Gli Proteins in Development and Disease

Chi-chung Hui[1,2] and Stephane Angers[3]

[1]Program in Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada; email: cchui@sickkids.ca

[2]Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5S 1A8 Canada

[3]Leslie Dan Faculty of Pharmacy and Department of Biochemistry, University of Toronto, Toronto, Ontario M5S 3M2 Canada; email: stephane.angers@utoronto.ca

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Abstract

Gli zinc-finger proteins are transcription factors involved in the intracellular signal transduction controlled by the Hedgehog family of secreted molecules. They are frequently mutated in human congenital malformations, and their abnormal regulation leads to tumorigenesis. Genetic studies in several model systems indicate that their activity is tightly regulated by Hedgehog signaling through various posttranslational modifications, including phosphorylation, ubiquitin-mediated degradation, and proteolytic processing, as well as through nucleocytoplasmic shuttling. In vertebrate cells, primary cilia are required for the sensing of Hedgehog pathway activity and involved in the processing and activation of Gli proteins. Two evolutionarily conserved Hedgehog pathway components, Suppressor of fused and Kif7, are core intracellular regulators of mammalian Gli proteins. Recent studies revealed that Gli proteins are also regulated transcriptionally and posttranslationally through noncanonical mechanisms independent of Hedgehog signaling. In this review, we describe the regulation of Gli proteins during development and discuss possible mechanisms for their abnormal activation during tumorigenesis.

Contents

INTRODUCTION

In mammalian cells, Gli1, Gli2, and Gli3 (glioma-associated oncogene family members 1, 2, and 3) are dedicated transcription factors for signal transduction of the Hedgehog (Hh) pathway, one of the key regulatory networks involved in animal development that is conserved from flies to humans (Jiang & Hui 2008). Not only are these zinc-finger proteins among the first human transcription factors to have their DNA-binding site determined (Kinzler & Vogelstein 1990) and the 3D structure of their DNA-binding domain resolved (Pavletich & Pabo 1993), the *Gli* genes were also the first developmental control genes implicated in human diseases including cancer (Kinzler et al. 1988, Ruppert et al. 1991, Vortkamp et al. 1991).

The Gli proteins, although highly related, have distinct biochemical properties and perform different functions in vivo. They act as bifunctional transcription factors through regulated proteolytic processing that converts some Gli proteins from a full-length transcriptional activator form into a truncated repressor. Because Hh signaling plays regulatory roles in almost every aspect of animal development, the Gli proteins are vital for embryogenesis and adult homeostasis. Many Gli target genes have been identified to date; they include regulators of cell determination, specification, proliferation, and death as well as stem cell renewal and differentiation. Misregulation of *Gli* genes therefore leads to unfavorable developmental and pathological consequences. Although the Hh pathway is central to the transcriptional control of *Gli* target genes and the posttranslational modification of Gli proteins, increasing evidence suggests that noncanonical mechanisms that are apparently independent of Hh signaling also regulate *Gli* target gene expression. This Hh-independent regulation of Gli function might represent cross talk between the Hh pathway and other signaling mechanisms during development and tumorigenesis and could offer opportunities to develop novel targeted therapy for disease conditions with aberrant Gli activities.

In this review, we focus primarily on mammalian Gli function and regulation in the context of Hh signaling. The roles of Gli proteins and their regulation during limb development and ventral neural tube patterning are described. We then summarize and discuss recent findings about the Hh-dependent and -independent regulation of Gli expression and function during tumorigenesis.

GLI TRANSCRIPTION FACTORS

Gli Proteins Act as Transcriptional Activators and Repressors of Hedgehog Signaling

The human *GLI1* gene (originally named *GLI* and pronounced "glee") was first identified by Vogelstein and colleagues (Kinzler et al. 1988) as a putative oncogene amplified in glioblastomas. Its protein product binds to a consensus sequence of 5′-GACCACCCA-3′ (Kinzler & Vogelstein 1990) through the DNA-binding domain, which consists of five C_2H_2-Krüppel-type zinc-finger motifs highly conserved among all Gli proteins (Hui et al. 1994, Orenic et al. 1990, Ruppert et al. 1988). The vertebrate *Gli* gene family comprises three members, *Gli1*, *Gli2*, and *Gli3*, which are thought to be derived from duplications of a single ancestral chordate *Gli* gene similar to those found in *Amphioxus* (Shimeld et al. 2007) and *Ciona* (Islam et al. 2010). The fruit fly *Drosophila melanogaster* has one Gli protein, Cubitus interruptus (Ci) (Orenic et al. 1990), which mediates all aspects of Hh signaling (Méthot & Basler 2001).

In addition to harboring three Gli proteins, mammals also have three Hh family members [Sonic hedgehog (Shh), Indian hedgehog, and Desert hedgehog] (Varjosalo & Taipale 2008). Sasaki et al. (1997) demonstrated that the Gli-binding site 5′-GACCACCCA-3′ can function as a Shh-responsive element in mammalian cells. Deletion analysis in cultured cells revealed that Gli1 contains only a C-terminal transcriptional activation domain, whereas both Gli2 and Gli3 possess C-terminal activation and N-terminal repression domains (Dai et al. 1999, Sasaki et al. 1999; see **Figure 1**). Mutant studies in mice demonstrate that Gli2 and Gli3 are the primary mediators of Shh signaling and are essential for embryogenesis (Ding et al. 1998, Mo et al. 1997, Motoyama et al. 1998a). By contrast, Gli1 is dispensable for development (Bai et al. 2002, Park et al. 2000) and appears to function as part of a positive feedback loop. Although Gli2 and Gli3 act as the principal transcriptional activator (Gli^A) and repressor (Gli^R), respectively, of the Hh pathway, all three Gli proteins share an overlapping activator function (Bai et al. 2004, Buttitta et al. 2003, Motoyama et al. 2003, McDermott et al. 2005), and Gli2 also contributes a minor repressor activity (Buttitta et al. 2003, McDermott et al. 2005, Pan et al. 2006). Response to Hh stimulation is lost in mouse embryos lacking both Gli2 and Gli3, which indicates that Gli proteins mediate most, if not all, of the transcriptional output of mammalian Hh signaling (Buttitta et al. 2003, Motoyama et al. 2003).

Functional studies in other vertebrate models also revealed the importance of Gli proteins in Hh signaling. However, changes in the regulation and role of Gli proteins are apparent in some vertebrate species. For example, in contrast to what is observed in mammals, Gli1 rather than Gli2 is the principal activator of Hh signaling in early zebrafish and *Xenopus* embryos (Karlstrom et al. 2003, Lee et al. 1997), and these species appear to have additional duplication and functional specialization of *Gli2* (Ke et al. 2008). As discussed below, the roles of other Hh pathway components in Gli regulation also appear to be significantly different between mice and zebrafish.

Hedgehog Signaling Regulates the Balance of Gli Activation and Repression

The intracellular machinery of Hh signal transduction is fundamentally similar between *Drosophila* and mice, although there are differences in several regulatory steps, and the mammalian pathway is more complex owing to the presence of multiple Gli proteins

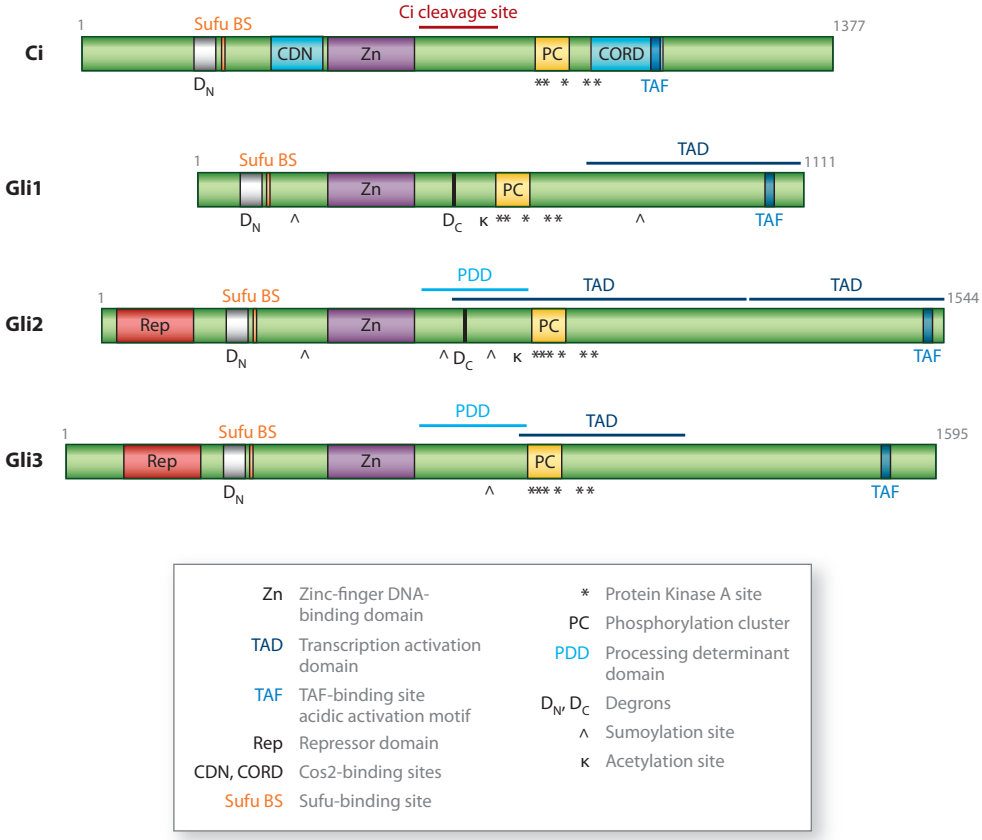

Figure 1

Schematic representation of domains and motifs in Gli proteins. Various protein domains and modification sites of the *Drosophila* Gli protein Cubitus interruptus (Ci) and mouse Gli1, Gli2, and Gli3 are depicted.

(Wilson & Chuang 2010). Hh activates signaling in target cells by binding and inactivating Patched (Ptc/Ptch), which unleashes the Smoothened (Smo) transmembrane protein to trigger downstream intracellular events (**Figure 2**). In *Drosophila* cells, Hh promotes endocytosis of Ptc and cell-surface accumulation of Smo. A similar reciprocal trafficking of Ptch and Smo also occurs in mammalian cells, where Hh promotes localization of Smo to the primary cilium, a microtubule-based cell-surface protrusion that is critical for Hh signaling in vertebrates (Goetz & Anderson 2010). Hh ultimately exerts its effects by influencing the balance between Gli^A and Gli^R. In the absence of Hh, Ptc blocks Smo activity, which allows the formation of Gli^R. Binding of Hh to Ptc activates Smo, which blocks Gli^R formation and promotes Gli^A function.

In *Drosophila* cells, Ci exists as both full-length (Ci/Gli) as well as processed (Ci^R/Gli^R) forms in the absence of Hh. Full-length Ci is rendered inactive by inhibitory mechanisms, whereas Ci^R represses a subset of Hh target genes. Low levels of Hh block Ci^R formation and unleash full-length Ci to become Ci^A/Gli^A. At higher levels of Hh signaling, Ci^R is completely absent, and Ci exists only as Ci^A. On the basis of this model, distinct sets of Hh target genes are switched on or off at different ratios

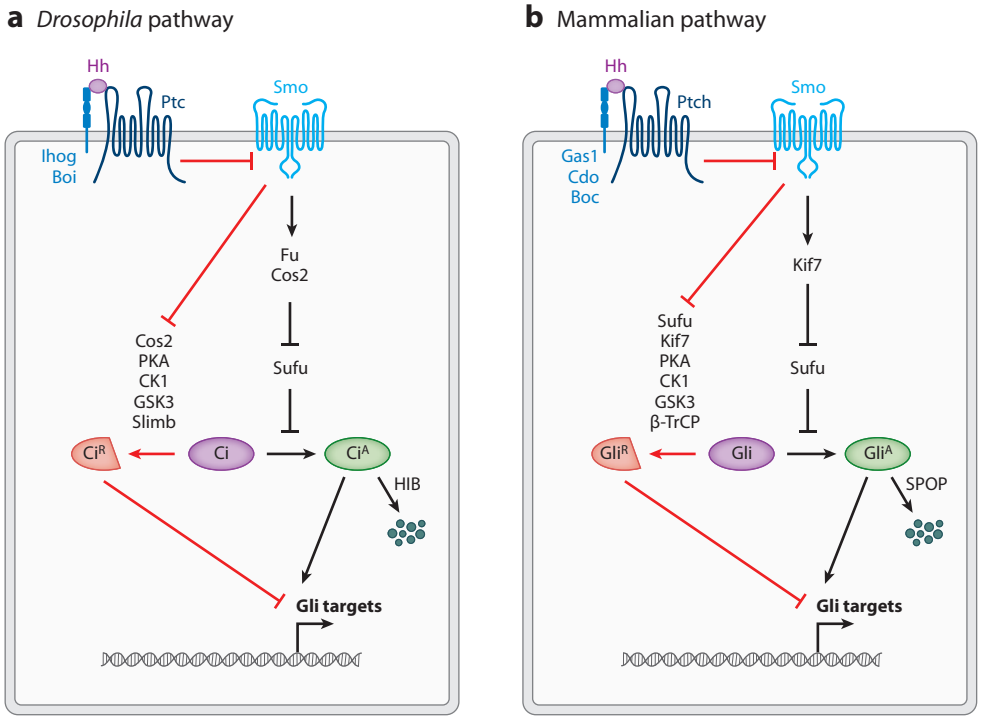

Figure 2

Schematic of the (*a*) *Drosophila* and (*b*) mammalian Hedgehog (Hh) pathways. The two pathways share many common features, including the binding of Hh to a receptor complex, which consists of Patched (Ptc/Ptch) and Ihog (Boi)/Cdo (Boc, Gas1). This binding releases the inhibition of Smoothened (Smo). Cubitus interruptus (Ci)/Gli is converted to a transcriptional repressor (Ci^R/Gli^R) via limited degradation by the proteasome through the actions of protein kinases Protein Kinase A (PKA), Casein Kinase 1 (CK1), and Glycogen Synthase Kinase 3 (GSK3), as well as the E3 ubiquitin ligase $SCF^{Slimb/\beta\text{-}TrCP}$. Active Smo promotes the formation of a Gli transcriptional activator (Ci^A/Gli^A) through the inhibition of Gli processing and negative regulation by two evolutionarily conserved regulators, Suppressor of fused (Sufu) and Cos2/Kif7. Upon Hh pathway activation, the activity of Ci^A/Gli^A is limited through its proteasome-mediated degradation via Hedgehog-induced BTB protein (HIB)/Speckle-type PDZ protein (SPOP).

of Ci^R/Gli^R and Ci^A/Gli^A (**Figure 3**). A similar but more complex scenario could be formulated for the mammalian system; Gli3 and, to a lesser extent, Gli2 lead to Gli^R, whereas all three Gli proteins contribute to Gli^A. Gli3, as the principal repressor of the mammalian Hh pathway, is mainly responsible for suppressing Hh target genes in the absence of Hh. In response to Hh stimulation, Gli2 acts as the principal activator to trigger the expression of *Gli1* and additional Gli targets. Gli1 is a robust activator and potentiates the transcriptional output of Hh signaling. However, the Hh signaling response is subject to additional levels of transcriptional regulation of the *Gli* genes. For example, in the embryonic kidney, Gli3 represses the transcription of *Gli1* and *Gli2* by binding to their promoters in the absence of Hh (Hu et al. 2006). Chromatin immunoprecipitation assays revealed that Gli2 normally binds to its own promoter as well as that of *Gli1*, and intriguingly, if *Gli3* is deleted, Shh is not required for expression of *Gli1* and *Gli2* in the kidney. These observations indicate that Hh-independent mechanisms can activate the expression of *Gli1* and *Gli2*.

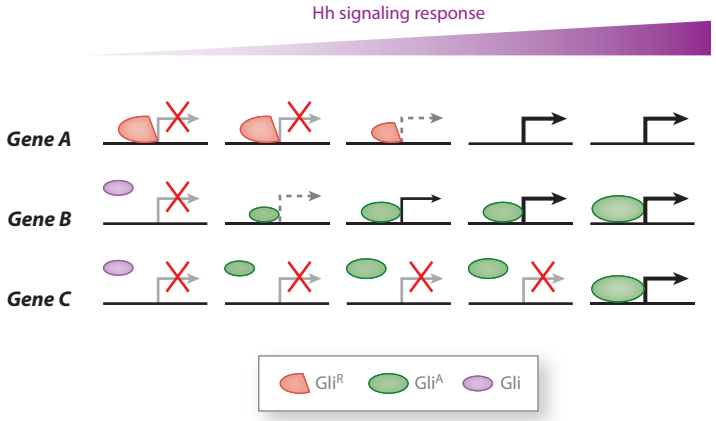

Figure 3

Schematic representation of differential Gli target gene expression. Hedgehog (Hh)-responsive cells harbor varying ratios of Gli^R and Gli^A, which results in the transcriptional activation of different Gli target genes. In the absence of Hh, the majority of Gli protein exists as Gli^R, which leads to the repression of a subset of Gli target genes (*Gene A*), and full-length Gli protein is kept inactive by inhibitory mechanisms. *Gene A* is derepressed when Gli^R levels are reduced in response to Hh stimulation. Hh signaling leads to the activation of preexisting full-length Gli protein (forming Gli^A), which results in the transcription of *Gene B*, which low levels of Gli^A can activate. *Gene C* is transcribed later when higher levels of Gli^A are present.

Different Modes of Gli Target Gene Regulation

Known Gli target genes include both universal targets that are activated by Hh signaling in most cell types as well as those that are regulated in specific contexts. Genome-scale analyses using chromatin immunoprecipitation and gene expression profiling have revealed many putative direct Gli target genes (Lee et al. 2010; Vokes et al. 2007, 2008). In addition, computational analysis together with high-throughput screening of high-affinity Gli protein–binding sites have been used to predict the location of Gli-responsive enhancer/promoter elements in the mammalian genome (Hallikas et al. 2006).

Two well-known universal targets are *Ptch1* (Agren et al. 2004) and *Gli1* (Dai et al. 1999). Consistent with its role as a receptor for Hh, *Ptch1* exhibits basal expression in the absence of Gli2 and Gli3 (Motoyama et al. 2003). In contrast, *Gli1* promoter activity in early mouse embryos is strictly dependent on Gli2 and Gli3 (Bai et al. 2004). However, accumulating evidence indicates that Hh/Smo-independent mechanisms can induce *Gli1* expression during development and tumorigenesis (Beauchamp et al. 2009, Hu et al. 2006, Nolan-Stevaux et al. 2009). Other pathway components, including many Hh-binding proteins, are also transcriptionally regulated by Hh signaling (Chuang & McMahon 1999, Martinelli & Fan 2007, Motoyama et al. 1998b, Tenzen et al. 2006). For example, Shh signaling induces the expression of *Ptch1*, *Ptch2*, and *Hhip*, which inhibit Hh pathway activation, and inhibits the expression of *Cdo*, *Boc*, and *Gas1*, which promote the reception of the Hh signal, thus illustrating a precise transcriptional control of Hh signaling through multiple negative feedback loops (**Figure 4*a***). Among these genes, *Ptch1*, *Ptch2*, *Hhip*, and *Boc* are likely direct targets because Gli proteins can bind to their chromatin sites (Lee et al. 2010; Vokes et al. 2007, 2008).

Shh signaling regulates the expression of many transcriptional regulators during ventral neural tube patterning (Briscoe et al. 2000, Dessaud et al. 2008). Among them, *Foxa2* was the first neural-specific Gli target gene identified (Sasaki et al. 1997). A Gli-binding site in the 3′ enhancer of *Foxa2* is essential for Shh-dependent transcription in the ventral neural tube. Loss of Gli2 and Gli3 completely abolishes *Foxa2* expression in the neural tube even when the pathway is constitutively activated by *Ptch1* deletion (Motoyama et al. 2003). Shh signaling induces the expression of *Nkx2.2* and *Nkx6.1* at the ventral midline of the developing neural tube. However, the expression of *Nkx2.2*, but not *Nkx6.1*, is lost in the $Gli2^{-/-}$; $Gli3^{-/-}$ neural tube (Bai et al. 2004, Lebel et al. 2007, Motoyama et al. 2003). Furthermore, *Nkx6.1*, but not *Nkx2.2*, expression is restored in the $Shh^{-/-}$;$Gli3^{-/-}$ or $Smo^{-/-}$;$Gli3^{-/-}$ neural tube (Lebel et al. 2007, Wijgerde et al. 2002). These observations suggest that Gli activation mediates *Nkx2.2* expression, whereas *Nkx6.1* expression depends upon loss of Gli repression (**Figure 4*b***). Consistent with this model, *Nkx2.2* is a direct target of Gli activators (Lei et al. 2006, Vokes et al. 2007). Chromatin immunoprecipitation assays have

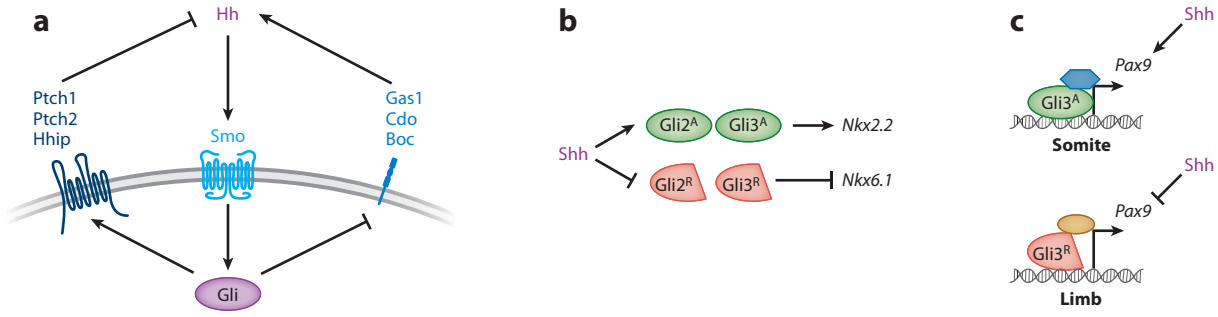

Figure 4

Different modes of Gli target gene regulation in mammalian cells. (*a*) Negative feedback control of signaling by transcriptional regulation of Hedgehog (Hh) receptor components. Gli transcription factors activate the expression of negatively acting Hh-binding receptors, including Patched 1 (Ptch1), Ptch2, and Hhip, and repress the expression of positively acting Hh-binding proteins, including Gas1, Cdo, and Boc. Abbreviation: Smo, Smoothened. (*b*) Activation as well as derepression can mediate Hh-induced gene expression. In the ventral spinal cord, Sonic hedgehog (Shh) induces *Nkx2.2* expression by promoting the formation of $Gli2^A$ and $Gli3^A$, whereas it induces *Nkx6.1* expression by blocking the formation of $Gli2^R$ and $Gli3^R$. (*c*) Shh activates *Pax9* expression in the somite but inhibits *Pax9* expression in the limb bud. How $Gli3^R$ contributes to the transcriptional activation of *Pax9* in the limb is not known.

identified *Pax9* as a putative target of Gli3 in the developing limb (Vokes et al. 2008). Indeed, *Pax9* expression in the anterior limb bud mesenchyme is $Gli3^R$-dependent (Hill et al. 2009, McGlinn et al. 2005). Intriguingly, although Shh signaling suppresses *Pax9* expression in the limb bud (McGlinn et al. 2005), it activates *Pax9* expression in the developing somite (Buttitta et al. 2003). Whether the same Gli-binding site is utilized in the transcriptional regulation of *Pax9* in both tissues and how $Gli3^R$ participates in the activation of *Pax9* expression remain to be determined (**Figure 4*c***).

Control of Gli Transcription Factors at the Chromatin Level

Several histone-modifying enzymes and chromatin remodeling proteins have been shown to influence the transcriptional activity of Gli proteins. Canettieri et al. (2010) showed that Gli1 and Gli2 are modified by acetylation and that class I histone deacetylases (HDACs) regulate their transcriptional activities. In cerebellar granule cell progenitors, Hh signaling promotes Gli transcriptional activity through upregulation of HDAC1 expression, and pathway activity is modulated by REN, an adaptor subunit of the Cullin-3 (Cul3)-based ubiquitin ligase complex, which targets HDAC1 for ubiquitination and proteasome degradation. Interestingly, Gli3 is apparently not affected by deacetylation resulting from HDAC1 and HDAC2 overexpression. Transcriptional activation by Gli3 involves the histone acetyltransferase (HAT) cAMP response element–binding protein (CREB)-binding protein (Dai et al. 1999), whereas Gli3's repressor activity is mediated by Ski-dependent recruitment of HDAC (Dai et al. 2002). Whether HAT and HDAC directly modify Gli3 or whether these histone-modifying enzymes modulate Gli3 activity via chromatin remodeling remains to be determined. Suppressor of fused (Sufu), a negative regulator of the Hh pathway (see below), also recruits the mSin3/mSin3-associated polypeptide 18 (SAP18) corepressor complex to Gli proteins, and SAP18 can synergize with Sufu in repressing Gli-dependent transcription in mammalian cells (Cheng & Bishop 2002). How this HDAC corepressor complex functions in Hh signaling is not known.

Two recent studies also have linked Gli proteins to chromatin remodeling. Snf5, a core member of the ATP-dependent Switch (SWI)/Sucrose Nonfermenting (SNF) chromatin remodeling complex, as well as several other SWI/SNF complex subunits were identified as Gli1-specific interacting proteins

(Jagani et al. 2010). Interestingly, inactivation of *Snf5* and *Brg1*, another core subunit of the SWI/SNF complex, resulted in a dramatic upregulation of *Gli1* expression. Because Snf5 localizes to Gli1-regulated promoters and loss of Snf5 function leads to activation of *Gli1* and other Gli1 target genes, these results suggest that the SWI/SNF complex is a regulator of Hh signaling that is normally involved in the suppression of Gli1 transcriptional activity. Cox et al. (2010) showed that all three Gli proteins are targets of small ubiquitin-related modifier (SUMO)-1 conjugation and that expression of the SUMO E3 ligase Pias1 promotes Gli transcriptional activity in cultured cells as well as chick neural tube. These observations suggest Gli protein sumoylation as a potential regulatory mechanism in Hh signaling. Interestingly, sumoylation can convert the function of transcription factors from activator to repressor and vice versa through HDAC recruitment (Garcia-Dominguez & Reyes 2009).

GLI PROTEIN MODIFICATION AND REGULATION

Gli Repressor Is Generated by the Proteasome Through Partial Degradation

Aza-Blanc et al. (1997) elegantly showed that Ci is proteolytically cleaved in the absence of Hh to form an N-terminal repressor form (Ci^R). Protein Kinase A (PKA), Glycogen Synthase Kinase 3 (GSK3), and Casein Kinase 1 (CK1) sequentially phosphorylate multiple sites in the C-terminal region of Ci, which results in the recruitment of the F-box-containing protein Slimb/β-TrCP, a substrate-specific receptor of the SCF-type E3 ubiquitin ligase (Jia et al. 2005, Jiang & Struhl 1998, Smelkinson & Kalderon 2006). A processing determinant domain (PDD) located between the zinc-finger DNA-binding domain and the Slimb/β-TrCP-binding domain of Ci (see **Figure 1**) is critical for limited proteolysis by the proteasome that selectively removes the C-terminal half of the protein containing the activation domain (Tian et al. 2005). In flies, deletion of the PDD from Ci blocks Gli^R formation (Methot & Basler 1999) and results in complete degradation of Ci (Smelkinson et al. 2007).

Similar to Ci, Gli3 is also phosphorylated by PKA, GSK3, and CK1 in the absence of Hh signaling and targeted to limited proteolysis by the proteasome to generate $Gli3^R$ (Tempé et al. 2006, Wang & Li 2006, Wang et al. 2000a). Interestingly, although these kinases phosphorylate Gli2 through similar sites, phosphorylation of Gli2 usually induces complete degradation by the proteasome; only a tiny fraction of $Gli2^R$ is formed (Pan et al. 2006, 2009). Pan & Wang (2007) showed that the difference in Gli^R formation between Gli2 and Gli3 is at least partly because of a more potent PDD in Gli3. Consistent with this model, Gli1 lacks a PDD and does not exhibit Gli^R activity.

Control of Gli Activity by Multiple Degradation Mechanisms

In addition to $SCF^{Slimb/\beta\text{-}TrCP}$-mediated processing and degradation, Gli proteins are also targeted to the ubiquitin-proteasome system through other mechanisms. For instance, in *Drosophila* cells, full-length Ci is subject to proteasome degradation through the action of *hib/roadkill*, which encodes a substrate-specific receptor for the Cul3-based E3 ubiquitin ligase (Kent et al. 2006, Zhang et al. 2006). Hh signaling induces *hib* expression, which serves as a feedback control limiting Ci activity after pathway activation. SPOP (speckle-type PDZ protein), a mammalian homolog of Hib, interacts with Gli2 and Gli3 and promotes their ubiquitin-mediated proteasome degradation (Chen et al. 2009, Zhang et al. 2009). Hh pathway activation leads to a rapid decrease in the half-life of $Gli3^A$, which is dependent on the activity of the SPOP-Cul3 E3 ligase complex (Wen et al. 2010). $Gli3^R$ is also degraded, albeit with longer kinetics, which suggests that yet another E3 ligase system may be involved in the control of $Gli3^R$ stability.

In contrast to Gli2 and Gli3, Gli1 is not a strong substrate for SPOP (Chen et al. 2009,

Zhang et al. 2009), but its levels are also regulated by Numb, which recruits Gli1 to the E3 ubiquitin ligase Itch (Di Marcotullio et al. 2006). Thus, multiple E3 ubiquitin ligases impinge on the Hh pathway at different levels to control the spatial and temporal activity of Gli proteins. In mammalian cells, many protein kinases also influence the activity of Gli proteins (Evangelista et al. 2008; Riobó et al. 2006a,b; Varjosalo et al. 2008). Of particular interest is the dual-specificity tyrosine (Y) phosphorylation-regulated kinase (DYRK) family. For example, DYRK1A phosphorylates Gli1 and promotes its nuclear localization and transcriptional activity (Mao et al. 2002), whereas DYRK1B inhibits Gli2 activator function and promotes Gli3 processing (Lauth et al. 2010). DYRK2 phosphorylates Gli2 and promotes Gli2 and Gli3 degradation by the proteasome (Varjosalo et al. 2008). Interestingly, DYRK2 acts as a scaffold for an E3 ubiquitin ligase complex (Maddika & Chen 2009). The control of Gli protein degradation might play a central role in preventing tumorigenesis (Di Marcotullio et al. 2006, Huntzicker et al. 2006).

Kif7 and Suppressor of Fused Are Evolutionarily Conserved Regulators of Gli Proteins

How Smo activation impacts Gli transcriptional activity is not completely established. Some insights into this process came with the realization that Ci forms a large protein complex with the kinesin-like protein Costal2 (Cos2) and the serine/threonine kinase Fused (Fu) (Robbins et al. 1997, Sisson et al. 1997). Cos2 serves as a molecular scaffold allowing efficient phosphorylation of Ci by CK1, GSK3, and PKA (Zhang et al. 2005). In the absence of Hh signaling, the Cos2-Fu-Ci complex is thus required for Ci^R formation. During pathway activation, Hh leads to the stabilization of and changes in the distribution of Smo from intracellular vesicles to plasma membrane (Denef et al. 2000) in a process that involves the phosphorylation of the Smo C-terminal tail by PKA and CK1 (Apionishev et al. 2005, Jia et al. 2004, Zhang et al. 2004). A prevailing model is that, upon pathway activation, Fu rapidly phosphorylates Cos2 within the complex, and Cos2 phosphorylation and/or recruitment to Smo then lead to the disassembly of the Smo-Cos2-Ci complex, thereby inhibiting Ci^R formation and promoting accumulation of Ci^A (Raisin et al. 2010, Ruel et al. 2007). Given that it is required for Ci^R formation in the off state of the pathway and is important to sense the activation of Smo during signaling, Cos2 performs both negative and positive regulatory functions in the Hh pathway.

Vertebrates contain two Cos2-like kinesin family proteins (Kif7 and Kif27), and recent studies indicate that Kif7 is the functional homolog of Cos2 in vertebrates (Cheung et al. 2009, Endoh-Yamagami et al. 2009, Liem et al. 2009, Tay et al. 2005). Kif7 interacts with all three Gli proteins, and *Kif7*-null mice display phenotypes, such as polydactyly, that are associated with compromised $Gli3^R$ function, which suggests that it functions similarly to Cos2 in the negative regulation of Gli proteins. These studies also revealed that Kif7 is required for generating a high Hh signaling response, which indicates a potential positive regulatory role in Hh signaling. However, whether Kif7 interacts with Smo remains controversial (Endoh-Yamagami et al. 2009, Varjosalo et al. 2006). A functional homolog of Fu has been identified in zebrafish (Wilson et al. 2009, Wolff et al. 2003). Analysis of mice lacking Stk36, which exhibits the highest sequence homology to Fu, however, did not reveal any phenotypes indicative of defective Hh signaling (Chen et al. 2005, Merchant et al. 2005). Interestingly, Stk36 interacts with Kif27, but not with Kif7, and mutant studies indicate that both Stk36 and Kif27 play critical roles in the formation of motile cilia in mice (Wilson et al. 2009; H. Cheung & C.-c. Hui, unpublished data). Ulk3, another Fu/Stk36-like kinase, was recently found to exert both positive and negative regulatory functions on Gli-dependent transcription in cultured cells (Maloverjan et al. 2010). It

remains to be determined whether Ulk3 is a functional homolog of Fu in the mammalian Hh pathway.

Sufu is another negative regulator of the Hh pathway (Preat 1992). Although it is dispensable for Hh signaling and development in *Drosophila*, it suppresses the phenotypes of *fu*-null flies and exacerbates the effects of *cos2* and *pka* mutations, which suggest a negative regulatory role in the Hh pathway. In contrast to *Drosophila*, Sufu is critical for mammalian Hh signaling, as inactivation of *Sufu* in mice leads to ectopic pathway activation and embryonic lethality (Cooper et al. 2005, Svärd et al. 2006). The reasons why Sufu is essential for mammalian, but not *Drosophila*, Hh signaling are still unclear. In the absence of Hh, Sufu binds directly to Gli proteins and anchors them in the cytoplasm to prevent spurious pathway activation in both *Drosophila* (Méthot & Basler 2000, Wang & Holmgren 2000, Wang et al. 2000b) and mice (Barnfield et al. 2005, Ding et al. 1999, Kogerman et al. 1999, Murone et al. 2000). In mammalian cells, Sufu may have assumed additional negative regulatory functions to control the activity of multiple Gli proteins, such as suppressing Gli^A function by recruiting a corepressor complex (Cheng & Bishop 2002) and promoting $Gli3^R$ formation through binding GSK3 (Kise et al. 2009). Furthermore, Sufu plays a key role in the stabilization of Gli2 and Gli3. In *Sufu*-null mouse embryonic fibroblasts, the half-life of Gli proteins is shortened dramatically by the activity of the SPOP-Cul3 E3 ligase complex (Chen et al. 2009, Wang et al. 2010a, Wen et al. 2010).

Primary Cilium Links Gli Activation to Transcriptional Activation

Hh signal transduction in vertebrates requires the integrity of a microtubule-based organelle called the primary cilium that bulges out of the surface of quiescent cells (for a review, see Goetz & Anderson 2010). To date, a variety of mouse mutants and human congenital syndromes with defective ciliary and basal body genes have been shown to exhibit phenotypes similar to those observed in Hh pathway mutant mice (Goetz & Anderson 2010, Huangfu et al. 2003). Many signaling components of the Hh pathway are localized within the cilia either under resting conditions or during pathway activation (Corbit et al. 2005, Haycraft et al. 2005, Huangfu & Anderson 2005, Liu et al. 2005). The levels of Gli2, Gli3, and $Gli3^R$ proteins are altered in many of these ciliary and basal body mutant mice, which suggests that the normal function of the primary cilium and its associated structures is crucial for Hh signal transduction, which eventually leads to proper control of Gli protein degradation and processing.

At the receptor complex level, Ptch1 localizes under resting conditions to the primary cilium, where it prevents the localization or limits the residency time of Smo within cilia. Upon Hh binding, Ptch1 exits and Smo accumulates in cilia to initiate signaling (Rohatgi et al. 2007). Gli2 and Gli3 have also been shown to translocate to and accumulate at the tip of primary cilia upon pathway activation (Chen et al. 2009, Kim et al. 2009, Wen et al. 2010), and this process appears to be Kif7 dependent (Endoh-Yamagami et al. 2009, Liem et al. 2009). Because Kif7 localizes at the base of cilia when the pathway is inactive, one possibility is that Gli proteins are processed to Gli^R within basal bodies where the ubiquitin-proteasome system is enriched (Wigley et al. 1999). Hh signaling may inhibit Gli^R formation and activate the pathway by promoting the translocation of Gli proteins away from this subcellular localization.

Because cilia are essential for Smo activation in *Ptch1*-null cells but dispensable for ligand-independent pathway activation in *Sufu*-null cells (Chen et al. 2009, Jia et al. 2009), a simple model is that active Smo at the primary cilium inhibits Sufu to promote Gli activation. Humke et al. (2010) and Tukachinsky et al. (2010) have uncovered a critical role for the Gli-Sufu complex in sensing pathway activation within the primary cilium as well as important biochemical properties of Gli3 that correlate with its conversion into a transcriptional activator

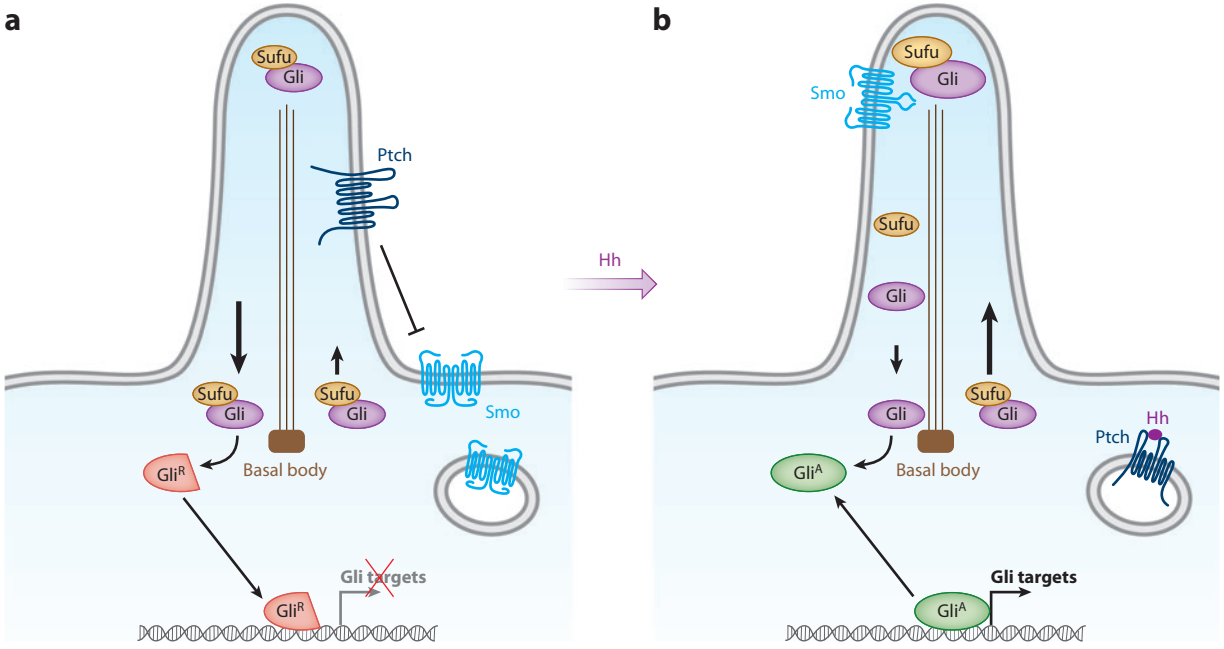

Figure 5

Hedgehog (Hh) signaling promotes Gli^A formation through dissociation of the Suppressor of fused (Sufu)-Gli complex in the primary cilium. (*a*) In the absence of Hh, Patched (Ptch) localizes to and prevents Smoothened (Smo) from entering primary cilia. Low levels of Sufu-Gli protein complexes enter the cilia, which promotes the formation of Gli^R by the ubiquitin-proteasome system. (*b*) Hh signaling leads to the accumulation of Smo and increases the translocation of Sufu-Gli complexes to the tip of the primary cilium. Active Smo promotes the dissociation of Sufu-Gli complexes, the activation of full-length Gli proteins to Gli^A, and the microtubule-dependent translocation of Gli^A into the nucleus.

(**Figure 5**). Within minutes of pathway activation, the Gli-Sufu complex localizes to cilia in a process negatively regulated by PKA activity. Upon continuous signaling, Gli proteins are progressively dissociated from Sufu and translocate to the nucleus to activate target gene expression. Once in the nucleus, Gli3 is phosphorylated by an unidentified kinase and then eventually degraded by the nuclear ubiquitin-proteasome system, likely via the SPOP-Cul3 E3 ligase. Whether phosphorylation, ubiquitination, and turnover of Gli proteins are linked and important for transcriptional activation remains to be tested directly. It will be important to determine how the dynamics of the Gli-Sufu interaction is controlled during pathway activation. In a short interfering RNA screen designed to identify kinases affecting Hh signaling, Cdc2l1 was both necessary and sufficient for signaling (Evangelista et al. 2008). Interestingly, Cdc2l1 can relieve the inhibitory effect of Sufu on Gli-dependent transcription by binding to Sufu and modulating the Gli-Sufu interaction. The nuclear kinases involved in Gli protein phosphorylation during pathway activation remain to be determined. DYRK1 is a nuclear kinase that promotes the transcriptional activity of Gli1 (Mao et al. 2002). However, inhibition of DYRK1 does not affect Gli3 phosphorylation during pathway activation, which suggests the involvement of a different nuclear kinase (Humke et al. 2010).

The Role of Dynamic Microtubules in Gli Activation

Disruption of microtubules using vinblastine and nocodazole recently highlighted a role for dynamic cytoplasmic microtubules in the

nuclear translocation of Gli proteins (Kim et al. 2009) and in activation of target gene transcription (Humke et al. 2010, Kim et al. 2009, Tukachinsky et al. 2010). Because these drugs do not disrupt the stable microtubules of primary cilia (Gerdes et al. 2007), these studies suggest that cytoplasmic microtubules are involved in either the recruitment of components within the cilia or processes occurring downstream. Intriguingly, although vinblastine inhibited the recruitment of Gli2 but not of Smo into cilia (Kim et al. 2009), nocodazole treatment prevented the nuclear translocation of Gli proteins without affecting the ciliary recruitment of Sufu, Smo, and Gli proteins in a different study (Tukachinsky et al. 2010). Similarly, nocodazole treatment blocked the pathway activation-dependent phosphorylation and destabilization of $Gli3^A$, which are thought to be nuclear events, but did not prevent the dissociation of Sufu from Gli3, which is believed to occur in the cilia (Humke et al. 2010). It is important to explore further how cytoplasmic microtubules contribute to Gli activation.

GLI FUNCTIONS IN DEVELOPMENT AND DISEASE

GLI Genes and Human Congenital Malformations

The role of *Gli* genes in development was first revealed by the discovery of deleterious mutations of *GLI3* in several human congenital malformations, including Greig cephalopolysyndactyly syndrome (GCPS) (Vortkamp et al. 1991), Pallister-Hall syndrome (PHS) (Kang et al. 1997), nonsyndromic polydactyly (Radhakrishna et al. 1997, 1999), and acrocallosal syndrome (Elson et al. 2002). GCPS, a dominant genetic disorder with polydactyly and craniofacial features, is frequently associated with large deletions or truncating mutations resulting in loss of *GLI3* function (Johnston et al. 2010, Shin et al. 1999). Truncating mutations in the middle third of *GLI3* generally cause PHS; patients display polydactyly, bifid epiglottis, and/or hypothalamic hamartoma owing to the persistent formation of a mutant GLI3 protein with constitutive repressor function (Kang et al. 1997, Shin et al. 1999). Mouse models of GCPS and PHS faithfully phenocopy the human syndromes (Hui & Joyner 1993, Böse et al. 2002). Loss-of-function as well as dominant-negative mutations in *GLI2* have also been identified in patients with holoprosencephaly-like features and pituitary anomalies (Roessler et al. 2003, 2005). Similarly, *Gli2*-null mice display midline anomalies (Mo et al. 1997) and pituitary defects (Wang et al. 2010b). Consistent with the observation that *Gli1* is not essential for mouse development (Park et al. 2000), *GLI1* mutations have not yet been reported in human congenital malformations.

In a recent study, Johnston et al. (2010) identified *GLI3* mutations in six of 21 patients with features of oral-facial-digital syndrome (OFDS). To date, only OFDS type I, of the 13 clinical types of OFDS, has a known molecular etiology. Intriguingly, the mutated gene *OFD1*, which encodes a protein localized to the centrosome and basal body, is required for primary cilia formation and Hh signaling (Ferrante et al. 2006). These observations provide additional support for the critical role of primary cilia in Gli3 regulation and suggest that GLI3 is involved in the pathogenesis of OFDS. *GLI3* is also linked to Opitz syndrome (OS), a genetically heterogeneous disorder characterized by defects of the ventral midline (Liu et al. 2001). The OS gene product MID1 is a microtubule-associated protein whose phosphorylation is regulated by protein phosphatase 2A (PP2A). Interestingly, MID1-PP2A regulates the nuclear localization as well as transcriptional activity of GLI3, and several GCPS-associated point mutations in the C-terminal half of GLI3 exhibit reduced transcriptional activity and nuclear localization, apparently owing to loss of this regulation (Krauss et al. 2009). These genotype-phenotype correlation and molecular analyses should provide useful information about GLI function and regulation in human development.

Sonic Hedgehog–Dependent and –Independent Functions of Gli3 in Limb Development

As illustrated by *GLI3* mutations in various human limb malformations, *Gli3* plays a pivotal role in limb development. Shh is expressed in the posterior limb bud mesenchyme and acts as the principal signal of the zone of polarizing activity (ZPA), which organizes anteroposterior (AP) patterning of the limb (for a review, see Bénazet & Zeller 2009). Wang et al. (2000a) showed that $Gli3^R$ exists as an $anterior^{high}$ to $posterior^{low}$ gradient in the developing chick and mouse limb buds. This $Gli3^R$ gradient is thought to establish polarized gene expression in the limb bud, including anterior expression of *Pax9* and posterior expression of several 5′*Hoxd* genes (McGlinn et al. 2005, te Welscher et al. 2002b). In the absence of Shh, elevated $Gli3^R$ levels in the posterior limb mesenchyme result in expanded expression of *Pax9* as well as loss of 5′*Hoxd* gene expression and lead to massive apoptosis and eventual loss of the posterior four digits (digits 2–5). Simultaneous inactivation of *Shh* and *Gli3* results in severe polydactyly and loss of AP identities, similar to the defects observed in *Gli3*-null limbs (Litingtung et al. 2002, te Welscher et al. 2002b), which indicates that one of the main functions of Shh is to counteract $Gli3^R$.

Although $Gli3^R$ levels are important for limb development, unexpectedly, Shh-dependent regulation of Gli3 processing appears not to be critical for the patterning of most limb elements. Mutant mice containing one copy of an unprocessed form of Gli3 with multiple PKA sites mutated ($Gli3^{P1-4}$) and one copy of a truncated Gli3 ($Gli3^{\Delta 699}$, which lacks the C-terminal half of the protein and mimics $Gli3^R$) develop grossly normal limbs with an extra digit 1 (Wang et al. 2007). Hill et al. (2009) showed that $Gli3^R$ is central to the establishment of AP limb asymmetry. $Gli3^{\Delta 699/-}$ mice, which express only a truncated $Gli3^R$-like mutant protein, develop limbs with AP asymmetry (Hill et al. 2009). Importantly, AP limb asymmetry in these animals apparently does not require Shh signaling. Prior to the onset of *Shh* expression in the ZPA, *Gli3* restricts *Hand2* expression in the posterior limb mesenchyme, and *Hand2* acts to exclude *Gli3* expression from the posterior limb mesenchyme (te Welscher et al. 2002a). This mutual antagonism of *Gli3* and *Hand2* is required for the establishment of AP limb asymmetry independent of Shh signaling (Galli et al. 2010). These observations indicate that $Gli3^R$ executes a Shh-independent function in the establishment of AP asymmetry during early limb development (**Figure 6*a*,*c*–*e***).

After the onset of *Shh* expression in the ZPA, Shh signaling influences both *Gli3* transcription and Gli3 processing (Wang et al. 2000a). Genetic ablation of *Shh* at different times during limb development revealed that Shh signaling is continuously required to promote growth (Zhu et al. 2008). As shown by the *Shh*- and *Shh*; *Gli3*-null limb phenotypes, elevated levels of $Gli3^R$ are deleterious to posterior limb mesenchyme cells and result in massive apoptosis (te Welscher et al. 2002b, Zhu et al. 2008). Therefore, continuous Shh signaling likely keeps $Gli3^R$ levels low to allow growth and proliferation of cartilage progenitor cells during later stages of limb development (**Figure 6*b***). Genome-scale analysis of Gli target genes should help decipher these Shh-dependent functions of Gli3 (McGlinn et al. 2005, Vokes et al. 2008). Although *Gli1* and *Gli2* are not essential for limb development (Park et al. 2000), Shh signaling may promote growth through Gli activator functions.

Gradient of Gli Activity in the Ventral Neural Tube Is Regulated by Transcriptional and Posttranslational Mechanisms

Notochord-derived Shh signals specify the floor plate (FP) at the ventral midline as well as five discrete domains of neural progenitors (p3, pMN, p2, p1, and p0) along the dorsoventral axis of the spinal cord (for a review, see Dessaud et al. 2008). Shh acts as a morphogen by regulating the expression of various transcription factors in distinct neural progenitor cell

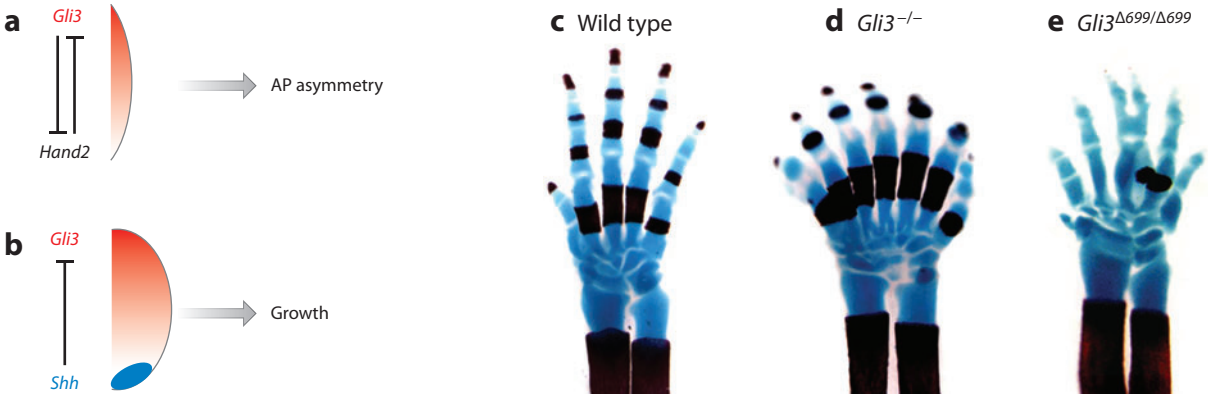

Figure 6

Shh-dependent and -independent functions of Gli3 in limb development. (*a*) Mutual antagonism of *Gli3* and *Hand2* is essential for the establishment of anteroposterior (AP) asymmetry of the limb. Independent of Shh signaling, $Gli3^R$ is required for specification of the anterior identity of the limb. (*b*) Later in development, *Shh* is expressed in the zone of polarizing activity (*blue*) in the posterior limb mesenchyme and promotes growth of chondrogenic progenitors by keeping $Gli3^R$ levels low through inhibition of *Gli3* transcription and Gli3 processing. (*c–e*) Skeletal staining revealed that the $Gli3^{-/-}$ forelimb is polydactylous and lacks AP asymmetry. In contrast, the $Gli3^{\Delta 699/\Delta 699}$ forelimb shows AP asymmetry even though it exhibits central polydactyly.

populations. It represses the expression of dorsally restricted genes, such as *Pax6* and *Irx3*, and induces the expression of ventrally expressed genes, including *Foxa2* and *Nkx2.2* (Briscoe et al. 2000). Mutual antagonism between pairs of these genes, such as *Pax6* and *Nkx2.2*, is thought to establish the boundary between distinct progenitor populations.

A gradient of Gli activity is likely responsible for specifying distinct gene expression patterns, as progressive changes in the level of Gli activator could recapitulate the patterning activity of graded Shh signaling (Stamataki et al. 2005). In mice, all three Gli proteins cooperate to establish the gradient of Gli activity. Gli2, as the principal activator of the pathway, is essential for generating the highest Shh signaling response, and *Gli2*-null embryos fail to specify $Shh^+/Foxa2^+$ FP (Ding et al. 1998). Although *Gli1*-null embryos do not display any phenotypes, FP defects are observed when one copy of *Gli2* is removed in the *Gli1* mutant background, and Nkx2.2-expressing p3 cells are also lost in *Gli1*; *Gli2*-null embryos (Park et al. 2000). Gli3 acts mainly as a repressor in the establishment of the Gli activity gradient, and the *Gli3*-null neural tube exhibits only a dorsal expansion of p0 and p1 cells (Persson et al. 2002). *Gli2*; *Gli3*-null embryos do not exhibit *Gli1* expression and display a more severe phenotype with overproliferation of ventral neural progenitors (Bai et al. 2004). Interestingly, although pMN and p0–p2 cells can be specified in the absence of all Gli functions, these progenitor populations lose their normal boundaries. These observations suggest that Gli^A is critical for the Shh-dependent specification of FP and p3 cells, whereas in the absence of Shh signaling, elevated Gli^R levels inhibit the formation of pMN and p2. Consistent with this notion, all ventral progenitor populations, except for FP and p3, can be specified in *Shh*- and *Smo*-null embryos when Gli3 function is abrogated (Litingtung & Chiang 2000, Wijgerde et al. 2002).

Recent studies indicate that Shh modulates the Gli activity gradient through transcriptional as well as posttranslational mechanisms during ventral neural tube patterning. On one hand, Lek et al. (2010) showed that Pax6 inhibits the Shh signaling response through increased *Gli3* transcription. When Pax6 function is abrogated, FP and p3 cells can be specified in embryos lacking Gli2, which is normally required for high Shh signaling response (Lei et al. 2006,

Lek et al. 2010). The repression of *Pax6* expression by Nkx2.2 thus strengthens the Shh signaling response and promotes specification of FP and p3 cells. On the other hand, another Shh-induced transcription factor, Foxa2, inhibits *Nkx2.2* expression and the Shh signaling response, and this downregulation of Shh signaling appears to be critical for FP differentiation (Cruz et al. 2010, Ribes et al. 2010). Mavromatakis et al. (2011) also recently reported that Foxa2 and its related family member Foxa1 attenuate Shh signaling in the ventral midbrain by inhibiting *Gli2* expression. They demonstrated by chromatin immunoprecipitation that Foxa2 binds to multiple sites in the genomic region of *Gli2*, which suggests that Foxa2 may directly repress *Gli2* transcription. These observations illustrate that transcriptional regulation of *Gli* genes plays a critical role in the fine-tuning of Shh signaling responses during ventral patterning of the spinal cord and midbrain. Similar regulatory mechanisms could contribute to the developmental changes in competence to Shh signaling in the developing forebrain (Sousa & Fishell 2010).

Similar to mice with defects in ciliary and basal body genes, mouse embryos lacking *Sufu* or *Kif7* also display altered levels of Gli^A and Gli^R as well as exhibit ventral neural tube defects. Inactivation of *Sufu* leads to loss of $Gli3^R$ and unleashing of Gli^A, which results in a severe ventral neural tube phenotype with expanded FP, p3, and pMN populations (Chen et al. 2009, Cooper et al. 2005, Jia et al. 2009, Svärd et al. 2006, Wang et al. 2010a). However, Kif7 plays dual regulatory roles in this process. It inhibits the formation of p3 and pMN cells but acts positively in FP specification, which requires the highest level of Shh signaling (Cheung et al. 2009, Endoh-Yamagami et al. 2009, Liem et al. 2009). Recent studies indicate that Kif7 promotes FP specification by controlling the inhibitory function of Sufu on Gli2 (K. Law & C.-c. Hui, unpublished observations). Because Hh signaling promotes Gli activation in the primary cilium through the dissociation of Gli-Sufu complexes, it will be important to determine whether Kif7 is involved in this process and whether it is the key that links Hedgehog signaling to the primary cilium.

Hedgehog-Dependent and -Independent Activation of Gli Proteins in Tumorigenesis

Hh pathway activation, as revealed by upregulation of *Gli1* and *Ptch1* expression, has been found in a wide variety of human cancers, including basal cell carcinoma, breast cancer, gastrointestinal cancer, glioma, leukemia, medulloblastoma, melanoma, and prostate cancer (for reviews, see Teglund & Toftgard 2010, Yang et al. 2010). Indeed, recent clinical trials with Hh pathway antagonists have validated this pathway as a promising anticancer target (Low & de Sauvage 2010, Merchant & Matsui 2010). Pathway activation through *Ptch1* deletion or overexpression of a constitutively active form of Smo (*SmoM2*) can induce basal cell carcinoma and medulloblastoma (see Mao et al. 2006, Villani et al. 2010, Yang et al. 2008 and references therein). Paradoxically, pathway activation at the level of Smo could not induce many cancers that are associated with elevated Gli activity (Mao et al. 2006). Although these observations suggest that the formation of basal cell carcinoma and medulloblastoma in these mouse models likely does not require mutational events other than Hh pathway activation, recent studies have provided clues to why activated Smo is not sufficient to induce other tumors with elevated Gli activity.

Many lines of evidence indicate that other signaling pathways can activate Gli transcription factors in cancer cells (Stecca & Ruiz i Altaba 2010). Dennler et al. (2007) showed that transforming growth factor-β (Tgf-β) activates *Gli1* and *Gli2* expression in various cell types in the presence of a Smo antagonist, cyclopamine. Importantly, pharmacologic inhibition of Tgf-β signaling leads to reduced *Gli* transcription and growth inhibition in several cyclopamine-resistant pancreatic carcinoma cell lines, which indicates that Gli transcription factors mediate the growth-promoting

activity of Tgf-β in these cancer cells. Although early studies suggested that epithelial tumors such as pancreatic cancer secrete and respond to Hh ligand in an autocrine-juxtacrine manner (Berman et al. 2003), Yauch et al. (2008) showed that Hh ligands fail to activate signaling in tumor epithelial cells but promote signaling in the stromal microenvironment. Intriguingly, they found that genetic ablation of *Smo* in the host stroma reduces growth of human tumor xenografts, which suggests that Hh-induced stroma-derived factors contribute to the growth of these tumor xenografts. Furthermore, Nolan-Stevaux et al. (2009) demonstrated that the expression of *Gli1* and its target genes in the pancreatic epithelium is not affected by deletion of *Smo* and that Tgf-β and Ras signaling regulates Gli1 activity in a Smo-independent manner. Interestingly, Ras signaling can exert both positive and negative effects on Gli-dependent transcription. Although Ji et al. (2007) and Nolan-Stevaux et al. (2009) found that oncogenic Kras induces Gli1 transcriptional activity in pancreatic cancer cells, Lauth et al. (2010) showed that similar Kras mutations result in Hh pathway inhibition. It is important, therefore, to determine how Ras signaling regulates the posttranslational modification of Gli proteins in various contexts and how it promotes tumorigenesis in cooperation with Gli proteins.

These and related studies have led us to a model in which the transcriptional activation of *Gli* genes as well as the posttranslational modification of Gli proteins by other signaling pathways contribute to the formation of many cancers with elevated Gli activity (**Figure 7**). These epithelial tumor cells secrete Hh ligand, which acts on the stromal microenvironment to elicit the production of growth factors such as Tgf-β and epidermal growth factor (Egf). These factors could promote Gli activity in the cancer cells through transcriptional activation of the *Gli* genes (**Figure 7**, path 1) and/or through Ras-mediated posttranslational modifications (**Figure 7**, path 2). This is contrary to the Gli activation found in basal cell carcinoma and medulloblastoma, where Gli activity is governed mostly by Hh-dependent activation of Smo (**Figure 7**, path 3). Indeed, the numbers of known noncanonical mechanisms of Gli activation in tumorigenesis are increasing. For example, the Ewing's sarcoma (EWS)-Friend's leukemia insertion (FLI) oncoprotein in EWS can activate *GLI1* transcription directly (Beauchamp et al. 2009), and p53 negatively regulates the levels as well as activities of GLI1 (Stecca & Ruiz i Altaba 2009). Gli1 activity also can be influenced through modulation of a regulator such as Sufu. Kasai et al. (2008) showed that SCL/TAL1-interrupting locus (SIL) is a cytoplasmic protein that binds directly to SUFU and can disrupt SUFU-GLI1 interactions, leading to elevated GLI1 transcriptional activity in pancreatic cancer cells. Given that Gli targets are involved in various cellular processes, including stem cell renewal (Po et al. 2010, Takanaga et al. 2009, Zbinden et al. 2010), any transcriptional and/or posttranslational events that lead to sustained Gli activity potentially could promote tumorigenesis.

Stromal-derived factors and transcriptional regulation of *Gli* genes could also contribute to the formation of conventional Hh-dependent tumors. EGF receptor signaling can synergize with Hh signaling to promote growth of basal cell carcinoma (Schnidar et al. 2009). The transcriptional activation of *Gli2* in granule neuron precursors (GNPs), which are likely the cell of origin of medulloblastoma, plays a critical role in tumorigenesis. Atoh1 activates *Gli2* transcription in GNPs, and its deletion results in loss of *Gli2* expression and prevents tumorigenesis in the *SmoM2* mouse model of medulloblastoma (Flora et al. 2009). Furthermore, disruption of primary cilia could promote the formation of basal cell carcinoma and medulloblastoma in transgenic mice with overexpression of $Gli2^A$ (Han et al. 2009, Wong et al. 2009). Apparently, the disruption of primary cilia alleviates some inhibitory actions on Gli^A. These observations indicate that, similar to the situation in development, *Gli* genes, their products, and Gli regulators are subject to multiple levels of control during tumorigenesis.

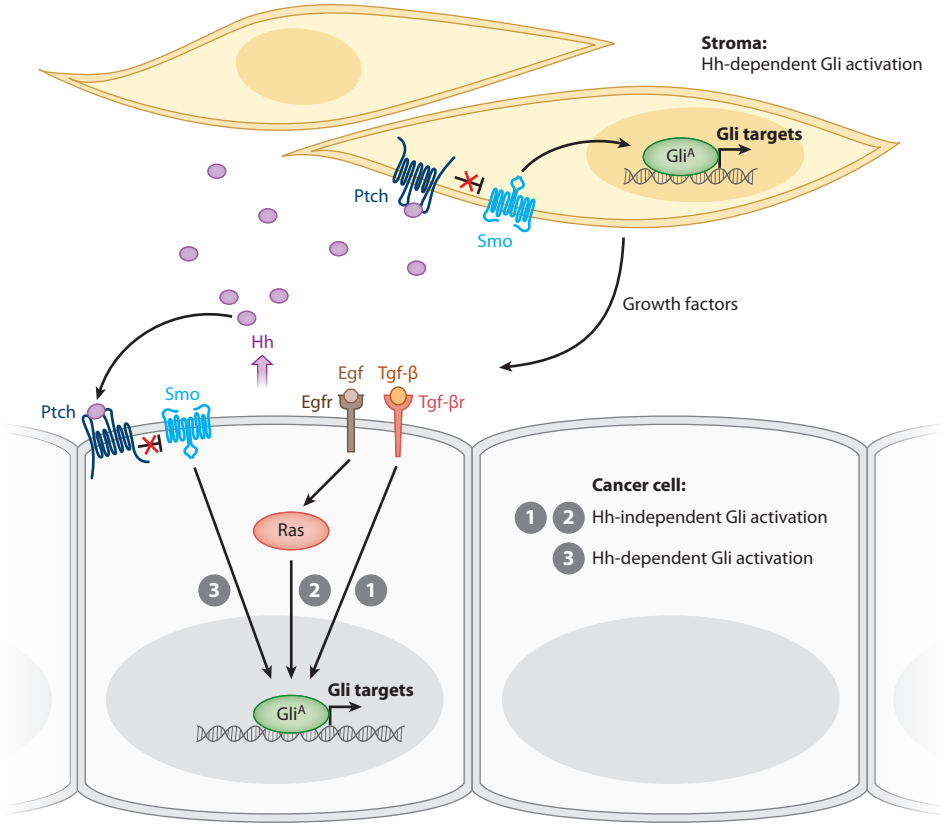

Figure 7

Hedgehog (Hh)-dependent and -independent Gli activation in tumorigenesis. In epithelial tumor cells, transforming growth factor β (Tgf-β) signaling has been shown to activate the transcription of *Gli1* and *Gli2* (①), whereas epidermal growth factor (Egf) and/or Ras signaling promotes Gli^A function through the kinase cascade (②). In certain circumstances, the Hh/Smoothened (Smo)-dependent mechanism (③) is not critical for activation of Gli function. For example, in pancreatic tumors (*bottom*), cancer cells express and secrete Hh ligand, but Smo is dispensable for Gli^A function. Instead, Hh signaling is required in the stromal fibroblasts (*top*) for the expression of growth factors, which are believed to maintain high Gli^A levels in the cancer cells. Abbreviation: Patched, Ptch.

SUMMARY AND CONCLUSION

Molecular genetic analysis in mice and other model systems has established the central role of Gli proteins in many Hh-dependent developmental processes (Jiang & Hui 2008, Varjosalo & Taipale 2008). However, Gli proteins also perform critical functions independent of Hh signaling during development and tumorigenesis. We have highlighted here some of these examples, including the Shh-independent function of $Gli3^R$ during limb development and the Smo-independent Gli activation in pancreatic cancer cells. Recent studies have begun to illustrate the regulation of *Gli* transcription as important steps in the fine-tuning of Hh signaling response during development. Furthermore, activation of *Gli1* and *Gli2* transcription also appears to play a key role in tumorigenesis. Thus, understanding both the transcriptional and posttranscriptional regulation of the three *Gli* genes will be necessary to further decipher the signal transduction mechanism of the Hh pathway.

Enormous efforts have gone toward developing Hh pathway inhibitors to target tumors caused by Hh pathway activation, or more appropriately Gli activation (Low & de Sauvage 2010, Merchant & Matsui 2010, Yang et al. 2010). To develop effective therapeutic approaches, the molecular basis underlying Gli activation in these tumors needs to be determined. This task could be challenging, as it is evident that many mechanisms underlie Gli activation. However, we should be optimistic because most signaling pathways known to be involved in Gli activation are quite established, and effective drugs against many of these pathways are available for clinical use.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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