detected will certainly aid in elucidating their structures, identifying their biosynthetic pathways, and determining their role in *M. tuberculosis* biology.

Importantly, the quantitative and reproducible data generated from this LC-MS approach will allow for future comparative studies to probe dynamic changes in the global lipidome. It is clear that changes in lipid content allow for *M. tuberculosis* to adapt to a variety of different conditions, including upon encountering stress (Cunningham and Spreadbury, 1998) and during infection (Kondo et al., 1970). The technology developed by Layre et al. (2011) will allow for a systems approach to study these types of dynamic changes. Indeed, the authors have demonstrated that this method can sensitively detect mycobacterial species even in extracts from *M. tuberculosis*-infected tissue "contaminated" with eukaryotic lipids. Understanding the changes that occur to the lipidome during infection or conditions that mimic infection such as reactive oxygen or nitrogen stress, low iron, and hypoxia, will be important to understand the role of lipids in virulence and the metabolic changes that occur during infection.

Approaches to globally monitor nucleic acids, proteins, and the water-soluble metabolites within cells have revolutionized our understanding of *M. tuberculosis* biology. The absence of similar methodologies to monitor hydrophobic lipids has been a glaring deficiency, especially given the important roles of these molecules during infection. The lipidomic platform pioneered by Layre et al. (2011) will allow researchers to finally probe this final frontier of the mycobacterial cell.

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A Wnt Inhibitor with a Twist

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Although the clinical safety of compounds targeting the core components of the Wnt signaling pathway remains to be determined, a simple in vivo chemical screen identifies small molecules that inhibit Wnt signaling in a cell type-specific manner (Ni et al., this issue of *Chemistry & Biology*).

The Wnt/β-catenin signaling pathway is undoubtedly one of the most prominent biological pathways. In 1987, Roel Nusse's group showed that the mouse mammary oncogene *int-1* was the homolog of the *Drosophila* segment polarity gene *wingless* (Rijsewijk et al., 1987). Thereafter, the name "Wnt," derived from the combined names of wingless and int-1, rightly signifies its myriad roles in regulating embryonic development and the homeostasis of adult tissues. In addition, aberrant Wnt signaling may cause cancers and other diseases (Clevers, 2006). Although inhibitors that target Wnt signaling ubiquitously may seemingly have the broadest utility, their potential for pleiotropic effects on adult tissues remains a concern. Conceivably, inhibitors that target cell type-specific components of the Wnt pathway may have better safety profiles. Why has such a compound not turned up in the screens that have been performed? Is discovery of tissue-specific Wnt inhibitors possible? In this issue, Ni et al. (2011) provide the first example of a tissue-specific Wnt inhibitor and demonstrate its potential for expanding cardiac progenitor cells.

Myocardial infarctions cause significant clinical problems in Western society. Thus, cardiogenic compounds are sought after for they may reactivate cardiac progenitors and repopulate infarcted myocardium. Several signaling pathways have been implicated in the genesis of cardiac progenitors from pluripotent stem cells. Moreover, a number of targeted and screening approaches for the identification of cardiogenic compounds have been reported (Hao et al., 2008;

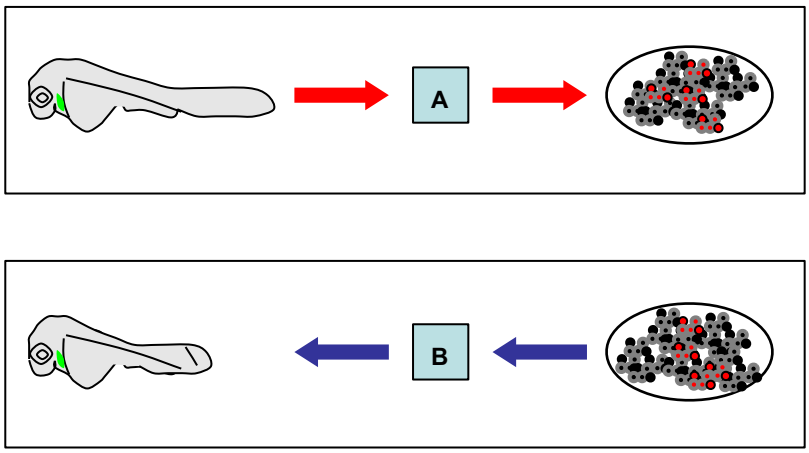

Figure 1. Two Ways to Discover Cardiogenic Small Molecules
The top panel shows the method utilized by Ni et al. (2011) for discovering cardiogenic small molecules. The bottom panel shows a more common method. In the top panel, cardiogenic compound A is identified via an in vivo chemical screen in embryonic zebrafish. It induces an enlarged heart phenotype without affecting any other aspects of embryonic development. Subsequently, compound A may show a similar cardiogenic effect on mouse ES cells. In the bottom panel, cardiogenic compound B is identified via a cell-based screen in mouse ES cells. Subsequently, compound B may show a similar cardiogenic effect in embryonic zebrafish while simultaneously inducing additional effects on some other aspects of embryonic development.

Wang et al., 2011; Willems et al., 2011). In the hope of discovering additional cardiogenic compounds, Ni et al. (2011) decided to shy away from the common approaches and conducted an unbiased chemical screen using embryonic zebrafish. Traditionally a favorable model for developmental biologists, zebrafish have gained popularity in the chemical biology field in the recent years. Embryonic or larval zebrafish can be adopted in various kinds of low to high-throughput screening methodology. Assays relying on more complex biological interactions may be developed in vivo. Furthermore, a whole-organism screening strategy enables the detection of desirable effects in the target tissues and any potential unwanted effects in other tissues simultaneously (Figure 1). In this study, Ni et al. (2011) conducted a simple chemical screen using an existing transgenic zebrafish line that exhibited green fluorescent cardiomyocytes to search for compounds that caused an enlarged heart phenotype. The authors identified three structurally-related compounds named Cardionogens that could do so without affecting any other aspects of embryonic development.

Ni et al. (2011) first confirmed that Cardionogen increased heart sizes by increasing cardiac progenitors and not by other less desirable mechanisms (such as increasing cardiomyocyte sizes). Next, an important hint of the mode of action of Cardionogen emerged from its effective time window during embryonic development. Although Cardionogen treated after gastrulation enhanced cardiogenesis, it adversely affected cardiogenesis if treated before gastrulation. Interestingly, the biphasic pattern of Cardionogen's effects is opposite to the known biphasic pattern of the Wnt effects on heart development (Naito et al., 2006; Ueno et al., 2007). This finding compelled the authors to perform a series of experiments, and they concluded that Cardionogen inhibited the Wnt/β-catenin signaling pathway in zebrafish heart and in mouse ES cells.

In the past two decades, most of the key players of the Wnt signaling pathway have been identified, such as the Wnt ligands, their transmembrane receptors, coreceptors, the β-catenin destruction complex and the Tcf/β-catenin transcription factors that ultimately activates the Wnt target genes (Clevers, 2006). In addition, Wnt signaling may be modified by other cell type-specific factors, though the identities and the regulation of these modifiers are far less clear. Several classes of small molecule Wnt inhibitors already exist today (Barker and Clevers, 2006; Chen et al., 2009; Huang et al., 2009). Previously, common strategies for discovering small molecule Wnt antagonists include in vitro assays to identify compounds that disrupt β-catenin-Tcf interaction and cell-based assays to identify compounds that inhibit Wnt/β-catenin-dependent gene activation. These methods have uncovered a number of potent Wnt antagonists that target the core components of the Wnt pathway (Barker and Clevers, 2006; Chen et al., 2009; Huang et al., 2009).

Although aberrant Wnt signaling is the key feature of some cancers and diseases, Wnt signaling also plays essential roles in renewing various adult tissues (Clevers, 2006). Thus, drugs that inhibit Wnt signaling ubiquitously may exert adverse effects on some renewing tissues. A line of evidence supporting this hypothesis came from the studies of a class of Wnt inhibitors called IWRs (Chen et al., 2009). It has been shown that, in addition to potently blocking Wnt/β-catenin activities in several types of cancer cells, IWRs also effectively blocked tailfin regeneration and renewal of intestinal epithelium in zebrafish (Chen et al., 2009). In this issue, Ni et al. (2011) also investigated the effects of IWR-1 on cardiogenesis. They showed that, like Cardionogen, IWR-1 induced cardiogenic effects on embryonic zebrafish and mouse ES cells. However, unlike Cardionogen, IWR-1 also disrupted development of embryonic posterior structures and resulted in a single-chambered heart (Figure 1). These results exacerbate the concerns for developing some existing Wnt inhibitors clinically.

From a clinical standpoint, regenerative medicines may require a more stringent safety profile than anticancer therapeutics do. In the report by Ni et al. (2011), the authors provided several pieces of information suggesting that Cardionogen antagonizes Wnt signaling in a cell type-specific manner, making it a desirable drug candidate. For example, Cardionogen inhibited Wnt3a-induced TOPflash luciferase activities in mouse ES cells but not in HEK cells. Moreover, Cardionogen reversed Wnt8-induced cardiac hypotrophy but not the small eye phenotype. Based on their results, the authors speculated that Cardionogen may target an intracellular or nuclear factor that modifies Wnt signaling. Unfortunately, the target of Cardionogen remains unidentified. Furthermore, it is not yet

known how similar embryonic and adult cardiac progenitors are. Although Cardionogen stimulates cardiac differentiation in mouse ES cells, until the ethical and practical issues of stem cell therapy are resolved, testing whether Cardionogen may directly induce the expansion of the adult cardiac progenitors in vivo is inevitably the final way to realistically assess its therapeutic potential.

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