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Identification of DNA Sequence Responsible for 5-Bromodeoxyuridine-Induced Gene Amplification

Abstract. *Bromodeoxyuridine (BrdUrd) treatment of the prolactin nonproducing subclone of GH cells (rat pituitary tumor cells) induces amplification of a 20-kilobase DNA fragment including all of the prolactin gene coding sequences. This amplified DNA segment, which is flanked by two unamplified regions, thus designates a unit of BrdUrd-induced amplified sequence. Cloned DNA segments, 10.3 kilobases long, from the 5′ end of the rat prolactin gene of BrdUrd-responsive and -nonresponsive cells, were ligated to the thymidine kinase gene of herpes simplex virus type 1 (HSV1TK), and the hybrid DNA was transferred to thymidine kinase–deficient mouse fibroblast cells by transfection. The HSV1TK gene and the rat prolactin gene were amplified together in drug-treated transfectants carrying the hybrid DNA HSV1TK gene and rat prolactin gene of BrdUrd-responsive GH cells. These results suggest that the 10.3-kilobase DNA segment at the 5′ end of the rat prolactin gene of BrdUrd-responsive GH cells carries the information for drug-induced gene amplification (amplicon) and that another gene, such as the HSV1TK gene, is also amplified when the latter is placed adjacent to this segment.*

Amplification of specific gene sequences during normal developmental processes or in response to cytotoxic agents are observed in several eukaryotic cell systems (*1–4*). The exact mechanism of such amplification of specific genes is not yet clear. A 20-kilobase (kb) length of DNA including all of the coding sequences and about 3.5 kb of the 5′ end and about 7 kb of the flanking regions of the 3′ end of the rat prolactin (rPRL) gene are amplified in bromodeoxyuridine (BrdUrd)-treated PRL-nonproducing (PRL^-) GH cells (rat pituitary tumor cells) (Fig. 1) (*5–8*). The reversible (*8*) nature of the amplification process suggests that BrdUrd treatment of cells generates a signal either within or in the immediate neighborhood of the 20-kb amplified sequences that leads to such preferential DNA replication in this region of the chromosome. To substantiate this concept, we ligated cloned DNA segments from the neighborhood of the rPRL gene of BrdUrd-responsive and -nonresponsive cells to the thymidine kinase (TK) gene of herpes simplex virus type 1 (HSV1TK) and transferred the hybrid DNA to thymidine kinase–deficient $LMTK^-$ mouse fibroblast cells by transfection (*9*).

The construction of hybrid DNA containing the rPRL and HSV1TK gene sequences is outlined in Fig. 1. Table 1 shows the characteristics of the hybrid DNA, which carries a constant region consisting of the HSV1TK gene and a variable region consisting of the 10.3-kb DNA fragment from the 5′ end of the rPRL gene of GH cell strains that are responsive and nonresponsive to BrdUrd (Fig. 1).

The HSV1TK gene does not show significant sequence homology to the mouse fibroblast TK gene (Figs. 2 and 3). However, DNA isolated from TK^+ transfectants derived with hybrid DNA generate positive signals in dot-hybridization analysis when probed with ^{32}P-labeled HSV1TK DNA (Fig. 2) (*10*). These results suggest that the HSV1TK gene is indeed transferred to mouse fibroblast cells. The hypoxanthine-aminopterin-thymidine (HAT)-resistant phenotype of the transfectants further substantiates that the HSV1TK gene is also

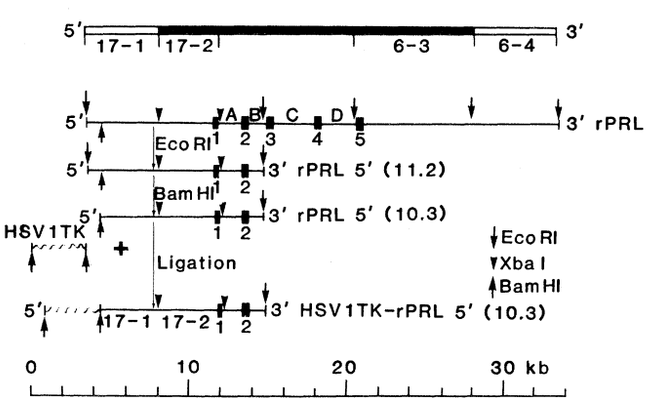

Fig. 1. Construction of hybrid DNA with the HSV1TK gene and the rPRL gene 5′ end fragment of PRL^+ and PRL^- GH cells. High molecular weight DNA (40 μg) (*12*) from PRL^+ (GH_3) and PRL^- ($F_1BGH_12C_1$) cells were digested with Eco RI, and fragments were separated on a 1 percent low-melting agarose gel. The 11-kb fragments of the genomic DNA were extracted from the gel and cloned in the Eco RI site of λ Charon 4A (*13*). Recombinant phage plaques were screened for the $[^{32}P]cDNA_{PRL}$-hybridizable sequences (*14*). The 11-kb DNA insert from the recombinant clones of PRL^+ and PRL^- GH cells were then digested with Bam HI and ligated to HSV1TK DNA to generate the hybrid DNA fragments for subsequent transfection. The HSV1TK DNA was released from the recombinant plasmid pBR322–HSV1TK (*15*) DNA by digestion with Bam HI followed by separation on 1 percent agarose gel and extraction (*7*). The top sketch shows the amplified (closed area) and unamplified (open area) regions of the PRL gene and its neighboring sequences in BrdUrd-treated GH cells. The second sketch from the top shows the organization of the rPRL gene. Closed blocks numbered 1 to 5 are exons, and letters A to D designate the four introns in the rPRL gene.

expressed in these cells. The identity of rPRL gene coding and flanking sequences in the TK^+ (mouse fibroblast) transfectants is established by hybridization with ^{32}P-labeled PRL complementary DNA ($cDNA_{PRL}$) and the genomic DNA_{PRL} probes (*11*).

Results presented in Fig. 2A indicate that the HSV1TK gene is amplified only in BrdUrd-treated transfectants (transfectants 11 and 26) that are obtained by transfer of the hybrid DNA containing the HSV1TK DNA ligated to the 5′ end of the 10.3-kb DNA fragment of PRL^- BrdUrd-responsive GH subclone. In contrast, the TK gene is not amplified in BrdUrd-treated TK^+ transfectants obtained with hybrid DNA containing HSV1TK gene ligated to the 10.3-kb DNA fragment from PRL^+ BrdUrd-nonresponsive GH cell strain (Fig. 2, transfectants 55 and 66) or the transfectants obtained with recombinant plasmid pBR322–HSV1TK DNA (Fig. 2A, transfectants 73 and 75). These results suggest that an unrelated gene such as the HSV1TK gene can be induced to amplify following BrdUrd treatment, when it is placed adjacent to the specific DNA sequence of BrdUrd-responsive PRL^- GH cells. The rPRL gene and its 5′ end flanking regions, which are transferred from PRL^- or PRL^+ GH cells strains

Table 1. Transfection of mouse fibroblast cells ($LMTK^-$) with HSV1TK-rPRL 5′ end hybrid DNA. Growth of $LMTK^-$ cells and transfection with hybrid DNA fragments (1 μg each per incubation) are carried out as described by Wigler *et al.* (*9*). Numbers in parentheses designate the length (kilobases) of rPRL 5′ end sequence in hybrid DNA. Isolation of the rPRL 5′ end DNA fragment is described in Fig. 1. The HAT selection medium is that of Wigler *et al.* (*9*).

Transfectants	GH cell phenotype		Hybrid DNA	Recipient cell	HAT-resistant TK^+ transfectants per microgram of DNA
	PRL synthesis	BrdUrd-induced amplification			
11	PRL^-	Yes	HSV1TK-rPRL 5′ (10.3)	$LMTK^-$	12
26	PRL^-	Yes	HSV1TK-rPRL 5′ (10.3)	$LMTK^-$	10
55	PRL^+	No	HSV1TK-rPRL 5′ (10.3)	$LMTK^-$	15
66	PRL^+	No	HSV1TK-rPRL 5′ (10.3)	$LMTK^-$	12
75			HSV1TK-pBR322	$LMTK^-$	10
CA			None	$LMTK^-$	0

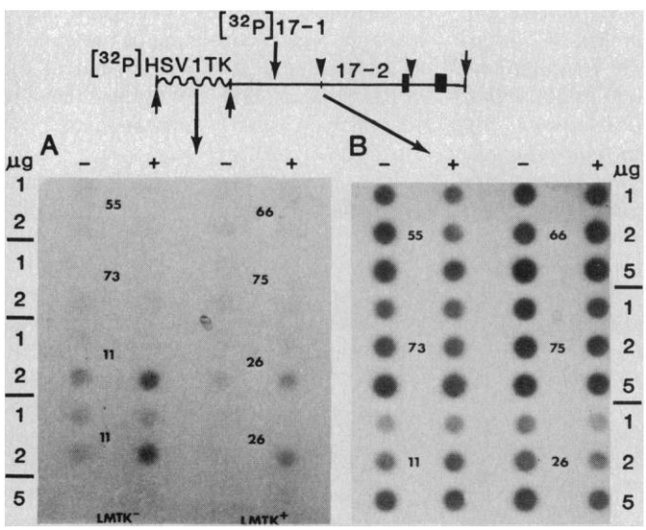

Fig. 2. Levels of HSV1TK gene and DNA sequences complementary to rPRL 17-1 in transfectants. Results from two of each class of transfectants are shown. The levels of HSV1TK gene (A) and DNA sequences complementary to 17-1 (B) were measured by the dot-hybridization technique (*7*) with ^{32}P-labeled (*10*) HSV1TK (inset) (A) and ^{32}P-labeled 17-1 (isolated from genomic clone λ17) (*11*) (B) as probes. Transfectants 55 and 66 were obtained with hybrid DNA carrying the rPRL 5′ 10.3-kb DNA segment from BrdUrd-nonresponsive PRL^+ cells; transfectants 73 and 75 were obtained with recombinant plasmid DNA HSV1TK–pBR322, and transfectants 11 and 26 were obtained with the rPRL 5′ 10.3-kb DNA segment from BrdUrd-responsive PRL^- cells. The indicated amount (micrograms) of DNA isolated from control (−) and BrdUrd-treated (10 μg/ml for 5 days) (+) transfectants are applied to a membrane filter (Zetabind, AMF) and prehybridized and hybridized as described (*7*). The probes [^{32}P]HSV1TK (specific activity, 5×10^7 count/min per microgram of DNA) and [^{32}P]17-1 (specific activity, 2×10^8 count/min per microgram) are used at 1×10^6 count/min per 5 ml of hybridization mixture. Hybridization was carried out for 72 hours and autoradiography for 72 hours under conditions described (*7*, *8*). The top sketch shows the structural organization of the hybrid DNA.

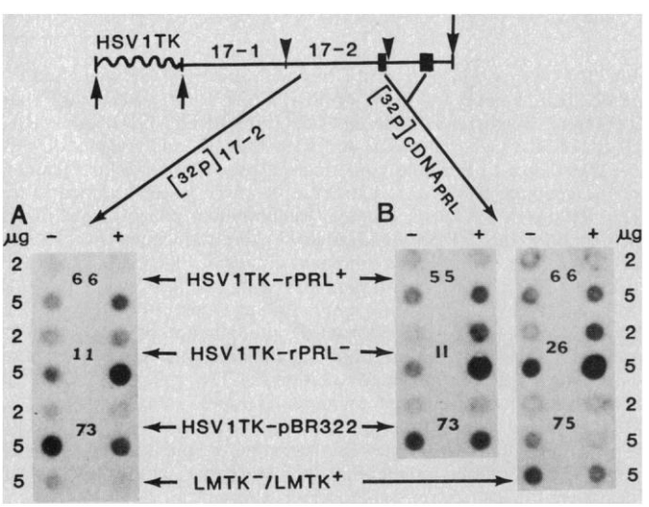

Fig. 3. The levels of rPRL structural gene and 5′ end flanking sequences in transfectants. The levels of rPRL gene 5′ end flanking sequences, [^{32}P]λ17-2–hybridizable (A), and rPRL gene structural gene sequences, [^{32}P]$cDNA_{PRL}$ hybridizable (B), in control (−) and BrdUrd-treated (10 μg/ml) (+) transfectants were measured by the dot-hybridization technique (*7*, *8*). Indicated amounts (micrograms) of DNA isolated from control (−) and drug-treated (+) transfectants and from $LMTK^+$ and $LMTK^-$ cells were applied to a membrane filter (Zetabind, AMF) and prehybridized, hybridized, and autoradiographed as described in the legend to Fig. 2. [^{32}P]$cDNA_{PRL}$ (specific activity, 2×10^8 count/min per microgram of DNA) and [^{32}P]λ17-2 (specific activity, 1×10^8 count/min per microgram) are used at 1×10^6 count/min per 5 ml of hybridization mixture.

(rat) into mouse fibroblast cells after ligation with the HSV1TK gene, show a pattern of gene amplification similar to that observed in the parent strains from which these DNA sequences were isolated. The region of the rPRL gene 5′ end flanking sequences designated 17-1 (Fig. 1) is not amplified in response to BrdUrd in either of the GH cell strains or in transfectants derived with hybrid DNA containing these sequences (Fig. 2B).

The 3.8-kb 5′ end PRL gene–flanking region complementary to 17-2 (Figs. 1 and 3A) and PRL structural gene regions ($cDNA_{PRL}$ hybridizable sequences) (Fig. 3B) are likewise amplified only in BrdUrd-treated transfectants derived with hybrid DNA containing the HSV1TK and 5′ end DNA segment from PRL^- BrdUrd-responsive GH cells (Fig. 3, transfectants 11 and 26). Transfer of the hybrid DNA, HSV1TK-$rPRL^+$ BrdUrd-nonresponsive cells does not affect the levels of these sequences in BrdUrd-treated transfectants (Fig. 3, A and B; transfectants 55 and 66). These results suggest that the basic difference between the PRL gene of PRL^- and PRL^+ GH cells, in relation to BrdUrd-induced PRL gene amplification, is linked to this region (10.3-kb fragment) of the cellular DNA.

A 20-kb length of DNA at the 3′ end of 17-1 is amplified in BrdUrd-responsive GH cells. Cloned 10.3-kb DNA sequences of this region—the sequences complementary to 17-1 and 17-2 of the BrdUrd-responsive GH cells—when transferred to mouse fibroblast cells, showed a similar pattern of gene amplification in the transfectants. The HSV1TK gene, which is ligated at the 5′ end of the 10.3-kb rat DNA sequence, is also amplified in the transfectants. This suggests that the HSV1TK gene is located in the transfectant close to the 10.3-kb DNA segment of BrdUrd-responsive cells. As the 10.3-kb DNA segment has a Bam HI site at the 5′ end and an Eco RI at the 3′ end, the HSV1TK gene with Bam HI sites at both ends would be expected to anchor only at the 5′ end of the 10.3-kb DNA segment in the transfectants. Thus it may be postulated that the hybrid DNA retains its organization in the transfectants. However, this has yet to be established. Our results show that information responsible for the BrdUrd-induced gene amplification, which we have termed "amplicon": (i) is located within the 10.3-kb 5′ end flanking region of the PRL gene of BrdUrd-responsive GH cells, (ii) can be transferred from one cell type to the other, and (iii) induces amplification of another gene placed adjacent to it. The results also suggest that the structure of the amplicon region in the 10.3-kb segment at the 5′ end of the PRL gene differs in cells responsive and nonresponsive to BrdUrd.

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Distinctive Termini Characterize Two Families of Human Endogenous Retroviral Sequences

Abstract. *Human DNA contains many copies of endogenous retroviral sequences. Characterization of molecular clones of these structures reveals the existence of two related families. One family consists of full-length (8.8 kilobases) proviral structures, with typical long terminal repeats (LTR's). The other family consists of structures which contain only 4.1 kilobases of* gag-pol *sequences, bounded by a tandem array of imperfect repeats 72 to 76 base pairs in length. Typical LTR sequences that exist as solitary elements in the genome were cloned and characterized.*

Like the DNA's of other vertebrates (*1*), human DNA contains multiple germline copies of retroviral sequences. These endogenous structures were first identified and selected from a genomic DNA library through the use of two cross-species, low-stringency DNA hybridizations: the first, using a subclone of a murine leukemia virus to screen an African green monkey genomic library (*2*), and the second, using a subclone of the African green monkey retroviral sequence to probe a human genomic library (*3*). The first clone obtained, which displays considerable deduced amino acid homology to murine retroviruses in *gag* and *pol*, was used to screen the human library (*4*) under high-stringency conditions to obtain highly related clones (*5–7*). Since retroviral sequences are bounded by long terminal repeats (LTR's), we sought to localize such structures at the 5′ and 3′ termini of the cloned retroviral sequences. We now describe the nucleotide sequence of two distinct types of termini that were found.

The location of cross-hybridizing termini within the human endogenous retroviral clones was determined by Southern blotting (*8*) (data not shown). One type of repeat discovered in clone 4-1 (Fig. 1A) resembled a typical LTR and defined a structure with the size expected for proviral DNA [8.8 kilobases (kb)] (*6*). Additional clones with typical LTR's were obtained by screening the human genomic library with a 1.1-kb restriction fragment containing the 3′ LTR of clone 4-1 (Fig. 1A, box a). Of numerous positive plaques picked, 82 percent reacted with only the 3′ flanking cellular DNA component of the probe. The other 18 percent were equally divided between clones of the full length type, including clone 4-14 (Fig. 1A), and solitary LTR clones, which reacted only with a specific LTR probe (*6*), but not with *gag*, *pol*, or *env* probes.

The second type of repeating element, present in clone 51-1 as well as other clones having a similar restriction map (*5*), defined a truncated 6-kb retroviral segment (Fig. 1B).

The full-length and truncated retroviral sequences (Fig. 1, A and B, respectively) share a common 4.1-kb stretch of *gag-pol* sequence (*5, 9*) (Fig. 1B). Highly conserved restriction sites that reflect this sequence similarity are shown in Fig. 1: a Hind III site at 2.8 kb, Eco RI