and are refractory to further stimulation or inhibition of proliferative activity in vitro. The living skin equivalent model lends itself to further studies of psoriasis since long-term cultures can be carried out. It is possible that in such cultures the typical hyperacanthosis of PP epidermis will be observed.

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Chromosomal Locations of Human Tissue Plasminogen Activator and Urokinase Genes

Abstract. *A panel of human-mouse somatic cell hybrids and specific complementary DNA probes were used to map the human tissue plasminogen activator and urokinase genes to human chromosomes 8 and 10, respectively. This result is in contrast to a previous assignment of a plasminogen activator gene to chromosome 6. As neoplastic cells produce high levels of plasminogen activator, it is of interest that aberrations of chromosome 8 have been linked to various leukemias and lymphomas and that two human oncogenes,* c-mos *and* c-myc, *have also been mapped to chromosome 8.*

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Plasminogen activators (PA's) are proteases that convert plasminogen, a plasma zymogen that is ubiquitous in the body, to plasmin, a protease of broad specificity. Plasminogen activation is thought to be involved in fibrinolysis and it has also been implicated in processes such as tissue remodeling and cell migration (*1*). High levels of PA production have characteristically been associated with primary malignant tumors (*2*), and PA may be required for the metastatic activity of a human tumor (*3*).

Mammalian cells produce two forms of PA: tissue PA (t-PA) and urokinase (u-PA). For gene mapping, in accordance with human gene nomenclature, the human genes for t-PA and u-PA will be designated *PLAT* and *PLAU*, respectively (*4*). The two PA's can be distinguished on the basis of their molecular weights, antigenic characteristics, and amino acid sequences (*5*). The complementary DNA (cDNA) for human t-PA (*6, 7*), u-PA (*8*), and part of the gene for human t-PA (*9*) have been cloned and characterized.

We used human-mouse somatic cell hybrids and specific cDNA probes for t-PA and u-PA to map the genes for t-PA and u-PA on human chromosomes. The human and mouse parental cells and the fusion, isolation, and characterization of human-mouse hybrids have been described (*10*).

A fragment of human t-PA cDNA which was synthesized from polyadenylated RNA that had been isolated from HeLa S3 cells and cloned into pBR322 (*7*) was used as the probe. It was an 800–base pair (bp) Sma I–Pst I fragment encoding the 20 COOH-terminal amino acids and all of the 3′ nontranslated region.

The t-PA probe hybridized to a 7-kilobase (kb) Hind III fragment from human DNA (Fig. 1A, lane 7). This result is in agreement with our previous characterization of the human t-PA gene (*11*). The t-PA probe hybridized to a band larger than 23 kb in mouse DNA (Fig. 1A, lanes 6 and 8). As expected, the mouse DNA–specific fragment was observed in DNA from all hybrid cells, whereas the 7-kb human DNA–specific band was found in DNA's from certain hybrids (Fig. 1A). Human chromosomes in the hybrid cells were identified by karyotyping (*12*) and by the examination of human chromosome-specific isozyme markers (*13*). In an analysis of DNA from 32 different cell hybrids, the human t-PA cosegregated with human chromosome 8 (Table 1). No other chromosome or chromosome-specific isozyme marker could be correlated with the presence of human t-PA.

The human u-PA gene was mapped in the same manner as the t-PA gene except that a 1.0-kb Bam HI–Eco RI fragment from a human u-PA cDNA, derived from RNA isolated from Hep3 cells and cloned into pUN121, was used as the probe. This fragment encoded the 13 COOH-terminal amino acids and all of the 3′ nontranslated segment. The u-PA probe hybridized to a Hind III fragment of 10 kb from human DNA (Fig. 1B, lane 8) and a 3-kb fragment from mouse DNA (Fig. 1B, lane 7). The same panel of 32 hybrid lines that served for t-PA localization (Table 1) was used to map the human u-PA gene. The presence of the 10-kb u-PA hybridizing human band correlated exclusively with the presence of human chromosome 10; no other chromosome or isozyme marker cosegregated with u-PA. Figure 1B shows DNA from chromosome 10-positive (lanes 2, 4, and 6) and -negative (lanes 1, 3, and 5) hybrids.

The genes for t-PA and u-PA are not syntenic. This is not surprising because the two proteins are genetically and immunologically distinct (*5–9*), and, although functionally alike, they appear to be regulated by different hormones and respond differently to tumor-promoting agents (*1, 7, 14*). Data obtained from genomic Southern hybridization analysis with human t-PA specific probes, as well as human t-PA cDNA and gene sequences, indicate that there is probably only one t-PA gene in the human haploid genome (*6, 7, 9, 11*); this also appears to be true for the human u-PA gene (*8*). Therefore, the results presented here

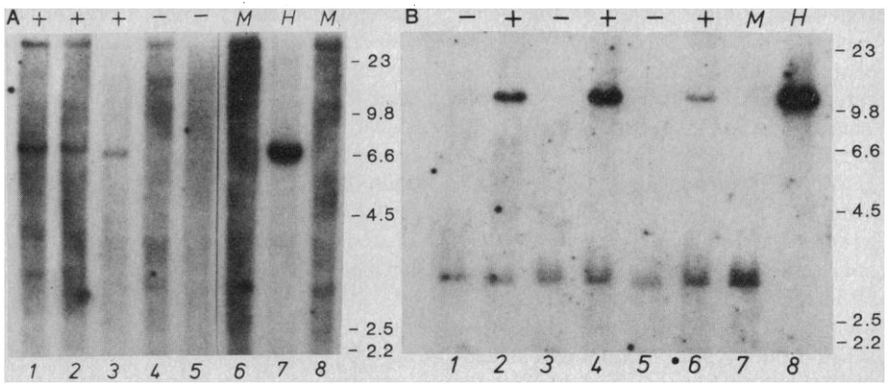

Fig. 1. Southern hybridization analysis of DNA from parental and hybrid cells, with human t-PA (A) or u-PA (B) cDNA as probe. Genomic DNA from human, mouse, and hybrid cells was isolated as described (*24*). Ten micrograms of each DNA was digested to completion with Hind III, fractionated electrophoretically on a 0.8 percent agarose gel, and transferred onto nitrocellulose (*25*). The filter was prehybridized in 2× Denhardt's solution plus 6× standard saline citrate (SSC) at 68°C for 15 to 24 hours. The filter-immobilized DNA was then hybridized with ^{32}P-labeled, nick-translated t-PA or u-PA probe in the prehybridization mixture plus 0.5 percent sodium dodecyl sulfate (SDS) and 1 m*M* EDTA at 68°C for 15 to 24 hours. After hybridization, the filter was washed three times in 1× SSC plus 0.5 percent SDS at 68°C for 1 hour each. The hybridized bands were visualized by autoradiography. (A) Lanes 1 to 3, human t-PA–positive hybrids (+); lanes 4 and 5, human t-PA–negative hybrids (−); lane 6, mouse parent RAG (M); lane 7, human parent W138 (H); lane 8, mouse parent LM/TK$^-$ (M). (B) Lanes 1, 3, and 5, human u-PA–negative hybrids (−); lanes 2, 4, and 6, human u-PA–positive hybrids (+); lane 7, mouse parent RAG (M); and lane 8, human parent W138 (H). Hind III–digested DNA was used as a size marker (kilobases).

Table 1. Segregation of t-PA and u-PA with human chromosomes in human-mouse cell hybrids. All analyses were done on the same cell passage. The human t-PA and u-PA genes were determined by scoring for the presence or absence of the 7-kb or 10-kb human DNA fragment, respectively, upon hybridization with the appropriate probe. Human female parental cells were used, thus eliminating the Y chromosome. Discordancy (+/−) indicates the presence of t-PA or u-PA but the absence of a specific chromosome, or the reverse. No discordancy indicates the concordant segregation (+/+ or −/−) of t-PA or u-PA and a specific human chromosome.

			Human chromosomes																							
Hybrid	t-PA	u-PA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Translocations
WIL−1	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−	−	+	−	+	
WIL−2	+	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	+	−	+	−	−	−	+	−	+	
WIL−7	+	+	−	+	+	−	+	+	−	+	−	+	+	−	+	+	−	−	+	+	−	−	+	−	+	
WIL−8	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	
WIL−8X	+	+	−	−	+	+	+	−	+	+	−	+	+	+	−	+	−	−	+	+	+	+	+	−	+	
WIL−13	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+	−	
WIL−14	+	+	+	−	+	−	+	−	+	+	−	+	−	+	−	+	+	−	+	−	−	−	−	−	+	
WIL−15	−	+	−	+	+	+	−	+	+	−	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	
REW−7	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	
REW−8D	+	−	−	−	−	+	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−	+	+	+	+	
REW−11	−	−	−	−	−	+	−	−	+	−	−	−	+	+	+	−	−	+	−	−	−	+	+	−	+	
ICL −15	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	+	+	−	−	
SIR−8	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	
SIR−11	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	
VTL−6	+	+	−	+	−	−	−	+	+	+	−	+	+	−	−	−	+	−	+	−	+	+	+	+	−	
VTL−8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−	−	+	+	+	−	
VTL−17	−	+	−	−	−	−	−	−	+	−	−	+	+	−	+	+	−	−	+	−	−	+	+	−	−	
TSL−1	−	+	−	−	+	+	−	−	−	−	−	+	+	−	+	+	−	+	+	+	−	+	−	−	−	
TSL−2	−	+	−	+	−	−	+	+	−	−	−	+	−	+	−	−	−	−	−	+	−	+	+	−	+	17/3 3/17
NSL−9	+	+	−	−	−	−	+	−	−	+	−	+	−	+	+	+	+	+	+	−	−	+	+	+	−	17/9
DUA−3 BSAgA	+	−	−	+	−	−	−	−	+	+	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	
REX−11 BSAgB	−	+	−	−	+	−	−	−	−	−	−	+	−	−	−	+	+	−	−	+	−	−	−	+	−	
REX−11 BSHF	−	+	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	22/X
REX−26	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	−	−	22/X
DUM−13	−	+	+	+	+	−	−	+	+	−	−	+	+	+	−	+	−	+	+	+	+	+	+	+	−	X/15 15/X
XER−11	+	+	+	−	+	+	−	+	+	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	−	11/X X/11
XTR−2	+	+	−	−	−	−	+	−	−	+	−	+	−	+	+	−	−	−	−	+	−	+	+	−	−	3/X
XTR−22	+	+	−	+	−	+	+	+	−	+	−	+	+	−	−	−	+	−	−	+	+	+	+	+	−	X/3
ATR−13	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	−	−	−	−	5/X
JSR−14	−	−	−	+	+	+	+	+	−	−	−	−	−	+	+	−	−	−	+	−	−	+	+	−	+	
JSR−17S	+	+	+	+	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	7/9
JWR−22H	−	+	−	−	−	+	−	+	−	−	−	+	+	+	−	+	+	−	+	+	−	+	+	−	−	2/1
%Discordancy t-PA			34	38	44	44	31	50	38	0	44	38	44	41	47	38	31	44	38	47	34	56	44	47	38	
%Discordancy u-PA			38	34	19	44	38	38	38	38	56	0	25	34	47	25	31	44	38	16	41	38	44	47	44	

show that single copies of the genes encoding the two PA's that have been characterized in mammalian cells are located on human chromosomes 8 and 10. This conclusion is inconsistent with the previous assignment by Kucherlapati *et al.* of a PA gene, probably the u-PA gene, to chromosome 6 (*15*). DNA from hybrids containing chromosome 6 but not 8 (TSL-2, DUM-13, JSR-14, and JWR-22H) did not show any hybridization with the t-PA probe. Similarly, DNA from hybrids retaining chromosome 6 but not chromosome 10 (JSR-14) did not hybridize with the u-PA probe, and, further, DNA from hybrid JSR-14 (which contains chromosome 6, but neither chromosome 8 or 10) also did not hybridize with either probe. Kucherlapati *et al.* (*15*) relied on the expression of a PA gene in hybrid cells as the means of determining the presence of the gene. Hybridization of structural gene sequences to specific cDNA probes is a more direct and sensitive technique that does not depend on the expression of the gene to be mapped in hybrid cells and hence avoids the uncertainty that a regulatory gene is being identified in hybrids. Blasi and his colleagues have independently localized the u-PA gene to human chromosome 10 (*16*).

Tissue-PA and u-PA belong to the family of serine proteases, a group of enzymes related by structure and function, that may have evolved from a common ancestral gene (*17*). The genes for trypsin; chymotrypsin B; elastase; coagulation factors IX, X, and XII; plasminogen; complement component C3; and haptoglobin are located on human chromosomes 7, 16, 12, X, 13, 6, 6, 19, and 16, respectively (*18*). Although haptoglobin is not a serine protease, it is thought to have evolved from the same ancestral gene as the serine proteases (*19*). The presence of serine protease genes on different chromosomes implies that the duplication and the divergence from the common ancestral gene occurred early in evolutionary history.

In view of the association of PA's with tumor formation and metastasis, it may be significant that two oncogenes, c-*mos* and c-*myc*, have been mapped to human chromosome 8 (*20*). A gain of chromosome 8 is the most common change observed in chronic myelogenous leukemia and acute nonlymphocytic leukemia, and translocations and rearrangements of chromosomes 8 have also been linked to Burkitt lymphoma and acute myelogenous leukemia (*21*). It is therefore of interest that circulating cells that secrete t-PA appear to be characteristic of a particular subpopulation of adult human leukemia patients who are refractory to conventional antileukemic chemotherapy (*22*). In addition, fragile sites have been found on chromosomes 8 and 10; fragile sites have been associated with breakpoints in chromosomal translocations (*23*). However, it is not known if the t-PA or the u-PA gene locus is translocated or rearranged in any human cancer.

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Phytochelatins: The Principal Heavy-Metal Complexing Peptides of Higher Plants

Abstract. *A set of novel heavy-metal complexing peptides was isolated from plant cell suspension cultures; the structure of the peptides was established as (γ-glutamic acid–cysteine)*n*-glycine* (n = *3 to 7*). *These peptides appear upon induction of plant cells with heavy metals and represent the principal metal-binding activities in the cells. The name phytochelatin is proposed for this new class of natural products.*

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Heavy metals in vertebrates and fungi are detoxified by the metallothioneins, which are sulfur-rich proteins 6.5 kilodaltons (kD) in size (*1*). Mammalian metallothioneins in general consist of a single polypeptide chain with 61 amino-acid residues (*1*). Metal chelating compounds have also been proposed for higher plants (*2*), and a metallothionein has been reported in a vascular plant (*3*). Further characterization of this protein revealed that it bears a close resemblance to vertebrate metallothioneins (*4*). Recently, several 10-kD metal-binding metallothionein-like proteins from differentiated tissues and cell cultures of higher plants have been reported (*5–9*).

Suspension cultures of *Rauvolfia serpentina* were grown under sterile conditions for 6 days in the medium described (*10*). The medium was then augmented with 200 μM $CdSO_4$, and the cell suspension was cultured for another 4 days. Cells were harvested by suction filtration, washed extensively with deionized water, dried by suction, and frozen in liquid nitrogen. All extraction and isolation procedures were carried out at 0° to 4°C under nitrogen.

A typical separation of a crude cell-free extract of Cd^{2+}-treated and untreated (control) cells on Sephadex G-50 is shown in Fig. 1. The extract from Cd^{2+}-treated cells (Fig. 1B) was resolved into high molecular weight material containing approximately 3 percent of the total