changes in the location of mobile genetic elements) (*9*) may be basic to the switching mechanism, although heritable extrachromosomal changes have not been ruled out.

The differences in colony morphology between smooth and variant phenotypes appear to be due to spatial, temporal, quantitative, and qualitative differences in bud and mycelium formation (*10*). However, the molecular or cytological basis for these developmental differences have not been elucidated. All of the seven switch phenotypes described here retain the basic capacity of dimorphism, and are therefore capable of forming buds and mycelia, but the environmental constraints, such as *p*H-regulated dimorphism (*11*), on the transitions between bud and hyphal forms vary markedly between o-smooth and the switch phenotypes (*10*).

Switching has been shown to occur not only in our standard laboratory strain but also in strains of *C. albicans* isolated from the mouths of healthy individuals (*12*) and in the related yeast *C. tropicalis* (*13*). It therefore seems reasonable to suggest that switching may provide *C. albicans* and related infectious yeasts with the diversity that is expected of such pervasive and successful pathogens. Switching may provide an organism with the capacity to (i) invade diverse body locations, (ii) evade the immune system in a fashion analogous to *Salmonella* (*14*) and *trypanosomes* (*15*), or (iii) change antibiotic resistance. It may also account for a significant portion of the 90 or more *Candida* "species" that have been described (*16*).

References and Notes

1. F. C. Odds, *Candida and Candidosis* (University Park Press, Baltimore, Md., 1978); G. P. Bodey and V. Fainstein, Eds., *Candidiasis* (Raven, New York, 1985).
2. A. Polak and H. J. Scholer, *Chemotherapy* **21**, 113 (1975); K. S. Defever *et al.*, *Antimicrob. Agents Chemother.* **22**, 810 (1982); E. F. Gale, A. M. Johnson, D. Kerridge, E. A. Miles, *J. Gen. Microbiol.* **109**, 191 (1978) ; M. Pesti, S. Paku, E. K. Novak, *Acta Microbiol. Acad. Sci. Hung.* **29**, 55 (1982); E. Sakellarides, *Schweiz. Med. Wochenschr.* **111**, 1367 (1981); P. Auger, C. Dumas, J. Joly, *J. Infect. Dis.* **139**, 590 (1979); J. D. Dick, W. G. Merz, R. Saral, *Antimicrob. Agents Chemother.* **18**, 158 (1980).
3. T. J. Rogers and E. Balish, *Microbiol. Rev.* **44**, 660 (1980); C. B. Smith, in *Candidiasis*, G. P. Bodey and V. Fainstein, Eds. (Raven, New York, 1985), pp. 53–70; D. W. R. MacKenzie, in *Microbiology—1983*, D. Schlessinger, Ed. (American Society for Microbiology, Washington, D.C., 1983), pp. 258–261.
4. G. P. Bodey and V. Fainstein, in *Candidiasis*, G. P. Bodey and V. Fainstein, Eds. (Raven, New York, 1985), pp. 135–168.
5. A. F. Olaiya and S. J. Sogin, *J. Bacteriol.* **140**, 1043 (1979); W. L. Whelan, R. M. Partridge, P. R. Magee, *Mol. Gen. Genet.* **180**, 107 (1980).
6. W. L. Whelan and D. R. Soll, *Mol. Gen. Genet.* **187**, 477 (1982); W. L. Whelan, in *Microbiology—1983*, D. Schlessinger, Ed. (American Society for Microbiology, Washington, D.C., 1983), pp. 227–229.
7. The agar that resulted in the best visualization of switch phenotypes was developed from the amino acid–rich defined medium of K. L. Lee, H. R. Buckley, and C. C. Campbell [*Sabouraudia* **13**, 148 (1975)], supplemented with arginine (70 μg/ml) and zinc (9 μ*M*) [D. R. Soll, G. W. Bedell, M. Brummel, *Infect. Immun.* **32**, 1139 (1981)]. Switch morphologies were best visualized at 24°C.
8. J. Brown-Thomsen, *Heredity* **60**, 355 (1968).
9. J. A. Shapiro, Ed., *Mobile Genetic Elements* (Academic Press, New York, 1983).
10. B. Slutsky, M. Staebell, D. R. Soll, in preparation.
11. J. Buffo, M. A. Herman, D. R. Soll, *Mycopathologia* **85**, 1 (1984); D. R. Soll, in *The Microbial Cell Cycle*, P. Nurse and E. Streiblova, Eds. (CRC Press, Boca Raton, Fla., 1984), pp. 143–162; L. Mitchell and D. R. Soll, *Exp. Cell Res.* **120**, 167 (1979).
12. S. MacKenzie, B. Slutsky, D. R. Soll, in preparation.
13. P. Davis, B. Slutsky, D. R. Soll, in preparation.
14. F. W. Andrews, *J. Path. Bacteriol.* **25**, 515 (1972); M. Silverman and M. Simon, in (*9*), pp. 537–557.
15. J. H. Hoeijmakers, A. C. Frasch, A. Bernards, P. Borst, G. A. Gross, *Nature (London)* **284**, 78 (1980); P. Borst (*9*), pp. 621–659.
16. N. van Uden and H. Buckley, in *The Yeasts*, J. Ladder, Ed. (North-Holland, Amsterdam, 1970), pp. 893–1087.

17. The authors are indebted to M. Staebell, P. Jennings, and Z. Sunleaf for technical assistance and to C. Newlon and J. Yarger for valuable discussions. Supported in part by NSF grant PCM-8202380, NIH grant GM25832, and a grant from the University of Iowa Research Foundation.

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Psoriatic Fibroblasts Induce Hyperproliferation of Normal Keratinocytes in a Skin Equivalent Model in Vitro

Abstract. *A skin equivalent model has been used to fabricate tissues with psoriatic and normal cells. Psoriatic fibroblasts can induce hyperproliferative activity in normal keratinocytes. The psoriatic epidermis from lesions continues to proliferate at high rates for at least 15 days in this model, and normal fibroblasts are unable to suppress this hyperproliferation. The primary defect in psoriatic skin may reside in the dermal fibroblast.*

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Psoriasis is a common skin disease characterized clinically by the appearance of sharply circumscribed scaly red plaques. Histopathological changes in established psoriatic plaques are essentially epidermal, with keratinocyte hyperproliferation in the basal layers and incomplete keratinization. The main changes in the dermis consist of papillary edema, dilatation of the papillary capillaries, and the appearance of a moderate infiltrate of inflammatory cells. The role of dermal fibroblasts in inducing plaque formation is the subject of this report.

There is strong evidence that the disease may not be of systemic origin but that the defect may originate within the skin (*1*). When full-thickness psoriatic skin is grafted to the nude mouse, the epidermis from lesions [involved psoriatic (PP) skin] maintains most of its pathological features for at least 6 weeks and the epidermis from apparently normal areas [uninvolved psoriatic (PN) skin] can develop certain features of the psoriatic lesion (*1*). However, the rates of proliferation and the patterns of differentiation of keratinocytes from PP or PN skin monocultured in vitro do not differ from each other or from the rates and patterns of normal cultured keratinocytes (*2*).

Keratinocytes cultivated without a living dermal component may be an incomplete model for studying interrelations within the skin that could explain the mechanism of plaque formation. To overcome this handicap we have adapted a model system (*3*), called a skin equivalent, made from matrix materials with dermal and epidermal cells to study this aspect of the disease. The dermal component, called a dermal equivalent, originates from the contraction of a collagen gel by dermal fibroblasts (*4*) that have first been grown as conventional monolayer cultures on plastic. In the original experiments, the epidermis was applied to the dermal equivalent as a cell suspension (*3*); in the experiments reported here a new procedure, the insertion of a small full-thickness punch biopsy into the dermal equivalent, was used. The multilayered epidermis that develops in vitro by a process of epiboly (Fig. 1, A through C) consists of tightly associated, well-differentiated keratinocytes emanating from an organized basal layer of cells (*5*). Using the skin equivalent model, we have studied the outgrowth of keratinocytes from punch biopsies of PP and PN skin harvested from the forearms of untreated patients afflicted with chronic plaques of psoriasis and from punch biopsies of normal skin (NN) from age- and sex-matched healthy volun-

teers. The biopsies were implanted in vitro into dermal equivalents made up with fibroblasts originating from PP and PN skin (FPP and FPN) and from NN skin (FNN). Informed consent was obtained from all subjects.

Skin biopsies from any source (PP, PN, or NN) exhibited a rate of epidermal outgrowth (area) that was linear between day 5 and 15. To minimize the number of biopsies required from each human volunteer, we used this area of epidermal outgrowth after 9 or 12 days in culture as a measure of the degree of keratinocyte proliferation since this parameter was linearly correlated with epidermal DNA content (Fig. 1D).

Epidermalization arising from NN biopsies was enhanced when either PP or PN was used as a source of fibroblasts for the dermal equivalent (Fig. 2). Our results suggest that the primary defect (or defects) leading to the hyperproliferation of keratinocytes in psoriasis may lie within the dermis, in fact, within the dermal fibroblasts. Without suggesting a mode of action, we propose that a diffusible factor from the fibroblasts is involved.

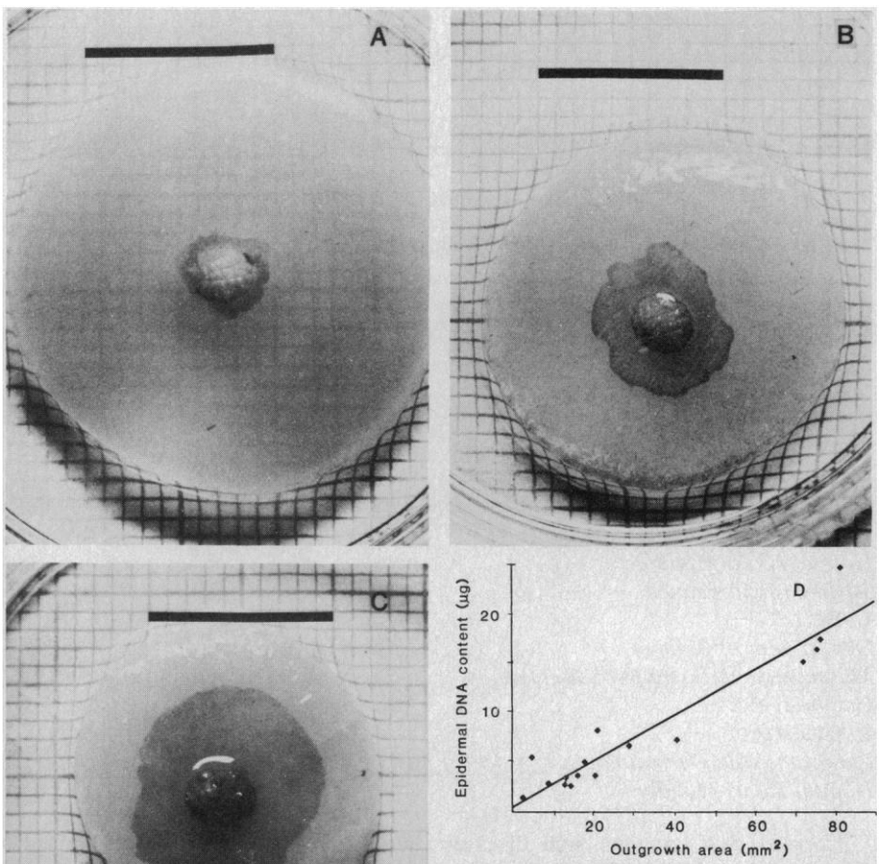

Fig. 1. Radial outgrowth of keratinocytes from three NN punch biopsies on dermal equivalents made up with FNN; the cultures were stained with Nile blue sulfate after 5 (A), 8 (B), and 12 (C) days in culture (bar, 1 cm). (D) Epidermal DNA content as a linear function of the area of outgrowth after 9 days in culture (slope ± standard error of the slope: 0.23 ± 0.02 μg/mm²). Skin equivalents were fabricated with normal or psoriatic fibroblasts and NN, PN, or PP biopsies. Dermal equivalent tissues were fabricated as described (*10*) with a few modifications (*13*). A full-thickness 3-mm skin punch biopsy was inserted, epidermis up, into the center of the uncontracted dermal equivalent (*5*). Biopsy samples were washed three times in culture medium and used within 1 to 3 hours. PN biopsies, taken at least 5 cm from the nearest psoriatic lesion, and PP biopsies harvested from the same psoriatic subject were incorporated into dermal equivalents. By day 5 after pouring, the dermal equivalent was contracted into a firm tissue disk about 20 mm in diameter (*4*). At this time, skin equivalents were positioned on a stainless steel grid and the culture medium was replaced with a mixture (*14*) that favors keratinocyte growth. The medium just covered the surface of the skin equivalent. Cultures were reincubated until assayed. To visualize the area of keratinocyte outgrowth, cultures were stained with Nile blue sulfate 1:10,000 (Sigma) for 30 minutes and washed in 10 ml of phosphate-buffered saline. The areas of epidermal outgrowth as well as those of the dermal equivalent were measured. The epidermal DNA content was assayed by a fluorimetric procedure (*15*) after outgrowths were separated from dermal equivalents with dispase (1.2 μg/ml) (Boerhinger) in serum-free medium. Purified fetal calf thymus DNA (Sigma) was used as standard, and hydrolyzed Nile blue sulfate solutions were used as controls. Histology was carried out after 10 days on tissues resulting from all combinations of psoriatic or normal fibroblasts and keratinocytes. No histological differences were observed, and the numbers of epidermal cell layers were not significantly different.

These results differ from those reported by Baden *et al.* (*6*), who showed no increase in keratinocyte growth from skin explants cultured on a feeder layer of normal or psoriatic dermal fibroblasts pretreated with mitomycin C. The dermal equivalent that we fabricated differs greatly from a feeder layer. One explanation for our finding that psoriatic fibroblasts induce hyperproliferation of normal epidermis is that the model in which they function provides a living, well-developed dermal equivalent that has many properties of actual dermis, in particular, a well-organized collagen network of high density. The requirement of living dermal elements for facilitating and directing growth and differentiation of epithelia has been demonstrated (*7*). Furthermore, when dermal fibroblasts are incorporated into a dermal equivalent, they express patterns of differentiation similar to those of cells in vivo. Dermal fibroblasts incorporated into a dermal equivalent exhibit collagen processing (*8*), growth regulation (*9*), and perinuclear peroxidase activity (*10*) like those of dermal cells in vivo and unlike those of fibroblasts cultured as monolayer.

To discover whether psoriatic keratinocytes maintained a high proliferative rate in our model, we implanted PP, PN, or NN punch biopsies into dermal equivalents made up with normal dermal fibroblasts. Outgrowth areas after 9 days in culture were significantly higher (Bonferroni correction applied throughout for all multiple comparisons) when keratinocytes originated from PP biopsies than from PN or NN biopsies (Fig. 3A). Since all tissues biopsied contained a dermal component, it is necessary to comment that fibroblasts from the biopsy probably do not migrate into the surrounding dermal equivalent. This would explain why epidermis from a PN biopsy is not hyperstimulated when it is implanted into an FNN dermal equivalent. The increased proliferative activity of keratinocytes from involved psoriatic epidermis, characteristic of cells in lesions in vivo, seems to be maintained in vitro in our model for at least 15 days.

Our findings differ from the results of others. It has been reported that primary cultures of psoriatic keratinocytes from

explants on an irradiated feeder layer of fibroblasts from NIH 3T3 mice grow more slowly than normal ones (*2*). Moreover, similar kinetics of DNA synthesis have been reported for normal and psoriatic keratinocytes in outgrowth cultures (*11*) and in cultures of keratinocytes passaged onto collagen surfaces without a feeder layer (*2*). Because in all of the foregoing studies fibroblasts were either absent or mainly moribund (*12*), as they may have been in the dermis of explanted skin biopsies (*11*), the significance of the results may be questioned.

The increased proliferative capacities acquired in vivo by keratinocytes from PP epidermis and maintained in vitro in our model can also be illustrated in other combinations of the skin equivalent components. Skin equivalents with FPP and FNN fibroblasts and with keratinocytes from PP, PN, or NN biopsies were fabricated; increased areas of keratinocyte outgrowth on dermal equivalents made up with FPP as compared with areas made up with FNN were observed only when PN or NN biopsies were used as a source of keratinocytes (Fig. 3B). Thus the keratinocytes taken from a lesion (PP) seem already to be maximally induced. Moreover, normal fibroblasts are unable to suppress the hyperproliferative growth of PP keratinocytes.

Not all the functions of psoriatic fibroblasts are abnormal. To examine the capacity of fibroblasts to remodel a collagen matrix, a useful wound-healing model, we studied the contraction rates of unepidermalized dermal equivalents made up with ten FPP and ten FNN strains when cells were four passages old. Arabinosylcytosine (Ara C), a blocker of cell division, was used since contraction rates depend on the fibroblast number in dermal equivalents (*4*). Since no statistically significant differences between the contraction rates with FPP and FNN were seen with or without Ara C (Fig. 4), we concluded that psoriatic fibroblasts do compact collagen fibrils as well as normal fibroblasts and that FNN and FPP growth regulation in dermal equivalents seems to be identical.

Our results demonstrate that hyperproliferation of normal epidermis can be induced by PP and PN fibroblasts and that NN fibroblasts seem unable to suppress the hyperproliferative activity of keratinocytes from lesions. On the basis of these results, we propose that a primary defect of psoriatic skin may reside in the dermal fibroblasts. The hyperproliferative activity of PP keratinocytes can be maintained in a model system in vitro. Our results indicate that these keratinocytes are maximally induced in vivo

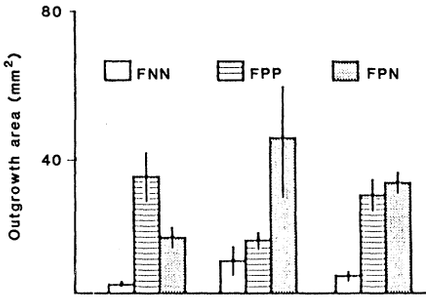

Fig. 2. Outgrowth of keratinocytes from NN biopsies taken from the same specimen of normal skin on dermal equivalents made up with FPP, FPN, or FNN strains (9 days in culture). Each of three psoriatic patients provided a strain of FPP and of FPN. Three normal volunteers each provided an FNN strain. The epidermal outgrowth on FPN and FPP dermal equivalents was significantly greater than that obtained with FNN ($P < 0.002$, Wilcoxon test) with one exception seen in the middle set of data for FPP (bar, ± standard error of the mean). In other experiments carried out for 9 or 12 days, six different sources of NN biopsies were tested on dermal equivalents made up with seven FPP and seven FNN strains. Outgrowth areas were significantly higher on FPP dermal equivalents than on FNN dermal equivalents ($P < 0.001$, Student's t test for paired values).

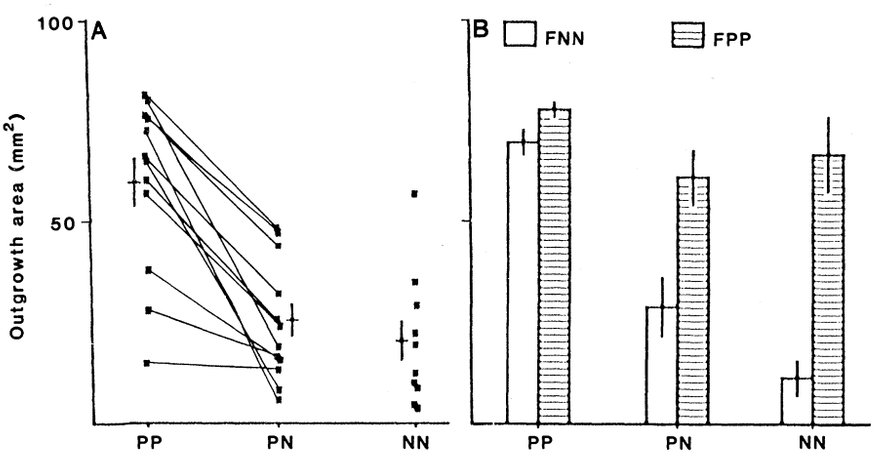

Fig. 3. (A) Areas of epidermal outgrowth at day 9 from PP, PN (harvested from 12 psoriatic patients 42 ± 12 years old), or NN (harvested from 10 healthy sex-matched individuals 36 ± 16 years of age) biopsies on dermal equivalents made up with FNN. Data obtained from PN and PP biopsies harvested from the same psoriatic patient were paired. Outgrowth areas from PP biopsies were significantly higher than with PN and NN biopsies ($P < 0.001$, Wilcoxon test). Dermal equivalent areas were not significantly different. Similar data were obtained with four different FNN strains. (B) Epidermal outgrowth at day 9 from PP, PN (harvested from five psoriatic patients), or NN (harvested from five normal volunteers) biopsies on dermal equivalents made up with FPP or FNN strains. One strain of FPP and one of FNN were used (bar, ± standard error of the mean).

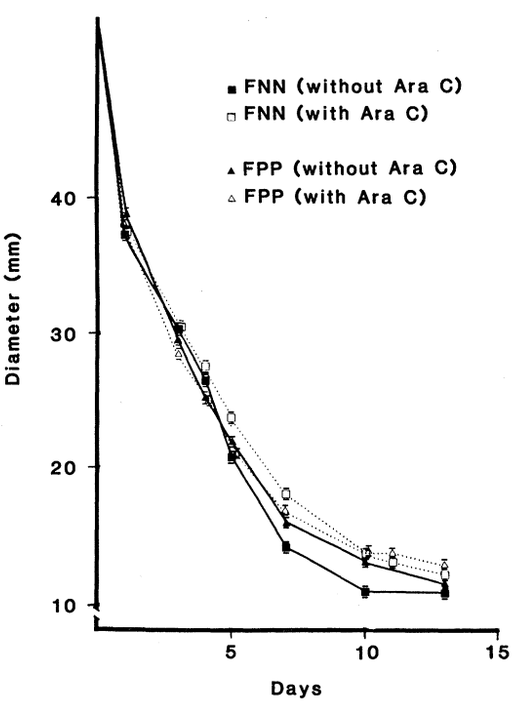

Fig. 4. Typical experiment comparing the contraction of unepidermalized collagen gels by one FPP and one FNN strain, with or without Ara C (1 μg/ml) (Sigma). Media were not renewed at day 5 (bar, ± standard error of the mean).

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.

References and Notes

1. J. E. Fraki, R. A. Briggaman, G. S. Lazarus, *J. Invest. Dermatol.* **80**, 31s (1983).
2. S. C. C. Liu and C. S. Parsons, *ibid.* **81**, 54 (1983).
3. E. Bell, H. P. Ehrlich, D. J. Buttle, T. Nakatsuji, *Science* **211**, 1052 (1981).
4. E. Bell, B. Ivarsson, C. Merrill, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1274 (1979).
5. B. Coulomb *et al.*, *Br. J. Dermatol.*, in press.
6. H. P. Baden, J. Kubilus, M. J. Macdonald, *J. Invest. Dermatol.* **76**, 53 (1981).
7. I. C. Mackenzie and N. E. Fusenig, *ibid.* **81**, 189s (1983).
8. B. Nusgens, C. Merrill, C. Lapière, E. Bell, *Collagen Relat. Res.* **4**, 351 (1984).
9. R. Sarber, B. Hull, C. Merrill, T. Soranno, E. Bell, *Mech. Ageing Dev.* **17**, 107 (1981).
10. B. Coulomb *et al.*, *J. Invest. Dermatol.* **81**, 75 (1983).
11. B. A. Flaxman and D. P. Chopra, *ibid.* **59**, 102 (1972).
12. K. A. Holbrook and H. Hennings, *ibid.* **81**, 11s (1983).
13. The total volume of a dermal equivalent was made 10 ml; 4×10^5 low-passaged fibroblasts were used to constitute a dermal equivalent; in all culture media, 10 percent human AB serum was used instead of fetal bovine serum.
14. B. E. Hull, St. E. Sher, S. Rosen, D. Church, E. Bell, *J. Invest. Dermatol.* **81**, 429 (1983).
15. B. Fiszer-Szafarz, D. Szafarz, A. Guevara de Murillo, *Anal. Biochem.* **110**, 165 (1980).

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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