Physica A 391 (2012) 6470–6483

Contents lists available at SciVerse ScienceDirect

Physica A

journal homepage: www.elsevier.com/locate/physa

Multi-scale interactions in *Dictyostelium discoideum* aggregation

James A. Dixon [a,b,*], Damian G. Kelty-Stephen [c]

[a] *Center for the Ecological Study of Perception and Action, 406 Babbidge Road, U1020, University of Connecticut, Storrs, CT 06269-1020, USA*

[b] *Department of Psychology, 406 Babbidge Road, U1020, University of Connecticut, Storrs, CT 06269-1020, USA*

[c] *Wyss Institute for Biologically Inspired Engineering, Harvard University, 60 Oxford St 4th Floor, Suite 403, Cambridge, MA 02138, USA*

ARTICLE INFO

Article history:
Received 5 July 2011
Received in revised form 30 May 2012
Available online 27 July 2012

Keywords:
Cellular aggregation
Dictyostelium discoideum
Multifractals
Vector autoregression
Multi-scale analysis
Pattern formation

ABSTRACT

Cellular aggregation is essential for a wide range of phenomena in developmental biology, and a crucial event in the life-cycle of *Dictyostelium discoideum*. The current manuscript presents an analysis of multi-scale interactions involved in *D. discoideum* aggregation and non-aggregation events. The multi-scale fractal dimensions of a sequence of microscope images were used to estimate changing structure at different spatial scales. Three regions showing aggregation and three showing non-aggregation were considered. The results showed that both aggregation and non-aggregation regions were strongly multi-fractal. Analyses of the over-time relationships among nine scales of the generalized dimension, $D(q)$, were conducted using vector autoregression and vector error-correction models. Both types of regions showed evidence that across-scale interactions serve to maintain the equilibrium of the system. Aggregation and non-aggregation regions also showed different patterns of effects of individual scales on other scales. Specifically, aggregation regions showed greater effects of both the smallest and largest scales on the smaller scale structures. The results suggest that multi-scale interactions are responsible for maintaining and altering the cellular structures during aggregation.

1. Introduction

Cell aggregation is an important precursor for a host of events in developmental biology, including cell-fate decisions [1] and differentiation [2]. Aggregation requires the coordinated activity of many levels of cell behavior, such as intra- and intercellular "signaling" [3], cell motility [4], and the adhesion of adjoining cell membranes [5]. As such, aggregation is a macro-level phenomenon that is supported by the activity of many levels within the system.

Aggregation is also a fundamental phenomenon in the study of complex physical materials, such as those found in cloud formation, electro- and magneto-rheological fluids, colloidal gases and gels, and polymers [6–9]. In these domains, aggregation often occurs at multiple scales. For example, in complex fluids, such as colloidal suspensions, individual particles coalesce to form mesostructures, which in turn link together to form clusters. These clusters can ultimately form a macrostructure that spans the entire sample. An important insight from this work is that the properties of the fluid in which the aggregates have formed are not reducible to the micro-properties of the particles. Rather, the aggregates themselves exert influence through mechanical interactions and through effects on the hydrodynamics [10–12].

* Corresponding author at: Center for the Ecological Study of Perception and Action, 406 Babbidge Road, U1020, University of Connecticut, Storrs, CT 06269-1020, USA. Tel.: +1 860 486 6880; fax: +1 860 486 2760.
E-mail addresses: james.dixon@uconn.edu (J.A. Dixon), foovian@gmail.com (D.G. Kelty-Stephen).

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doi:10.1016/j.physa.2012.07.001

The current study addresses cellular aggregation in *Dictyostelium discoideum* from the perspective of multi-scale structures and their interactions. Causal physical structures in the *Dictyostelium* system exist at many scales [13,14]. Some of these structures are well known, such as cell membranes and cAMP gradients [15,16]. Others are less obvious, such as the adhesion complexes of pseudopods [17], but all must have measurable effects on the system, if they affect aggregation. Importantly, recent work shows that mechanical forces are not just the distal outcome of signaling processes that control cellular behavior. Rather, mechanical forces feed back into these signaling pathways through processes such as protein stretching [1] and stretch-activated calcium channels [18]. Thus, the activity generated by multi-scale processes within the system, also affect those self-same processes.

Because it is not known how many structures are in play during cell aggregation or at what scales they operate, our strategy is to measure the system across a wide range of scales. Each measurement quantifies the structures in that region of scales in a way that relates to their thermodynamic and hydrodynamic properties. We then use these measures to predict the changing structures at multiple scales. Our goal here is to show how interactions across scales drive aggregation (and non-aggregation) in *Dictyostelium*. The current approach treats the structured matter that constitutes the collection of cells as a multifractal field and proposes that interactions within that field are causally related to the macroscopic behavior of the system. A complementary approach is to extrapolate models from known processes at multiple scales (e.g., individual cells, tissues) and eventually model their interplay. See Refs. [19,20] for excellent examples of this latter approach.

For relatively simple, homogeneous media with a single type of particle structure (or very few structures), it is possible to determine hydrodynamic and thermodynamic properties analytically, given the fractal dimension [21]. For more complex media with heterogeneous structures, it is necessary to empirically determine their fractal dimensions, and use those measures as predictors of the behavior of the system, relying on their known links to hydro- and thermodynamics [10]. Given the very complex nature of cellular structures, we estimated the fractal dimensions across a range of different magnitudes (via a multifractal formalism) and used those estimates as measures of structure at multiple scales. We expected that structures at each scale would potentially have causal effects on all other scales, and that regions in which aggregation occurs would have different patterns of interaction compared to regions in which aggregation did not ultimately occur.

Carrillo et al. [22] utilized a related approach in their investigation of two different types of rheological dispersions, an electro-rheological fluid and a magneto-rheological fluid. Each dispersion began with a single type of particle ($Bi_4Ti_3O_{12}$ and Fe_3O_4, respectively) suspended in silicone oil at low concentration (volume fraction <0.05). They filmed the process of pattern formation as a field (electric or magnetic) was applied. From the resulting images, Carrillo et al. calculated the mass fractal dimension, D. Their results showed three distinct scaling regions, indicative of aggregations with three different characteristic scales. The first of these aggregates to emerge (i.e., the smallest scale) is driven primarily by dipolar interactions. The second-order aggregate (i.e., the middle scale) depends on both liquid viscosity and mechanical interactions among the first-order clusters. The formation of the third-order aggregate is slower, because of the shorter interaction time among second-order clusters and effects of viscosity.

Like Carrillo et al., we calculate the fractal dimension, D, from images taken during aggregation. We sampled six regions from a 541 frame (eight-bit, 672×512 pixel) recording of a *Dictyostelium* aggregation event. Each sampled region was 220×230 pixels. We selected three of these regions because they became aggregation sites. The remaining three were selected because they showed considerable activity, but aggregates did not form there. We performed multi-fractal analysis on each frame for each of the six regions. Multifractal analysis estimates the fractal dimension, D, across a spectrum of scales. For mono-fractals, the estimated values of D will vary little across scales. For multi-fractals, D will decrease monotonically with an increase in scales. The value of D within the spectrum provides a measure of the local fractal dimension for that scaling region. The details of the analysis are presented below, but here we note that the fractal dimension of structures at any particular scale relates to its energetic properties. For example, the degree to which a structure interacts with neighboring structures depends on its fractal dimension; greater fractal dimension increases the strength of interactions [10,11]. Similarly, the degree to which particles can move (e.g., via diffusion) near the structure is determined by the fractal dimension of the structure. The fractal dimension of a structure also affects the osmolar pressure within the bounded region in which the structure sits [21].

It is perhaps worth noting here that unlike Carrillo et al.'s rheological fluids in which there is initially very little structure (i.e., the particles are homogeneously distributed at first), the *Dictyostelium* images reveal considerable structure from the start. There is obviously structure within the cells themselves, but also among the cells.

To model the effects of D at each scale on future states of all other scales, we adopted a standard set of methods from econometrics for addressing systems in which all variables are treated as potentially endogenous and mutually affecting (i.e., each variable may affect each other variable) [23,24]. This family of methods, called vector autoregression and vector error-correction models, allows us to treat the fractal dimension at each scale as both a predictor (at a particular lag) and an outcome. We show that all scales have significant effects on the system. We also show that none of the scales functions as an exogenous variable. Finally, we show that aggregation and non-aggregation sites have different patterns of across-scale effects.

In the next sections, we describe the microscopy used to obtain the images during *Dictyostelium* aggregation and then review the multifractal analysis used to quantify the structures in those images. Finally, we explain the vector autoregression/error correction methods used to model the system-level effects and across-scale interactions.