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Transcripts of human *HLA* gene fragments lacking the 5′-terminal region in transfected mouse cells

(promoters/recombination/gene conversion/interferon action)

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ABSTRACT **Clones of mouse L cells transfected with a human *HLA-B7* gene fragment lacking the 5′ segment of exon 2 and all upstream sequences express HLA-specific transcripts of various lengths. These include species that correspond in size to full-length HLA-B7 mRNA. The level of these transcripts is increased in cells treated with interferon. It is probable that the full-length transcripts arise as a result of the linkage of the *HLA-B7* gene fragments with DNA segments providing transcription initiation or polyadenylylation signals.**

The highly polymorphic proteins specified by the class I genes of the major histocompatibility complex (designated as HLA in humans and as H-2 in mice) are guides and regulators of the immune response (1, 2).

We reported earlier that interferons can enhance the level of a human class I antigen and mRNA in mouse cells transfected with a cloned human *HLA-B7* gene (3). We wanted to establish which part of the clone is needed to make the expression of the gene responsive to interferons. For this purpose we generated various deletions in the *HLA-B7* gene and tested the expression of the gene fragments in transfected mouse cells in the presence and in the absence of interferons. In the course of these studies we observed in the transfected mouse cells transcripts from gene fragments lacking the 5′-terminal segments of the coding region and all upstream sequences.

RESULTS

Preparation of *HLA-B7* Gene Fragments and Transfection into Mouse Cells. The *HLA-B7* gene fragments were obtained by digesting the complete gene with *Bal* 31 exonuclease and inserting the resulting gene fragments into plasmids for propagation (4, 5). The sizes of the 5′- and 3′-end deletions in the various gene fragments were determined by restriction mapping, and five of the gene fragments were selected for further study (Fig. 1).

Plasmids carrying one of these five were introduced into cells of a mouse line (Ltk^-) by cotransfection with the herpes simplex virus *tk* gene (8). Six to eight clones were chosen from each of the five sets of transfected cells for testing the expression on the cell surface of the HLA-B7 antigen. The tests involved the determination of the extent of the binding to the cells of a monoclonal anti-HLA antibody (3).

HLA or HLA-Like Antigens on the Transfected Cells. In the case of cells transfected by gene segments with about 670-, 440-, 340-, or 240-nucleotide-long 5′ flanking regions (in plasmids pJY150R1.1, pΔ −440, pΔ −340, or pΔ −240), a high proportion (56% in one experiment) of the clones transfected to tk^+ expressed the HLA-B7 antigen. Moreover, this expression was enhanced (3- to 4-fold) in cells exposed to mouse interferon at 1,000 units/ml for 48 hr (the data for one cell clone transfected with pΔ −240 are shown in Table 1).

As a control, we also tested for the presence of the HLA antigen on cell clones transfected with plasmids containing *HLA-B7* gene fragments (i.e., pΔ +30 or pΔ +240). The gene fragment in pΔ +30 lacks all the 5′-flanking sequences together with the sequence specifying the 5′-untranslated segment of the mRNA upstream from the initiation codon. The gene fragment in pΔ +240 lacks all 5′-flanking sequences together with the sequences specifying the 5′-untranslated segment of the mRNA and the regions specifying the entire first exon and the 5′ end of the second exon (Fig. 1).

Two (of six) cell clones transfected with the gene fragment iΔ +30 and three (of seven) cell clones transfected with the gene fragment iΔ +240 were found unexpectedly to express surface protein(s) binding a monoclonal anti-HLA antibody (W6/32) (10). On none of these clones was the amount of the bound antibody increased after exposing the cells to interferons. (The data for one cell clone each from sets of clones transfected with pΔ +30 and pΔ +240 are shown in Table 1.)

HLA-B7-Specific DNA in the Transfected Cells. The unexpected presence of the HLA-like antigen in cells transfected with iΔ +30 and iΔ +240 gene fragments prompted us to characterize the transfected DNA segments and the RNAs specified by these. For this purpose the DNA from the cells was cleaved with the restriction enzyme *Eco*RI, fractionated by gel electrophoresis, blotted to nitrocellulose filters, and tested by hybridization with labeled HLA-B7-specific probes (11) (Fig. 2).

Hybridization with the cDNA probe iDP001 revealed that the most prevalent of the HLA-B7-specific DNA segments in the transfected cells are indistinguishable in size from the corresponding segments used for the transfection (Fig. 2). Thus the bulk of the transfected DNA appears to have been integrated without major rearrangement. A minority of the hybridizing DNA segments are, however, of a different size from the transfecting segments, revealing that some DNA rearrangements have taken place. DNA hybridizing to the probe was not detected in the DNA preparation from cells transfected with the *tk* gene only (lane 5).

The copy numbers of HLA segments in those lines of transfected cells that bound the most anti-HLA antibodies were determined by comparing the densities of the hybridized major (unrearranged) bands with those of bands containing known amounts of HLA DNA segments on appropriately exposed autoradiographs (not shown). The results indicate that the number of HLA-specific segments (of unrearranged DNA) are about 20 copies per cell in cells of a clone transfected with pΔ −240, about 50 in cells of a clone transfected with pΔ +30, and about 150 in cells of a clone transfected with pΔ +240.

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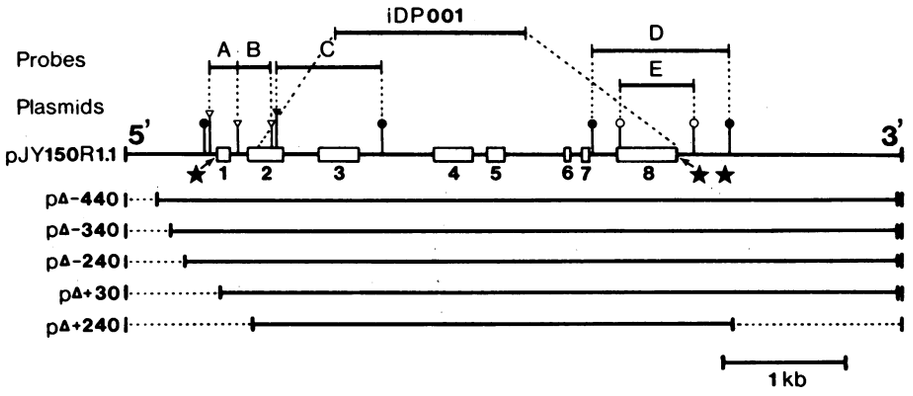

FIG. 1. Subcloning gene fragments of the *HLA-B7* gene iJY150R1.1 (a 6.0-kilobase *Eco*RI fragment including the *HLA-B7* gene, a 670-base-pair sequence upstream from the segment coding for the leader peptide and a >2-kilobase sequence downstream from the gene in pBR328) (3) was excised and digested with *Bal* 31 exonuclease (New England BioLabs). The resulting DNA segments were cloned in the *Eco*RI site of pBR322. The plasmid preparation obtained was transfected into *Escherichia coli*. *E. coli* colonies (200) were screened for plasmids with *HLA-B7* gene fragments of the required size by restriction mapping with *Eco*RI and *Bgl* II and in some cases by sequence analysis. The plasmids containing HLA-B7 fragments in pBR322 are designated according to the 5'-terminal nucleotide of the HLA-B7 insert (—, gene fragments; ----, deleted segments). The nucleotide in the DNA where RNA synthesis is initiated (cap site) is taken as nucleotide +1. Thus, for example, pΔ −440 and pΔ +30 indicate that the 5'-terminal nucleotide of the insert in the plasmid is ≈440 nucleotides upstream or 30 nucleotides (±4; estimated by primer extension) downstream from the cap site, respectively. At their 3' ends the inserts iΔ −440, iΔ −340, iΔ −240, and iΔ +30 are ≈10 nucleotides shorter than iJY150R1.1, and iΔ +240 is about 1,200 nucleotides shorter. The 5' end of the iΔ +30 insert was determined by sequence analysis. The 5' and 3' termini of all other gene fragments are approximations, with an accuracy of about ±25 nucleotides, based on restriction mapping. The orientations of the inserts in the plasmids are as follows. In pΔ −440, pΔ −340, pΔ −240, and pΔ +30 the end of the insert specifying the 5' end of the transcript is adjacent to the *Hin*dIII site of pBR322. The orientation of the insert in pΔ +240 is the opposite. DNA segments used as hybridization probes are also shown. iDP001 is a 1.5-kilobase cDNA segment of HLA-B7 mRNA in the *Pst* I site of pBR322 (pDP001) (6). Probes A, B, C, and D were obtained by digestion of iJY150R1.1 with *Bgl* II and *Xba* I. These procedures result in probes C and D and in a segment comprising both probes A and B. The latter were separated by cleavage with *Ava* II. Probe E was obtained by cleaving iJY150R1.1 with *Pvu* II, inserting the products into the *Hin*cII site of the M13mp8 phage vector (7) (New England BioLabs), and transfecting *E. coli* JM103. Sequence analysis identified which of the M13 phage vectors with the probe E segments contained the DNA strand analogous in sequence to the HLA-B7 mRNA (JY 24) and which contained the DNA strand complementary in sequence to the HLA-B7 mRNA (JY 27). Only the restriction enzyme cleavage sites used to generate the probes are shown. The cleavage sites are *Ava* II (▽), *Bgl* II (▼), *Xba* I (●), and *Pvu* II (○). The boxes represent the exons. ★, Cap site; ★★, polyadenylylation site. kb, Kilobases.

Not more than 5% of the HLA-specific DNA was found as low molecular weight DNA in extracts ("Hirt lysates") (14) from the transfected cells (data not shown). This indicates that the large majority of the HLA-specific DNA segments became integrated into large DNA structures.

HLA-B7-Specific Transcripts in the Transfected Cells. For the characterization of HLA-B7-specific transcripts in the transfected cells, RNA preparations were fractionated according to size by gel electrophoresis, blotted to nitrocellulose filters, and tested for HLA-B7-specific components by hybridization with labeled probes (15) (Fig. 3). The first hybridization probe used (the cDNA segment iDP001) covers the entire region specifying the HLA-B7 RNA with the exception of the first 250 5'-terminal nucleotides (Fig. 1). RNA blots hybridized with this probe (Fig. 3*a*) reveal that in cells transfected with the plasmid pΔ −240 the bulk of the HLA-B7-specific RNA is "full size"—i.e., indistinguishable in length from the bulk of that from cells transfected with pJY150R1.1 (lanes 1 and 2). Some of the HLA-B7-specific RNAs in cells transfected with either of these plasmids are shorter (by about 300–600 nucleotides, as judged from their mobility) and appear to be heterogeneous.

HLA-B7-specific RNA also occurs in cells transfected with *HLA-B7* gene fragments pΔ +30 or pΔ +240 in spite of the fact that these gene fragments lack the normal transcription initiation sites together with all upstream sequences. The bulk of these RNAs are, however, shorter than full size, and only a discrete minor species comigrates with the full-sized RNAs (lanes 3 and 4). In all of these cases, the amounts of HLA-B7-specific full-sized and shorter RNAs are more abundant in cells exposed to interferon than in control cells.

Some hybridizing RNA about the same length as full-sized RNA is detected on long exposures in cells transfected with the *tk* gene only and exposed to interferon (lane 5+). However, this band in the tk^+ cells is much weaker than the full-sized bands from cells transfected with pΔ +30 or pΔ +240 (see lanes 3 and 4). The labeling of this band in tk^+ cells (lane 5+) may be a consequence of a cross-hybridization of mouse H-2 RNA with the HLA probe. Experiments involving the use of shorter and more specific HLA-B7 probes (and sequence analysis of cDNA clones; data not shown) clearly indicate the presence of HLA-B7 RNA sequences in cells transfected with pΔ +30 or pΔ +240. Such hybridizing transcripts are absent from cells transfected with the *tk* gene only.

The bulk of the transcripts from transfected *HLA-B7* gene fragments lacking the normal transcription initiation site are not initiated from sites in the vector pBR322 because (*i*) similar amounts of (both full-sized and short) transcripts are present in different transfectants in which *HLA-B7* gene fragments are inserted into pBR322 in opposite orientation (see legend to Fig. 1) and (*ii*) in several RNA blots pBR322 probes did not hybridize appreciably to the transcripts (data not shown).

The large majority, if not all, of both the full-sized and short HLA-B7-specific RNAs in cells transfected with the gene fragments pΔ +30 and pΔ +240 have poly(A) segments: 4 μg of poly(A)$^+$ RNA from either cell line gives darker bands than 20 μg of total cytoplasmic RNA (compare lane 2 to 1 and lane 4 to 3 in Fig. 3*b*).

For a characterization of the HLA-B7-specific transcripts, we used hybridization probes from different regions of the *HLA-B7* gene (Fig. 1). One of these, probe A, covers the first exon extending approximately from nucleotide −5 to nucleotide +185 of the transcribed region of the *HLA-B7* gene. As expected, the full-sized transcripts from cells transfected with pJY150R1.1 hybridize with this probe, whereas tran-

Table 1. Effect of mouse interferon on expression of an HLA or HLA-like antigen on mouse cells transfected with the *HLA-B7* gene or its fragments

Transfecting plasmid	Exposure of cells to interferon	Anti-HLA antibody binding assay	
		Exp. 1, cpm per culture	Exp. 2, cpm per culture
pX-1	−	2,064	1,452
	+	1,707	1,137
pΔ −670	−	18,930	11,589
	+	38,754	25,641
pΔ −240	−	14,028	4,905
	+	64,425	18,291
pΔ +30	−	5,292	2,853
	+	3,537	2,685
pΔ +240	−	9,012	4,476
	+	7,905	4,347

Mouse Ltk$^-$ cells (lacking thymidine kinase activity) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (GIBCO). Ltk$^-$ cells (10^6) were transfected with 0.5 μg of DNA of pX-1 (a plasmid with a herpes simplex virus *tk* gene in pBR322) (9) and 5 μg of salmon sperm DNA or with 0.5 μg of DNA of pX-1 and 5 μg of DNA from one of the following plasmids—pΔ −670 (i.e., pJY150R1.1), pΔ −240, pΔ +30, or pΔ +240 (see Fig. 1)— and 5 μg of salmon sperm DNA according to Wigler *et al.* (8). tk$^+$ transformants were selected in a culture medium supplemented with hypoxanthine (15 μg/ml)/aminopterin (1 μg/ml)/thymidine (5 μg/ml) and isolated colonies were grown (3). The expression of the HLA antigen was assayed on cells that had been exposed to mouse interferon (1,000 units/ml) (specific activity, 7×10^8 NIH standard reference units per mg of protein) for 48 hr and on control cells by binding to ($\approx 2 \times 10^5$) cells of W6/32 (a monoclonal anti-HLA framework antibody) (10) and detecting the bound antibody with ^{125}I-labeled protein A (3). Cells transfected to tk$^+$ with the plasmid pX-1 served as negative controls. The amounts of ^{125}I-labeled protein A bound to such cells was not increased when the cells were treated with interferon. The amount of ^{125}I-labeled protein A bound was increased by exposure to interferon of cells in every line transfected with the plasmids pΔ −440, pΔ −340, and pΔ −240 and expressing HLA or HLA-like antigens, but in none of the lines transfected with the plasmids pΔ +30 or pΔ +240.

scripts (full-sized or short) from cells transfected with pΔ +240 or the *tk* gene (in pX-1) do not (Fig. 3*c*). Probe A does not appear to hybridize detectably to RNA from cells transfected with pΔ +30 either. This indicates that the transcription of the HLA-B7-specific RNA in cells transfected with pΔ +30 is not initiated in the 135-nucleotide-long 5′ segment that is present both in the iΔ +30 and the A probe.

Probe B covers the 5′-terminal two-thirds of exon 2 and some of the intervening sequences (Fig. 1). This probe hybridizes only with full-sized transcripts from cells transfected with any one of the plasmids pJY150R1.1, pΔ +30, or pΔ +240 (Fig. 3*d*). The fact that the hybridization is much more extensive to poly(A)$^+$ RNA than to total cytoplasmic RNA seems to indicate that most of the hybridizing transcripts are polyadenylylated.

Probe C covers the 3′-terminal one-quarter of exon 2 and all of exon 3 (Fig. 1). It hybridizes both to full-sized and to short transcripts from cells transfected with plasmids pΔ +30 or pΔ +240, and again most of the hybridizing transcripts appear to be polyadenylylated (Fig. 3*e*).

Probe D covers exon 8 together with most of the intervening sequence between exons 7 and 8 and a long untranscribed downstream sequence, whereas the two probes E are shorter, covering most of exon 8 and a short segment of downstream untranscribed sequence (Fig. 1). The two probes E (cloned in the single-stranded DNA phage M13) are strand specific.

Experiments with probes D (not shown) and E (Fig. 3*f*) reveal that: (*i*) none of the three probes hybridizes with full-sized transcripts in cells transfected with pΔ +30 or pΔ +240

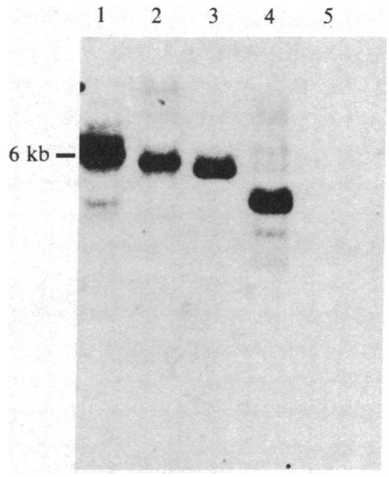

FIG. 2. Characterization of the HLA-B7 DNA segments in the DNA from mouse cell lines transfected with the *HLA-B7* gene or its fragments. Assay by Southern blot hybridization. DNA was extracted (12) from 4×10^7 mouse cells transfected to HLA-B7$^+$ with plasmids pJY150R1.1, pΔ −240, pΔ +30, and pΔ +240 or to tk$^+$ with plasmid pX-1. The DNA was digested with *Eco*RI, subjected to electrophoresis on 0.8% agarose, and transferred to nitrocellulose filter papers (Schleicher & Schuell) (11). These were hybridized with ^{32}P-labeled DNA probes (10^6 cpm/ml) in the presence of 50% formamide at 42°C for 48 hr, washed (with 15 mM sodium chloride/1.5 mM sodium citrate and 0.1% NaDodSO$_4$ at 65°C), and exposed to x-ray film for 1–5 days. The DNA probe used was iDP001 (see Fig. 1), an HLA-B7-specific cDNA segment in plasmid pDP001 (6). This had been nick-translated to a specific activity of $1–5 \times 10^8$ cpm/μg (13). DNA from cells transfected to HLA-B7$^+$ with plasmids pJY150R1.1 (lane 1), pΔ −240 (lane 2), pΔ +30 (lane 3), pΔ +240 (lane 4), and to tk$^+$ with pX-1 (lane 5). The position of the HLA-B7-specific *Eco*RI cleavage product from pJY150R1.1 is indicated by 6 kb (kilobases) (3).

(*ii*) probe D and one of the two E probes (the one that also hybridizes with the HLA-B7 transcripts in cells transfected with pJY150R1.1) hybridizes with the short transcripts in cells transfected with pΔ +30 or pΔ +240.

None of the probes A, B, C, D, or E hybridizes with transcripts from cells transfected with the *tk* gene only.

These results seem to indicate that: (*i*) the full-sized transcripts from the *HLA-B7* gene fragments in pΔ +30 and pΔ +240 share sequences with at least exons 2 and 3 or their flanking sequences (but not with exons 1 or 8). They are polyadenylylated but do not seem to make use of the normal polyadenylylation site of the *HLA-B7* gene. (*ii*) The short transcripts share sequences with exons 3 and 8 (but not with exons 1 or 2). They are polyadenylylated and might use normal polyadenylylation sites on the *HLA-B7* gene.

DISCUSSION

The above results describe several unexpected characteristics of HLA-B7-related transcripts in Ltk$^-$ mouse cells transfected with *HLA-B7* gene fragments. Shorter RNA species, which are only minor components in cells transfected with the intact *HLA-B7* gene, are predominant among the HLA-B7-related transcripts in cells transfected with gene fragments lacking the conventional promoter region and upstream sequences. Furthermore, polyadenylylated RNA indistinguishable in size from the major transcript of the intact gene is present in cells transfected with a gene fragment lacking a contiguous region, including the 5′ segment of exon 2 and all upstream sequences. Finally, the concentration of both the full-length and the short transcripts is modulated by mouse interferon even in cells transfected with the incomplete genes.

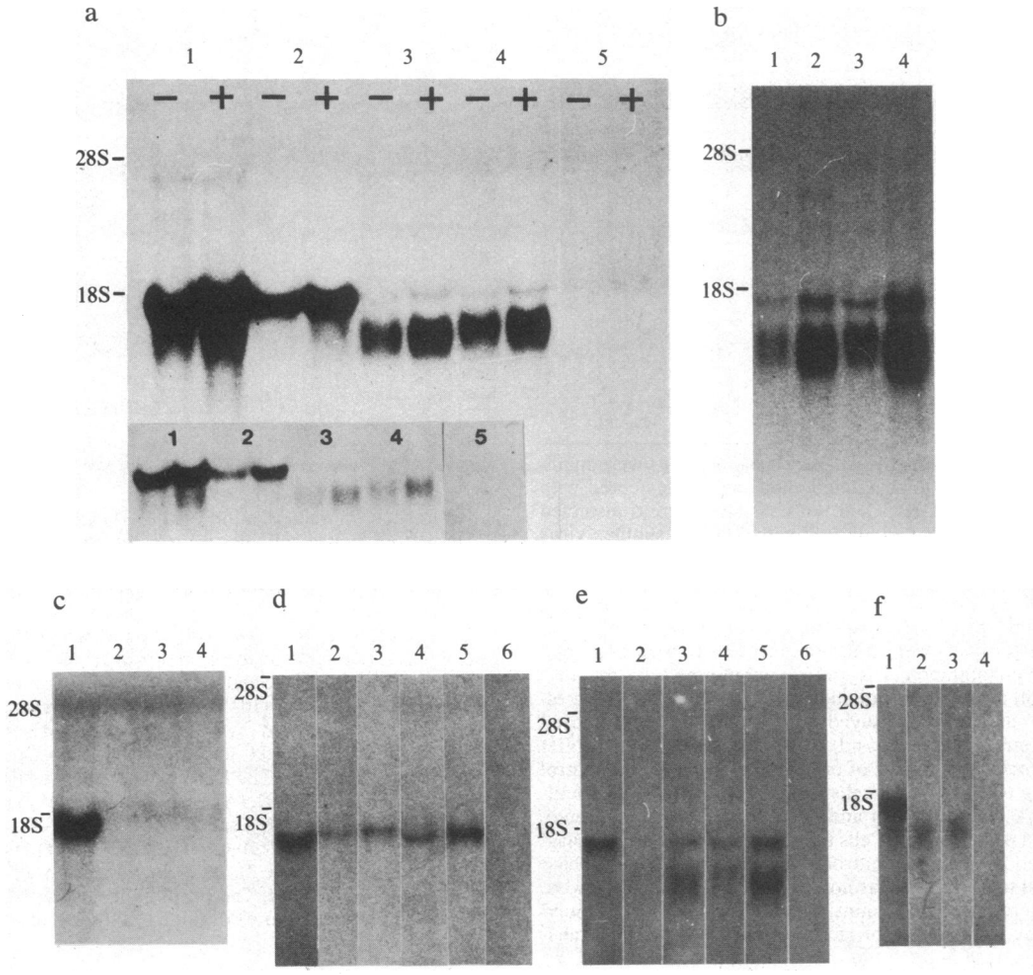

FIG. 3. Characterization of *HLA-B7* gene-specific transcripts in mouse cells transfected with the *HLA-B7* gene or its fragments. Assay by RNA blot hybridization. Cells cultured for 24 hr without or with mouse interferon (1,000 units/ml) were lysed, and the nuclei and the cell debris were removed by centrifugation at 10,000 × *g* for 2 min. The supernatant fractions were supplemented with 0.5% $NaDodSO_4$ and treated with proteinase K at 0.2 mg/ml (Merck) at 37°C for 60 min, and the RNA was extracted from the supernatant fractions. Poly(A)$^+$ RNA was isolated as described (16). Unless otherwise specified, all RNA samples were from cells treated with interferon (1,000 units/ml) for 24 hr. Cytoplasmic RNA (20 μg) or poly(A)$^+$ RNA (4 μg) was suspended in 10 mM phosphate buffer, pH 7.0/50% (vol/vol) formamide/2.2 M formaldehyde, heated at 65°C for 5 min, and fractionated by electrophoresis through 1.2% agarose formaldehyde gel (17). The gels were blotted onto nitrocellulose filter papers (15), which were hybridized with ^{32}P-labeled probes (10^6 cpm/ml) in the presence of 50% formamide at 42°C for 48 hr, washed (with 15 mM sodium chloride/1.5 mM sodium citrate and 0.1% $NaDodSO_4$ at 42°C), and exposed to x-ray film for 2–7 days (17). The positions of the 18S and 28S rRNA markers were established by acridine orange staining. (*a*) Hybridization probe used was ^{32}P-labeled iDP001, an HLA-B7-specific cDNA probe (see Fig. 1). Cytoplasmic RNA from cells transfected with the *HLA-B7* gene pJY150R1.1 (lane 1), the *HLA-B7* gene fragments pΔ −240 (lane 2), pΔ +30 (lane 3), pΔ +240 (lane 4), and the *tk* gene pX-1 (lane 5). RNA from cells not treated with interferon (−); RNA from cells treated with interferon (+). (*Inset*) Shorter exposure of the same RNA blot. It shows that the bulk of the HLA-B7-specific RNA in lanes 1 and 2 consists of full-sized species, and no hybridizing RNA is detectable in this exposure in lane 5+, whereas the full-sized RNA species remain detectable in all other lanes. (*b*) Hybridization probe used was ^{32}P-labeled idP001. Cytoplasmic RNA from cells transfected with pΔ +30 (lane 1) and pΔ +240 (lane 3), poly(A)$^+$ RNA from cells transfected with pΔ +30 (lane 2) and pΔ +240 (lane 4). (*c*) Hybridization probe used was ^{32}P-labeled probe A (see Fig. 1). Cytoplasmic RNA from cells transfected with pJY150R1.1 (lane 1), pΔ +30, (lane 2) pΔ +240 (lane 3), or pX-1 (lane 4). (*d*) Hybridization probe used was ^{32}P-labeled probe B (see Fig. 1). Cytoplasmic RNA from cells transfected with pJY150R1.1 (lane 1), pΔ +30 (lane 2), pΔ +240 (lane 4), or pX-1 (lane 6), poly(A)$^+$ RNA from cells transfected with pΔ +30 (lane 3), or pΔ +240 (lane 5). (*e*) Hybridization probe used was ^{32}P-labeled probe C (see Fig. 1). Cytoplasmic RNA from cells transfected with pJY150R1.1 (lane 1), pΔ +30 (lane 2), pΔ +240 (lane 4), or pX-1 (lane 6), poly(A)$^+$ RNA from cells transfected with pΔ +30 (lane 3) or pΔ +240 (lane 5). (The bands of full-sized and short RNAs that are not discernible in lane 2 are clearly visible in the original autoradiograph.) (*f*) Hybridization probes used were ^{32}P-labeled probes E, two HLA-B7-specific and strand-specific probes (see Fig. 1). The M13 phage JY24 (with the DNA strand analogous in sequence to that of HLA-B7 mRNA) and the M13 phage JY27 (with the DNA strand complementary in sequence to that of HLA-B7 mRNA) were labeled with ^{32}P to a specific activity of 10^8 cpm per μg using [α-^{32}P]dCTP, the hexadecamer hybridization probe primer (New England BioLabs), and the Klenow fragment of DNA polymerase I (18). Cytoplasmic RNA from cells transfected with pJY150R1.1 (lane 1), pΔ +30 (lane 2), pΔ +240 (lane 3), or pX-1 (lane 4).

The shorter RNAs could not be derived by degradation from the full-length RNA, because the latter lacks the sequences complementary to the 3′-untranslated region of the *HLA-B7* gene, whereas these sequences are present in the shorter RNA. To elucidate the origin of the shorter (and full-length) RNAs, we have prepared and are analyzing the sequence of several appropriate cDNA clones. It may be relevant that shorter c-*myc* transcripts were observed in plasma cytomas carrying a c-*myc* gene truncated at its 5′ end (19–23).

The full-length HLA-B7-specific RNA in cells transfected with plasmids pΔ +30 and pΔ +240 is polyadenylylated, but

it lacks sequences from the 3'-terminal exon (exon 8) of the *HLA-B7* gene. Therefore, this transcript must use a polyadenylylation signal other than the physiologic HLA-B7 signal. In addition, this RNA is devoid of sequences from exon 1 even when the templates for these sequences are present in the transfecting DNA plasmid (i.e., pΔ +30). This suggests that their transcription does not initiate in sequences immediately upstream from the DNA template encoding the first exon.

The simplest explanation for these observations is that HLA-B7 sequences from the central portion of the gene (including in some cases exon 2) have become associated with other non-HLA-B7-specific DNA segments that provide both transcription initiation and polyadenylylation signals (24, 25).

The identical electrophoretic mobilities of the full-length RNA and normal HLA-B7 mRNA and the observation of material on the surface of some of the transfected cells that is reactive with the anti-HLA antibody W6/32 are intriguing (10). Hood and colleagues (26) have recently reported the interesting observation that mouse cells transfected with truncated *H-2* genes synthesize proteins containing antigenic determinants crossreacting with those normally encoded by the gene from which the transfecting fragments were derived. Such antigenic determinants were noted in only a small portion of the transfected cells. A substantial fraction of our cells transfected with the HLA-B7 DNA fragments reacted with the anti-HLA antibody W6/32: RNA from four cell lines transfected with *HLA-B7* gene fragments and reactive at various levels with W6/32 antibody have been examined. All were found to contain full-length RNA (this paper and unpublished results). This high frequency of occurrence of the full-length transcript suggests that efficient special mechanisms or structures (or both) are responsible for the generation of their DNA template. Limited sequence analysis of two cDNA clones complementary to such transcripts reveals that homologous recombination with known *H-2* genes does not account for at least some of the HLA-B7-specific RNA (data not shown). We have not excluded the possibility that some of this RNA may arise as a result of a recombination of HLA-B7 with carrier salmon sperm DNA.

An additional curious feature of the HLA-B7-related transcripts is that their levels are modulated by interferon even when the normal HLA-B7 promoter is missing. It remains to be determined if interferon modulates the level of these RNAs by affecting the frequency of transcription initiation or the rate of mRNA turnover or both.

It cannot be excluded that the appearance of some (although certainly not all) of the full-sized transcripts in the cells transfected with the *HLA-B7* gene fragments is a consequence of the following unlikely sequence of events: the transfection of *HLA-B7* gene fragments lacking 5'-flanking sequences together with exon 1 (but not of the herpes *tk* gene alone) would have induced the transcription of mouse genes normally not expressed at a detectable level. These induced genes would have to exhibit more sequence homology to the *HLA-B7* gene in the regions of probes B and C (but not in the regions of probes A and D) than is found in normally expressed mouse genes.

The characterization of HLA-B7-specific cDNA clones may reveal those features of the gene fragments that make them efficient in generating transcribed genes when transfected into cells.

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1. Ploegh, H. L., Orr, H. T. & Strominger, J. L. (1981) *Cell* **24,** 287–299.
2. Bodmer, W. F. & Bodmer, J. G. (1978) *Br. Med. Bull.* **34,** 309–316.
3. Yoshie, O., Schmidt, H., Reddy, E. S. P., Weissman, S. & Lengyel, P. (1982) *J. Biol. Chem.* **257,** 13169–13172.
4. Legerski, R. J., Hodnett, J. L. & Gray, H. B., Jr. (1978) *Nucleic Acids Res.* **5,** 1445–1464.
5. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 394–397.
6. Sood, A. K., Pereira, D. & Weissman, S. M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 616–620.
7. Messing, J. (1981) in *Third Cleveland Symposium on Macromolecules: Recombinant DNA*, ed. Walton, A. (Elsevier, Amsterdam), pp. 143–153.
8. Wigler, M., Pellicer, A., Silverstein, S., Axel, R., Urlaub, G. & Chasin, L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1373–1376.
9. Enquist, L. W., Van de Woude, G. F., Wagner, M., Smiley, J. R. & Summers, W. C. (1979) *Gene* **7,** 335–342.
10. Barnstable, C. J., Bodmer, W. F., Brown, G., Galfre, G., Milstein, C., Williams, A. F. & Ziegler, A. (1978) *Cell* **14,** 9–20.
11. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
12. Mantei, N. & Weissmann, C. (1982) *Nature (London)* **297,** 128–132.
13. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
14. Hirt, B. (1967) *J. Mol. Biol.* **26,** 365–369.
15. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
16. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 202–203.
18. Hu, N. & Messing, J. (1982) *Gene* **17,** 271–277.
19. Harris, L. J., D'Eustachio, P., Ruddle, F. H. & Marcu, K. B. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6622–6626.
20. Calame, K., Kim, S., Lalley, P., Hill, R., Davis, M. & Hood, L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6994–6998.
21. Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aronson, S. & Leder, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7837–7841.
22. Mushinski, J. F., Bauer, S. R., Potter, M. & Reddy, E. P. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1073–1077.
23. Marcu, K. B., Harris, L. J., Stanton, L. W., Erikson, J., Watt, R. & Croce, C. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 519–523.
24. Proudfoot, N. J. & Brownlee, G. G. (1974) *Nature (London)* **252,** 359–362.
25. Fitzgerald, M. & Shenk, T. (1981) *Cell* **24,** 251–260.
26. Goodenow, R. S., Stroynoiwski, I., McMillan, M., Nicolson, M., Eakle, K., Sher, B. T., Davidson, N. & Hood, L. (1983) *Nature (London)* **301,** 388–394.