Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 6989–6993, September 1986
Immunology

Negative trans-regulation of T-cell antigen receptor/T3 complex mRNA expression in murine T-lymphoma somatic cell hybrids

(gene regulation/somatic cell genetics)

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Communicated by Helen M. Ranney, May 27, 1986

ABSTRACT **The antigen-specific T-cell receptor (TCR) is composed of variable antigen-recognition chains TCR-α and TCR-β in noncovalent association with the invariant T3 multimer. The TCR-α and TCR-β chains are encoded by gene segments that must be juxtaposed by rearrangement in order to be expressed. To examine whether mechanisms other than gene rearrangement might regulate TCR/T3 gene expression, somatic cell hybrids were formed among closely related murine SL12 T-lymphoma clones that differ in TCR/T3 mRNA levels. In hybrid cells formed between cell clones in which one parent is TCR-β^+ and the other is TCR-β^-, the resultant hybrid cells lack detectable TCR-β transcripts. Since the protein synthesis inhibitor cycloheximide partially reverses TCR-β repression in the hybrid cells, we postulate that a labile repressor protein is involved. The amount of mRNA encoding one of the T3 polypeptide chains, T3-δ, is also strongly negatively trans-regulated in the same hybrid cells in which TCR-β mRNA expression is repressed. The negative trans-regulation of TCR-β and T3-δ mRNA expression is relatively specific, since the levels of TCR-α mRNA and several thymocyte surface antigens are not repressed in somatic cell hybrids. Our results indicate that rearrangement of the TCR genes alone is not sufficient for TCR-β expression and that trans-acting factors regulate the amounts of both TCR-β and T3-δ mRNA in this system.**

Recently, the T-cell receptor (TCR) for antigen has been identified as a multimeric glycoprotein consisting of variable TCR-α and TCR-β chains associated with invariant T3-γ, T3-δ, T3-ε, and T3-ζ chains (1–7). The TCR-α/TCR-β heterodimer has been strongly implicated as the antigen recognition unit of the complex (reviewed in ref. 8). The T3 chains may function to transduce signals initiating T-cell activation events (6). cDNAs encoding the TCR-α, TCR-β, T3-δ, and T3-ε subunits have been molecularly cloned and characterized (9–12), and the expression of those genes appears to be limited to the T-cell lineage. During thymocyte differentiation a sequence of events occurs that culminates in the constitutive expression of TCR/T3 complex in mature T cells (reviewed in ref. 8). The available evidence indicates that TCR-β genes are rearranged and transcribed prior to the expression of TCR-α genes (13–15, 27). TCR-β, T3-δ, and T3-ε transcripts become dectectable at about the same time (27, 32).

The rearrangement of TCR-α and TCR-β gene segments plays an important regulatory role in their transcription (8, 13, 16, 17), whereas T3-δ and T3-ε genes do not undergo rearrangements and therefore must be regulated in a different manner (11, 12). At present, the mechanism(s), other than rearrangement, involved in the activation and regulation of TCR/T3 gene expression is unknown.

In order to examine TCR/T3 gene regulation it is helpful to have cell lines that differ in the expression of the encoded products. To this end, clones that differ in several characteristics of thymocyte maturation were chosen from the murine T-lymphoma cell line SL12 (18, 19) to examine whether they might also differ in TCR/T3 gene expression. The cell clones were obtained by limiting dilution of the parental heterogeneous SL12 cell line (18, 19). In some but not all respects, the cell clones have characteristics in common with thymocytes at early stages of differentiation (18, 19). The clones display different thymocyte surface antigens and differ in their response to glucocorticoid hormones; these differences are unlikely to have a mutational basis (18). The clones have a stable phenotype *in vitro* and a near diploid karyotype [clones SL12.1, SL12.3, and SL12.4 have 40, 41, and 39 chromosomes, respectively, with no detectable rearrangements (18–20)]. SL12.3 is trisomic for chromosome 15, and SL12.4 is monosomic for chromosome 12 (E. Hays and C.L.M., unpublished data). The stable, near diploid characteristics of the clones make them particularly useful for genetic studies of the regulation of TCR/T3 complex gene expression.

We show here that these cell clones differentially express TCR-α, TCR-β, T3-δ, and T3-ε mRNA. Examination of somatic cell hybrids formed between SL12 T-lymphoma clones revealed the presence of trans-acting factors that strongly repress TCR-β and T3-δ mRNA expression, moderately inhibit T3-ε expression, but have no detectable effect on TCR-α expression. These trans-acting factors may play a regulatory role in the repression of TCR/T3 message levels early in thymocyte differentiation.

MATERIALS AND METHODS

Materials. Restriction enzymes were obtained from New England Biolabs; cycloheximide and acridine orange, from Sigma; and [α-^{32}P]dCTP, from Amersham.

Cell Lines and Cell Hybrids. The isolation of SL12.4 and SL12.1 (18) and of SL12.3 (19) has been described. SAK8 is an AKR spontaneous T-lymphoma cloned cell line that was established by R. Hyman (Salk Institute) and characterized by Gasson and Bourgeois (21). The isolation of the somatic cell hybrid clones used in this study has been reported (20). The cells were maintained in suspension culture in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum.

RNA and DNA Blot Analysis. Cytoplasmic RNA was prepared from 10^7 cells and denatured by heating to 65°C in formamide. Ten micrograms of RNA was applied to a small (25-ml) 1% agarose/formaldehyde gel and electrophoresed for 3–5 hr. After staining and destaining with acridine orange to visualize the 18S and 28S ribosomal markers, the RNA was

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Abbreviations: TCR, T-cell antigen receptor; kb, kilobase(s).

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transferred to nitrocellulose and the filters were washed, baked, and hybridized (22). The molecular sizes of the RNAs were estimated from the relative migration of the 18S and 28S ribosomal markers. Total cellular DNA was prepared from the parental and hybrid cell clones (23) and digested with *Eco*RI or *Pvu* II according to the supplier's conditions. Twenty micrograms of digested DNA was applied to each lane of a large (250-ml) agarose gel and electrophoresed for at least 48 hr. Southern blots (22, 23) were prepared and hybridized at 42°C in the presence of 50% formamide and 10% dextran sulfate (22). Nick-translated inserts of each of the following probes were prepared: TCR-α (pαDO, ref. 24), TCR-β (86T5, ref. 9), TCR-$J_\beta 2$ (33), T3-δ (pPEM-T3δ, ref. 11), and T3-ε (pDJ1, ref. 12). The specific activities of the insert DNA were $\approx 10^8$ cpm/μg; 5×10^6 cpm/ml were used to probe RNA blots, and $1–2 \times 10^7$ cpm/ml were used to probe DNA blots. The RNA blots were washed with 1% $NaDodSO_4$ at 98°C to remove the labeled probe, allowed to cool to room temperature, air-dried, and stored under vacuum until hybridized with the next cDNA probe.

Measurement of Surface Antigens. Flow cytometric analysis of the Thy-1, ThB and H-2K^k surface expression was done with the Salk Institute flow microfluorimeter (18, 26), using monoclonal antibodies directed against ThB (18), Thy-1 (18, 26), and H-2K^k (18) provided by R. Hyman. Signals from 50,000 cells per sample were collected; dead cells were gated out; and the remaining data were plotted as relative fluorescence, presented on a log scale, standardized to a mean background fluorescence of 1 (26).

RESULTS

Expression of TCR/T3 mRNAs in Cloned Lymphoma Cell Lines and Hybrids. Cloned SL12 cell lines were examined for the presence of TCR/T3 transcripts by RNA blot analysis. Cytoplasmic RNA was probed with nick-translated cDNA inserts complementary to sequences encoding TCR-α, TCR-β, T3-δ, and T3-ε. Fig. 1 shows that SL12.4 cells lack detectable TCR-α mRNA but contain the 1.0-kb TCR-β transcript, an immature form of the message which lacks the variable region (25, 27). An independently derived AKR T-lymphoma cell line, SAK8 (21), expresses both TCR-α and the full-size (1.3-kb) TCR-β transcript, which is likely to contain the variable "antigen-recognition" sequences (8, 25). In contrast, SL12.1 cells and SL12.3 cells have TCR-α but no TCR-β transcripts. T3-δ mRNA is present only in SL12.4; all the other clones lack detectable T3-δ mRNA. T3-ε transcripts have been found in all the clones except SL12.3 (Fig. 1 and Table 1).

The cell lines described above clearly differ in TCR/T3 mRNA expression. To investigate the genetic nature of the regulation of TCR/T3 gene expression, we chose to examine the amount of those mRNAs in somatic cell hybrids formed among the different clones (20). The hybrids are particularly useful for this analysis because they have an unusually stable and near-tetraploid chromosome number, ranging from 77 to 81 with an average of 79–80 for each hybrid clone (20). Fig. 1 shows that the amount of TCR-β mRNA is substantially reduced when either SL12.3 or SL12.1 (TCR-β^-) is fused with either SL12.4 (TCR-β^+) or SAK8 (TCR-β^+); e.g., SL12.3–SL12.4, SL12.3–SAK8, and SL12.1–SL12.4. Both the 1.0-kb (found in SL12.4) and 1.3-kb (found in SAK8) TCR-β transcripts are negatively regulated in trans. A plausible explanation for this repression is that cell lines SL12.1 and SL12.3 (TCR-β^-) make diffusible products that "shut off" TCR-β expression in hybrids containing SL12.4 or SAK8 parent genomes.

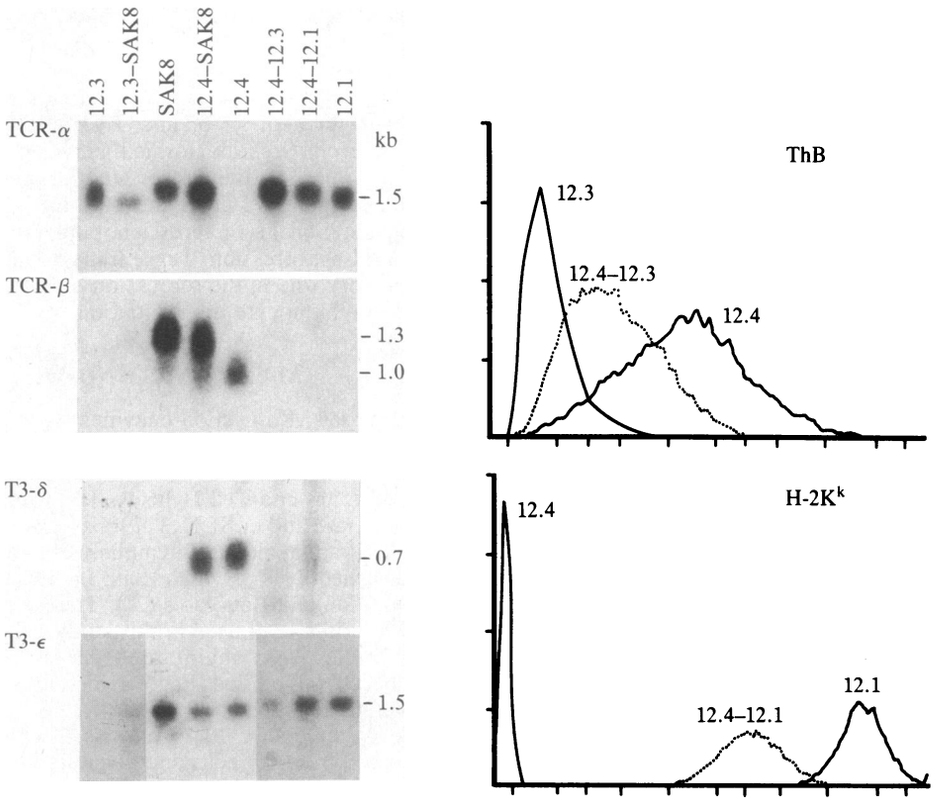

FIG. 1. Expression of T-cell-related gene products in SL12 clones and hybrid cells. (*Left*) Cytoplasmic RNA from the indicated cell lines and hybrid clones was electrophoresed (10 μg per lane) and analyzed by blot hybridization. All the data were obtained from the same blot washed and probed sequentially, except three lanes inserted in the T3-ε panel, which were from another blot. kb, Kilobase(s). (*Right*) Flow cytometric analysis of ThB expression on SL12.4 and SL12.3 parental clones (solid lines) and hybrid clone (dotted line) (*Upper*) and of H-2K^k expression on SL12.4 and SL12.1 parental clones (solid lines) and hybrid clone (dotted line) (*Lower*). Abscissae represent fluorescence intensity measured on a log scale in arbitrary units; ordinates represent cell number in arbitrary units.

Table 1. Summary of TCR/T3 mRNA and surface antigen expression in T-lymphoma clones

	mRNA							
		TCR-β				Surface antigen		
	TCR-α	1.0 kb	1.3 kb	T3-δ	T3-ε	ThB	H-2K^k	Thy-1
SL12.3	+	−	−	−	−	−	−	+
SL12.1	+	−	−	−	+	−	+	+
SL12.4	−	+	−	+	+	+	−	+
SL12.4–12.3	+	−*	−	−*	±	+	−	+
SL12.4–12.1	+	−*	−	−*	+	+	+	+
SL12.3–SAK8	+	−	−*	−	±	−	+	+
SL12.4–SAK8	+	+	+	+	+	+	+	+
SAK8	+	−	+	−	+	−	+	+

Expression of TCR and T3 mRNA was determined by blot-hybridization analysis as illustrated in Fig. 1. An asterisk (*) indicates negative trans-regulation of mRNA expression in hybrids formed between a clone that lacks detectable expression and a clone with easily detectable amounts. ± indicates less than codominant regulation, but not negative trans-regulation of T3-ε mRNA. The cell surface expression of ThB and H-2K^k was determined as illustrated and detailed in Fig. 1. A plus indicates that >90% of the cells were at least 2 SD more fluorescent than the background fluorescence (ref. 26). A minus indicates that >80% of the cells were less than 2 SD below the mean background fluorescence.

All of the hybrid cell clones that lack detectable TCR-β transcripts also lack measurable T3-δ mRNA (Fig. 1); e.g., hybrids formed between SL12.4 (T3-δ$^+$) and either SL12.1 (T3-δ$^-$) or SL12.3 (T3-δ$^-$). The repression of TCR-β and T3-δ is not the result of cell fusion itself, since SL12.4–SAK8 hybrid cells contain easily detectable amounts of T3-δ mRNA and both size classes of TCR-β mRNA (Fig. 1). It should be noted that although SAK8 cells lack detectable amounts of T3-δ mRNA, they do not appear to contain the trans-acting repressors present in both SL12.1 and SL12.3 cells, which negatively regulate T3-δ message levels. Therefore, the absence of T3-δ mRNA in SAK8 cells remains unexplained.

In contrast to the negative trans-regulation of TCR-β and T3-δ described above, hybrid cells express intermediate levels of TCR-α mRNA and surface antigens ThB and H-2K^k when compared to their respective parental clones (Fig. 1 and Table 1). The amount of T3-ε mRNA, although still detectable, is less than intermediate in hybrids formed with T3-ε$^-$ SL12.3 and T3-ε$^+$ cells (Fig. 1). The amounts of TCR-β and T3-δ mRNAs are more substantially reduced than T3-ε in hybrid cells, so that the transcripts are only detectable upon prolonged exposure of the autoradiogram (Fig. 1). These data are consistent with the proposal that SL12.1 and SL12.3 contain trans-acting repressors that strongly inhibit the production of TCR-β and T3-δ mRNA, less strongly inhibit the expression of T3-ε, and have no apparent effect on TCR-α mRNA levels or the level of expression of several thymocyte surface antigens (Fig. 1).

Southern Analysis of TCR in Parental and Hybrid Cells. The lack of TCR-β and T3-δ mRNAs in the hybrid cells could be due to the loss of the chromosomes encoding their respective genes, even though the hybrid cells are near tetraploid and unusually stable (20). Since TCR-β genes are uniquely rearranged in each cell clone, these genes provide an unusual opportunity to document the presence of both sets of parental genes in the hybrid cells. Southern blots of the parental cell DNA show that all the cell clones possess rearrangements of the TCR-β genes (Fig. 2). Southern blots of hybrid cell DNA show that the rearranged bands from SL12.4 and SAK8 are present in hybrids formed with either SL12.1 or SL12.3. These results demonstrate that the lack of TCR-β mRNA is not due to the loss of TCR-β genes from the hybrid cells. In addition, it is likely that T3-δ and T3-ε genetic information is present in the hybrids, since the Thy-1 structural gene is closely linked to T3-δ and T3-ε genes on chromosome 9 (28, 29) and all the hybrids display Thy-1 surface antigen (Table 1). Furthermore, several different clones from each hybrid cross were examined. In no case was any evidence obtained that would suggest the loss of genetic information encoding TCR or T3 (data not shown).

Although TCR-α mRNA is not expressed in SL12.4 cells, Southern analysis of *Pvu* II-digested SL12.4 DNA does not reveal any loss of TCR-α gene(s) (data not shown). Furthermore, TCR-α transcripts are inducible in SL12.4 cells (M.W. and C.L.M., unpublished observation), demonstrating that the cell line possesses at least one functional TCR-α gene.

Cycloheximide Induces the Expression of TCR-β mRNA. In order to gain more information on the repression of TCR-β and T3-δ mRNA expression, the hybrid and parental cells

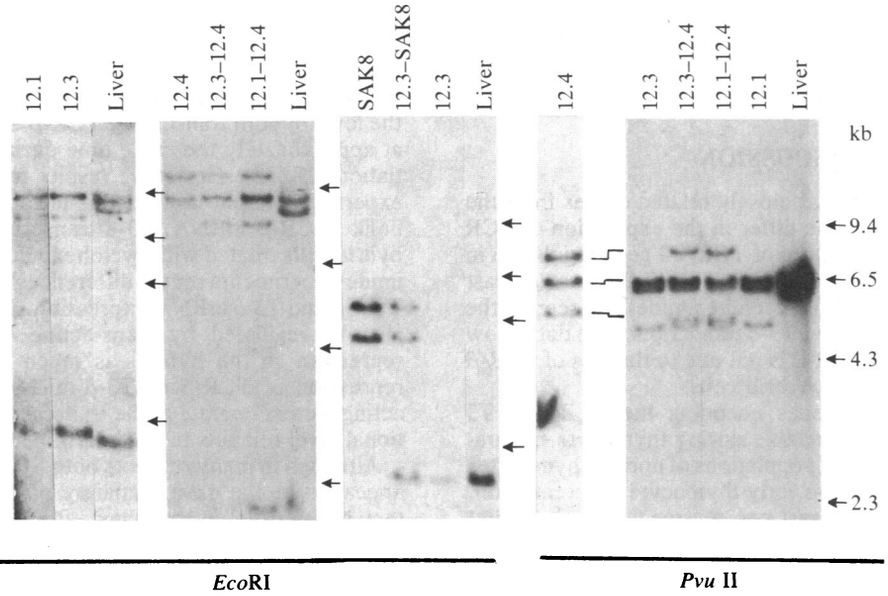

FIG. 2. Southern analysis of the TCR-β gene in hybrid and parental cells. Total DNA from the parental and hybrid clones was digested with *Eco*RI or *Pvu* II. Numbers at right indicate the molecular sizes of fragments of *Hin*dIII-digested λ DNA. The center panel was probed with a genomic clone recognizing the $J_\beta 2$ region; the outer panels were probed with TCR-β cDNA clone 86T5.

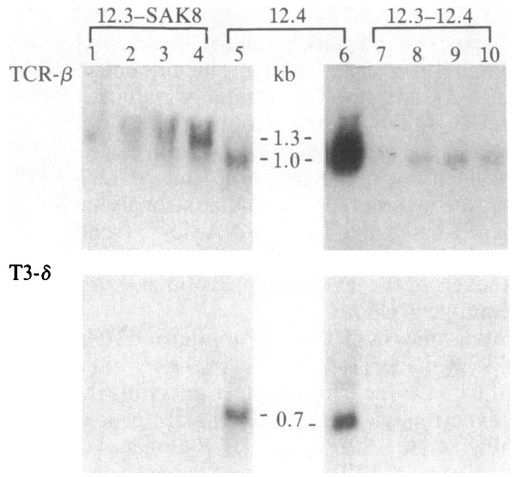

FIG. 3. Blot-hybridization analysis of 10 μg of total cellular RNA from the indicated cells treated with cycloheximide as follows. Lanes 1, 5, 6, and 7, no cycloheximide. Lane 2: 10 μg/ml for 8 hr. Lane 3: 100 μg/ml for 8 hr. Lane 4: 100 μg/ml for 3 hr. Lanes 8–10: 10 μg/ml, 30 μg/ml, and 100 μg/ml, respectively, for 7 hr. Probes were as in Fig. 1. Left panels were from the same blot, right panels were from a different experiment; each blot was probed sequentially.

were treated with the protein synthesis inhibitor cycloheximide. At concentrations sufficient to inhibit >95% of cellular protein synthesis, cycloheximide partially reverses the inhibition of TCR-β mRNA expression in SL12.3–SL12.4 and SL12.3–SAK8 somatic cell hybrids (Fig. 3). Cycloheximide treatment results in an increase only of the 1.0-kb TCR-β mRNA in SL12.3–SL12.4 hybrid cells, whereas in 12.3–SAK8 fusions only the 1.3-kb TCR-β transcript is induced. Cycloheximide does not induce detectable amounts of TCR-β mRNA in SL12.3 parental cells (data not shown). From the size of the transcripts induced and the absence of cycloheximide-induced expression of TCR-β in SL12.3 cells, it seems likely that cycloheximide derepresses TCR-β mRNA expression only from SAK8 and SL12.4 genetic information. Cycloheximide did not reverse the inhibition of T3-δ mRNA expression in any of the hybrids under conditions that induce detectable TCR-β (Fig. 3). Furthermore, even when several different cycloheximide concentrations and various durations of treatment were tested, cycloheximide failed to increase TCR-δ mRNA content (data not shown). It therefore seems likely that TCR-β and T3-δ mRNA are regulated by different trans-acting molecules.

DISCUSSION

We have demonstrated that closely related clones from the SL12 T-lymphoma cell line differ in the expression of TCR and T3 transcripts. The study of TCR/T3 gene regulation in somatic cell hybrids has revealed trans-acting factors (at least one of which is inhibitable by cycloheximide) that repress the accumulation of TCR-β and T3-δ transcripts. The data show that the repression of TCR-β is not due to the loss of TCR-β genetic information in the hybrid cells.

The order in which genes encoding the TCR and T3 polypeptides become detectable during thymocyte maturation has been studied using populations of normal thymocytes (reviewed in ref. 8). During early thymocyte differentiation, TCR-β genes undergo at least two rearrangements: the first rearrangement juxtaposes the diversity (D) and joining (J) regions allowing the expression of a truncated 1.0-kb TCR-β transcript which lacks variable (V) sequences, and the second rearrangement fuses the D-J segment with a V sequence, allowing the transcription of full-length 1.3-kb TCR-β mRNA (13, 25, 27). T3-δ and T3-ε transcripts become detectable at about the same time (27, 32). TCR-α message is the last to be detected and follows full expression of the other transcripts (reviewed in refs. 8 and 32).

Although the SL12 clones were derived from a T-lymphoma cell line, they have some characteristics of discrete stages in early thymocyte differentiation (18–20). Clone SL12.4 is similar to some immature thymocytes in that it expresses only the 1.0-kb TCR-β transcript and lacks detectable TCR-α message. SL12.1 and SL12.3 may represent even more immature thymocytes, in that they display abundant amounts of Thy-1 and Pgp-1 surface antigen (18, 19). Thy-1 is present in large amounts on cortical thymocytes and in lower amounts on medullary cells and mature T cells (31). Pgp-1 is known to be present on prothymocytes and on thymus-homing progenitors (16). Roughly 25% of the Pgp-1$^+$ thymocytes have TCR-β gene rearrangements, and the majority of the genes are in the germ-line configuration, attesting to their immature status (16). SL12.3 cells have rearranged TCR-β genes but express no detectable TCR-β transcripts, nor do they contain T3-δ or T3-ε mRNA. SL12.1 cells also lack detectable TCR-β and T3-δ mRNA, but unlike SL12.3, they express T3-ε message.

Thus, the cell clones that display the most immature phenotype, SL12.1 and SL12.3, both produce the trans-acting factors that repress TCR-β mRNA expression. Such factors could function at early stages of thymocyte differentiation to repress the expression of TCR-β message in cells that possess functional TCR-β gene rearrangements. Although these cell lines are similar to normal thymocytes, they may not represent normal thymocyte developmental stages. Our data show that rearrangement of the TCR-β gene alone is not sufficient for TCR-β mRNA expression. The amount of both the truncated 1.0-kb TCR-β mRNA (found in immature thymocytes) and the full-length 1.3-kb TCR-β mRNA (found in more mature thymocytes and in T cells) are repressed by trans-acting factor(s). Thus, such factors might function at several stages during T-cell maturation. The fact that the 1.0-kb and 1.3-kb TCR-β transcripts use different promoters raises questions regarding the mechanism of their common repression in hybrid cells. Common negative trans-regulation of both transcripts could result from the action of a factor that prevents the use of a putative enhancer or acts on both promoters; alternatively, it could result from the action of more than one factor.

It is intriguing that T3-δ mRNA expression is negatively trans-regulated in the same cell hybrids as is TCR-β. The repression of both TCR-β and T3-δ mRNA in the same hybrid cells suggests that a common trans-acting factor could control the level of both transcripts. TCR-β and T3-δ mRNA appear at approximately the same time during thymocyte differentiation (27, 32). However, results from the cycloheximide experiments argue against this attractive possibility, since, unlike TCR-β mRNA, T3-δ transcripts are not induced in hybrid cells treated with cycloheximide. Thus, the cycloheximide experiments reveal differences between the control of TCR-β and T3-δ mRNA expression. T3-ε mRNA levels may also be regulated by trans-acting factors; however, the repression in the hybrids is much less marked than the repression of TCR-β or T3-δ mRNA. Whether the trans-acting factors operate at the transcriptional or posttranscriptional level remains to be determined.

Although in many respects both SL12.3 and SL12.1 clones appear to be the most immature of the cell clones studied, they are unusual in that they express abundant amounts of TCR-α transcripts and yet lack detectable TCR-β and T3-δ mRNA. The fact that both SL12.3 and SL12.1 cells transcribe TCR-α in the absence of any detectable TCR-β mRNA is different from the accepted order of TCR gene activation in normal thymocytes (8). This discrepancy could be because (*i*) SL12.3 and SL12.1 represent a rare thymocyte lineage with

a reverse order of TCR-α/β expression, (*ii*) these lymphoma cloned cell lines do not represent normal thymocyte differentiation, or (*iii*) the TCR-β genes are rearranged in a fashion that is not productive, resulting in the repression of TCR-β expression that is not reversible by cycloheximide.

Recently, the TCR-β gene was transfected into a mutant T-leukemia cell line that expresses very low amounts of both TCR-α and TCR-β mRNA and lacks detectable TCR/T3 surface expression (31). Following transfection of the TCR-β gene, the cells displayed surface TCR/T3 protein and expressed a larger amount of both TCR-α and TCR-β mRNA, suggesting that TCR-α mRNA expression was probably regulated by TCR-β transcripts. In contrast, our results with the SL12.1 and SL12.3 cell lines clearly show that in some T-cell lines, TCR-α mRNA is not dependent upon the presence of either the 1.0-kb or the TCR-$J_\beta 2$ 1.3-kb TCR-β message.

C.L.M. is indebted to Dr. Stephen Hedrick for the TCR-β cDNA probe 86T5 and the TCR-$J_\beta 2$ genomic probe; Dr. Edward Palmer for the TCR-α cDNA probe pαDO; Dr. Peter Van den Elsen for the T3-δ cDNA probe; Dr. Robert Hyman for monoclonal antibodies to Thy-1, H-2K^k, and ThB as well as helpful comments on the manuscript; Dr. Judith Gasson for the SAK8 cell line; Dr. Robert Tukey for suggesting the cycloheximide experiments; and especially to Dr. Esther Hays, who was a collaborator in the construction of the hybrids (20), for AKR liver DNA and many helpful discussions. The work was conducted in part by the Clayton Foundation for Research, California Division, and supported by National Institutes of Health Grants CA37778 (C.L.M.) and AI17651 (C.T.) and CA12386 (Esther Hays and C.L.M.). C.L.M. is a Clayton Foundation Investigator, D.P.G. is a Cancer Research Institute/Eleanor Naylor Dana Charitable Trust Fellow, C.T. is a Leukemia Society of American Scholar, and M.W. is supported by National Institutes of Health Training Grant HL07107.

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