Emergence of polarity in early C. elegans Embryo

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Abstract

The establishment of cell polarity during embryogenesis is a crucial process. The molecular basis for polarity in C. elegans embryos involves a set of partitioning-defective (PAR) polarity proteins that segregates into anterior and posterior domains to form the basis of a stable front-back axis. While experimental evidence suggested that external signals trigger an anterior-directed cortical flow to transport the anterior PAR proteins, it has also been shown that a reaction-diffusion system without coupling to advective flow is sufficient to generate stable partitioning. In this report, I will try to summarize some models that explains the spontaneous emergence of cell polarity by reaction-diffusion patterns. I will also try to discuss the effect of coupling cortical flow to the reaction-diffusion models.

I. INTRODUCTION

The establishment of cell polarity is an important process for the development of embryos. An unpolarized cell is often triggered by some sort of stimulus to establish polarity. In the embryo case, it is triggered by the entry of sperms. After polarity is established, cell polarity can be maintained even after the stimulus is removed. After cell division, the polarity direction will eventually point at the head-tail direction of the embryo.

The nematode worm Caenorhabditis elegans (C. elegans) is one species that has been frequently used to study cellular development because of its simple structure. Experimental findings[1] show that the C. elegans zygote is already highly polarized. At the moment of fertilization, the homogeneous zygote is quickly reorganized and the polarity domain will soon be established. This process is highly related to two groups of partitioning-defective (PAR) protein, which mutually exclude one another on the cell membrane. Another important process required to understand the polarization process is the cortical flow. Cortical flows are thought to be driven by myosin motors acting along actin filaments on the cell cortex and generate forces and tension. During polarization, the cortex consistently moves and rearranges, suggesting that fluid dynamics plays a crucial role to this problem.[2]

However, Filipe Tostevin and Martin Howard proposed that it is the cytoskeletal asymmetry in cytoplasm and cortex that is responsible for the establishment of polarization.[3] This model proposes a bistable switch which allows PAR proteins to occupy the anterior and posterior domains in C. elegans embryo. To maintain stable domains of polarity, Dawes and Munro [4] consider the binding of the PAR proteins and establish a stable domain model. To stop the drift of domain boundary, they proposed that the previously studied wave-pinning model proposed by Mori [5] may help explain the mechanism. The modeling approaches can be summarized by the reaction-diffusion approach.[6] Combining reaction-diffusion dynamics of the PAR proteins coupled to a simple model of actin and myosin contraction is used to study this phenomenon.

II. MODELING THE ESTABLISHMENT OF PAR PROTEIN POLARITY

The mathematical model of this kind of flow can be modeled by reaction-diffusion pattern. The biochemically bistability plays an essential role in this model. According to Tostevin

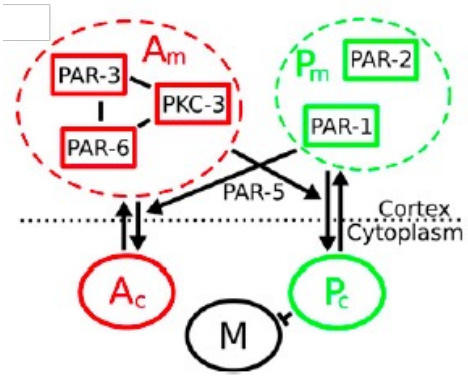

FIG. 1. Summary of reaction processes of the basic model.Figure taken from [3]

and Howard's paper[3], we first consider only the posterior and anterior PAR porteins in both cytoplasmic and cortex as well as the coupling to cortical actomyosin contraction. We denote our variable as: 1. contractile actomyosin a, cortically localized anterior PAR proteins A_m, cortically localized posterior PAR proteins P_m, anterior PAR proteins in the cytoplasm A_c, posterior PAR proteins in the cytoplasm Pc, and cytoplasmic MEX-5/6 M, which is essential to cell division. The reactions is summarized in Fig.1 and the resulting reaction-diffusion equations are

$$\frac{\partial A_m}{\partial t} = D_m \frac{\partial^2 A_m}{\partial x^2} + (c_{A1} + c_{A2} a) A_c - c_{A3} A_m - c_{A4} A_m P_m, \tag{1a}$$

$$\frac{\partial A_c}{\partial t} = D_c \frac{\partial^2 A_c}{\partial x^2} + (c_{A1} + c_{A2} a) A_c + c_{A3} A_m + c_{A4} A_m P_m, \tag{1b}$$

$$\frac{\partial P_m}{\partial t} = D_m \frac{\partial^2 P_m}{\partial x^2} + c_{P1} P_c - c_{P3} P_m - c_{P4} A_m P_m, \tag{1c}$$

$$\frac{\partial P_c}{\partial t} = D_c \frac{\partial^2 P_c}{\partial x^2} - c_{P1} P_c + c_{P3} P_m + c_{P4} A_m P_m. \tag{1d}$$

The first terms on the right hand side of Eq. 1 are diffusion terms. Other terms are reactions in the model, such as cortical association and spontaneous dissociation. Since the proteins are exchanging between cytoplasm and cortex, the signs are opposite. The set of equations ignores the degradation or production of PAR proteins. Next, we consider the effect of the contractile actomyosin, which is crucial to PAR protein density evolution. Sperm, acts as the stimulus of polarization, firstly inhibits of actomysin network around the tail of the zygote, causing symmetry breaking. The postive feedback of the head of zygote, the tension on the system varies with A_m. This can be simplified as a usual spring.

Since the viscous forces dominate all other forces inside the cell, it can be viewed as an overdamped spring with large damping and is determined by the following properties: the Youngs modulus E; the crosssectional area α ; the damping coefficient γ; and the natural length λ. Assuming that α and γ are constants, the length of the spring, $l(t)$, will be given by

$$\frac{dl}{dt} = -\frac{E\alpha}{\gamma\lambda(t)}(l(t) - \lambda(t)) \tag{2}$$

And assuming the density of contractile actomyosin ,$a(x,t)$, is uniform all across l. Also, A_m varies natural length λ. Let $m(t)$ be a function of time associated with the contractile activity stimulated by anterior PAR proteins and depends on A_m. Therefore, we have arrived a coarse-graining model which matches the expermintal data of cortical dynamics.

$$m(t) = \frac{1}{L}\int_0^{l(t)} A_m(x,t)dx \tag{3}$$

$$\lambda(t) = \lambda_0 - \lambda_1 m(t) \tag{4}$$

and

$$a(x,t) = \begin{cases} a(x,0)\frac{L}{l(t)}, & 0 \le x \le l(t) \\ 0, & l(t) \le x \le L \end{cases} \tag{5}$$

However, this kind of model exhibits the domain drifting of the PAR proteins. To prevent domain drift of simulation, Dawes and Munro[4] proposed that considering the mutually interactions between the anterior and posterior PAR protein groups. Here are the six assumptions they made:

Assumption 1: The PAR proteins can be categorized into two groups based on their localization in embryos that are polarized: anterior PAR proteins (ParA, including PAR-3, PAR-6) and posterior PAR proteins (ParP, including PAR-1, PAR-2).

Assumption 2: ParA can homodimerize.

Assumption 3: The concentrations of ParA and ParP in the cytoplasm are at steady state.

Assumption 4: All kinematic reactions are linear.

Assumption 5: Cortical ParA can promote the dissociation of cortical ParP into the cytoplasm, and vice versa.

Assumption 6: ParA and ParP both dissociates rapidly.

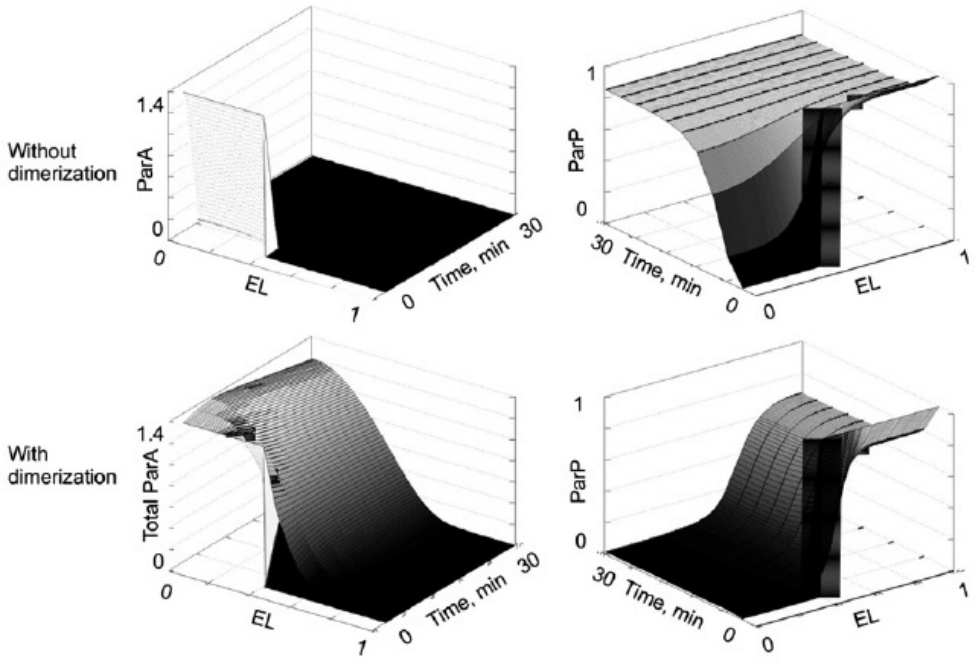

FIG. 2. Modeling result considering with or without dimerization. When dimerization is not considered (top row), boundaries of PAR proteins disapperas during time evolution. However, in experiment, PAR proteins can matian unique domains, which agrees with considering dimerization simulation result (bottom row). Figure taken from [4]

The set of new equations comes out a bistable solution of six freely-chosen parameters associated with the association and dissociation of the PAR proteins. The time course of PAR proteins, considering with or without dimerization, are shown in Fig.2 However, for larger timescales, the stable boundary still requiers additional buffering mechanism against drifting of boundaries.[4] The Wave-pinning model may explain the stability of PAR protein boundary. The fast exchange between slow-diffusing active and fast-diffusing inactive proteins with conserved total numbers results in a stable boundary position in 1D geometry. Since the evolving behavior of PAR proteins is similar to a model of Rho-family guanosine triphosphatases (GTPases)[7], the model proposed by [5] is able to compare with this case. To simplify the problem, consider on 2 types of proteins, a and b. $f(a,b)$ is like dl/dt in Eq.2, meaning the rate of activation minus the rate of inactivation proteins and can be writtern as

$$f(a,b) = b\left(k_0 + \frac{\gamma a^2}{K^2 + a^2}\right) - \delta a \tag{6}$$

where δ is the rate constant of inactivation, k_{ab}, and the term in the bracket of first term on the right hand side of this equation means the rate constant of activation, k_{ba}. The postive feedback and is characterized by a Hill function, with maximal rate γ and saturation parameter K. Steady state solution requires $f(a,b) = 0$.

The set of diffussion euqations in 1D will become

$$\frac{\partial a}{\partial t} = D_a \frac{\partial^2 a}{\partial x^2} + f(a,b), \tag{7a}$$

$$\frac{\partial b}{\partial t} = D_b \frac{\partial^2 b}{\partial x^2} - f(a,b), \tag{7b}$$

Since it's natural to assume that no external flux at boundary, which means

$$\frac{\partial a}{\partial x}|_{x=0,L} = 0, \frac{\partial b}{\partial x}|_{x=0,L} = 0 \tag{8}$$

And the number of proteins are conserved, so

$$\int_0^L (a+b)dx = const. \tag{9}$$

From these equations we can summarize to obtain wave-pinning result. We need:

1. The added stimuli does not affect the total amount of proteins.
2. The inactive protein acts as a global varible.
3. The whole system is bistable. For b in some range, $\partial a/\partial t = f(a,b)$ are stable at $a = a_-$ and $a = a_+$

And this mechanism is largly affected by propagation of wave. Fig. 3 shows a wavefront of high concentration propagating in the cell. At $t = 0$, the shaded areas , reprensents bk_{ba} and ak_{ab}, are different. As the wavefront propagates, the area of two shaded areas become the same, and maintain at this state. As a result, the wave-pinning model may be able to explain the large timescale maintenance of polarity domains in C. elegans embryo.

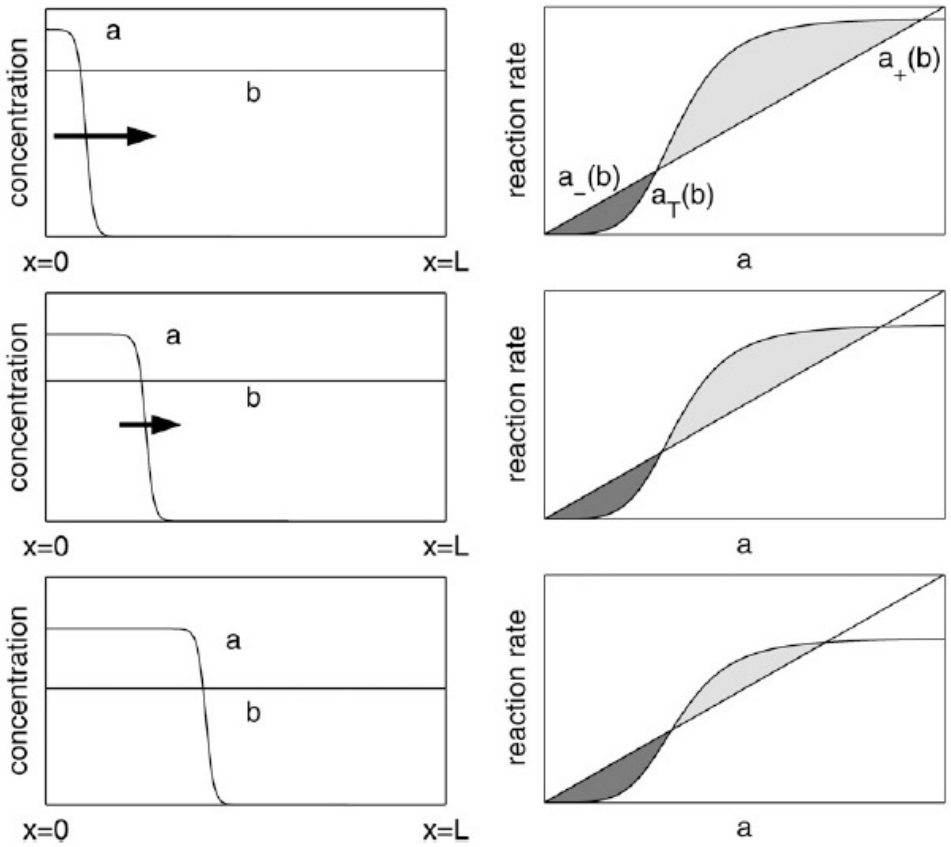

FIG. 3. The diagram of wave-pinning behavior. Figure taken from [5]

III. COMPARISON WITH EXPERIMENTAL RESULTS

Fig. 4 shows both experimental and calculated result using the simplest model. The left column in Fig. 4 is the calculated concentration profiles, and the center column is the experimental data, along with the fluorescence intensity of posterior and anterior PAR porteins under microscope, where PAR-6 is a type of anterior protein and PAR-2 is a type of posterior protein. The x-axis of the concentration profiles denotes the time (in min:sec) of evolution. In Fig. 5, it has summarizedfrom (A) and (B).

The long maintaince (over 35 minutes) of domain polarity of C. elegans zygote is shown in Fig. 6, which are the snapshots of different stage of zygote development from a single cell. From top to down, they are from pronuclear formation to two-cell stage. In this series of snapshots, the domain of protein boundaries are well maintained.

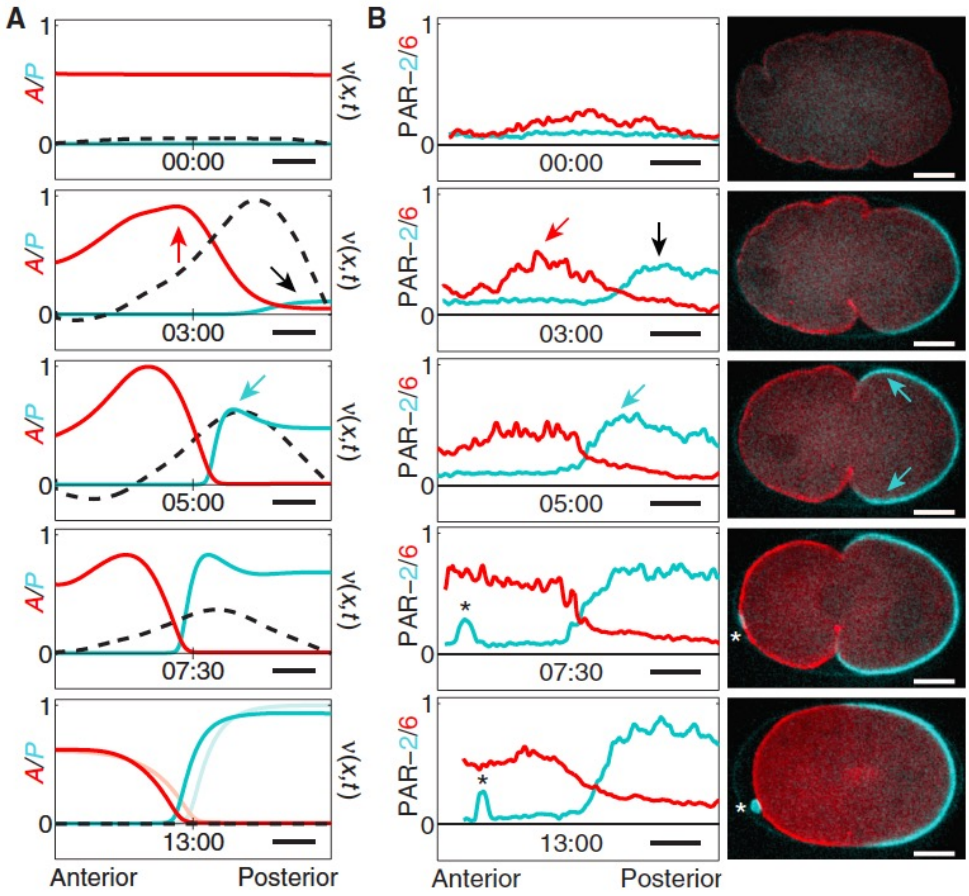

FIG. 4. Reaction diffusion coupled with advection can act as a mechanism for polarity establishment. (a) Calculated concentration profiles for ParA (red) and ParP (cyan) and its evolution over time. (b) Fluorescence intensity profile (left) of PAR-6 (red) and PAR-2 (cyan), calculated from fluorescence microscopy images. Figure taken from [7]

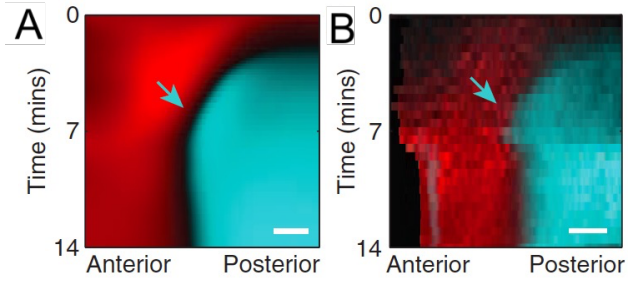

FIG. 5. (A and B) Kymograph of Fig. 4 (A) and (B), where ParA/Par-6 is in red and ParP/Par-2 is in cyan. Figure taken from [7]

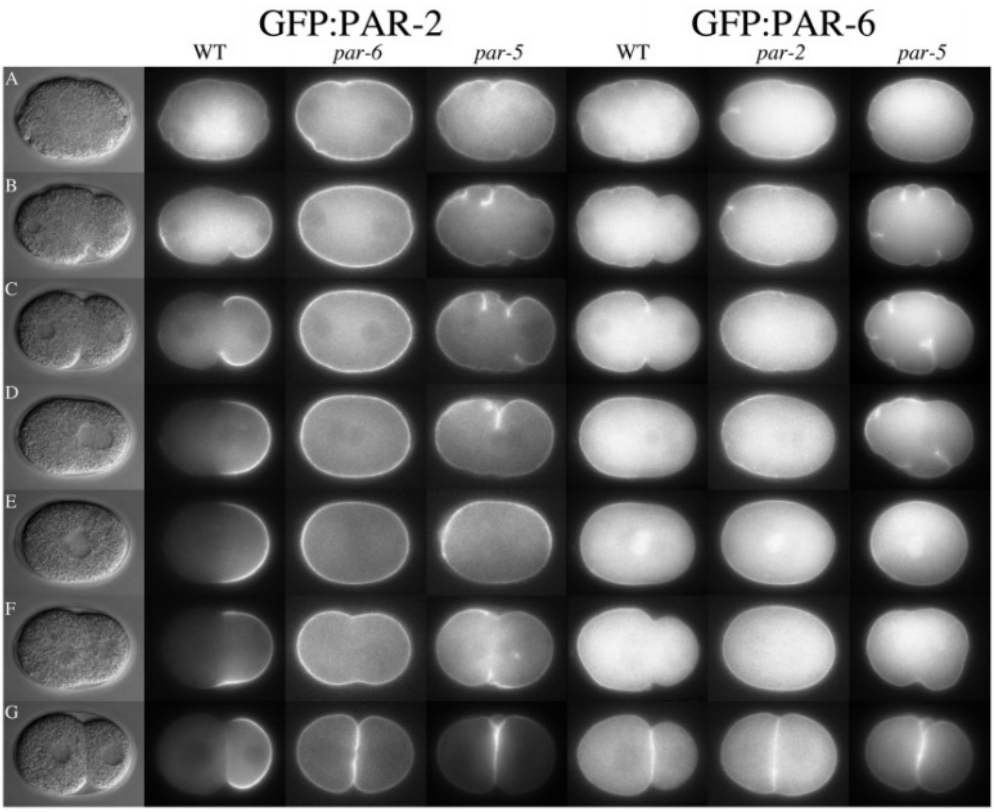

FIG. 6. Snapshot of different stage of zygote development from a single cell. The first row denotes which kind of protein is marked by green fluorescent protein (GFP). The second row denotes which kind of protein is inactivated, while PAR-2 is a posterior protein, PAR-6 is an anterior protein and PAR-5 is a uniform protein. WT means control experiment. Figure taken from [8]

IV. CONCLUSION

From these research, we have concluded that how the polarity establish at early development of embryo. Firstly, the concentration difference of reaction-diffusion relations in the cortex and the cytoplasm is the core physics model of whole system. Secondly, the association and dissociation of PAR proteins plays a role on mataining polarity domain boundary in short time scales, and the wave-pinning nature also helps preventing drift of boundary by rapid exchanges of two kinds of proteins. Along these 3 assumptions, the model strongly agrees with the experimental results. For further research, it is expected to use this mechanism to understand the development of embryo of other speices. This model is also expected

used to explain other cell polarity phenomena, such as the direction of cell migration.

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