Tissue self-organization based on collective cell migration by contact activation of locomotion and chemotaxis

Taihei Fujimori*, Akihiko Nakajima, Nao Shimada, and Satoshi Sawai

*Graduate School of Arts and Sciences, University of Tokyo, Komaba, 153-8902 Tokyo, Japan; and †Research Center for Complex Systems Biology, University of Tokyo, Komaba, 153-8902 Tokyo, Japan

Edited by Herbert Levine, Rice University, Houston, TX, and approved January 22, 2019 (received for review September 14, 2018)

Despite their central role in multicellular organization, navigation rules that dictate cell rearrangement remain largely undefined. Contact between neighboring cells and diffusive attractant molecules are two of the major determinants of tissue-level patterning; however, in most cases, molecular and developmental complexity hinders one from decoding the exact governing rules of individual cell movement. A primordial example of tissue patterning by cell rearrangement is found in the social amoeba Dicyostelium discoideum where the organizing center or the “tip” self-organizes as a result of sorting of differentiating prestalk and prespore cells. By employing microfluidics and microsphere-based manipulation of navigational cues at the single-cell level, here we uncovered a previously overlooked mode of Dicyostelium cell migration that is strictly directed by cell–cell contact. The cell–cell contact signal is mediated by E-set Ig-like domain-containing heterophilic adhesion molecules TgrB1/TgrC1 that act in trans to induce plasma membrane recruitment of the SCAR complex and formation of dendritic actin networks, and the resulting cell protrusion competes with those induced by chemoattractant cAMP. Furthermore, we demonstrate that both prestalk and prespore cells can protrude toward the contact signal as well as to chemotax toward cAMP; however, when given both signals, prestalk cells orient toward the chemotaxtractant, whereas prespore cells choose the contact signal. These data suggest a model of cell sorting by competing juxtacrine and diffusive cues, each with potential to drive its own mode of collective cell migration.

Significance

Migration of cells as a group is pivotal to the making of various tissues in developing embryos; however, their complexity hinders one from identifying the exact rules. We exploited relatively simple and conditional multicellularity of the social amoeba Dicyostelium to analyze tissue patterning from bottom up by identifying the navigation rules at the individual cell level. We uncovered a guidance mechanism directed by cell–cell contact which gives rise to collective migration and competes with diffusive attractant molecules. Competition of the two directional cues forms the basis of how cells position themselves in the multicellular aggregate according to cell type. A similar scheme of collective migration and sorting may underlie patterning of other developing tissues as well as cancer invasion.

Author contributions: T.F., A.N., and S.S. designed research; T.F. performed research; A.N. and N.S. contributed new reagents/analytic tools; T.F. analyzed data; and T.F. and S.S. wrote the paper.

The authors declare no conflict of interest. This article is a PNAS Direct Submission.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

*To whom correspondence should be addressed. Email: cssawai@mail.ecc.u-tokyo.ac.jp.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1815063116/-/DCSupplemental.

Published online February 19, 2019.

www.pnas.org/cgi/doi/10.1073/pnas.1815063116

PNAS | March 5, 2019 | vol. 116 | no. 10 | 4291–4296
Movies S1 and S2). Addition of purified TgrC1ext resulted in a then formed tips as cAMP waves ceased (Fig. 1C, +PDE; Movies S1 and S2). Prestalk cells sorted out to the periphery but never collected to form the apical tip (Fig. 1C, +PDE; SI Appendix, Fig. S1G). The rotational movement was not correlated with a few passages of residual waves (Fig. 1H and I), suggesting that cell migration, despite being highly coordinated, was not chemotactically oriented. When both purified TgrB1ext and PDE were applied, prestalk cells were completely stalled, while prespore cells retained some movement but were less coordinated (Fig. 1C, +TgrB1ext/+PDE; Movie S1). These observations indicate that, in addition to chemotaxis toward cAMP, there is an additional guidance cue mediated by cell–cell contact that directs collective cell movement.

TgrB1/C1 are essential for postaggregative gene regulation and are differentially expressed in pretalk and prespore cells (SI Appendix, Fig. S2) (26, 28). To avoid developmental effects and clarify the basic rule of cell movement, we first analyzed migration of cells immediately before prestalk/prespore diversification ("streaming-stage" cells; see Materials and Methods) using a microfluidic gradient chamber (SI Appendix, Fig. S3 A and B). The leader cells moved toward the cAMP source, cells at 0.52(−) contacted leading edge compared with 1.00(−) cells (Fig. 2B). While further away toward the cytosolic region, filaments are highly enriched in Arp2/3 (Fig. 2G and Movie S5). The cell–cell contact was persistent, long (≈5µm), and appeared most strongly at the outer edge (Fig. 2F, Right, magenta, SI Appendix, Fig. S6A, and Movie S5). The cell–cell contact was also observed in naturally streaming cells (SI Appendix, Fig. S6B). The contact region was highly enriched in Arp2/3 (Fig. 2G), indicating that actin filaments form dendritic networks (37). These observations are compatible with the general feature of a leading edge of migrating cells where dendritic F-actin networks grow mainly by side-branching nucleation mediated by the Arp2/3 complex, while further away toward the cytosolic region, filaments are severed and depolymerized. The other widespread feature of the leading edge in various cells is the so-called "retrograde" flow of F-actin due to excess filament growth relative to the speed of membrane expansion. Although solitary migrating Dictyostelium cells are known to lack obvious retrograde flow at the leading edge (38), time-lapse images of F-actin at the cell–cell contact region were indicative of such flow (Movie S5). To quantitate the speed of retrograde flow of the F-actin network, GFP-Arp2 incorporated in dendritic filaments was photobleached partially, and dislocation of the bleached region was followed over time. After photobleaching of GFP-Arp2, the nonfluorescent region proximal and distal to the bleached region was photobleached partially, and dislocation of the bleached region was followed over time. After photobleaching of GFP-Arp2, the nonfluorescent region moved backward (Movie S6A, and Movie S5). The F-actin pattern was also observed in naturally streaming cells (SI Appendix, Fig. S6B). The contact region was highly enriched in Arp2/3 (Fig. 2G), indicating that actin filaments form dendritic networks (37). These observations are compatible with the general feature of a leading edge of migrating cells where dendritic F-actin networks grow mainly by side-branching nucleation mediated by the Arp2/3 complex, while further away toward the cytosolic region, filaments are severed and depolymerized. The other widespread feature of the leading edge in various cells is the so-called "retrograde" flow of F-actin due to excess filament growth relative to the speed of membrane expansion. Although solitary migrating Dictyostelium cells are known to lack obvious retrograde flow at the leading edge (38), time-lapse images of F-actin at the cell–cell contact region were indicative of such flow (Movie S5). To quantitate the speed of retrograde flow of the F-actin network, GFP-Arp2 incorporated in dendritic filaments was photobleached partially, and dislocation of the bleached region was followed over time. After photobleaching of GFP-Arp2, the nonfluorescent region moved backward (Movie S6A, and Movie S5). The F-actin pattern was also observed in naturally streaming cells (SI Appendix, Fig. S6B). The contact region was highly enriched in Arp2/3 (Fig. 2G), indicating that actin filaments form dendritic networks (37). These observations are compatible with the general feature of a leading edge of migrating cells where dendritic F-actin networks grow mainly by side-branching nucleation mediated by the Arp2/3 complex, while further away toward the cytosolic region, filaments are severed and depolymerized. The other widespread feature of the leading edge in various cells is the so-called "retrograde" flow of F-actin due to excess filament growth relative to the speed of membrane expansion. Although solitary migrating Dictyostelium cells are known to lack obvious retrograde flow at the leading edge (38), time-lapse images of F-actin at the cell–cell contact region were indicative of such flow (Movie S5). To quantitate the speed of retrograde flow of the F-actin network, GFP-Arp2 incorporated in dendritic filaments was photobleached partially, and dislocation of the bleached region was followed over time. After photobleaching of GFP-Arp2, the nonfluorescent region moved backward (Movie S6A, and Movie S5). The F-actin pattern was also observed in naturally streaming cells (SI Appendix, Fig. S6B). The contact region was highly enriched in Arp2/3 (Fig. 2G), indicating that actin filaments form dendritic networks (37). These observations are compatible with the general feature of a leading edge of migrating cells where dendritic F-actin networks grow mainly by side-branching nucleation mediated by the Arp2/3 complex, while further away toward the cytosolic region, filaments are severed and depolymerized. The other widespread feature of the leading edge in various cells is the so-called "retrograde" flow of F-actin due to excess filament growth relative to the speed of membrane expansion. Although solitary migrating Dictyostelium cells are known to lack obvious retrograde flow at the leading edge (38), time-lapse images of F-actin at the cell–cell contact region were indicative of such flow (Movie S5). To quantitate the speed of retrograde flow of the F-actin network, GFP-Arp2 incorporated in dendritic filaments was photobleached partially, and dislocation of the bleached region was followed over time. After photobleaching of GFP-Arp2, the nonfluorescent region moved backward (Movie S6A, and Movie S5). The F-actin pattern was also observed in naturally streaming cells (SI Appendix, Fig. S6B). The contact region was highly enriched in Arp2/3 (Fig. 2G), indicating that actin filaments form dendritic networks (37). These observations are compatible with the general feature of a leading edge of migrating cells where dendritic F-actin networks grow mainly by side-branching nucleation mediated by the Arp2/3 complex, while further away toward the cytosolic region, filaments are severed and depolymerized. The other widespread feature of the leading edge in various cells is the so-called "retrograde" flow of F-actin due to excess filament growth relative to the speed of membrane expansion. Although solitary migrating Dictyostelium cells are known to lack obvious retrograde flow at the leading edge (38), time-lapse images of F-actin at the cell–cell contact region were indicative of such flow (Movie S5). To quantitate the speed of retrograde flow of the F-actin network, GFP-Arp2 incorporated in dendritic filaments was photobleached partially, and dislocation of the bleached region was followed over time. After photobleaching of GFP-Arp2, the nonfluorescent region moved backward (Movie S6A, and Movie S5). The F-actin pattern was also observed in naturally streaming cells (SI Appendix, Fig. S6B). The contact region was highly enriched in Arp2/3 (Fig. 2G), indicating that actin filaments form dendritic networks (37). These observations are compatible with the general feature of a leading edge of migrating cells where dendritic F-actin networks grow mainly by side-branching nucleation mediated by the Arp2/3 complex, while further away toward the cytosolic region, filaments are severed and depolymerized. The other widespread feature of the leading edge in various cells is the so-called "retrograde" flow of F-actin due to excess filament growth relative to the speed of membrane expansion. Although solitary migrating Dictyostelium cells are known to lack obvious retrograde flow at the leading edge (38), time-lapse images of F-actin at the cell–cell contact region were indicative of such flow (Movie S5). To quantitate the speed of retrograde flow of the F-actin network, GFP-Arp2 incorporated in dendritic filaments was photobleached partially, and dislocation of the bleached region was followed over time. After photobleaching of GFP-Arp2, the nonfluorescent region moved backward (Movie S6A, and Movie S5). The F-actin pattern was also observed in naturally streaming cells (SI Appendix, Fig. S6B). The contact region was highly enriched in Arp2/3 (Fig. 2G), indicating that actin filaments form dendritic networks (37). These observations are compatible with the general feature of a leading edge of migrating cells where dendritic F-actin networks grow mainly by side-branching nucleation mediated by the Arp2/3 complex, while further away toward the cytosolic region, filaments are severed and depolymerized. The other widespread feature of the leading edge in various cells is the so-called "retrograde" flow of F-actin due to excess filament growth relative to the speed of membrane expansion. Although solitary migrating Dictyostelium cells are known to lack obvious retrograde flow at the leading edge (38), time-lapse images of F-actin at the cell–cell contact region were indicative of such flow (Movie S5). To quantitate the speed of retrograde flow of the F-actin network, GFP-Arp2 incorporated in dendritic filaments was photobleached partially, and dislocation of the bleached region was followed over time.
Fig. 2. Microfluidics single-cell level analysis of train migration and contact-induced leading edge dynamics. (A) Train migration. GFP-Lifeact/AX4 (green), Lifeact-RFP/AX4 (magenta), 0- to 10-nM cAMP gradient (blue, ATTO425). (Scale bar, 100 μm.) (B) Snapshots of solitary (Left) and two-cell train (Right). (Scale bar, 10 μm.) (C) Cell contours (arrows, lateral pseudopods). (Scale bars, 10 μm.) (D) Response to reversal of cAMP gradient (0–1 μM; magenta, Lifeact-RFP; green, fluorescein). (Scale bars, 10 μm.) (E) Fraction of cells with (+) or without (−) an immediate response (solitary: n = 73 cells, leader: n = 28 cells, follower: n = 97 cells). (F) A contact-free (Left) and a cell-cell contact (Right) leading edge (green, HSPC300-GFP and magenta, Lifeact-RFP) in a 0–1 μM cAMP gradient. (Scale bars, 2 μm.) (G) Fluorescence recovery after photobleaching in GFP-Arpa2/3 cells (yellow boxed region) in a contact-free (Left) and contacted (Right) leading edge. White dashed line, a leading cell contour; 0–10-nM cAMP gradient. (Scale bars, 1 μm.) (H) Scatterplot of nucleation and protrusion speed. (I) Slip speed of actin filaments. Mean ± SEM, contact-free: n = 18 cells, cell-cell contact: n = 23 cells. (J) Immobilization of a follower (Upper) or a leader (Lower) by UV irradiation; 0- to 10-nM cAMP gradient. (Scale bars, 10 μm.)

contact-free leading edge, suggesting an enhanced nucleation at the contact site.

The enhanced F-actin formation at the cell-contacted leading edge suggests that there is up-regulation of Arp2/3 activity. In solitary migrating Dictyostelium cells, the SCAR complex, which is required for full activation of the Arp2/3 complex, translocated to the membrane in small patches that lasted no longer than ~10 s (36) (Fig. 2F, Left, magenta). In contrast, at the cell-contacted leading edge, there was markedly enhanced localization of the SCAR complex (Fig. 2F, SI Appendix, Fig. S6 C and D, and Movie S5). Transient localization of myosin II during the short-term retraction of the leading edge in solitary cells (SI Appendix, Fig. S7A) was completely absent from the leading edge of follower cells (SI Appendix, Fig. S7 B–E). Major leading-edge signals such as Ras-GTP, PI(3,4,5)P3, and Rac-GTP were also present at the contacted front and appeared similar to contact-free leading edge (SI Appendix, Fig. S6 E–J). These observations suggest that the follower cells have a leading edge with a persistent dendritic F-actin network that generates unidirectional propulsive force that pushes the plasma membrane forward. Accordingly, when leader cells were immobilized by UV irradiation, the follower cells continued to move and push the leader cells (Fig. 2J and Movie S6). The observation indicates contact-dependent protrusive activity that is independent of pulling by the front cell in contact. The cell–cell contact mode of migration was also evident in the slug-stage cells, as they exhibited train migration in a microchamber with the characteristic SCAR complex localization at the contact site and the ballistic motion of follower cells (SI Appendix, Fig. S8A). In addition to head-to-tail contact as observed in streaming-stage cells, head-to-head was frequently observed in slug-stage cells (SI Appendix, Fig. S8B). As in streaming-stage cells, transient localization of myosin II at the contact-free front was absent from the cell-contacted front in slug-stage cells (SI Appendix, Fig. S8C).

TgrB1/C1 and Contact-Induced Front Protrusion. The molecular basis of cell-train formation was further analyzed by studying binary mixtures of WT, tgrB1, and tgrC1. First, streaming-stage cells were employed to circumvent the inability of the Tgr-null cells to differentiate after aggregation (28). We found that in cells that followed tgrC1, F-actin formation failed to become persistent (Fig. 3 A and B and SI Appendix, Fig. S9 A and B). The pairwise frequency of the contact itself was also low when cells being followed were tgrC1 or when the following cells were tgrB1 (Fig. 3C), which is consistent with a recent study suggesting that TgrB1 and TgrC1 act as a receptor and a ligand, respectively, for allorrecognition (31). TgrB1/C1 are developmental stage-specific genes, hence the protrusions and the elongated shape in developing cells were never induced by contact with vegetative cells unless TgrC1 was overexpressed (Fig. 3 D and SI Appendix, Fig. S9 C and D). Moreover, streaming-stage cells were able to follow TgrC1 over extended time (vegetative cells that migrated toward the attractant folate, contact-dependent front protrusion and guidance are mediated primarily by TgrB1/C1 and do not require chemotraction. In the case of slug-stage cells, cells formed small clusters without a stable contact-free front and rotated in random directions (SI Appendix, Fig. S10). Application of purified TgrB1ext in a cAMP gradient extinguished the cell clusters and cells migrated directly along the cAMP gradient (SI Appendix, Fig. S104 and Movie S8). Moreover, when purified TgrC1ext and lectin wheat germ agglutinin (WGA) which binds to cell surface glycoproteins such as the homophilic adhesion protein CsA (39) were able to induce an extensive protrusion at the site of cell–microsphere contact (Fig. 3F). Much like in cell trains, the cell–microsphere contact site was intensely decorated with the SCAR complex and F-actin (Fig. 3F and Movie S9). Microsphere coated with WGA alone or TgrC1ext alone or TgrB1ext/WGA was unable to induce the characteristic protrusion (SI Appendix, Fig. S114). The results indicate that juxtacrine signaling between TgrC1 at the tail of a cell and TgrB1 at the front of a cell activates the SCAR complex and induces a highly enhanced formation of F-actin at the cell–cell contact site. The induction by the TgrC1ext/WGA-coated beads was also observed in streaming-stage cells (SI Appendix, Fig. S11 B and C) but was less frequent.

Despite their spatially restricted mechanism of action, TgrB1 was localized to the front and the back of collectively moving cells in a mound, whereas TgrC1 was observed uniformly at the cell-train leading edge (Fig. 3G and SI Appendix, Fig. S12 A–C). Lack of front/back symmetry breaking was puzzling, considering that, in cell trains and mounds, cells did not form two fronts in the opposite directions. In cells attached to two beads, cells indeed formed a front protrusion on one bead, and the other bead was attached to their tail except in cases where protrusions faced the same directions and hence cells were double headed.
delineate the roles of chemotaxis and contact-dependent protrusion in tip formation. It has been hypothesized that prestalk cells sort to the tip by migrating fast and winning the chemotaxis race against prespore cells (22, 32). When assayed at the single-cell level, however, prespore cells migrated faster than prestalk cells in a 0- to 1-μM cAMP linear gradient (Fig. 4A). Similar results were obtained from tracking well-isolated dissociated cells in the initial phase of reaggregation mitigated of contact signal by TgrB1mut (Fig. 4B). In a 0- to 10- or 0- to 50-μM linear gradient, both cell types halted at the same location in the channel, indicating a similar response range (SI Appendix, Fig. S13). How then can cell–cell contacts facilitate cell segregation? When attached to TgrClmut/WGA-coated microsphere (Fig. 4C), a marked protrusion was observed in about one-third of the cell–bead interface regardless of the cell type (Fig. 4D). While the prespore cells exhibited a single protrusion at the contact site, many of the prestalk cells had auxiliary protrusions (Fig. 4C, arrows, Fig. 4E, and Movie S11). Accordingly, in two-cell clusters, Arp2 accumulated at multiple contact sites in prestalk cells, whereas in prespore cells it was confined to a single contact site (SI Appendix, Fig. S5D). The difference in the number of protrusions between prespore and prestalk cells was also evident in vivo and in isolation without cell–cell contact (SI Appendix, Fig. S14). When exposed to a cAMP gradient, the protrusion of prestalk cells at the bead–cell interface often disappeared and new pseudopods formed toward the cAMP source, whereas prespore cells remained attached to the beads and retained the polarity (Fig. 4 F and G and Movie S12). The results indicate that prespore and prestalk cells can be oriented by cAMP and TgrB1/C1; however, when both signals are presented, there is a dominance as to which directs their leading edge. Prestalk cells are

(SI Appendix, Fig. S12D). These results suggest that a protrusion is inhibited from forming at the tail once cells polarize. Interestingly, the choice of a bead to which protrusion formed sometimes swapped between the two (SI Appendix, Fig. S12E), suggesting that contact-mediated polarity is dynamically maintained and possibly mechanosensitive. Similar front competitions were often observed in a monolayer aggregate where TgrB1 first accumulated toward two cells which then split as the cells deviated, and a contact with one cell was selected (Fig. 3H and Movie S10).

Single-Cell-Level Response to Navigational Cues. Lastly, slug-stage cells were analyzed in details according to the cell types to
less polarized, continue to form random pseudopods even in the presence of a Tgr-induced protrusion, and thus can be navigated chemotactically, whereas prespore cells are highly polarized and locked in toward the cell–cell contact. Taken together with the results obtained from the competition for two cues TgrC1 and cAMP for tip formation (Fig. 1 C–H), the radial trajectories and the head-to-tail alignment of prespore cells is best explained by the Tgr-mediated navigation, whereas prestalk cells deviate from the contact-mediated collective migration and chemotax to extracellular cAMP (Fig. 4H).

Discussion

A propensity of Dictyostelium cells to follow cells in contact was suggested by a classic work by Shaffer who coined the term “contact following” (40); however, it has remained heretofore unclear (41, 42). In the present work, we conclude that the following behavior is driven by “contact activation of locomotion”—an induction of leading edge by cell–cell contact and the accompanying forward propulsion. The cell–contacted leading edge was highly enriched in a SCAR complex subunit HSPC300 and dendritic F-actin. Since the same response was observed in cells attached to TgrC1/WGA-coated microspheres, there appears to be a mechanism whereby TgrB1/C1 interaction induces accumulation of the SCAR complex at the cell–cell contact site. Lack of apparent features in the cytosolic residues in TgrB1 and requirement for lectin WGA for the response points to a possibility that the interaction between TgrB1 and the SCAR complex is indirect and that there is clustering of adhesion and signaling complex at the contact site. The contact site appears distinct from that forming a phagocytic cup, since the contact area appears to extend narrowly instead of expanding and engulfing. A PI3(3,4,5)P3 marker, PHcrac-RFP, known to be enriched in phagocytic cups (43) was not localized at the cell–bead interface (SI Appendix, Fig. S15 A and B). Addition of latrunculin A shortened the protrusion; however, it did not promote contact activation (SI Appendix, Fig. S15 C–E). Retrograde flow of F-actin has been suggested to play a major role in determining front–back polarity and its persistence during cell migration (44). In addition, it has been shown that F-actin flow induced by confinement and decrease in cell–substrate adhesiveness helps to establish persistent cell polarity by rearward transfer of inhibitors for protrusion such as myosin II (45). The observations of the retrograde flow, absence of myosin II at cell–contacted front, monopodal morphology, and loss of cell–substrate adhesion in the cell anterior are in line with the current understanding of cell polarity (47). Indeed, in cells that dance of myosin II-enriched actin cortex (47). Indeed, in cells that lack cell–cell contact signal mediated by cadherin activate RhoA, inhibits protrusion, and facilitate cell repulsion (54). On the other hand, migration toward self-secreted chemotactic C3a keeps neural crest cells together (55, 56). In Dictyostelium, cell–cell contact mediated by TgrB1/C1 promotes protrusion, and chemotaxis rather is disruptive to otherwise more tightly packed cell mass as evidenced by mounds becoming spherical in the absence of the chemotactic cue. The present findings raise many open questions for future work. Besides prestalk segregation, myosin-mediated motility appears to play an important role in Dictyostelium slug migration, culmination as well as kindiscriminatory segregation (25, 30). Also of note is a striking evolutionary convergence of collective cell migration, despite no homologs of TgrB1/C1 existing in metazoans. Are there parallelsisms to protocadherin-dependent SCAR complex recruitment and enhancement of migration in cultured cells (57) or similar enhancement of F-actin by atypical cadherin in rotating the Drosophila egg chamber (58)? Are other cell-streaming behaviors such as those observed in human breast cancer cells (59) driven by a related mechanism? Drawing from a recent demonstration of contact following in tissue culture cells (60), it is tempting to speculate that contact activation of locomotion is at work in systems outside of Dictyostelium. Further investigations in these phenomena should clarify common rules and logical necessities for cellular collective movement.

Materials and Methods

Cell Preparation, Microfluidics, and Live Cell Imaging. To obtain the streaming-stage cells, cells were synchronously differentiated by 6-h cAMP pulsing. To obtain the slug-stage cells, cells incubated for 12–17 h on agar were collected, suspended in PB for dissociation by passing them through a syringe needle. Polydimethylsiloxane (PDMS) chambers were fabricated and used as described previously (61, 62). All fluorescence images were obtained by confocal microscopy. For details, see SI Appendix.

Plasmids, Protein Purification, and Microsphere Coating. His-tagged TgrB1* and TgrC1* expression vectors were constructed by inserting genomic DNA fragments of extracellular domain of tgrB1 or tgrC1 into the vector harboring act15 promoter and His-tag sequence. Cells harboring pA15-tgrB1*ext-His6-2H3term or pA15-tgrC1*ext-His6-2H3term were designed to secrete extracellular domain of TgrB1 and TgrC1, respectively (SI Appendix, Fig. S1C). TgrB1*ext and TgrC1*ext were purified from the respective cultured cells by affinity chromatography using a Ni2+–NTA column. Elution buffer was exchanged with PB by ultrafiltration. Purified TgrC1*ext was immobilized to functionalized silica beads (5 μm diameter, Sumitomo Bakelite BS-X9905) by covalent bond according to the manufacturer’s protocol. For details, see SI Appendix.

Fujimori et al.

PNAS | March 5, 2019 | vol. 116 | no. 10 | 4295

Constructs by guest on April 8, 2021

Downloaded by guest on August 3, 2021