© 2017. Published by The Company of Biologists Ltd | Journal of Cell Science (2017) 130, 97-103 doi:10.1242/jcs.179952

SPECIAL ISSUE: 3D CELL BIOLOGY

SHORT REPORT

Myosin II governs collective cell migration behaviour downstream of guidance receptor signalling

Anne Combedazou[1], Valérie Choesmel-Cadamuro[1], Guillaume Gay[2], Jiaying Liu[1], Loïc Dupré[3,4,5], Damien Ramel[1,*] and Xiaobo Wang[1,*,‡]

ABSTRACT

Border cell migration during *Drosophila* oogenesis is a potent model to study collective cell migration, a process involved in development and metastasis. Border cell clusters adopt two main types of behaviour during migration: linear and rotational. However, the molecular mechanism controlling the switch from one to the other is unknown. Here, we demonstrate that non-muscle Myosin II (NMII, also known as Spaghetti squash) activity controls the linear-to-rotational switch. Furthermore, we show that the regulation of NMII takes place downstream of guidance receptor signalling and is critical to ensure efficient collective migration. This study thus provides new insight into the molecular mechanism coordinating the different cell behaviours in a migrating cluster.

KEY WORDS: Myosin II, Border cells, Collective cell migration, Guidance receptor

INTRODUCTION

Collective cell migration plays a key role in normal development and in pathological conditions such as metastasis formation (Thiery, 2009). This mode of migration applies to different sizes of cell cohorts and can adopt various organisations such as sheets, strands or small clusters (Friedl and Gilmour, 2009; Malet-Engra et al., 2015). However, how cell dynamics are coordinated during collective migration in response to extracellular cues remains an intriguing question. Border cell migration during *Drosophila* oogenesis is a simple model to study collective cell migration *in vivo* (Montell et al., 2012). These groups of cells migrate between nurse cells to reach the oocyte (Fig. 1A). The directionality of border cell migration is defined by the polarised activity of guidance receptors, the epidermal growth factor receptor (EGFR) and the platelet-derived growth factor (PDGF)- and vascular endothelial growth factor (VEGF)-receptor related (PVR) (Duchek and Rørth, 2001; Duchek et al., 2001). Studies of border cell movements have revealed that migration is divided into two phases: a linear movement characterises the early phase (the first half) with a single cell leading the others, whereas later, the forward migration appears less organised with rotational (also called tumbling) movement in which each cell of the cluster is able to take the lead to drive the migration (Bianco et al., 2007; Poukkula et al., 2011). Although PVR and EGFR seem redundant, PVR mostly controls the first step of migration and EGFR the second step (Poukkula et al., 2011). However, apart from this implication of guidance receptors and potentially the Hippo pathway (Lucas et al., 2013), nothing is known about the molecular mechanisms that drive the switch between the two modes of migration. In this study, we show that non-muscle Myosin II (NMII, also known as Spaghetti squash) is necessary to promote a rotational behaviour in addition to its role in the detachment process (Majumder et al., 2012). Moreover, we show that EGFR regulates positively NMII, whereas PVR has an inhibitory impact on NMII activity. Taken together, these data demonstrate the central role of NMII in the control of the migratory behaviour of border cells.

[1]LBCMCP, Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, UPS, 31062 Toulouse, France. [2]DamCB, Data Analysis and Modelling for Cell Biology, Marseille F-13005, France. [3]INSERM, UMR 1043, Centre de Physiopathologie de Toulouse Purpan, 31024 Toulouse, France. [4]Université Toulouse III Paul-Sabatier, 31062 Toulouse, France. [5]CNRS, UMR 5282, 31204 Toulouse, France.
*These authors contributed equally to this work

‡Author for correspondence (xiaobo.wang@univ-tlse3.fr)

Received 1 September 2015; Accepted 18 March 2016

RESULTS AND DISCUSSION

Control of NMII activation is crucial for ensuring collective migration

Given that NMII is highly expressed in border cells (Edwards and Kiehart, 1996) and that actin dynamics is the main driving force during border cell migration (Majumder et al., 2012; Murphy and Montell, 1996), we asked whether the actomyosin cytoskeleton could be involved in the switch between these two behaviours. First, we tested the role of NMII regulation in the efficiency of collective migration in terms of speed and ability of the cells to reach their destination. We calculated the average speed of each type of migration in NMII-activated [overexpression of the active version of its kinase ROCK (ROCK CA) or overexpression of a constitutively active version of Rho1 (RhoV14)] or -inactivated (expression of RNAi against ROCK or NMII or overexpression of the Mbs phosphatase, a negative regulator of NMII activity) clusters. In both cases, we observed a decrease in the speed of border cell migration (Fig. 1B). A decrease in migration speed would theoretically result in a migration delay. In order to quantify whether such a delay occurs, we took advantage of the fact that wild-type border cells perform a stereotypical migration that is completely achieved by stage 10 of oogenesis. Therefore, we could precisely determine the migration index (i.e. mean migrated distance) and completion index (i.e. the proportion of clusters having reached the oocyte). To do so, the egg chamber was divided into five sections corresponding to different positions along the migration path (no migration, 0%; full migration, 100%). The number of border cell clusters in each section was counted on fixed egg chambers at stages 9 and 10 of oogenesis (Fig. S1A). Preventing NMII activation led to lower migration and completion indexes compared to the control in both stages 9 and 10 egg chambers due to slower migration and detachment defects (Fig. 1C–F; Fig. S1A,B). However, locking NMII signalling in an activated state led to only moderate (stage 9) and surprisingly weak (stage 10) migration defects (Fig. 1C–F; Fig. S1A,B). Taken together, these results reveal that tight regulation of NMII is essential for optimised border cell migration.

Journal of Cell Science (2017) 130, 97-103 doi:10.1242/jcs.179952

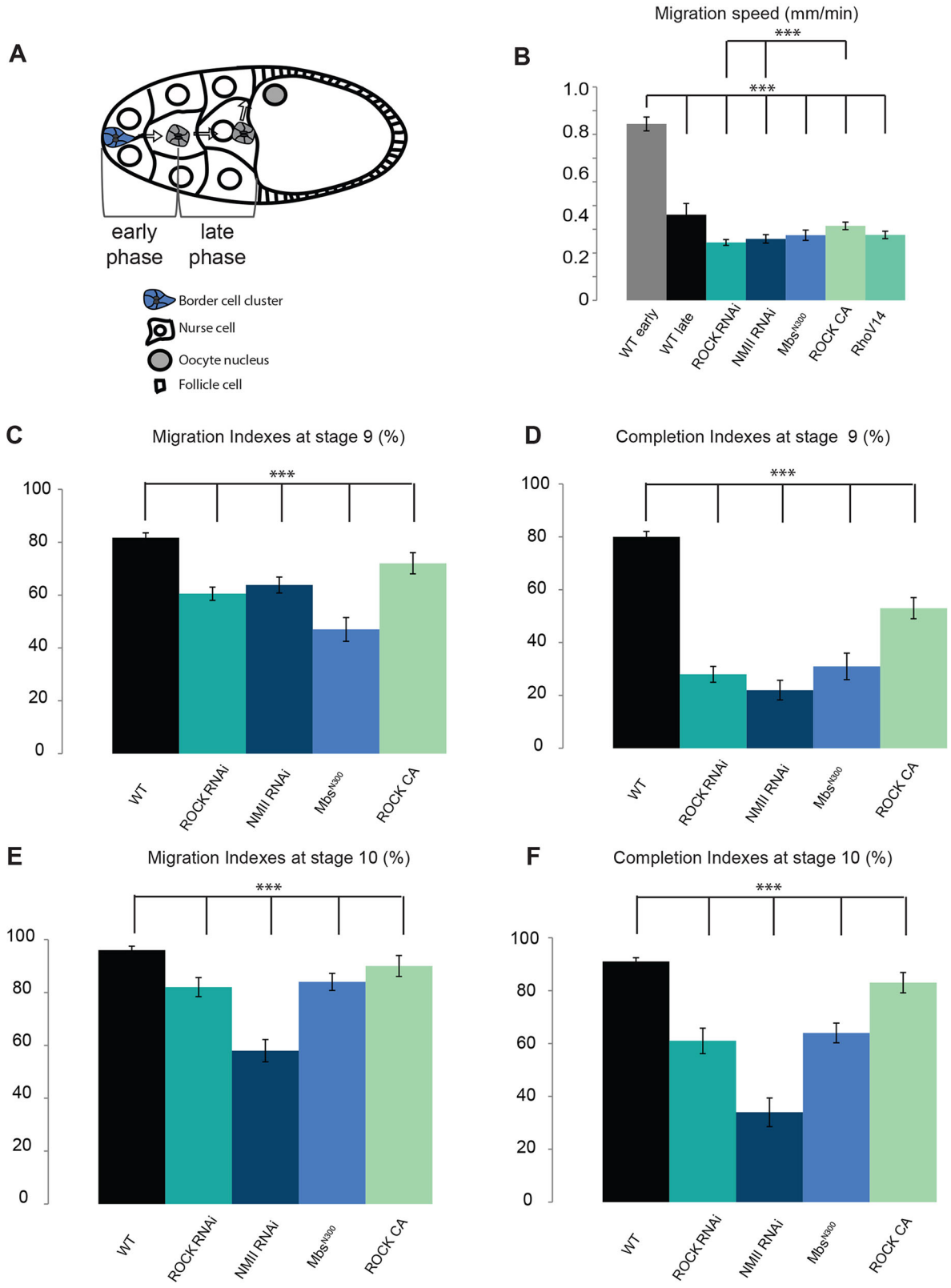

Fig. 1. Rotational movement is crucial for collective cell migration. (A) Schematic representation of border cell migration. (B) Migration speed in the indicated genotypes [wild-type (WT), *n*=11; ROCK-RNAi, *n*=11; NMII-RNAi, *n*=13; Mbs^{N300}, *n*=10; ROCK CA, *n*=14; RhoV14, *n*=10]. (C) Migration indexes at stage 9 of the indicated genotypes (WT, *n*=78; ROCK-RNAi, *n*=69; NMII-RNAi, *n*=82; Mbs^{N300} *n*=61; ROCK CA, *n*=72). (D) Completion indexes at stage 9 of the indicated genotypes (WT, *n*=78; ROCK-RNAi, *n*=69; NMII-RNAi, *n*=82; Mbs^{N300}, *n*=61; ROCK CA, *n*=72). (E) Migration indexes at stage 10 of the indicated genotypes (WT, *n*=109; ROCK-RNAi, *n*=81; NMII-RNAi, *n*=171; Mbs^{N300}, *n*=65; ROCK CA, *n*=83). (F) Completion indexes at stage 10 of the indicated genotypes (WT, *n*=109; ROCK-RNAi, *n*=81; NMII-RNAi, *n*=171; Mbs^{N300}, *n*=65; ROCK CA, *n*=83). Results are mean±s.e.m. ***P<0.0001 (*t*-test for migration indexes; Chi-squared test for completion indexes).

Journal of Cell Science (2017) 130, 97-103 doi:10.1242/jcs.179952

Myosin activation is required to induce rotation

Next, we investigated the NMII distribution pattern in border cells during migration. We used live imaging of border cell clusters expressing NMII fused to GFP (Royou et al., 2002) and NLS-dsRed (to visualise nuclei). We first verified that accumulation of NMII–GFP signal colocalised with staining of phosphorylated NMII (phospho-NMII), showing that NMII–GFP was a good readout for NMII activity *in vivo* (Fig. S2A,B). Our analysis revealed an accumulation of NMII–GFP signal at the periphery of the cluster that correlated with the occurrence of rotation (Fig. 2A,B; Fig. S2C), suggesting a link between NMII activity and the linear-to-rotational migration switch. Moreover, we asked whether NMII accumulation occured in specific regions of the cluster. We divided the group of cells in four sections and measured NMII levels in each sector (Fig. 2C). This analysis showed that NMII accumulation could occur in any cells of the clusters. These data indicate that NMII accumulation is highly dynamic throughout the cluster, which was confirmed by live analysis (Movie 1). To further study the role of NMII regulation during the linear-to-rotational switch, we manipulated the level of NMII activity and assessed its impact on the migratory behaviours. Decreasing NMII activity, through expression of ROCK RNAi in border cells, inhibited NMII accumulation at the cluster periphery in both the early and late phases of migration (Fig. 3A,B). Moreover, as compared to control (Fig. 2A), NMII-depleted border cell clusters mostly migrate in a linear fashion regardless of their position in the egg chamber and without affecting individual cell dynamics (Movies 2, 3, Fig. S2D,E). Indeed the linearity index and rotation speed (for an explanation of the method, see Fig. S3) of clusters expressing ROCK RNAi in the late migration phase was comparable to that of wild-type early phase clusters (Fig. 3C,D; Movie 2). Consistent with this, as NMII activity is already weak in the early phase, ROCK RNAi had no impact on the migratory behaviour in this phase (Fig. S4A,B). Overexpression of the NMII phosphatase Mbs or NMII RNAi had a similar impact on migration behaviour (Fig. 3C,D; Figs S2D, S4A,B and Movie 3). Thus, inhibiting NMII activation prevents the switch from linear to rotational migration. In contrast, ectopic activation of NMII, using ionomycin drug treatment, or through overexpression of ROCK CA or RhoV14, led to a strong peripheral accumulation of NMII (Fig. 3A,B). Except for RhoV14, this correlates with rotational migration because the beginning of migration was characterised by a low linearity index and a high rotational speed from early to late

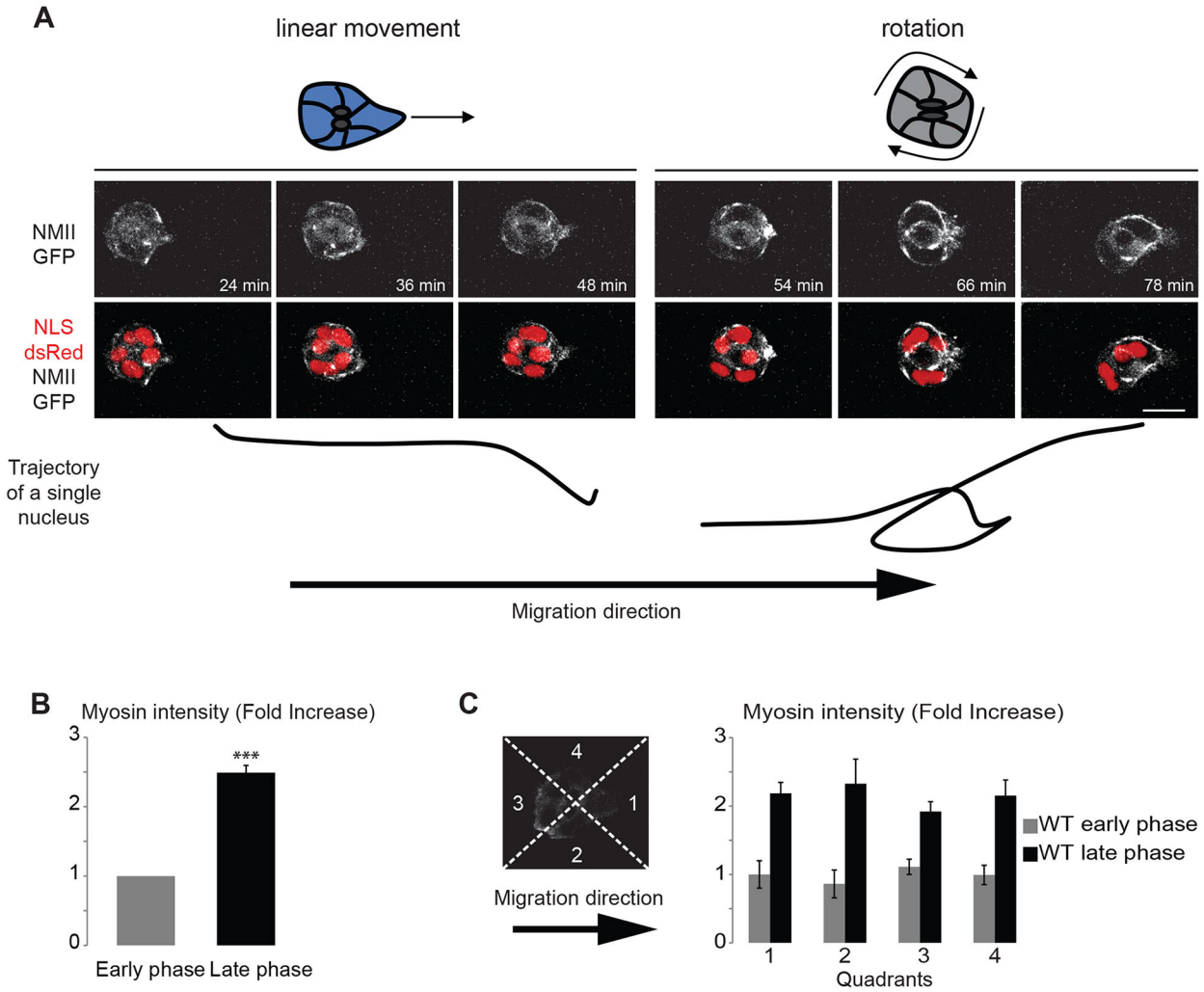

Fig. 2. Myosin accumulates during rotation. (A) Images from a time-lapse movie of WT clusters expressing NLS-dsRed and NMII–GFP. The trajectory of one nucleus is shown below the images. Scale bar: 10 µm. (B) Quantification of NMII–GFP intensity according to the migration phase (n=9). Intensity values are normalised to the early wild-type (WT) value. (C) Quantification of NMII–GFP intensity from 3D clusters divided in four sections (n=39). Intensity values are normalised to the early WT quadrant 1. Results are mean±s.e.m. ***P<0.0001 (t-test).

Journal of Cell Science (2017) 130, 97-103 doi:10.1242/jcs.179952

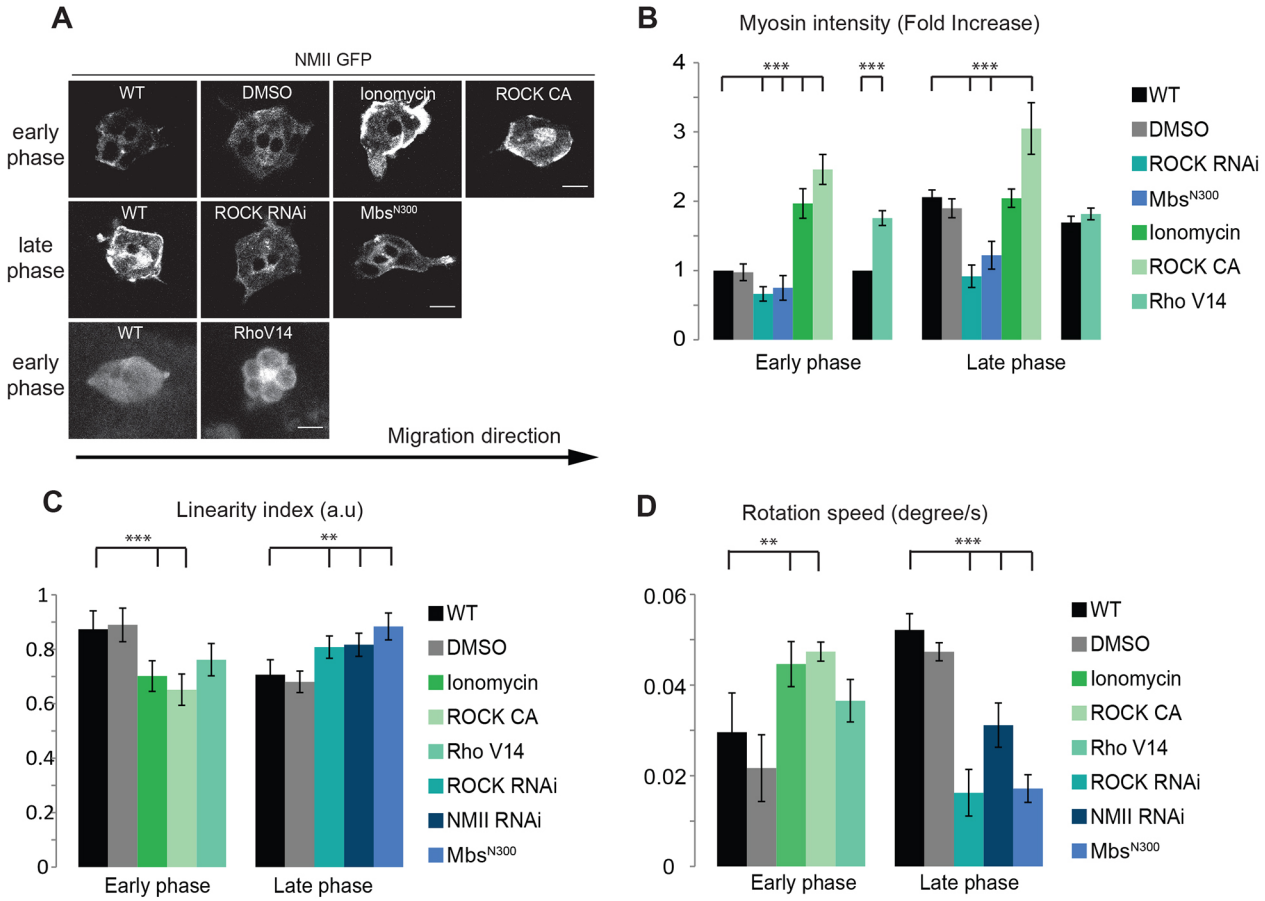

Fig. 3. Myosin controls rotational behaviour. (A) Confocal images showing NMII–GFP in the indicated backgrounds. WT, wild-type. Scale bar: 10 µm. (B) Quantification of NMII–GFP intensity in the above contexts (n=45). Intensity values are normalised to the early WT value. (C) Linearity index quantifications (WT, n=11; ROCK-RNAi, n=11; NMII-RNAi, n=13; Mbs^{N300}, n=12; ionomycin, n=10; ROCK CA, n=14; RhoV14, n=10). (D) Rotation speed quantifications (WT, n=11; ROCK-RNAi, n=11; NMII-RNAi, n=13; Mbs^{N300}, n=12; ionomycin, n=10; ROCK CA, n=14; RhoV14, n=10). Results are mean±s.e.m. **P<0.001; ***P<0.0001 (t-test).

phases, as for the wild-type late phase migration (Fig. 3C,D; Fig. S4A,B, Movies 4, 5). The paradoxical effect of RhoV14 could be due to the myriad of signalling cascades activated by Rho that could affect cell dynamics. Taken together, these data indicate that NMII activation is necessary and might be sufficient to induce rotational migration, and that regulation of NMII is essential for the linear-to-rotational switch as locking NMII in one state leads to a uniform mode of migration (linear when NMII activity is low, and rotational when NMII activity is high).

EGFR and PVR oppositely control NMII activity

Guidance receptors PVR and EGFR have been shown to play a pivotal role in the control of early and late border cell migration (Poukkula et al., 2011). Thus, we asked whether these guidance receptors were responsible for the regulation of NMII activity during border cell migration. We first explored the relationship between guidance receptors and NMII by scoring for migration efficiency in clusters where the receptor tyrosine kinase (RTK) and NMII activities had been manipulated. We found that reducing NMII by RNAi in clusters overexpressing PVR^{WT} or $EFGR^{WT}$ modified the migration index of these clusters to the level of NMII loss of activity (Fig. S4C,D). These data indicate an epistatic relationship between guidance receptors and NMII. As rotational movement occurs in the second phase of migration when EGFR is predominant, we hypothesised that EGFR could activate NMII. To test this, we first overexpressed the wild-type form of EGFR. Overexpression of EGFR induced an increase of NMII activity in the early phase of migration (Fig. 4A,B). Consistent with this, under this condition, rotation was also increased in the early phase (Fig. 4C,D; Fig. S4E,F, Movie 6). We then investgated the impact of PVR on the migratory behaviour. Overexpression of a wild-type form of PVR reduced the NMII signals as well as rotational movement in the late phase of migration (Fig. 4A–D; Fig. S4E,F, Movie 7). These data are consistent with a permanent Rac polarisation signal due to PVR expression (Fernandez-Espartero et al., 2013; Poukkula et al., 2011) and indicate that guidance receptors regulate NMII activity in order to control border cell behaviour. It can be inferred from these data that PVR contributes to inhibition of NMII activity, which prevents rotation in the early phase. Such inhibition could be mediated through Rac signalling. In a second phase, EGFR activates NMII to promote the switch from linear-to-rotational movement. To confirm this model, we decided to inhibit guidance receptors by overexpressing their dominant-negative form. Inhibition of PVR induced an increase in NMII–GFP in the early phase of migration concomitantly with a rotational movement (Fig. 4A–D; Fig. S4E,F, Movie 8). This result is consistent with a model stating that in the absence of PVR signalling in the first phase, EGFR activity becomes dominant, leading to an overt rotational behaviour as in a control late phase. Moreover, it confirms that PVR has a negative impact on NMII activity. Then,

Journal of Cell Science (2017) 130, 97-103 doi:10.1242/jcs.179952

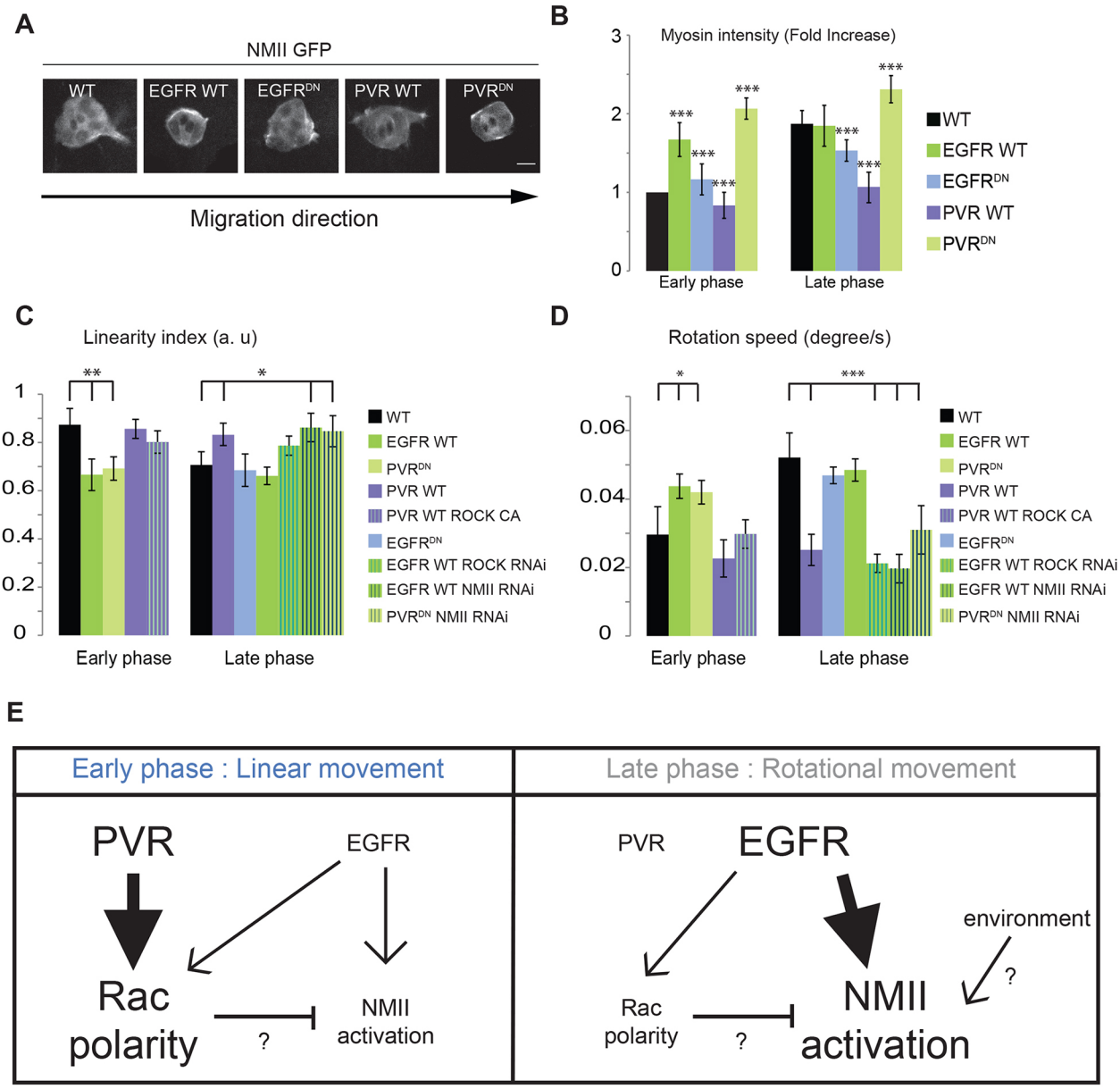

Fig. 4. EGFR controls Myosin activity. (A) Confocal images showing NMII–GFP in the indicated genotypes. Scale bar: 10 µm. WT, wild-type; DN dominant negative. (B) Quantification of NMII–GFP intensity in the indicated genotypes ($n \geq 31$). Intensity values are normalised to the early WT value. (C) Linearity index quantification in the indicated genotypes (WT, n=11; $EGFR^{WT}$, n=15; PVR^{DN}, n=14; PVR^{WT}, n=13; PVR^{WT} ROCK CA, n=10; $EGFR^{DN}$, n=15; $EGFR^{WT}$ ROCK-RNAi, n=11; $EGFR^{WT}$ NMII-RNAi, n=12; PVR^{DN} NMII-RNAi, n=13). (D) Rotation speed quantification in the indicated genotypes (WT, n=11; $EGFR^{WT}$, n=15; PVR^{DN}, n=14; PVR^{WT}, n=13; PVR^{WT} ROCK CA, n=10; $EGFR^{DN}$, n=15; $EGFR^{WT}$ ROCK-RNAi, n=11; $EGFR^{WT}$ NMII-RNAi, n=12; PVR^{DN} NMII-RNAi, n=13). (E) Model for the regulation of border cell migration behaviour. Results are mean±s.e.m. *P<0.05; **P<0.001; ***P<0.0001 (t-test).

the inhibition of EGFR in the early phase of migration decreases the linearity index without affecting the rotation speed (Fig. S4E,F). This data confirms that EGFR and PVR have partially redundant functions on the regulation of movement linearity (Poukkula et al., 2011). Surprisingly, $EGFR^{DN}$ overexpression had a moderate effect on NMII activity in the second phase and no effect on the migratory behaviour (Fig. 4C,D; Movie 9). This result suggests that NMII regulation in the second phase of migration could also depend on other internal or external cues. Alternatively, this effect could be explained by the decrease of PVR activity in the second phase, which would lead to a decreased inhibition of NMII activity, and thus to a normal tumbling behaviour. Finally, we asked whether NMII activation was crucial in EGFR-induced rotational behaviour. To do so, we inhibited NMII activation in the clusters overexpressing EGFR using ROCK or NMII RNAi. Strikingly, impairing NMII activation in this context was sufficient to prevent the switch to rotational movement (high linear index and low rotational speed) (Fig. 4C,D; Movies 10, 11). Consistent with this, inhibition of NMII activity in PVR^{WT}-expressing clusters did not modify the migratory behaviour (Fig. 4C,D). Taken together, these experiments demonstrate that PVR signalling inhibits, whereas EGFR activates, NMII activity in the early and late phases, respectively (Fig. 4E). Furthermore, we conclude that NMII regulation is the key molecular event controlling the linear-to-rotational switch downstream of guidance receptor signalling during border cell migration.

NMII – a key player that controls collective movement behaviours

Our data reveal the molecular mechanisms that control the linear-to-rotational switch occurring during collective cell migration. We show that border cell migration requires finely tuned NMII regulation during both linear and rotational motility modes. Furthermore, our results suggest that EGFR controls the linear-to-rotational switch upstream of NMII signalling. Our hypothesis is that guidance receptor signalling controls rotation. Indeed, it has been shown that PVR controls the first phase of migration, whereas EGFR controls the second phase (Poukkula et al., 2011). Accordingly, our data demonstrate that PVR and EGFR act antagonistically on the NMII-dependent linear-to-rotational switch. Indeed, PVR activates Rac signalling, which could be responsible for NMII inhibition in the early phase (Bianco et al., 2007; Fernandez-Espartero et al., 2013), whereas EGFR activates NMII during the second half of migration, thus promoting rotational migration. Moreover, we demonstrate that overexpression of $EGFR^{DN}$ reduces early linear migration, which suggests that EGFR is playing a role in the first phase of migration. Consistent with this, Poukkula et al. have demonstrated that EGFR DN overexpression combined to reduce the level of the PVR effects on the early phase of migration even though $EGFR^{DN}$ overexpression has only a small effect on the early movement by itself (Poukkula et al., 2011). These data show that PVR and EGFR have partially redundant functions, explaining the impact of $EGFR^{DN}$ on the early phase of migration. Finally, it has been demonstrated that clusters lacking EGFR are not able to migrate more than 50% of the migration distance, suggesting that EGFR is absolutely required for the late migration phase (Duchek and Rørth, 2001). Taken together, these data allow us to propose a model where the early migration phase is mainly controlled by PVR signalling through Rac polarisation. In this phase, EGFR could act redundantly to control migration. In late migration phase, EGFR alone controls the migration behaviour by regulating both Rac and NMII (Fig. 4E).

We show that locking NMII into either an inactivated or activated state affects the speed of migration. The fact that linear migration efficiency is also affected when NMII is inhibited suggests that, rather than total inhibition, linear migration might require restricted activation of NMII. The linear-to-rotational switch could thus be linked to a change in NMII activity level from the single cell to the cluster level. Indeed, localised NMII signalling could be required during the initial polarised migration, whereas collective NMII regulation would dominate the later phase for more efficient migration through the nurse cells (Majumder et al., 2012). We suggest that rotation and activation of NMII could be a response to environmental changes. Indeed, border cell clusters meet different types of nurse cell organisation, which could impact on their morphology directly and/or indirectly through modification of the local distribution of the gradient or mechanical stress.

Using NMII regulation to switch from one mode to the other ensures a rapid, coordinated cell adaptation that might be crucial for optimal migration both during development and cancer metastasis. Future work should further unravel the complexity of guidance-receptor-dependent NMII regulation during collective cell migration.

MATERIALS AND METHODS

Fly genetics

slbo-Gal4, *UAS-mCD8::GFP UAS-NLS-dsRed* drives UAS transgene expression. Other stocks used were: *UAS-PVR^{DN}*, *UAS–EGFR^{DN}*, *UAS-PVR*, *UAS-EGFR*; *UAS-ROCKcat* (from Bloomington), *UAS-MbsN300* (from Jessica E. Treisman, New York School of Medicine, Skirball Institute of Biomolecular Medicine, USA); *Sqh-GFP* (*myosin-GFP*, from Eric F. Wieschaus, Department of Molecular Biology, Princeton Univeristy, USA). UAS-RNAi lines were from either Bloomington or Vienna *Drosophila* Centers (ROCK-RNAi, BL28797; Sqh-RNAi, V109493). All stocks were maintained at room temperature. Before dissection, flies were flattened at 30°C overnight with dry yeast.

Time-lapse imaging and immunofluorescence

Time-lapse imaging was performed as previously described (Prasad et al., 2007). *z* stacks with a 1.5-µm interval were taken every 3 min for 180 min. Ionomycin (3 µM, from Invitrogen 20 min) treatment and immunofluorescence imaging was carried out as previously described (Zhang and Ward, 2011). Antibodies used were anti-phospho-NMII (from Robert Ward, University of Kansas, USA; 1:1000) and Alexa-Fluor-555-conjugated anti-guinea-pig-IgG antibodies (Fischer Scientific, 1:400). Images were captured using a Zeiss 710 microscope or a Leica DM6000 and processed with Image J.

Mathematic analysis of rotational and linear behaviour

Manual tracking

We tracked each nucleus within a cluster in 3D through the plugin MtrackJ from ImageJ software.

Measurement of rotation speed

The 2D trajectories of individual cells are corrected to the cluster centre. We measured the angle between the positions of an individual border at two different time points and the cluster centre. Rotation speed was calculated using the following equation:

$$\text{Rotation speed (degree/s)} = \frac{atan(y_n/x_n) - atan(y_{n-1}/x_{n-1})}{t_n - t_{n-1}} \times \frac{180}{\pi} \times 60,$$

where x_n and y_n are nuclei position in x and y at the time n, x_{n-1} and y_{n-1} are nuclei position in x and y at the time $n-1$, and t is the time. This calculation is done for any time point of a movie and the final result represents the mean of the rotation speeds.

Measurement of linearity index

The linearity index was calculated as the average of the cumulative migrated distance of the cluster centre divided by the cumulative distances migrated by each nucleus.

Quantification of Myosin II intensity

NMII–GFP intensity levels at the 2D and 3D cluster cortex region were quantified by Image J software. The cytoplasmic signals were excluded for the analysis.

Migration speed

The distance of the cluster centre between the first and last time points was divided by the elapsed time.

Migration speed of individual border cell

The distance covered by individual border cell between each time point was measured and divided by the elapsed time.

Quantification of border cell phenotypes

Migration phenotypes were calculated as described previously (Assaker et al., 2010).

Statistical analysis

Data are presented as mean±s.e.m. A *t*-test was used except for completion indexes where we used Chi-squared test.

Acknowledgements
We thank the VDRC and Bloomington Stock Centers, and E. Wieschaus and J. E. Treisman for fly stocks.

Competing interests
The authors declare no competing or financial interests.

Author contributions
A.C., V.C.-C., L.D., X.W. and D.R. designed and performed experiments. G.G. and J.L. developed the data analysis methods. D.R. wrote the paper.

Funding
This work was supported by the Institut National de la Santé et de la Recherche Médicale [the ATIP-Avenir program (2012–2016)]; Région Midi-Pyrénées Excellence program (2013–2016); the French Agence Nationale de la Recherche [grant number ANR-13-BSV1-0031]; the Ministère de l'Enseignement Supérieur, de la Recherche, de la Science et de la Technologie; and Fondation ARC pour la Recherche sur le Cancer.

Supplementary information
Supplementary information available online at
http://jcs.biologists.org/lookup/doi/10.1242/jcs.179952.supplemental

References

Assaker, G., Ramel, D., Wculek, S. K., Gonzalez-Gaitan, M. and Emery, G. (2010). Spatial restriction of receptor tyrosine kinase activity through a polarized endocytic cycle controls border cell migration. *Proc. Natl. Acad. Sci. USA* **107**, 22558-22563.

Bianco, A., Poukkula, M., Cliffe, A., Mathieu, J., Luque, C. M., Fulga, T. A. and Rørth, P. (2007). Two distinct modes of guidance signalling during collective migration of border cells. *Nature* **448**, 362-365.

Duchek, P. and Rørth, P. (2001). Guidance of cell migration by EGF receptor signaling during Drosophila oogenesis. *Science* **291**, 131-133.

Duchek, P., Somogyi, K., Jékely, G., Beccari, S. and Rørth, P. (2001). Guidance of cell migration by the Drosophila PDGF/VEGF receptor. *Cell* **107**, 17-26.

Edwards, K. A. and Kiehart, D. P. (1996). Drosophila nonmuscle myosin II has multiple essential roles in imaginal disc and egg chamber morphogenesis. *Development* **122**, 1499-1511.

Fernandez-Espartero, C. H., Ramel, D., Farago, M., Malartre, M., Luque, C. M., Limanovich, S., Katzav, S., Emery, G. and Martin-Bermudo, M. D. (2013). GTP exchange factor Vav regulates guided cell migration by coupling guidance receptor signalling to local Rac activation. *J. Cell Sci.* **126**, 2285-2293.

Friedl, P. and Gilmour, D. (2009). Collective cell migration in morphogenesis, regeneration and cancer. *Nat. rev. Mol cell biol.* **10**, 445-457.

Lucas, E. P., Khanal, I., Gaspar, P., Fletcher, G. C., Polesello, C., Tapon, N. and Thompson, B. J. (2013). The Hippo pathway polarizes the actin cytoskeleton during collective migration of Drosophila border cells. *J. Cell Biol.* **201**, 875-885.

Majumder, P., Aranjuez, G., Amick, J. and McDonald, J. A. (2012). Par-1 controls myosin-II activity through myosin phosphatase to regulate border cell migration. *Curr. Biol.* **22**, 363-372.

Malet-Engra, G., Yu, W., Oldani, A., Rey-Barroso, J., Gov, N. S., Scita, G. and Dupré, L. (2015). Collective cell motility promotes chemotactic prowess and resistance to chemorepulsion. *Curr. Biol.* **25**, 242-250.

Montell, D. J., Yoon, W. H. and Starz-Gaiano, M. (2012). Group choreography: mechanisms orchestrating the collective movement of border cells. *Nat. Rev. Mol. Cell Biol.* **13**, 631-645.

Murphy, A. M. and Montell, D. J. (1996). Cell type-specific roles for Cdc42, Rac, and RhoL in Drosophila oogenesis. *J. Cell Biol.* **133**, 617-630.

Poukkula, M., Cliffe, A., Changede, R. and Rørth, P. (2011). Cell behaviors regulated by guidance cues in collective migration of border cells. *J. Cell Biol.* **192**, 513-524.

Prasad, M., Jang, A. C.-C., Starz-Gaiano, M., Melani, M. and Montell, D. J. (2007). A protocol for culturing Drosophila melanogaster stage 9 egg chambers for live imaging. *Nat. Protoc.* **2**, 2467-2473.

Royou, A., Sullivan, W. and Karess, R. (2002). Cortical recruitment of nonmuscle myosin II in early syncytial Drosophila embryos: its role in nuclear axial expansion and its regulation by Cdc2 activity. *J. Cell Biol.* **158**, 127-137.

Thiery, J. P. (2009). Metastasis: alone or together? *Curr. Biol.* **19**, R1121-R1123.

Zhang, L. and Ward, R. E. T. (2011). Distinct tissue distributions and subcellular localizations of differently phosphorylated forms of the myosin regulatory light chain in Drosophila. *Gene. Expr. Patterns* **11**, 93-104.