

## A New Smurf in the Village

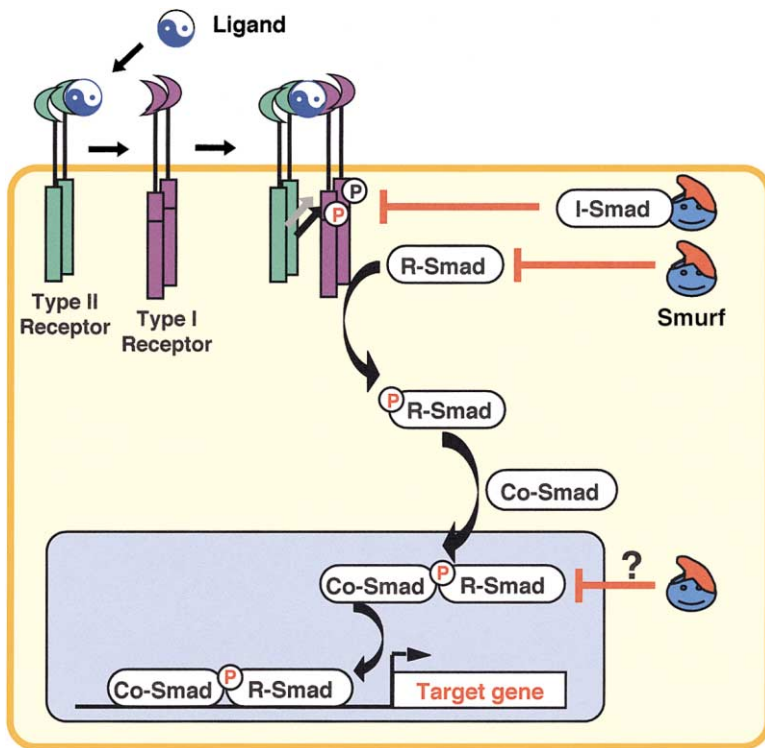
**TGF- $\beta$  signaling is modulated by Smurfs, E3-ubiquitin ligases that selectively target the receptors and Smad proteins for degradation. New evidence from *Drosophila* suggests that Smurfs regulate the amplitude and the duration of the cellular response to signaling in vivo.**

Say the word “Smurfs” to most scientists of a certain age and it elicits a momentary cringe and memories of blue cartoon characters with strange sock-like headgear. For investigators studying transforming growth factor  $\beta$  (TGF- $\beta$ ) signal transduction, however, the word has a different connotation—it is the whimsical abbreviation for the *Smad* ubiquitin regulatory factors (Zhu et al., 1999). These latter-day Smurfs have been implicated in targeting components of the TGF- $\beta$  signaling pathway for ubiquitin-mediated degradation. A flurry of recent papers utilizing primarily biochemical, cell culture, and overexpression assays has outlined several mechanisms through which Smurfs could modulate TGF- $\beta$  signaling (Ebisawa et al., 2001; Kavsak et al., 2000; Lin et al., 2000; Zhang et al., 2001; Zhu et al., 1999). Despite the insight provided by these studies, the importance and overall biological impact of Smurf activity has remained unclear. This lacuna is now partially filled by the report presented in this issue of *Developmental Cell* that the *DSmurf* gene in *Drosophila* is essential for embryonic development and viability (Podos et al., 2001). Podos and coworkers demonstrate that loss of *DSmurf* activity affects both the amplitude and duration of bone morphogenetic protein (BMP) signaling. Thus, Smurfs may be critical in determining the competence of cells to respond to TGF- $\beta$  signaling as well as in ensuring that the signal is temporally restricted.

A striking feature of the TGF- $\beta$  pathway is that information is transmitted from the cell surface to the nucleus in a remarkably direct fashion. TGF- $\beta$ , activin, and BMP ligands that constitute this superfamily of related growth factors initiate signaling by inducing the formation of a heteromeric complex of type I and type II receptor serine-threonine kinases. This allows the type II receptor to activate the type I kinase that in turn phosphorylates a receptor-specific Smad (R-Smad). The modified R-Smad associates with the common co-Smad, translocates into the nucleus, and directly binds DNA to regulate target gene expression with other transcription factors, coactivators, and corepressors. Despite this apparent simplicity, several negative feedback loops have been identified that regulate the activity of the pathway at multiple levels (Massagué and Chen, 2000). One of these involves inhibitory Smads (I-Smads) that are induced in response to signaling and interfere with type I receptor activity, while another strategy involves signaling-dependent receptor turnover. Recent studies indicate that Smurfs function in both these processes, in addition to regulating the cytoplasmic pool of R-Smads.

Two closely related Smurfs (Smurf1 and 2) that contain HECT catalytic domains characteristic of E3-ubiquitin ligases have been identified in vertebrates. These enzymes mediate the final step in the ubiquitination of target proteins and display a high degree of substrate specificity, thus ensuring the selectivity of the process (Hershko and Ciechanover, 1998). Among a multitude of other functions, E3 ligases are known to play critical roles in modulating the outcome of NF $\kappa$ B, Hedgehog, Wingless, EGFR, and Notch signaling (Maniatis, 1999). One way in which Smurfs antagonize TGF- $\beta$  signaling is by interacting with R-Smads and regulating their destruction in a ligand-independent process, thus controlling their basal levels and the sensitivity of cells to incoming signals. A second mechanism involves association of Smurfs with I-Smads (Smad6 and 7) in a signaling-dependent manner. The I-Smads function as adaptors, permitting the Smurfs to target the activated type I receptor to the proteasome (Ebisawa et al., 2001; Kavsak et al., 2000; see Figure). In cell culture studies, Smurf1 primarily affects the response to BMPs while Smurf2 displays a broader specificity that allows it to interfere with both BMP and activin signaling. However, the results from injection assays in *Xenopus* suggest that the BMP pathway is more sensitive to overexpression of both Smurf1 and 2 (Zhang et al., 2001; Zhu et al., 1999).

The study by Podos et al. represents a significant advance since it analyzes the biological consequences of loss of Smurf activity. Mutations in *DSmurf* were isolated based on their ability to suppress the lethality resulting from partial loss of Decapentaplegic (Dpp) signaling. The BMP2/4 ortholog Dpp acts in a dose-dependent manner to pattern embryonic and adult structures, and is essential for viability. Strong evidence that *DSmurf* antagonizes BMP signaling in vivo comes from the fact that *DSmurf* mutants are rescued to viability by decreasing the *dpp* copy number, indicating that their lethality results from an elevated level of Dpp signaling. The authors were able to distinguish two primary patterning defects in *DSmurf* embryos—alterations in Dpp target gene expression during early development, and a temporal delay in downregulation of Dpp signaling at later stages of gastrulation. At cellular blastoderm, a gradient of Dpp activity specifies multiple thresholds of gene expression in the dorsal region of the embryo. Using phosphorylated MAD (P-MAD) as a read-out for Dpp signaling, Podos and colleagues show that at blastoderm stages, P-MAD accumulates in a wider band of dorsal nuclei in *DSmurf* embryos compared to wild-type animals. Consistent with this increase in the amplitude of the Dpp signal, the domains of several target genes are expanded. Surprisingly, these changes do not have any significant effect on late cuticular patterning. A second striking defect in *DSmurf* mutants is that the initial accumulation of P-MAD in dorsal cells is not downregulated during gastrulation. This abnormal perdurance of MAD in the hindgut primordium results in misregulation of gene expression and consequent disruption of gut development. The authors favor the idea that the early



#### Downregulation of TGF-β Signaling through Smurf Function

Smurfs are E3-ubiquitin ligases that antagonize TGF-β signal transduction through several mechanisms. Interaction with the R-Smads through a specific PY motif targets their degradation to maintain of a low basal level in the absence of signaling. In an alternate process that is signaling dependent, the Smurfs can target activated type I receptors and enhance receptor turnover. Inhibitory Smads that are induced by signaling serve as adaptors in this process. The consequence of both these activities is an attenuation of phosphorylated Smad accumulation in the nucleus. It appears that nuclear Smads are also sensitive to signaling-dependent ubiquitin-mediated degradation, although thus far there is no evidence to link Smurfs to this mode of negative feedback.

defects encountered in *DSmurf* mutants result from an increased cellular pool of MAD, while the later loss of temporal regulation predicts a role for DSmurf in receptor turnover. The first model assumes that the cytoplasmic pool of MAD is limiting during early embryogenesis. There is some evidence that activin signaling is sensitive to dosage of Smad2 in *Xenopus*. However, it is not clear that similar conditions prevail in *Drosophila*. In injection experiments, excess MAD does not elicit a response, while overexpression of constitutively activated Dpp receptor Thickveins results in hyperphosphorylation of MAD, arguing that MAD protein is not the limiting component (Hudson et al., 1998). An alternative mechanism not considered here, but consistent with the mutant phenotype, is that DSmurf enhances the nuclear turnover of Smads. Future mechanistic studies will no doubt resolve this issue and whether DSmurf has any effect on activin signaling in flies. Finally, the *DSmurf* phenotype strongly suggests that additional Smurf-like activities remain to be discovered. As the authors point out, P-MAD is eventually cleared from the nucleus even in *DSmurf* mutants, and the few escaper flies that survive to adulthood lack overt phenotypic defects despite the extensive requirement for *dpp* in growth and patterning of imaginal discs. Thus, it seems likely that the world will be an even "Smurfier" place in years to come.

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#### Selected Reading

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