refill the docking sites. Their interaction with the cytoskeleton supposedly is mediated by fodrin, as anti-fodrin antibodies introduced into detergent-permeabilized chromaffin cells partially inhibit exocytosis[17]. The rapid depolymerization of submembranous actin filaments observed on stimulation[18] might liberate vesicles tethered in the cytoskeletal meshwork and allow their advance and docking to the plasma membrane.

Our results establish a functional role for the plasma membrane-located 51K CGBP in exocytosis in PC12 and adrenal chromaffin cells. Although the inhibition experiments reported here do not reveal the precise biochemical function of the CGBP, they indicate that the 51K CGBP is part of an intracellular recognition site for chromaffin granules on the plasma membrane. The search for molecules on the chromaffin granules complementary to 51K CGBP could lead to identification of an intracellular receptor-ligand pair suggested by Palade[4] to be involved in exocytosis. □

Received 27 April; accepted 17 May 1989.

1. Burgess, T. L. & Kelly, R. B. *A. Rev. Cell Biol.* **3,** 243-293 (1987).
2. Douglas, W. W. *Br. J. Pharmac.* **34,** 451-474 (1968).
3. Augustine, G. J., Charlton, M. P. & Smith, S. J. *A. Rev. Neurosci.* **10,** 633-693 (1987).
4. Palade, G. *Science* **189,** 347-358 (1975).
5. Meyer, D. I. & Burger, M. M. *J. biol. Chem.* **254,** 9854-9859 (1979).
6. Heumann, R., Schwab, M. & Thoenen, H. *Nature* **292,** 838-840 (1981).
7. Neher, E. & Marty, A. *Proc. natn. Acad. Sci. U.S.A.* **79,** 6712-6716 (1982).
8. Lindau, M. & Neher, E. *Pflügers Arch. ges. Physiol.* **411,** 137-146 (1988).
9. Joshi, C. & Fernandez, J. M. *Biophys. J.* **53,** 885-892 (1988).
10. Creutz, C. E. *et al. J. biol. Chem.* **262,** 1860-1868 (1987).
11. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch. ges. Physiol.* **391,** 85-100 (1981).
12. Fenwick, E. M., Marty, A. & Neher, E. *J. Physiol., Lond.* **331,** 599-635 (1982).
13. Pusch, M. & Neher, E. *Pflügers Arch. ges. Physiol.* **411,** 204-211 (1988).
14. Bourne, H. B. *Cell* **53,** 669-671 (1988).
15. Kelly, R. B. *Neuron* **1,** 431-438 (1988).
16. Schäfer, Th., Karli, U. O., Schweizer, F. E. & Burger, M. M. *Biosci. Rep.* **7,** 269-279 (1987).
17. Perrin, D., Langley, O. K. & Aunis, D. *Nature* **326,** 498-501 (1987).
18. Cheek, T. R. & Burgoyne, R. D. *J. biol. Chem.* **262,** 11663-11666 (1987).
19. Schäfer, Th., Karli, U. O., Grathwohl, E. K.-M., Schweizer, F. E. & Burger, M. M. *J. Neurochem.* **49,** 1697-1707 (1987).
20. Patzak, A. *et al. J. Cell Biol.* **98,** 1817-1824 (1984).
21. Porter, R. R. *Biochem. J.* **73,** 119-126 (1959).

ACKNOWLEDGEMENTS. We thank Dr W. B. Adams for help with the capacitance measurements and Drs J. G. Nicholls, D. Dagan, J. Hagmann and R. G. Johnson Jr for discussions and support.

Diversity of $\gamma\delta$ T-cell receptors on murine intestinal intraepithelial lymphocytes

Yohtaroh Takagaki, Amy DeCloux, Marc Bonneville & Susumu Tonegawa

Howard Hughes Medical Institute and Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

THE search for the genes encoding the T-cell receptor (TCR) α- and β-subunits revealed a third gene γ which shares with the α- and β-genes several properties including somatic rearrangement[1,2]. This gene, together with a fourth rearranging gene δ[3,4], encodes a second type of T-cell receptor, TCR $\gamma\delta$[5-8]. Although TCR $\gamma\delta$-bearing T cells constitute a relatively minor subpopulation in the thymus and in peripheral lymphoid organs[8,9], they are the major lymphocytes of epidermis (dendritic epidermal cells or DEC)[10] and of intestinal epithelium (intestinal intraepithelial lymphocytes or IEL) in mice[11,12], suggesting that at least some $\gamma\delta$ T cells are important in the surveillance of a variety of epithelia[13]. It was recently reported, however, that the TCR $\gamma\delta$ on DEC has essentially no structural diversity, implying that the putative ligand is monomorphic[14]. As this finding, if generally applicable, poses severe restrictions on the origin of the ligand, we investigated the diversity of the TCR on the second major epithelium-associated $\gamma\delta$ T cells, namely IEL from mice. We report here that by contrast with the DEC $\gamma\delta$, the IEL $\gamma\delta$ TCR are structurally diverse.

To synthesize DNA encoding the *V-J* or *V-D-J* junctional regions of TCR γ- or δ-chains, RNA extracted from IEL was

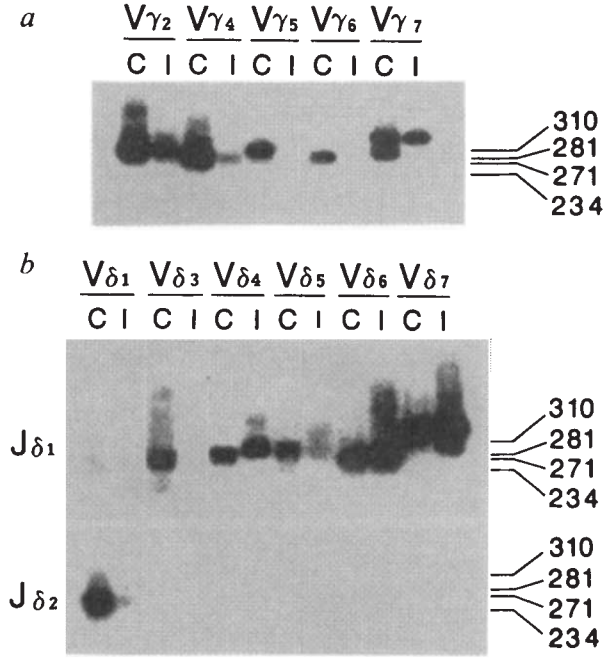

FIG. 1 Detection of TCR γ and TCR δ RNA in IEL by Southern blot analysis of PCR-amplified cDNA. cDNA synthesis was initiated with a C_γ- or C_δ-primer. The sequence of the C_γ primer is shared by all three functional C_γ-gene segments (C_1, C_2, and C_4; refs 2, 23 & 24). For the subsequent PCR we added a variety of *V*-specific primers listed in Table I. $V_{\gamma3}$ was not used because it rearranges only to the non-functional $C_{\gamma3}$-gene segment[2]; $V_{\gamma1}$ was also not used because the IEL γ protein (relative molecular mass (M_r 34-35K)[11,12] was clearly different from the $V_{\gamma1}$-$C_{\gamma4}$ protein (M_r 37-42K)[10,25]. *a*, γ RNA. The Southern blot of γ-PCR products was hybridized with random-primed $C_{\gamma1}$ cDNA (*Sfa*NI-*Sfa*NI, 650 base pairs (bp)). The controls (lanes C) were RNA fragments extracted from the following hybridomas: $V_{\gamma2}$ and $V_{\gamma4}$, KN6 (ref. 18); $V_{\gamma5}$, KI129 (ref. 26); $V_{\gamma6}$, KN25 (ref. 18); and $V_{\gamma7}$, KN106 (ref. 18). The products from IEL RNA were run in lanes I. *b*, δ RNA. The Southern blots of δ-PCR products were hybridized with oligonucleotide probes for $J_{\delta1}$ or $J_{\delta2}$ as indicated on the left side of the blots. For the controls (lanes C), RNA fragments extracted from the following hybridomas were used: $V_{\delta1}$, KI129 (ref. 26); $V_{\delta4}$, KN12 (ref. 18); $V_{\delta5}$, KN106 (ref. 18); $V_{\delta6}$, 66-33B (gift of I. Ishida); $V_{\delta7}$, KN25 (ref. 18). For the $V_{\delta3}$ control, 0.1 ng of Z68 cDNA[19] cut with *Eco*RI was used. The products from IEL RNA are shown in lanes I.

METHODS. IEL were isolated from adult (15-20-week-old) C57BL/6J mice as described by Petit *et al.*[20] and RNA from the lymphocytes in the 50% Percoll fraction was extracted by the guanidinium isothiocyanate/CsCl method[27]. 10 μg of RNA was incubated with 0.5 μM *C*-primer, 0.5 mM deoxynucleotides, 50 mM Tris-HCl (pH 8.3), 10 mM $MgCl_2$, 8 mM dithiothreitol, 2 units of human placental ribonuclease inhibitor (Amersham) and 15 units of reverse transcriptase (Seikagaku) in a total volume of 20 μl. After 45 min at 43 °C, 2.5 μl of the mixture was brought to 25 μl in 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 2.5 mM $MgCl_2$, 1 μM *V*-primer, dNTPs at 250 μM and 0.5 units of *Taq* polymerase (Perkin Elmer Cetus). PCR cycles were run essentially as described by Saiki *et al.*[15] with 1 min at 92 °C, 2 min at 50 °C and 3 min at 72 °C. A 10-μl aliquot of the reaction mixture was applied to a 1.5% Agarose gel in 1 × TBE[27], and Southern transfers were performed, as described previously[27] on Nitro-plus 2000 cellulose nitrate membranes (MSI). The $J_{\delta1}$ probe was the oligonucleotide 5′-CTACCGACAAACTCGTCTTTGGACAAGGAACCCAAGTGACTGTGGAACCA-3′, labelled by Klenow enzyme with the primer 5′-TGGTTCCACAGTCAC-3′. The $J_{\delta2}$ probe was the oligonucleotide 5′-CTCCTGGGACACCCGACAGATGTTTTTTGGAACTGGCATAGAGCTCTTTGTGGAGCCCC-3′, labelled by Klenow enzyme with the primer 5′-GGGGCTCCACAAAGAGCT-3′. After standard hybridization and washing[27], an autoradiogram was generated using the Fujix BA100 Bio-image analyser (Fuji Photo Film Co., Ltd) after exposure for 15 min[28].

subjected to the polymerase chain reaction (PCR)[15] (see Table 1 for the synthetic primers used), and subsequently analysed by agarose gel electrophoresis (Fig. 1). Concordant with a previous RNA blotting analysis[11], the $V_{\gamma 7}$-primer gave a strong DNA band of the expected length. DNA bands of appropriate lengths were also observed with the $V_{\gamma 2}$- and $V_{\gamma 4}$-primers, but not with the $V_{\gamma 5}$- or $V_{\gamma 6}$-primers. As all IEL γ-chains are N-glycosidase-sensitive (data not shown), the $\gamma 2$ chain ($V_{\gamma 2}J_{\gamma 2}C_{\gamma 2}$) which lacks the site for N-linked carbohydrates[1] was not analysed. In the case of TCR δ-chains, a given V_δ segment can rearrange to either $J_{\delta 1}$- or $J_{\delta 2}$-gene segments[4]: strong PCR bands corresponding to $V_{\delta 4}$-$J_{\delta 1}$, $V_{\delta 6}$-$J_{\delta 1}$, and $V_{\delta 7}$-$J_{\delta 1}$ rearrangements and faint bands corresponding to $V_{\delta 5}$-$J_{\delta 1}$ and $V_{\delta 1}$-$J_{\delta 2}$ rearrangements were detected (Fig. 1*b*).

Both the γ and δ PCR products from the IEL RNA were cloned into a plasmid and their nucleotide sequences determined (Fig. 2). Of the 13 DNA clones obtained from the $V_{\gamma 7}$ PCR products, 10 had in-frame V-J joints, and three had out-of-frame joints (Fig. 2*a*), all with N-nucleotide additions[16]. Additionally, the joining ends of both the $V_{\gamma 7}$- and $J_{\gamma 1}$-gene segments varied among clones. Although the $V_{\gamma 4}$ PCR products also exhibited high junctional variability, all three in-frame clones had a translational termination codon (TAG) indicating that they cannot code for the surface-expressed γ chains. This is consistent with our previous immunoprecipitation analysis[11]. The nucleotide sequences of the δ PCR products show all but one, pd5-100, to be joined in-frame and therefore have the potential to be expressed on the IEL surface (Fig. 2*b*). It is evident that all mechanisms responsible for the junctional diversity of TCR δ-genes are utilized in IEL δ chains, namely the use of two known D_δ gene segments, the use of D_δ gene segments in all three reading frames, the occurrence of N nucleotides in most junctions, and the imprecise joining[17] of the V_δ and J_δ gene segments.

a

	V_γ	N	$J_{\gamma 1}$	*In frame?*
germline $V_{\gamma 7}$	-TGT GCC TCC TGG GCT GG *cacaatg.....*			
germline $J_{\gamma 1}$			*.....cactgtg* AT AGC TCA GGT-	
g7- 19	-TGT GCC TCC TGG GC	CCGAT	AT AGC TCA GGT-	*Yes*
g7- 30	-TGT GCC TCC TGG G	GGAGGGGGT	AT AGC TCA GGT-	*Yes*
g7- 42	-TGT GCC TCC TGG GC	AT	AT AGC TCA GGT-	*Yes*
g7- 51	-TGT GCC TCC TGG GC	GGAAT	AT AGC TCA GGT-	*Yes*
g7- 52	-TGT GCC TCC TGG G	TTCTAT	AT AGC TCA GGT-	*Yes*
g7- 70	-TGT GCC TCC TGG GCT GG	CCACGG	T AGC TCA GGT-	*Yes*
g7- 97	-TGT GCC TCC TGG GCT GG	C	AGC TCA GGT-	*Yes*
g7-108	-TGT GCC TCC TGG GCT GG	AT	AT AGC TCA GGT-	*Yes*
g7-133	-TGT GCC TCC TGG GCT	ACAT	AT AGC TCA GGT-	*Yes*
g7-134	-TGT GCC TCC TGG GCT G	CT	AGC TCA GGT-	*Yes*
g7- 56	-TGT GCC TCC TGG GCT GG	TAT	AT AGC TCA GGT-	*No*
g7- 57	-TGT GCC TCC TGG GCT	ACGGAT	AT AGC TCA GGT-	*No*
g7- 91	-TGT GCC TCC TGG	CCCTAT	AT AGC TCA GGT-	*No*
germline $V_{\gamma 4}$	-TGT TCC TAC GGC TAA AG *cacagca.....*			
germline $J_{\gamma 1}$			*.....cactgtg* AT AGC TCA GGT-	
g4- 7	-TGT TCC TAC GGC TA	G	AGC TCA GGT-	*Yes*
g4- 22	-TGT TCC TAC GGC TA	GAAG	AGC TCA GGT-	*Yes*
g4- 25	-TGT TCC TAC GGC TA	GCGG	AGC TCA GGT-	*Yes*
g4- 16	-TGT TCC TAC GGC TAA A	CCAGATAAGG	CA GGT-	*No*
g4- 21	-TGT TCC TAC GG	AG	GGT-	*No*
g4- 23	-TGT TCC TAC GGC T	TACGTC	TCA GGT-	*No*
g4- 55	-TGT TCC TAC GGC TAA	GAGGA	AGC TCA GGT-	*No*
g4- 70	-TGT TCC TAC GGC TAA A	TAGACC	TCA GGT-	*No*

FIG. 2 V-J junctional sequences of γ and δ transcripts from IEL. *a*, $V_{\gamma 7}$-$J_{\gamma 1}$ and $V_{\gamma 4}$-$J_{\gamma 1}$. The V-J junctional DNA sequences are aligned with V_γ germline sequence (Y.T., unpublished observations) or germline $J_{\gamma 1}$ sequence[23]. *N*-regions due to nucleotide additions are shown with normal letters, germline coding sequences are shown in bold letters and heptamer sequences for recombination are indicated in italics. *b*, V_δ-$J_{\delta 1}$. Junctional DNA sequences are aligned with published germline sequences[19] for $D_{\delta 1}$, $D_{\delta 2}$, and $J_{\delta 1}$ and published V_δ sequences with estimated boundaries[18,19]. *N*-regions (N_1, N_2 and N_3) corresponding to the nucleotide additions at *VD*, *DD* and *DJ* junctions are shown in normal letters, germline coding sequences are shown in bold letters and germline heptamer sequences are shown in italics. pd6-92 has the same V_δ sequence as M23 (ref. 4). pd7-30 and pd7-33 both have V-region sequences that are slightly different from the other $V_{\delta 7}$ sequences.
METHODS. Amplified cDNA from IEL (see Fig. 1) was treated with kinase, purified by 1.5% agarose gel electrophoresis and cloned into the *Sma*I site of the pUC13 vector. After transformation into *E. coli* cells, ampicillin-resistant colonies were screened with labelled $V_{\gamma 7}$ or $V_{\gamma 4}$ probes described previously[18], and plasmid DNA from positive colonies was sequenced by the dideoxy method[27] using Sequenase (U.S. Biochemical).

b

	V_δ	N_1	$D_{\delta 1}$	N_2	$D_{\delta 2}$	N_3	$J_{\delta 1}$	*In frame?*
germline $V_{\delta 4}$	-TGT GCT CTC ATG GAG CG							
pd4-203	-TGT GCT CTC ATG GAG CG	CGGGC	GCATAT	TGGGC	CGGAGGGATACGAG	A	ACC GAC AAA-	*Yes*
pd4-213	-TGT GCT CTC ATG GAG C	ACACA	TGGC	TCTCAAT	ATCGGAGGGATACGA	CCCTC	CT ACC GAC AAA-	*Yes*
pd4-276	-TGT GCT CTC ATG G			GAGG	GGAGGGATACG		CC GAC AAA-	*Yes*
pd4-305	-TGT GCT CTC ATG GAG				ATCGGAGGGATA	AAG	ACC GAC AAA-	*Yes*
pd4-318	-TGT GCT CTC ATG GAG C	TA	CAT		ATCGGAGGG	CA	T ACC GAC AAA-	*Yes*
germline $V_{\delta 5}$	-TGT GCC TCG GGG TAT							
pd5- 17	-TGT GCC TCG GGG TAT	TGG	ATATC		GAGGGATACGAG	CTTGG	CC GAC AAA-	*Yes*
pd5- 86	-TGT GCC TCG GGG TAT	CCC	GCAT		CGGAGGGA	G	CT ACC GAC AAA-	*Yes*
pd5-100	-TGT GCC TCG GGG	CTC	CATAT	GGCA	ATCGGAGGGATAC	AGG	ACC GAC AAA-	*No*
pd5-187	-TGT GCC TCG GGG	GAA	ATAT		GG	CCCT	CT ACC GAC AAA-	*Yes*
pd5-238	-TGT GCC TCG GG	CTTC			GAGGG	CCCCA	CT ACC GAC AAA-	*Yes*
germline $V_{\delta 6}$	-TGC GCT CTC TCG GAA CT							
pd6- 32	-TGC GCT CTC TCG GAA CT	TGGA	ATAT		ATCGGAGGGATACG	T	CT ACC GAC AAA-	*Yes*
pd6-138	-TGC GCT CTC TCG GAA CT	G		ACC	AGGG		CT ACC GAC AAA-	*Yes*
pd6-201	-TGC GCT CTC TCG GAA C	GTGGGAGGCACCA	CATATC	GG	AGGGATACGAG	CTGGG	C GAC AAA-	*Yes*
pd6-203	-TGC GCT CTC TCG G	TC GT		T	ATCGGAGGGATACGAG		CT ACC GAC AAA-	*Yes*
pd6-204	-TGC GCT CTC TCG GAA CT			G	TCGGAGGGATACG	GGG	CT ACC GAC AAA-	*Yes*
pd6- 92*	-TGT GCT CTC TGG GAG C	CT	TAT		ATCGGAGGGATACG	GA	CT ACC GAC AAA-	*Yes*
germline $V_{\delta 7}$	-TGT GCT ATG G							
pd7- 37	-TGT GCT ATG G		ATC	C	ATCGGAGGGATACGAG	CTGACG	GAC AAA-	*Yes*
pd7- 43	-TGT GCT ATG G	AAC	GTGG	G	CGGAGGG	AC	ACC GAC AAA-	*Yes*
pd7- 51	-TGT GCT A	GGAGGG	ATATC		GGAGGGATACGAG		CT ACC GAC AAA-	*Yes*
pd7-152	-TGT GCT ATG		GTGG	G	CGGAGGGATACGAG	AGG	CT ACC GAC AAA-	*Yes*
pd7- 30#	-TGT GCT AT	A			GGAGGGATACGA	CT	C GAC AAA-	*Yes*
pd7- 33#	-TGT GCT A	GA			ATCGGAGGGATAC	ACC	CT ACC GAC AAA-	*Yes*

germline $D_{\delta 1}$ .....*cactgtg* GTGGCATATCA *cacaggt.....*
germline $D_{\delta 2}$ .....*caccgtg* ATCGGAGGGATACGAG *cacagtg.....*
germline $J_{\delta 1}$ .....*agctgtg* CT ACC GAC AAA-

TABLE 1 PCR primers

Region	Name	Sequence 5′-3′	Position	Ref.
C_γ	STP. 120	CTTATGGAGATTTGTTTCAGC	139-145	1
$V_{\gamma 2}$	STP. 121	CGGCAAAAAACAAATCAACAG	37-43	1
$V_{\gamma 4}$	STP. 073	TGTCCTTGCAACCCCTACCC	49-56	29
$V_{\gamma 5}$	STP. 094	TGTGCACTGGTACCAACTGA	35-42	23
$V_{\gamma 6}$	STP. 107	(GGAA)TTCAAAAGAAAACATTGTCT	55-62	23
$V_{\gamma 7}$	STP. 102	AAGCTAGAGGGGTCCTCTGC	18-24	30
C_δ	STP. 110	CGAATTCCACAATCTTCTTG	158-165	3
$V_{\delta 1}$	STP. 111	(GGA)ATTCAGAAGGCAACAATGAAAG	79-86	4
$V_{\delta 3}$	STP. 119	TTCCTGGCTATTGCCTCTGAC	65-72	19
$V_{\delta 4}$	STP. 075	CCGCTTCTCTGTGAACTTCC	61-68	18
$V_{\delta 5}$	STP. 082	CAGATCCTTCCAGTTCATCC	42-49	18
$V_{\delta 6}$	STP. 113	TCAAGTCCATCAGCCTTGTC	72-78	3
$V_{\delta 7}$	STP. 076	CGCAGAGCTGCAGTGTAACT	18-25	18

The position of the nucleotide sequence of the primer is indicated by the corresponding amino-acid number counted from the putative N-terminal cleavage site in each reference. For the $V_{\delta 6}$ primer, a sequence common to pλ12, Z53 and Z49 was chosen. The 3′ 15 bases of this primer are also common to M23 (ref. 4).

The amino-acid sequences deduced from the junctional nucleotide sequences indicate that IEL γδ TCR would have a high degree of structural diversity in the V-J junctional regions (data not shown); but diversity is not limited to these regions because the $V_{\gamma 7}$-coded γ-chain can pair with either the V_4, V_5, V_6 or V_7 δ-chain. This diversity of the IEL γδ TCR is reminiscent of that observed for the γδ TCR expressed on the thymocytes of adult mice[18,19]. The IEL γδ TCR, however, clearly comprise a unique subset distinct from those on adult thymocytes which use $V_{\gamma 4}$ and $V_{\delta 5}$ gene segments predominantly.

The γδ TCR expressed on DEC, the other known epithelium-associated γδ T-cell subset, utilize a single V_γ ($V_{\gamma 5}$) and a single V_δ ($V_{\delta 1}$) gene segment and have no junctional diversity[14]. This suggests that the ligand for DEC γδ TCR is monomorphic unlike those of αβ TCR[14]. By contrast, IEL certainly have the capacity to recognize structurally diverse ligands with their highly diverse γδ TCR. This, plus the fact that IEL are CD8-positive[20,21] strongly suggests that their ligand is composed of a structurally variable peptide presented by a class I or class I-like protein of the major histocompatibility complex (MHC). The high level of diversity concentrated in the V-(D)-J junctions is consistent with the recognition of variable peptides, if the folding of polypeptide chains is similar for TCR γδ and immunoglobulin molecules[22]. The origin of the postulated peptides is a matter of speculation. One possibility is that they originate from a relatively large set of self proteins whose syntheses are induced when the epithelial cells are under stress. Another possibility is that the peptides arise from viruses, bacteria and other microorganisms that are prone to infect the intestinal epithelium cells. The preferential usage of the $V_{\gamma 7}$ segment may reflect its affinity for a limited number of class I or class I-like protein(s) that may be expressed on intestinal epithelial cells. □

Received 22 March; accepted 19 May 1989.

1. Saito, H. *et al. Nature* **309,** 757-762 (1984).
2. Hayday, A. C. *et al. Cell* **40,** 259-269 (1985).
3. Chien, Y-h., Iwashima, M., Kaplan, K. B., Elliott, J. F. & Davis, M. M. *Nature* **327,** 677-682 (1987).
4. Chien, Y-h. *et al. Nature* **330,** 722-727 (1987).
5. Brenner, M. B. *et al. Nature* **322,** 145-149 (1986).
6. Lew, A. M. *et al. Science* **234,** 1401-1405 (1986).
7. Nakanishi, N., Maeda, K., Ito, K., Heller, M. & Tonegawa, S. *Nature* **325,** 720-723 (1987).
8. Maeda, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **84,** 6536-6540 (1987).
9. Bluestone, J. A., Pardoll, D., Sharrow, S. O. & Fowlkes, B. *J. Nature* **326,** 82-84 (1987).
10. Koning, F. *et al. Science* **236,** 834-837 (1987).
11. Bonneville, M. *et al. Nature* **336,** 479-482 (1988).
12. Goodman, T. & Lefrancois, L. *Nature* **333,** 855-858 (1988).
13. Janeway, C. A. Jr *Nature* **333,** 804-806 (1988).
14. Asarnow, D. M. *et al. Cell* **55,** 837-847 (1988).
15. Saiki, R. *et al. Science* **239,** 487-491 (1988).
16. Alt, F. & Baltimore, D., *Proc. natn. Acad. Sci. U.S.A.* **79,** 4118-4122 (1982).
17. Tonegawa, S. *Nature* **302,** 575-581 (1983).
18. Takagaki, Y., Nakanishi, N., Ishida, I., Kanagawa, O. & Tonegawa, S. *J. Immunol.* **142,** 2112-2121 (1989).
19. Elliott, J. F., Rock, E. D., Pattern, P. A., Davis, M. M. & Chien, Y-h. *Nature* **331,** 627-631 (1988).
20. Petit, A. *et al. Eur. J. Immunol.* **15,** 211-215 (1985).
21. Ernst. P. B., Befus, A. D. & Bienenstock, J. *Immunol. Today* **6,** 50-55 (1985).
22. Davis, M. M. & Bjorkman, P. J. *Nature* **334,** 395-402 (1988).
23. Garman, R. D., Doherty, P. J. & Raulet, D. H., *Cell* **45,** 733-742 (1986).
24. Iwamoto, A. *et al. J. Expl. Med.* **163,** 1203-1212 (1986).
25. Koning, F. *et al. J. Immunol.* **141,** 2057-2062 (1988).
26. Ito, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 631-635 (1989).
27. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
28. Amemiya, Y. & Miyahara, J. *Nature* **336,** 89-90 (1988).
29. Heilig, J. S. & Tonegawa, S. *Nature* **322,** 836-840 (1986).
30. Pelkonen, J., Traunecker, A. & Karjalainen, K. *EMBO J.* **6,** 1941-1944 (1987).

ACKNOWLEDGEMENTS. We thank Y. Chien for the Z68 cDNA, K. Ito for the preparation of IEL, Elly Basel for the preparation of the manuscript, H. N. Eisen, D. Gerber and P. Mombaerts for comments on the manuscript, and Fuji Photo Film Co. for the use of BA100-BioImage Analyser. This work was supported by a fellowship (to M.B.) from the Association pour la Recherche Contre le Cancer and Ligue Nationale Contre le Cancer and research grants from the HHMI, the NIH, the American Cancer Society and Ajinomoto Co., Ltd.

Cloning of murine α and β retinoic acid receptors and a novel receptor γ predominantly expressed in skin

Arthur Zelent, Andrée Krust, Martin Petkovich, Philippe Kastner & Pierre Chambon*

Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Institut de Chimie Biologique, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg-Cédex, France

In addition to having profound effects on embryonic pattern formation[1–5], retinoic acid (RA) has striking effects on differentiation and maintenance of epithelial cells *in vivo* and *in vitro* (reviewed in refs 6 and 7). Skin is a major target organ for retinoids both in its normal[6–9] and pathological states[10]. The discovery of two human nuclear receptors for RA (hRARα and hRARβ) acting as transcriptional RA-inducible enhancer factors[11–14] has provided a basis for understanding how RA controls gene expression[15,16]. To investigate the specific role that RARs might play during development and in adult tissues, we have cloned the mouse RARα and RARβ (mRARα and mRARβ). Their amino-acid sequences are much more homologous to those of hRARα and hRARβ, respectively, than to each other, which suggests strongly that RAR α- and β-subtypes have different functions. Most interestingly we have discovered a novel RAR subtype (mRARγ) whose expression in adult mouse seems to be highly restricted to skin, whereas RARα and RARβ are expressed in a variety of adult tissues. Furthermore, both mRARα and mRARγ RNAs are readily detected in undifferentiated F9 embryocarcinoma (EC) cells, whereas mRARβ messenger RNA is induced at least 30-fold in RA-differentiated F9 cells.

* To whom correspondence should be addressed.