[14]. This motif resides in the first 231 amino acids of Lig1, which were removed for crystal structure analysis, but Figure 2B illustrates how these two proteins would be juxtaposed while on DNA. From studies with *E. coli* [15], the PCNA clamp is presumed to be left behind on an Okazaki fragment after the DNA polymerase has finished extending it to a nick. The abandoned PCNA clamp may act as a marker to recruit Lig1 and may help orient it as well. Lig1 may also employ the sliding clamp to track along DNA until it locates a nick.

Provided Lig1 binds PCNA while both proteins encircle DNA, as modeled in Figure 2B, Pascal *et al.* [3] make the point that Lig1 would effectively mask all three protein binding sites on the PCNA trimer, thereby excluding other proteins from binding the clamp. This steric exclusion argues against the 'toolbelt' model in which trimeric PCNA simultaneously binds three different replication proteins [16]. For example, instead of FEN1 and Lig1 both binding PCNA simultaneously during Okazaki fragment maturation, FEN1 would need to be dislodged from PCNA in order for Lig1 to interact with the clamp. How FEN1 and Lig1 switch places on PCNA, and whether Lig1 has a specific mechanism to displace FEN1 from PCNA must await future studies.

The structural snapshot of the ligase ring about to seal the nick brings to mind new questions. How does a protein encircling DNA dissociate after sealing the nick? Perhaps the rigidity of the fully double-stranded DNA product provides energy to open the Lig1 ring. How does Lig1 assemble onto DNA? Does the enzyme adenylation step destabilize and open the ring? Alternatively, ligase may not be a stable ring without DNA. Indeed, the *T. filiformis* ligase–AMP structure requires large conformational changes to bind DNA as a ring. Finally, how does ligase integrate its actions with other binding partners? These questions and many more suggest that exciting studies of this fascinating enzyme will continue well into the future.

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Cell Signaling: Wingless and Glypicans Together Again

The role of the Glypican proteoglycans in Wingless signaling has been controversial. New studies show that the Glypican Dally-like can have both positive and negative effects on Wingless signaling; moreover, signaling can be regulated by removing Dally-like from the cell surface.

Seth S. Blair

The proteoglycans are a major component of cell surfaces and the extracellular matrix [1]. They are made from a core protein decorated with one or more glycosaminoglycan side chains, unbranched carbohydrate polymers made of disaccharide subunits. The two major families of cell surface proteoglycans are the transmembrane Syndecans, which are decorated with the glycosaminoglycans heparan sulfate and chondroitin sulfate, and the Glypicans, which are decorated with heparan sulfate and are anchored to the cell surface via a glycosylphosphatidylinositol (GPI) linkage.

Proteoglycans play a number of different roles, but one of the most intriguing is the regulation of signaling between cells. Specific glycosaminoglycans can recognize and bind members of several different families of signals and, in humans, defects in Glypicans induce Simpson-Golabi-Behmel

syndrome, associated with tissue overgrowth.

A number of researchers have therefore been examining how proteoglycans regulate developmental signals. Examples include the Syndecans, which can form a complex with fibroblast growth factor (FGF) and its high-affinity receptor, and the two *Drosophila* Glypicans, Dally and Dally-like protein (Dlp), which have been shown to strongly affect signaling via FGF, Hedgehog (Hh) and the BMP family member Decapentaplegic (Dpp).

The Wnts are another family of widely used developmental signaling molecules, including the *Drosophila* protein Wingless (Wg). Do proteoglycans also affect Wnt signaling? *In vitro*, both heparan sulfate and chondroitin sulfate have been shown to bind Wg, and treatments that remove heparan sulfate or chondroitin sulfate from cells reduce Wg reception [2]. *In vivo*, the levels of Wg signaling and extracellular Wg are reduced by mutations that block the synthesis of heparan sulfate chains [3–7].

But it is not known which heparan sulfate proteoglycans (HSPGs) are responsible for these effects, and the role of the *Drosophila* Glypicans has been controversial. Wg accumulates around cells misexpressing Dlp, and it was claimed that reduction of Dally or Dlp from the embryo resulted in mild reductions in Wg signaling [3,4,8]. But a more recent study [9] found no evidence of such reductions.

If the Glypicans do play a role in Wg signaling, it is clear that they cannot account for all the effects of removing heparan sulfate. A further problem encountered by the embryonic studies is that the continued expression of *wg* in the embryo requires Hh signaling; as Dally and Dlp are required for strong Hh signaling, it becomes difficult to tease apart the two pathways.

One group [10] has avoided these problems by using an *in vitro* signaling assay. Two other groups [11,12] have examined Wg signaling instead in the wing imaginal disc, the tissue that gives rise to the adult wing of *Drosophila*. Wg is secreted by a narrow stripe of cells along the prospective margin of the wing, helping to set up positions along the proximo-distal axis of the wing blade. Hh and Dpp signaling, on the other hand, set up positions along the anterior–posterior axis of the wing, and do not obviously influence Wg signaling. Wg also acts over a longer range in the wing disc than in the embryo, and thus the disc might provide the more sensitive signaling assay.

The effects of Dlp loss turn out to be surprisingly complex. In the wing disc, Wg signaling was reduced distant from *wg*-expressing cells of the wing margin, but closer to the wing margin it was enhanced [11,12]. Clones lacking Dlp had slight reductions in reception, but at clone boundaries reception was higher. These effects correlated with changes in the distribution of extracellular Wg; in Dlp mutant discs levels were higher near the margin, and levels were also higher at the boundaries of clones.

Similar effects were observed *in vitro* [10]; Dlp suppressed high levels of Wg signaling, but enhanced low levels. The reductions in Wg signaling are probably due to a slight reduction in the accumulation of Wg when levels are lower, while Dlp may be sequestering a significant fraction of Wg from the Wg receptors when levels are higher.

However, the buildup of excess Wg observed after loss of Dlp also suggests a failure in the movement of Wg away from the wing margin, and is reminiscent of effects of HSPGs on Hh and Dpp movement in the wing. How might the HSPGs stimulate the movement of ligands?

In the case of Hh, a skeptic might argue that HSPGs affect the stability of Hh, rather than its movement *per se*, as the absence of HSPGs reduces Hh levels even around Hh-secreting cells [6,7]. Thus, in the absence of HSPGs the ligands may be broken down, and their failure to signal over long distances could simply reflect their loss en route. But this does not fit the Wg data, as in the absence of Dlp the levels of Wg appear higher than normal [11,12]. Moreover, if the levels of free ligand were lowered in clones lacking HSPGs, the clone should act as a sink for diffusible ligand. In fact just the opposite is observed, as abnormally high levels of Wg, Hh and Dpp can build up at the boundaries of clones lacking HSPGs [7,12,13].

So it appears that ligands cannot move as easily into and through such clones. One explanation for this relies on a variation of a ‘bucket-brigade’ model of ligand movement, with the ligands being handed from one cell-bound HSPG to the next, moving along cell surfaces and between adjacent cells [5,7]. However, HSPGs can also be shed from cells [1], raising the possibility that a fraction of Dlp is free to diffuse.

New evidence provides a mechanism for freeing Dlp from the cell surface. Notum (a.k.a. Wingful) is a protein secreted by the edge cells of the wing margin; it has similarity to α/β-hydrolases, and can affect Wg signaling and modify Dlp [12,14,15]. The evidence suggests that Notum stimulates cleavage of the GPI linkage that anchors Dlp to the cell membrane [11]. Intriguingly, in *notum*$^-$ mutants, excess Wg and Wg signaling build up near the wing margin [14,15].

Another model that has been proposed to explain the movement of ligands is that they are passed through cells via a process termed planar transcytosis; the ligand is endocytosed and re-secreted on another surface of the cell. Mutations that block endocytosis can reduce the range of Wg and Dpp movement (although apparently not that of Hh [5,16]). Moreover, it was reported a lack of endocytosis limits Dpp signaling through a cell clone, throwing a ‘shadow’ of low signal in the wild-type cells on the far side of the clone [17]. In this model, HSPGs might be required for normal endocytosis or transport of ligand. Alternative explanations of the shadow phenotype have been debated pro and con [18,19]. But a recent study [13] on Dpp signaling in the

wing has also called the existence of such shadows into question.

In fact, it may not be necessary to invoke either bucket-brigade or transcytosis to explain the effects of HSPGs on ligand movement; simple diffusion may be sufficient. The predicted effects of HSPGs on ligand diffusion depend in part on whether the HSPG-bound ligand has access to the higher-affinity receptors. Receptor binding can limit the range of ligand movement by lowering the levels of unbound, diffusible ligand and, via endocytosis, clearing ligand from the extracellular space. Binding to HSPGs might protect the ligand from the receptor, giving it a chance to diffuse over a longer range. Similar models have been proposed to explain the positive effects of the Dpp-binding Chordin homolog Short gastrulation on long-range Dpp signaling in the *Drosophila* embryo (for example, see [20]).

All of this serves to remind us how poorly we understand the movement of signaling molecules through tissues. Add a few more wrinkles, such as the signaling-dependent and endocytosis-dependent changes in the levels of receptors, HSPGs, and their modulators, and testing alternative hypotheses becomes a difficult problem for experimentalist and theorist alike.

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Photoreceptor Evolution: Ancient Siblings Serve Different Tasks

Photoreceptor cells of vertebrate eyes are fundamentally different from those of invertebrate eyes. New work on the brain of a ragworm now suggests that ancestral bilaterians possessed both types of photoreceptor cell.

D-E. Nilsson

Our view of eye evolution has changed several times in the past 30 years. In 1979, Hansjochem Autrum [1] argued that all eyes share an evolutionary connection through the consistent use of membrane-bound rhodopsin as a photopigment. He also noted that, throughout the animal kingdom, photoreceptors are primarily of two different kinds, rhabdomeric and ciliary, coexisting in the major branches of the phylogenetic tree.

The first serious challenge to this view was a survey of photoreceptor cell ultrastructure which claimed independent evolution in 40 to 65 cases in separate phyletic lines [2]. More than a decade later, the discovery of homologous genes controlling eye development in vertebrates, insects and several other animals seemed to suggest that all eyes of recent animals can be traced back to the eyes of a common ancestor [3,4].

The monophyletic eye hypothesis received justified criticism [5–7], because it did not account for the fundamentally different transduction mechanisms in the ciliated photoreceptors of vertebrates and rhabdomeric photoreceptors of invertebrates,