synthesis of histone proteins in G_1 HeLa cells is the inability to detect histone messenger RNA sequences in the cytoplasm during G_1. When cytoplasmic RNA of G_1 and S phase cells was fractionated electrophoretically in methyl-mercury-agarose gels, transferred electrophoretically to diazotized cellulose (*20*), and analyzed by hybridization with ^{32}P-labeled cloned human H3 and H4 histone DNA sequences (*21*, *22*), annealing with the S phase but not the G_1 RNA's was observed (Fig. 4). The validity of using cytosine arabinoside as an inhibitor of HeLa cell histone and DNA synthesis is substantiated by the obvious loss of more than 95 percent of the histone messenger RNA sequences from S phase polysomes after drug treatment for 1 hour (Fig. 4). We observed a similar absence of H1 and H2B histone messenger RNA sequences from G_1 Hela cell cytoplasmic RNA and a loss of H1 and H2B histone RNA sequences from polysomes after treatment of S phase cells with cytosine arabinoside (data not shown).

Taken together, results from our analyses of newly synthesized histone polypeptides and of the representation of cytoplasmic histone messenger RNA sequences in G_1 and S phase HeLa cells are consistent with cell cycle stage specific synthesis of the histone proteins. Thus, while it appears from our results that the control of histone gene expression during the cell cycle in HeLa cells is different from that in mouse lymphoma cells (*19*), such an observation would not be unprecedented. For example, it has been well documented that histone proteins are synthesized in the absence of DNA replication during oocyte maturation. The emerging picture may be one in which the level at which regulation of histone gene expression is mediated can vary depending on the cell type and the biological circumstance.

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26 August 1981; revised 29 October 1981

Uninvolved Skin from Psoriatic Patients Develops Signs of Involved Psoriatic Skin After Being Grafted onto Nude Mice

Abstract. *Clinically involved psoriatic epidermis maintains its histological appearance, increased labeling index, and increased level of plasminogen activator after being grafted onto athymic nude mice. Uninvolved psoriatic epidermis develops increases in plasminogen activator activity after being grafted onto athymic nude mice; this is accompanied by an increased labeling index. Thus, psoriatic skin can develop markers of psoriasis independent of the host.*

Psoriasis vulgaris, a common skin disease, is characterized by circumscribed scaly plaques. Epidermal cells in psoriatic lesions are hyperproliferative (*1*), and psoriatic epidermis is thickened and papillomatous (*2*). The transit time of cells through the epidermis is markedly shortened (*3*), and the epidermis does not attain differentiated characteristics. The cause of epidermal hyperproliferation is unknown. Numerous hypotheses have been suggested, including intrinsic dysfunction of epidermal keratinocytes, alterations in cyclic nucleotide and prostaglandin metabolism, aberrations of beta-adrenergic receptor function (*4*), and abnormalities of the immune system (*5*). A major difficulty in investigating this disease in vitro is that psoriatic epidermis is not distinctive in tissue or cell culture. However, it was recently shown that clinically involved psoriatic skin maintains its morphological characteristics and accelerated mitotic rate when transplanted onto athymic nude mice (*6*).

Psoriatic scales (*7*) and psoriatic epidermis (*8*) have greater levels of plasminogen activator than normal epidermis. The activity of this enzyme increases in numerous situations in which cell transformation or activation occurs (*9*). We report here that clinically involved epidermis from psoriatic patients maintains its high plasminogen activator level when grafted onto nude mice. We also report that plasminogen activator activity in clinically uninvolved psoriatic epidermis increases significantly after the tissue is grafted onto nude mice; this is associated with an increased labeling index. These findings suggest that psoriatic epidermis can manifest markers of psoriasis independent of the host.

Specimens were removed from psoriatic plaques and from uninvolved (normal-appearing) skin on the hip and proximal thigh in nine male patients with psoriasis. Three normal males provided control skin (informed consent was obtained from all the donors). The tissue was removed with a Castroviejo dermatome to a depth of 0.4 mm for uninvolved skin and 0.6 to 0.7 mm for lesions. Half of each specimen was immediately grafted onto athymic nude mice. Each animal received two grafts 6 mm in diameter (10 to 18 animals per specimen). The remaining half of each specimen was incubated in 2*M* KBr for 30 minutes at 37°C, permitting mechanical separation of epidermis from the dermis. The harvested epidermis was washed in sterile saline, mixed into 2*M* KCl and 0.01*M* NaH_2PO_4 and Na_2HPO_4 buffer (*p*H 7.0), and frozen at −70°C. The brief potassium bromide treatment did not affect the level of plasminogen activator or of the lysosomal proteinase cathepsin D in separated epidermis.

The transplanted skin was harvested from the nude mice 6 weeks after grafting. One hour before the mice were killed, [^3H] thymidine (Schwarz/Mann; specific activity, 7 to 10 mCi/mole) was

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injected into the peritoneal cavity (1 μCi/g). A portion of each harvested specimen was prepared for histological examination and autoradiography. Labeling index was determined by counting the number of labeled cells (criterion: five or more grains per cell) per 1000 basal cells and expressing the result as a percentage. The remainder of the harvested tissue was used for the preparation of epidermis, as described.

Plasminogen activator activity was measured by a modification of the method described by Burger *et al.* (*10*). Purified dog plasminogen was used as the substrate, and activated plasmin was quantitated by measuring the release of radioactive peptides from ^{125}I-labeled fibrinogen attached to Linbro tissue culture plates (Flow). Bovine fibrinogen was purified (*11*) from a crude fibrinogen preparation (Calbiochem) and labeled with ^{125}I (Bio-Rad). Appropriate controls without plasminogen or sample were used, and all assays were performed in the linear dose range of the assay. All specimens were assayed for cathepsin D (*12*), protein concentration (*13*), and DNA content (*14*). Enzyme activities are expressed as hydrolytic rate of substrate per quantity of DNA in the sample.

The involved psoriatic skin maintained its psoriasiform histology, with papillomatosis, acanthosis, and variable parakeratosis; no granulocytes were found. Uninvolved and control skin appeared histologically normal. The thymidine labeling index for basal epidermal cells in involved epidermis was 6.9 ± 2.6 percent, which is significantly higher than the index in normal controls (3.5 ± 0.9 percent) ($P < .001$, two-tailed *t*-test for independent variables). The labeling index for uninvolved epidermis (5.5 ± 2.5 percent) was similar to that for involved epidermis and significantly different from that for control skin ($P < .05$).

The specific activity of plasminogen activator in uninvolved psoriatic epidermis before grafting was similar to that in control epidermis. By contrast, epidermis from psoriatic plaques had a mean plasminogen activator activity 17 times higher than that in uninvolved or control epidermis ($P < .008$, two-tailed *t*-test for paired samples) (Fig. 1). Involved epidermis maintained its high level of plasminogen activator after grafting. By contrast, the level of plasminogen activator in uninvolved skin after grafting was as high as that in involved epidermis ($P < .012$). The plasminogen activator level in control epidermis did not change after grafting.

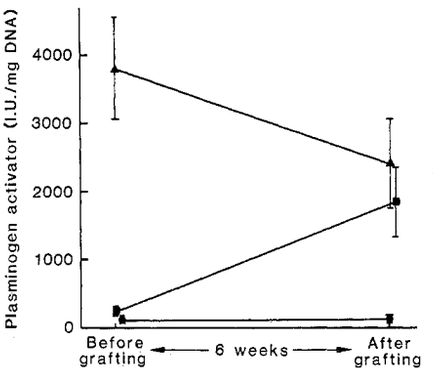

Fig. 1. Plasminogen activator activity (equivalent to Abbokinase activity) in involved psoriatic epidermis (▲), uninvolved epidermis (■), and control epidermis (●) before grafting onto athymic nude mice and after 6 weeks of grafting.

Extracts of normal and uninvolved epidermis before grafting inhibited the exogenous plasminogen activator urokinase; 8 I.U. of Abbokinase was inhibited by 1 mg of protein from the epidermal extract. By contrast, extracts of involved epidermis did not inhibit urokinase. Plasminogen activator in the psoriatic epidermis was inhibitable with the serine proteinase inhibitor diisopropyl fluorophosphate (1 m*M* final concentration, 30 minutes incubation), indicating that bacteria were not the source of the enzyme. The specific activity of cathepsin D was similar in involved, uninvolved, and control epidermis and did not change after grafting.

Our data demonstrate that involved psoriatic epidermis contains markedly higher levels of plasminogen activator than uninvolved psoriatic epidermis or epidermis from normal individuals. This confirms earlier findings that the level of plasminogen activator is increased in psoriatic scales (*7*) and supports the hypothesis that plasminogen activator is a marker of cell activation in psoriatic epidermis (*15*). After grafting, involved psoriatic epidermis maintains its increased level of plasminogen activator, increased labeling index, and psoriasiform histology for at least 6 weeks, and uninvolved skin develops the markers of involved epidermis. The increase in plasminogen activator activity in uninvolved epidermis after grafting appears to be characteristic because the specific activity of cathepsin D did not change. Furthermore, there was no detectable inflammatory infiltrate in the epidermis. Thus it appears that uninvolved psoriatic skin can display markers of psoriasis independent of the host. The histology of uninvolved epidermis did not change markedly after grafting, suggesting that morphological changes may require more time to develop or that they are not possible in nude mice.

A high labeling index for epidermal basal cells from uninvolved psoriatic skin after grafting onto nude mice was recently reported by Krueger *et al.* (*16*). The correlation of plasminogen activator activity with labeling index suggests that this enzyme may be a reliable marker for cell activation in psoriasis. The increase in plasminogen activator activity in psoriatic epidermis may be explained by increased synthesis or activation of the enzyme or by decreased production of the inhibitor of epidermal plasminogen activator (*17*).

The ability of uninvolved psoriatic skin to display markers of involved epidermis when transplanted onto nude mice might be similar to the Koebner phenomenon. In this unexplained phenomenon, psoriatic plaques form at sites of trauma to uninvolved skin in certain patients with active psoriasis. Recently, several investigators demonstrated that stimulation of uninvolved psoriatic skin induces epidermal kinetics similar to those of involved skin (*18, 19*). It seems that skin itself can develop psoriasiform changes without interaction with serum factors or blood cells.

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9 September 1981; revised 30 October 1981

Cytoplasmic Inclusion Bodies in *Escherichia coli* Producing Biosynthetic Human Insulin Proteins

Abstract. Escherichia coli *that has been genetically manipulated by recombinant DNA technology to synthesize human insulin polypeptides (A chain, B chain, or proinsulin) contains prominent cytoplasmic inclusion bodies. The amount of inclusion product within the cells corresponds to the quantity of chimeric protein formed by the bacteria. At peak production, the inclusion bodies may occupy as much as 20 percent of the* Escherichia coli *cellular volume.*

Manipulation of the bacterial genome through recombinant DNA technology to produce organisms capable of forming pharmacologically useful peptides is an area of intense research activity. As part of an ongoing project for the production of human insulin in bacteria, we have examined cultures of *Escherichia coli*, using scanning and transmission electron microscopy at intervals during the culture cycle for the production of insulin chain polypeptides. We have observed a morphologically characteristic intracellular product whose accumulation in the form of inclusion bodies corresponds to the formation of chimeric protein. The product was present in cells bearing plasmids containing gene sequences coding for insulin A chain, insulin B chain, or proinsulin fused to portions of either the lactose or the tryptophan operons. Similar inclusion bodies were not evident in the nonplasmid-containing strain of *E. coli* or in *E. coli* containing the parental plasmid vector pBR322.

The construction of plasmids coding for the expression of insulin A and B chains has been described (*1*). These plasmids contain DNA sequences coding for A or B chain fused to the major portion of the β-galactosidase gene from λ plac 5. In addition, a second series of plasmids in which the β-galactosidase gene fragment has been replaced with an analogous fragment of the tryptophan operon has been constructed. In this series, the formation of product is under the control of the trp promoter, and the chimeric protein consists of insulin chains (A chain, B chain, or proinsulin) fused at the COOH-terminus of a tryptophan operon gene product.

Escherichia coli containing fused gene-bearing plasmids were cultivated in shake flasks in either L broth with ampicillin (500 μg/ml) or in modified M-9 salts medium containing tetracycline (1.5 μg/ml). Growth was obtained with vigorous agitation at 37°C. When the appropriate cell density was achieved, the culture was used to inoculate similar medium in a small fermentor. Samples were taken at intervals throughout the fermentation for evaluation. Typically, cultures grown under these conditions completed the logarithmic phase of growth in about 15 hours. The insulin chimeric proteins were produced as insoluble products by the bacterial cell. They accumulated within the cell, with peak levels being reached at varying times depending on which chimeric protein was being formed and on culture conditions.

Samples taken during fermentation for morphological study were monitored by sodium dodecyl sulfate–polyacrylamide gel electrophoresis for chimeric protein production. The band identified as chimeric protein increased in density as the fermentation progressed (Fig. 1). The morphology of *E. coli* producing the insulin chain chimeric proteins is illustrated in Figs. 2 to 4. The structure of the chimeric protein producing cells was distinct from that of the nonplasmid-containing cells (Fig. 5) in that a large proportion of the cell was filled with a finely granular, moderately electron-opaque material. The material lacked an obvious boundary such as an enclosing membrane or the "membrane coat" that has been observed with poly-β-hydroxybutyrate and polyglucose granules (*2*). The product, nevertheless, was segregated from the rest of the bacterial cytoplasm. At moderately high magnifications (> 150,000×) fine fibers with diameters in the 2- to 4-nm range could be resolved within some areas of the granular matrix. We have consistently observed inclusion bodies in *E. coli* cultures producing the insulin chain chimeric proteins. On the basis of our observations with transmission electron microscopy and with light microscopy by dark-field and differential

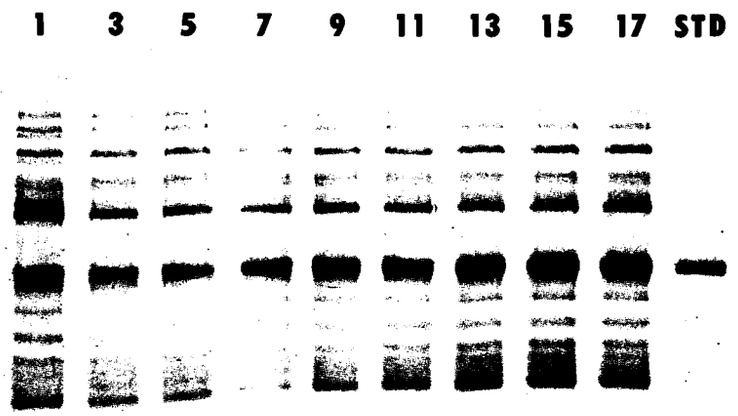

Fig. 1. Sodium dodecyl sulfate (SDS) gel electrophoresis of chimeric protein. Broth samples were collected at the stated times after initiation of fermentation and the cells were centrifuged. The supernatant was removed and the cell pellet was frozen. The pellet was dissolved in a buffered solution of 2.3 percent SDS and 5 percent β-mercaptoethanol and heated at 90° to 100°C for 5 minutes (*8*). A portion of the sample was analyzed with a 7.5 percent polyacrylamide gel prepared wth imidazole buffer (*p*H 7.35) containing 0.2 percent SDS. Electrophoresis was performed on an LKB Multiphor apparatus with an ISCO power supply. Gels were stained with Coomassie blue R-250. The standard (*STD*) is a sample of partially purified chimeric proinsulin. The numbers indicate the hour of collection.

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