Morphogen Gradients, in Theory

The idea that morphogen gradients are established by a process of repeated cycles of exocytosis and endocytosis—so-called planar transcytosis—has been gaining acceptance. This is now challenged by a theoretical approach that experimental biologists should not dismiss; diffusive mechanisms of gradient formation may be correct after all.

That concentration gradients of signaling molecules known as morphogens organize and pattern tissue in developing animals is now well established; but the mechanism by which the gradients form has become increasingly controversial. Support for the simplest model, that morphogen gradients arise by passive diffusion (for example, recently supported by Strigini and Cohen, 2000; McDowell et al., 2001), has been waning in favor of a model where morphogens move by repeated cycles of endocytosis and exocytosis in the plane of an epithelium, so-called planar transcytosis (Moline et al., 1999; Entchev et al., 2000; Greco et al., 2001). A variation on this theme is the so-called "bucket brigade" model, in which morphogens are handed between receptors on neighboring cells (Kerszberg and Wolpert, 1998). The arguments against diffusive mechanisms are derived from three principle types of observation. First, unoccupied receptors very efficiently bind the morphogen, thereby impeding its progress beyond a cell or two from its source; second, much of the morphogen can be seen inside cells—much less is detectable in the extracellular space; and third (and most convincing) are a number of experiments that show that blocking endocytosis prevents morphogen transport and gradient formation.

A paper in this issue of *Developmental Cell* (Lander et al., 2002) challenges the notion that these results support transcytosis over diffusive mechanisms, but in a way that many of its protagonists may tend to dismiss as largely irrelevant: a theoretical treatment of the issue, with no "wet" experiments at all. Although pattern-forming mechanisms, including gradients, have been a significant focus of theoretical biology from at least the days of Turing, the startling successes of experimental developmental biology over the last decades have led to a major dislocation between the two sides of the field. The questions may be the same, but many of the questioners pay little attention to each other—in fact, they hardly speak the same language. The experimentalists dismiss the theoretical treatment as bearing no necessary link to reality, and the theoreticians are no doubt frustrated by the experimentalists' inability or unwillingness to provide hard quantitative data that can be plugged into numerical models. As an experimental biologist, I confess to sharing the prejudice against a theoretically driven approach, preferring to analyze what actually happens in nature. But this chauvinist position is becoming increasingly unreasonable. The quantity and quality of the experimental data now available is beginning to allow a "joined-up" approach to biology: for example, the emergent properties of signaling systems can now begin to be analyzed (Bhalla and Iyengar, 1999), allowing a much fuller picture of how myriad signals are integrated. The paper by Lander et al. illustrates another reason for taking theoretical biology seriously (at least when it is firmly rooted in experimental data): the "obvious" interpretations of apparently straightforward experimental results are sometimes wrong.

Lander et al. directly tackle the objections to a passive diffusion process and test their validity. They conclude that almost all the data that has been published to date is, in many cases counter-intuitively, better explained by diffusion than by transcytosis or bucket brigades. A striking example of a nonobvious conclusion is the interpretation of a number of experiments that have shown the need for endocytosis in morphogen gradient formation (Moline et al., 1999; Entchev et al., 2000). The obvious interpretation is that an active transcytotic mechanism transports the morphogen, but Lander et al. make a convincing case that this result is exactly what is predicted for diffusion mechanisms. Their calculations show that endocytosis is necessary to solve a dilemma for the receiving cell—how to have enough receptors available so that signaling can be strong enough to reach a response threshold, without having so many receptors on the cell surface that the morphogen becomes trapped close to its source, preventing it from diffusing any further. Endocytosis allows the internalized receptor/ligand complexes to continue to signal while the surface receptor population remains low. Another nonobvious conclusion is that the "shadow" of reduced morphogen concentration just distal to a patch of cells in which endocytosis is blocked (Moline et al., 1999; Entchev et al., 2000), which has been taken as strong evidence that there is a failure of transcytosis through that patch, is in fact equally predicted by diffusive mechanisms. They argue that time is on the side of diffusion, too. The rate of gradient formation in some tissues has been directly measured (Gurdon et al., 1994; Entchev et al., 2000; Strigini and Cohen, 2000; Teleman and Cohen, 2000) and the equations in this paper strongly imply that there would simply be no time for transcytosis or bucket brigades to get the morphogen across the field of cells (although this may be challenged by the apparent rate of movement of argosomes, vesicles proposed to be involved in Wingless signaling; Greco et al., 2001).

There are more of the same kind of provocative insights into morphogen gradient establishment in the paper by Lander et al., and overall they build a strong case that most current evidence can be interpreted within a diffusion-based model. In fact they go further, claiming that other models require biologically implausible conditions. An important feature of this paper is that it explicitly discusses (in nonmathematical language) its assumptions, and the conditions and values that give the final answers; they are there for any experimental biologist to judge and criticize. They also openly discuss results that appear not to be consistent with their conclusions; in some cases they reinterpret these previous experiments, while in other cases they can't and they are refreshingly honest about it. Although I don't understand the differential equations that lie at the heart of the paper, the authors succeed in making me feel able to form an opinion about the solidity of their conclusions.

Furthermore, they are sensibly circumspect about how generalized their conclusions are. The paper takes as its prime example signaling by the BMP-like molecule Dpp in the *Drosophila* wing (Entchev et al., 2000; Teleman and Cohen, 2000). They and others have noted that signaling characteristics may be quite different in different developmental and cellular contexts. Transcytosis may not occur in the wing, but perhaps it does elsewhere. Even more plausibly, different morphogens may well use different transport mechanisms. The main signals that work as morphogens are members of the TGF-β, Hedgehog, and Wnt pathways. These proteins have very different biochemical and biophysical properties, so although, for example, Lander et al. note characteristics of diffusive transport in the data corresponding to Wnt gradients, more experimental work will be needed to test this.

Of course the big question is: are the theoretically derived conclusions of Lander et al. correct? What really happens in developing organisms? And there's the rub. Theoretical biology alone cannot provide experimental tests of the predictions and assumptions on which the models are based. This is not, and could not be, the final word. Lander et al. go a considerable way toward opening a useful dialog between the two cultures of biology, but their paper also emphasizes that the separation of these cultures slows progress toward a true mechanistic understanding of the complexity of living organisms.

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Selected Reading

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The Undiscovered Country: Chromosome Territories and the Organization of Transcription

The interchromosome domain (ICD) model proposes that genes are selectively positioned at the surfaces of chromosome territories to facilitate their regulation. A paper in the May 13 issue of the *Journal of Cell Biology* provides evidence that supports a reinterpretation of this model.

Fluorescence in situ hybridization (FISH) has become an experimental guide in the growing exploration of the functional organization of the interphase nucleus. The use of FISH to detect both genomic loci and RNA, in conjunction with 3- and 4D microscopy, has greatly expanded our understanding of the spatial compartmentalization of inactive and active genes. For example, a number of recent studies have demonstrated that inactive genes selectively colocalize with two characterized transcriptionally repressive subcompartments, centromeric heterochromatin and the nuclear periphery (Francastel et al., 2000). Importantly, the combination of FISH with whole chromosome paints in mammalian cells led to the proposal of a unifying paradigm for the nuclear organization of active transcription: the interchromosome domain (ICD) model (Cremer et al., 1993).

The ICD model is based upon the observation that chromosomes exist as discrete territories, which results in an intervening interchromosome compartment that runs throughout the nucleus. Postulated nearly 10 years ago, the ICD model predicted that genes, in order to make them accessible to the transcription and splicing apparatus confined to the ICD, would be preferentially localized to the periphery of chromosome territories (CTs) (see Figure) (Cremer et al., 1993). Supporting a growing body of evidence, a recent paper in the *Journal of Cell Biology* by Bickmore and colleagues suggests that the conception of the interchromosome compartment as a channel between the surfaces of CTs is more convoluted than expected (Mahy et al., 2002).

Immunolabeling and electron microscopy (EM) examination of chromatin structure had revealed that interchromatin granules ("nuclear speckles") rich in splicing components colocalize with perichromatin fibrils (decondensed chromatin), while RNA FISH had detected transcript "tracks" from the site of transcription to the nuclear periphery (see Figure) (reviewed in Cremer et al., 1993 and Misteli and Spector, 1998). The foundation for the ICD model came when Lichter and colleagues determined the relationship between transcripts from an integrated virus to its respective chromosome territory, demonstrating that the RNA (as well as splicing factors) were excluded from the CT (Zirbel et al., 1993). The correlation was extended by an analysis of the relative position of genes and CTs, which revealed the preferential localization of genes, and not intergenic DNA, to the territorial surfaces (Kurz et al., 1996). More recently, Sheer and colleagues demonstrated that the human major histocompatibility complex (MHC) region displays a cell- and activity-dependent organization in a large loop