Degrading Ci: who is Cul-pable?

Jin Jiang

Center for Developmental Biology and Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, Texas 75390-9133, USA

Proper cell fate specification and pattern formation rely on a cell's ability to sense and interpret graded spatial information. One class of spatial signals widely used in animal development is the Hedgehog (Hh) family. Hh family members govern numerous developmental processes in both vertebrates and invertebrates, and in many instances act as morphogens that specify distinct developmental outcomes as a function of their concentrations (for review, see Ingham and McMahon 2001). How different thresholds of Hh are perceived and transmitted by signal-receiving cells to elicit distinct responses remains largely unknown, however, recent studies on the Drosophila Hh signaling effector, Cubitus interruptus (Ci), provide important insights.

Ci exists in two distinct forms: a full-length form (Ci155) and a C-terminally truncated form (Ci75) that is derived from Ci155 by partial degradation (Fig. 1B; Aza-Blanc et al. 1997). These two forms of Ci play distinct roles in Drosophila appendage development. In developing wings, for example, Ci155 is processed to generate Ci75 in anterior (A) compartment cells distant from the anteroposterior (A/P) compartment boundary, where Ci75 acts as a transcriptional repressor to block the expression of a subset of Hh-responsive genes including decapentaplegic (dpp; Methot and Basler 1999). In A-compartment cells near the A/P boundary, Hh inhibits Ci processing and thus the generation of its repressor form. In addition, Hh stimulates the activity and nuclear translocation of the accumulated Ci155, which acts as a transcriptional activator to turn on other Hh-responsive genes including patched (ptc) and engrailed (en; Alexandre et al. 1996; Methot and Basler 1999). The regulation of Ci processing and activity has been subject to numerous studies in the past several years, ranging from early genetic identification of Hh pathway components to more recent biochemical characterization of large signaling complexes (Ingham and McMahon 2001). Strikingly, almost all the intracellular Hh signaling components identified so far are involved in Ci processing, forecasting the complexity of this process. Here, I briefly review several recent findings that provide new insights into how Ci processing is accomplished, including a paper in this issue of Genes & Development (Ou et al. 2002) that uncovers a novel regulatory pathway of Ci degradation.

Ci processing: a tale of three kinases

An initial clue about what triggers the proteolytic processing of Ci came from studying Drosophila cAMP-dependent protein kinase [PKA]. Loss-of-function mutations in the catalytic subunit of PKA lead to constitutive Hh signaling (Jiang and Struhl 1995; Lepage et al. 1995; Li et al. 1995; Pan and Rubin 1995). Subsequent work indicated that PKA phosphorylates a C-terminal region of Ci at multiple Ser/Thr residues essential for its processing (Y. Chen et al. 1999; Price and Kalderon 1999, Wang et al. 1999). Two recent studies identified Glycogen synthase kinase 3 (GSK3) and Casein kinase I (CKI) as two PKA partners that act cooperatively with PKA to promote Ci processing (Jia et al. 2002, Price and Kalderon 2002). The new link of GSK3 to Ci processing came as a surprise as this kinase had been extensively studied in Drosophila, both genetically and biochemically. Nevertheless, different paths taken by those two groups converged on the role of GSK3 in regulating the Hh pathway.

GSK3 controls numerous cellular and physiological processes, and is best known for its role in the Wnt/Wingless (Wg) pathway, where it phosphorylates β-catenin/Armadillo (Arm) and targets it for degradation (for review, see Kim and Kimmel 2000). Loss-of-function mutations in the Drosophila GSK3, Shaggy (Sgg)/Zeste-White 3 (Zw3), result in the accumulation of Arm and constitutive Wg signaling in both embryos and adult structures. In a recent study, Jia et al. (2002) further explored the role of Sgg in Drosophila appendage development using a null mutation that lacks all Sgg isoforms. To their surprise, they found that sgg mutant clones showed phenotypes indicative of mild gain of Hh signaling activity in multiple tissues, as manifested by the accumulation of Ci155, ectopic expression of a subset of Hh target genes including wg and dpp, and anterior wing duplication. Further, these phenotypes can be enhanced by removal of Suppressor of fused [Su[fu]], a negatively acting component in the Hh pathway. Along a different vein, Price and Kalderon (2002) sought to identify additional kinases that phosphorylate Ci in embryonic extracts. Using GST–Ci fusion protein as a substrate, they found that PKA-prephosphorylated Ci was further phosphorylated when incubated with embryonic extracts. By aligning sequences between Ci and its vertebrate homolog Gli proteins, both groups noticed the presence of a consensus sequence, SxRRXS_{PKA}, at two previously identified PKA sites that matches the GSK3 consensus.
site (Fig. 1A; Kim and Kimmel 2000). Using an in vitro kinase assay, both groups showed that GSK3 phosphorylates Ci at SN following PKA phosphorylation at SPKA. Further evidence that GSK3 phosphorylates Ci came from experiments using a selective GSK3 inhibitor, LiCl (Klein and Melton 1996). For example, treating embryonic extracts with LiCl abolished PKA-primed phosphorylation at SN sites (Price and Kalderon 2002), whereas treating cultured cells with LiCl led to a reduction in the levels of Ci phosphorylation (Jia et al. 2002). To address whether phosphorylation of Ci by GSK3 is essential for its processing, both groups found that alteration of multiple GSK3 sites abolished Ci processing and the generation of the repressor form in vivo. Mutating individual GSK3 sites greatly compromised although did not completely abolish Ci processing, suggesting that GSK3 phosphorylation at multiple sites is essential for efficient processing of Ci (Jia et al. 2002). In agreement with the genetic analysis of sgg by Jia et al., Price and Kalderon observed that a null mutation in sgg resulted in ectopic but modest activation of Hh signaling. Both groups also noticed that hypomorphic alleles of sgg, which were widely used in previous studies, only caused partial blockage of Ci processing. The levels of Ci155 in these hypomorphs were thus insufficient for triggering the ectopic expression of Hh-responsive genes, possibly explaining why hh-related phenotypes had not been observed previously.

In addition to GSK3, Price and Kalderon (2002) provided evidence implying that Casein kinase I (CKI) is a third kinase that phosphorylates Ci and promotes its processing. In pursuing the nature of PKA-primed phosphorylation of GST–Ci fusion proteins by embryonic extracts, they also noted the existence of a consensus sequence SPKAXXSC at three PKA sites among Ci and some Gli proteins (Fig. 1A), which is a preferred phosphorylation site for kinases of the CKI family (Umphress et al. 1992). Indeed, an in vitro kinase assay coupled with the results of mutagenesis studies suggested that CKI phosphorylated GST–Ci at SC sites following PKA phosphorylation at SPKA sites. Treating embryonic extracts with a CKI inhibitor, CKI-7, abolished PKA-primed phosphorylation at SC sites, suggesting that enzymes with the specificity of CKI are active in embryonic extracts and are responsible for the observed phosphorylation at SC sites. Alteration of multiple CKI sites in Ci, as defined by the in vitro kinase assay, abolished Ci processing and the generation of its truncated repressor, supporting the argument that phosphorylation of Ci by CKI is also essential for its processing. However, a role for CKI in the Hh pathway has not been established by a loss-of-function study, which is complicated by the presence of eight predicted CKI family members in the Droso phila genome (Morrison et al. 2000). These CKI family members may have redundant function in regulating Ci, because members of the CKI family appear to have similar substrate specificity and function when overexpressed (Tuazon and Traugh 1991; McKay et al. 2001).

Nevertheless, these new studies suggest that Ci processing requires sequential phosphorylation by three kinases. PKA phosphorylation at multiple sites in the C-terminal region of Ci promotes further phosphorylation by GSK3 and CKI at adjacent sites, resulting in the hyperphosphorylation of Ci, which is essential for Ci processing to generate its repressor form (Fig. 1B).

**Ci processing and the SCF connection**

How does phosphorylation target Ci for processing? One mechanism that couples phosphorylation with proteolysis is the ubiquitin (Ub)/proteasome pathway mediated by SCF complexes that constitute a large family of Ub
ligases [E3] and contain the core subunits Skp1, Cullin [Cul], Roc1/Rbx1 RING finger protein, and a variable substrate-recognition subunit F-box protein [Deshaies 1999]. SCF complexes prefer to bind phosphorylated substrates through cognate F-box proteins, thus bridging the phosphorylated substrates to a nearby E2 that has been recruited to the SCF complexes through Roc1 [Deshaies 1999]. What is the evidence for the involvement of an SCF complex in regulating Ci proteolytic processing? The first hint came from a study showing that the F-box-containing protein Slimb is required to generate the repressor form of Ci [Jiang and Struhl 1998]. Remarkably, Slimb and its vertebrate homolog β-TRCP are also involved in Wnt/Wg and NF-κB/Dorsal pathways, where they target β-catenin/Arm and IκB, respectively, for ubiquitination and proteasome-mediated degradation after these protein substrates are phosphorylated by cognate kinases [for review, see Maniatis 1999]. These observations led to the proposal that an SCF^{Slimb} complex targets phosphorylated Ci for ubiquitination, followed by proteasome-mediated processing. Consistent with this hypothesis, proteasome inhibitors block Ci processing in cultured c8 cells [C.H. Chen et al. 1999].

In this issue of Genes & Development, Ou et al. (2002) fill an important gap in the SCF hypothesis by identifying the Cullin responsible for Ci processing. They conducted a series of elegant genetic analyses addressing the physiological roles of Drosophila cullin family members (particularly Cul1 and Cul3) and their modification by Nedd8, a small ubiquitin-like protein. The authors found that loss of Nedd8 results in the accumulation of high levels of Ci155, Arm, and cyclin E. As Nedd8 covalently modifies Cul1 and regulates its activity in other systems, they speculated that Nedd8 mutations affect the activity of Cul1-based SCF E3 complexes required for the processing of Ci, or the degradation of Arm and cyclin E. In support of this, they showed that Drosophila Cul1 is neddylated in vivo and that Cul1 neddylation is abolished in Nedd8 mutants. More importantly, they showed that Cul1 mutant cells accumulate high levels of Ci155, Arm, and cyclin E, as is the case with Nedd8 mutant cells. The authors further characterized the role of the Nedd8/Cul1 pathway in regulating Hh signaling activity using developing eyes [or eye imaginal discs] as a model system. The eye disc is divided by a line of indentation in the disc epithelium called the morphogenetic furrow [MF] into an anterior region, where cells undergo rapid division, and a posterior region, where cells enter differentiation programs [for review, see Heberlein and Treisman 2000]. During eye development, the MF is initiated at the posterior margin of eye discs and propagates anteriorly. hh is expressed posteriorly to the MF and is required for both the initiation and progression of the MF. The Ou group showed that Nedd8 acts genetically downstream of Hh signaling components Smootherned (Smo) and PKA to regulate Ci processing in cells anterior to the MF. In addition, loss of Nedd8 in anterior cells close to the MF results in ectopic expression of several Hh-responsive genes including dpp and atonal. Taken together these results strongly support the argument that an SCF complex containing Slimb and Cul1 is required for Ci processing and that Nedd8 stimulates the activity of this E3 complex through covalent attachment to Cul1.

A distinct pathway for Ci proteolysis

Whereas the study of Cul1 provided further support for the role of an SCF^{Slimb} E3 in Ci processing, the study of another Cullin family member, Cul3, uncovered a distinct pathway for Ci degradation (Fig. 2). Unlike the situation in the developing wing, where Ci is not expressed in P-compartment Hh-producing cells, Ci is expressed nearly uniformly in the entire eye disc, including cells posterior to the MF that produce Hh. However, high levels of Ci155 are accumulated in cells in the MF in response to Hh. Cells posterior to the MF appear to downregulate the levels of Ci155 even though they are exposed to Hh. How Ci155 is regulated posterior to the MF has long been elusive. In the course of analyzing Nedd8 mutants, Ou et al. (2002) noticed that Nedd8 mutant cells situated posterior to the MF accumulate high levels of Ci155, suggesting that down-regulation of Ci155 posterior to the MF may also depend on Cullin-mediated proteolysis. The authors showed that posterior degradation of Ci155 is distinct from the anterior one, as it is independent of Slimb, Cul1, and PKA, and it results in complete degradation of Ci as opposed to selective removal of the C-terminal half of Ci. More intriguingly, posterior degradation of Ci depends on Hh signaling, as removal of Smo results in the accumulation of high levels of Ci155. To explore which Cullin participates in the posterior degradation of Ci, the authors turned to Cul3. They found that loss of Cul3 leads to the accumulation of Ci155 in cells posterior but not anterior to the MF, suggesting that Cul3 is specifically required for the posterior degradation of Ci, whereas Cul1 is only involved in anterior processing of Ci. This specificity does not seem to be determined by tissue-specific distribution of Cul1 and Cul3, as both proteins are produced uniformly in eye discs. Rather, Cul1 and Cul3 appear to have distinct biochemical properties, as Cul1 but not Cul3 binds Skp-1 [Michel and Xiong 1998]. Thus, Cul1 and Cul3 may form distinct E3 complexes, one targeting phosphorylated Ci for processing and the other targeting Ci for complete degradation in a phosphorylation-independent manner [Fig. 2].

Dual pathways for regulating protein degradation have also been observed for cyclin E, which is regulated by a Cul1-based SCF E3 in a phosphorylation-dependent manner and by a Cul3-based E3 in a phosphorylation-independent fashion [Singer et al. 1999; for review, see Schwab and Tyers 2001]. The latter pathway has been shown to specifically attack unbound cyclin E [Singer et al. 1999]. It has been shown that Ci155 forms a complex with the positively acting Hh-signaling component Sufu and that high levels of Hh signaling activity convert Ci155 into a labile, hyperactive form, possibly by dissociating Sufu from Ci155 [Ohlmeyer and Kalderon 1998]. By analogy to the regulation of cyclin E, Cul3 may
specifically attack the unbound hyperactive form of Ci after it dissociates from Su(fu) in response to Hh, which could account for the dependence of this pathway on Smo activity (Fig. 2).

What could then be the physiological function of the posterior degradation of Ci? Ou et al. propose that complete degradation of Ci provides an efficient way to turn off Hh signal transduction in cells posterior to the MF, which might be essential for these cells to enter appropriate differentiation programs. Consistent with this hypothesis, they found that cone cell differentiation is affected in Cul3 mutants. A critical test of this model would be to simultaneously remove Cul3 and Ci function in posterior cells and examine if Cul3 mutant phenotypes can be rescued by removal of Ci, which is predicted by the model.

Future directions
The new findings raise many important questions that need to be addressed in the future. For example, how extensively is Ci phosphorylated in vivo prior to its processing? Mutagenesis studies suggest that alteration of each of the four PKA consensus sites [sites 1–4] appears to abolish Ci processing [Y. Chen et al. 1999, Methot and Basler 1999]. Similarly, mutating individual GSK3 consensus sites greatly impairs Ci processing [Jia et al. 2002]. Although individual CKI consensus sites have not been evaluated, mutating multiple putative CKI sites completely blocks Ci processing [Price and Kalderon 2002]. Thus, it appears that phosphorylation of Ci at multiple clusters of Ser/Thr is essential for its processing in vivo. It has been previously shown that inhibition of the 26S proteasome in cultured cl8 cells leads to the accumulation of hyperphosphorylated forms of Ci with slow electrophoretic mobility (C.H. Chen et al. 1999). The extensive mobility shift of the hyperphosphorylated forms of Ci cannot be accounted for simply by PKA phosphorylation alone, and thus is likely caused by phosphorylation by additional kinases such as GSK and CKI. Consistently, treating cells with both GSK3 and proteasome inhibitors increases the mobility of phosphorylated forms of Ci, probably through a reduction in the extent of Ci phosphorylation [Jia et al. 2002]. Nevertheless, future experiments with more sophisticated tools such as mass spectrometry and phospho-specific antibodies are required to map the in vivo phosphorylation sites in Ci and monitor how individual phosphorylation events change in response to Hh stimulation.

Why must Ci be phosphorylated at multiple sites to be targeted for processing? In the case of β-catenin or IκB, phosphorylation at a single cluster of Ser/Thr residues appears to be sufficient to confer Slimb/β-TRCP binding [Liu et al. 1999; Spencer et al. 1999; Winston et al. 1999]. Comparison of the Slimb/β-TRCP binding motifs in IκB and β-catenin reveals a consensus sequence, DSGΦXS,
which is not present in Ci. In contrast, the multiple clusters of phosphorylation sites in Ci and Gli proteins define a distinct consensus, [S]RRS/DSXXS (Fig. 1A). It is possible that Slimb/β-TRCP could recognize more than one motif, and that the motif present in β-catenin and IκB may confer high-affinity binding to Slimb/β-TRCP, whereas those present in Ci may confer low-affinity binding. In this regard, cooperative binding among multiple low-affinity sites is essential for Slimb recognition, which may explain why Ci must be phosphorylated at multiple clusters of Ser/Thr residues to be processed. A similar mechanism has been shown for the regulation of yeast Sic1, which is phosphorylated at multiple low-affinity sites to be recognized by the F-box protein Cdc4 (Nash et al. 2001). A second possibility is that multiple phosphorylation sites may confer binding to accessory proteins in addition to Slimb, which may act in conjunction with Slimb to regulate Ci processing. A third possibility is that hyperphosphorylation of Ci may regulate its interaction with an unknown protein, such as a processing protease, rather than Slimb. In this scenario, Ci might not be the direct substrate for SCP3/Slimb-mediated ubiquitination. Indeed, ubiquitinated forms of Ci were not observed in c8 cells treated with proteasome inhibitors (C.H. Chen et al. 1999). However, one cannot rule out the possibility that a Ci-specific deubiquitination enzyme is highly active in c8 cells, rendering it difficult to detect the ubiquitinated forms of Ci. Future studies using in vitro reconstituted ubiquitination systems may help resolve this issue.

What is the advantage of using multiple kinases, each of which phosphorylates Ci at multiple sites, to regulate its processing? One possibility is that cooperative binding among multiple low-affinity sites may confer a sharp response to a shallow Hh activity gradient, as a small decrease in the Ci phosphorylation levels could result in complete blockage of Ci processing. A similar mechanism has been implicated in the regulation of Sic1, where the requirement for multiple phosphorylations prior to its destruction confers a switch-like response to a steady change in the upstream kinase activity (Nash et al. 2001). Another advantage of using multiple phosphorylation events is that it may allow the levels of Ci phosphorylation to be fine-tuned by different thresholds of Hh signaling activity, leading to differential regulation of the two forms of Ci. It has been previously shown that phosphorylation of Ci by PKA plays dual roles in that it not only targets Ci for processing but also inhibits the activity of full-length Ci independent of its processing (Wang et al. 1999). Moreover, the activity of Ci155 and the production of the Ci75 repressor appear to be governed by different levels of Ci phosphorylation, as the alteration of individual PKA sites only abolishes Ci processing but does not activate Ci155, whereas the alteration of multiple PKA sites confers constitutive activity to Ci155 (Y. Chen et al. 1999; Price and Kalderon 1999; Wang et al. 1999). In addition, low levels of Hh appear to be sufficient to block Ci processing to generate the repressor form, whereas high levels of Hh are required to activate Ci155 (Wang and Holmgren 1999). Thus, it is tempting to speculate that different thresholds of Hh may differentially regulate Ci, at least in part, by controlling its level of phosphorylation. Low levels of Hh induce partial dephosphorylation of Ci, which blocks Ci processing to generate the Ci75 repressor, leading to the depression of Hh-responsive genes normally blocked by Ci75. High levels of Hh cause more complete dephosphorylation of Ci, which stimulates the activity of Ci155, resulting in the expression of Hh-responsive genes controlled by Ci155. It would be important, therefore, to measure the levels of Ci phosphorylation in cells stimulated with different concentrations of Hh, and to determine if distinct thresholds of Hh induce dephosphorylation of Ci to different extents.

The finding that Ci is phosphorylated by multiple kinases also raises an important question of how Ci phosphorylation is regulated by Hh. A more general question is how kinases such as GSK3, which are involved in numerous signaling pathways, are regulated in a pathway-specific manner. An emerging theme is that GSK3 is present, together with its substrates, in distinct complexes that are regulated by different upstream signals. Such a mechanism has been implicated in the regulation of GSK3 by Wnt (Kim and Kimmel 2000). Ci forms a large complex with Costal2 (Cos2) and Fused (Fu), which is essential for its processing (Fig. 2; Robbins et al. 1997; Sisson et al. 1997; Wang and Holmgren 1999; Lefers et al. 2001). It is tempting to speculate that GSK3 may also be part of the Ci complex in order to phosphorylate Ci effectively, and that Hh may antagonize GSK3 activity by regulating the dynamics of the complex, for example, by dissociating GSK3 from the complex. Alternatively, Hh may antagonize GSK3 through regulating a phosphatase (C.H. Chen et al. 1999).

Is the mechanism underlying Ci regulation conserved? All Gli proteins contain multiple PKA, GSK3, and CKI consensus sites arranged in a similar fashion to that in Ci, suggesting that vertebrate homologs of Ci are also regulated by these kinases (Fig. 1A) Jia et al. 2002; Price and Kalderon 2002). Among the Gli proteins, Gli3 has been shown to undergo proteolytic processing to generate a truncated repressor form (Wang et al. 2000). In addition, alteration of any one of the six putative PKA sites in Gli3 completely blocks it processing in cultured cells, suggesting that Gli3 processing also requires extensive phosphorylation at multiple sites (Wang et al. 2000). It remains to be determined if the Slimb homolog β-TRCP and its associated SCF complex as well as other kinases are also involved in Gli3 processing.

Finally, it is not clear what determines complete versus partial degradation of Ci if both events are controlled by the ubiquitin/proteasome pathway. Although in most cases, the ubiquitin/proteasome pathway targets proteins for complete degradation, limited proteolysis by this pathway has been implicated in regulating several other proteins (Palombella et al. 1994; Hoppe et al. 2000). However, the rules governing complete versus partial degradation remain poorly understood. Further study on Ci regulation should therefore not only provide insight into how different thresholds of Hh signaling activity are
transduced but also shed light on how the degradation of a key signal-dependent transcriptional effector is tightly controlled in cells.

Acknowledgments
I thank Keith Wharton, James Chen, Jon Graff, and members of the Jiang lab for critical reading of the manuscript. This work is supported by the NIH (GM61269-01), the Scarle Scholar program, and the University of Texas Southwestern Endowed Scholar program.

References
Alexandre, C., Jacinto, A., and Ingham, P.W. 1996. Transcriptional activation of Hedgehog target genes in Drosophila is mediated directly by the Cubitus interruptus protein, a member of the GLI family of the zinc finger DNA-binding proteins. Genes & Dev. 10: 2003–2013.

Aza-Blanc, P., Ramirez-Weber, F., Laget, M., Schwartz, C., and Kornberg, T. 1997. Protein kinase A and Hedgehog signaling in Drosophila. Results Probl. Cell Differ. 31: 37–50.

Hoppe, T., Matuschewski, K., Rape, M., Schlenker, S., Ulrich, H.D., and Jentsch, S. 2000. Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. Cell 102: 577–586.

Ingham, P.W. and McMahon, A.P. 2001. Hedgehog signaling in animal development: Paradigms and principles. Genes & Dev. 15: 3059–3087.

Jiang, J., Amanai, K., Wang, W., Tang, J., Wang, B., and Jiang, J. 2002. Shaggy/GSK3 antagonizes Hedgehog signaling by regulating Cubitus interruptus. Nature 416: 548–552.

Jiang, G. 1995. Protein kinase A and Hedgehog signalling in Drosophila limb development. Cell 80: 563–572.

Lefers, M.A., W. Ohlmeyer, J.T., Lane, M.E., and Kalderon, D. 1995. Functional activation of protein kinase A in hedgehog signal transduction and Drosophila imaginal disc development. Cell 80: 553–562.

Liu, C., Kato, Y., Zhang, Z., Do, V.M., Yankner, B.A., and He, X. 1999. β-TRCP couples β-catenin phosphorylation–degradation and regulates Xenopus axis formation. Proc. Natl. Acad. Sci. U.S.A. 96: 6273–6278.

Manniatis, T. 1999. A ubiquitin ligase complex essential for the NF-κB, Wnt/Wingless, and Hedgehog signaling pathways. Genes & Dev. 13: 505–510.

Mckay, R.M., Peters, J.M., and Graff, J.M. 2001. The casein kinase I family in Wnt signaling. Dev. Biol. 235: 388–396.

Michel, J.J. and Xiong, Y. 1998. Human CUL-1, but not other cullin family members, selectively interacts with SKP1 to form a complex with SKP2 and cyclin A. Cell Growth Differ. 9: 435–449.

Morrison, D.K., Murakami, M.S., and Cleghon, V. 2000. Protein kinases and phosphatases in the Drosophila genome. J. Cell Biol. 150: F57–F62.

Nash, P., Tang, X., Orlicky, S., Chen, Q., Gertler, F.B., Mendenhall, M.D., Sizheng, F., Pawson, T., and Tyers, M. 2001. Mutisite phosphorylation of a CDK inhibitor sets a threshold for the onset of DNA replication. Nature 414: 514–521.

Oliveira, M.A., Donaldson, T.D., Thacker, S.A., and Duro, R.J. 2002. Drosophila Roc1a encodes a RING-H2 protein with a unique function in processing the Hedgehog signal transducer Ci by the SCF E3 ubiquitin ligase. Dev. Cell 2: 757–770.

Ohlmeyer, J.T. and Kalderon, D. 1998. Hedgehog stimulates maturation of Cubitus interruptus into a labile transcriptional activator. Nature 396: 749–753.

Ou, C.-Y., Lin, Y.-F., Chen, Y.-J., and Chien, C.-T. 2002. Distinct protein degradation mechanisms mediated by Cul1 and Cul3 controlling Ci stability in Drosophila eye development. Genes & Dev. [this issue].

Palombella, V.J., Rando, O.J., Goldberg, A.L., and Maniatis, T. 1994. The ubiquitin–proteasome pathway is required for processing the NF-κB precursor protein and the activation of NF-κB. Cell 78: 773–785.

Pan, D. and Rubin, G.M. 1995. cAMP-dependent protein kinase and hedgehog act antagonistically in regulating decapentaplegic transcription in Drosophila imaginal discs. Cell 80: 543–552.

Price, M.A. and Kalderon, D. 1999. Proteolysis of cubitus interruptus in Drosophila requires phosphorylation by protein kinase A. Development 126: 4331–4339.

Price, M.A. and Kalderon, D. 2002. Proteolysis of the Hedgehog signaling effector Cubitus interruptus requires phosphorylation by Glycogen Synthase Kinase 3 and Casein Kinase 1. Cell 108: 823–835.

Robbins, D.J., Nybakken, K.E., Kobayashi, R., Sisson, J.C., Bishop, J.M., and Therond, P.P. 1997. Hedgehog elicits signal transduction by means of a large complex containing the kinesin-related protein costal2. Cell 90: 225–234.

Schwab, M. and Tyers, M. 2001. Cell cycle. Archipelago of destruction. Nature 413: 268–269.

Singer, J.D., Gurian-West, M., Clurman, B., and Roberts, J.M. 1999. Cullin-3 targets cyclin E for ubiquitination and controls S phase in mammalian cells. Genes & Dev. 13: 2375–2387.

Sisson, J.C., Ho, K.S., Suyama, K., and Scott, M.P. 1997. Costal2, a novel kinesin-related protein in the Hedgehog signaling pathway. Cell 90: 235–245.
Spencer, E., Jiang, J., and Chen, Z.J. 1999. Signal-induced ubiquitination of IκBα by the F-box protein Slimb/β-TrCP. *Genes & Dev.* 13: 284–294.

Tuazon, P.T. and Traugh, J.A. 1991. Casein kinase I and II—multipotential serine protein kinases: Structure, function, and regulation. *Adv. Second Messenger Phosphoprotein Res.* 23: 123–164.

Umphress, J.L., Tuazon, P.T., Chen, C.J., and Traugh, J.A. 1992. Determinants on simian virus 40 large T antigen are important for recognition and phosphorylation by casein kinase I. *Eur. J. Biochem.* 203: 239–243.

Wang, B., Fallon, J.F., and Beachy, P.A. 2000. Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb. *Cell* 100: 423–434.

Wang, G., Wang, B., and Jiang, J. 1999. Protein kinase A antagonizes Hedgehog signaling by regulating both the activator and repressor forms of Cubitus interruptus. *Genes & Dev.* 13: 2828–2837.

Wang, Q.T. and Holmgren, R.A. 1999. The subcellular localization and activity of *Drosophila* cubitus interruptus are regulated at multiple levels. *Development* 126: 5097–5106.

Winston, J.T., Strack, P., Beer-Romero, P., Chu, C.Y., Elledge, S.J., and Harper, J.W. 1999. The SCFβ-TRCP–ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IκBα and β-catenin and stimulates IκBα ubiquitination in vitro. *Genes & Dev.* 13: 270–283.
Degrading Ci: who is Cul-pable?

Jin Jiang

*Genes Dev.* 2002, 16:

Access the most recent version at doi:10.1101/gad.1027902

References

This article cites 40 articles, 14 of which can be accessed free at:

http://genesdev.cshlp.org/content/16/18/2315.full.html#ref-list-1

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.