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Bilaterally symmetric facial morphology simulated by diffusion-controlled chemical precipitation in gel

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ABSTRACT

Reactions controlled by diffusion create self-organizing periodic patterns in chemical systems and play a key role in biological pattern formation through diffusion-generated morphogen gradients. To better understand the organizing ability of diffusion-controlled assembly, we investigated the formation of bilaterally symmetric morphologies by simple inorganic precipitation reactions in neutral agarose gel. Our results reveal that bilaterally symmetric 'face-like' deposits of insoluble products reproducibly form in gel, by reactions governed by the properties and concentrations of soluble reactants placed symmetrically relative to a midline and by the temporal and spatial distributions arising by diffusion from initial times and positions of reactant placement.

1. Introduction

In developmental biology, gradients of diffusible biochemicals called morphogens control cell growth and differentiation by binding to cell-membrane receptors in complex biochemical signaling pathways that regulate cellular gene expression and resultant products [1,2]. Diffusion-controlled biochemical reactions are thought to play a key role in the development of many biological patterns [3], e.g., periodic segmentation in *Drosophilia* embryos, limb segmentation in vertebrates [4], and striped or spotted patterns in feather or hair growth [5,6]. In the case of vertebrate skeletal growth and facial features, bilateral symmetry seems to require retinoic acid, a vitamin A-derived amphipathic molecule able to diffuse through cell membranes [7–9]. Perhaps such a morphogen can stimulate the combination of intracellular and extracellular activities needed for fibroblasts to abundantly produce and secrete the triple-helical protein, collagen, which aggregates (i.e. precipitates) in extracellular space to form the connective tissue fibers of skeleton and skin. How collagen fiber production is regulated to achieve bilateral symmetry in the whole body plan has yet to be determined.

Symmetric patterns can arise in simple chemical precipitation systems when soluble reactants, such as inorganic anions and cations, move only by diffusion while forming insoluble ionic combinations (precipitates) in an aqueous gel. The formation of an insoluble precipitate occurs when the combination of interacting anion and cation concentrations exceeds a certain threshold (the solubility product), whereupon precipitation may spontaneously yield crystals or organized particulate deposits, such as periodic (Liesegang) bands or rings, held by the gel fibrous network [10–12]. Previously we have shown that periodic banding in agarose gel, observed for inorganic precipitates such as silver chromate or dichromate, is caused by soluble ionic impurities that can be removed by extracting agarose powder with pure water [13]. In the resultant neutral agarose gel, we can study the precipitation properties of inorganic ions in the absence of ionic impurities that induce the periodic banding observed in commercial agarose [13] and animal gelatin [13–16].

By using water-extracted agarose to avoid the effects of ionic impurities, we have begun to examine how diffusion-controlled patterns of ionic precipitation in neutral agarose gel depend on (i) the choice of ionic reactants and their concentrations, and (ii) where and when each reactant is introduced in the system. In this Letter we illustrate some examples of bilaterally symmetric 'facial' patterns formed by diffusion-controlled precipitation of inorganic ions in a thin layer of neutral agarose gel formed in a standard Petri dish. Such a gel allows anionic (**a**) and cationic (**c**) reactants to diffuse freely in two dimensions, while holding in place the insoluble (**ac**) products formed. Bilateral symmetry of insoluble product deposition is achieved in a reproducible fashion simply by using diffusion to regulate the ionic reactant concentrations as a function of distance and time from initial sites of highest concentration symmetrically placed with respect to a midline.

2. Experimental

Gels of neutral agarose (0.5% by weight) were prepared by using electrophoresis grade agarose powder (Bio Rad), which was extracted with ultrapure water to remove ionic impurities [13],

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before being dissolved with stirring in hot water or aqueous solution at 99–100 °C. In each case, a 10-ml aliquot of hot agarose solution was transferred to a Petri dish (100 × 10 mm) on a level plate warmer (70 °C) and kept there for 10–15 min before slowly cooling to room temperature, in order to eliminate any pouring-induced effects in the gel. After cooling, gels were stored in a cold room at 4 °C in the dark to avoid light-induced conversion of Ag^+ ion to Ag metal (photographic effect).

To obtain the bilaterally symmetric diffusion-controlled morphologies shown here (Fig. 1), we used *localized* and *extended* competition on the midline, between two anionic reactants, **a** and **b**, competing to form an insoluble precipitate with symmetrically placed cationic reactant **c** (Fig. 1A and D, respectively). It should be noted that each Petri dish of gel was placed on top of a graph paper template showing the positions of drops to be added, to ensure accurate geometry for bilateral symmetry.

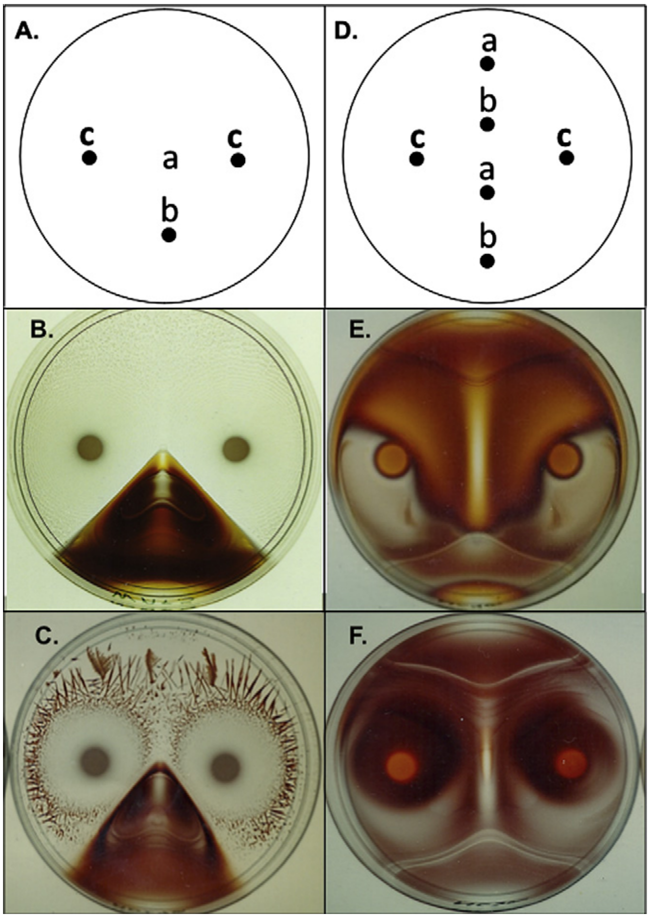

Figure 1. Experimental setups and resulting diffusion-controlled precipitation patterns obtained with one cationic reactant and two competing anionic reactants. (A) First experimental setup, where colored anionic reactant **a** (potassium chromate or dichromate) was uniformly distributed in the agarose gel at low concentration, before a drop of competing colorless anionic reactant **b** (potassium iodide) at higher concentration was placed at one spot marked by a dot on the gel, along with two symmetrically placed drops of colorless cationic reactant c (silver nitrate) at even higher concentration. (B and C) are the resultant diffusion-controlled precipitation patterns for colored silver chromate and dichromate, respectively. (D) The second experimental setup, starting with plain agarose gel with alternating drops of reactants **a** and **b** at equal concentration placed as indicated by dots and allowed to diffuse for 5 days to create diffusion gradients of **a** and **b** before drops of reactant c were added in the same two symmetric locations. These produced the very different precipitation patterns (E) and (F) for colored silver chromate and dichromate, respectively. (The concentrations and volumes of the **a**–**c** drops are detailed in the Section 2).

For *localized* competition (Fig. 1A), the agarose was dissolved in a hot solution of anionic reactant **a** (3 mM potassium chromate or potassium dichromate). After this gel containing uniformly distributed **a** had cooled to 4 °C, competing anionic reactant **b** was added as a 50 μl drop of 0.3 M potassium iodide, placed on spot marked **b** on the midline (Fig. 1A). Then a 50 μl drop of 4.0 M silver nitrate solution was placed at each of the two spots marked **c**, equidistant from the midline.

For *extended* competition (Fig. 1D), the agarose gel was prepared without any added chemical and cooled to 4 °C, before placing a 50 μl drop of 0.3 M potassium chromate or dichromate at each of the two positions marked **a** on the midline and a 50 μl drop of 0.3 M potassium iodide at each of the two **b** positions on the same midline. These drops were allowed to diffuse into the gel for 5 days in the dark at 4 °C, to create bilaterally symmetric concentration gradients of chromate (or dichromate) and iodide within the gel. Then a 50 μl drop of 4 M silver nitrate was carefully placed at each of the two spots marked **c**, equidistant from the midline (Fig. 1D).

In each experimental setup, after the silver nitrate drops were added, the gel was kept for at least three more days at 4 °C in the dark, before being photographed. As silver ions diffused into the gels, the following reactions occurred to produce insoluble precipitates (↓) with the chromate, or dichromate, and iodide anions: $2Ag^+ + CrO_4^{2-} \rightarrow Ag_2CrO_4 \downarrow$, or $2Ag^+ + Cr_2O_7^{2-} \rightarrow Ag_2Cr_2O_7 \downarrow$, and $Ag^+ + I^- \rightarrow AgI \downarrow$.

3. Results

3.1. Facial morphologies formed by bilaterally symmetric diffusion-controlled precipitation

The two 'eyes' in our simulated facial morphologies (Fig. 1) are defined by the equidistant placement, relative to the midline, of two small (50 μl) drops of concentrated reactant **c**, in the form of colorless silver cation (Ag^+) at 4 M concentration, as its highly soluble silver nitrate salt ($AgNO_3$). These drops are added only after all the anionic reactants (**a** and **b**) have been placed to define 'face with nose' centered on the midline, as indicated in Figure 1A and D. As reactant **a** for 'face' formation, we chose the colored anion of chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$), which might be viewed as a colored mimic of the dominant but colorless phosphate or pyrophosphate anion in biology. As reactant **b** that competes with **a** for 'nose' formation, we chose the colorless iodide anion (I^-), also as potassium salt, after finding that chloride (Cl^-) and bromide (Br^-) gave less satisfactory results (data not shown). The results shown here (Fig. 1B, C, E and F) clearly reveal that even simple chemical precipitation reactions between inorganic ions, when controlled by diffusion from symmetric reactant arrangements, can produce insoluble product morphologies resembling those made by complex biological organisms.

3.2. Morphologies formed with localized competition on the midline

In our first experimental setup (Fig. 1A), used to obtain the results in Figure 1B and C, anionic reactant **a** was uniformly distributed at low concentration (3 mM) in the hot agarose gel solution before a 10 ml aliquot was pipetted into a Petri dish and then covered on a plate warmer. After the gel had been cooled slowly to 4 °C, anionic reactant **b** and cationic reactant **c** were carefully placed as 50 μl drops on top of the solidified gel, as indicated by the dots in Figure 1A. The covered gel was then kept in the dark at 4 °C for at least two days to obtain complete diffusion-controlled precipitation of both silver iodide and silver chromate or dichromate.