A23187 (Ref. 27). In addition, transgenic mice carrying N-*myc* under the control of the Ig heavy chain enhancer continue to express *RAG1* and *RAG2* in their mature B cells that carry Ig on the surface. *RAG* expression in these cells is turned off in response to crosslinking of the surface Ig, but is restored after the crosslinking reagent is removed[28]. Understanding the signals required to turn *RAG* transcription on and off *in vivo* in B and T cells could lead to an understanding of some of the complex regulatory events that take place during lymphocyte maturation.

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Creation of immunoglobulin diversity by intrachromosomal gene conversion

CRAIG B. THOMPSON

Not all vertebrates create an immunoglobulin repertoire through the recombination of individual members of variable (V), diversity (D) and joining (J) gene segment families. In chickens, for example, a diverse set of immunoglobulins is created by intrachromosomal gene conversion of the single variable gene segments of the immunoglobulin heavy and light chain genes. Recent evidence from other species such as the rabbit suggests that gene conversion may be a more widespread mechanism for the creation of immunologic diversity than previously supposed.

A central feature of the vertebrate immune system is the ability to produce a specific antibody response to a wide variety of foreign antigens. The genetic information that encodes this diversity is stored in a relatively small number of germ-line immunoglobulin (Ig) gene segments. During B cell development, these elements undergo somatic recombination to generate a primary immunoglobulin repertoire[1–3]. An Ig heavy chain gene (IgH) is created through recombination of individual variable (V), diversity (D) and joining (J) elements. A functional Ig light chain gene (IgL) is assembled from a similar assortment of V and J gene segments. Because of the large number of individual V, D and J elements in the germ line, and additional diversity created by the variation in the position of joining of individual elements, V(D)J recombination can potentially give rise to more than 10^{11} distinct antibody molecules during human B cell development[4,5]. Thus, in some species, the diversity created by the V(D)J recombination mechanism is sufficient to lead to the generation of a significant immune repertoire.

However, not all species create their immunoglobulin repertoires by V(D)J recombination. In some species, V(D)J recombination appears to serve only to activate Ig gene expression in a B cell lineage-specific fashion. In these species, an immunoglobulin repertoire is created during the subsequent stages of B cell development by gene conversion. Gene conversion has been documented as a mechanism for the generation of immunologic diversity in rabbits and in a variety of avian species[6–11]. The study of B cell development in these species provides an opportunity to study the molecular regulation of somatic gene conversion in higher eukaryotes.

Avian B cell development

The chicken Ig heavy and light chain loci each contain only single V and J gene segments, which undergo recombination between days 10 and 15 of

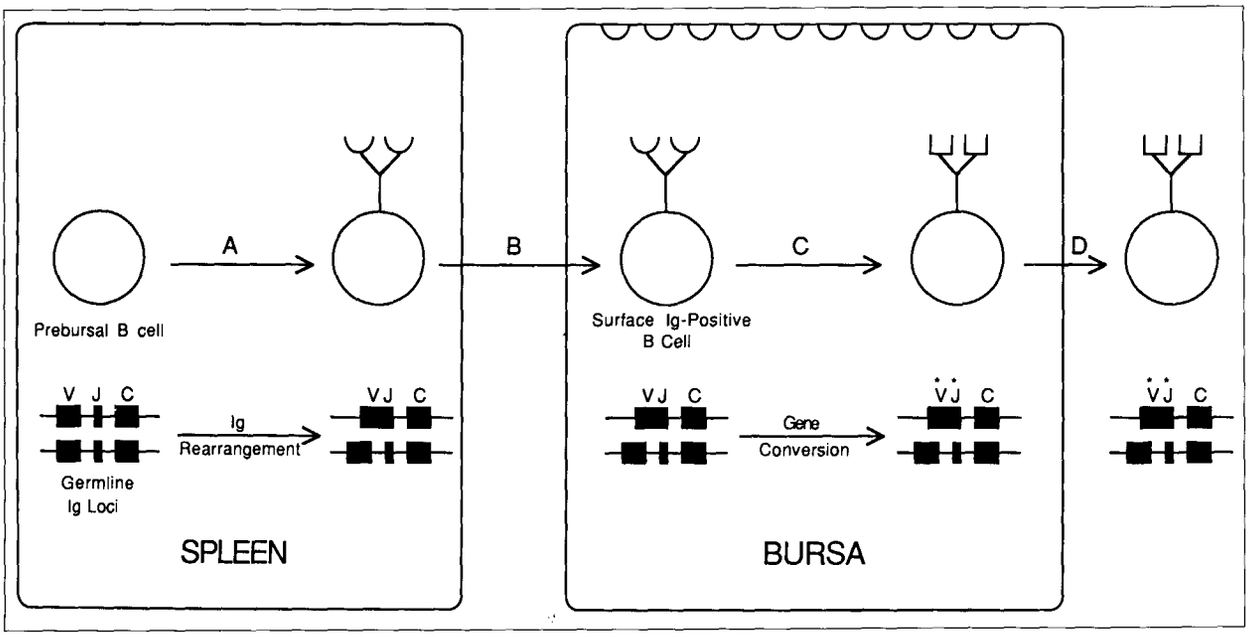

FIG 1

A model for B cell development in the chicken. Prebursal stem cells become committed to B cell development in the splenic anlage between days 10 and 15 of embryogenesis. These cells initiate rearrangement of their immunoglobulin genes (A) and migrate to the bursa of Fabricius (B). Once in the bursa, cells that express a functional immunoglobulin gene on their surface are induced to proliferate. The immunoglobulins expressed on the surface of these B cells have the specificity of the single functional germ-line encoded V and J gene segments. It is suggested, therefore, that this antibody may recognize a self-antigen present in the bursa and initiate B cell proliferation in an antigen-dependent fashion. Within the bursa, proliferating B cells undergo gene conversion of their rearranged IgL and IgH V gene segments (C), thus altering the antigenic specificity of their surface immunoglobulin. B cells that have lost specificity for the bursal self-antigen or any other self-antigen expressed in the bursa cease proliferation and acquire the ability to migrate to the periphery (D). Peripheral B cells collectively produce a diversified set of non-self-reactive immunoglobulins which provide the bird with a primary immunologic repertoire for responding to foreign antigens.

embryogenesis at the time when splenic lymphoid progenitor cells differentiate into B cells[6,7,12–14]. By day 18 of embryogenesis, all lymphoid progenitor cells capable of differentiating into B cells have completed V(D)J recombination[12,15,16]. Cells that undergo immunoglobulin gene recombination also acquire the ability to migrate to the bursa of Fabricius, a developmental lymphoid organ in the posterior cloaca of avian species (Fig. 1).

Within the environment of the bursa of Fabricius, B cells expand in number from 3–5 × 10^4 Ig-positive progenitor cells to 1–2 × 10^9 mature B cells. All of the progenitors that undergo this proliferative expansion express an Ig molecule encoded by the same V_H and J_H and V_L and J_L elements[7,13,17]. However, during the bursal phase of B cell development, the rearranged V gene segments of both the heavy and light chain genes in each cell undergo progressive sequence diversification by intrachromosomal gene conversion using sequence information derived from pseudo-V (ΨV) gene segments located 5' of the functional V element[7,17,18]. This process is restricted to the rearranged V gene segment, does not occur in the unrearranged V gene segment of cells in which the other parental allele is in the germ-line configuration, and cannot be detected at other genetic loci within developing B cells in the bursa of Fabricius[19,20]. During B cell development, both the IgL and IgH genes in each cell will undergo 4–10 of these intrachromosomal gene conversion events before the cell completes the bursal-dependent phase of development and migrates to the peripheral lymphoid organs[7,17]. Theoretically, this process of segmental gene conversion can result in the creation of an immunoglobulin repertoire of at least 10^{11} distinct antibody molecules. Thus, gene conversion can be an effective strategy for the development of a primary immune repertoire.

Rabbit immunoglobulin gene diversity

The use of gene conversion to generate an immunoglobulin repertoire is not restricted to avian species. Recent studies have demonstrated that 70–90% of rabbit immunoglobulin heavy chains are encoded by the same V_H gene segment[21,22]. The preferential use of a single V_H gene segment explains the mendelian inheritance of allotypic markers linked to the rabbit V_H region, an observation that has long puzzled immunologists. Nevertheless, the rabbit has several hundred additional V_H gene segments. Sequence analysis of the rearranged V_{H1} gene segment from adult B cells shows that it has acquired nucleotide substitutions and codon insertions and deletions from these other V_H gene segments[10]. This suggests that, just as in the chicken Ig loci, somatic gene conversion is used to generate diversity within the preferentially rearranged V_{H1} gene segment in the rabbit. Thus, the use of gene conversion to generate somatic diversity appears to be more widely distributed among vertebrates than at first supposed. Ongoing studies in several laboratories should soon provide us with additional developmental and

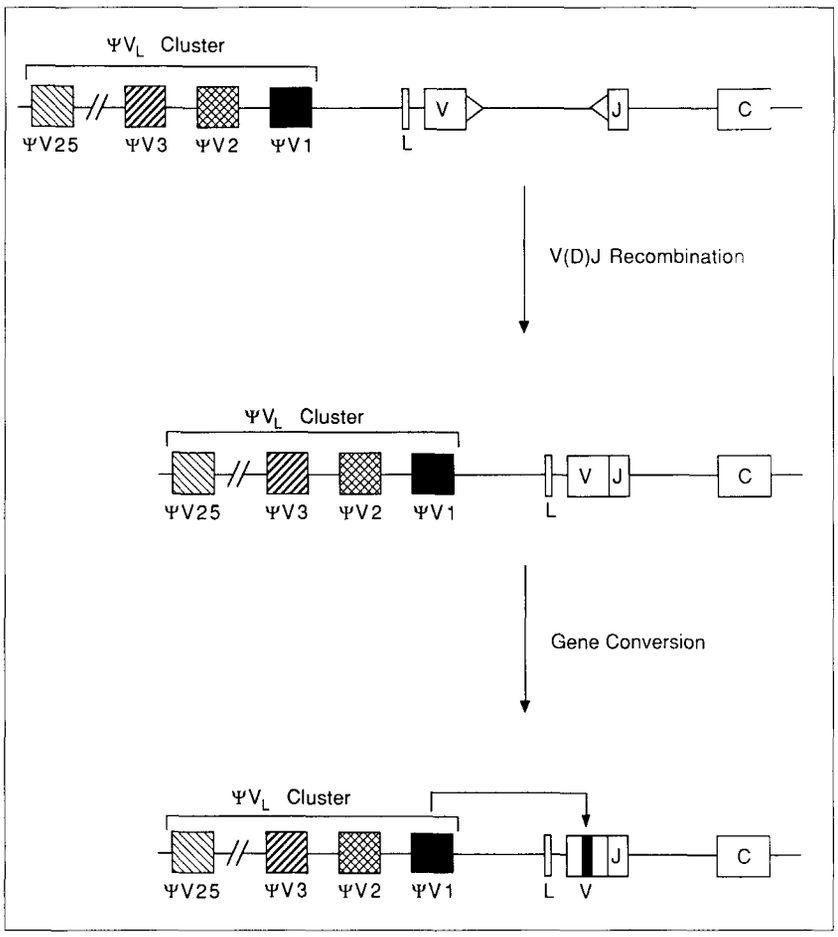

FIG 2

The chicken IgL locus. The unrearranged IgL locus is composed of single functional V, J and C elements. Upstream of the functional V element are a set of 25–27 ΨV gene segments. Triangles represent recombination signal sequences. These recombination signal sequences direct V(D)J recombination of the single unique V and J gene segments. Within the bursa of Fabricius, functionally rearranged immunoglobulin light and heavy chain genes undergo progressive sequence diversification. The rearranged V gene segments are somatically diversified by sequence substitutions copied from the ΨV gene segments.

molecular details concerning how this process is regulated in rabbits (for review see Ref. 23).

The chicken IgL locus

Most work on the molecular regulation of somatic gene conversion has been carried out on the chicken Ig light chain gene, because of its size and organization (Fig. 2). The entire chicken Ig light chain locus occupies just over 25 kbp of DNA[17]. The locus is flanked by clusters of lymphoid-specific DNase I hypersensitive sites[19]. The single germ-line V_L, J_L and C_L elements are linearly arranged in the same transcriptional orientation at the 3' end of the locus. In the germ line, the single V_L and J_L gene segments are separated by 1.8 kbp of DNA which contains the recombination signal sequences necessary to direct the V–J joining reaction during development. Located 5' of the germ-line V_L element is a small leader exon and the promoter region, which is similar in organization to mammalian Ig promoters. In contrast, the ΨV gene segments lack Ig-specific promoter elements and recombination signal sequences[17].

ΨV gene segments as gene conversion donors

Rearranged V–J segments have been sequenced from B cells isolated from the bursa of Fabricius at various stages of development[9,17,24], and sequences of the rearranged V_L gene segments compared with those of the germ-line V_L and ΨV gene segments. Studies of gene conversion in the F_1 progeny of two inbred chicken strains demonstrate that gene conversions are limited to ΨV and V_L gene segments present on the same parental allele[9]. It has also been shown that there is no reciprocal transfer of genetic information, and that sequence transfer does not require the presence of a duplicated parental allele. Thus, the process by which sequence transfer occurs during IgL diversification appears to be intrachromosomal gene conversion.

Several observations concerning the frequency with which an individual pseudogene is used as a gene conversion template have been reported[17,25]. The pseudogenes located close to the rearranged V_L gene segment were more likely to be used as donors than those at the distal end of the locus. In addition, pseudogenes with greater sequence similarity to the V_L gene segment are used more frequently than those that have a lower percentage of sequence identity or a shorter length of similarity with the V_L gene segment. In fact, ΨV gene segments that have less than 150 bp of sequence similarity with the V_{L1} gene segment do not appear to be used to create diversity during B cell development. This finding suggests that a minimal length of homology may be required for the alignment of the donor and recipient sequences.

Finally, the ΨV gene segments are organized in both the sense and antisense orientation relative to the transcription of the V_L gene segment. Surprisingly, pseudogenes in the antisense orientation are used three to five times more frequently than pseudogenes in the sense orientation, even when distance and homology are taken into consideration. The reason for this bias is not immediately obvious. However, if the donor and recipient segments need to be aligned during the process of gene conversion, then segments on the same chromosomal segment that are in the antisense orientation can be aligned by a simple fold back of the DNA, whereas segments in the same orientation on a chromosomal segment require more complex folding to achieve alignment.

Characteristics of individual gene conversion events

Gene conversion tracts can begin and end at multiple positions within the rearranged V gene segments (Fig. 3). No consistent nucleotide sequence is observed at either end of a gene conversion tract[17,25]. However, several observations suggest that gene conversions occur in a directional manner[25]. Relative to the transcriptional orientation of the V_L gene segment, the 5' ends of gene conversion tracts always begin in regions of sequence identity between the V_L segment and the ΨV templates. Gene conversions rarely occur within the first 45 bp of 5' homology between V_L and the ΨV gene segments. In contrast, 3' ends of gene conversion tracts often occur at positions where the donor and recipient gene segments are not identical. The 3' ends of gene conversion tracts occasionally extend into the J_L segment. Gene conversion can also add or delete codons from the rearranged V_L gene segment, a process that occurs primarily at the 3' end of gene conversion tracts. Together, these observations suggest that gene conversions occur in a 5'→3' direction relative to the transcriptional orientation of the rearranged V_L gene segment.

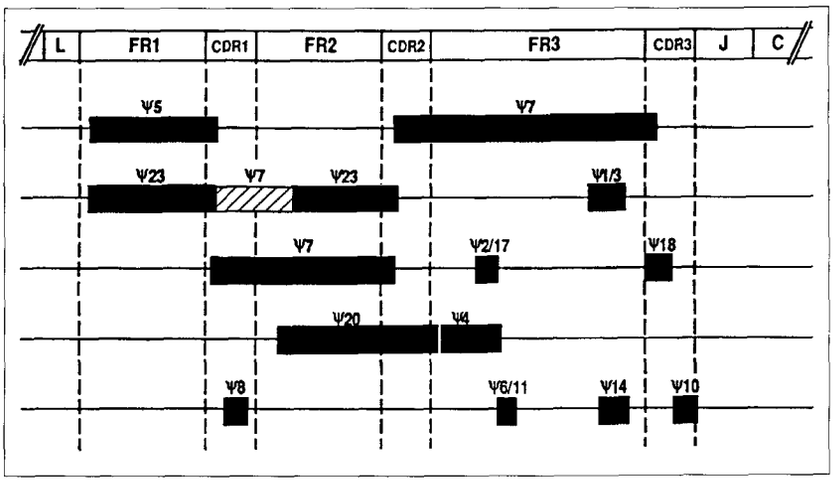

FIG 3

The position and sizes of gene conversion events in five rearranged V_L gene segments isolated from the bursa of an 18-day embryo. The complementarity-determining regions (CDR1, CDR2, CDR3), which are predicted to determine the antigen-binding specificity of the V region, and the framework regions (FR1, FR2, FR3), which encode conserved structural components of the V region, are indicated. As few as 10 bp or as many as 125 bp are involved in the gene conversions depicted.

Within the Ig light chain locus, gene conversion is targeted to the rearranged V_L gene segment. In B cells undergoing development in the bursa of Fabricius, the V_L gene segments of parental alleles that are in a germline configuration do not undergo gene conversion or transcription[19]. Gene conversion and recombination of the ΨV gene segments has not been observed[9]. ΨV segments lack the promoter elements normally associated with immunoglobulin-specific promoters and do not appear to be transcribed in bursal lymphocytes.

Box 1. Molecular characteristics of the Ig gene conversions that occur in the bursa of Fabricius

(1) Gene conversions occur in *cis*.

(2) The frequency with which a pseudogene is used as a conversion template is determined by:
(i) distance from V_L,
(ii) orientation,
(iii) length of homology.

(3) Gene conversions occur in a directional manner.
(i) 5' ends begin in regions of sequence identity between the V_L and the ΨV templates and rarely occur within the first 45 bp of homology between V_L and the ΨV segments.
(ii) 3' ends often occur at positions where donor and recipient are not identical, occasionally extend into J sequences, and can result in loss or duplication of base pairs.

(4) The activation of the bursal-specific gene conversion is associated with transcription of the rearranged IgL gene.
(i) The V_L segment of the unrearranged allele is neither transcribed nor undergoes gene conversion.
(ii) Gene conversion and/or recombination within the ΨV region has not been observed.
(iii) Crossovers between alleles have not been observed.

(5) Gene conversion is limited to the Ig loci.

A molecular model of somatic immunoglobulin gene conversion

A molecular model for chicken V gene segment conversion has been proposed[25] (Fig. 4) that accounts for the novel features of the somatic gene conversion observed in the chicken immunoglobulin loci (summarized in Box 1). Displacement (D) loop formation and strand extension by a DNA polymerase would explain why 5' ends of gene conversion events always begin in regions of sequence identity between the donors and recipients. Most DNA polymerases that have been described to date require several base pairs of sequence identity to initiate strand extension from a 3' end on a homologous template. If stable D loop formation requires 35–50 bp of homology between the heteroduplexed strands, then initiation of gene conversion by D loop formation would explain why there is no detectable gene conversion within the first 45 bp of homology between the V region and the ΨV gene segments. Finally, the structural features unique to the 3' ends of gene conversion events could be caused by imprecise strand ligation. The duplication or loss of codons at the 3' ends of gene conversion events argues against sequence transfer resulting from D loop formation and heteroduplex repair within the D loop.

A single-strand model for the transfer of information from the pseudogene donors to the rearranged V gene segment is favored over models involving crossovers of both strands of DNA because no recombination products can be detected as a byproduct of gene conversion (Ref. 9; L. Tjoelker and C. Thompson,

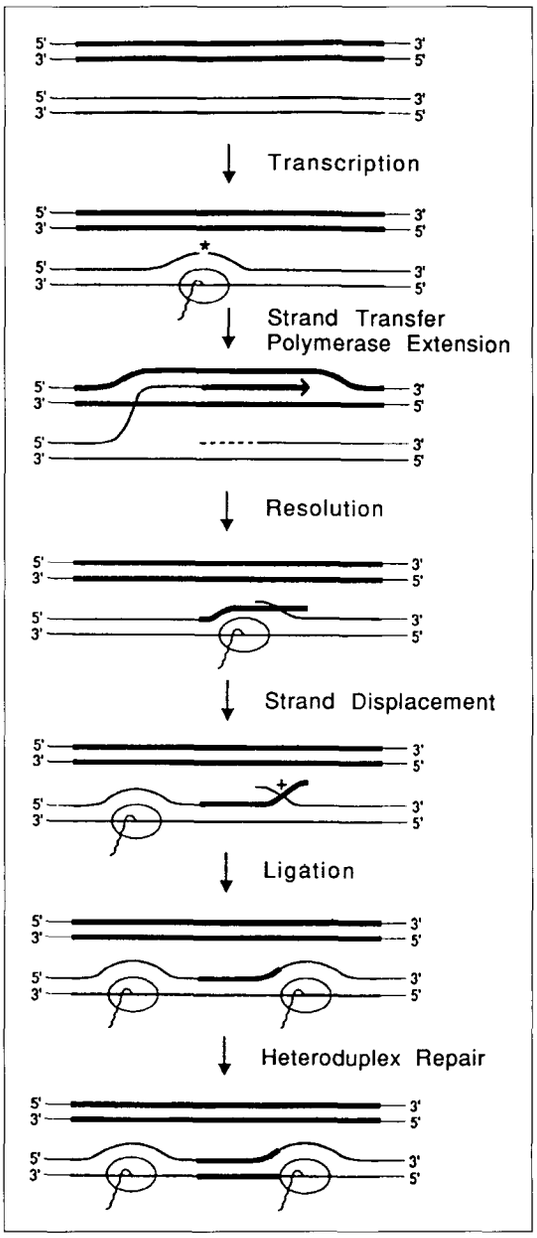

FIG 4

A hypothetical model for chicken immunoglobulin gene conversion. At the top, a ΨV and the rearranged V_L segments on the same parental allele are shown aligned in the 5'→3' orientation. The ΨV_L donor is represented by thick lines, the recipient V_L is represented by medium weight lines, and flanking nonhomology is shown as thin lines. This model proposes that sequence transfer is initiated by transcription, which exposes single-strand breaks in the nontranscribed strand of the V_L gene segment (•). The unwound free 3' end is then transferred into a homologous duplex, creating a displacement (D) loop by strand displacement in a 3' to 5' direction. The intercalated 3' end acts as a primer to initiate DNA synthesis using the ΨV_L segment as a template. After the D loop has been dissolved by the action of RNA polymerase, this extended strand is then rewound into the recipient locus. Ligation of the extended strand product to the remaining nontranscribed strand in the recipient locus results in formation of heteroduplex DNA, and the conversion is completed by either heteroduplex repair or DNA replication. Figure adapted from Ref. 25.

unpublished). This suggests that a Holliday junction is not formed during somatic Ig gene conversion. Although this model is consistent with existing information on somatic Ig gene conversion, it has been most useful in making several testable predictions for the gene conversion process. The model suggests that (1) the gene conversion is dependent on strand breaks that occur specifically within the rearranged immunoglobulin gene locus; (2) the process is dependent on transcription; and (3) cells undergoing gene conversion must be proficient in homologous recombination.

Evidence for Ig-specific strand breaks

The specificity with which the Ig genes undergo gene conversion during B cell development in the bursa of Fabricius implies that there is a mechanism to target the Ig loci for gene conversion. One mechanism that would target the Ig genes for recombination is the creation of Ig-specific strand breaks that would leave free ends of DNA necessary for the initiation of recombination. Such a mechanism is used during mating-type switching in *Saccharomyces cerevisiae*: a double-strand break created by the HO endonuclease targets the mating-type locus for gene conversion (see review by Haber[26] in this issue). To date, there is no definitive evidence that similar single- or double-strand breaks occur within the rearranged immunoglobulin loci. However, there are several major DNase I hypersensitive sites that are bursal-specific and localized within the rearranged V gene segment[19]. These DNase I hypersensitive sites are not present on the unrearranged V gene segment of bursal lymphocytes nor in developing T lymphocytes. Strand breaks at these sites can be observed in DNA isolated from bursal lymphocytes, but it is not clear whether these strand breaks are created during DNA preparation as a result of the action of endogenous nucleases, or represent authentic strand breaks at these sites within some cells developing within the bursa of Fabricius.

Sequence analysis has suggested that certain stretches of DNA within the V region are involved in conversion tracts more frequently than others[25]. These tracts are found primarily within the complementarity-determining regions (CDR) of the rearranged V gene segment, regions predicted by X-ray crystallography to form the antigen-binding pocket of the immunoglobulin molecule. It is possible that the increased frequency in gene conversion of these sites results from immunological selection. An alternative possibility is suggested by the fact that these sites are flanked by evolutionarily conserved sequences resembling the heptamer sequence that targets immunoglobulin V(D)J recombination[17]. Consistent with a role for this heptamer sequence in gene conversion, a bursal-derived cell line that constitutively undergoes immunoglobulin gene conversion in culture[27,28] and that has acquired a point mutation in the heptamer sequences 5' of the CDR3 domain, no longer undergoes gene conversion within the CDR3 domain. Together, these data suggest that the activities that normally recognize and cleave the heptamer sequence of the V(D)J recombination signal sequence may be involved in targeting gene conversion to the rearranged immunoglobulin locus.

It is unlikely, however, that the full V(D)J recombinase is active during the bursal phase of B cell development, since ongoing rearrangement of immunoglobulin genes cannot be detected at this stage[12,13]. To address the potential role of the V(D)J recombinase in Ig gene conversion, the expression of the recombinase-activating genes, *RAG1* and *RAG2*, has been studied during avian lymphoid development[29]. In contrast to

cells undergoing V(D)J recombination, which always coexpress *RAG1* and *RAG2*[30], chicken B cells undergoing gene conversion in the bursa of Fabricius fail to express *RAG1* but express high levels of *RAG2*. This selective expression of *RAG2* appears to be unique to the avian bursa of Fabricius, since chicken T cells stop expressing *RAG1* and *RAG2* at the same time upon completion of thymic development. Thus, the selective expression of *RAG2* during B cell development in the bursa of Fabricius suggests that *RAG2* or genes under the control of *RAG2* may play a role in the regulation of immunoglobulin gene conversion. Alternatively, selective expression of *RAG2* may be merely a marker of an intermediate stage of B cell development that exists in avian species. *RAG2* does not appear to be absolutely required for gene conversion, as a bursal-derived cell line that undergoes Ig gene conversion *in vitro* maintained the ability to undergo gene conversion even after both copies of *RAG2* were deleted by homologous recombination[31]. Additional studies to determine the physiological role of *RAG2* and to determine if it can mediate, or interact with genes that can mediate, DNA strand cleavage should help to clarify this issue.

IgL gene conversion is dependent on transcription

As depicted in Fig. 4, the proposed model for immunoglobulin-specific gene conversion is dependent on the transcription of the recipient sequence. However, a rearranged V_L gene segment differs in two ways from the ΨV gene donors and unrearranged V_L gene segments. First, the rearranged V_L gene segment has been juxtaposed to the J_L gene segment, and second, IgL transcription is activated by the rearrangement process. In an attempt to determine whether transcription is in fact required for the initiation of V_L gene conversion, we have been studying gene conversion within the IgL gene of the Muscovy duck (Ref. 8; L. Tjoelker and C. Thompson, unpublished). Several of the pseudogene segments in the Muscovy duck V_L locus retain recombination signal sequences at their 3' flank. These ΨV segments are capable of undergoing V(D)J recombination and are retained within some developing B cells because of productive V(D)J rearrangement on the other parental allele in these cells. Such rearranged ΨV segments, which lack immunoglobulin promoter sequences, fail to undergo sequence diversification during B cell development in the bursa of Fabricius. In contrast, rearranged ΨV gene segments that have promoters are capable of undergoing gene conversion, even though they cannot lead to a functional gene product because of internal stop codons.

Thus, the ability to undergo V–J recombination is not sufficient to activate the gene conversion process. Either sequences present in the immunoglobulin promoter region or the act of transcription itself is required to initiate gene conversion. The promoter sequences that are missing from the ΨV gene segments are outside the stretch of homology between the V region and the ΨV gene segments. Thus, it seems unlikely that these sequences act as an initiation point for gene conversion tracts since sequence transfer appears to occur exclusively in regions of high sequence similarity. These data suggest that transcription plays a fundamental role in the mechanism of gene conversion.

Avian B cells are proficient at homologous recombination

The ability of avian B cells to carry out homologous recombination has recently been examined. Buerstedde and Takeda have shown that chicken B cell lines have a much higher ratio of targeted to random integration events than other avian cell types or cell lines from other complex eukaryotes[32]. Furthermore, this process showed cell type specificity in that it was observed in a variety of B cell lines but not in three non-B cell lines. This high rate of homologous recombination was not specific for the immunoglobulin locus, as a high ratio of targeted to random integration was also observed at the β-actin locus. This B cell-specific homologous recombination process does not appear to require transcription, as a construct containing flanking sequences derived from the non-transcribed vitellogenin locus also underwent targeted integration. Thus, avian B cells appear to have a specialized form of homologous recombination that may facilitate gene conversion, but this does not explain the selectivity for the immunoglobulin locus that is observed during B cell development. Homologous recombination was also observed in several B cell lines that do not undergo gene conversion *in vitro*. The presence of homologous recombination in B cells that have completed the bursal stage of development suggests that homologous recombination is not itself the regulated component that targets the immunoglobulin locus for gene conversion during the bursal stage of B cell development. However, avian B cells do appear to provide a unique opportunity to study the genes involved in targeted integration (i.e. homologous recombination) in higher eukaryotic cells.

Conclusions

Studies of the avian immunoglobulin genes have demonstrated that gene conversion can play an important role in the somatic diversification of DNA. Rabbits also appear to use gene conversion to create much of their immunoglobulin diversity. The continued study of the immunoglobulin loci in these species may provide additional insights into how the processes of immunoglobulin site-specific recombination and gene conversion are regulated.

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Genetic variation in pathogenic bacteria

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In contrast to textbook ideas of pure cultures and defined strains, genetic variation is a fact of life in the microbial world. It not only allows pathogens to establish themselves in their chosen host, but also allows them to resist that host's subsequent attempts to evict them. Here we review some of the mechanisms that bring about this variation, and some of the functional consequences that result from it.

Although seemingly homogeneous, microbial populations representing a single strain are almost always heterogeneous at the cellular and genetic levels. This remarkable phenomenon, which has been intensively studied in a small number of model microorganisms, particularly pathogens, is probably widely distributed in the prokaryotic kingdom. It results from spontaneous changes in the genetic information of a species, here termed genetic variation*, which occur at relatively high frequencies and thus affect even small populations. Often such changes lead to the phenotypic variation of surface components. This is particularly important in the case of pathogenic bacteria because most of the variable structures studied so far have turned out to be essential either for the colonization of the host, or for the survival of the pathogen within that host. The ability to vary the immunogenicity of important structures and to 'fine tune' the specificities of receptors or adhesins is of considerable evolutionary advantage to pathogens, which must encounter unpredictable changes in their environment.

This review will cover the functional consequences and advantages of phenotypic variation, and the mechanisms that produce it in *Neisseria gonorrhoeae* and other pathogenic bacteria, concentrating on some of the newer discoveries in the field. Phenotypic variation includes phase and antigenic variation. For the purposes of this review we will define phase variation as the reversible loss or gain of a molecule or defined structure. Antigenic variation concerns the organization or composition of that molecule or structure, and is sometimes defined in terms of the presence or absence of epitopes; such epitopes can be referred to as 'phase variable'. Genetic variation is not limited to bacteria but is also found in a wide variety of eukaryotic organisms and viruses (discussed elsewhere in this issue). Detailed reviews on bacterial variation, emphasizing different aspects of this subject, have been published[1,2].

*Such changes have been described both as 'random' processes[1], and also as 'programmed rearrangements'[2]. This apparent terminological contradiction is resolved by considering the temporal and spatial contexts of the terms: genetic variation is random with regard to the time of occurrence but nonrandom with regard to the genetic information involved; there appear to be genetic programs that affect loci in distinct regions of the chromosome.

Functional consequences of variation

Genetic variation as a means of escaping the immune response

The function of the immune system in higher organisms depends on extensive genetic rearrangements, which are crucial for maintaining the diversity of antibodies and the T cell receptor (see review by Gellert, this issue). One of the first examples of molecular variation described, for the African trypanosome (see Van der Ploeg *et al.*, this issue), is a response to this enormously diverse immune response. As far as is known, a single variable protein covers the entire surface of this pathogen, allowing it to avoid the host immune response. The spirochaete *Borrelia hermsii*, which causes relapsing fever, may also use antigenic variation primarily to escape the immune response. This bacterium produces large quantities of a variable lipoprotein, which could be sufficient for a complete surface coat.

In contrast, the surface of *N. gonorrhoeae* is composed of both variable and conserved surface components, and the major invariant surface antigens are also targets for the host immune response. Therefore surface protein variation in the case of *N. gonorrhoeae* is unlikely to be a way of avoiding the immune response in the conventional sense. The fact that gonorrhoea is a persistent infection probably results