Text S1: Methods Supplement

Linking morphodynamics and directional persistence of T lymphocyte migration

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Note: all pertinent references are cited in the main text.

Computational model basics

The model is implemented in the freely available, spatial stochastic simulator, Smoldyn v2.31, which treats molecular species as particles. The code is provided as a separate, supplemental text file so that others may tweak the structure of the model and/or the values of the parameters. The code is annotated, but the same information may be found here along with additional comments.

The idealized geometry of the cell is a two-dimensional disk of radius 10; although length, time, and concentration units are relative, length units are roughly μm. The circular boundary is continuous and represents the outer membrane of the cell, whereas the interior of the domain represents the cytoplasm. The simulation is configured to run for 10,000 time units (roughly, seconds), with a time step of 0.05 units. Movies are constructed by exporting 1 frame out every ten (0.5 time units).

Because of the stochastic nature of the model, not every simulation produces a polarized cell of sufficient stability. Simulations that failed to produce a defined cell front and rear for a substantial period of time were discarded.

Model species, initial conditions, and mobilities

Model species are either membrane-associated or cytosolic and move in space — along the boundary contour or within the interior of the domain, respectively — by diffusion. Besides the membrane/cytosol distinction, species may be classified by their association with either the *F-actin Circuit* or the *Cell Rear*. Species separated according to these two distinctions and their associated diffusivity values (roughly, in $\mu m^2/s$) are given below, starting with those of the F-actin Circuit.

F-actin Circuit		
Membrane species	**Description**	**Diffusivity**
Mem	Discrete points on the membrane boundary	0
F	F-actin barbed ends at the boundary	0.3
Ri	Inactive receptors at the boundary	0.01
Ra	Active receptors at the boundary	0.01
P	‘Poison’ molecules recruited to the boundary	0.01
Cytosolic species	**Description**	**Diffusivity**
Si	Inactive signaling molecules	30

Sa	Active signaling molecules	0.3

The immobile species, Mem, simply provides discrete points on the membrane where certain reactions can occur. Its purpose in the model is to serve as a catalyst for 'source' or 'sink' reactions. The boundary is initially seeded with a sufficient number of copies of Mem (2000 was the number used in our simulations).

F-actin barbed ends, F, represent the appearance of membrane protrusion and thus this species provides the primary connection to experimental observations. In the simulations, F appear spontaneously and are not present initially. The diffusivity of 0.3 does not represent diffusion on the membrane but rather the expansion of the dendritic F-actin network due to the action of the Arp2/3 complex. This value was set after some experimentation to establish a reasonable velocity of wave propagation.

Inactive receptors, Ri, are initially seeded in the membrane (2000 was the number used in our simulations). This species represents unligated chemokine receptors and, as integral membrane proteins, diffuse slowly. A reasonable diffusivity value of 0.01 was chosen. Active receptors, Ra, are converted from/to inactive receptors and are not present initially. This species has a diffusivity of 0.01, same as Ri. The membrane-associated 'poison' molecules, P, are produced by the active receptors and are not present initially. This species has a diffusivity of 0.01, same as Ra.

Inactive signaling molecules, Si, are initially seeded in the cytosol and were assigned a reasonably rapid diffusivity of 30. The initial number of Si in our simulations was 1000; use of a higher number (e.g., 10000; with appropriate adjustment of the rate constant for activation of the signaling molecule) does not affect the results but makes the simulations run much slower. Active signaling molecules, Sa, are converted from/to inactive signaling molecules (by Ra at the membrane) and are not present initially. They reside in the cytosol but technically represent a transiently membrane-bound state. Therefore the diffusivity of Sa is much lower than that of Si, satisfying a known requirement of this class of pattern formation models. The diffusivity value (0.3) was chosen to match that of F.

The following is a list of species associated with the Cell Rear. As explained in the main text, this module was added after formulation of the F-actin Circuit to enforce a cell polarity.

Cell Rear		
Membrane species	**Description**	**Diffusivity**
Mem	Discrete points on the membrane boundary	0
Rear	Structural component of the cell rear	0.3
Amp	Amplifier of the cell rear	0.01
Cytosolic species	**Description**	**Diffusivity**
Xi	Inactive 'messenger' for the rear	100
Xa	Active 'messenger' for the rear	0.3

L	Long-range inhibitor of the rear from the front	3

Here, the aforementioned species Mem is used for the Cell Rear module as well. The species Rear and Amp are analogous to F and Ra in the F-actin circuit, respectively (Mem is adopted as the equivalent of Ri in the cell rear), and the diffusivity values were assigned to match. Xi and Xa are analogous to Si and Sa, respectively. To speed up the simulations, a lower initial number of Xi was used (100); to reduce the associated 'noise', a higher diffusivity of 100 was used for this species, whereas the diffusivity of the active form, Xa, was kept the same as that of Sa (0.3).

The long-range inhibitor, L, is produced in proportion to F, together with the local destruction of F by Rear, provides the antagonism that allows relative movements of the rear and the F-actin patch(es). L also helps prevent the spontaneous formation of a 'second rear'. Its diffusivity value (3) and turnover rate constant are such that L covers the F-actin patch(es) and short spaces in between but does not accumulate near the center of the rear domain.

Simulated reactions

Each reaction in the model is handled according to the mass-action, particle-based Smoluchowski formalism upon which Smoldyn is based. The reactions are listed below along with comments about their formulation and associated rate constants. In each, membrane-associated species are indicated in **bold**, and the associated mass-action rate constant is specified and explained as needed. When a species appears on both sides of the reaction with equal stoichiometry, that species acts as a catalyst. When a species appears on both sides of the reaction but with a higher stoichiometry on the product side, it is an autocatalytic transformation. The symbol Ø on the product side indicates irreversible destruction of the species on the reactant side.

Reactions 1-10 apply to the F-actin Circuit.

Reaction 1

Mem → **Mem** + **F** $1x10^{-5}$

Spontaneous appearance of F-actin. The frequency is set so that the event is rare (but eventually happens during a typical simulation). One can adjust the likelihood by changing either the rate constant or the initial density of Mem.

Reaction 2

F → Ø 0.02

First-order loss of F-actin barbed ends, e.g. by capping. The first-order rate constant is ~ 1/min, or approximately 0.02 s^{-1}.

Reaction 3

F + **Ri** → **F** + **Ra** $1x10^{-7}$

Protrusion, represented by the density of F-actin barbed ends, promotes receptor activation, e.g. by spreading over immobilized ligands on the surface. This is the first step in the amplification of the F-actin circuit. The rate constant is tuned so that

activated receptors tend to appear following the spontaneous appearance of F, but its value should not be so high that Ri is dramatically depleted (which affects the sensitivity of the amplifier). One can also tune this rate by changing the initial density of Ri.

Reaction 4
Ra → **Ri** 1
First-order deactivation of the receptor. The rate constant is set somewhat arbitrarily so that receptor activation equilibrates quickly enough relative to the dynamics of F; i.e., its value is significantly greater than that of Reaction 2.

Reaction 5
Ra + Si → **Ra** + Sa 100
This heterogeneous reaction describes the efficient activation of the signaling molecule by active receptors. Its rate constant should be sufficiently high so that F-actin patches are in competition with one another; i.e., Si must be partially depleted.

Reaction 6
Si → Sa 1
First-order deactivation of the signaling molecule. As in Reaction 4, the rate constant is set to be arbitrarily high; however, once it is set, there are two important considerations. First, the rate constant for Reaction 5 should be of a certain magnitude to affect global depletion of Si, and second, the spatial range of Sa (affected also by the diffusivity of Sa) should be low enough that each Sa remains localized in the vicinity of the F-actin patch where it was generated.

Reaction 7
Sa + **F** → Sa + 2**F** 0.3
This heterogeneous reaction describes the autocatalytic growth of F-actin barbed ends (involving the Arp2/3 complex), promoted by the active signaling molecule. Given the inherent nonlinearity of this process, the dynamics of the circuit are sensitive to the value of the associated rate constant. The value is tuned so that F-actin waves propagate, but not to the extent that a single F-actin patch fills the entire membrane.

Reaction 8
Ra → **Ra** + **P** 0.3
Active receptors also generate a poison. The value of the rate constant is set so that the poison regularly appears during growth of an F-actin patch.

Reaction 9
P + **F** → **P** 1
The poison molecule reduces the number of F-actin barbed ends. The rate constant is tuned along with that of Reaction 8 so that F-actin patches reliably split into two and then propagate in opposite directions.

Reaction 10
P → *Ø* 1
First-order loss of P. The value of the rate constant is arbitrarily set to 1 as in Reactions 4 and 6.

The remaining reactions apply to the Cell Rear and its interactions with the F-actin Circuit.

Reaction 11
Mem → **Mem** + **Rear** $1x10^{-5}$
Spontaneous appearance of the 'Rear' component, akin to Reaction 1 for F-actin. The value of the rate constant is the same as for Reaction 1.

Reaction 12
Rear → *Ø* 1
Rear has a finite lifetime. The value of the rate constant is arbitrarily set to 1.

Reaction 13
Rear + **Mem** → **Rear** + **Amp** $1x10^{-7}$
Rear promotes the generation of Amp, the local amplifier of the Cell Rear module. The reaction is analogous to Reaction 3 of the F-actin Circuit, with the same value of the rate constant.

Reaction 14
Amp → **Mem** 1
Amp has a finite lifetime. The value of the rate constant is arbitrarily set to 1.

Reaction 15
Amp + Xi → **Amp** + Xa 10
Amp promotes activation of the messenger molecule for the rear. After some experimentation, its rate constant value was set along with that of Reaction 17 so that the rear domain would grow robustly and at a reasonable rate.

Reaction 16
Xa → Xi 1
Deactivation of the messenger molecule for the rear. The value of the rate constant is arbitrarily set to 1.

Reaction 17
Xa + **Rear** → Xa + 2**Rear** 1
Autocatalytic growth of Rear, promoted by Xa, analogous to Reaction 7 of the F-actin Circuit. Its rate constant value was set along with that of Reaction 15 so that the rear domain would grow robustly and at a reasonable rate.

Reaction 18
Rear + **F** → **Rear** 0.3
Rear acts as a sink for F-actin. For example, Myosin II could act to crush the F-actin network. The value of the rate constant was optimized along with that of Reaction 21 so that F-actin patches can exist adjacent to, but do not encroach significantly upon, the rear domain.

Reaction 19
F → **F** + L 1
F-actin produces a long-range inhibitor, L. The value of the rate constant is arbitrarily set to 1.

Reaction 20
L → Ø 1
L has a finite lifetime. The value of the rate constant is arbitrarily set to 1 (see previous comment about the diffusivity of L).

Reaction 21
L + **Rear** → L 0.2
L consumes Rear. The value of the rate constant was optimized along with that of Reaction 18 so that F-actin patches can exist adjacent to, but do not encroach significantly upon, the rear domain. The rear domain is forced to move in concert with the wave(s) of F-actin.