Skinny Hedgehog, an Acyltransferase Required for Palmitoylation and Activity of the Hedgehog Signal

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One of the most dominant influences in the patterning of multicellular embryos is exerted by the Hedgehog (Hh) family of secreted signaling proteins. Here, we identify a segment polarity gene in *Drosophila melanogaster*, *skinny hedgehog* (*ski*), and show that its product is required in Hh-expressing cells for production of appropriate signaling activity in embryos and in the imaginal precursors of adult tissues. The *ski* gene encodes an apparent acyltransferase, and we provide genetic and biochemical evidence that Hh proteins from *ski* mutant cells retain carboxyl-terminal cholesterol modification but lack amino-terminal palmitate modification. Our results suggest that *ski* encodes an enzyme that acts within the secretory pathway to catalyze amino-terminal palmitoylation of Hh, and further demonstrate that this lipid modification is required for the embryonic and larval patterning activities of the Hh signal.

In a genetic screen for components of the *Drosophila* Hh signaling pathway (*1*) we found that mutations in the *skinny hedgehog* (*ski*) gene produced phenotypes typical of those resulting from loss of *hh* or *wingless* (*wg*) function (*2*). Mutant individuals lacking zygotic function of *ski* survive until early pupal stages. However, lethality during the embryonic period and a strong segment polarity phenotype (Fig. 1, A to C) result from additional loss of the maternal component of *ski* function. To determine whether *ski* is required specifically for either the Hh or Wg pathway, we analyzed larval tissues in which the two pathways function independently of each other in distinct subpopulations of cells (*3*). Mutant imaginal discs show wild-type levels of Wg target gene expression but are abnormally small (Fig. 1, D and E). The expression of the Hh target genes *decapentaplegic* (*dpp*) and *patched* (*ptc*), respectively, is strongly reduced or absent in these discs (Fig. 1, F to I) (*4*–*6*). Thus, we conclude that *ski* acts in the Hh signaling pathway and that *ski* mutant discs are undersized because *dpp* is expressed at abnormally low levels.

To determine whether *ski* is required for the production of active Hh signal or for transduction of this signal, we assayed Hh target gene expression in genetic mosaics. Clones lacking *ski* function in the anterior compartment of wing imaginal discs show no effect on *dpp* or *ptc* expression (Fig. 1, J and

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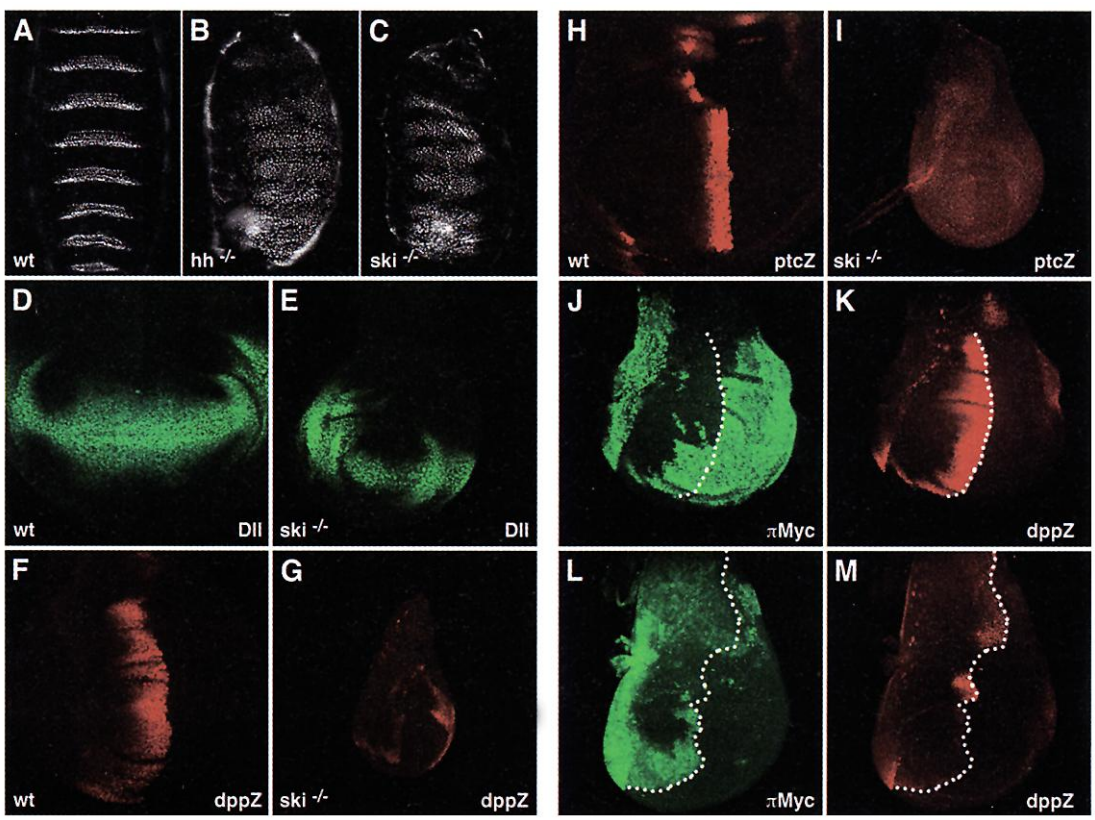

Fig. 1. The *ski* product is a component of the Hh signaling system and is required in Hh producing cells for target gene expression in Hh transducing cells. (**A** to **C**) Cuticle preparations of a wild-type embryo (A), a hh^{AC} mutant embryo (B), and a $ski^{-/-}$ embryo (C) derived from a $ski^{-/-}$ germline clone. All further micrographs (with the exception of Fig. 2A) are confocal images of third instar wing imaginal disc preparations stained with antibodies against the proteins indicated on the lower right of each panel. Genotypes are indicated on the lower left of each panel. (**D** to **I**) Expression of Wg target gene Distalless (Dll) and the Hh targets, *ptc-lacZ* or *dpp-lacZ* in wild-type (D, F, and H) and *ski* mutant (E, G, and I) discs. (**J** to **M**) Large clones of *ski* mutant cells marked by the absence of πMyc expression [green, (J) and (L)] and double-stained for the expression of *dpp-lacZ* [red, (K) and (M)]. The approximate position of the anteroposterior compartment boundary is indicated by a dotted line. Clones were induced in a *Minute* background.

K). In contrast, discs with large clones of posterior compartment cells lacking *ski* display reduced expression of Hh target genes in adjacent anterior cells (Fig. 1, L and M). As this requirement for *ski* function is similar to that of *hh* itself (*7*), we interpret these results as evidence that Ski is essential for effective production of the Hh signal. Ski appears not to be required for expression of the *hh* gene, as wild-type levels of *hh* transcription are observed in mutant discs (Fig. 2, A and B). Neither does Ski appear to be required for

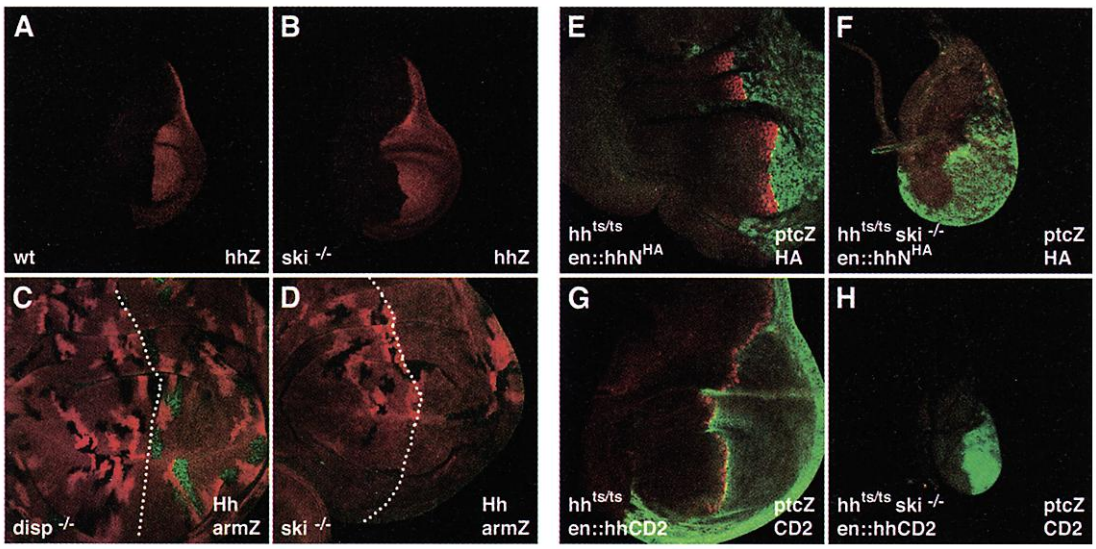

Fig. 2. Ski is required for a Hh modification that is essential for the activities of Hh, HhN, and HhCD2. (**A** and **B**) Early third instar wild-type (A) and late third instar *ski* mutant (B) discs stained for the expression of *hh-lacZ*. (**C**) Cells mutant for *dispatched* (*disp*, marked by the absence of *armadillo-lacZ* expression shown in red) fail to secrete Hh protein (green) properly (*7*). In contrast, cells mutant for *ski* (**D**) do not show abnormally high levels of Hh protein. Discs in (C) and (D) were prepared and stained simultaneously under identical conditions. The approximate position of the anteroposterior compartment boundary is indicated with a dotted line. (**E** and **F**) Expression of a *UAS-hhN^{HA}* transgene under control of *engrailed-Gal4* (en::hhN^{HA}) in a *hh^{ts}* background under nonpermissive conditions causes overgrowth in the anterior compartment and a stripe of *ptc-lacZ* expression (red). In a *ski* mutant background (F), neither of these activities of HhN can be detected. HhN protein is visualized with an antibody against the HA epitope. (**G**) Expression of a HhCD2 fusion protein (*12*) in a *hh^{ts}* background under nonpermissive conditions results in a narrow stripe of *ptc-lacZ* expressing cells (red). HhCD2 (green) has no detectable activity when produced by *ski* mutant cells (**H**).

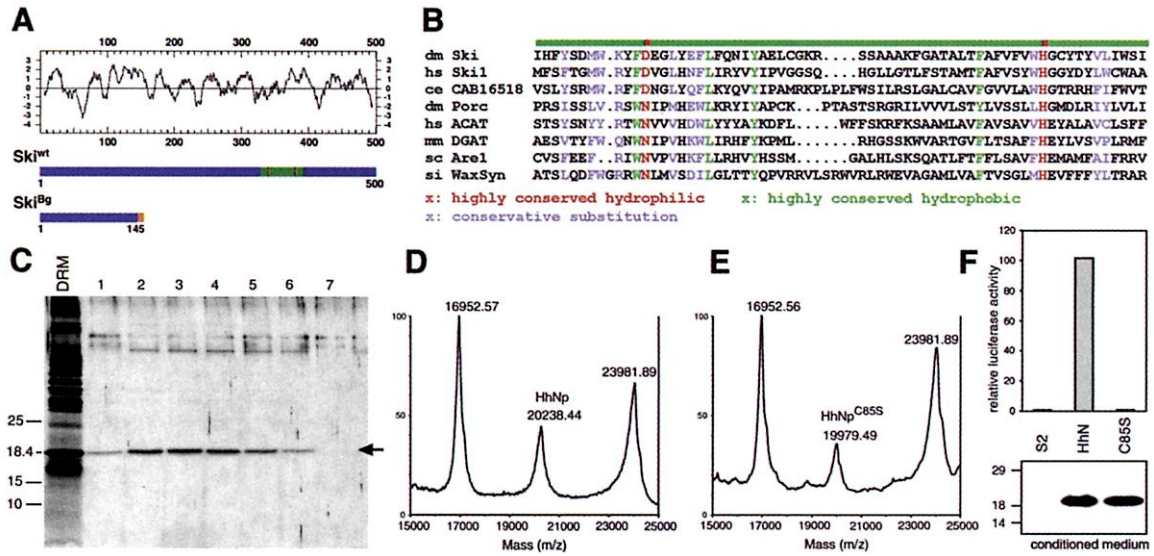

Fig. 3. Properties of the *Drosophila* Ski and Hh proteins. (**A**) Hydrophobicity plot of the 500–amino acid Ski protein sequence. Positive values represent hydrophobic regions. Schematic representation of the proteins encoded by the wild-type and *ski^{Bg}* mutant allele, indicating the truncation caused by a frame shift (orange). Position and extent of the conserved stretch of residues shown in (B) is highlighted in green. (**B**) Sequence alignment of conserved residues in Ski and several other putative membrane-bound *O*-acyltransferases (*16*). The residues in red correspond to highly conserved polar residues, D341 and H381, of Ski that have been mutated to enzymatically inactivate Ski. Highly conserved hydrophobic residues and conservative substitutions are shown in green and blue, respectively. The human homolog (hs Ski1) can be aligned throughout most of its extent to *Drosophila* Ski (Z. Chamoun *et al.*, data not shown). Abbreviations: hs Ski1, human Skinny hedgehog protein 1 (*31*); ce CAB16518, closest homolog of Ski in the *Caenorhabditis elegans* genome. dm Porc, *Drosophila porcupine* gene product (*25*); hs ACAT, human acyl-CoA:cholesterol acyltransferase-1 (*19*); mm DGAT, mouse acyl CoA:diacylglycerol acyltransferase (*18*); sc Are1, yeast alpha 2 repression gene 1 (*32*); si WaxSyn, jojoba embryo wax synthase (*17*); (**C** through **E**) Purification and mass determination of HhNp and HhNp^{C85S}. Following initial enrichment by preparation of detergent-resistant membranes (DRM), HhNp and HhNp^{C85S} expressed in *Drosophila* S2 cultured cells were further purified by binding to and elution from an antibody affinity column [HhNp elution shown in (C), arrow] (*33*). Masses of these proteins were determined by MALDI-TOF mass spectrometry (*35*) with horse skeletal myoglobin and bovine trypsinogen as internal standards (D and E). The average of two mass determinations for HhNp was 20,235 daltons; the average of three determinations for HhNp^{C85S} was 19,982 daltons. (**F**) Signaling activity of HhN and HhN^{C85S}. Media conditioned by S2 cells with stably integrated constructs for expression of HhN and HhN^{C85S} were tested for activity in a cl-8 cell reporter assay (*36*). HhN induced reporter expression by more than 100-fold whereas HhN^{C85S} had no inducing activity, despite similar protein levels in the medium (see Western blot in panel below; S2 denotes medium conditioned by the untransfected parental cell line).

secretion of the Hh protein, as abnormal Hh protein accumulation in *ski* mutant cells is not observed (Fig. 2, C and D). One remaining possibility is that the *ski* gene product controls a maturation event critical for activity of the Hh signal.

A critical step in maturation of the Hh protein is autoproteolytic cleavage at an internal site, during which the bioactive NH_2-terminal product of cleavage acquires a COOH-terminally attached cholesterol (*8, 9*). This modification can be bypassed by the expression of truncated or chimeric Hh proteins, such as HhN (*10, 11*) or HhCD2 (*12*), which are produced in bioactive form without cleavage or cholesterol addition. Neither of these proteins is active in the absence of *ski* function (Fig. 2, E to H), indicating that Ski must control a different property of the Hh signal, one that is shared by HhNp, HhN, and HhCD2.

To investigate the role of Ski, we localized the *ski* gene to cytological position 63B6 (*13*) and used double-stranded RNA injections and genomic transgenes to identify a single transcript encoding *ski* function (*14*). This transcript contains an open reading frame encoding a protein of 500 amino acids (*15*). A single base pair in codon 145 is deleted in the *ski* allele ski^{Bg} resulting in a frameshift mutation and a truncation of the presumptive protein product (Fig. 3A). Definitive identification of the *ski* gene was obtained from the complete rescue of all phenotypes associated with *ski* mutations by a transgenic wild-type open reading frame expressed under control of the ubiquitously active promoter of the *tubulinα1* gene (Fig. 4C). The Ski protein sequence exhibits an extraordinarily high content of hydrophobic amino acids, which fall into 10 to 12 putative membrane-spanning domains (Fig. 3A). There is a short but significant sequence homology between Ski and members of a diverse superfamily of membrane-associated acyltransferases (Fig. 3B) (*16*). The biochemically characterized proteins of this superfamily transfer fatty acids onto hydroxyl

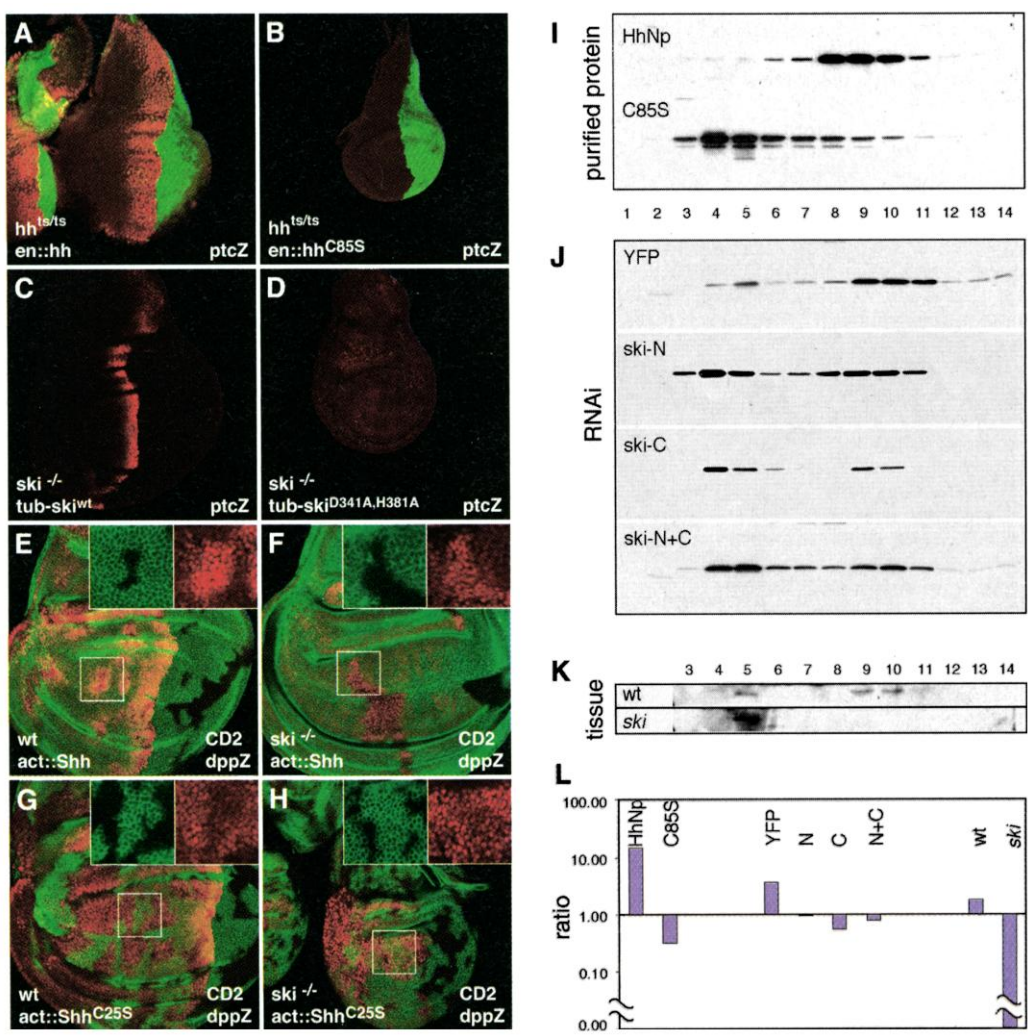

Fig. 4. Genetic and biochemical evidence that Ski functions as an acyltransferase for Hh proteins. (**A**) A transgene expressing wild-type Hh protein in P compartment cells (marked by GFP expression in green) rescues disc growth and *ptc-lacZ* expression of hh^{ts} mutant discs. Neither activity is observed with transgenes expressing Hh^{C85S} (**B**). (**C** and **D**) One copy of a transgene encoding wild-type Ski fully restores viability and Hh target gene expression in *ski* mutant animals (C). Ski protein with two amino acid substitutions at the presumed catalytic sites (D341 and H381) shows no detectable restoration of Hh target gene expression (D). (**E** to **H**) Wild-type or C25S mutant forms of mouse Shh were produced in cells of *actin5c>Gal4* expressing clones (marked by the absence of green CD2 staining). Shh induces high levels of *dpp-lacZ* expression in a wild-type background (E), and reduced, intermediate levels of *dpp-lacZ* (and only low levels of *ptc-lacZ*, not shown) in *ski* mutant discs (F). Equal intermediate levels of *dpp-lacZ* (or low levels of *ptc-lacZ*, not shown) are induced by Shh^{C25S} in a wild-type (G) or *ski* mutant background (H). *dpp-lacZ* expression is shown in red. Twofold magnifications of selected clones (small white squares) are shown in the upper right corner of panels (E) to (H) as single-labeled images to indicate that wild-type Shh protein produced from *ski* mutant cells and mutant Shh protein produced from wild-type cells retain the ability to induce *dpp-lacZ* nonautonomously. (**I** through **L**) Reversed-phase HPLC analysis of HhNp proteins. (I) Partially purified HhNp and $HhNp^{C85S}$ from S2 cells (Fig. 3, C to E) were analyzed by RP-HPLC in a C4 column developed with a gradient of increasing acetonitrile:*n*-propanol (1:1) (*37*). The $HhNp^{C85S}$ protein elutes primarily in fractions 3 through 6, whereas the HhNp protein elutes primarily in fractions 8 through 11. (J) HhNp proteins from cells (*33*) incubated with dsRNA sequences (*22*) corresponding to yellow fluorescent protein (YFP) as a control and NH_2- and COOH-terminal regions of the Ski coding sequence were fractionated by RP-HPLC in a C4 column. HhNp protein from YFP-treated cells showed a distribution indistinguishable from untreated S2 cells (R. K. Mann *et al.*, data not shown), whereas HhNp from cells incubated with dsRNA corresponding to NH_2- or COOH-terminal regions of Ski, or both, displayed a significant shift toward earlier elution. (K) HhNp protein from wild-type larval tissue (*33*) behaved similarly to that expressed in S2 cells, whereas homozygous *ski* larvae exclusively produced HhNp protein with an early pattern of elution. (L) On the basis of densitometric scanning, the ratio of late-eluting (fractions 8 through 11) to early-eluting (fractions 3 through 6) HhNp is plotted on a log scale. A ratio significantly greater than 1 is observed for HhNp in purified form, from YFP dsRNA-treated cells, and from wild-type third instar larvae, and less than 1 for samples from cells with impaired *ski* function.

groups of membrane-embedded nonproteinaceous targets (*17–19*).

It has recently been demonstrated that in addition to COOH-terminal addition of cholesterol, the human Sonic hedgehog (Shh) protein is further modified by NH_2-terminal attachment of a palmitoyl adduct in a manner dependent upon the NH_2-terminal cysteine residue (*20*). Our finding that *ski* encodes a putative acyl transferase raises the possibility that it could function as an enzyme for the palmitoylation of Hh protein. To determine whether *Drosophila* Hh is NH_2-terminally acylated, we purified the protein from *Drosophila* tissue culture cells and subjected it to mass spectrometric analysis. The experimentally determined mass of HhNp (20,235 daltons; Fig. 3C) exceeds by 238 daltons the average mass expected for the amino-terminal signaling domain linked to cholesterol alone (19,996.8 daltons). The difference between these values corresponds closely to the expected mass of a palmitoyl adduct in ester or amide linkage (238.4 daltons). Furthermore, substitution of a serine in place of the NH_2-terminal cysteine results in a protein modified by cholesterol alone (Fig. 3D; average experimental mass, 19,982; 19,980.7 daltons expected). These results support the conclusion that *Drosophila* HhNp undergoes NH_2-terminal palmitoylation.

If Ski functions as the Hh palmitoyl-transferase, then the absence of Ski should result in Hh protein that is not NH_2-terminally modified, with a consequent loss or reduction of Hh signaling activity. Genetic evidence for this possibility derives from the functional equivalence between mutations that abolish the enzymatic activity of Ski and alterations in Hh proteins that prevent their NH_2-terminal acylation. If the wild-type *ski* alleles are replaced by a *ski* transgene in which two presumptive active-site residues (*16*) of Ski are mutated, Hh signaling activity is greatly reduced (Fig. 4, C and D). The same phenotypes are observed when posterior wing cells express the NH_2-terminally mutant form of Hh (Hh^{C85S}) instead of wild-type Hh protein (Fig. 4, A and B). Interestingly, although mouse Shh protein also depends on *ski* function for maximal activity, it retains some signaling activity if secreted from *ski* mutant cells (Fig. 4, E and F). The same partial reduction in Shh activity is observed with a mutant form of Shh in which the NH_2-terminal cysteine residue is mutated (Fig. 4G). The activity of Shh^{C25S} (*21*) is not further reduced when secreted from *ski* mutant cells (Fig. 4, F and H). These results suggest that Ski is specifically required for the NH_2-terminal addition of palmitate to Hh.

Biochemical evidence for Ski's role in Hh palmitoylation was obtained by examination of Hh protein produced from cells with reduced *ski* function. Hh proteins were analyzed by reversed-phase high pressure liquid chromatography (RP-HPLC), which resolves doubly-modified HhNp from proteins lacking the NH_2-terminal palmitate adduct (compare HhNp to $HhNp^{C85S}$; Fig. 4I). Note that a small proportion of HhNp in normal cells and tissues displays the earlier elution profile characteristic of the $HhNp^{C85S}$ protein, suggesting that autoprocessing and cholesterol modification precede palmitoylation during Hh biogenesis. *Drosophila* cultured cells expressing HhNp were treated with double-stranded RNA corresponding to two regions of the *ski* coding sequence (*22*). A significantly increased proportion of the protein from these cells eluted at a position indicative of absence of the NH_2-terminal palmitate (Fig. 4, J and L). A similar shift in elution was observed for HhNp from tissues of mutant third instar larvae, except that all of the HhNp eluted earlier, as would be expected for a complete loss of palmitate transfer (Fig. 4, K and L). Reduction or loss of Ski function thus reduces the hydrophobicity of HhNp to that of $HhNp^{C85S}$, consistent with a loss of NH_2-terminal palmitoylation and with a role for Ski function in palmitate transfer.

It has been proposed that the linkage of palmitate to the NH_2-terminus of ShhNp is via an amide bond (*20*), but the mechanism of amide formation is not clear. The membership of Ski in a family of enzymes catalyzing *O*-linked acyl transfers strongly argues for a mechanism in which a thioester intermediate is formed with the side chain of the NH_2-terminal cysteine, followed by a rearrangement through a five-membered cyclic intermediate to form the amide (*20*). It is curious that addition of cholesterol, the other lipid modification of the mature Hh signal, also involves a cyclic intermediate and thioester chemistry but proceeds in reverse, from amide to ester (*9, 11*).

In summary, *ski* encodes an apparent acyltransferase critically required for Hh palmitoylation, a modification vital for signaling activity in vivo. Our analysis indicates that nonacylated Hh protein is secreted efficiently and reaches target cells (*23*). The severe *hh*-like phenotypes of *ski* mutant animals demonstrate that the NH_2-terminal acyl-group plays a critical role in enabling Hh to gain access to and inactivate Ptc. Finally, we note that Porcupine, required for production of active Wnt signal (*24, 25*), is a member of the same family of acyl transferases as Ski (*16*) (Fig. 3B), raising the possibility of a more widespread role for acylation of secreted protein signals in development.

References and Notes

1. Approximately 2000 lethal P-element insertions (*26*) were recombined onto FRT80 or FRT82 chromosomes to allow the generation of germline clones by Flp-mediated mitotic recombination (*27*). The P element *l(3)S050006* was identified by screening these lines for *hh*-like phenotypes. However, the single P element present in *l(3)S050006* maps at cytological position 63C6 and is not responsible for the *ski* mutant phenotype (*13*); it was removed from the chromosome resulting in allele ski^{50006} that was used for genetic experiments throughout this study.
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13. Using deficiencies Df(3L)M21, Df(3L)HR370, Df(3L)-HR218, Df(3L)HR218-Dp(3,3)pdp7, Df(3L)HR232, Df(3L)HR119, and Df(3L)HR298, *ski* was mapped to the cytological interval 63B6-B8. In situ hybridizations with end probes of unordered contigs of the *Drosophila* genome project were used to define a region of >20 kb from this cytological region, which included 10 genes.
14. Genomic transgenes were used to narrow the number of candidate genes. A 17.7-kb genomic fragment was identified that completely rescued *ski* mutant animals. This fragment contains the genes CG12079, CG15812, CG11495, CG14962, CG12032, and CG14963. Injections into wild-type embryos of RNAi corresponding to CG11495 produced a weak segment polarity phenotype. A full-length cDNA for CG11495 was isolated from an imaginal disc library. The open reading frame of CG11495 was sequenced from DNA of larvae hemizygous for the ski^{Bg} allele, which was isolated as *l(3)63Bg* (*28*). The sequence of allele ski^{50006} is not known. Both alleles show the same genetic behavior over a deficiency for the *ski* locus.
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22. The RNAi experiments followed methods described in (*29*). The dsRNA corresponding to the NH_2-terminal half of Ski begins at the start codon and extends to base pair 641 (relative to ATG). The COOH-terminal dsRNA spans base pairs 841 through 1498. After a 3-day incubation of the cells with dsRNA, we induced Hh protein expression by 24 hours of additional culture in the presence of $CuSO_4$.
23. Hh^{C85S} exerts a nonautonomous dominant-negative activity in the presence of wild-type Hh [Z. Chamoun *et al.*, data not shown; see also (*30*)]. In addition, the level of processed Hh protein released from S2 cells into the culture medium is greatly increased by treatment with RNAi to inactivate *ski* (R. K. Mann *et al.*, data not shown). These observations suggest that loss of Ski-mediated palmitoylation leads to greater release of Hh signal, and that nonacylated Hh reaches target cells.
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33. Hedgehog protein expression in S2 cells was as described in (*11*). Detergent-resistant, membrane-associated HhNp proteins were isolated as previously described for mouse Shh-Np (*34*), except that immu-

noaffinity chromatography was with an affinity-purified anti-Hh-N polyclonal antibody (*11*). Whole-cell protein extracts were prepared by solubilization in radioimmunoprecipitation assay buffer [1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, and a protease inhibitor cocktail of phenylmethylsulfonyl fluoride, leupeptin, aprotinin, pepstatin, and E64]. *Drosophila* larval extracts were prepared by dounce homogenization of dissected, inverted larvae in RIPA. All incubations and centrifugations were at 0° to 4°C.

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35. Masses of HhNp and HhNp^{C85S} were determined by MALDI-TOF mass spectrometry on a Voyager DE STR Biospectrometry Workstation (PerSeptive Biosystems) in linear mode. Affinity-purified protein samples were desalted and concentrated using C4 ZipTips (Millipore), with binding taking place in the presence of 10% acetonitrile. Eluted samples and standards were mixed 1:1 with 10 mg/ml sinapinic acid (Sigma) in 50% acetonitrile/0.3% (v/v) trifluoroacetic acid and spotted. Horse skeletal myoglobin {[M+H]1+ (avg.) = 16952.56; Sigma} and bovine trypsinogen (23981.89; Sigma) were included in samples for internal mass calibration.

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37. HhNp-containing protein samples were prepared for C4 reversed-phase chromatography by acetone precipitation (six to eight volumes, –20°C), resuspension in hexafluoroisopropanol (neat; with brief water-bath sonication) and solubilization with 70% formic acid (2 volumes); samples were immediately injected. Gradient fractionations were performed at 60°C with acetonitrile and *n*-propanol (1:1) as organic modifiers and 0.15% (w/v) TFA in mobile phases; 1-min fractions were collected during a 1% gradient developed from 40 to 54% (total organic). HhNp-containing fractions were lyophilized and analyzed on Western blots. Film densities of early- and late-eluting peaks were determined and summed for ratio analysis.

38. Supported by the Swiss National Science Foundation and the Howard Hughes Medical Institute. We thank E. Frei for the genomic *Drosophila* library, A. McMahon for the mouse Shh cDNA, and A. Erkner for generating UAS-Shh transformants.

16 July 2001; accepted 24 July 2001
Published online 2 August 2001;
10.1126/science.1064437
Include this information when citing this paper.

Requirement of Tissue-Selective TBP-Associated Factor $TAF_{II}105$ in Ovarian Development

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Transcription factor TFIID, composed of TBP and TAF_{II} subunits, is a central component of the RNA polymerase II machinery. Here, we report that the tissue-selective $TAF_{II}105$ subunit of TFIID is essential for proper development and function of the mouse ovary. Female mice lacking $TAF_{II}105$ are viable but infertile because of a defect in folliculogenesis correlating with restricted expression of $TAF_{II}105$ in the granulosa cells of the ovarian follicle. Gene expression profiling has uncovered a defective inhibin-activin signaling pathway in $TAF_{II}105$-deficient ovaries. Together, these studies suggest that $TAF_{II}105$ mediates the transcription of a subset of genes required for proper folliculogenesis in the ovary and establishes $TAF_{II}105$ as a cell type–specific component of the mammalian transcriptional machinery.

The control of transcription in a spatial and temporal fashion is essential for the proper development and differentiation of specialized cell types that define multicellular organisms. The intricate regulation of transcription relies on the coordinate assembly of large multiprotein complexes at promoter and enhancer regions of specific genes. The transcription factor TFIID is responsible for core promoter recognition and for directing RNA polymerase II to select genes in response to upstream activators (*1*, *2*). Although TFIID was initially thought to be ubiquitous in expression and universal in function, the discovery of putative tissue-restricted components of TFIID prompted us to reevaluate the gene-specific function of this transcription complex. The first cell type–specific subunit of TFIID, called $TAF_{II}105$, was identified as a polypeptide that coprecipitated with TATA-binding protein (TBP) and the other TAF_{II}s from a highly differentiated human B cell line but not other cell lines (*3*). The primary amino acid sequence of $TAF_{II}105$ revealed that it is related to the more broadly expressed human $TAF_{II}130$ (*4*, *5*) and its *Drosophila* homolog $dTAF_{II}110$ (*6*). The recent identification of yeast $TAF_{II}48$ has revealed a weak similarity to the COOH-terminal third of $TAF_{II}105$; however, the NH_2-terminal coactivator domain is absent in $TAF_{II}48$, suggesting that the metazoan subunits function in regulating programs of gene expression specific to multicellular organisms (*7*). Indeed, the circumstances of $TAF_{II}105$'s discovery suggest that it might be involved in regulating B cell–specific gene expression. Recent experiments demonstrating that human $TAF_{II}105$ can interact with nuclear factor kappa B and OCA-B (also called OBF-1) support this hypothesis (*8*, *9*). To examine further whether $TAF_{II}105$ indeed functions in a tissue- and gene-specific manner in mammals, we set out to characterize the biological role of $TAF_{II}105$ in the mouse.

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To determine the tissue-selective nature of $TAF_{II}105$ expression in mice, we measured the relative levels of $TAF_{II}105$ and $TAF_{II}130$ mRNA in selected tissues. Ribonuclease (RNase) protection assays (Fig. 1A) revealed high levels of $TAF_{II}130$ in all tissues examined, except for the liver. In contrast, $TAF_{II}105$ transcripts were expressed most highly in the testes and ovary, whereas lower levels were detected in most other tissues. On the basis of restricted expression of $TAF_{II}105$ mRNA largely in the gonads, we turned to identifying tissue-selective functions of $TAF_{II}105$.

First, we confirmed that $TAF_{II}105$ is a component of mouse TFIID in ovaries (Fig. 1B). Total cell extracts prepared from mouse ovaries were precipitated with monoclonal antibodies to TBP (anti-TBP); the presence of $TAF_{II}250$, $TAF_{II}105$, and TBP was confirmed by Western blot analysis (Fig. 1B, lane 2) (*10*). No TFIID subunits were detected in the control precipitation with a nonspecific antibody (lane 1). These data establish that $TAF_{II}105$ is a bona fide TFIID component in murine ovaries.

Given the highly restricted pattern of $TAF_{II}105$ expression in mice, it seemed plausible that disruption of the endogenous $TAF_{II}105$ gene by homologous recombination would not compromise viability. A $TAF_{II}105$ genomic targeting vector (Fig. 2A) was constructed carrying the neomycin (NEO) resistance gene inserted in reverse orientation to the 3′ end of the $TAF_{II}105$ gene (*11*). This construct was transfected into mouse embryonic stem (ES) cells, generating several clones containing one copy of the wild-type (WT) gene and one copy of the mutant $TAF_{II}105$ gene (Fig. 2B). Heterozygous ES cells were injected into mouse blastocysts, and chimeric mice were established that transmitted a mutant copy of the $TAF_{II}105$ gene through the germ line. A polymerase chain reaction (PCR)–based genotyping assay (Fig. 2C) was performed on the progeny of heterozygous matings to identify homozygous knockout (KO) animals. Western blot analysis of B cell extracts derived from mice of each genotype (Fig. 2D)