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Activation of Vκ gene rearrangement in pre-B cells follows the expression of membrane-bound immunoglobulin heavy chains

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During B cell development Vκ gene rearrangement seems to occur only in μ-positive pre-B cells. To study the role of the μ chain in the activation of the Igκ locus, we introduced expression vectors carrying different forms of the μ gene into null pre-B cells. The activation of the Igκ locus followed the expression of the membrane form (μm) of the μ chain. The expression of the secreted form (μs) did not result in the activation of the Igκ locus. We further show that both forms of the μ chain differ in their intracellular transport in pre-B cells.

Key words: V gene rearrangement/B cell development/membrane-bound immunoglobulin chain/transmembrane signal

Introduction

The genes for the variable part (v) of the immunoglobulin (Ig) heavy and light chains are somatically assembled from different V gene segments which are V_H, D and J_H for the heavy chain and V_L and J_L for the κ or λ light chain (Tonegawa, 1983). The V gene segments are organized in multiple copies at the three Ig loci (IgH, Igκ, Igλ), each of which is situated on a different chromosome. During B cell development, V gene rearrangements occur at the three different Ig loci in an ordered fashion (Yancopoulos and Alt, 1986). They begin at the IgH locus with a D to J_H joint followed by a V_H to DJ_H rearrangement (Yaoita *et al.*, 1983; Alt *et al.*, 1984); and occasionally by a V_H to V_HDJ_H joint (Kleinfield *et al.*, 1986; Reth *et al.*, 1986a). Vκ to Jκ rearrangements occur later and are mostly found in pre-B cells which carry a functional V_HDJ_H complex and express a μ chain (Lewis *et al.*, 1982). Rearrangements at the third locus, Igλ, occur after Vκ gene assembly and most λ-positive B cells carry non-functionally rearranged Vκ genes (Moore *et al.*, 1985; Siminovitch *et al.*, 1985).

The order of Ig V gene rearrangement was first deduced from immunofluorescence studies on fetal liver pre-B cells (Gathings *et al.*, 1977; Siden *et al.*, 1981). These cells frequently express only μ chains, whereas κ light chains are mostly found in μ-positive pre-B cells. Further evidence for the ordered rearrangement of Ig V genes came from studies of fetal liver hybridomas (Burrows *et al.*, 1979) or Abelson murine leukaemia virus (A-MuLV)-transformed pre-B cell lines (Alt *et al.*, 1981). These lines have already rearranged both J_H alleles but only rarely show a Vκ to Jκ rearrangement.

Abelson cells are useful tools for the study of early events of pre-B cell development (Alt *et al.*, 1981, 1982, 1984). We have studied in recent years the bone marrow-derived Abelson line, 300-19 (Rosenberg *et al.*, 1975), which differentiates *in vitro* from an early μ-negative pre-B cell into a μ- and κ-positive B cell (Reth *et al.*, 1985). 300-19 parent cells originally carried a DJ_H3 complex on both J_H alleles and expressed a Dμ chain from one of these alleles (Reth and Alt, 1984). Daughter cells of 300-19 frequently performed secondary J_H rearrangements either as a D to J_H4 joint or as a V_H to DJ_H3 rearrangement (Reth *et al.*, 1986b). Roughly 30% of the assembled V_HDJ_H complexes in 300-19 subclones were productive and resulted in the expression of a μ chain. With the exception of a Dμ-producing line, Vκ gene rearrangements were found only in μ-positive 300-19 cells. This analysis suggested that the μ chain may provide a positive signal for the activation of the Igκ locus. To prove the regulatory role of the μ chain in pre-B cells, we introduced various μ expression vectors into null pre-B cells, which harboured unproductive V_HDJ_H rearrangements or two DJ_H alleles and thus did not produce μ chains by themselves. Vκ to Jκ rearrangements started in several μ-positive transformants, which had not assembled a functional V_H gene. The activation of the Igκ locus required the expression of membrane-bound μ chains suggesting that the μm chain is generating the signal which leads to Igκ activation.

Results

Vκ gene rearrangement occurs only in μ-producing pre-B cells

A study of many independent subclones of the 300-19 pre-B cell lineage demonstrated that Vκ to Jκ rearrangements occurred only in μ-producing cells (Reth *et al.*, 1985). This is also seen in the analysis of the 300-19 subclone P17 and its derivatives. P17 carries unproductive V_HDJ_H complexes on both J_H alleles which are seen on a Southern blot as J_H-hybridizing *Eco*RI fragments of 2.2 and 2.0 kb (Figure 1A). Both $V_HDJ_H^-$ alleles of P17 have been sequenced and found to have an out-of-phase V_H to DJ_H joint (Reth *et al.*, 1986a). P17 cells are not producing μ chains and κ light chain; nor do they show any indication for Vκ gene rearrangements (see Figure 1C,B). However, P17 can perform further J_H rearrangements by joining an upstream V_H segment to one of the $V_HDJ_H^-$ complexes (Reth *et al.*, 1986a). Such a V_H replacement joint occurred in subclone P17-32 resulting in a productive V_HDJ_H complex which is detected as a rearranged J_H fragment of 3.5 kb (Figure 1A). P17-32 cells produce μ chains and submolar amounts of κ light chains (Figure 1C), suggesting that part of the P17-32 culture is activating the Igκ locus. This activation cannot yet be seen in a Southern blot analysis of P17-32 DNA which still shows the Jκ allele in germline configuration (Figure 1B).

To prove the activation of the Igκ locus in cells of the P17-32 population we subcloned this line and analyzed seven subclones for J_H and Jκ rearrangement as well as for Ig chain production. Three of the subclones had deleted the productive $V_HDJ_H^+$ allele (Figure 1A, lanes 1, 2 and 10); these subclones were μ- and κ-negative and did not carry any Jκ rearrangement (Figure 1C,B). The four other subclones, however, retained the productive 3.5-kb $V_HDJ_H^+$ allele of the P17-32 parent and pro-

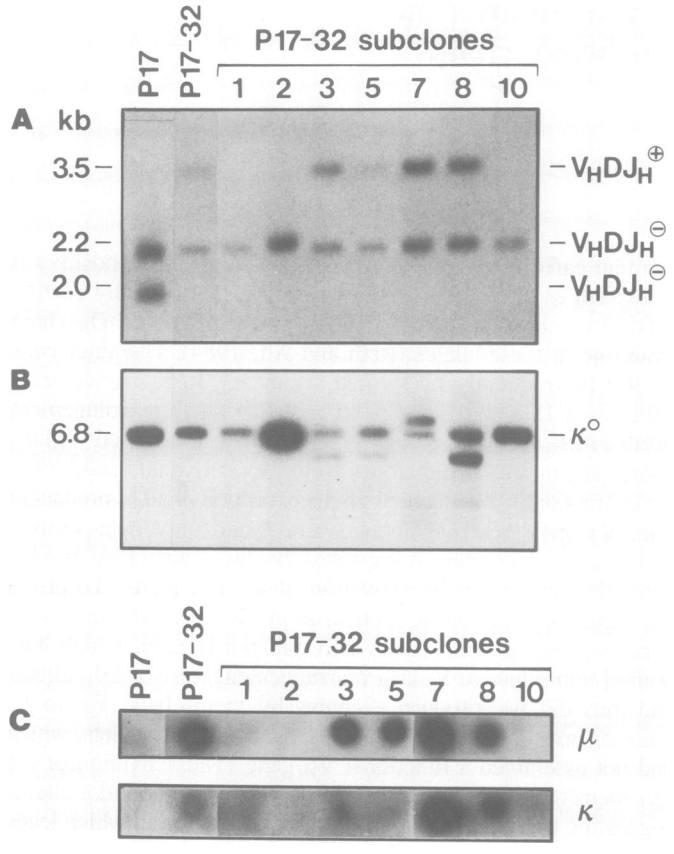

Fig. 1. Analysis of P17-32 subclones. (**A**) Approximately 10 μg of DNA of each subclone were digested with *Eco*RI and assayed for hybridization to a J_H-specific probe (a *Hin*dIII–*Eco*RI J_H fragment, see Figure 6) by Southern blotting. The parental cell lines P17 and P17-32 were analyzed in parallel. The size and position of *Eco*RI fragment carrying the $V_HDJ_H^-$ and $V_HDJ_H^+$ complexes are indicated. (**B**) The same DNA shown in (A) was digested simultaneously with *Bam*HI and *Eco*RI and assayed for hybridization to a Jκ-specific probe. The position of the 6.8-kb *Bam*HI–*Eco*RI Jκ germline fragment (κ^0) is indicated. (**C**) Analysis of μ and κ light chain production of each subclone in a protein dot assay.

duced high amounts of μ chains (Figure 1A,C, lanes 3, 5, 7 and 8). Vκ to Jκ rearrangements occurred in all four subclones, two of which also produce κ light chain. The P17-32 analysis stresses again the strict correlation between μ production and the onset of Vκ gene rearrangement in the 300-19 pre-B cell lineage. It also suggests that prolonged μ production is required for the activation of the Igκ locus.

Introduction of expression vectors carrying the μ gene into null pre-B cells

To analyze further the regulatory role of μ chains we introduced expression vectors carrying the μ gene into null pre-B cells which did not produce any μ chain from their own JH alleles. In doing so we wished to investigate whether the expression of the μ chain could activate Vκ gene assembly in these cells. Expression of the Cμ gene can give rise to two different μ mRNAs coding for either the membrane-bound (μm) or the secreted (μs) μ chain (Alt *et al.*, 1980a; Early *et al.*, 1980). The production of the two different mRNAs is regulated during B cell development. Small B cells contain predominantly the μm RNA, while plasma cells express only the μs mRNA (Kemp *et al.*, 1980; Nelson *et al.*, 1983). In μ-producing Abelson lines both types of mRNAs are present in similar amounts (Alt *et al.*, 1982) and these cells also contain both forms of the μ protein (M.Reth *et al.*, unpublished).

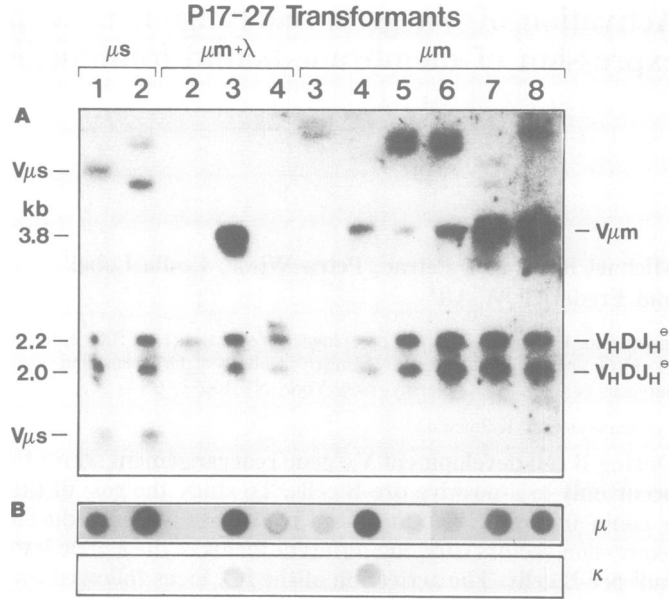

Fig. 2. Analysis of P17-27 transformants. (**A**) Approximately 10 μg of each transformant were digested with *Eco*RI and assayed for hybridization to a J_H-specific probe on a Southern blot. J_H fragments carrying the two $V_HDJ_H^-$ alleles as well as those characteristic of the integrated pSVμs and the pSVμm vector are indicated. The vectors used in the transformation experiments are indicated above the lanes. (**B**) Analysis of μ and κ light chain production in P17-27 transformants in a protein dot assay.

To distinguish in the transformation experiments between the two forms of the μ chains we constructed the vectors pSVμs and pSVμm, which specifically express either the secreted or the membrane-bound μ chain. These vectors were constructed from cDNAs derived from μs or μm mRNA (Rogers *et al.*, 1980; Bothwell *et al.*, 1981) (Figure 6c; for details see Materials and methods). Both vectors contained the IgH enhancer (Banerji *et al.*, 1983; Gillies *et al.*, 1983; Neuberger, 1983) and the productively rearranged V_HDJ_H complex of the hybridoma B1-8, which secretes a λ1-bearing IgM anti-NP antibody (Reth *et al.*, 1978). Thus, all μ chains expressed from the vectors should be able to form an anti-NP antibody, provided a λ1 light chain is present. The pSVμs and pSVμm vectors were tested for the production of the expected μ chain by introducing them into the λ1-producing myeloma line 558L. All 558Lμs transformants expressed a full-length μs chain (see Figure 5, lane 1) and secreted large amounts of anti-NP antibodies into the culture medium (data not shown). In contrast, the 558Lμm transformants did not secrete any anti-NP antibodies. However, lysates of these transformants contained μ proteins with a mol. wt of 78 kd, a size expected for the mature μm chain (see Figure 5, lane 2).

For our transformation experiments we chose the null pre-B cell line P17-27 which was a sister clone of P17-32. P17-27 retained the $V_HDJ_H^-/V_HDJ_H^-$ genotype of the P17 parent and did not produce any μ or κ protein. In three different experiments, we transformed the P17-27 line with pSVμs, pSVμm alone and pSVμm along with pIXE, a vector which expresses λ_1 light chains (Picard and Schaffner, 1983). From these experiments we obtained two P17-27μs transformants, three P17-27μmλ transformants and six P17-27μm transformants (see Figure 2). Each transformant was analyzed for J_H rearrangement and Ig chain production by Southern and Western blotting.

All transformants retained the two $V_HDJ_H^-$ alleles of the P17-27 recipients (Figure 2A). Integration of the pSVμs and pSVμm vector in P17-27 cellular DNA is indicated on a Southern

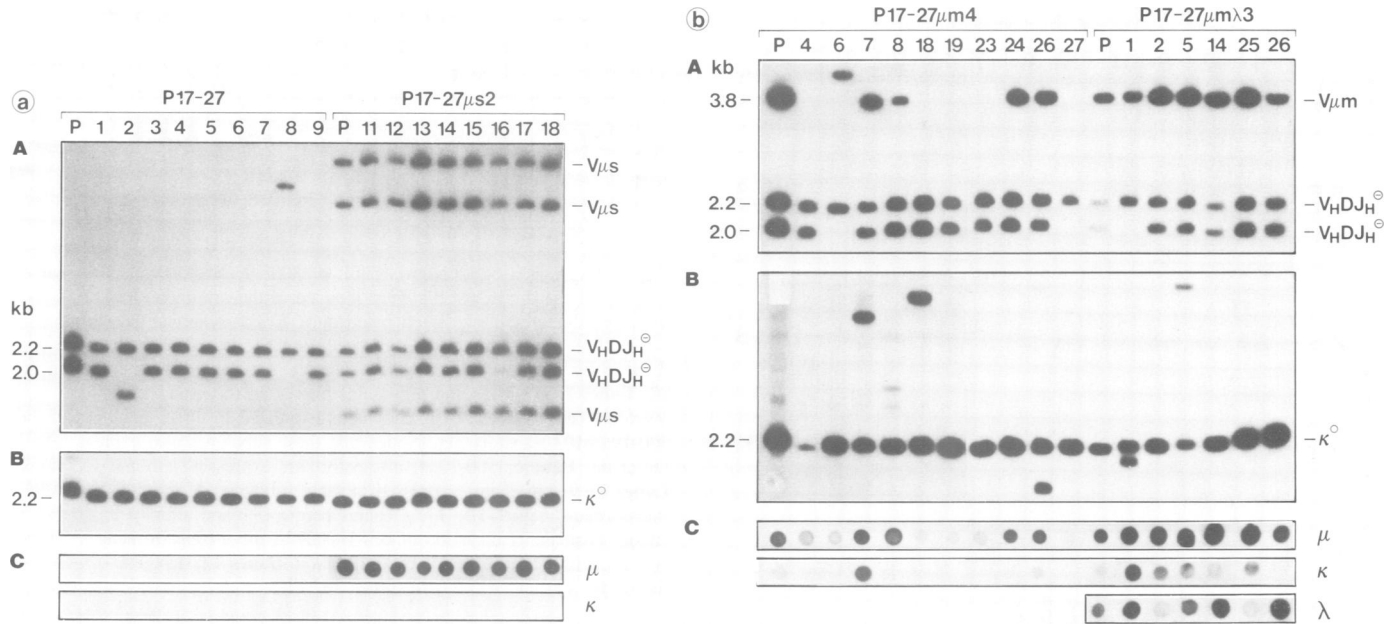

Fig. 3. Analysis of subclones of P17-27 and P17-27 transformants. (**A**) Genomic DNA of the different subclones was digested with *Eco*RI and assayed for hybridization to a J_H-specific probe. The name of the parental population from which the subclones are derived is indicated above the panels, and the analysis of the parental population is shown in the lanes marked **P**. The different J_H-hybridizing fragments are labelled as in Figure 2. (**B**) The same DNA as used in (A) was digested with *Hin*dIII and assayed for hybridization to a Jκ-specific probe. The position of the 2.2-kb *Hin*dIII−Jκ germline (κ^0) fragment is indicated. (**C**) Analysis of μ, κ and λ chain production in the different subclones in a protein dot assay.

blot by the presence of J_H-hybridizing *Eco*RI fragments of 1.5 and 3.8 kb respectively (see Figure 6 for restriction map of the two μ vectors). Of the three P17-27μmλ transformants only one, P17-27μmλ3, contained J_H-hybridizing vector DNA and produced the vector derived μm chain (Figure 2A). Interestingly, P17-27μmλ3 contained submolar amounts of κ light chain indicating the onset of Vκ gene assembly in this transformant (Figure 2B). Among the P17-27μm transformants, P17-27μm4, which produces the highest amount of μm, also contains submolar amounts of κ light chains. The second strongest μm producer of this group, P17-27μm8, also revealed κ light chains in a longer exposure of the κ dot blot (data not shown) but all other μm transformants — which did not produce high amounts of μm chains — did not contain any κ protein. Thus the P17-27μm transformants behaved similarly to the P17-32 subclones in that the activation of their Igκ locus required μ expression. The two P17-27μs transformants, however, behaved differently. Even though both μs transformants produced high amounts of μs chains, they did not show any indication for κ light chain production (Figure 2, lanes 1 and 2).

Only μm-producing transformants activate the Igκ locus

To study the activation of the Igκ locus in more detail, one μ producer of each group was subcloned and isolated subclones were analyzed for J_H and Jκ rearrangement as well as for Ig chain production. The analysis of these subclones is partially shown in Figure 3 and summarized in Table I. As a control, we subcloned the recipient line P17-27. None of the isolated P17-27 subclones produced μ or κ light chains, nor did they show any Jκ rearrangement (Figure 3a, panels B and C) although in two subclones a further JH rearrangement occurred on one of the $V_HDJ_H^-$ alleles (Figure 3a, panel A, lanes 2 and 8). Thus, in the μ-negative P17-27 line, rearrangements of the Vκ gene did not occur spontaneously. Vκ gene rearrangement or κ light chain were also absent among the P17-27 μs2 subclones, although all of them produced high amounts of μs chain (Figure 3a, panels B and C). The absence of Vκ gene assembly in P17-27μs2 does not seem to be due to the inactivation of the recombinase system because one of the P17-27μs2 subclones performed a further J_H rearrangement (see Table I).

Table I. Summary of the subcloning analysis

Cell line	Allele A	Allele B	Vector	Clones[a]	J_H[b]	Jκ[c]
P17	$V_HDJ_H^-$	$V_HDJ_H^-$	−	17	5	1[d]
P17-32	$V_HDJ_H^+$	$V_HDJ_H^-$	−	7	0	4
P17-27	$V_HDJ_H^-$	$V_HDJ_H^-$	−	11	2	0
P17-27μs2	$V_HDJ_H^-$	$V_HDJ_H^-$	pSVμs	19	1	0
P17-27μm4	$V_HDJ_H^-$	$V_HDJ_H^-$	pSVμm	39	1	9
P17-27μmλ3	$V_HDJ_H^-$	$V_HDJ_H^-$	pSVμm + pIXE	11	0	3
300-19	DJ_H3	DJ_H3	−	30	12	0
300-19μm103	DJ_H3	DJ_H3	pSVμm	10	1	0
300-19μm130	DJ_H3	DJ_H3	pSVμm	10	1	0
300-19μmλ12	DJ_H3	DJ_H3	pSVμm + pIXE	11	0	0
300-19μmλ36	$V_HDJ_H^-$	DJ_H4	pSVμm + pIXE	11	0	2

[a]Number of subclones of the indicated transformants.
[b]Clones showing J_H rearrangement.
[c]Clones showing Jκ rearrangement.
[d]Jκ rearrangement occurred in one of the μ-positive P17 subclones carrying a V_H replacement.

The Igκ locus, however, was activated in both P17-27μm transformants analyzed. Four of ten P17-27μm4 and two of six P17-27μmλ3 subclones (Figure 3b, panel B) carried Jκ rearrangement and many subclones also contained κ light chains (Figure 3b, panel C). The 3.8-kb *Eco*RI fragment of the pSVμm vector was deleted in some of the subclones of the P17-27μm4 transformant resulting in the loss of μ production in these subclones (Figure 3b, panels A and C). Interestingly, in the P17-27μm4 series, three of the four cases of Jκ rearrangements occurred in μm-positive subclones which retained the pSVμm vector (Figure 3b, lanes 7, 8 and 26), stressing the importance of continuing

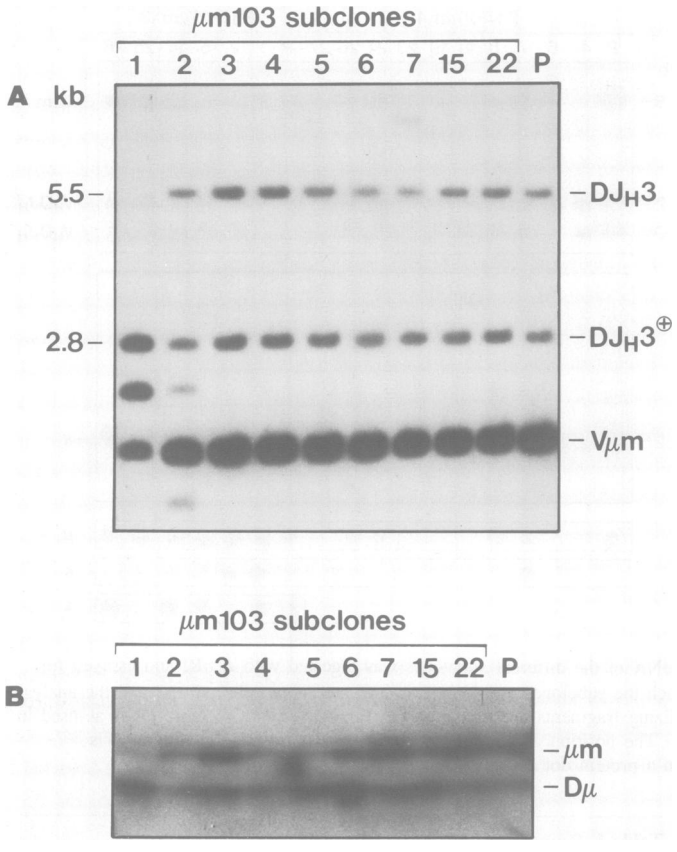

Fig. 4. Analysis of subclones of the 300-19μm103 transformants. (**A**) Approximately 10 μg of DNA of each subclone were simultaneously digested with *Bam*HI and *Eco*RI and assayed for hybridization to a J_H-specific probe. J_H fragments carrying the two DJ_H alleles as well as those characteristic of the integrated pSVμm vector are indicated. **Lane P** shows the analysis of the parental 300-19μm103 cell population before subcloning. (**B**) Western blotting analysis of μ chain production in subclones of the 300-19μm103 transformants. Forty microliters of cell lysates were fractionated by electrophoresis over a 10% acrylamide gel, transferred onto nitrocellulose and tested for bound μ chain by incubation with ^{125}I-labelled antibodies. The position of the 78 kd and 73 kd μm and 56 kd Dμ chain is indicated.

μ production for the activation of the Igκ locus. In summary, the P17-27 experiments demonstrate that Vκ gene rearrangements can indeed be induced in null pre-B cells by the expression of a μ chain vector. However, only the μm but not the μs chain seems to be active in this process.

Early pre-B cells do not activate the Igκ locus upon μm production but show evidence for allelic exclusion

In another series of transformation experiments we introduced the pSVμm vector either alone or together with the λ1 vector pIXE into the 300-19 parental cell line. 300-19 cells carry two DJH alleles and thus represent an early stage of pre-B cell development (Alt *et al.*, 1984). From these experiments we obtained twelve 300-19μm and nine 300-19μmλ transformants. Interestingly, the Igκ locus was activated in only one of these transformants, 300-19μmλ36. Both J_H alleles of this transformant underwent secondary J_H rearrangements which were characterized as an unproductive V_H to DJ_H joint and as a D to J_H4 rearrangement (see Table I and Figure 6). All other 300-19μm and 300-19μmλ transformants retained the early DJ_H3/DJ_H3 genotype and did not activate the Igκ locus (see Table I).

However, the μm chain still seems to have a regulatory influence on the 300-19μm transformants. The expression of the

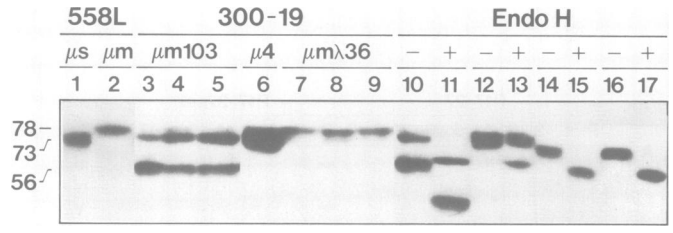

Fig. 5. Expression of μ chain in 558L or 300-19 transformants. Internal proteins of the different cells were reduced in the presence of mercaptoethanol and size fractionated on a 10% acrylamide gel. Proteins were subsequently blotted onto nitrocellulose and bound μ chains were detected by ^{125}I-labelled antibodies. The sizes of the μ chains are given in kd. **Lanes 1** and **2**: μs and μm chains expressed in transformed 558L myeloma cells. **Lanes 3–5**: μm chains expressed in three subclones of the 300-19μm103 transformant. Notice that these subclones also express a 56 kd Dμ chain. **Lane 6**: 300-19μ4, a transformant expressing μm and μs chains. **Lanes 7–9**: μm chains expressed in three subclones of the 300-19μmλ36 transformant. **Lanes 10–17**: μ chains before (–) and after (+) endoglycosidase H digestion of cell lysates of the μ transformants 300-19μm103 (**lanes 10, 11**), 300-19μmλ36 (**lanes 12, 13**), P17-27μs1 (**lanes 14, 15**) and P17-27μs2 (**lanes 16, 17**).

μ chain in pre-B cells is thought to exercise allelic exclusion control in preventing V_H gene assembly on the second J_H allele (Alt *et al.*, 1981). Four of the 300-19μm transformants were tested for J_H rearrangement in a subcloning analysis. Whereas secondary J_H rearrangements are generally found in 10–30% of the subclones of 300-19 or its derivatives still carrying DJ_H alleles (Reth *et al.*, 1986b), J_H rearrangements did not occur in 11 subclones of the transformants 300-19μmλ12 and 300-19μmλ36 and occurred once in ten subclones of each of the transformants 300-19μm103 and 300-19μm130 (see Table I). These J_H rearrangements, however, do not violate allelic exclusion because in the case of 300-19μm103 an unproductive V_H to DJ_H joint (Figure 4A, lane 1) occurred in a subclone which barely expressed the μm chain (Figure 4B, lane 1, compare μm with internal Dμ production), and in the case of 300-19μm130 the subclone carried out a secondary D to J_H4 joint (data not shown), a rearrangement which does not seem to be strictly controlled by the μ chain feedback signal (see Discussion). Thus the μm production in the 300-19μm transformant seems to prevent functional V_H to DJ_H rearrangement.

Evidence for two pools of μm chain in pre-B cells

Production of μm chains in the different μm transformants was analyzed by SDS–PAGE electrophoresis and Western blotting (Figure 5). To our surprise, we found two different sizes of the μm chain in all Abelson μm transformants: a 78 kd form and a 73 kd form. The 73 kd protein was the predominant form in 300-19μm transformants, where the 78 kd μm chain was only hardly visible (Figure 5, lanes 3–5). The same pattern of expression of μm was also found in P17-27μm4 subclones (data not shown). In 300-19μmλ36 cells, however, the predominant species was the 78 kd μm protein and not the 73 kd μ protein. 300-19μmλ36 differed from the other 300-19μm transformants, because they contained λ light chains and expressed a complete antibody molecule on the cell surface (data not shown). We thus interpreted the two different μm pools in 300-19μm transformants as being two different glycosylation forms which a μm chain goes through on its way to the cell surface: a 73 kd high-mannose form found in the rough endoplasmic reticulum, and a complexly glycosylated 78 kd form found in the Golgi apparatus. This interpretation was shown to be correct by digestion of cell lysates with endoglycosidase H, an enzyme which only cleaves sugars

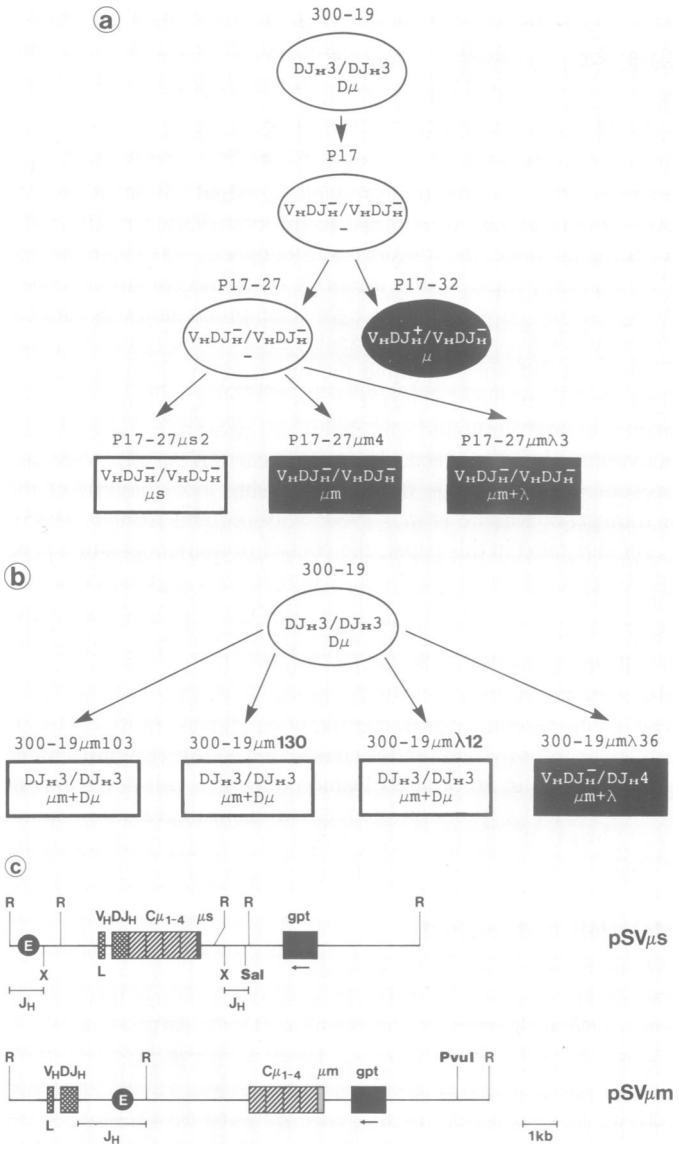

Fig. 6. Summary of the subcloning and transformation experiments and maps of the two vectors used. (a, b) Generation tree of P17 and 300-19 subclones (drawn as circles) and transformants (drawn as boxes). The name of the cell line is written above and its genotype and μ expression inside each box or circle. Cell lines which are activating the Igκ locus are drawn in black. The vector usage is indicated by the name of the transformant, i.e. 300-19μmλ36 is transformed by pSVμm and the λ vector pIXE. All cell lines shown were studied by a subcloning analysis (see Table I).
(c) Restriction map of the μ vectors pSVμs and pSVμm. The vectors are derivatives of the pSV2gpt vector (Mulligan and Berg, 1981). The V_H and the Cμ exons are drawn as stippled boxes and the *gpt* gene as a black box. The position of the J_H-hybridizing region and of the IgH enhancer (E) is indicated. Restriction sites used for vector linearization are drawn with capital letters. R = *Eco*RI; B = *Bam*HI; X = *Xba*.

of the high-mannose form but not the complexly glycosylated form. After endoglycosidase H digestion, the 73 kd μm form of 300-19μm103 was reduced to a band of 60 kd, while the faint 78 kd band was resistant to this digestion (Figure 5, lanes 10 and 11). Notice that the 56 kd Dμ protein expressed in 300-19μm103 is also trimmed by the enzyme. In 300-19μmλ36, which predominantly expressed the 78 kd μm form, most μm chains were resistant to endoglycosidase H digestion (Figure 5, lanes 12 and 13). The analysis of the μs transformants, P17-27μs1 and P17-27μs2, showed that all of the μs chains are sensitive to the endoglycosidase H digestion (Figure 5, lanes 14–17), suggesting that in pre-B cells μs chains are only expressed in the rough endoplasmic reticulum and not transported into the Golgi. The data of the transformants as well as a similar analysis of μ positive Abelson lines (data not shown) can be summarized as follows: Abelson pre-B cell lines produce μm and μs chains. Both μ chains are predominantly found in the endoplasmic reticulum, where they both have a mol. wt of 73 kd and thus co-migrate on an acrylamide gel. A few μm but not μs chains are transported into the Golgi, where they are transformed into the complexly glycosylated form of 78 kd. This transport into the Golgi seems to be considerably increased once a light chain has bound to the μm chain.

Discussion

Signal function of the μm chain

In a highly regulated fashion the Abelson pre-B cell line 300-19 activates the Igκ locus only in μ-producing cells. We demonstrate here that the activation of the Igκ locus can be induced in null pre-B cells by a μ chain encoded by an expression vector (see summary in Figure 6a and b). This finding suggests that the μ chain plays a role in the activation process, and the same may be true in normal pre-B cells because staining experiments detect κ light chain dominantly in μ-producing pre-B cells (Gathings *et al.*, 1977; Siden *et al.*, 1981). Thus the order of V gene rearrangements during B cell development does not seem to be only the result of a predetermined differentiation programme but also to be the consequence of a specific regulation in which the μ chain gives a signal for the activation of the Igκ locus.

The finding that the μm chain but not the μs chain is able to activate Vκ rearrangements in μ transformants may provide some insight on the nature of the μ signal. Both μ chains differ only in the C terminus: the μs chain carries 20 amino acids specific for the secreted form whereas the μm chain terminus contains 41 amino acids some of which form the membrane-spanning region. It is possible that the transmembrane part of the μm chain is directly involved in the generation of the signal resulting in activation of the Igκ locus. In contrast to the transmembrane part of most other membrane proteins, the transmembrane part of IgH chains contains not only hydrophobic but also the polar amino acids serine and threonine (Early *et al.*, 1980). Most of the transmembrane sequence, including the position of some of the polar amino acids, is highly conserved between different IgH classes and between μm chains of different mammalian species (Rabbitts *et al.*, 1981; Bernstein *et al.*, 1984). The conservation of the transmembrane sequence has been considered as evidence for a functional role of the transmembrane region not only as a membrane anchor but also as a transmembrane signalling device (Rogers and Wall, 1984).

The transmembrane region may form an ion channel in the membrane of an activated B cell once crosslinking of surface-bound antibodies by antigen has resulted in the aggregation of the polar transmembrane regions. An ion flux has indeed been observed during antigen stimulation (Monroe and Cambier, 1983; Cambier and Ransom, 1986) although it has not been directly shown that it is derived from the membrane-bound antibody. The signal generated by the μm chain on the surface of an activated B cell may be qualitatively similar to that generated inside a pre-B cell and it is possible that the heavy chain binding protein BiP or GRP78 (Haas and Wabl, 1983; Munro and Pelham, 1986) takes part in the crosslinking of the internally expressed μm chain.

It is, however, also possible that the μm chain interacts with another receptor which upon binding to μm will generate the

activation signal. This receptor may not bind to μs chains or alternatively may be actively expressed only in a cellular compartment which is not reached by the μs chain. As indicated by the endoglycosidase H sensitivity experiments (see Figure 5), some of the μm but none of the μs chains reach the Golgi, a finding which was also made in human pre-B cell lines and normal resting B cells (Hendershot and Levitt, 1984; Sidman, 1981). If the signal for the activation of the Igκ locus is generated inside the Golgi or after leaving this compartment, the differences of the intracellular transport could explain the different regulatory functions of the two μ chains.

How the μm signal is transported into the nucleus and its action in the nucleus are presently unknown. The recombinase system itself is unlikely to be the target of the μm signal because the same recombinase seems to be active throughout B cell development (Blackwell *et al.*, 1986; Yancopoulos *et al.*, 1986). Thus it is more likely that the μm signal is mediated by factors binding to the different Ig loci. This binding may be dependent on the accessibility of the Ig loci (Yancopoulos and Alt, 1985). The Igκ locus may, for example, not be accessible in early pre-B cells carrying two DJ_H alleles but may be gradually opened during further development and V_H gene assembly. This could explain why P17-27μm transformants but not the DJ_H/DJ_H-carrying 300-19μm transformants activate rearrangement at the Igκ locus although in the latter cells the μm signal seems to act on the 'open' IgH locus.

Feedback regulation of μ chains

Each B cell produces only one functional heavy chain and thus expresses only one of its two IgH alleles, a phenomenon known as 'allelic exclusion' (Pernis *et al.*, 1965). To explain this control, it is thought that once a functional $V_HDJ_H^+$ complex is formed, the expressed μ chain generates a signal to stop rearrangement of the second J_H allele (Alt *et al.*, 1981; Alt, 1984). Evidence for such a stop signal was derived from studies of transgenic mice carrying a complete μ gene (Rusconi and Köhler, 1985; Weaver *et al.*, 1985). Recently transgenic mice were analyzed which carry either the μs or the μm gene (Storb *et al.*, 1986; Nussenzweig *et al.*, 1987). In these experiments only the expression of the transgenic μm chain seems to stop J_H rearrangements at the endogenous IgH alleles (Nussenzweig *et al.*, 1987). The signal generated by the μm chain may thus have a dual regulatory function: the activation of the Igκ locus and the cessation of V_H gene assembly at the IgH locus.

Evidence for the feedback control of the μm chain was also seen in the analysis of the 300-19μm transformants carrying DJ_H alleles. Many subclones of these transformants did not show any further J_H rearrangements as long as they produced the μm chain. In one transformant (300-19μm130), however, a secondary D to J_H4 rearrangement occurred in a μm-producing subclone (see Table I). Secondary D to J_H rearrangements occurred also on the DJ_H allele of μ-positive 300-19 subclones (P.Wiese and M.Reth, unpublished). D to J_H rearrangements did not in these cases result in the assembly of a functional V_H gene and thus did not violate allelic exclusion. These data suggest that, in the 300-19 line, the feedback control of the μm chain does not *per se* stop rearrangement at the J_H locus but rather seems to interfere with the activation of V_H segments for the V_H to DJ_H joint. The finding that the V_H locus becomes transcriptionally inactive and inaccessible in μ-producing pre-B cells may reflect this regulation (Yancopoulos and Alt, 1985).

A regulatory function is also assigned to the complete antibody molecule. Its role seems to be the arrest of further VL gene assembly at the IgL loci once a functional light chain is produced which can bind to the heavy chain (Alt *et al.*, 1980b). Experiments with transgenic mice carrying a κ transgene demonstrate this regulation. Vκ gene rearrangements were not found in B cells where the transgenic κ chain is bound by the endogenous heavy chain (Ritchie *et al.*, 1984; Storb *et al.*, 1985). We have addressed this question by introducing μm and λ light chains into 300-19 derivatives. In contrast to the observation made in the κ transgenic mice, the transformants obtained, 300-19μmλ36 and P17-27μmλ3, expressed an antibody molecule but still activated the Igκ locus. However, the λ light chain production was variable in the subclones of two μmλ transformants (see Figure 3b, panel C) and we did not determine the molar ratio of the μm and λ light chains produced. Thus it is possible that, in most transformants, enough unbound μm chains are present to generate the activation signal. Indeed, Vκ gene rearrangements were not prevented in transgenic mouse expressing low amounts of the κ transgene (Ritchie *et al.*, 1984; Rusconi and Köhler, 1985).

As illustrated in this paper, the control of immunoglobulin gene rearrangements seems to be an example of a regulatory circuit where the product of a given locus can either activate rearrangements at another locus or stop rearrangements of the second allele. It will therefore be of interest to study whether this control is also a model for the controlled expression of other heterodimers. The T cell receptor chains are likely candidates in this respect. Similarly to immunoglobulin chains, the T cell receptor chains are expressed in an ordered fashion during T cell development where β chain expression precedes α chain expression (Raulet *et al.*, 1985; Snodgrass *et al.*, 1985). It is thus possible that the β chain also generates a signal for the activation of the α locus.

Materials and methods

Cell culture and transformant experiments

Abelson cells were grown in RPMI culture medium containing 10% fetal calf serum, 2 mM L-glutamine, 50 U/ml penicillin, 50 mg/ml streptomycin and 2×10^{-5} M mercaptoethanol. Cells were subcloned by limiting dilution by plating 50 cells in a 96-well plate. The cloning efficiency was 20–50%. Prior to electroporation, cells were washed in PBS. One milliliter of the cell suspension (10^7 cells) was then mixed with 10–20 μg linearized vector DNA, transferred to a cold plastic cuvette containing two aluminium electrodes to which was applied an electric pulse of 2000 V, as previously described (Potter *et al.*, 1984). After the pulse, the cells were kept in the cuvette for 10 min, transferred to a 10-ml plastic dish containing warm medium, and finally plated in two 24-well plates. The selection medium was culture medium containing 250 μg/ml xanthine, 15 μg/ml hypoxanthine and 0.1–1 μg/ml mycophenolic acid. Selection started 2 days after the electroporation and was continued for 2–3 weeks under rising concentrations of mycophenolic acid. Transformants were grown in selection medium containing 1 μg/ml of mycophenolic acid.

Vector construction

The pSV-Vμ1 and pIXE vectors (Neuberger, 1983; Picard and Schaffner, 1983) were kindly provided to us by M.Neuberger and C.Burger. The pSVμs vector was constructed from plasmid pABμ11 containing the μs cDNA clone from the cell line B1-8, and from plasmid p186-2 containing the germline VH gene segment which is productively rearranged in B1-8 (Bothwell *et al.*, 1981). A 500-bp *Hin*cII–*Pst* fragment containing the VH promoter and leader was isolated from p186-2 and cloned into pUC8 cut with *Hin*cII and *Pst* to obtain plasmid pVNP. pVNP was linearized by double-digestion with *Pst* and *Hin*dIII and used for the insertion of the 600-bp *Pst*–*Bgl*II and the 2-kb *Bgl*II–*Hin*dIII μ fragment of pABμ11. The plasmid p5.1 thus obtained contained a functional μs gene on an *Eco*RI fragment which we cloned into the retroviral vector pVcos7, a kind gift of S.Goff. The μs gene was cut out of the retroviral vector as a *Xba* fragment and cloned into *Xba*-cut pSV2gptE, a derivative of the pSV2gpt vector (Mulligan and Berg, 1981) carrying a 2.0-kb J_H-positive *Hin*d–*Eco*RI fragment which includes the IgH enhancer and the *Xba* site used for cloning. The expression vector obtained was called pSVμs (see Figure 6c).

For the construction of pSVμm a *Kpn* fragment of pSVμ containing the two μm exons and part of the *gpt* gene was cloned into pUC19 cut with *Kpn*. The plasmid pμm1 thus obtained was linearized by double-digestion from *Pst* and *Xba*

and used to insert the 500-bp *Pst*−*Xba* fragment from pU6, a vector containing a cloned cDNA of μm mRNA (Rogers *et al.*, 1980). The vector obtained (pμm1-1) carried μm cDNA in conjunction with sequences lying 3′ of the μm exons including the poly(A) addition site of the μm RNA. The 1.5-kb *Pst* fragment carrying part of the VH gene and Cμ gene of pABμ11 (see above) was inserted into *Pst*-cut pμm1-1 to obtain plasmid pμm1-2. The *BglII*−*Kpn* fragment of pμm1-1 reaching from Cμ1 exon to the *gpt* gene was finally cloned into *Kpn* and partially *BglII*-cut pSVμ to obtain the pSVμm vector (see Figure 6c).

Protein and DNA analysis

Approximately 10^7 cells were washed once with PBS and then lysed with 200 μl phospholysis buffer (1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, 10 mM sodium phosphate buffer pH 7.5, 100 mM NaCl). For the dot assay 3 μl of each lysate was spotted onto nitrocellulose filters as described previously (Reth *et al.*, 1985). Filters were incubated in PBS containing 1% casein, and filter-bound immunoglobulin chains were detected by a sandwich technique employing a specific antiserum and a radiolabelled monoclonal antibody. For the μ test the filter was first reacted with a goat anti-μ antiserum for 8 h, washed in PBS and incubated overnight with ^{125}I-labelled B1-8, a λ-bearing IgM antibody. Filter-bound λ light chains were detected similarly by incubating the filter first with a goat anti-λ serum and then with ^{125}I-labelled B1-8 (see above). The κ test employed a monoclonal rat anti-mouse-κ antibody (R33-101) and a ^{125}I-labelled mouse anti-rat-κ antibody (MARK1). All antisera were diluted in PBS−casein buffer to a concentration of 10 μg/ml. For the SDS−PAGE and Western blotting analysis, 20−40 μl of each cell lysate was loaded on a 10% polyacrylamide gel and size-separated proteins were transferred onto nitrocellulose filter by electroblotting. The transfer buffer was SDS running buffer containing 20% methanol. Filter-bound immunoglobulin chains were detected as described above. For the endoglycosidase H digestion, 4 μl of cell lysate was diluted with 16 μl of 50 mM sodium citrate buffer (pH 5.5) and incubated for 2.5 h at 37°C with 6 mU endoglycosidase H. All techniques involving genomic DNA isolation and Southern blotting and the J_H probe used have been described previously (Alt *et al.*, 1984; Reth and Alt, 1984). The Jκ probe is a *Hin*dIII Jκ fragment derived from plasmid pHJκ (Lewis *et al.*, 1982).

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