# New Tools to Engineer Tissues for Research and Therapy

Bruce Conklin of the Gladstone Institute of Cardiovascular Disease.

**While stem cell biology has made incredible advances** in creating pluripotent cells, the next challenge is how to use these cells for research and therapy. To advance tissue engineering, we need ways to regulate how the pluripotent stem cells can be building blocks for making tissues for experimental or therapeutic purposes. Exciting recent advances in stem cell technology have great promise, making the need for advanced control mechanisms even greater.

Engineered receptors are one solution to this problem. Bruce Conklin of the Gladstone Institute of Cardiovascular Disease has developed a unique strategy to produce them. Called RASSLs, these designer receptors provide a valuable tool for dissecting the intricate pathways of normal cell signaling while filling a fundamental niche in tissue engineering by giving scientists the ability to control the signaling pathways within a cell.

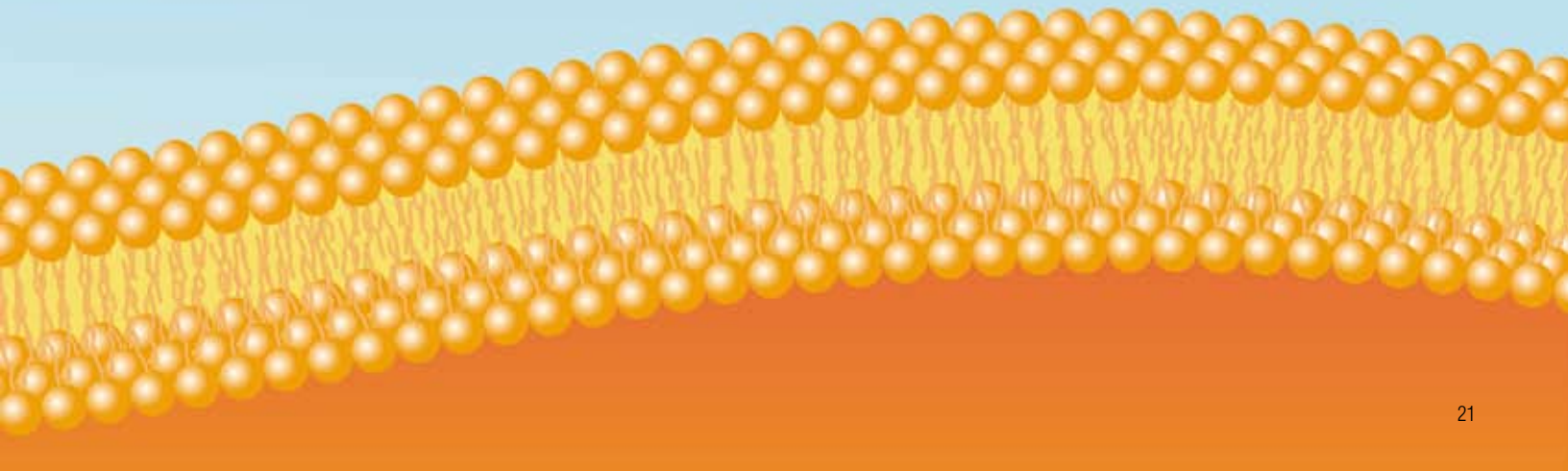
Cells must be poised to respond to a dizzying variety of extracellular signals, including smells, tastes, light, and hormones. Many of these signals are transmitted by G protein–coupled receptors (GPCRs). Within cells, GPCRs are regulated through effects on their location, stability, and signal duration—all of which are key components of the signaling process. This group is the largest family of cell-surface receptors in mammals. Because of their specificity, diversity, and physiological importance, GPCRs are targets for drugs that are used to treat disorders ranging from heart disease and chronic pain to schizophrenia.

For several reasons, GPCRs are an ideal base for building synthetic signaling systems. They have a relatively simple design that allows them to be transferred to different tissues and species. GPCRs control many processes (e.g., muscle contraction, heart rate, and cell growth) that are essential for tissue engineering. Some GPCRs act very rapidly—for example, they can decrease the heart rate in transgenic mice within seconds. Other control systems act deep inside the cell and may complement the unique level of control orchestrated by a GPCR expressed on the cell surface. Thus, GPCRs are the focus of intense pharmaceutical development, and potentially, any fundamental observation can be applied directly to them.

## RASSLs Provide Control over GPCR Signals with Established Drugs

The key to Dr. Conklin's strategy for making RASSLs was to take advantage of potent synthetic drugs (e.g., spiradoline) that had been developed as potential analgesics. His group made a RASSL by mutating an existing GPCR so that it no longer responded to its normal relatively large natural peptide ligands, but still responded to the drug spiradoline.

The first in this series, RASSL opioid 1 (RO1), has been expressed in at least six different tissues in transgenic animals to control



several physiological processes, such as heart rate and bitter and sweet taste sensations. These initial in vivo experiments yielded exciting results and fueled their efforts to develop more RASSLs with different properties.

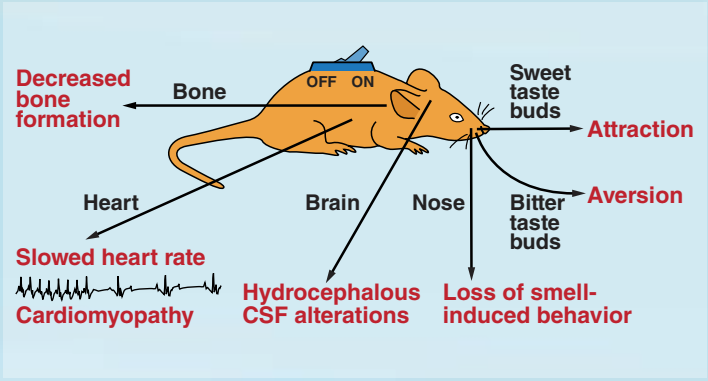
Dr. Conklin and other scientists have made other RASSLs that work with existing drugs and a wide variety of receptors. The use of existing drugs allows them to take full advantage of known pharmacology, and in several cases, the side effects have been negligible in animals.

## Controlling Tissue Engineering for Growth and Development

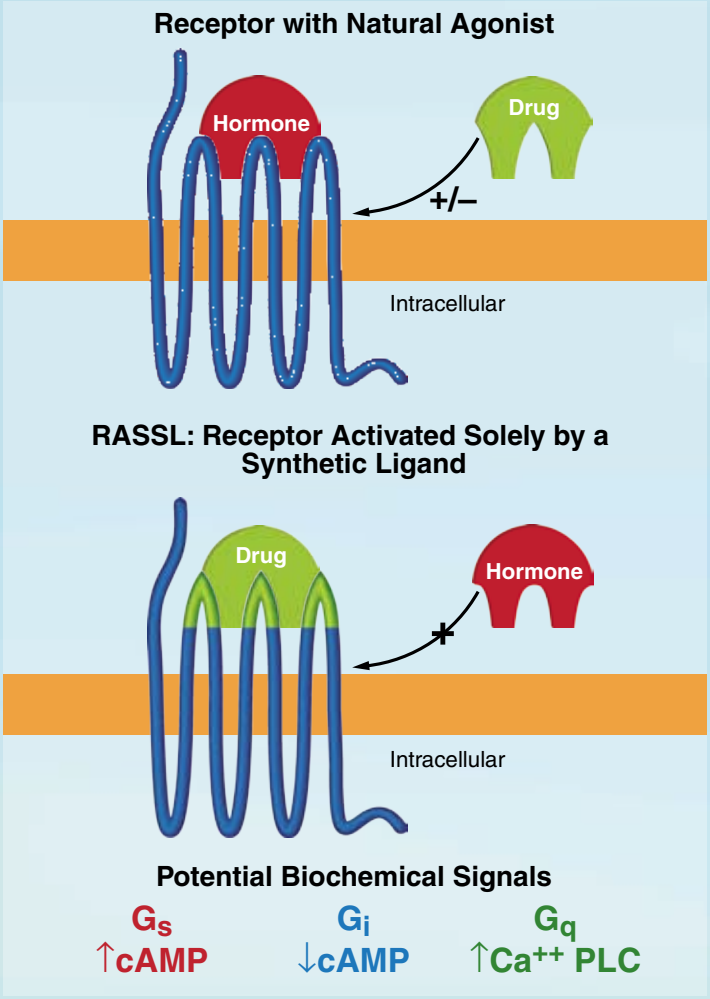
Because GPCR signaling is important for growth and differentiation of many tissues, RASSLs may be valuable in tissue engineering to repair damaged organs. In humans, mutations in signaling pathways cause multiple endocrine disorders, mental retardation, and severe bone deformities. GPCR pathway genes participate in many aspects of heart development. Likewise, several GPCRs have been implicated in the formation of vascular tumors, suggesting that this class of receptors is involved in vascular development. With this variety of potent responses, RASSLs can be used to direct the growth and development of many types of tissues. Ultimately, RASSLS may prove useful for rebuilding damaged hearts, livers, bones, and other organs.

## The Heart, Bone, and Nerves as Model Systems

The Conklin laboratory uses multiple model systems that each provide unique proving grounds for the RASSL technology. The pacemaker of the heart is particularly ideal for detecting rapid responses since it is exquisitely sensitive to RASSL signals that can control beat rate. Studies of heart rate control have great medical relevance since abnormal heart beat ("arrhythmia") and/or a failing heart ("cardiomyopathy") are leading causes of death worldwide, and GPCRs are already the targets of many heart drugs.




**Tissue-specific effects of RO1, a $G_i$-RASSL.**




Bone tissue is an ideal system for testing long-term signaling by RASSLs, since there is cumulative build-up of bone with every day the RASSL is signaling. In one experiment with the "Rs1" RASSL, the Conklin laboratory recently showed dramatic in-

creases in the bones of mice. Controlling bone formation could be useful in understanding and treating diseases characterized by bone loss such as osteoporosis or by abnormal bone growth such as heart valve calcification.

RASSLs could be particularly useful to grow engineered tissues toward a target (chemotaxis) to form a chain of cells such as blood vessels or nerve cells, since we know that GPCRs control similar processes in nature. The nervous system offers particularly challenging opportunities because RASSLs can deliver precise biochemical responses to discrete locations to identify the wiring diagrams of the brain. RASSLs have been used to dissect the pathways that enable mice to distinguish between "sweet" and "bitter" tastes. Similarly, RASSLs are being tested to control the movement disorders in animal models of Parkinson's disease. Although the potential applications are exciting to consider, RASSL technology is so new that it is impossible to predict all of the potential uses.

RASSLs have already proven to be useful in revealing potential mechanisms that native GPCR signals may contribute to therapeutic and disease states. Dr. Conklin hypothesizes that better GPCR drugs could be created if we understood more definitively how each G-protein pathway contributes to heart and bone physiology through acute and normal baseline signaling. RASSLs could prove to be an ideal tool to match discrete biochemical responses to specific physiological responses. This knowledge could be applied to our basic understanding of how drugs work as well as tissue engineering.

## The Future

The recent exciting advances with stem cells have great promise for biomedicine in terms of a better understanding of disease processes and potentially for therapeutic applications. For example, stem-cell transplants into heart or brain tissue (animal models and human clinical trials) may usher in a new era of regenerative therapy. As these studies continue, RASSLs will be a key tool for developing a greater understanding of how cells can be induced to proliferate, grow, and differentiate to form viable tissue.