most completely reepithelialized. Epithelial hyperthickening was less pronounced in heterozygous transgenic animals (Fig. 3B), and reepithelialization was much less complete. This finding suggested that migration and proliferation of the keratinocytes in these mice were impaired. The reduced reepithelialization of the wound was most obvious in homozygous transgenic mice, where almost no hyperthickening of the epidermis at the wound edge was observed (Fig. 3C). The phenotypic abnormalities seen in the wound epithelium were consistent with high expression of the transgene in the keratinocytes of the migrating epithelium (Fig. 3D) and the hypertrophic epithelium at the wound edge (Fig. 3E).

To quantify the difference in the proliferation rate of the keratinocytes, we labeled the proliferating cells with 5-bromodeoxyuridine (BrdU) in vivo and subsequently stained them with an antibody to BrdU (*13*). At day 5 after injury, a large number of cells in the epidermis at the wound edge of control mice had incorporated BrdU (Fig. 4, A and B), whereas the number of proliferating keratinocytes at the wound edge of transgenic mice was 95 to 99% lower compared to that of control mice (Fig. 4, C and D). This reduced proliferation rate was seen in all transgenic mice tested. As an indication of specificity, the number of proliferating cells in the underlying granulation tissue was similar in transgenic and control mice at this stage of wound healing, demonstrating that transgene expression had only blocked proliferation of keratinocytes. The reduced proliferation rate of the keratinocytes that express the dominant-negative KGF receptor demonstrates that KGF receptor signaling is essential for wound reepithelialization.

The data presented here reveal a direct role of KGF receptor signaling in the morphogenesis of the epidermis and the hair follicles and an indirect role of KGF receptor function in the control of connective tissue formation in the dermis, indicating a complex epidermal-dermal interaction during morphogenesis of the skin. Our results demonstrate that KGF is essential for wound reepithelialization and that a blockade of KGF function is associated with wound-healing abnormalities.

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12. Homozygous and heterozygous transgenic mice as well as control littermates (3 months old) were used for wound-healing experiments. Mice were anesthetized with a single dose of Avertin. A single full-thickness wound of 5-mm diameter was made in the middle of the back by excising skin and panniculus carnosus. Animals were killed with ether at day 5 after injury and the complete wound was isolated. All animal experiments were carried out with permission from the local government of Bavaria (permission number 211-2531-16/93).
13. For BdUr-labeling studies, wound-healing experiments were carried out as described in (*12*). At day 5 after injury, mice were injected intraperitoneally with BrdU (Sigma; 250 mg per kilogram of body weight) and killed 2 hours after injection. Wound tissue was fixed in 70% ethanol and paraffin embedded.

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Determination of Intrinsic Transcription Termination Efficiency by RNA Polymerase Elongation Rate

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Transcription terminators recognized by several RNA polymerases include a DNA segment encoding uridine-rich RNA and, for bacterial RNA polymerase, a hairpin loop located immediately upstream. Here, mutationally altered *Escherichia coli* RNA polymerase enzymes that have different termination efficiencies were used to show that the extent of transcription through the uridine-rich encoding segment is controlled by the substrate concentration of nucleoside triphosphate. This result implies that the rate of elongation determines the probability of transcript release. Moreover, the position of release sites suggests an important spatial relation between the RNA hairpin and the boundary of the terminator.

Termination of RNA synthesis by *E. coli* RNA polymerase (RNAP) at intrinsic (or ρ-independent) terminators is a property of the core enzyme and probably of the highly conserved large subunits β and β′ (*1*, *2*). Essential elements of intrinsic terminators include DNA specifying an RNA hairpin, followed by a DNA segment encoding RNA rich in uridines at the end of the terminated RNA (*1*, *2*). How these elements act is unknown, although a terminal U-encoding segment is also essential in eukaryotic RNAP III terminators (*3*), and U-encoding sequences cause stalling by eukaryotic RNAP II (*4*). Terminator function may reflect the available energy of RNA and DNA strand associations: It has been suggested that RNA hairpin formation destroys part of an RNA-DNA hybrid that is essential for the stability of the elongating complex, after which the instability of the remaining U-rich RNA-DNA hybrid allows the complex to dissociate (*1*, *5*). Alternatively, or in addition, the hairpin may interact with RNAP and promote termination either by disrupting RNA-enzyme associations required for complex stability or by inducing conformational changes that lead to termination (*6*, *7*).

The efficiency of intrinsic termination may reflect competition between the rate of elongation and the rate of RNA (and eventually enzyme) release. As is true at ρ-dependent terminators (*8*), RNAP at or near the release site of an intrinsic terminator may be in a "pause," meaning a condition in which elongation is slowed or

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halted temporarily; the duration of the pause would determine the efficiency of RNA release. The pause might be induced by the RNA hairpin (*1*, *2*, *9*, *10*), the U-encoding segment, downstream DNA, other unidentified elements (*2*, *9*), or a combination of these. Pausing and intrinsic termination have been associated experimentally in two ways: through the existence of mutant RNAPs that simultaneously enhance or diminish both pausing and termination (*11*) and through the antipausing activity of the transcription antiterminators encoded by phage λ genes *Q* and *N* (*12*, *13*), which act on both intrinsic and ρ-dependent (*13*, *14*) terminators.

We used two mutationally altered RNAP enzymes, RpoB2 and RpoB8, which affect both termination and pausing, in order to investigate the relation between elongation rate and termination efficiency. RpoB2 reads through intrinsic terminators more efficiently, and RpoB8 reads through intrinsic terminators less efficiently than does wild-type enzyme ($RpoB^+$), both in vivo and in vitro (*11*). RpoB2 enzyme also pauses less efficiently than $RpoB^+$ does at the *trp* attenuator and other pause sites, whereas RpoB8 pauses more efficiently than wild-type enzyme does (*11*) and elongates more slowly (*15*). The rate of elongation of these mutant enzymes may affect their susceptibility to termination at ρ-dependent terminators (*16*).

We first examined termination in vitro by purified mutant and wild-type enzymes during synthesis at high (1 mM) and low (25 μM) nucleoside triphosphate (NTP) concentrations at several well-characterized intrinsic terminators (Table 1). For all terminators except t_{22}, readthrough was higher for RpoB2 than for $RpoB^+$ at 1 mM NTP—as high as 49%. Furthermore, readthrough by RpoB2 could be raised for each terminator by raising the concentration of a single NTP to 1 mM. For most terminators, this nucleotide is uridine triphosphate (UTP); but for $t_{R'}$ it is adenosine triphosphate (ATP); and for t_c, high guanosine triphosphate (GTP) slightly but significantly increases readthrough. For RpoB8 enzyme, we detected less than 1% readthrough at several terminators at both high and low concentrations of NTP (*17*).

Because different nucleotides induce readthrough of different terminators, we determined the relation between sites of RNA release and the surrounding sequence for two terminators. RNAs formed by the t_{21} (*18*) and t_{22} terminators are short, so that the detailed pattern of terminated ends can be examined directly by gel electrophoresis. Figure 1 shows the lengths of RNAs terminated at t_{21} in synthesis by the two mutant and wild-type enzymes (*19*).

Termination at t_{21} was generally heter-

Table 1. Termination efficiencies of intrinsic terminators vary among RNA polymerase mutants and with NTP concentration. Termination efficiency is expressed as percent of readthrough, which is calculated as the percentage of the total RNA (readthrough + terminated RNA) that is present as readthrough RNA. The terminators are described (Fig. 3); the promoter was either the natural promoter of the terminated transcript (t_{21}, t_{22}, *ilv-leu*) or $p_{R'}$ (*12*) [t_o, t_o (21 imm), t_{82}, and t_c]. $RpoB^+$ is wild-type RNAP, and RpoB2 is mutationally altered RNAP that terminates less efficiently than does wild-type enzyme in vivo. All four NTPs were present at the indicated concentrations. High A, C, G, or U means that the indicated NTP was present at 1 mM and the other three nucleotides at 25 μM. RNA was synthesized and analyzed as described (*23*).

Terminator	RNApol	1 mM NTPs	25 μM NTPs	High A	High C	High G	High U
t_{82}	$RpoB^+$	9	5	2	2	4	5
	RpoB2	49	10	3	8	8	76
$t_{R'}$	$RpoB^+$	2	2	2	1	1	1
	RpoB2	9	4	19	4	3	5
t_c	$RpoB^+$	1	<1	<1	<1	<1	<1
	RpoB2	10	1	1	1	2	1
t_o	$RpoB^+$	7	3	2	3	3	3
	RpoB2	31	8	5	5	7	26
t_o (21 imm)	$RpoB^+$	10	3	2	2	2	6
	RpoB2	22	6	5	6	5	28
t_{21}	$RpoB^+$	1	<1	<1	<1	<1	3
	RpoB2	14	5	5	2	4	33
ilv-leu att.	$RpoB^+$	25	7	23	2	4	13
	RpoB2	32	16	35	15	13	26
t_{22}	$RpoB^+$	<1	<1	<1	<1	<1	<1
	RpoB2	1	<1	<1	<1	<1	1

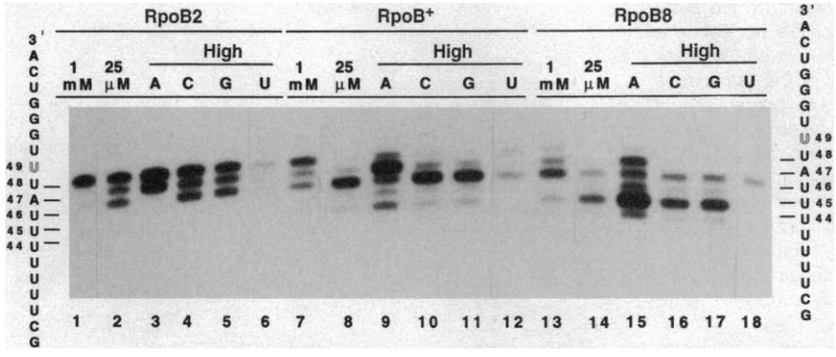

Fig. 1. The pattern of RNAs released at terminator t_{21} is both RNAP- and substrate-dependent. Lanes 1 to 6, RpoB2; lanes 7 to 12, wild-type enzyme; and lanes 13 to 18, RpoB8. Lanes representing reactions with high UTP concentration are from an exposure ~25-fold longer because of lower specific activity of the products. The sequence of the terminator region, shown as RNA, is aligned with the released RNAs at the left. Transcription reactions were carried out as described (*23*) with the following nucleotide concentrations: In lanes 1, 7, and 13, all four nucleotides were at 1 mM, and in lanes 2, 8, and 14, all four nucleotides were at 25 μM. In lanes 3, 9, and 15, ATP was at 1 mM, whereas C, G, and U were at 25 μM. Similarly, in lanes 4, 10, and 16, CTP was at 1 mM, whereas A, G, and U were at 25 μM; lanes 5, 11, and 17 contained 1 mM GTP and 25 μM A, C, and U; and lanes 6, 12, and 18 contained 1 mM UTP and 25 μM A, C, and G.

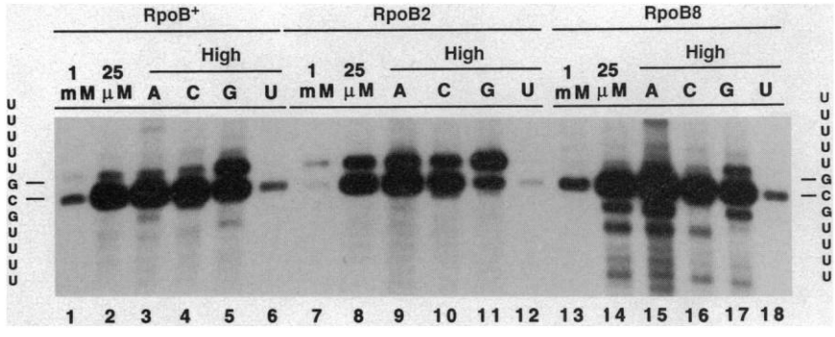

Fig. 2. Pattern of RNAs released at terminator t_{22}. The RNA is the late gene leader RNA of phage P22; RNA synthesis and analysis were done as described in Fig. 1.

ogeneous and included up to five adjacent ends. Both NTP concentration and type of enzyme affected the pattern of termination. The effect of synthesis with one NTP at high concentration showed exactly where release occurs at the distal end of the U-rich segment; thus, high ATP concentration increased ends at A47, the only A in the region, and decreased the fraction of ends at the preceding U46; high concentrations of cytidine triphosphate (CTP) or of GTP had no effect. The terminal sequence of RNA released at t_{21} has been confirmed by direct sequencing with terminating NTPs (*17*).

The pattern of termination correlated with the termination phenotype of each enzyme. Thus, during synthesis at a particular NTP concentration, the population of RNA ends was shifted toward the promoter relative to wild-type enzyme for RpoB8, which terminates very efficiently, and the ends were shifted away from the promoter for RpoB2, which terminates less efficiently.

The pattern of released RNAs also varied with NTP concentration for all three enzymes in a rational manner: A higher concentration favored longer RNAs, whereas a lower concentration favored shorter RNAs. In fact, the change from 25 μM to 1 mM NTP effectively simulated the change between mutants: The pattern at 1 mM NTP for RpoB8 was similar to that for wild-type enzyme at 25 μM NTP, and the pattern for wild-type enzyme at 1 mM NTP was similar to that at 25 μM NTP for RpoB2. For each enzyme, the pattern shifted continuously as concentration was raised and reached a final distribution at 250 to 500 μM NTP (*17*).

For a different terminator, t_{22}, we detected no significant readthrough under any conditions (Table 1), but there was a similar skewing of ends, depending on the enzyme and NTP concentration (Fig. 2). Thus, the effect of high concentrations of GTP and CTP assigns the major ends to C61 and G62; G62 is favored over C61 at higher GTP concentration and in the progression RpoB8→RpoB$^+$→RpoB2.

The pattern of RNA release at t_{21} and its control by NTP concentration suggest the existence of a critical nucleotide that defines a boundary of the terminator. High UTP concentration gave high readthrough of t_{21} by RpoB2 (14% and 33%; Table 1) and also left terminated RNA predominantly at the extreme position of the set of RNAs, U48. We suggest that a critical position is the next nucleotide, U49. By hypothesis, if UTP concentration is high, transcription proceeds with significant probability to 49, which is beyond the limit of the terminator. This boundary is sharp at the promoter distal end of the terminator; thus, even under conditions that gave 95% termination and left a large fraction of ends at U48, we detected no RNA at U49 (see Table 1 and the distribution of ends in Fig. 2).

These results have implications for both the mechanism of transcription termination and the nature of its control by antiterminators. First, we conclude that an extended portion of the U-rich encoding segment of t_{21} is characterized by an elongation-limiting Michaelis constant (K_s) for NTPs and that the rate of elongation through this region determines release efficiency. We cannot directly show that this is a pause, in the sense of slower-than-average elongation; however, pausing is suggested by the dependence of the extent of elongation on NTP concentration and the equivalence of this effect to mutational changes that affect transcription pausing. Prolonged pausing would increase the probability of release and reduced pausing would diminish it (*20*).

Second, these results suggest that a modest effect of a transcription antiterminator on the elongation rate at a critical pause can explain its activity (*21*). RpoB2 reads through terminators as much as 50% at high NTP concentration in vitro. Although we used 1 mM NTP (Fig. 1), 250 to 500 μM NTP gave as much shift in the pattern. Therefore, RpoB2 gives efficient termination and produces a pattern of RNA release

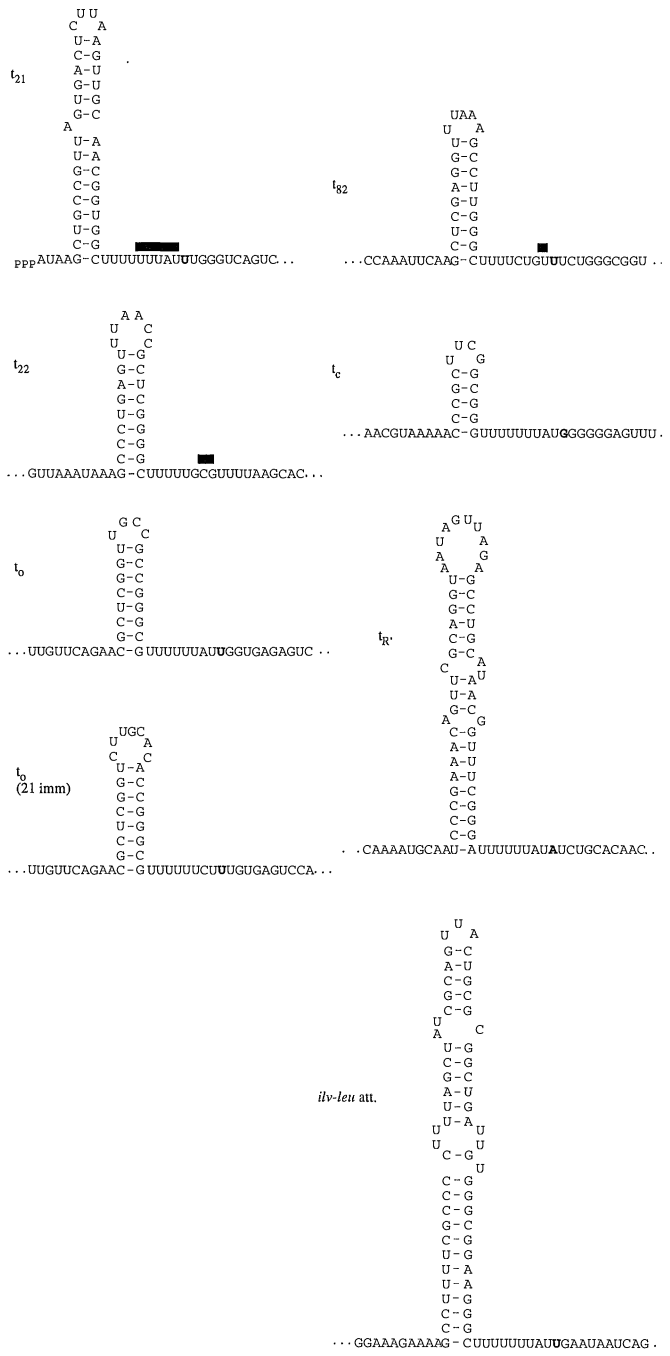

Fig. 3. Sequences of the terminator regions, shown as theoretical hairpin loop structures of the RNA. We do not include in the hairpin additional A-U base pairing that could exist at the bottom of the stem (for many of the terminators). The position corresponding to the putative critical nucleotide is shown in bold. The identities of the terminators are: t_{82}, phage 82 late terminator; $t_{R'}$, phage λ late terminator; t_c, *rpoC* terminator; t_o, phage λ *oop* terminator; t_o (21 imm), *oop* terminator of λ-21 hybrid; t_{21}, phage 21 late terminator; *ilv-leu* att., *B. subtilis ilv-leu* attenuator; and t_{22}, phage P22 late terminator. Black rectangles designate regions of RNA release determined by sequencing.

like that of wild-type enzyme if the concentration of NTP, and thus the rate of elongation through the release region, is reduced by no more than 10 to 20 times. The λ gene Q transcription antiterminator increases the rate of elongation at a pause near the λ late gene promoter region by about fivefold (*17*), although Q does not necessarily affect the same pause-inducing elements as do the RNAP mutants or the changes in NTP concentration.

Figure 3 shows the presumed structures of the terminators we used. We illustrate the RNA hairpins closed only with stable G-C base pairs (*17*), although each could be extended by two to four A-U base pairs. The actual RNA ends are shown for t_{21} and t_{22}. In these cases, release still occurs after eight to nine nucleotides are transcribed beyond the base of the G-C closed hairpin, conditions under which most of the putative DNA-RNA hybrid would remain. We also show in bold a hypothetical critical nucleotide for other terminators for which increased NTP concentration induces readthrough by RpoB2. Except for t_{21} and t_{22}, the critical nucleotide is assigned only by the criteria of distance and sensitivity to NTP concentration, although direct sequencing indicates that the last released RNA does precede the designated "U" for t_{82} (*17*). In all cases, the position of the critical nucleotide is consistent with the ability of the terminating complex to release RNA transcribed eight to nine bases beyond the end of the stable RNA stem. For the relatively inefficient *Bacillus subtilis ilv-leu* attenuator, readthrough even by wild-type RNAP is controlled by ATP and UTP, nucleotides encoded by the expected region of release.

Although we find no evidence to dispute the conventional view that the RNA hairpin disrupts the RNA-DNA hybrid, or, in particular, that the structure or stability (or both) of terminal rU-dA base pairs are involved in termination (*5*, *22*), it is difficult to explain our results by these properties alone. Release still can occur at a distance from the bottom of the stable RNA stem—eight to nine nucleotides—nearly the length of the putative RNA-DNA hybrid, where the hairpin should have little effect in disrupting the hybrid. Furthermore, for several terminators the abrupt downstream boundary of the release region has no obvious source in the stability of the RNA-DNA hybrid (*17*); for example, extending the transcript a few nucleotides beyond the terminal position for t_{21} and t_{22} only adds rU-dA base pairs to the putative hybrid and thus should add no stability if the hybrid had a constant length. Furthermore, t_{22} releases efficiently with three terminal G-C base pairs and not at all beyond them. These results are consistent with aspects of a model (*6*, *7*) that envisions distinct conformational states of RNAP—perhaps a repeating cycle of such states—as it progresses through elongation, and that proposes interaction between RNAP and transcript to be particularly important to the stability of the complex. One view of the release process according to this model, which also considers the extended zone of RNA release shown by t_{21}, is as follows: The release potential persists while the enzyme is in a state, presumably corresponding to a transcription pause, that allows it to bind the RNA hairpin as it synthesizes the U-rich region. When eight to nine nucleotides of RNA have been made (a length that reflects the geometry of the elongation complex) a conformational shift occurs that prevents hairpin binding. The distinct downstream boundary of the terminator would reflect this shift. The mechanism by which antiterminators like λ Q and *N* exert their antipausing and antitermination effects could be to disfavor a conformational state of RNAP that is associated with pausing and RNA release.

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19. By transcribing DNA attached to paramagnetic beads that could be removed from the solution, we have shown that these RNAs are released from the transcription complex (*17*).
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