

# Do Morphogen Gradients Arise By Diffusion?

Arthur D. Lander

Dept. of Developmental & Cell Biology  
University of California, Irvine  
Irvine, CA 92697

adlander@uci.edu

Qing Nie

Dept. of Mathematics  
University of California, Irvine  
Irvine, CA 92697

qnie@uci.edu

Frederic Y.M. Wan

Dept. of Mathematics  
University of California, Irvine  
Irvine, CA 92697

fwan@uci.edu

## ABSTRACT

A major goal of developmental biology is to understand how the locations of structures and cell types in the embryo are specified. In systems as diverse as fly wings, frog embryos, vertebrate limbs and spinal cords, tissue patterning is orchestrated by morphogens, diffusible molecules that specify distinct cell fates depending on their concentration. Each of these systems behaves as though it is patterned by concentration gradients of morphogens, among which are the polypeptides decapentaplegic (dpp), wingless, hedgehog, and activin

That morphogens are indeed distributed in gradients in developing organisms has recently been established, but how such gradients arise is the subject of ongoing controversy. Although an obvious possibility is that morphogens simply diffuse through tissues, both theoreticians and experimentalists have argued that the functions and distributions of morphogen gradients *in vivo* can only be explained if other transport mechanisms are invoked. For example, Kerszberg and Wolpert [1] have asserted, based on modeling studies, that the morphogen receptors in tissues should so impede transport as to preclude the formation of useful spatial gradients by simple diffusion. In contrast, they argue that gradients could be formed by a novel "bucket brigade" transport mechanism, in which receptor-bound morphogen on one cell is handed off directly to receptors on an adjacent cell.

More recently, two groups have studied the formation of dpp gradients in the developing *Drosophila* wing by expressing dpp-green fluorescent protein (GFP) fusion proteins in the normal dpp domain (a stripe-shaped source in the center of the larval wing disc) [2,3]. Both groups measured the profile of the ensuing dpp gradient, and studied how it responded to experimental manipulations. Some of the reported data fit very well with the notion that the dpp gradient is established by diffusion (e.g. the rapidity with which it was established), but other findings seemed problematic: much labeled dpp was found not around cells but within them; genetic blockade of endocytosis by responding cells caused defects in dpp transport; and genetic ablation of receptors in small clones of cells resulted in accumulation of morphogen at the side of the clone facing the morphogen source. These results were taken by one group [3] as evidence that morphogen transport occurs not by diffusion, but by transcytosis—the coupled endocytosis, intracellular transport, and exocytosis of bound ligands. Indeed, the notion that not only dpp, but also the morphogens wingless and hedgehog, move through tissues by transcytosis is becoming increasingly accepted by many investigators.

In contrast, we show here that, when the dynamics of morphogen transport, diffusion, binding, dissociation, and internalization are appropriately modeled—through mathematical analysis and numerical solution of the relevant differential equations—the very data that seem to speak against diffusive morphogen transport actually speak in favor of it.

Among other things, we show that there is no *a priori* reason why stable, useful morphogen gradients cannot form by diffusion in tissues that contain morphogen receptors, but doing so requires that receptor numbers, receptor kinetics, and receptor internalization and degradation meet certain conditions. Intriguingly, these conditions fit biological observations well.

We also show that the inhibitory effects of endocytic blockade on morphogen transport cannot be taken as an indication that endocytosis is involved in the transport process itself. Indeed, such data are well fit by a model in which diffusion is the only transport mechanism. Finally, we illustrate that, in order to explain the establishment of known morphogen gradients by non-diffusive mechanisms—e.g. "bucket brigades" and transcytosis—it is necessary for certain cell biological processes to occur at rates that are implausibly fast.

Overall, we find that the establishment by diffusion of morphogen gradients in receptor-containing tissues is a process that is both possible and useful, but one that nevertheless constrains biological parameters in interesting ways.

## REFERENCES

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