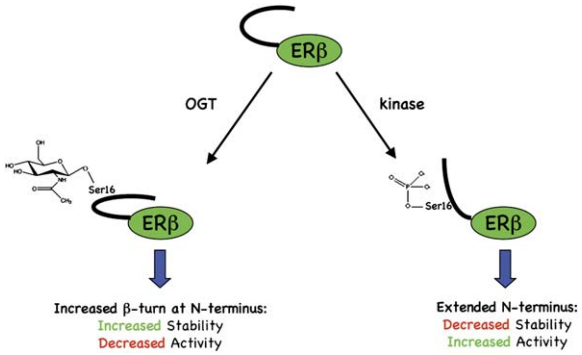

Figure 1. Regulation of Estrogen Receptor β by Alternate O-GlcNAcylation or O-Phosphorylation at Ser^{16}

O-GlcNAcylation by O-linked N-acetylglucosamine transferase (OGT) at Ser^{16} enhances the β-turn conformation at the N terminus, leading to decreased ER-β activity, but it also results in stabilization of the protein, increasing its half-life within the cell. In contrast, O-phosphorylation at Ser^{16} causes the N terminus to adopt a more extended conformation, leading to increased transcriptional activity, but also causing the protein to become rapidly degraded within the cell.

amenable to widespread use. Despite the considerable chemical and methodological challenges remaining, sorting out the distinctive functions of O-GlcNAcylated or O-phosphorylated forms of regulatory molecules will be key to understanding the regulation of signaling and transcription and the assembly of the cytoskeleton, as well as being important for developing treatments for diabetes, neurodegenerative disorders, and cancer.

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A Noncanonical Path to Mechanism of Action

Improved methods for discovering small-molecule mechanisms of action are needed. In this issue of *Chemistry & Biology*, Zhang et al. [1] make clever use of the zebrafish to study the mechanism of the angiogenesis inhibitor fumagillin and reveal that it targets the noncanonical Wnt pathway.

Few things are more intriguing in the world of chemical biology than a small molecule with pronounced biological activity and therapeutic importance for which little is known of its mechanism of action. Deciphering these molecules' mechanisms of action can provide surprising biological insights and avenues for novel therapeutic approaches. One such small molecule is fumagillin, a natural product serendipitously discovered to inhibit endothelial cell proliferation. Fumagillin and its derivative TNP-470 are potent inhibitors of angiogenesis and have been the focus of numerous basic and clinical studies [2]. Although fumagillin has been shown to target methionine aminopeptidase 2 (MetAP-2) [3], little is known about how MetAP-2 inhibition exerts its antiangiogenic influence.

In this issue of *Chemistry & Biology*, Zhang et al. [1] employ a clever combination of in vivo and in vitro experiments to uncover an unexpected connection between MetAP-2 and noncanonical Wnt signaling. Their approach takes advantage of the zebrafish, a genetic model organism for which thousands of mutations and their phenotypes have been characterized and cataloged [4, 5]. Each mutant zebrafish embryo can be thought of as providing a three-dimensional readout of a gene's biological activity. The rich variety of zebrafish phenotypes makes it possible to characterize in detail the effects of disrupting a single gene product and to link a specific embryonic phenotype with the molecular disruption that causes it. The authors reasoned that treating zebrafish embryos with TNP-470 might also produce a distinct phenotype, and that by comparing the resultant phenotype with those cataloged for zebrafish gene mutations, they might be able to learn what pathway is targeted by TNP-470 (Figure 1).

In many cases, zebrafish embryos can be treated with small molecules simply by adding the molecules to the water in which the embryos are developing [6]. When the authors exposed developing zebrafish embryos to fumagillin or TNP-470, the embryos developed a defect in the anterior-posterior axis that resulted in a severely truncated tail. They also observed the same developmental defect in zebrafish embryos in which MetAP-2

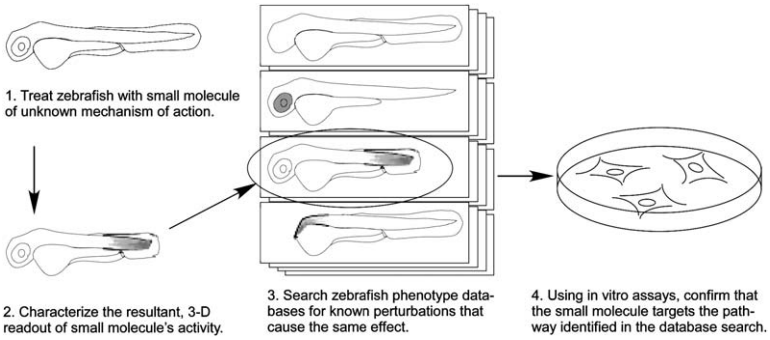

Figure 1. Use of Zebrafish for Deciphering Small-Molecule Mechanisms of Action

Treating zebrafish embryos with a small molecule often generates a distinctive phenotype. By comparing the phenotype with databases of existing zebrafish phenotypes, candidate targets for the small molecule can often be identified. As described in this issue, fumagillin causes a truncated tail phenotype similar to that caused by Wnt5 mutation and other perturbations of noncanonical Wnt signaling.

expression was knocked down using antisense morpholino oligonucleotides (morpholinos). By examining descriptions of previously characterized zebrafish phenotypes, the authors recognized that the fumagillin/MetAP-2 phenotype was similar to that of the Wnt5 mutant *pipetail* and to embryos injected with Wnt5 morpholinos [7]. These observations suggested an unexpected link between MetAP-2 and Wnt5. The idea of a MetAP-2/Wnt5 connection was reinforced when the authors injected zebrafish with lower doses of morpholinos targeting MetAP-2 and Wnt5. Doses that produced only mild tail phenotypes individually produced a severe tail truncation phenotype when combined, suggesting an interaction between MetAP-2 and Wnt5.

The fact that fumagillin-treated zebrafish phenocopy Wnt5 mutants suggests that fumagillin may exert its biological effects through inhibition of Wnt signaling. The Wnt proteins are a large family of diffusible extracellular ligands that perform central functions during numerous developmental and disease processes [8]. The various Wnt proteins share some common downstream effectors, but two rather different classes of signal can be transduced. The canonical Wnts signal through the receptor Frizzled1, resulting in the stabilization of β-catenin, which translocates to the nucleus and promotes T cell factor (TCF)-dependent transcription [8]. Noncanonical Wnt signaling, by contrast, is mediated through the receptor Frizzled2 and results in intracellular calcium release and actin cytoskeleton rearrangement (see Zhang et al. [1], Figure 1).

After their zebrafish experiments suggested Wnt signaling in the fumagillin mechanism of action, the authors turned to cultured cells to distinguish between effects on canonical and noncanonical signaling. A mouse teratocarcinoma system was used that enables specific activation of canonical or noncanonical Wnt signaling. Activation of either pathway leads to differentiation of the cells into primitive endoderm. Crews and colleagues showed that TNP-470 treatment or siRNA-mediated knockdown of MetAP-2 could block primitive endoderm differentiation mediated through noncanonical Wnt signaling, but not through canonical Wnt signaling. This remarkable result suggests that TNP-470 blocks noncanonical Wnt signaling without affecting the canonical pathway, making it the first small molecule known to specifically target this pathway.

Zhang et al. went on to localize TNP-470's point of action further, placing it downstream of Frizzled2 and upstream of the noncanonical Wnt effectors CamKII, c-Jun, and RhoA. They stopped short, however, of identifying a relevant enzymatic substrate for MetAP-2 itself, a point that will undoubtedly receive significant future attention. Additional effort is also needed to solidify the importance of noncanonical Wnt signaling in mediating TNP-470's antiangiogenic effects. The authors take a step in that direction by demonstrating that an activated form of the Wnt effector Disheveled-2 can partially rescue endothelial cells from the cytostatic effect of TNP-470.

The identification of fumagillin/TNP-470 as specific inhibitors of noncanonical Wnt signaling will likely be of great interest to those studying the Wnt pathway. Substantial overlap between the canonical and noncanonical pathways has made it difficult, in many instances, to distinguish between canonical and noncanonical signals [8]. A small molecule capable of specific inhibition of noncanonical signaling may be helpful in this regard. In addition, the Wnt pathway is required for multiple processes at numerous stages of development. A small molecule capable of inactivating the pathway conditionally (e.g., with temporal control) should facilitate study of later processes without the complications of disrupting earlier Wnt signals.

Beyond fundamental research, the discovery of a link between noncanonical Wnt signaling and angiogenesis may have therapeutic implications. If inhibition of noncanonical Wnt signaling blocks angiogenesis, then components of the noncanonical Wnt pathway might be viewed as potential new targets for antiangiogenesis. Given that pathway inhibition causes a distinctive tail truncation phenotype in zebrafish, it is easy to envision roles for the zebrafish in discovery of novel noncanonical Wnt inhibitors. For example, large-scale small-molecule screens are feasible in the zebrafish [6], and one could screen for small molecules that induce a tail truncation phenotype like that seen with fumagillin treatment, MetAP-2 knockdown, and Wnt5 mutation. Alternatively, traditional angiogenesis assays could be used to identify active small molecules, with the zebrafish used to identify those that function via the noncanonical Wnt pathway.

Could the combination of zebrafish phenotyping and cellular assays employed so effectively by Crews and coworkers become a standard approach for deciphering small-molecule mechanisms of action? The authors of this report clearly benefited from the fact that MetAP-2 inhibition causes a distinctive phenotype that was represented in existing zebrafish databases. It is unlikely that every small molecule would cause such a distinctive phenotype, or that every phenotype could be so readily associated with a specific biological pathway. Nevertheless, the report by Zhang et al. clearly demonstrates the

power of the high-resolution phenotyping afforded by zebrafish and the value of the large-scale databases linking phenotype and genotype in this organism. As these databases progress toward completeness, the validity of the approach will continue to increase. Although this strategy is unlikely to be universally effective, the fumagillin success makes a strong case for considering the approach as a potent, albeit "noncanonical," path to deciphering small-molecule mechanisms of action.

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A Stability Switch for Proteins

A paper published in the September 8 issue of *Cell* [1] describes a generally applicable approach for chemical control of protein stability, with potential for broad use in chemical genetics.

An oft-stated goal for chemical biology is to provide new tools for analyzing gene and protein function that complement classical genetics. This aspiration, encapsulated in the phrase "chemical genetics," is increasingly being fulfilled as chemical probes are used to inhibit proteins of interest and generate chemically-induced alleles of protein function [2].

What chemistry has to offer genetics above all else is the dimension of time [2, 3]. Essentially, small molecules can be used to effect "instant mutagenesis" by specifically inhibiting (or activating) a protein of interest in real time. By contrast, generation of knockout mice to look at the functional consequences of protein loss is a slow process, often stymied by embryonic lethality or confounding compensatory changes in gene expression. RNAi [4] provides a new way to knock down protein function, but is still slower to take effect as it does not directly target the protein. Thus, chemical approaches have the potential to illuminate otherwise invisible areas of biology.

Unfortunately, as a field we are not yet at a point where we can routinely generate suitable chemical inhibitors for every protein of interest. This is particularly true if in vivo studies are contemplated, meaning that the additional hurdle of pharmacology needs to be cleared. An alternative strategy is to "tag" a protein of interest in such a way that it can be targeted by a generic ligand—for example by fusing it to a heterologous protein [3]. This powerful approach in principle allows any protein of interest to be chemically manipulated in real time without the need for a specific inhibitor. "Tagging" strategies have both drawbacks and advantages. They assume that protein function is unaffected by the tag, and they also require genetic steps to express the modified protein in cells. In addition, because endogenous, unmodified protein molecules will be unaffected by the generic ligand, either the protein of interest needs to have a dominant effect, or the study needs to be performed in the background of a knockout of the endogenous protein. On the other hand, since the effects of the small molecule are restricted to cells expressing the tagged protein, these approaches can often be more precise than the use of a direct chemical inhibitor.

Many successful "tagging" strategies have been described [3]. For example, dimeric small molecules can be used to inducibly dimerize tagged proteins, allowing study of proteins and pathways controlled by protein-protein associations [5, 6]. Expression of protein kinases that are modified to bind certain inhibitors uniquely has allowed very precise delineation of the roles of individual kinases and substrates in cell biology [7]. Several of these technologies have "crossed over" from the field of chemical biology to become established mainstream biological research tools. However, they are generally restricted to subsets of proteins that share a certain mechanism of action. A truly versatile technology for chemically regulating protein function, generally applicable to any protein, is a highly desirable goal.

Such an approach was described a few years ago by Stankunas et al. [8], who developed a system for controlling protein stability—a shared property essential to all proteins (see Figure 1). These authors exploited the chance observation that a certain mutated protein