2003; Moloney et al., 2000). Moreover, there are other proteins with multiple EGF domains, but Notch appears to be unique in its functional requirement for *Ofut1* and *rumi*. Once the structural basis for their influence is determined, the logic behind this may become clear. For now, the discovery of the *rumi O*-glucosyltransferase is another sweet success from studies of Notch signaling in the fly.

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A New Look at Cytokine Signaling

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Signal transduction is initiated when a cytokine binds to the extracellular domains of its receptors, bringing them together and triggering a complicated sequence of events inside the cell. In this issue, LaPorte et al. (2008) present crystal structures of three signaling complexes of the cytokines interleukin-4 and interleukin-13 with their receptors, showing how events taking place outside the cell may affect the specificity of signal transduction.

Signaling by the cytokines interleukin-4 (IL-4) and interleukin-13 (IL-13) is critical for T cell development and T cell-mediated immune responses, including allergy. IL-4 and IL-13 signal through a heterotrimeric complex consisting of the cytokine and two receptors. The receptor complexes for IL-4 come in two varieties, type I and type II. Type I receptor complexes comprise the IL-4Rα and γ-chain (γ_c) subunits. The presence of the γ_c subunit defines type I complexes and γ_c is present in receptor complexes for at least six different cytokines (Kelly-Welch et al., 2003). The type II receptor for IL-4 is a complex of IL-4Rα and IL-13Rα1, which also serves as a receptor for IL-13. However, despite signaling through the same type II receptor, IL-4 and IL-13 initiate distinct signaling responses even in the same cell types. In this issue, LaPorte et al. (2008) present crystal structures of the complexes of IL-4 and IL-13 with their soluble receptors. Their findings reveal how the γ_c receptor might be able to function in multiple cytokine signaling complexes and show how different ligands can mediate distinct signaling responses from a shared set of receptors.

The first picture of an interaction between a growth factor and a receptor was revealed more than 15 years ago with the pioneering publication of the structure of a signaling complex of human growth hormone (hGH) and the extracellular soluble domains of its receptor GHR (de Vos et al., 1992). A single molecule of hGH binds to equivalent regions of two receptor molecules, forcing them to form dimers. Structural and biochemical data supported the notion that the signaling complex formed sequentially. The first step involved binding of hGH to one receptor molecule, forming the ligand-receptor interface I (Figure 1A). In the second step, another receptor molecule binds to the ligand through the interface site II. Only a few other crystal structures before or since have had such an impact and explained as much.

With the availability of the hGH/GHR structure, as well as crystal structures of free cytokines (Rozwarski et al., 1994), a plethora of models of other complexes have been generated, including that of IL-4 with its receptors (Gustchina et al., 1995). Although these models explain at least some of the signaling properties correctly, subsequent crystal structures, including a binary complex of IL-4 and IL-4α (Hage et al., 1999), have shown them to be oversimplified. In addition, understanding the close structural and biological relationships between different cytokines, such as IL-4 and IL-13, has

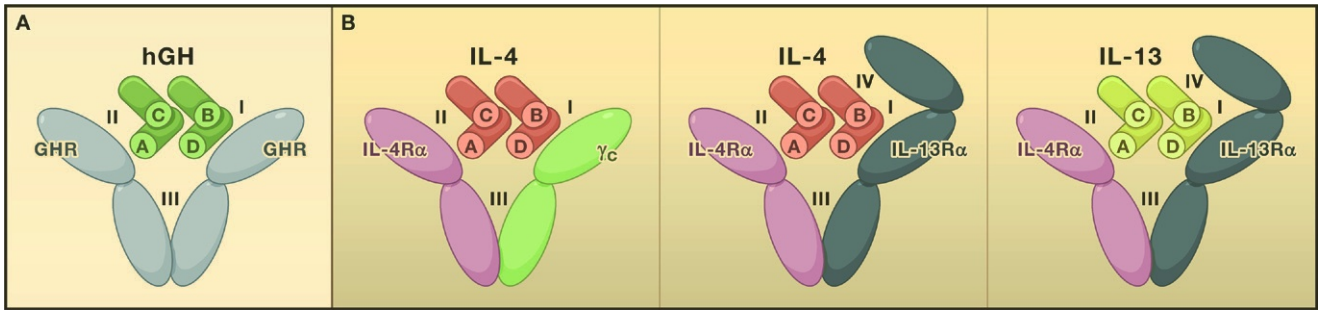

Figure 1. Complexes of Helical Cytokines and Their Receptors

(A) Complex of human growth hormone (hGH) with two identical receptor (GHR) molecules. The complex contains two ligand-receptor interfaces (I and II) located on the opposite sides of hGH, as well as the third inter-receptor interface (III) formed by the C-terminal regions of the receptors located in the proximity of the plasma membrane. The arrangement of the receptor molecules in the complex shows approximate two-fold symmetry, although the ligand itself is completely asymmetric.

(B) IL-4 and IL-13 are short four-helix bundle cytokines (Rozwarski et al., 1994; Presnell and Cohen, 1989) sharing 25% sequence identity. Depicted are complexes studied by LaPorte et al. (2008). (Left) The type I complex of IL-4 with IL-4Rα and γ_c; (middle) the type II complex of IL-4 with IL-4Rα and IL-13Rα1; (right) the type II complex of IL-13 with IL-4Rα and IL-13Rα1.

been slow to emerge (Obiri et al., 1995). That situation, however, is now changing with the publication of the new structures of the ternary complexes of IL-4 and IL-13 with their receptors (LaPorte et al., 2008), which markedly improves our understanding of how these cytokines induce their signaling cascades.

One of the structures presented by LaPorte et al. depicts the complete type I complex consisting of IL-4, IL-4Rα1, and γ_c (Figure 1B). The other structures represent type II ternary complexes of IL-4 and IL-13 bound to the same two receptors, IL-4Rα and IL-13Rα1 (Figure 1B). In all three complexes, IL-4Rα binds to the ligand interface, which is structurally equivalent to site II of the hGH/GHR complex, whereas γ_c and IL-13Rα bind to the equivalent of site I of hGH/GHR (Figure 1B). In the structure of hGH/GHR, site I is the high-affinity (primary) receptor binding site, whereas site II is the weaker (secondary) receptor binding site. IL-13 behaves in the same way as hGH in the hGH/GHR complex. However, in the complexes of IL-4, both type I and type II, it is site II that exhibits high affinity and site I exhibits low affinity. Interactions between the two receptor molecules in each of the three complexes are similar to those in the hGH/GHR complex. IL-13Rα1 is a unique molecule that consists of three fibronectin type III domains instead of the usual two found in IL-4Rα and γ_c. This additional domain was also found to interact with the ligands in type II complexes through binding site IV (Figure 1B). Similar to the hGH/GHR complex, the formation of the ternary complexes was also found to be sequential. At first, the ligand binds to the high-affinity receptor (IL-4Rα in the case of IL-4 complexes, and IL-13Rα1 in the case of IL-13), and only then does the low-affinity receptor complete the signaling complex.

A structure of the quaternary complex of IL-2 with IL-2Rα, IL-2Rβ, and γ_c was previously determined by the same laboratory (Wang et al., 2005). The interfaces of IL-2 and IL-4 with γ_c are very similar. The presence of the structurally equivalent amino acid residues found on the surfaces of IL-2 and IL-4 suggests that these residues very likely constitute the γ_c recognition motif, which also should be present on other cytokines that use the γ-chain for signaling.

The structures of LaPorte et al. provide a new opportunity to analyze the influence of the interactions occurring outside the plasma membrane on the signaling that occurs inside the cell. It has been commonly accepted that the principal role of the ligand is simply to bring together the appropriate receptors, with the subsequent chain of events inside the cell already predetermined. Such a concept is based on the fact that several chimeras of cytokine receptors, with swapped extracellular domains, still initiate proper signaling. Examples include replacement of the extracellular domain of erythropoietin by an equivalent domain of prolactin, with the resulting chimera providing signals directing formation of red blood cells (Socolovsky et al., 1998). Even small polypeptides that are able to bind to the erythropoietin receptor can initiate signaling (Wrighton et al., 1996). However, this mode of receptor activation does not seem to apply to the type II complexes of IL-4 and IL-13. Although the complexes of these cytokines with their receptors appear to be structurally similar, their biological role is quite different. Binding of both cytokines results in formation of the same IL-4Rα/IL-13Rα1 heterodimer, but in one case it is IL-4Rα that binds to the ligand first and in the other it is IL-13Rα1 that binds first. The different order of association in the complex, the different affinities, as well as variations in the concentration of the ligand and the receptors on the plasma membrane affect the whole signaling process and influence its specificity. Understanding exactly how that happens on a molecular level remains a challenge for both cell and structural biologists.

There is so much new that we can learn from the work of LaPorte et al., yet the overall picture is still not completely clear. And we thought we understood it all 15 years ago!

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Mps1 Checks Up on Chromosome Attachment

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The protein kinase Mps1, a crucial regulator of the spindle-assembly checkpoint, now turns out to be essential for correcting errors in chromosome attachment (Jelluma et al., 2008). Mps1 exerts this effect by regulating the activity of the Aurora B kinase through phosphorylation of its partner protein Borealin.

Cell division is a tricky business. The goal is to produce healthy daughter cells containing the same genetic information as the mother cell, but the road to accurate cell division is riddled with obstacles. Assaults from outside the cell, such as radiation or chemicals that cause DNA damage, or occasional stochastic errors inside the cell threaten the required preciseness of this intricate molecular process. To cope with these obstacles to faithful division, cells have evolved surveillance mechanisms called "checkpoints" (Hartwell and Weinert, 1989). Checkpoints operate at certain key transitions, where they delay subsequent events unless all prior steps have been executed correctly. The spindle-assembly checkpoint is one such checkpoint (Musacchio and Salmon, 2007). It only allows cells to proceed to anaphase once all chromosomes have become correctly attached to the microtubules of the mitotic spindle, ensuring their accurate distribution to the two daughter cells. The core components of the spindle-assembly checkpoint include the proteins Mad1-3, Bub1, and Bub3 as well as the kinase Mps1. Now, Jelluma et al. (2008) report in this issue that Mps1 is not only a crucial regulator of the spindle-assembly checkpoint but is also essential to correct chromosome attachment errors through its effect on the chromosomal passenger complex (CPC) protein Borealin (Figure 1).

According to the strict definition, checkpoints, being mere surveillance mechanisms, should not be essential for the proper execution of cell division in the absence of any perturbation. This is true for the spindle-assembly checkpoint in yeast, where depletion of the core component Mad2 has hardly any effect on unperturbed cells. However, some of the classical checkpoint proteins do not meet this definition. Their absence clearly impairs cell function, even without any additional perturbation to challenge the checkpoint. The reason is that these proteins go "moonlighting;" that is, they carry out additional cellular functions. One well-known example is the budding yeast checkpoint kinase Mps1, which is required for the duplication of the spindle-pole bodies (the yeast equivalent of centrosomes) that organize the mitotic spindle.

In their new work, Jelluma and colleagues (2008) now add another moonlighting job to the list for human Mps1 (Jelluma et al., 2008). Like its yeast counterpart, human Mps1 is essential for the spindle-assembly checkpoint (Musacchio and Salmon, 2007), but, as the authors report, Mps1-deficient human cells also fail to properly attach all of their chromosomes to the mitotic spindle. The type of attachment defect observed after blocking Mps1 activity is reminiscent of the defect observed after inhibition of another mitotic kinase, Aurora B. This kinase associates with INCENP, Survivin, and Borealin/Dasra to form the CPC, which has a well-established role in correcting chromosome attachment errors (Ruchaud et al., 2007) (Figure 1). Current evidence suggests that correctly