Review

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Creating gradients by morphogen shuttling

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Morphogen gradients are used to pattern a field of cells according to variations in the concentration of a signaling molecule. Typically, the morphogen emanates from a confined group of cells. During early embryogenesis, however, the ability to define a restricted source for morphogen production is limited. Thus, various early patterning systems rely on a broadly expressed morphogen that generates an activation gradient within its expression domain. Computational and experimental work has shed light on how a sharp and robust gradient can be established under those situations, leading to a mechanism termed 'morphogen shuttling'. This mechanism relies on an extracellular shuttling molecule that forms an inert, highly diffusible complex with the morphogen. Morphogen release from the complex following cleavage of the shuttling molecule by an extracellular protease leads to the accumulation of free ligand at the center of its expression domain and a graded activation of the developmental pathway that decreases significantly even within the morphogen-expression domain.

Morphogen gradients

A central feature in the patterning of multicellular organisms is the ability to provide a group of cells with information regarding their global position within the developing embryo or organ. This is achieved by morphogen gradients, which are typically represented by secreted proteins that trigger cellular signaling by activating specific receptors. The term 'morphogen' was originally coined by Alan Turing [1], and elaborated on by Lewis Wolpert, who provided the French flag analogy to the outcome of morphogen-induced signaling [2]. In essence, each color within the flag represents a distinct domain that is characterized by the target genes it will express. Morphogens are used in a variety of tissue settings to pattern a field of cells according to their concentration gradients. The classical model of patterning by morphogen gradients involves three cardinal aspects: (i) release of the morphogen from a restricted source; (ii) graded distribution of the morphogen across a field of cells; and (iii) the ability of cells to respond to different concentrations of the morphogen in discrete ways, such that distinct target genes will be induced by different morphogen levels [3].

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Because the responding cells monitor the level of the morphogen and differentiate accordingly, the change in morphogen levels over space should be sufficiently steep, such that distinct levels can be reproducibly sensed at different positions. In addition, the distribution of the morphogen should not fluctuate markedly when the production rates of the morphogen and the components it triggers are altered by asymmetric segregation, genetic perturbations such as heterozygosity to mutations, or changing environmental conditions. Collectively, these features are termed 'robustness'.

Two different configurations have been shown to provide a source for the localized release of a morphogen. One setup involves the juxtaposition of two distinct types of tissues, one of which produces the morphogen but does not respond to it, whereas the other tissue does [through tissue-specific expression of a downstream factor(s)]. Alternatively, a select group of cells that was defined by an earlier signaling event could release the morphogen to the extracellular milieu (Figure 1a,b). The ability to define the interface between two distinct compartments, or to allocate a defined fate to a small group of cells that will serve as the source of the morphogen is feasible when patterning a tissue at an advanced stage of development, when sufficient cues are in place to clearly demarcate these cells. Thus, in the *Drosophila* wing imaginal disc, production of Hedgehog in the posterior compartment (which does not respond to the ligand) and its restricted range of signaling within the adjacent anterior compartment will induce production of the morphogen Dpp in only a few rows of cells [4]. Once produced, Dpp diffuses and activates its receptors symmetrically [5–7], thus providing an example of the successive utilization of the two mechanisms of local morphogen release.

The need for morphogen shuttling

During early stages of embryogenesis, coarse symmetry-breaking events provide the initial patterning cues. Morphogen-based mechanisms are then recruited for elaboration of the patterning scheme. However, because the initial asymmetry is only roughly defined, the capacity to delineate a signaling interface between two cell types or to induce a narrow group of morphogen-producing cells in the center of the region that will be patterned is limited. This means that patterning systems in early embryos are more likely to rely on broadly expressed morphogens. How then can a gradient be generated within a field of

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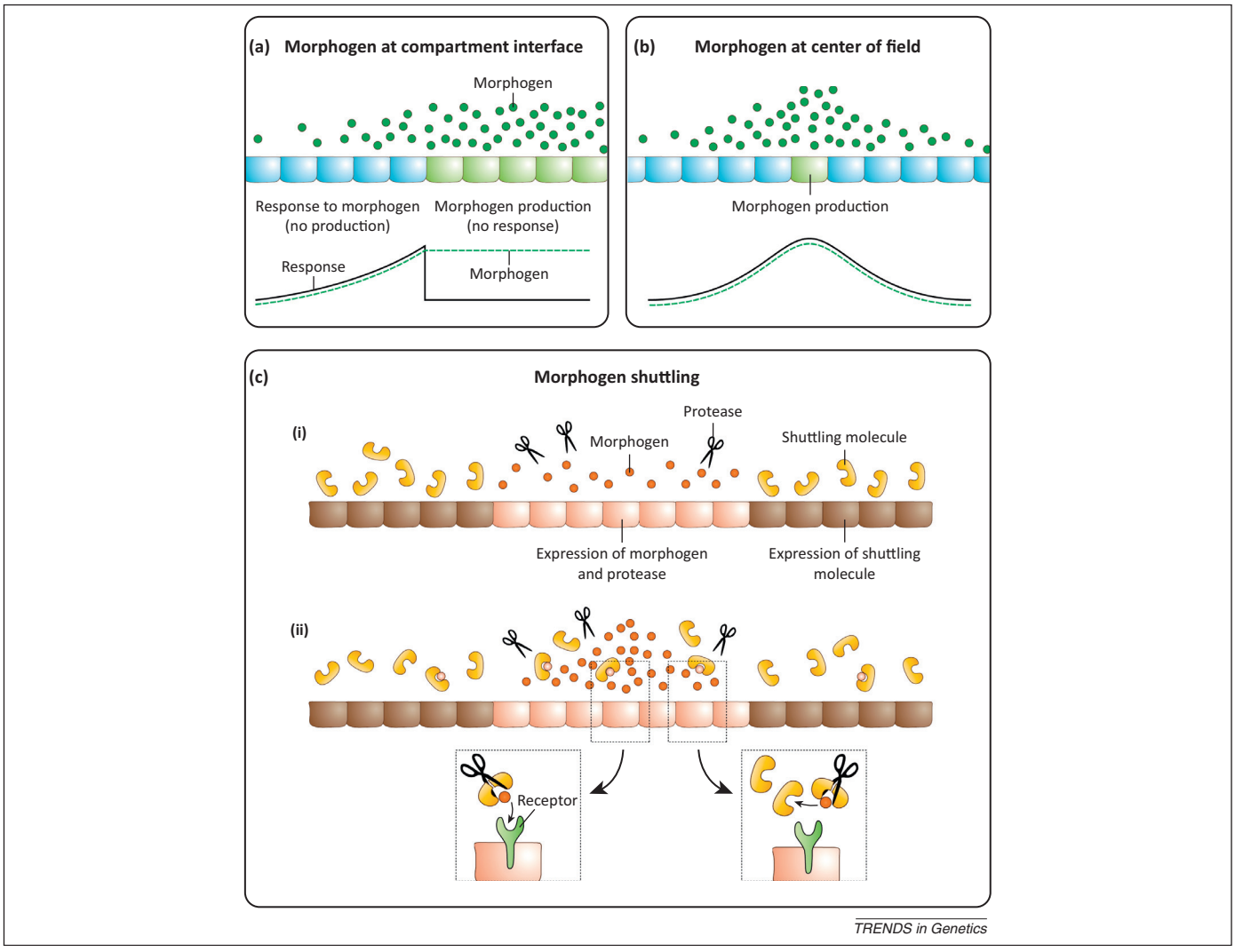

Figure 1. Mechanisms for generating morphogen gradients. **(a)** Morphogen gradient at the compartment interface. A tissue that produces the morphogen, but does not respond to it, is positioned next to a tissue that can respond to the morphogen, but does not produce it. Graded distribution of the morphogen is generated within the responding tissue, leading to a graded activation pattern within this compartment. **(b)** Morphogen release from a restricted source. When a restricted group of cells produces the morphogen within a field of responsive cells, the morphogen will spread symmetrically, leading to a corresponding graded activation pattern. **(c)** Morphogen shuttling. (i) Shuttling of the morphogen is initiated by broad expression of the morphogen within the patterned region, flanked by domains expressing the shuttling molecule. The morphogen and shuttling molecule can associate, to generate a biologically inactive and highly diffusible complex. (ii) Due to the activity of a protease within the patterned region, the shuttling molecule in the complex will be cleaved and release the ligand. When cleavage takes place within the lateral region, the released ligand will bind another shuttling molecule, due to the graded distribution of the free shuttling molecule within the patterned region. Conversely, cleavage at the center of the patterned region will preferentially lead to binding of the free ligand to the receptor. This will give rise to physical concentration of the free ligand towards the center. A low diffusion rate of the free ligand is essential to preserve the graded distribution of morphogen.

responding cells, if the morphogen-expression domain is not spatially restricted? Moreover, how can this gradient be sufficiently steep and robust?

Nevertheless, sharp and robust gradients exist during early embryogenesis and enable later establishment of classical morphogen patterning. This seeming paradox has inspired a combination of computational and experimental approaches to elucidate the patterning scenarios at work. A mechanistic solution to the generation of early gradients has recently been obtained, defining a process termed 'morphogen shuttling', which is the focus of this review. This mechanism was proposed based on its ability to define sharp gradients under conditions where the morphogen is broadly expressed, as well as its relative insensitivity to the morphogen production rate and other fluctuating biochemical rate constants, thereby ensuring that the final gradient will be robust and reproducible from one embryo to another.

The concept of shuttling

The profile of signaling activation by the morphogen gradient determines the developmental responses of the cells within the patterned region. Thus, it was logical to assume that the solution to graded patterning within a broad region with uniform morphogen expression should be executed within the extracellular milieu, where the morphogen exerts its activity. Genetic studies of each morphogen pathway have identified a limited number of molecules that function extracellularly. A computational approach that focused on these extracellular components was taken to search for parameters that would not only allow the extracellular components to form a sharp gradient, but also