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A Stochastic Model for Patterning of the Cytoplasm by the Saltatory Movement

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In various cases of importance for animal physiology and development, a specific distribution of cellular components is achieved through the active transport of these components along cytoskeletal fibres by molecular motors. The pattern-generating transport is stochastic; it is commonly referred to as the saltatory movement which means frequent, random change of direction of movement of individual transported particles. Inference of the distribution of the cellular components and kinetics of transitions between different patterns from parameters of the saltatory movement is the goal of the proposed theory. The theory is presented by developing a sample model for the redistribution of lipid droplets at early stages of *Drosophila* development, a process well studied at the quantitative level. The saltatory movement is modelled at the fundamental level as a stochastic velocity jump process. A diffusion (in the mathematical sense) model is derived from the fundamental velocity jump description as its simple and accurate approximation. This approximation reduces the number of parameters, simplifies the methods of their measurement and clarifies the relationship between the kinetics and the resulting pattern.

Introduction

A remarkable feature of cell structure that has been fully recognized only recently as a result of developments in experimental techniques is that what appears as a steady arrangement of components is in many cases brought about by a continuous active transport of these components. Examples include such phenomena of importance as the polarization of mRNA distribution that determines anterio-posterior and dorso-ventral axes in early embryos (Wilhelm & Vale, 1993; St. Johnston, 1995; King, 1996; Karlin-McGinness *et al*., 1996), and the redistribution of pigment granules in skin cells of fish and frogs, which adaptively changes the colour of the animal (Bagnara & Hadley, 1973; Haimo & Thaler, 1994; Rogers *et al*., 1997; Rodionov *et al*., 1998). The active intracellular transport in its turn displays another counter-intuitive property. The direction of movement of an individual transported particle frequently and randomly changes or reverses. Such particles are said to undergo the "saltatory movement" (Rebhun, 1967; Freed & Lebowitz, 1970; Rogers *et al*., 1997; Wacker *et al*., 1997; Welte *et al*., 1998; Suomalainen *et al*., 1999; Gross *et al*., 2000). The theoretical approach to the problem of pattern generation by the active intracellular transport should thus consist of developing a

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stochastic model of the transport, and then testing whether the model predicts patterns and their dynamics in agreement with experimental data when the parameters take on their measured values.

In the present paper I propose a deductive theory which proceeds from kinetics of the transport at the molecular level to quantitatively infer the distribution of the transported substances in the cell. Deriving a general formalism would be overly theoretical now that there is little quantitative knowledge of the intracellular transport. Instead, I illustrate the possible general approach to the problem on a specific example of a well-studied phenomenon. The kinetics of the transport of lipid droplets in early embryos of *Drosophila* has been exceptionally well studied, owing to the development of sophisticated techniques for the quantitative microscopic observation of this object that take advantage of its favourable geometry and optical properties (Welte *et al*., 1998; Gross *et al*., 2000). Despite the apparent insignificance of the lipid transport for the development of the embryo (Welte *et al*., 1998), the amenability to precise observation and measurement has made this phenomenon a unique experimental model for studying the intracellular transport, which is likely to yield results of general importance (Jäckle & Jahn, 1998). At the early stages of *Drosophila* development, numerous lipid droplets, approximately 0.5 μm in diameter, are in continuous motion along microtubules that stretch in the baso-apical direction in the blastoderm (Fullilove & Jacobson, 1971; Foe & Alberts, 1983; Welte *et al*., 1998; Gross *et al*., 2000). At the stage of syncytial blastoderm, the motile droplets concentrate near the surface of the embryo. From cycle 14 till the end of cellularization the distribution of droplets is reversed so that they occupy the basal parts of cells forming in the blastoderm. At the onset of gastrulation, the droplets move again to the periphery of the embryo. The movement of the droplets is saltatory and continues while their stationary distribution is maintained. The droplets are transported apically (toward the surface of the embryo) by dynein; the most likely candidate motor for their basal transport (away from the surface) is kinesin (Welte *et al*., 1998; Gross *et al*., 2000). Experiments have suggested the existence of a specific molecular switch that coordinately regulates the motors of opposite polarity and is responsible for the random alternation of the direction of movement of individual droplets (Gross *et al*., 2000). A number of parameters were measured for a kinetic description of the transport as alternating basal and apical runs (Welte *et al*., 1998; Gross *et al*., 2000). The model proposed here infers the stationary patterns at two developmental stages and the kinetics of the transition between these patterns from the parameters of the saltatory movement.

I model the active intracellular transport as a stochastic velocity jump process. Velocity jump processes are a class of dispersal processes in biological systems (Othmer *et al*., 1988). Following Rebhun (1967) and Freed & Lebowitz (1970), one can define the saltatory movement as such a movement that consists of periods, during which the velocity of the transported particle is constant and which are interspersed by abrupt and unpredictable changes in the velocity. Then, in principle, all the features of this type of movement can be incorporated in the stochastic velocity jump description. However, the complexity of the saltatory movement urges the modeller to reduce the dimensionality of the parameter space by looking for an adequate approximation; otherwise, the model can be intractable even if all the multiple parameters have been measured. Reduction of the number of parameters also facilitates quantitative experimentation. In this paper, I demonstrate that the complex stochastic process of the saltatory movement has a simple diffusion (in the mathematical sense) approximation.

Model

In the saltatory movement of lipid droplets in *Drosophila* embryos, six states of a droplet alternate stochastically (Gross *et al*., 2000). The states are the short-slow displacement in the basal direction (state 1), the long-fast displacement in the basal direction (state 2), the short-slow displacement in the apical direction (state 3), the long-fast displacement in the apical direction (state 4), the pause after a basal

displacement (state 5), and the pause after an apical displacement [state 6, Fig. 1(a)]. The distribution of the duration of each state is exponential, which implies first-order kinetics of transitions between the states (Gross *et al.*, 2000). A kinetic diagram showing the possible transitions between the states is presented in Fig. 1(b). The measurements (Welte *et al.*, 1998; Gross *et al.*, 2000) do not indicate the dependence of the kinetics of transport upon the position of the droplet, which is consistent with the uniform baso-apical arrangement of microtubules in the blastoderm (Fullilove & Jacobson, 1971). Since the distribution of the droplets themselves is not uniform, the constancy of the kinetics also justifies the assumptions that, first, the kinetics are independent of the droplet density, and, second, that the movements of individual droplets are statistically independent.

Let us introduce x, the distance from the embryo surface, and the density functions $p_i(t,x)$, such that $p_i(t,x)\,\mathrm{d}x$ is the probability that a given droplet is in the state i at the position x at the time moment t. In general, the pattern of the droplet distribution can be described by $n(t,x)$, the number density of droplets at (t,x). Under the assumption of the independent motion, $n(t,x) = Np(t,x)$, where N is the total number of droplets and $p(t,x) = \Sigma_i\, p_i(t,x)$. The practical goal of the theory is therefore the deduction of the density functions $p_i(t,x)$ from the kinetics of the saltatory movement. To formalize the above kinematics, one can write

$$\frac{\partial p_i(t,x)}{\partial t} = -v_i\frac{\partial p_i(t,x)}{\partial x} - \sum_j k_{ji}\,p_i(t,x) + \sum_j k_{ij}\,p_j(t,x), \tag{1}$$

where v_i is the velocity of the droplet in the state i, and k_{ij} is the rate constant of the transition to the state i from the state j. Velocities are equal to zero in the states of pause (states 5 and 6), and the rate constants are non-zero only for the transitions shown in Fig. 1(b). According to the measurements, the motile droplets are distributed between $x = 0$ and $x = a = 50\,\mu\mathrm{m}$ below the surface of the embryo (Welte *et al.*, 1998), hence the normalization

$$\sum_i \int_0^a p_i(t,x)\,\mathrm{d}x = 1. \tag{2}$$

A simple assumption is that of immediate reversal of the direction of the movement of a droplet upon the droplet reaching a boundary. If

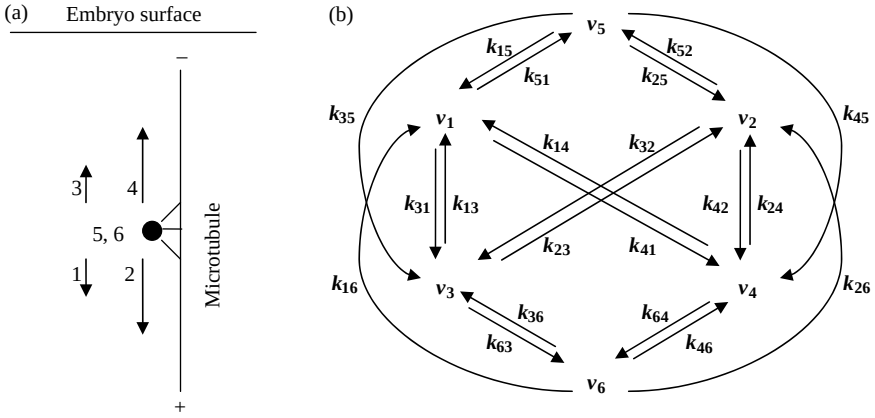

FIG. 1. Diagram of the velocity jump model of the saltatory movement of lipid droplets in *Drosophila* blastoderm. (a) A cartoon showing the orientation of a microtubule in the blastoderm (+, plus end of the microtubule, –, minus end), a droplet (●) attached to a microtubule through molecular motors, and the directions and the relative velocities of movement of the droplet in the six kinematical states. State 1 is short-slow basal movement, state 2 is long-fast basal movement, state 3 is short-slow apical movement, state 4 is long-fast apical movement, state 5 is pause after basal movement, state 6 is pause after apical movement. (b) Kinetic diagram of transitions between the kinematical states of a droplet. The velocity in state i ($i = 1, 2, \ldots, 6$) is v_i, the rate constant of the transition to state i from another state (j) is k_{ij}. Only transitions with non-zero rate constants are shown. The kinetic scheme is postulated here on the basis of the observations made by Gross *et al.* (2000).

in such a reversal the long-fast, rather than short-slow, mode of the movement is chosen with the probability p_l^+ for a basal run, and with the probability p_l^- for a apical run, then

$$\begin{aligned} v_1 p_1(t,0) &= (1 - p_l^+)[|v_3| p_3(t,0) + |v_4| p_4(t,0)], \\ v_2 p_2(t,0) &= p_l^+[|v_3| p_3(t,0) + |v_4| p_4(t,0)], \\ |v_3| p_3(t,a) &= (1 - p_l^-)[v_1 p_1(t,a) + v_2 p_2(t,a)], \\ |v_4| p_4(t,a) &= p_l^-[v_1 p_1(t,a) + v_2 p_2(t,a)]. \end{aligned} \tag{3}$$

Since the processive runs of droplets are much shorter than a, the dependence of the density functions upon the laws of behaviour of the droplets at the boundaries should be weak, justifying the assumptions made. Equations (1)–(3) constitute the velocity jump model of the droplet motion and can be solved by the finite difference method (Mitchell & Griffiths, 1980). In the steady state,

$$\frac{\partial p_i(t,x)}{\partial t} = 0, \tag{4}$$

the dynamically maintained distribution of the droplets is given by the stationary variants of eqns (1)–(3),

$$\begin{aligned} v_i \frac{d p_i(x)}{dx} &= -\sum_j k_{ji} p_i(x) + \sum_j k_{ij} p_j(x), \\ v_1 p_1(0) &= (1 - p_l^+)[|v_3| p_3(0) + |v_4| p_4(0)], \\ v_2 p_2(0) &= p_l^+[|v_3| p_3(0) + |v_4| p_4(0)], \\ |v_3| p_3(a) &= (1 - p_l^-)[v_1 p_1(a) + v_2 p_2(a)], \\ |v_4| p_4(a) &= p_l^-[v_1 p_1(a) + v_2 p_2(a)], \end{aligned} \tag{5}$$

$$\sum_i \int_0^a p_i(x)\,\mathrm{d}x = 1.$$

In practice, eqns (5) are more convenient to solve numerically.

Approximation

As suggested by the observations (Welte *et al.*, 1998; Gross *et al.*, 2000), the above model describes the droplet motion as a process in which the velocity of a droplet changes abruptly at unpredictable time moments. This type of a stochastic process is widespread in biological systems; it has been called velocity jump process (Othmer *et al.*, 1988), and diffusion approximations of this process have been proposed to simplify the treatment of such phenomena as cell locomotion and chemotaxis (Alt, 1980; Othmer *et al.*, 1988; Grünbaum, 2000). In the present case of the droplet motion, one can predict, in accordance with the central limit theorem (Feller, 1968) and neglecting the boundary constraints, that the stochastic displacement of a droplet during the time much longer than the characteristic time of transitions between the states will make the distribution of the position of the droplet Gaussian with the mean $u_\infty t$ and variance $2 s_\infty t$. Then by analogy with the theory of diffusion (Keizer, 1987) the parameters u_∞ and s_∞ can be called, respectively, the asymptotic drift coefficient and the asymptotic diffusivity of the droplets. These parameters will be functions of the fundamental parameters of the droplet motion, which are velocities and frequencies of the transitions between the states (the derivation is given in Appendix A). Whether this approximation is valid for the physiological times and for the constrained movement of the droplets should be tested. If it is valid, then by analogy with the diffusion proper (Keizer, 1987), the density function of position of the droplet will comply with the diffusion equation

$$\frac{\partial p(t,x)}{\partial t} = s_\infty \frac{\partial^2 p(t,x)}{\partial x^2} - u_\infty \frac{\partial p(t,x)}{\partial x}. \tag{7}$$

The normalization and the boundary conditions for the diffusion approximation that are analogous to eqns (2) and (3) are

$$\int_0^a p(t,x)\,\mathrm{d}x = 1 \tag{8}$$

and

$$\begin{aligned} s_\infty \frac{\partial p(t,x)}{\partial x}\bigg|_{x=o} &= u_\infty p(t,0), \\ s_\infty \frac{\partial p(t,x)}{\partial x}\bigg|_{x=a} &= u_\infty p(t,a). \end{aligned} \tag{9}$$

Equations (7)–(9) constitute the diffusion approximation model for the droplet motion. They can be solved by the finite difference method (Mitchell & Griffiths, 1980). The stationary solution to eqns (7)–(9) is

$$p(x) = \frac{\xi}{e^{\xi a} - 1} e^{\xi x}, \qquad (10)$$

where $\xi = u_\infty / s_\infty$.

Estimation of Parameters

The measured parameters of the movement of droplets at two stages of development of wild-type *Drosophila* embryos (Welte *et al.*, 1998; Gross *et al.*, 2000) are collected in Table 1. The phases in the droplet distribution are enumerated following Welte *et al.* (1998). Phase II spans from cycle 14 till the end of cellularization, and phase III corresponds to gastrulation. The parameters in Table 1 are only a selected subset of all the parameters measured by Welte, Gross, and co-authors, namely, those that are necessary for the estimation of the parameters of the present model. Yet they are numerous (14), and not all of their values have been reported for phase III. To complete the description of the phase III droplet motion, I assume that three unknown parameters of the plus end movement retain in phase III the values they had in phase II (Table 1). In this paper I use the terminology of Gross *et al.* (2000), who called pauses of a droplet after its travel in the direction of the microtubule minus end (apically, Fig. 1(a)) "minus-pauses", and, similarly, pauses after plus end (basal) travel, "plus-pauses".

To fully characterize the kinetics of transitions between the states of a droplet we have to know the probabilities that govern the choice of the next state in the stochastic sequence of the states. The probabilities that a plus- or minus-pause ends in a reversal of the direction of movement have been estimated experimentally (Table 1). Let us compute from the measured parameters other probabilities that characterize the transitions between the states. First, I estimate the probability p_p^+ that a plus end run of a droplet is succeeded by a pause, rather than by an immediate reversal of the direction of travel. Suppose there were N plus end runs of a droplet. Then the expected number of those which were followed by an immediate reversal is $N(1 - p_p^+)$. In $Np_p^+ p_r^+$. cases, where p_r^+ is the directly estimated probability that a plus-pause ends in a reversal (Table 1), the reversals were preceded by a pause. The fraction f_p^+ of plus–minus

TABLE 1
Measured parameters of the droplet motion

Parameter	Symbol	Value	
		Phase II	Phase III
Mean duration of a plus-pause	t_5	0.55 s	0.55 s*
Mean duration of a minus-pause	t_6	0.62 s	0.60 s
Probability that a plus-pause ends in a reversal	p_r^+	0.270	0.270*
Probability that a minus-pause ends in a reversal	p_r^-	0.346	0.432
Fraction of plus–minus reversals involving a pause	f_p^+	0.117	0.117*
Fraction of minus-plus reversals involving a pause	f_p^-	0.138	0.139
Mean displacement in short-slow plus end travel	d_1	0.067 μm	0.096 μm
Mean displacement in long-fast plus end travel	d_2	1.144 μm	0.780 μm
Mean displacement in short-slow minus end travel	d_3	–0.098 μm	−0.083 μm
Mean displacement in long-fast minus end travel	d_4	−1.068 μm	−1.048 μm
Number ratio of short runs and long runs, plus end travel	r_{sl}^+	1.05	2.22
Number ratio of short runs and long runs, minus end travel	r_{sl}^1	2.15	2.15
Average velocity of plus end travel	$\overline{v^+}$	0.407 μm s^{-1}	0.285 μm s^{-1}
Average velocity of minus end travel	$\overline{v^-}$	−0.475 μm s^{-1}	−0.378 μm s^{-1}

Note: Average velocities are from Welte *et al.*, 1998; other parameter values are taken from Gross et al., 2000, assuming that the relative frequency of an outcome gives its probability. Displacements and velocities of minus end travel are negative in the coordinate system of the present model.

*These parameters are assumed to retain in phase III the values that they had during phase II.

TABLE 2
Additional probabilistic parameters of the droplet motion

Parameter	Symbol	Expression	Value	
			Phase II	Phase III
Probability that a plus end run is succeeded by a pause	p_p^+	$f_p^+/(p_r^+ - f_p^+ p_r^+ + f_p^+)$	0.329	0.329
Probability that a minus end run is succeeded by a pause	p_p^-	$f_p^-/(P_r^- - f_p^- p_r^- + f_p^-)$	0.316	0.272
Probability that a plus end run is long-fast	p_l^+	$1/(1 + r_{sl}^+)$	0.488	0.311
Probability that a minus end run is long-fast	p_l^-	$1/(1 + r_{sl}^-)$	0.317	0.317

reversals involving a pause among all plus–minus reversals is known from measurements (Table 1). Hence, we obtain an equation $Np_p^+ p_r^+/[Np_p^+ p_r^+ + N(1 - p_p^+)] = f_p^+$ that can be solved for the unknown p_p^+ (Table 2). The probability p_p^- that a minus end run is succeeded by a pause is calculated analogously (Table 2). Whether the reversal occurs or not specifies only the direction of the movement in the next state, providing no information on whether the movement will be long-fast or short-slow. For the purposes of the present model, I assume that the observed number ratio of short runs and long runs (Table 1) results from a probabilistic choice of the short-slow vs. long-fast mode of movement that is made each time the movement begins. Under this assumption, if there are r_{sl} times more short runs than long runs in a particular direction, then the probability p_l that a run in that direction will be long-fast satisfies the equation $(1 - p_l)/p_l = r_{sl}$. The solutions of this equation, in the cases of the plus and minus end movement at the two stages of the *Drosophila* development, are collected in Table 2 and are used in the estimation of the model parameters as explained below.

The parameters of the velocity jump model of the saltatory movement are velocities and rate constants of transitions between the states with the different velocities, eqn (1). With the use of the probabilities derived above, I estimate the velocities first. The measured average velocity of plus end travel ($\overline{v^+}$) is a weighted sum of the velocities of the short-slow (v_1) and long-fast (v_2) runs with the weights equal to the probabilities that a plus end run is long-fast (p_l^+) or short-slow ($1-p_l^+$). We know, in addition, that a "fast" run is approximately twice as fast as a "slow" run (Gross *et al.*, 2000). This consideration yields two simultaneous equations, $\overline{v^+} = v_1(1 - p_l^+) + v_2 p_l^+$ and $v_2 = 2v_1$, that can be solved for the unknown velocities in states 1 and 2 of a droplet (Table 3). The velocities in the states of the minus end movement, 3 and 4, are estimated analogously (Table 3). The so far derived parameters allow us to calculate the rate constants of transitions between the states of the droplet, and so to complete the estimation of the parameters of the velocity jump model of the droplet motion. The exponential distribution of the lifetime of state i with the mean t_i is consistent with a first-order kinetics of a process that leads to exiting this state if the rate constant of such a process is equal to $1/t_i$. The lifetime of every state of the droplet in our model is distributed exponentially according to the measurements made by Gross *et al.* (2000). The rate constant of the transition to state i from state j will be equal to p_{ij}/t_j, where p_{ij} is the probability that after exiting state j the droplet will enter state i. Let us compute, for example, the rate constant of the transition from state 3 to state 1. The mean lifetime t_3 can be computed as the quotient of the measured mean distance d_3 covered by a droplet in state 3 (Table 1) and the estimated velocity v_3 of the droplet in this state (Table 3). The probability p_{13} that state 3 is succeeded by state 1 [rather than by state 2 or 6, Fig. 1(b)] can be computed as follows. State 3 is the state of the short-slow minus end movement, and state 1 is the state of the short-slow plus end movement. We have already estimated the probability p_p^- that a minus end run is succeeded by a pause and the probability p_l^+ that a plus end run is long-fast (Table 2). In the transition from state 3 to state 1 the droplet reverses the

TABLE 3
Parameters of the velocity jump model of the droplet motion

Parameter	Expression	Value	
		Phase II	Phase III
v_1	$\overline{v^+}/(1+p_l^+)$	0.274 μm s^{-1}	0.217 μm s^{-1}
v_2	$2v_1$	0.547 μm s^{-1}	0.435 μm s^{-1}
v_3	$\overline{v^-}/(1+p_l^-)$	–0.361 μm s^{-1}	–0.287 μm s^{-1}
v_4	$2v_3$	–0.721 μm s^{-1}	–0.574 μm s^{-1}
k_{13}	$\frac{v_3}{d_3}(1-p_p^-)(1-p_l^+)$	1.288 s^{-1}	1.735 s^{-1}
k_{14}	$\frac{v_4}{d_4}(1-p_p^-)(1-p_l^+)$	0.236 s^{-1}	0.275 s^{-1}
k_{15}	$\frac{1}{t_5}(1-p_r^+)(1-p_l^+)$	0.680 s^{-1}	0.915 s^{-1}
k_{16}	$\frac{1}{t_6}p_r^-(1-p_l^+)$	0.286 s^{-1}	0.496 s^{-1}
k_{23}	$\frac{v_3}{d_3}(1-p_p^-)p_l^+$	1.227 s^{-1}	0.781 s^{-1}
k_{24}	$\frac{v_4}{d_4}(1-p_p^-)p_l^+$	0.225 s^{-1}	0.124 s^{-1}
k_{25}	$\frac{1}{t_5}(1-p_r^+)p_l^+$	0.647 s^{-1}	0.412 s^{-1}
k_{26}	$\frac{1}{t_6}p_r^-p_l^+$	0.272 s^{-1}	0.224 s^{-1}
k_{31}	$\frac{v_1}{d_1}(1-p_p^+)(1-p_l^-)$	1.905 s^{-1}	1.126 s^{-1}
k_{32}	$\frac{v_2}{d_2}(1-p_p^+)(1-p_l^-)$	0.223 s^{-1}	0.277 s^{-1}
k_{35}	$\frac{1}{t_5}p_r^+(1-p_l^-)$	0.335 s^{-1}	0.335 s^{-1}
k_{36}	$\frac{1}{t_6}(1-p_r^-)(1-p_l^-)$	0.720 s^{-1}	0.646 s^{-1}
k_{41}	$\frac{v_1}{d_1}(1-p_p^+)p_l^-$	0.869 s^{-1}	0.482 s^{-1}
k_{42}	$\frac{v_2}{d_2}(1-p_p^+)p_l^-$	0.102 s^{-1}	0.119 s^{-1}
k_{45}	$\frac{1}{t_5}p_r^+p_l^-$	0.156 s^{-1}	0.156 s^{-1}
k_{46}	$\frac{1}{t_6}(1-p_r^-)p_l^-$	0.335 s^{-1}	0.301 s^{-1}
k_{51}	$\frac{v_1}{d_1}p_p^+$	1.344 s^{-1}	0.746 s^{-1}
k_{52}	$\frac{v_2}{d_2}p_p^+$	0.157 s^{-1}	0.184 s^{-1}
k_{63}	$\frac{v_3}{d_3}p_p^-$	1.164 s^{-1}	0.940 s^{-1}
k_{64}	$\frac{v_4}{d_4}p_p^-$	0.214 s^{-1}	0.149 s^{-1}

Note: v_i is the velocity in state i of the droplet, k_{ij} is the rate constant of transition to state i from state j. Velocities in states 5 and 6 equal zero. Rate constants other than listed in this table equal zero, because the corresponding transitions do not take place in the model.

direction of its movement without going through a pause. The probability of this is $(1-p_p^-)$. Then the movement in the plus-end direction becomes short-slow rather than long-fast, which outcome has the probability $(1-p_l^+)$. If the two choices are made independently, the probability p_{13} will

be $(1-p_p^-)(1-p_l^+)$. The rate constant k_{13} is then obtained by dividing this probability p_{13} by the lifetime of state 3 (Table 3). The expressions of the other rate constants are derived similarly and are listed in Table 3 along with the estimated values of these constants at the two stages of the *Drosophila* development, which completes the estimation of the parameters of the velocity jump model of the droplet motion. The parameters of the diffusion approximations are functions of the parameters of the velocity jump model. Their analytical expressions would be too complex to derive, instead, the diffusion parameters are computed numerically as explained in Appendix A, and their values are listed in Table 4.

TABLE 4
Parameters of the diffusion approximation of the droplet motion

Parameter	Symbol	Value Phase II	Phase III
Drift coefficient	u_∞	$0.094\,\mu m\,s^{-1}$	$-0.025\,\mu m\,s^{-1}$
Diffusion coefficient	s_∞	$0.341\,\mu m^2\,s^{-1}$	$0.219\,\mu m^2\,s^{-1}$
Distribution constant	$\xi = u_\infty/s_\infty$	$0.274\,\mu m^{-1}$	$-0.114\,\mu m^{-1}$

Computation and Comparison with Experiment

Figure 2 shows the density functions of the baso-apical distribution of droplets in the blastoderm that have been calculated for two stages of the *Drosophila* development and for the transition between these stages. During cellularization (phase II), the stationary density function increases with the distance from the embryo surface [Fig. 1(a)]. This is consistent with the "clearing" of the periphery of the embryo of the light-scattering droplets that was observed at this stage (Welte *et al.*, 1998; Gross *et al.*, 2000). When the parameter values are changed from those of phase II to those of phase III (gastrulation), a net flux of the droplets toward the periphery is predicted in agreement with the observation (Welte *et al.*, 1998; Gross *et al.*,

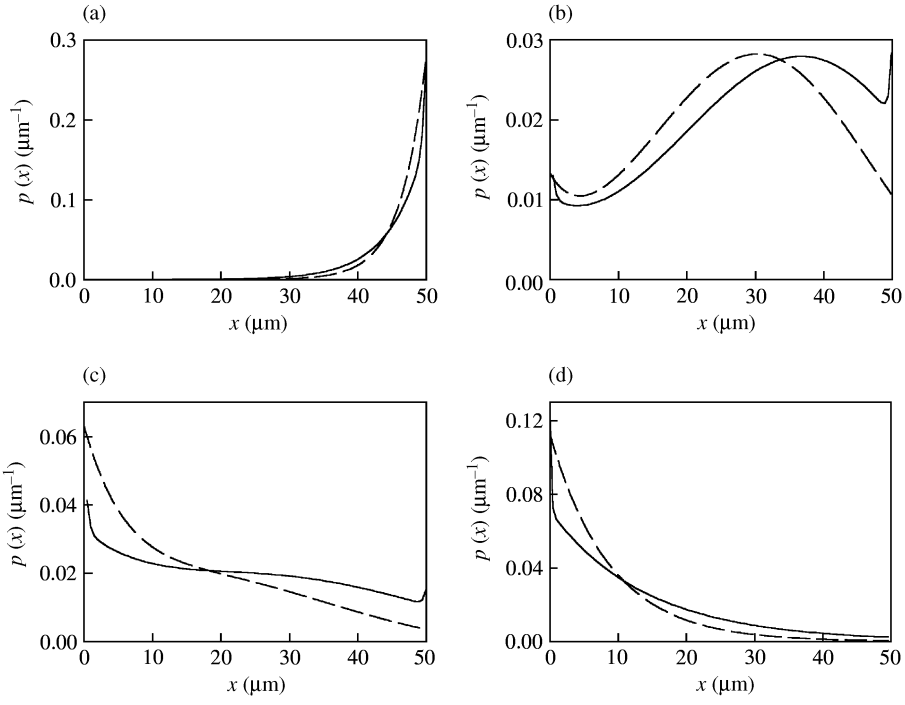

FIG. 2. Density function $p(x)$ of the distribution of droplets in *Drosophila* blastoderm. x, the distance from the embryo surface. Predictions of the velocity jump model are shown by (—) curves; predictions of the diffusion approximation are shown by (- - -) curves. (a) In the steady state in phase II of development (cell cycle 14 through the end of cellularization). (b) In 10 min after the transition from phase II to phase III (gastrulation). (c) In 20 min after the transition to phase III. (d) In the steady state of phase III.

2000). In this transition, the density function gradually adopts the form of the decreasing stationary density function [Fig. 1(b–d)]. This corresponds to the "clouding" of the periphery of the embryos because of the accumulation of the light-scattering droplets that was observed in phase III (Welte *et al.*, 1998; Gross *et al.*, 2000). In agreement with the observation (Welte *et al.*, 1998), the major increase in the density at the periphery of the embryo occurs in the model within 10 min [Fig. 2(b)], and the transition is close to completion in 20 min [Fig. 2(c)].

The density functions predicted in the diffusion approximation are essentially same as the functions predicted by the velocity jump model. The discrepancy between the predictions as seen in Fig. 2 is most likely smaller than the discrepancy between any of them and the reality. For example, the histograms obtained by analysing electron micrographs all show modal distributions of the droplets (Welte *et al.*, 1998), whereas both the velocity jump model and its diffusion approximation predict that the modal distributions are only transient [Fig. 2(b)]. Notwithstanding, the gross features of the droplet distribution, such as the direction of the accumulation of the droplets (basal or apical) and the rate of their redistribution at the onset of gastrulation, are adequately reproduced in the diffusion approximation as well as in the velocity jump model. The model therefore provides the quantitative framework, into which additional experimental data can be incorporated to reach a better agreement of the prediction with the more precise observation, and employing the diffusion approximation will greatly simplify such a study.

Discussion

The theory of patterning of the cytoplasm by saltatory movement is presented here as a sample model for a specific phenomenon, the redistribution of lipid droplets in an early *Drosophila* embryo. In the experimental work by Welte *et al.* (1998), the measured parameters of the saltatory movement of the droplets were used to calculate the quantity called "bulk displacement rate", whose meaning is close to that of the asymptotic drift coefficient u_∞ of the present diffusion approximation model. The directions of the bulk displacement were found to be in agreement with the directions of macroscopic fluxes of droplets during transitions between the phases of development. In the present work, the asymptotic drift coefficient is computed from the parameters of a more detailed description of the droplet motion (Gross *et al.*, 2000). The present model is designed to predict not only the direction of the fluxes, but also the entire kinetics of the transitions and the steady-state distributions of the droplets. Although the analysis can be carried out at the "fundamental" level of the saltatory movement as a velocity jump process, it becomes much simpler in the diffusion approximation. In this approximation, the asymptotic diffusivity s_∞ reflects the dispersion of displacements of individual droplets around the bulk displacement, whose rate is given by u_∞. Together, these two parameters suffice to predict the entire dynamics of the distribution of droplets during the transition. The steady-state distribution is predicted as an exponential function of the distance from the embryo surface with the increment equal to the ratio of the two parameters $\xi = u_\infty/s_\infty$. So, the stationary density of the droplets is predicted to increase or decrease away from the embryo surface depending on the sign of u_∞. Unlike u_∞ alone, the ratio ξ determines the entire shape of the steady-state distribution, not only whether the density increases or decreases. It is important to note that in spite of the fact that the interpretation of the quantity u_∞ as the "bulk displacement rate" is not useful in the steady state, the ratio $\xi = u_\infty/s_\infty$ is the parameter that explains the stationary distribution of droplets in terms of the kinetics of their continuing motion.

Regarding the proposed diffusion approximation of the saltatory movement it is necessary to note that the word "diffusion" is used here in its mathematical sense (e.g. Bharucha-Reid, 1960) and only to reflect the theoretical result that the density function of the droplet distribution complies with the diffusion equation. Applications of the stochastic diffusion processes are various even within biology; e.g. in population genetics (Crow & Kimura, 1970) or bacterial locomotion (Berg, 1993). It should be especially emphasized that in such applications neither the

usage of the word "diffusion" nor the description by the diffusion equation stipulates the thermal motion as the mechanism of the phenomenon in hand. In the present case of the saltatory movement, the active fundamental mechanism makes the phenomenological asymptotic diffusivity of the droplets several orders of magnitude higher than possible for the thermal motion. For the thermal diffusion, the diffusivity of a particle whose diameter is d can be calculated as $D = kT/3\pi\eta d$, where $kT = 4.1 \times 10^{-21}$ N m is the thermal energy, and η is the viscosity of the medium (Berg, 1993). The diameter of a droplet is $d \approx 0.5\,\mu$m (Welte *et al.*, 1998). Due to the complex structure of the cytoplasm, its apparent viscosity depends strongly on the size of the probe used in the experiment, varying between about $0.004\,\mathrm{kg\,m^{-1}\,s^{-1}}$ for macromolecules and $\sim 10^5\,\mathrm{kg\,m^{-1}\,s^{-1}}$ for particles $\sim 10^2\,\mu$m in size (Luby-Phelps *et al.*, 1986; Sato *et al.*, 1984). Using probes with $d \approx 0.5\,\mu$m yields the apparent viscosity of the cytoplasm of lung macrophages in the range 254–2745 $\mathrm{kg\,m^{-1}\,s^{-1}}$ (Valberg & Feldman, 1987). Assuming $\eta = 250\,\mathrm{kg\,m^{-1}\,s^{-1}}$, we can estimate the thermal diffusivity of the droplets as $D = 3.5 \times 10^{-6}\,\mu\mathrm{m^2\,s^{-1}}$. The computed asymptotic diffusivity of the droplets at the two stages of *Drosophila* development is 0.219 and $0.341\,\mu\mathrm{m^2\,s^{-1}}$, which reflects the active mechanism of the transport.

The proposed model reproduces the essential features of the reversible redistribution of droplets in the early development of *Drosophila* in agreement with the experimental data (Welte *et al.*, 1998; Gross *et al.*, 2000). At present, one cannot formulate any substantial discrepancy between the theory and experiment in quantitative terms. Undoubtedly, this should be attributed to the relative insufficiency of the quantitative data even on this exceptionally well-studied object rather than to the perfection of the model. On the experimental part, the exact distributions of droplets under the (quasi) steady-state conditions as well as the exact kinetics of the transitions between the steady states remain to be quantified. Some parameters of the plus-end movement during phase III, although likely to retain their values in phase II as assumed here (Table 1), remain to be measured, as well as the parameters during phase I (syncytial blastoderm). Knowledge of these parameters and distributions would allow us to compare the predictions and the experimental data more precisely and in more diverse situations. On the theoretical part, microtubules spanning the entire region where the droplets move and the absence of interference between the movement of individual droplets are the two major idealizations behind the proposed model that are likely to be abandoned when the model does not explain a more precise observation. Certainly, such a refinement of the theory would by itself require more measurements of the cytoskeleton structure and the droplet kinematics.

Generalization

The main purpose of the development of the model of the droplet motion in this work was to present the general concepts of modelling the patterning of the cytoplasm by the saltatory movement on a well-studied and simple example. This way of presenting the theory was chosen in the belief that the derivation of a general formalism, when the quantitative empirical knowledge is sparse, would be overly theoretical, whereas generalization of a specific model would be obvious. A more complex transport than described in the present model can be accounted for by the introduction of more states and by the corresponding increase in the number of equations of type (1). In general, every possible velocity of a transported particle should be represented in the velocity jump model by a separate state. This means that in the model there should be a state to represent every possible combination of a motor (motors) that is ferrying the particle and a filament along which the particle is being ferried. The position of the particle and its velocity have to be given by vectors. The kinetics of transitions between the states are, in the mechanistically simplest case, the kinetics of the motor–filament association and dissociation reactions. So the rates of such transitions will be functions of position of the particle when the cytoskeleton is inhomogeneous and the availability of filaments differs at different locations in the cytoplasm. It can be seen that the complete description of the

saltatory movement at the fundamental level requires specifying velocities for every state and rates of transitions for every pair of states. Keeping such a fundamental model tractable and the set of its parameters measurable will therefore involve neglecting some of the possible states and transitions.

The proposed velocity jump model of the saltatory movement of lipid droplets is very simple, yet it contains 24 parameters. The proposed diffusion approximation of the saltatory movement has only two parameters regardless of the complexity of the velocity jump model for the same phenomenon. As the number of parameters in the detailed description of a more complex motility explodes, the advantage of the simple approximation will increase. It should be emphasized that the approximation relies upon additional assumptions. It is based on equilibration of probabilities of the states of the transported particle. Therefore, if the region within which the particles move is not significantly larger than the distance a particle travels continuously in a certain state, the diffusion approximation will be inaccurate. Similarly, in the inhomogeneous case definite diffusion parameters can be assigned to a given position in space only if the fundamental kinetics does not differ substantially within a certain neighbourhood of that position. Such a neighbourhood has to be larger than the characteristic continuous displacement of the particle in any state for the diffusion parameters to converge to determinate asymptotic values. At the same time, the accuracy of the diffusion approximation of the real saltatory movement is expected to be higher than the accuracy of the diffusion approximation of the velocity jump model of that movement because many unaccounted irregularities in the real process additionally randomize it. Provided that the diffusion approximation is valid, two- or three-dimensional movement can be described as a diffusion process as well. In these cases, the asymptotic drift will be given by a vector, and the asymptotic diffusivity will be given by a tensor, whose components can be derived from the velocity jump parameters of the saltatory movement like the one-dimensional diffusion parameters in the present model.

Implications for Experimentation

Measurement of the velocity jump parameters of the saltatory movement requires tracking the transported particles with a high time resolution and parsing the trajectories into segments of continuous movement (Welte *et al.*, 1998; Gross *et al.*, 2000). This is a time-consuming and technically demanding experimental procedure, and the multiple parameters can be especially difficult to measure when the movement is more complex than in the case of the lipid droplets and when the particles are harder to monitor. Measurement of the asymptotic diffusion parameters is much less demanding, and the methods of the measurement themselves allow one to evaluate the validity of the diffusion approximation in the case under study. According to the definition, the drift coefficient can be measured as the slope of the plot of the mean displacement of particles vs. time. Analogously, the diffusivity is given by one-half of the slope of the plot of variance in the displacement of particles vs. time. In the two- or three-dimensional case, such linear regression has to be applied to the mean components of the displacement along the coordinate axes to obtain the components of the drift vector. Analogously, the rates of increase of elements of the covariance matrix of components of displacement will determine the corresponding elements of the matrix representing the diffusivity tensor. If the plots are substantially nonlinear, the diffusion approximation is evidently inaccurate. This method of measurement does not involve determining the beginning and the end of each segment of a continuous movement. Consequently, it does not require the fine time resolution in tracing the particles. Recently, we have performed such measurements of the asymptotic diffusion parameters of the saltatory movement and made use of them in our experimental research (Maly & Vorobjev, unpublished results). Although the diffusion parameters do not provide information on the detailed mechanism of the motion, the present analysis demonstrates that they can be sufficient to explain kinetically the patterns, which the transported particles form in the cytoplasm.

In conclusion, the proposed model for patterning of the cytoplasm treats the saltatory

movement of the cytoplasmic components as a stochastic velocity jump process. It infers the stationary distribution of the components and kinetics of the transitions between the steady patterns from the detailed kinetic characteristics of the saltatory movement. The diffusion approximation simplifies this kinetics–pattern relationship, reduces the number of parameters and makes their measurements easier.

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Appendix A

Computation of the Diffusion Parameters

Following the principles of the diffusion approximation of a velocity jump process (Alt, 1980; Othmer *et al.*, 1988; Grünbaum, 2000), our recent approach (Maly, 2002) can be generalized to compute the asymptotic diffusion parameters from the parameters of the velocity jump model

of the droplet motion. Consider the alternation of the states of the droplet regardless of its position (this implies neglecting the forced change of state that occurs at the boundaries). Let us arrange the probabilities of the states in a vector $\mathbf{p}$ and the rate constants of the transitions between the states in a matrix $\mathbf{K}$. Then the evolution of the probabilities is given by

$$\dot{\mathbf{p}} = \mathbf{K}\mathbf{p}. \tag{A.1}$$

The asymptotic drift is the weighted sum of the velocities in the states, with the steady-state probabilities of the states as the weights. Let $\mathbf{p}^{(\infty)}$ be the steady-state solution to eqn (A.1) and $\mathbf{v}$ be the vector of velocities in the states. Then the asymptotic drift of the droplets will be

$$u_\infty = \mathbf{v} \cdot \mathbf{p}^{(\infty)}. \tag{A.2}$$

The diffusivity is the integral of the velocity autocovariance function (Maly, 2002). Let $\mathbf{p}^{(j)}$ be the solution to eqn (A.1) with the initial condition $p_i = \delta_{ij}$, where δ is the Kronecker delta. Then the asymptotic diffusivity of the droplet will be

$$s_\infty = \sum_i p_i^{(\infty)}(v_i - u_\infty) \int_0^\infty (\mathbf{v} \cdot \mathbf{p}^{(i)} - u_\infty)\, \mathrm{d}t. \tag{A.3}$$