Only three principal components account for inter-embryo variability of the spindle length over time.

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Abstract

How does inter-individual variability emerge? When measuring a large number of features per experiment/individual, this question becomes non-trivial. One challenge lies in choosing features to recapitulate high-dimension data. In this paper, we focus on spindle elongation phenotype to highlight how a data-driven approach can help. We showed that only three typical elongation patterns could describe spindle elongation in C. *elegans* one-cell embryo. We called them archetypes. These archetypes were automatically extracted from the experimental data using principal component analysis (PCA) rather than defined a priori. They accounted for more than 85% of inter-individual variability in a dataset of more than 1600 experiments across more than 100 different experimental conditions (RNAi, mutants, changes in temperature, etc.). The two first archetypes were consistent with standard measures in the field, namely the average spindle length and the spindle elongation rate both in late metaphase and anaphase. However, our archetypes were not strictly corresponding to these manually-set features. The third archetype, accounting for 6% of the variance, was novel and corresponded to a transient spindle shortening in late metaphase. We propose that it is part of spindle elongation dynamics in all conditions. It is reminiscent of the elongation pattern observed upon defects in kinetochore function. Notably, the same archetypes emerged when analysing non-treated embryos only at various temperatures. Interestingly, because these archetypes were not specific to metaphase or anaphase, it implied that spindle elongation around anaphase-onset is sufficient to predict its late anaphase length. We validated this idea using a machine-learning approach.

Despite the apparent variability in phenotypes across the various conditions, inter-individual differences between embryos depleted from one cell division-related protein have the same underlying nature as inter-individual differences naturally arising between wild-type embryos. The same conclusion holds when analysing embryos dividing at various temperatures. We thus propose that beyond the apparent complexity of the spindle, only three independent mechanisms account for spindle elongation, and contribute differently in the various conditions, meanwhile, no mechanism is specific to any condition.

1 Introduction

While cell division is remarkably faithful, the mitotic spindle, key to ensuring a correct partitioning of the chromosomes, can take variable trajectories to achieve its task [Fonseca et al., 2019, Farhadifar et al., 2016]. We have previously reported such variability in spindle positioning [Bouvrais et al., 2018]. Differences in protein quantities may cause variability but may also reveal a loosely stereotypical system [Doncic et al., 2006, Zhang et al., 2013, Barkai and Shilo, 2007, Montevil et al., 2016]. Variability in spindle trajectories was often viewed as noise. Still, it also fosters the spindle's ability to resist or adapt to internal defects like chromosome misat-

tachment and external perturbations like changes in tissue environment [Shahrezaei and Swain, 2008, Knouse et al., 2018, Oegema et al., 2001, Itabashi et al., 2012, Bloomfield et al., 2020, Knouse et al., 2017, Heinrich et al., 2013]. Consistently, variability may increase cellular fitness in cancer [Sarkar et al., 2021].

Phenotype variation between genetically identical cells can arise from multiple causes, from random transcription rates to stochastic variations in internal chemical reactions [Snijder and Pelkmans, 2011, Niepel et al., 2013, Raj and van Oudenaarden, 2008]. This emergent variability can translate either into a gradation of phenotypes across cells, e.g. in expression levels of a fluorescent dye [Elowitz et al., 2002], or radically new cell behaviours, e.g. responsiveness to the induction of apoptosis [Spencer et al., 2009]. Threshold effects can induce the latter type of variability. Along that line, Raj et al. [Raj et al., 2010] investigated the development of the C.*elegans* intestine: high fluctuations in genes expression can lead to either viable or impaired intestinal development, even in a clonal population [Raj et al., 2010]. However, while evident in this study, it is generally challenging to identify whether a perturbation leads to quantitative or qualitative changes in phenotype, akin to a switch in phenotypes. This paper proposes a methodology to characterise the nature of variability (qualitative versus quantitative) with a thorough quantitative analysis, using the well-studied and stereotypical cell division of C.*elegans* one-cell embryo as a representative example [Pintard and Bowerman, 2019].

To quantify cell-to-cell variability, one can choose a specific quantitative feature and display the variance over a given population, e.g. size of internal structures such as spindle or centrosomes [Farhadifar et al., 2015], or expression of a given gene of interest [Elowitz et al., 2002]. Studying variability in dynamical systems, like spindle kinematics during cell division, raises subsequent technical difficulties, namely measuring variability between trajectories. Disregarding whether investigating mechanics or biochemistry, mathematical modelling can be instrumental [Tonn et al., 2019, Honegger and de Bivort, 2018, Acar et al., 2008]. The issue then shifts from comparing trajectories to comparing parameters from the fitting of the mathematical model to the experimental data. That is, measuring the variance of each model parameter across a population of cells to capture the overall cell-cell variability. Although attractive, such an approach requires an established and integrative model, which recapitulates all the phenotypes observed experimentally.

Numerous models describe how wild-type cell divisions occur. However, because of the complexity resulting from both numbers and families of molecular actors involved, it is often required to focus on one part of cell division [Sönnichsen et al., 2005, Prosser and Pelletier, 2017, Guilloux and Gibeaux, 2020, Cai et al., 2018]. In particular, the correct partitioning of the chromosomes critically depends on the metaphasic spindle. Its functioning, especially the role of mitotic motors and microtubules, received much attention [Kapoor, 2017, Oriola et al., 2018, Elting et al., 2018]. The spindle length is a classic and suitable entry point [Goshima and Scholey, 2010, Valfort et al., 2018]. It was already investigated through biological or modelling means [Needleman and Farhadifar, 2010, Wollman et al., 2008, Blackwell et al., 2017, Ward et al., 2014]. However, its mere observation left open many potential mechanisms: the complexity led to a broad range of non-consensual spindle elongation models, none recapitulating its dynamics fully. Moreover, tractable models usually rely on simplifying assumptions to capture the core principles of the biological system they represent. By its reductionist approach, modelling could overlook specific cell-cell differences that may account for unusual phenotypes.

In this paper, we present a complementary approach to model fitting. We classified spindle-elongation cell-cell variability using a data-centred approach, a common paradigm in biology [Greener et al., 2022]. From the spindle length over time measured during mitosis of the C.*elegans* one-cell embryo, we extracted principal component analysis (PCA) projection as a blueprint of cell division. We asked how each embryo may depart from this blueprint in genetically perturbed conditions. We aimed at a quantitative classification of variability observed across different embryos and derived interpretable descriptors of such variability. It is classically achieved by recapitulating phenotype through manually-selected features. For instance, to study the genetic grounds of cell-division variability, Farhadifar and colleagues pre-computed about 20 features, such as embryo size, division duration or centrosomes oscillation duration and frequency, before applying a PCA [Farhadifar et al., 2015, Farhadifar et al., 2016]. In contrast, we propose a hypotheses-free approach. We thus focused on data projection methods that reduce dimensionality, the most famous being Principal Com-

ponent Analysis (PCA) [Moon et al., 2019]. We expected the PCA to extract cell division's key features and highlight biologically relevant mechanisms while discarding noise. Such an approach has been used to classify cell phenotypes according to expression or shapes [Park et al., 2014, Wang et al., 2020]. At the organism scale, it enabled describing nematode shapes and movements quantitatively across various strains and provided a list of descriptors to do so [Yemini et al., 2013]. We set to apply a similar approach to spindle dynamics during the division of the C.*elegans* one-cell embryo. This critical difference allows us to account for more than 85% of cell-to-cell heterogeneity with only three descriptors, rather than about 40% with about 20 pre-selected features [Farhadifar et al., 2016]. As we study trajectories over time, our automatically derived descriptors of spindle elongation can be seen as typical trajectories. Experimental elongations are then a linear combination of these archetypes. Interestingly, our hypotheses-free descriptors partly meet the features largely adopted by the community, like the average spindle length.

2 Results

To understand the grounds of spindle behaviour variability, we derived descriptors of variability without a priori using PCA. Advantageously, it required no interpretative biophysical model. Key to such a data-centred approach was the choice of the dataset. We monitored the spindle elongation in the C. *elegans* one-cell embryo at typically 30 frames per second. We tracked the spindle poles with a 10 nm accuracy relying on our previously published automatic tracking methods [Pecreaux et al., 2006a, Pécréaux et al., 2016]. We analysed a set of published and unpublished experiments from the lab, corresponding to 1627 experiments covering 79 gene depletions (see table S1). These experiments corresponded firstly to various non-treated conditions, varying the temperature or labelled proteins. Secondly, they comprised hypomorphic or more penetrant depletions using RNAi and mutations, genes involved in spindle positioning and mechanics. In particular, we tested all kinesins, that is, the 19 ones with interpro *Kinesin motor domain* homology [Blum et al., 2021, Siddiqui, 2002], microtubule-binding proteins (29 over the 80 with *microtubule-binding* GO term [Carbon et al., 2009, Srayko et al., 2005]. They also feature subunits *dli-1*, *dylt-1*, *dyci-1*, *dhc-1* from dynein complex and *dnc-1* from dynactin [Vaughan, 2012]. Although we were not covering all early embryonic mitosis-related genes – we counted 1260 genes with cell-division-variant phenotype or one of its descendants in the worm phenotype ontology [Sönnichsen et al., 2005, Kamath et al., 2003, Schindelman et al., 2011] – we suggest that this is enough to challenge our approach as our dataset is diverse enough. Indeed, the recording of embryo movies suitable for this study had to be manually performed to reach the required accuracy.

2.1 Cell-cell variability in spindle elongation is recapitulated into three archetypes.

Diversity of spindle elongation phenotypes. After filtering out the tracking outliers (Methods 4.5), we computed the spindle length as the distance between the two centrosomes (Fig 1A-C). In the conditions tested here, we observed a variety of phenotypes departing from the average behaviour of the whole dataset (Fig 1D), even at identical temperatures. Interestingly, a diversity of phenotypes was also visible within the non-treated condition alone, as exemplified by γTUB::GFP strain (TH27) embryos in Fig 1E. The averaged spindle elongation for non-treated embryos (blue dashed curve) displayed two-step dynamics during metaphase and anaphase: A first mild increase in spindle length started about one hundred seconds before anaphase onset (referred hereafter as metaphase elongation), followed by a second quicker increase (referred hereafter as anaphase elongation), corresponding to the elongation in the literature. These two-step dynamics were observed in both the average elongation over the whole dataset and wild-type embryos. It is reminiscent of the "biphasic metaphase" observed by Goshima and Scholey [Goshima and Scholey, 2010]: a relatively constant spindle length in early metaphase followed by a rapid increase in late metaphase. When depleting proteins regulating microtubule stability and dynamics, trajectories could depart from the wild type, as when depleting CLS-2. This CLASP protein stabilises microtubules, particularly at the kinetochore [Cheeseman et al., 2005, Srayko et al., 2005, Espiritu et al., 2012]. A partial *cls-2(RNAi)* treatment cancelled out the metaphase elongation (Fig 1F) and displayed a clear spindle shortening prior to anaphase onset instead. It was consistent with the known defects in microtubule attachment at the kinetochore during metaphase [Lewellyn et al., 2010, Cheerambathur et al., 2017, Edwards et al., 2018]. The anaphase elongation also departed from the control

to a variable extent: the two upper trajectories in Fig 1E were typical of the so-called "spindle weakening" phenotype, resulting in a fast elongation of the spindle, much faster than the control one. From the mere pole-pole distance, it was impossible to state whether it was happening by breaking the spindle because of the defective attachment of the microtubules at the kinetochore [Cheeseman et al., 2005] or during anaphase due to a weakened central spindle [Maton et al., 2015]. In a broader take, protein depletions caused a variety of phenotypes, raising two questions: (i) how to describe such a wide inter-individual variability beyond selecting a few features manually and taking the risk of missing key aspects of variability, and (ii) how to relate such a classification of variability to meaningful biological mechanisms.

Using projection methods to obtain a model-free description of variability. To extract suitable descriptors of the variability in our dataset, we set to use Principal Component Analysis (PCA) as a projection method, which performed best in describing between-condition variability (suppl. mat. § 1). We set to use a linear method to ease the interpretability of the prototype into biological features and account for the interplay between the various time stages of the division. We also aimed to perform an analysis without priors, so we focused on feature extraction parameters and unsupervised algorithms [Jia et al., 2022]. Along with projecting trajectories, PCA identified a few archetypical elongation patterns and allowed to compute their dosage in each embryo spindle-length curve. Surprisingly, the first three descriptors of the PCA were necessary but also sufficient to provide most of the information needed to describe variability in our dataset, about 95% (Fig S1A). We further used three components, as it appears to be the corner value of the L-curve, in an analogy to finding an optimum number of clusters in k-means algorithm or hierarchical clustering [Hastie et al., 2009]. Each elongation pattern could be recapitulated into the weight given to each archetype, i.e. the dosage of each of the three descriptors. Importantly, these descriptors, further termed "main archetypes", were reminiscent of elongation patterns found in some mutants (Fig 2A), suggesting they could have a biological interpretation. An exemplar reconstruction illustrates how three components were enough to account for a given single-cell spindle elongation (Fig S1B). Contrasting with previous studies based on pre-extracted features, we extracted the archetypes from the dataset rather than predefining them arbitrarily [Farhadifar et al., 2015, Farhadifar et al., 2016]. Our method thus provides hypothesis-free information regarding variability in spindle elongation.

Three main archetypes of spindle elongation. These main archetypes accurately describe the diversity in spindle elongation. The first archetype explained 70% of the overall variability in spindle elongation trajectories (blue curve, Fig 2A, S1B). Its roughly flat dynamics suggested that it accounted for the average spindle length and its coefficient shifted up or down the spindle-length curve (Fig 2B). Interestingly, spindle length was a feature used in many studies interested in the diversity of spindle elongation [Goshima and Scholey, 2010, Farhadifar et al., 2015, Dumont and Mitchison, 2009, Brown et al., 2007, Lacroix et al., 2018]. The second archetype explained 19% of the variability (red curve, Fig 2A, S1B) and comprised both the metaphase elongation (-100 to 0 s from anaphase onset) and the anaphase elongation (0 to 50 s). Therefore, the second archetype mainly captured the dynamics of spindle elongation: a high coefficient for the second axis corresponded to a fast elongating spindle (Fig 2C). Such a feature was also previously identified as important to classify spindle elongation phenotypes, although only the anaphasic elongation was considered [Goshima and Scholey, 2010, Farhadifar et al., 2015, Scholey et al., 2016, Hara and Kimura, 2009]. The third archetype explained 6% of the variability (green curve, Fig 2A, S1B) corresponded to the spindle shortening just before anaphase onset (Fig 2D). It accounted for the spindle-length plateau observed in non-treated embryos during late metaphase and shortening in some conditions like *cls-2(RNAi)* (Fig 1F). In a broader take, such a phenotype was observed upon misattachment of kinetochores by the microtubules [Lewellyn et al., 2010, Cheerambathur et al., 2017, Edwards et al., 2018] but not reported as a classifier of phenotype in previous studies. Interestingly, this phenotype was present in all conditions, including the non-treated embryos, with various weights (dosages). Beyond proposing late metaphase shortening as a novel feature, we found objectively two archetypes that roughly match spindle length both in metaphase or at anaphase onset and elongation rate.

In a reversed perspective, we compared them to arbitrarily-chosen features used in previous studies to understand the corresponding biological mecha-

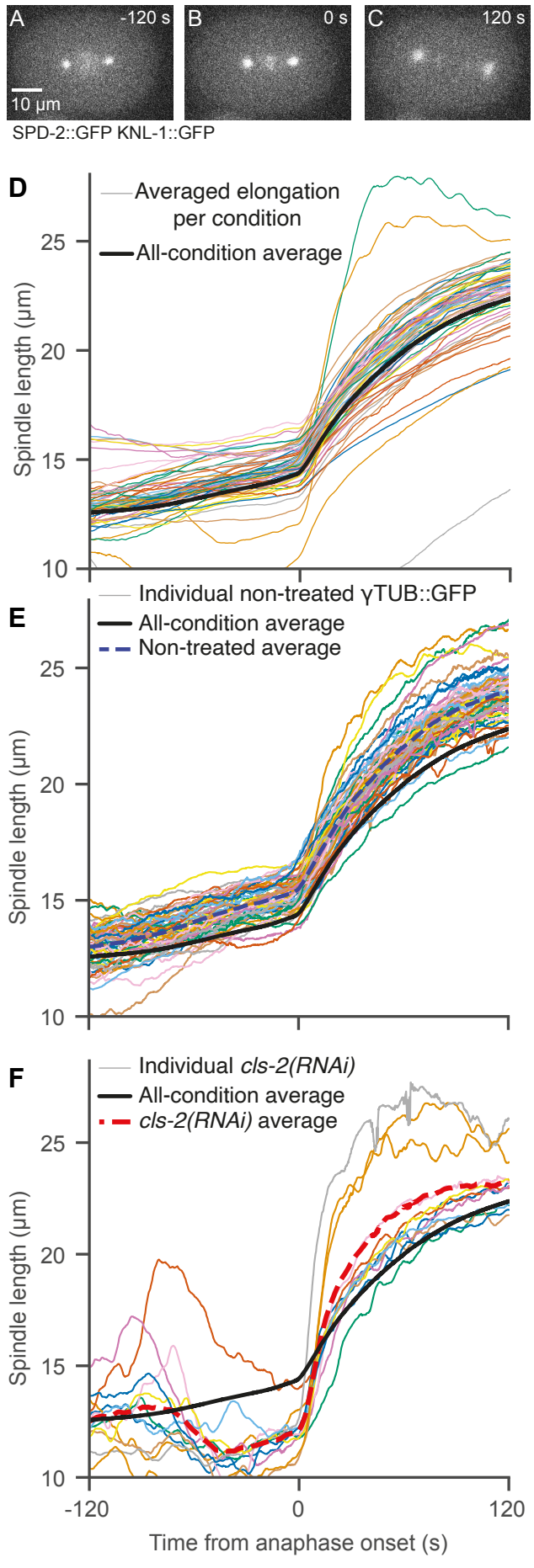

Figure 1: Diversity of the spindle length phenotypes. (**A-C**) Exemplar stills of a typical embryo of strain JEP29 imaged at 18°C, 120 s before, at anaphase onset and 120 s after. Centrosomes were labelled through SPD-2::GFP and kinetochore through KNL-1::GFP. The scale bar represents 10 µm. (**D**) (thin coloured lines) Pole-pole distance (spindle length) averaged per condition and plotted during metaphase and anaphase for each of the 67 genes targeted. We kept only conditions with at least 6 embryos (see Suppl Table S1). Multiple conditions treating the same gene by RNAi or mutating it are merged. (**E**) (thin coloured lines) Spindle length for individual non-treated TH27 embryos ($N = 58$). The thick blue dashed line corresponds to the average spindle length over these embryos. (**F**) (thin green lines) Spindle length for individual *cls-2(RNAi)* treated embryos ($N = 12$). The thick red dashed line corresponds to the average spindle length over these embryos. In panels D-F, individual embryos and averaged tracks were smoothed using a 1.5 s-running-window median. Black thicker line correspond to the average over the whole dataset, including all conditions. All considered embryos were imaged at 18°C.

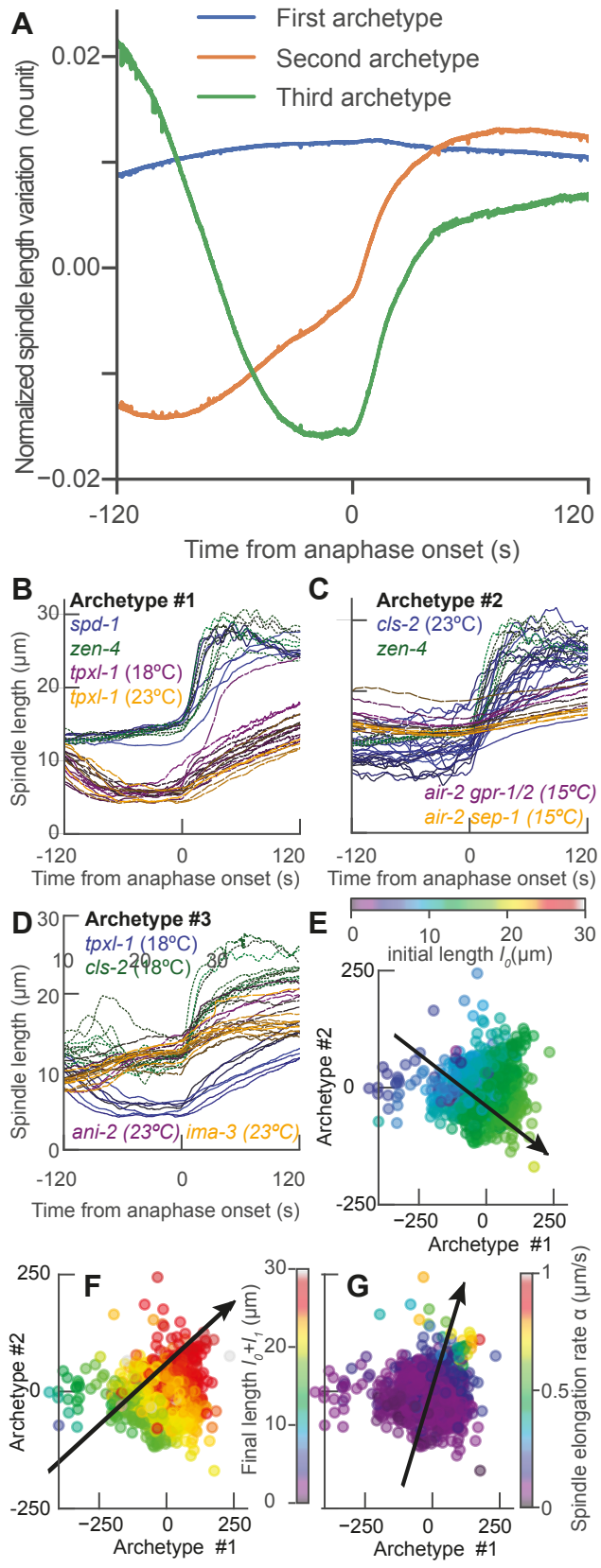

Figure 2: The three main archetypes (eigen-vectors) account for more than 95% of cell-cell variability in spindle elongation. (**A**) Archetypes extracted by PCA over all conditions. (**B**) Spindle elongations from individual experiments for the two conditions resulting in the highest coefficients for archetype 1 during PCA projection, namely γ-TUB::GFP (TH27) embryos treated with *spd-1(RNAi)* (N=7) and *zen-4(RNAi)* (N=7). The lowest coefficients for archetype 1 were obtained by treating the same strain with *tpxl-1(RNAi)* and imaging at 23°C (N=12) or 18°C (N=7). (**C**) Similar assay for archetype 2. Highest values were obtained with γ-TUB::GFP (TH27) embryos treated with *cls-2(RNAi)* and imaged at 23°C (N=22) and *zen-4(RNAi)* (N=7) imaged at 18°C. The lowest coefficients for archetype 2 were obtained treating the *air-2(or207)* mutant labelled with KNL-1::GFP and SPD-2::GFP (JEP31) strain either with *sep-1(RNAi)* (N=6 or *gpr-1/2(RNAi)* (N=9), and imaging at 15°C. (**D**) Similar assay for archetype 3. Highest values were obtained with γ-TUB::GFP (TH27) embryos treated with *tpxl-1(RNAi)* (N=7) and *cls-2(RNAi)* (N=12) while lowest coefficients resulted from treating with *ima-3(RNAi)* (N=5) and *ani-2(RNAi)* (N=13). (**E**) Average spindle length in early metaphase l_0 and (**F**) in anaphase $l_0 + l_1$, and (**G**) elongation rate α as defined in [Farhadifar et al., 2015] (see main text). These features are colour-coded, and an arrow depicts the axis along which these quantities vary while we plotted the values of the two first components of PCA in all conditions. 16/1627 embryos elongation cannot be fitted. In (FG) 10 additional fits were excluded because of aberrant values, that is final length larger than 55 µm or elongation rate larger than 1 µm s^{-1}, respectively. All considered embryos were imaged at 18°C except otherwise stated.

nisms better. We fitted individual embryo elongation curve with equation $l(t) = l_0 + \frac{l_1}{1+\exp\left(\frac{-(t-t_0)}{\tau}\right)}$ after [Farhadifar et al., 2015] using non-linear least-squares, namely the Levenberg-Marquardt algorithm. We excluded the poorly fitted embryos, where the standard error over one of the four fitted variables was higher than 50% of the nominal value. It corresponded to 16/1627 individual experiments. It led us to map on the two first PCA dimensions the variations of the average spindle length at early metaphase l_0 (Fig S1E) and final spindle length in anaphase $l_0 + l_1$(Fig S1F) (Supplemental method § 2). We found a good alignment of these traditional features with a combination of the two first components of the PCA, as depicted by the black arrows. However, it suggests that the first component cannot barely be associated with the spindle's initial or final length. We also considered the elongation rate $\alpha = l_1 / 4\tau$ (Fig S1G) and found a good alignment of this feature with the second component of the PCA, as expected. In a broader take and beyond proposing archetypes, we found that only three of them are needed suggesting that few mechanisms underlie the complexity of spindle phenotypes.

Anaphase onset as a turning point. Because the two first archetypes, which spanned over metaphase and anaphase accounted for 89% of the variability, we asked whether the spindle length in metaphase could correlate with the anaphasic one. We computed the spindle length in late anaphase (l_{LA}), as the average of the 300 last data points spanning between 111.7 s and 120 s after anaphase onset. Notably, beyond being a marker of correct spindle functioning, this feature correlates with spindle final position and, therefore, cytokinetic furrow positioning [White and Glotzer, 2012, Rappaport, 1971, Knoblich, 2010, Bringmann and Hyman, 2005, Pintard and Bowerman, 2019]. We used a machine learning approach to investigate whether late anaphase spindle length was set early during division (Suppl Text §3). Spindle length during a 25 s interval starting 5 s before anaphase onset provided an accurate prediction of length minutes later (green shading and red lines, respectively, Fig 3A). Comparing predicted spindle late-anaphase length and experimental one, we obtained a Pearson coefficient $R = 0.82$ with a p-value too low to be measured (Fig 3B). We next wondered how this correlation depended on the starting time of the 25 s interval. Considering Pearson R as the *predictive power*, we computed it when sliding the interval (Fig 3C). We observed a steep increase in the predictive power around anaphase onset. In other words, 80% of late-anaphase spindle length variability depends on the mechanisms already present at anaphase onset.

We next asked whether this information announcing the late-anaphase spindle length was captured by PCA projection. We this trimmed the elongation to the (-5, 20) s interval and projected, fed in the PCA as described above and kept only three components. We then performed a similar machine learning assay (Suppl Text §3). We again obtained a Pearson coefficient $R = 0.82$ with a p-value too low to be measured (Fig 3D) when comparing predicted and measured late-anaphase length. We concluded that the spindle characteristics supporting the prediction of the late-anaphase length were recapitulated by the coefficient of the three archetypes.

2.2 Robustness of the main archetypes.

Robustness to dataset composition. The main archetypes are automatically extracted from the dataset to describe its heterogeneity best. Therefore, one can wonder whether the rather extreme phenotypes identified on each axis, and described in the previous sections, are defining the archetypes alone (Fig 2A-D). In other words, how robust are these archetypes with respect to the composition of the dataset? To investigate this issue, we first performed a bootstrap sampling of the experimental dataset. We randomly selected 500 experiments among the 1627 of the dataset and repeatedly computed the archetypes (eigenvectors of the PCA). It provided a measure of their robustness to changes in the dataset size and composition (Fig 4A). Because the high-frequency noise in the elongations is unimportant, we applied a running-window median filtering with size 49 points, corresponding to 1.5 s. It showed reduced changes despite considering only 31% of the dataset. It suggests that our archetypes are present in most of our embryos rather than set by a few extreme phenotypes.

Going further, we focused on non-treated embryos. These embryos demonstrate a spindle elongation close to the average behaviour, that is the (0,0) position in the PCA plan (as shown in figure S2). Considering only these embryos can be seen as a more extreme version of bootstrap, in which most extreme phenotypes are indeed removed. Strikingly, we found similar archetypes (Fig 4B). We also tested the use of only the treated

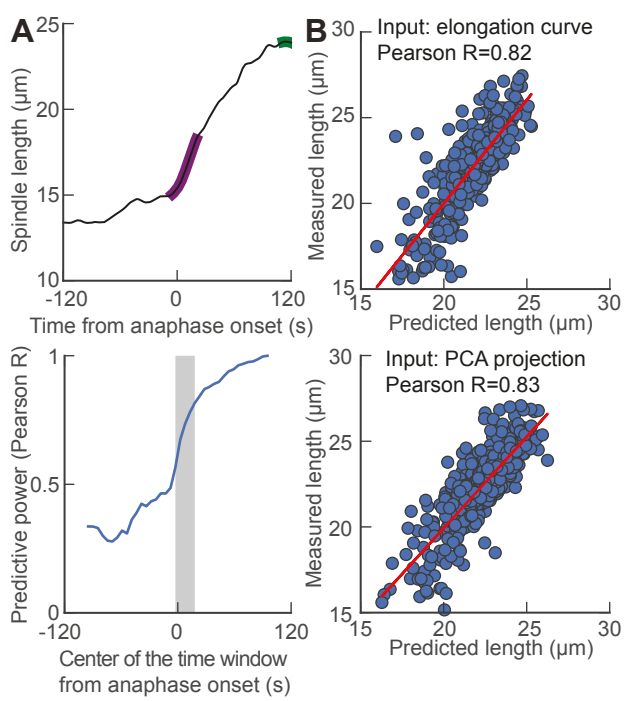

Figure 3: Machine learning (ML) predicts the spindle late-anaphase length l_{LA}. (**A**) (black line) elongation of an exemplar non-treated γ-TUB::GFP embryo, imaged at 18°C, highlighting (purple) the $(-5, 20)$ s interval used as input of algorithm and (green) the $(111.7, 120)$ s interval from anaphase-onset to compute the average spindle length at late-anaphase. (**B**) Using embryos in the testing set (32%, i.e. 521), we plotted the measured spindle late-anaphase length versus the predicted final length and obtained a high correlation (R=0.82, p value too low to be measured) when inputting an elongation curve over the (-5, 20) s interval as input to the ML network. (**C**) We slide this 25 s interval by 5 s starting at -120 s and ending at 120 s and computed the Pearson coefficient, as above, denoted predictive power. The grey shading region corresponds to the interval used in other panels. (**D**) Using embryos in the testing set (32%, i.e. 521), we plotted the measured spindle late-anaphase length versus the predicted final length and obtained a high correlation (R=0.82, p-value too low to be measured) when inputting the PCA projection (coefficient) computed on the elongation curve over the (-5, 20) s interval as input to the ML network. Details about the algorithmic approach is provided in Suppl Text §3.

embryos (excluding the control experiments using L4440 RNAi) and again found similar archetypes (Fig 4C).

Therefore, disregarding the subset of the dataset, the same overall interpretations remain: the first archetype accounts for average spindle length, the second for spindle elongation and the third for a spindle temporary shortening. While a shortening is not visible at first sight in most control embryos (Fig 1B), the PCA reveals that this phenomenon is still critical to explain cell-to-cell heterogeneity in these embryos. More generally, this suggests that the genetic perturbations explored in this study, while covering a broad range of documented molecular actors involved in spindle elongation, do not trigger a qualitative change in the spindle elongation phenotype. Moreover, the contributions of each archetype to describe the overall variability of wild type embryos are comparable to those obtained with the whole dataset (Suppl Table S3). Overall, it suggests that the PCA archetypes account for both the phenotypes of extreme RNAi or mutant, and the ones of non-treated embryos.

Robustness to experimental conditions. Variability can also arise from changes in the environment to which the organisms might respond to. Worms in their natural environment experience various kinds of stress, among which changes in the temperature has received attention [Pecreaux et al., 2006a, Begasse et al., 2015]. We wondered whether variability in spindle elongation would have the same underlying archetypes at two different temperatures since they correspond to two different spindle elongation phenotypes. We thus repeated the PCA projection considering only non-treated embryos at 18°C and 23°C and observed once again quite close archetypes compared to the ones extracted from the whole dataset (Fig 4D). Interestingly, the contribution of each of them to explain variance is more variable than before (Suppl Table SS3). The persistence of archetypes across temperatures suggested that while dynamics of elongation are influenced by temperature changes, the nature of variability, as captured by the archetypes, does not change under mild temperature changes.

2.3 From archetypes to phenotypes.

Interplay between the spindle elongation phenotype and the projection. We used partial RNAi in many cases (Suppl Table S1). We reckoned that the shift of PCA coefficients from control is dependent on the penetrance of the RNAi. Providing a general demonstration would be out of the scope of this paper. We instead offered an example: we varied the amount of a known microtubule-associated protein, the depolymerising kinesin KLP-7. Its depletion caused decreased growth and shrinkage rates, and reduced rescue and catastrophe rates [Srayko et al., 2005, Lacroix et al., 2014], causing chromosome attachment defects [Wordeman et al., 2007, Ems-McClung and Walczak, 2010]. It is reported to cause only mild spindle length defect upon hypomorphic treatment, while spindle breakage happens only upon penetrant one [Greenan et al., 2010, Grill et al., 2001]. It made KLP-7 an excellent candidate to test the link between penetrance and PCA coefficients. Thus, we investigated embryos where this protein was labelled with mNeonGreen and depleted by RNAi. Doing so, we observed a displacement mostly along the first axis (Fig 5). In a broader take, it suggests that variability of penetrance likely results in spreading the coefficient values within a given condition cloud. Other causes of spreading are likely present. Indeed, the non-treated condition also inherently displays variability (Suppl. Fig. S2) corresponding to the distribution of elongation curves (Fig 1B).

From spindle elongation pattern to gene function. Having established that the position of an experiment in the space of PCA coefficients is a robust and *bona fide* representation of its phenotype of elongation, we reckoned that computing the median position over all replicas of the same condition, we could map them in the PCA plane and link gene functions and the axis of the projection. We computed the median coefficient for each condition and mapped them in the PCA plane. We suggest that genes with similar functions will mostly cluster together. To test this assertion, we used the well-known example of the trimeric complex including GPR-1/2^{LGN}, LIN-5^{NuMA} and dynein, anchored by GOA-1$^{\mathrm{G\alpha}}$ and GPA-16$^{\mathrm{G\alpha}}$ and observed such a clustering mainly on components 1 and 3 [Nguyen-Ngoc et al., 2007] (Fig 6). Along the second axis, the spreading is primarily due to variations in the imaging temperature, known to change the elongation rate. We concluded that our PCA analysis could be instrumental in investigating gene function by looking at their neighbours in PCA space. Thus, we offer a complete mapping of the conditions used in this paper in 3D PCA space as an interactive 3D scatter plot (Suppl File 3).

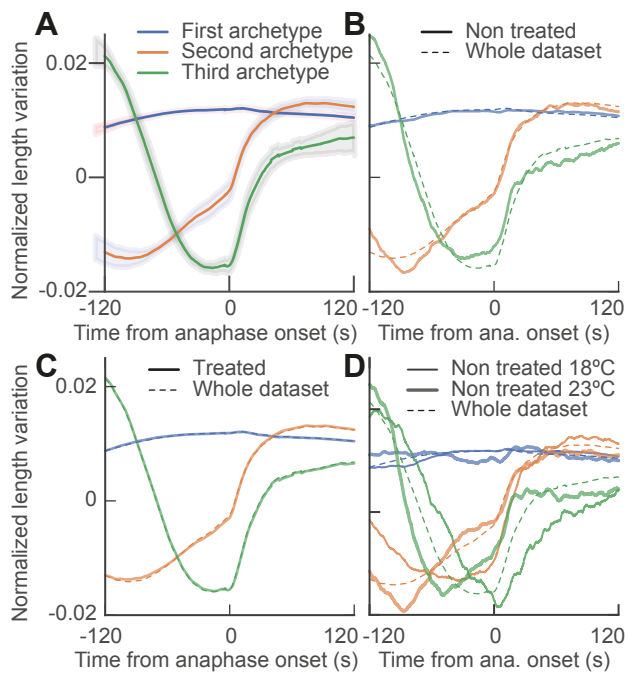

Figure 4: Variations of the archetypes upon dataset changes. (**A**) (lines) Average of the three first PCA archetypes (eigenvectors) ± (shading) two times their standard deviation, computed over a 500 embryos subset of the data (disregarding conditions). We repeated this bootstrapping 500 times to obtain standard deviations. (**B**) (lines) Three first PCA archetypes computed considering only the non-treated conditions (N=129) and compared to (dashed lines) archetypes extracted from the whole set of conditions (N=1627). (**C**) (lines) Three first PCA archetypes computed considering only the treated conditions (N=1317) without L4440 controls and compared to (dashed lines) archetypes extracted from the whole set of conditions. **D**) three first PCA archetypes computed considering only the non-treated conditions and control conditions images at (thick lines) 23°C (N=71) and (thin lines) 18°C (N=58), compared to (dashed lines) archetypes extracted from the whole set of conditions. In all cases, the elongation curves were smoothed with a 1.5 s running-median filtering before computing PCA.

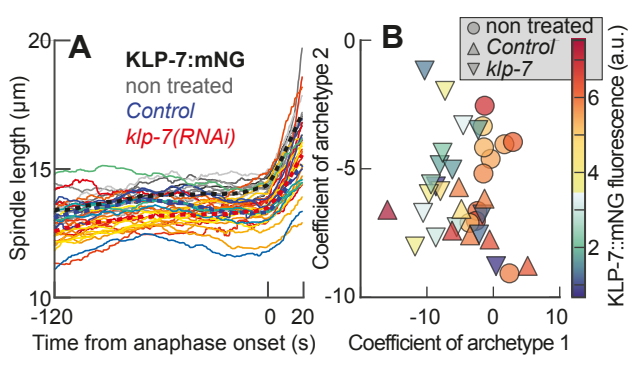

Figure 5: PCA coefficients depend on the penetrance of RNAi. (**A**) Spindle elongation trajectories for the depletion of KLP-7^{MCAK} in KLP-7::mNG background (strain LP447): (red/orange lines) N=18 *klp-7(RNAi))* treated embryos compared to (blue/green lines) N=8 control embryos (L4440 treated) and (grey lines) N=11 non-treated embryos. The thick dashed lines report the average of each condition. (**B**) Projection of these conditions on the PCA established on the whole dataset. We plotted the coefficients corresponding to the two first archetypes. The marker colour encodes the fluorescence level of KLP-7::mNG (Methods §4.6). The three conditions reported here were not included in the initial dataset. Acquisitions were performed at 18°C.

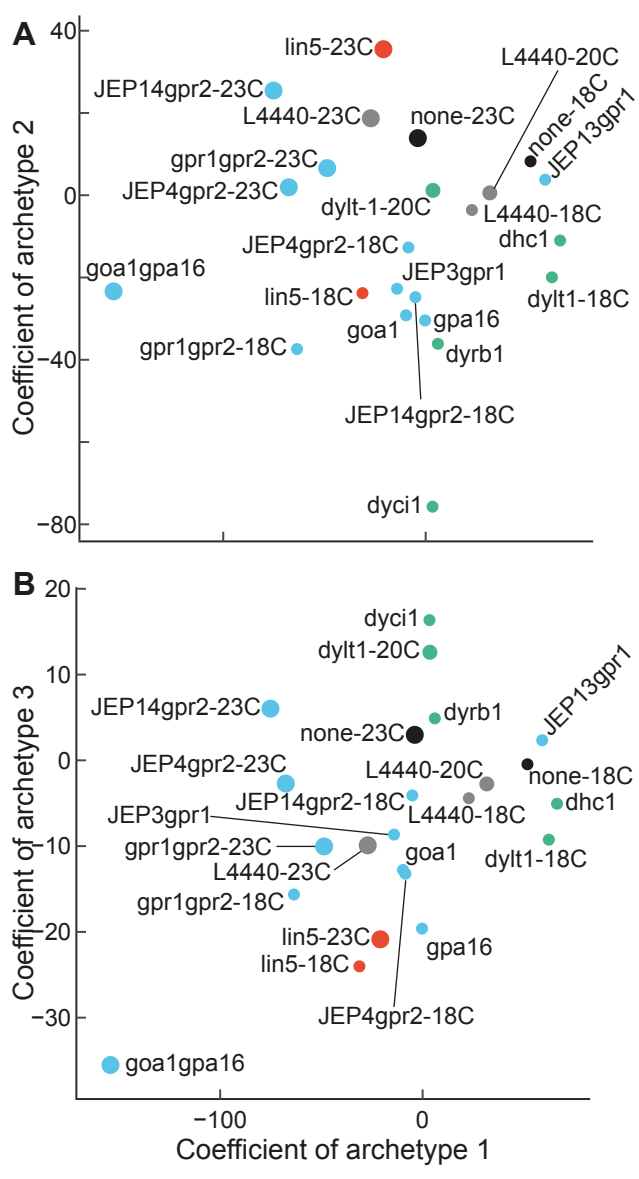

Figure 6: PCA coefficients as a quantitative phenotype. Each experiment is projected by PCA, and then a median is computed per condition (Suppl Table S1). The resulting scatter plot is attached as an interactive plot. For clarity, we display only genes related to the cortical pulling force, control and non-treated embryo. (**A** and **B**) report on the coefficients 1 and 2, 1 and 3, respectively. We colour-coded the protein depleted or mutated in each condition: blue: GPR-1/2, red: LIN-5, green: dynein sub-units, grey: control (L4440) and black: non-treated. The marker size scales with the temperature of imaging. Details of conditions and number of embryos can be found in Suppl table S1. Acquisitions were performed at 18°C when no other temperature is stated.

3 Discussion

The very question of extracting quantitative descriptors (in this study, the archetypes) of inter-individual variability has received interest from the cellular level [Farhadifar et al., 2015, Valfort et al., 2018] to the individual level [Cronin et al., 2005, Gyenes and Brown, 2016, Yemini et al., 2013] in the C.*elegans* research community. Our work complement and depart from these studies in several ways. At the cellular level, it has been shown that between-strains variability translates into variability in spindle elongation dynamics, measured on pre-selected features [Farhadifar et al., 2015, Needleman and Farhadifar, 2010, Valfort et al., 2018]. In their study, Farhadifar et al. aimed at linking phenotypic diversity in spindle elongation with mutation accumulation over several generations or with evolutionary divergences between various strains. In contrast, rather than tackling evolutionary genetics, we focus here on linking spindle elongation phenotypes with mild perturbations of key molecular players, in the most standard C.*elegans* lab strain, to shed light on the molecular mechanisms underlying variability in spindle elongation. Moreover, we do not extract pre-selected features to describe spindle elongation but rather let the projection method extract the most suited descriptors of variability for our dataset. It enabled us to uncover the third archetype, displaying a shortening during late metaphase, present in all conditions, including the non-genetically perturbed ones. Interestingly using PCA directly on time-series provide interpretable descriptors of variability (archetypes). Our two first archetypes, reminiscent of the spindle length and the elongation rate, strongly correlate with intuitively-set features [Farhadifar et al., 2015]. However, extracting the archetypes without a priori and making them independent allowed us to focus on more subtle aspects. For instance, the second axis of the PCA did not perfectly aligns with the spindle elongation rate (Fig 2F), albeit being correlated with it. It suggests that a slightly different descriptor is more accurate in describing the dynamics of spindle elongation. The second archetype displays two different slopes (Fig 2A, orange curve), one for the metaphase elongation - roughly starting 100 seconds before the metaphase-anaphase transition - and one for the anaphase elongation - starting at metaphase-anaphase transition. Our second archetype relies on both these slopes and not only the second one, starting at anaphase onset. This hints at mechanisms in spindle dynamics which would be conserved before and after anaphase onset. Consistently, we found that spindle length around anaphase onset is a predictor of spindle length in late anaphase.

Even more surprising, the third descriptor identified by this study highlights a transient spindle shortening phenotype during late metaphase. Such shortening has been described in particular conditions with defective kinetochore-microtubule attachment dynamics [Lewellyn et al., 2010, Cheerambathur et al., 2017, Edwards et al., 2018]. Yet, it has never been considered as a widespread phenomenon, which is instrumental in describing variability in spindle elongation in all conditions. At first glance, it might indeed seem paradoxical to include such a spindle shortening phenotype observed in only some specific experimental conditions. While this phenomenon is always present as suggested by PCA on non-treated only (Fig 4B, Suppl Table S3), its magnitude remains reduced in non-treated embryos (Fig S2). Yet, this phenomenon *naturally* occurs in wild-type embryos, but on a smaller magnitude than in genetically perturbed ones.

This statement raises the fundamental question of defining what is a phenotype in our biological system. Indeed, our archetypes can be described as phenotypes for extreme embryos (Fig 2B-D), complying with the usual definition of the term: observable characteristics. Previous papers have indeed reported shorter spindles, spindle weakening leading to fast elongation or spindle shortening [Goshima and Scholey, 2010, Wuhr et al., 2009, Barisic et al., 2021, Edelmaier et al., 2020, Scholey et al., 2016, Kapoor, 2017]. Yet, we show that wild embryo phenotype is a mixture of all three archetypes. It suggests that rather than describing an experiment with discrete descriptors, that is, whether a phenotype is absent or present, it would be more accurate to adopt a continuous description, namely the relative weights of archetypes. In a broader take, such a continuous description of experiments could lead to more straightforward interpretations. Indeed, a phenotype would most likely not disappear completely when mildly changing experimental conditions but diminish in intensity. Along that line, we mapped our different experiments using the proposed PCA to determine closely related gene through their quantitative phenotypes (Fig 6).

Using a dimensionality reduction approach without a priori also enabled us to investigate the complex spindle and propose only three descrip-

tors [Kapoor, 2017, Oriola et al., 2018, Elting et al., 2018]. Thus, the variability can be described by a small number of descriptors. It is likely that in many cases, the use of data-driven descriptors could reduce the number of descriptors from hundreds or thousands to a handful. For instance, quantifying C.*elegans* shape and movements is a difficult question, given the large apparent diversity in a given population. While previous studies have undergone a meticulous study of variability between individual shapes and behaviour by selecting up to several hundreds of features, unsupervised projection methods have identified four archetypes which recapitulate 97% of the observed variability in the dataset [Gyenes and Brown, 2016, Stephens et al., 2008]. Because variability relies on a small number of descriptors in these both very different cases suggests that beyond the apparent complexity, only a few independent mechanisms may be at work.

A small number of descriptors of variability, spanning across metaphase and anaphase, can also be understood as strong constraints for the biological system studied. As experimental data can be rebuilt only using these few descriptors, it follows that the range of possible experimental observations is bounded. For instance, the second archetype shows a first pre-anaphase elongation as well as anaphase elongation at anaphase onset. It means that a given experiment with a high coefficient along the second axis would then have both a first and anaphase elongation faster than average. This link between spindle elongation dynamics occurring before and after sister chromatin separation is in itself interesting to highlight. Indeed, the establishment of the central spindle, although not fully understood, requires a re-assembly from scratch of the microtubule [Nahaboo et al., 2015, Maton et al., 2015, Laband et al., 2017, Yu et al., 2019, Khmelinskii and Schiebel, 2008, Scholey et al., 2016]. While the central spindle only appears after anaphase onset, we still found that strong correlations exist between spindle elongation dynamics before and after chromatin separation because the second archetype contains both and because we can predict spindle late anaphase length from anaphase-onset one. This highlights that our approach not only quantitatively detects variability but also reveals robust aspects of spindle elongation.

Finally, mapping conditions in the PCA plane (Fig 6) provides indications of similarity between conditions. Such similar displacements along one of the axis of the PCA often indicates some commonality in the underlying molecular mechanisms. As such when investigating a spindle-related gene with little to no documented function, one could partially impair its expression, monitor spindle elongation and project the result in the PCA map. Therefore, our PCA analysis could turn into a prospective tool in finding gene candidates for a mechanisms by similarity to known players. This approach could also be instrumental in comparing various strains of C.*elegans* or even various nematode species. Indeed, distance in the PCA plane can be seen as a phenotypic distance between conditions [Xu et al., 2013, Sheehan et al., 2008, Mathur and Dinakarpandian, 2012, Kulmanov et al., 2021, Gan et al., 2013], more quantitative than the ones using phenotype ontology [Schindelman et al., 2011] and also enabling an analysis over separated phases of mitosis.

4 Material and Methods

4.1 Culturing C. *elegans*

This study partly re-used experiments, which we have previously published, as referenced in Table S1. In all cases, C. *elegans* nematodes were cultured as described in [Brenner, 1974] and dissected to obtain embryos. The strains were maintained and imaged at temperatures between 16°C and 25°C (Table S1). The strains were handled on nematode growth medium (NGM) plates and fed with OP50 *E. coli* bacteria.

4.2 Strains used in this study

Strains carrying mutations or fluorescent labels in use in this study are detailed in Table 1. Some strains were obtained by crossing as detailed in this table.

4.3 Gene inactivation through protein depletion by RNAi feeding

RNA interference (RNAi) experiments were performed by feeding using either Ahringer-Source-BioScience library [Kamath et al., 2003] with the bacterial strains detailed in Suppl Table S1, either bacteria transformed to express dsRNA targeting the desired gene. The bacterial clone (bact-16) targeting *par-4* is a kind kift from Anne Pacquelet and was in turn obtained from [Lee et al., 2008]. These latter sequence are either from Cenix bioscience and are documented in wormbase [Schindelman et al., 2011] or were designed in the framework of this project (see Table 2). JEP:vec-33 and JEP:vec-35 were designed against *ebp-1* and *ebp-1/3*, re-

spectively, and were disclosed in [Rodriguez-Garcia et al., 2018]. JEP vectors were constructed using Gateway technology (Invitrogen). Most RNAi were performed by feeding the worm with specific bacterial clones. Feeding plates were either obtained by growing a drop of bacteria mixed with IPTG at the center of a standard NGM plate, or laying bacteria on a feeding plate, i.e. a NGM plate with indicated IPTG concentration in the agar. In all cases, plates were incubated overnight [Kamath et al., 2001]. Alternatively, RNAi were obtained by injection of dsRNA in the gonads after [Timmons and Fire, 1998]. The dataset includes control embryos for the RNAi experiments, obtained by feeding with bacteria carrying the empty plasmid L4440. We did not notice any phenotype suggesting that the meiosis was impaired during these various treatments.

4.4 Embryos preparation and imaging

Embryos were dissected in M9 buffer and mounted on a pad (2% w/v agarose, 0.6% w/v NaCl, 4% w/v sucrose) between a slide and a coverslip. Embryos were observed at the spindle plane using a Zeiss Axio Imager upright microscope (Zeiss, Oberkochen, Germany) modified for long-term time-lapse. First, extra anti-heat and ultraviolet filters were added to the mercury lamp light path. Secondly, to decrease the bleaching and obtain optimal excitation, we used an enhanced transmission 12-nm bandpass excitation filter centred on 485 nm (AHF analysentechnik, Tübingen, Germany). We used a Plan Apochromat 100/1.45 NA (numerical aperture) oil objective. Images were acquired with an Andor iXon3 EMCCD (electron multiplying charge-coupled device) 512×512 camera (Andor, Belfast, Northern Ireland) at 33 frames per second and using Solis software. Embryos were imaged at various temperatures as reported in Suppl Table S1. To confirm the absence of phototoxicity and photodamage, we checked for normal rates of subsequent divisions [Riddle, 1997, Tinevez et al., 2012] in our imaging conditions. Images were then stored using Omero software [Li et al., 2016] and analysed from there.

4.5 Centrosome-tracking assay, filtering and getting spindle length

The tracking of labelled centrosomes and analysis of trajectories were performed by a custom tracking software developed using Matlab (The MathWorks) [Pecreaux et al., 2006a, Pécréaux et al., 2016]. Tracking of -20°C methanol-fixed γ-tubulin labelled embryos indicated accuracy to 10 nm. Embryo orientations and centres were obtained by cross-correlation of embryo background cytoplasmic fluorescence with artificial binary images mimicking the embryo or by contour detection of the cytoplasmic membrane using background fluorescence of used dye with the help of an active contour algorithm [Pecreaux et al., 2006b]. In this work, we accurately monitor spindle elongation from about 120 seconds before anaphase onset and up to 120 seconds after, which corresponds to the most dynamic phases of spindle elongation.

To exclude the rare tracking outliers, i.e. timepoints at which the centrosome was confused with a transient bright spot briefly appearing, we compared the raw data points to median-track computed by a running median over a 3 s window. Each point at a distance larger than 3 µm was excluded. It could correspond to a displacement at $1\,\mu m\,s^{-1}$ or faster, which is about one order of magnitude larger than the maximum speed observed for centrosome during elongation or when spindle breaks (targeting *cls-2* e.g.). We repeat this filtering procedure twice. Finally, we applied quality control and ensured that no more than 1 % of the points were removed along the whole trajectory. Embryos not complying with these conditions were excluded from further analysis. They correspond to acquisition issues like poor focus. In other embryos, the remaining raw data points after filtering are used for subsequent analysis. The spindle length is computed as the Euclidean distance between the two centrosomes.

4.6 Quantifying KLP-7 expression level by fluorescence

The expression level of KLP-7 was assessed by quantifying fluorescence using Image J software. From the equatorial section of the zygote, at the anaphase onset, the average fluorescence intensity of the cell was measured, and the average fluorescence outside the embryo (background) was subtracted.

5 Acknowledgements

Strains TH27, TH231, TH290, TH291, TH243 were a kind gift from Prof Anthony A. Hyman. Some strains were provided by the Caenorhabditis Genetics Center (CGC), which is funded by National Institutes of Health Office of Research Infrastruc-

ture Programs (P40 OD010440; University of Minnesota). Strain ANA019 was kindly offered by Dr Marie Delattre. Some strains were provided by NBRP, which is funded by the Japanese government. The bact-16 bacteria to perform *par-4(RNAi)* is a kind gift from Dr Anne Pacquelet. We thank Dr. Gregoire Michaux for the feeding clone library and technical support. We also thank Drs. Grégoire Michaux, Anne Pacquelet, Sébastien Huet, Marc Tramier and Olivier Dameron for discussions about the project. RRG and JP were supported by a Centre National de la Recherche Scientifique (CNRS) ATIP starting grant and La Ligue nationale contre le cancer. HB was supported by EMBO through a long-term postdoctoral fellowship (ALTF 326-2013). We also acknowledge Plan Cancer grant BIO2013-02, COST EU action BM1408 (GENiE), RTR siscom, La Ligue contre le cancer (comités d'Ille-et-Vilaine, des deux Sèvres, et du Maine-et-Loire), Rennes Métropole (AIS JP, HB and YLC), Region Bretagne (SAD AniDyn-MT grant and pRISM). Microscopy imaging was performed at the Microscopy Rennes Imaging Center, UMS 3480 CNRS/US 18 INSERM/University of Rennes 1.

5.1 Author contributions

Conceptualisation: YLC, JP; Experimental Data: LC, SP, XP, NS, DF, BM, RRG, ZA, AA, GDN, VC, MA, JP; Data curation: LC, SP, HB, JP; Formal analysis: YLC, JP; Funding acquisition: HB, JP, YLC; Investigation: YLC, LC, JP; Methodology: YLC, JP; Project administration: YLC, JP; Software: YLC, HB, JP; Supervision: JP; Validation: YLC, HB, CH, JP; Visualization: YLC, JP; Writing – original draft: YLC, JP; Writing – review and editing: YLC, CH, HB, JP.

5.2 Conflict of interest

The authors declare that they have no conflict of interest.

Table 1: Fluorescently tagged strains used in this study and their detailed genotype. Original strains are referenced by each of the crossed strains, whereas previously disclosed ones are referenced by the corresponding publication.

Strain	**Genotype**	**Crossing**	**Origin and Reference**
ANA019	C. *briggsae pie-1::Ce-tbg-1::GFP; Ce-sid-2*		[Riche, 2015]
JEP1	*unc-119(ed3) III; ddIs6 [Ppie-1::GFP::tbg-1; unc-119(+)] V; klp-13(tm3737) X*	TH27 x TM3737	[Oegema et al., 2001, elegans Deletion Mutant Consortium, 2012]
JEP3	*ddIs6 [Ppie-1::GFP::tbg-1; unc-119(+)] V; gpr-1(ok2126) III*	TH27 x VC1670	[Oegema et al., 2001, Barstead et al., 2012]
JEP4	*ddIs6 [Ppie-1::GFP::tbg-1; unc-119(+)] V; gpr-2(tm964) III*	TH27 x TM964	[Oegema et al., 2001, elegans Deletion Mutant Consortium, 2012]
JEP5	*unc-119(ed3) III; ddIs6 [Ppie-1::GFP::tbg-1; unc-119(+)] V; mbk-2(ne992) IV*	TH27 x WM73	[Oegema et al., 2001, Pang et al., 2004]
JEP6	*unc-119(ed3) III; ddIs6 [Ppie-1::GFP::tbg-1; unc-119(+)] V; lin-5(ev571) II*	TH27 x SV124	[Oegema et al., 2001, Lorson et al., 2000]
JEP10	*such-1(h1960) III; unc-46(e177) mdf-1(gk2) V. ddIs180[WRM062cF05 spd-2::2xTY1 GFP FRT 3xFlag;unc-119(+)]*	KR4012 x TH231	[Tarailo et al., 2007, Decker et al., 2011]
JEP13	*gpr-1(ok2126) III. unc-119(ed3) III (?); ddIs6 [Ppie-1::GFP::tbg-1; unc-119(+)] V*	TH27 x TH290	[Pécréaux et al., 2016]
JEP14	*gpr-2(ok1179) III. unc-119(ed3) III (?); ddIs6 [Ppie-1::GFP::tbg-1; unc-119(+)] V*	TH27 x TH291	[Pécréaux et al., 2016]
JEP15	*ddIs180[WRM062cF05 spd-2:: 2xTY1 GFP FRT 3xFlag;unc-119(+)] ltIs37 [pie-1p::mCherry::his-58 (pAA64) + unc-119(+)] IV*	JEP10 x OD56	[Bouvrais et al., 2018]
JEP16	*such-1(h1960) III; ddIs180[WRM062cF05 spd-2:: 2xTY1 GFP FRT3xFlag;unc-119(+)]; ltIs37 [pie-1p::mCherry::his-58 (pAA64) + unc-119(+)] IV*	JEP10 x OD56	[Bouvrais et al., 2018]
JEP17	*such-1(h1960) III; unc-46(e177) mdf-1(gk2) V. ddIs180[WRM062cF05 spd-2:: 2xTY1 GFP FRT 3xFlag;unc-119(+)] ltIs37 [pie-1p::mCherry::his-58 (pAA64)/unc-119(+)] IV*	JEP10 x OD56	[Bouvrais et al., 2018]
JEP25	*air-2(or207) unc-13(e51) I ddIs153[WRM064C_D03::unc-119-Nat([18578] knl-1::2xTY1wEGFP3xflag)]*	EU707 x TH243	[Severson et al., 2000, Sarov et al., 2012]
JEP27	*ebp-2(gk756) II. ddIs6 [Ppie-1::GFP::tbg-1; unc-119(+)] V*	TH27 x VC1614	[Rodriguez-Garcia et al., 2018]
JEP29	*unc-119(ed3)III; ddIs153[WRM064C_D03::unc-119-Nat([18578] knl-1::2xTY1wEGFP3xflag); ddIs180[WRM062cF05 spd-2:: 2xTY1 GFP FRT 3xFlag;unc-119(+)]*	TH231 x TH243	[Decker et al., 2011, Sarov et al., 2012]

Strain	Genotype	Crossing	Origin and Reference
JEP31	*air-2(or207) unc-13(e51) I; ddIs153[WRM064C_D03::unc-119-Nat([18578] knl-1::2xTY1wEGFP3xflag); ddIs180[WRM062cF05 spd-2:: 2xTY1 GFP FRT 3xFlag;unc-119(+)]*	JEP25 x JEP29	[Severson et al., 2000]
JEP32	*clip-1(gk470) III; ddIs6 [Ppie-1::GFP::tbg-1; unc-119(+)] V*	TH27 x VC1071	[Rodriguez-Garcia et al., 2018]
JEP46	*ebp-2(gk756) II; clip-1(gk470) III; ddIs6 [Ppie-1::GFP::tbg-1; unc-119(+)] V*	JEP27 x JEP32	this work
TH27	*unc-119(ed3) III; ddIs6 [Ppie-1::GFP::tbg-1; unc-119(+)] V*		[Oegema et al., 2001]
TH65	*unc-119(ed3); ddIs15 [pPIE-1::YFP::tba-2(genomic);unc-119(+)]*		[Srayko et al., 2005]
TH102	*N-YFP::spd-5*		[Greenan et al., 2010]
TH231	*unc-119(ed3)III; ddIs180[WRM062cF05 spd-2:: 2xTY1 GFP FRT 3xFlag;unc-119(+)]*		[Decker et al., 2011]
LP447	*klp-7(cp178[klp-7::mNG-C1^3xFlag]) III*		[Heppert et al., 2018]

Table 2: Bacterial clones designed for this study to silence genes by RNAi.

Name	Target	Primers (forward / reverse)	Reference
JEP:vec-6	*klp-20*	5'-AGTACATTCCGGTGGAGCAC-3' 5'-TAGGCAATTGCTTTGAGCTG-3'	this work
JEP:vec-7	*ubxn-2*	5'-AAAGTGAACCGCCACCAC-3' 5'-CAACATTTCCCAAACGGACT-3'	this work
JEP:vec-9	*clip-1*	5'-TCCCGATGGTTCAATCAGTTT-3' 5'-GCATCCTCCCTTTCTTTTCA-3'	this work
JEP:vec-11	*spn-4*	5'-GAGCGACACCAACCCGCAGA-3' 5'-ATCTGGTCACGAAGATGATGTGGGA-3'	this work
JEP:vec-37	*let-99*	5'-CCACCAAAGGCAAG-3' 5'-AAGTGATCTGTTCAAAATCTTCGGA-3'	this work

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