, the spatial distances separating the distal V_H regions from the C_H regions were substantially increased in Pax5-deficient pro-B cells [19]. Thus, Pax5 may act in pro-B cells, at least in part, by increasing the frequency by which distal V_H regions encounter D_HJ_H elements.

Recent observations have suggested another player, YY1, involved in the control of Igh V(D)J gene rearrangement. YY1 is a zinc-finger containing protein, that has remained conserved throughout invertebrate and vertebrate evolution. It is ubiquitously expressed but since it interacts with elements present in the Ig enhancers, it has been suggested to play critical roles in immunoglobulin gene regulation [45,46,47]. Recent data have shown that B cell development in YY1-deficient mice is blocked prior to the onset of Igh V(D)J gene rearrangement [21]. As described for Pax5-ablated mice, the defect in V(D)J gene rearrangement is most severe in the distal V_H regions. Furthermore, the spatial distances separating the distal V_H regions from the C_H elements are substantially increased in YY1 pro-B cells when compared to wild type pro-B cells [21]. YY1 does not modulate the expression of Pax5, indicating that YY1 acts independently of Pax5 to control locus contraction and V(D)J gene rearrangement [21].

How do Pax5 and YY1 modulate IgH topology? Although still to be proven, it is conceivable that the Igh locus is organized in clouds of loops. Pax5 and YY1 may act to modulate either loop size or the spacing between putative loops. Alternatively, the Igh locus might be organized into clusters of loops and Pax5 and/or YY1 may modulate the spacing between clusters of loops, perhaps through the induction of de novo loops (Figure 3). Obviously, it will be important to determine how the Igh locus is organized in 3D-space.

Allelic exclusion and nuclear geography

The analysis of the nuclear organization of Igh genes has also provided insight into the mechanisms that underlie the allelic exclusion process [33]. Mono-allelic looping involving Igh V_H and D_HJ_H elements occurs with substantially higher frequencies in committed pro-B cells as compared to non-B lineage cells (Figure 3) [44]. Mono-allelic looping might be caused by the low probability of V_H regions to encounter D_HJ_H elements in pro-B cells and/or allelic differences in chromatin structure [44]. Similarly, allelic differences and rare induction of

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enhancer activity may also contribute to the allelic exclusion mechanism in the IgK locus [48].

Other levels of allelic exclusion of antigen receptor genes involve feedback mechanisms, mediated by pre-BCR and pre-TCR signaling. Pre-BCR mediated signaling results in the decontraction of the Igh locus [41]. Spatial distances separating the V_H regions from the D_HJ_H elements in B cells expressing a pre-BCR have not been directly measured, but it seems likely that these distances would be increased as well upon pre-BCR signaling. As described above, larger spatial separation would result in decreasing probabilities for V_H regions to encounter D_HJ_H elements, contributing to the allelic exclusion mechanism. It has been proposed that additional mechanisms act to regulate the Igh allelic exclusion process. Once a functional V(D)J joint has been generated, the second Igh allele relocates to the heterochromatic region, possibly preventing continued Igh V(D)J gene rearrangement [41]. Mono-allelic activation and mono-allelic silencing have also been suggested to play critical roles in the mechanisms that control TCR allelic exclusion [49]. The TCRβ and TCRα loci show substantial locus contraction in the DN and DP cell stage, respectively. Decontraction of the TCRβ locus was observed in DP thymocytes. As described for the Ig loci, mono-allelic association of the TCRβ locus with heterochromatic regions was observed [44]. The critical issue is now to determine whether mono-allelic looping involving variable and DJ elements is controlled by epigenetic mechanisms or alternatively by mono-allelic activation of enhancer activity. It is conceivable that the probability of V regions to encounter DJ elements in developing lymphocytes is low preventing bi-allelic rearrangements. Statistical analysis describing cumulative frequency distributions of spatial distances separating the V and DJ elements should provide insight into the probabilistic nature of antigen receptor gene rearrangement.

Consistent with a probabilistic nature of antigen receptor gene rearrangement are the observations that the dosage of E2A proteins is rate limiting with regard to TCRγ VJ and TCRδ V(D)J gene rearrangement [50]. Similarly, in pre-B lineage cells, receptor editing is highly sensitive to the dosage of the E2A proteins [25]. Thus the low dosage of the E2A protein may prevent bi-allelic Igh enhancer/promoter activation [48]. Epigenetic mechanisms likely contribute to the allelic exclusion process by allowing or suppressing the ability of transcriptional regulators to interact with regulatory elements in antigen receptor loci [33].

Collectively, these studies suggest that allelic exclusion is regulated, at least in part, by four distinct epigenetic mechanisms: (1) Mono-allelic looping. (2) Mono-allelic activation of enhancer activity. (3) De-contraction of antigen receptor genes mediated by pre-BCR or pre-TCR signaling. (4) Association of antigen receptor genes with hetero-chromatic regions.

Organization of antigen receptor loci

Much has been learned during the past decade about the nuclear localization and contraction mechanisms of antigen receptor genes. However, how the Ig and TCR genes are organized in 3D-space is unknown. Understanding the topology and mechanics of antigen receptor assembly will require a statistical description focused on the average size and shape rather than a static structure. Ultimately, it should be possible to describe the spectrum of antigen receptor conformations in terms of statistical mechanics, providing a physical explanation for the mechanisms that underlie the assembly of antigen receptor genes.

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underwent long-range contraction in the DP and DN compartment, respectively. It is suggested that locus decontraction contributes to the allelic exclusion mechanism of TCR genes

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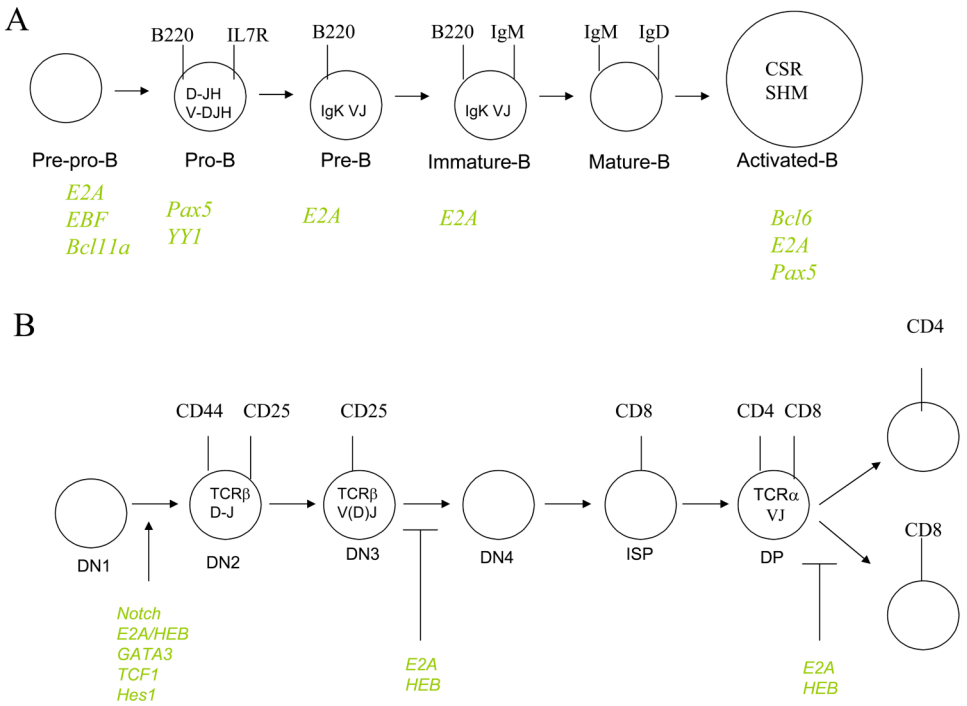

Figure 1.

(A) Diagram depicting B cell development. Ig D_HJ_H and $V_HD_HJ_H$ gene rearrangements are indicated. Transcriptional regulators controlling distinct checkpoints during B-lineage maturation are shown. (B) Diagram depicting T-lineage development. TCR gene rearrangements are indicated. Notch signaling and transcriptional regulators controlling distinct checkpoints during T-lineage maturation are shown.

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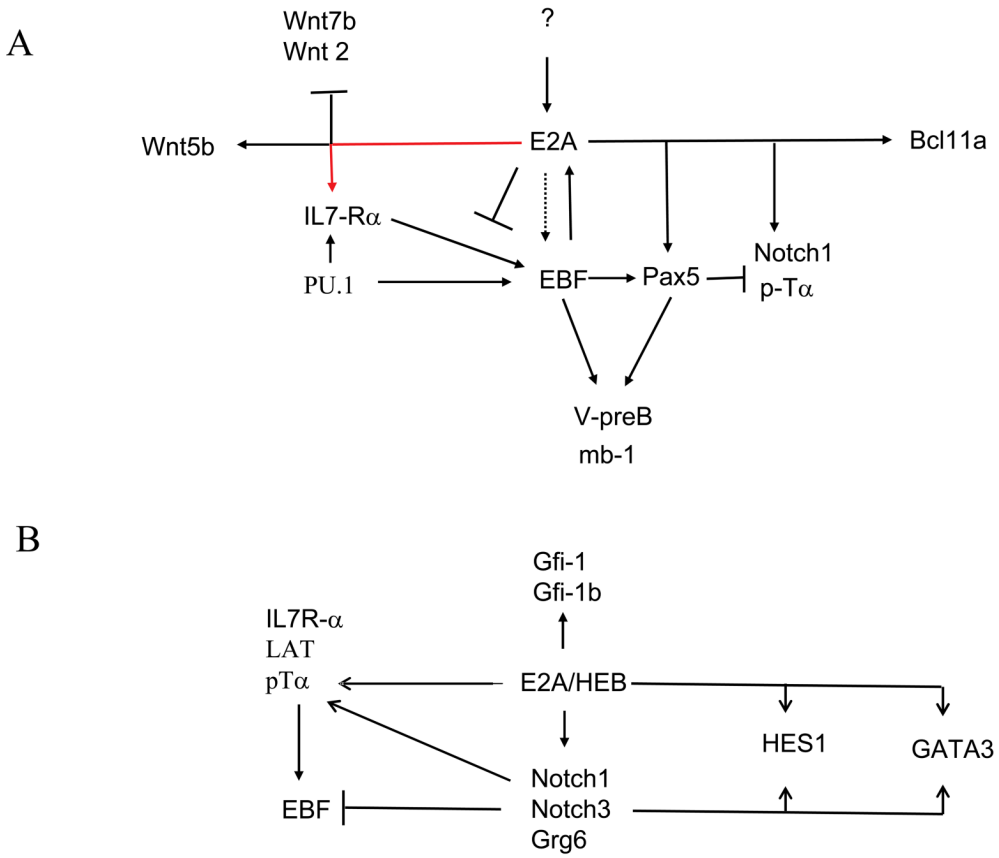

Figure 2.
(A) Regulatory network controlling B-lineage specification and commitment is shown. (B) Regulatory network controlling T-lineage development is indicated.

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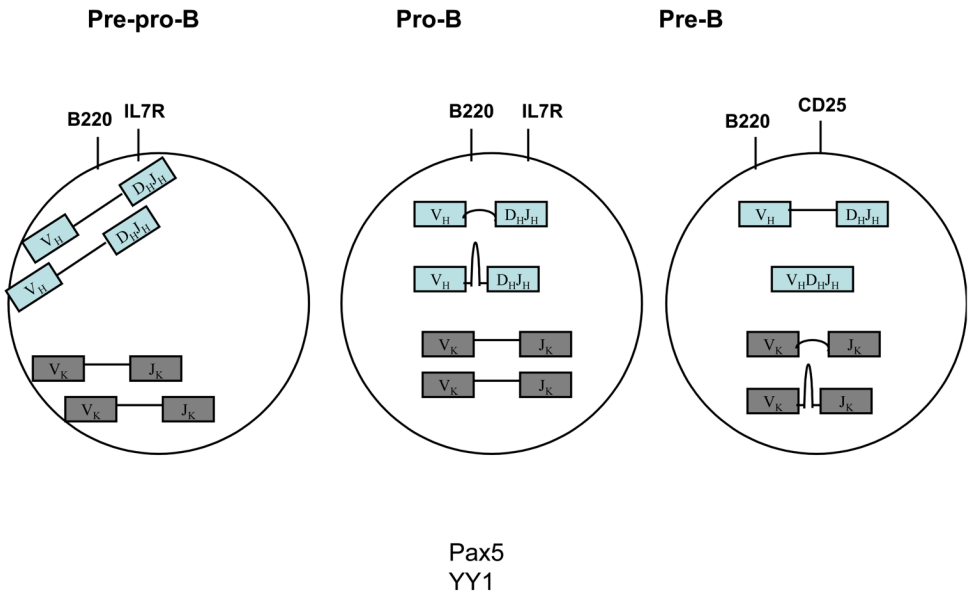

Figure 3.

Spectrum of Igh and Igl topologies and nuclear location in pre-pro-B, pro-B and pre-B cells. Indicated are the nuclear membrane localization of the Igh and Igl loci in pro-B cells. Igh locus contraction is observed on both alleles in pro-B cells, presumably mediated by looping of the intervening sequences. Mono-allelic looping bringing the Igh V, D and J elements into close proximity has been observed at the pro--B cells but not at the pre-pro-B cell stage. The IgL loci are also proposed to undergo mono-allelic looping (note that this still has to be demonstrated).