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Report

Live Imaging of Bicoid-Dependent Transcription in *Drosophila* Embryos

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Summary

The early *Drosophila* embryo is an ideal model to understand the transcriptional regulation of well-defined patterns of gene expression in a developing organism [1]. In this system, snapshots of transcription measurements obtained by RNA FISH on fixed samples [2, 3] cannot provide the temporal resolution needed to distinguish spatial heterogeneity [3] from inherent noise [4, 5]. Here, we used the MS2-MCP system [6, 7] to visualize in living embryos nascent transcripts expressed from the canonical *hunchback* (*hb*) promoter [8, 9] under the control of Bicoid (Bcd) [10]. The *hb-MS2* reporter is expressed as synchronously as endogenous *hb* in the anterior half of the embryo, but unlike *hb* it is also active in the posterior, though more heterogeneously and more transiently than in the anterior. The length and intensity of active transcription periods in the anterior are strongly reduced in absence of Bcd, whereas posterior ones are mostly Bcd independent. This posterior noisy signal decreases progressively through nuclear divisions, so that the MS2 reporter expression mimics the known anterior *hb* pattern at cellularization. We propose that the establishment of the *hb* pattern relies on Bcd-dependent lengthening of transcriptional activity periods in the anterior and may require two distinct repression mechanisms in the posterior.

Results and Discussion

Recently, the simple Bcd/*hunchback* gene network in the *Drosophila* embryo has been quantitatively investigated with the aim to uncover the transcriptional dynamics of Bcd target genes [10, 11]. No more than 30 min after the steady establishment of the Bicoid gradient [12], most nuclei in the anterior half of the embryo express synchronously both alleles of the main Bcd target gene *hb* [3]. Based on Bcd physical parameters [3, 13], theoretical models predict a realistic timing from the Bcd concentration measurement to the establishment of a robust *hb* expression pattern [3, 14]. However, given the absence of FISH signal for *hb* during the successive mitoses occurring in this short developmental period, it remains difficult to understand how such spatial homogeneity is achieved so rapidly in light of the challenge imposed by nuclear division [3].

To answer this question, we needed access to the temporal dimension lacking in the fluorescence in situ hybridization (FISH) approach on fixed samples. We used the MS2-MCP system, which allows fluorescent labeling of RNA in living cells and relies on the interaction between GFP-tagged MS2 coat protein (MCP-GFP) and MS2 RNA stem loops [6]. To adapt this system to the Bcd/*hb* network in the *Drosophila* embryo, we generated transgenic flies for single insertions of a P element carrying the canonical Bcd-dependent *hb* promoter [8, 9] upstream of a CFP-SKL-MS2 cassette carrying 24 MS2 repeats [15] (Figure 1A). Periods of transcriptional activity were observed in nuclei as time persistent green dots likely corresponding to the on-going production of several fluorescently labeled mRNA at the *hb-MS2* locus (Movie S1 available online and Figure 1). Movie S1 demonstrates the possibility to collectively monitor transcriptional dynamics at a given locus in most nuclei of the living embryo, from the onset of zygotic expression (nuclear cycle 10) to the beginning of cellularization (nuclear cycle 14). Remarkably, in an embryo carrying a single *hb-MS2* transgene, we never detect more than one period of intense transcriptional activity per nucleus during a given interphase (10 to 13). During interphases 10 to 12, transcription at the *hb-MS2* transgene is detected in both anterior and posterior nuclei. During interphase 13, the periods of transcriptional activity are intense and long-lasting in the anterior domain limited by a well-defined boundary, whereas fewer, shorter and weaker fluorescent events are seen in the posterior.

One of the most striking differences between live *hb-MS2* reporter expression and endogenous *hb* FISH data [3] are the unexpected *hb-MS2* transgene activity events in the posterior during interphases 10, 11, and 12. This posterior expression is observed with several independent insertions (Movie S1) and is thus intrinsic to the transgene. It is also observed with RNA FISH using a *CFP-MS2* probe on embryos carrying two copies of the *hb-MS2* transgene (Figures 2E–2H). Double RNA FISH using *hb* and *CFP-MS2* probes on the same embryo indicates that the *hb-MS2* transgene nicely recapitulates endogenous *hb* expression at interphase 13 and for the anterior but not the posterior at earlier interphases (Figures 2A–2H). To assess the probability for a locus to be activated, we considered the coarse temporal activity of the transgene by spatial averaging the fraction of active nuclei in four regions of equal amplitude along the anterior-posterior (AP) axis. Consistently with FISH data on endogenous *hb* [3], a large proportion of nuclei in the anterior express the *hb-MS2* allele in live embryos (Figure 2I). In the posterior, the proportion of active *hb-MS2* loci detected by both the FISH signals and live imaging is lower, more scattered when compared to the anterior, and reduced to only 10% at interphase 13 (Figure 2). Since consistent expression patterns are observed with FISH and live imaging, we conclude that the unexpected expression of the *hb-MS2* transgene during interphases 10 to 12 is not due to the MS2-MCP experimental system and is therefore mechanistically meaningful.

To characterize the temporal and spatial features of these GFP signals (Figure S1), we performed an in-depth three-dimensional (3D) image analysis (Supplemental Experimental Procedures). For each nucleus, we quantified the period of

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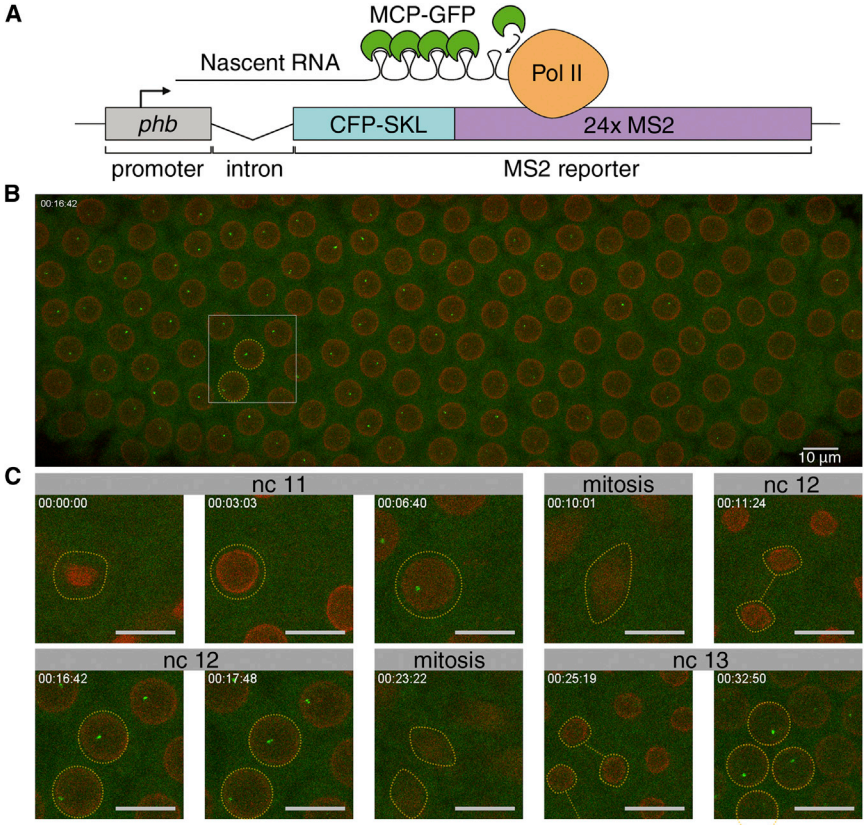

Figure 1. The MS2-MCP System to Monitor Transcription at the *hb* Promoter in Living *Drosophila* Embryos

(A) Schematic view of the *hb-MS2* transgene. The fluorescent detection of MS2-containing mRNAs was obtained with a second transgene expressing maternally the MCP-GFP [7]. The fluorescent labeling of Nucleoporin (mRFP-Nup) [16] allowed the detection of nuclear envelops. MCP-GFP (green) and mRFP-Nup (red) signals were imaged simultaneously using 3D confocal time-lapse microscopy.

(B) A 2D maximal projection snapshot from Movie S1 (clip 1) reveals periods of intense transcriptional activity in an embryo heterozygous for the *hb-MS2* transgene. In green, MCP-GFP proteins are recruited at the nascent MS2-containing mRNA accumulating at the *hb-MS2* locus (bright spots). In red, the mRFP-Nup proteins localize at nuclear envelops.

(C) Snapshots of a nucleus selected at cycle 11 that divides twice in the movie, giving rise to four daughter nuclei at interphase 13. Panels were extracted at different time points (indicated at the top left) and correspond to a magnification of the white square in (B). In agreement with FISH data [3], the GFP signals disappear during mitosis (Movie S1). Anterior is left.

See also Figure S1 and Movie S1.

transcriptional activity at the *hb-MS2* locus (Figure S1) fitting 2D Gaussians (Supplemental Experimental Procedures). We distinguished four parameters: (1) the delay of signal detection after mitosis (initiation time, T_{in}), (2) the intensity of the signal at each time point (instantaneous activity), (3) the duration of the period during which the fluorescent signal is continuously detected (persistence), and (4) the total activity integrated in time, which is likely proportional to the total amount of RNA produced over the whole interphase per nucleus (integral activity). Except for the posterior events at interphase 13, initiated randomly throughout interphase, the initiation time (T_{in}) depends neither on the nuclear cycle nor the position along the AP axis (Figure 3A); this surprising invariability in time and space suggests a universal delay to initiate transcription after mitosis. Because activities in the anterior were qualitatively different from posterior ones (Movie S1 and Figures S1 and S3B), we computed mean activities for these two groups of signals separately, using T_{in} to synchronize the various events. Both mean activities and duration of activity periods show a dependence on the AP axis (Figures 3B and 3C). Thus, the difference between the anterior and posterior expression greatly increases over the cycles. The regulation of *hb-MS2* expression during interphase 10 to 13 is principally controlled in the anterior through the scaling of the persistence of activity periods in relation to the interphase duration unlike in the posterior where arrests of transcription rapidly occur. Integrated activities per nucleus over each interphase estimate the total amount of mRNA produced in each nucleus per cycle along the AP axis (Figure 3D). We discern two groups of nuclei: anterior nuclei, expressing an increasing amount of mRNA over the cycles, and posterior nuclei, expressing on average a decreasing amount of mRNA over the cycles. Therefore, the border separating those two groups sharpens and steepens from cycle 11 to 13 and is shifted to the anterior from 50% egg length (EL) at interphase 11 to 40% EL at interphase 13 (Figure 3D).

To determine whether expression of the *hb-MS2* reporter depends on Bcd, we analyzed its expression in embryos from *bcd* mutant females. Surprisingly, we observed a significant number of transcriptional activity events in this mutant during early interphases indicating that the *hb* canonical promoter in the *hb-MS2* transgene can be transcribed without Bcd (Movie S2). However, in contrast to wild-type, the expression of the transgene in this mutant showed no significant AP axis dependence: T_{in} (Figure S3A), persistence of activity (Figures 3C and S3C), and integral activity per cycle (Figure 3D) are not statistically different between the anterior and posterior groups. Comparison of activity features between wild-type and *bcd* backgrounds indicates that the persistence of activity periods in the anterior strongly depends on Bcd at each interphase (Figure 3C). Additionally, in the *bcd* mutant background, the fractions of active nuclei are very similar in the four regions along the AP axis, following the same increase of repression strength over the cycles (Figure 2J). Importantly, once a locus has been turned ON in the anterior to produce mRNA, its probability to be ON remains high during the whole interphase and this occurs in a Bcd-dependent manner. Altogether, our data indicate that the *hb* canonical promoter is expressed efficiently and homogenously in the anterior half of the embryo from interphase 10 to interphase 13. Unexpectedly, it is also expressed in the posterior region in a more scattered manner. This posterior expression is efficiently repressed at cycle 13, and it is mostly Bcd independent. It can be due either to a general activator homogeneously expressed along the AP axis or to leakiness of the promoter at which PolII can fire without specific triggers.

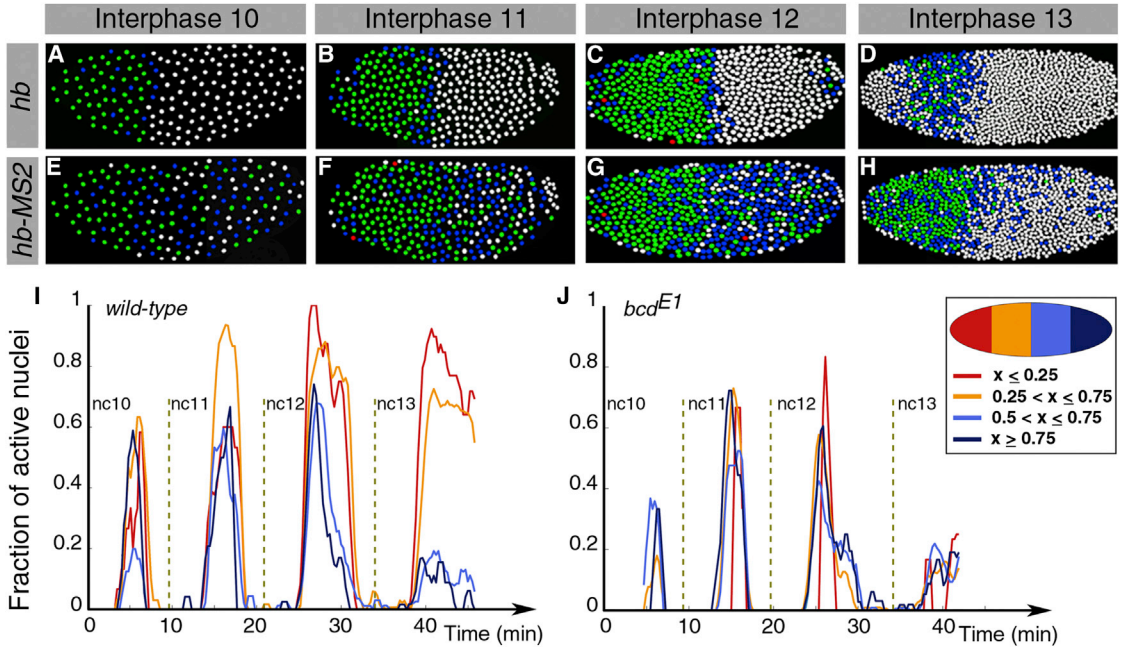

Figure 2. Spatiotemporal Activity of the *hb* Promoter at the Scale of the Whole Embryo

(A–H) Transcription fate maps of embryos carrying a *hb-MS2* homozygous insertion costained by FISH for endogenous *hb* (A–D) and for *CFP-MS2* (E–H). Each nucleus is colored according to its transcriptional status [3]: two active loci (green), one active locus (blue), or no detectable activation (white). Nuclei with more than two bright dots were sometimes observed (red); these were observed either after the replication of the *hunchback* locus or when the threshold for spot detection was below a lower limit. In contrast to endogenous *hb*, which is likely repressed in the most anterior region by the Torso terminal system (D), the *hb-MS2* reporter remains strongly expressed in this region (H) [17]. Anterior is left.

(I and J) The fraction of activated nuclei is shown as a function of developmental time. At each cycle, time zero or the vertical dashed lines indicate the mean of interphase onset. Nuclei are grouped in spatial regions according to their position along the AP axis (cartoon on the right): from 0% to 25% EL in red, from 25% to 50% EL in yellow, from 50% to 75% EL in light blue, and from 75% to 100% EL in dark blue. The fraction of active nuclei is calculated for each time point, as the ratio of active nuclei to the total number of nuclei followed by an averaging window of three time points. Data from a wild-type embryo (I) and an embryo from a *bcd* female (J) are shown. Similar results were obtained with movies of embryos carrying the same *hb-MS2* transgenic insertion (Figure S2). 0% EL is anterior.

See also Figure S2 and Movie S2.

Our data indicate that the establishment of the precise border of endogenous *hb* gene expression results from at least three distinct processes. First, Bcd is responsible for a strong and persistent expression in nuclei localized in the anterior half of the embryo. This Bcd-dependent expression does not seem to control the instantaneous activity of the gene in a spatially graded fashion but sets a rough boundary of maximal activation. Interestingly, the transcription initiation time is constant at each interphase and along the AP axis. This observation suggests that the postmitotic delay of transcription reactivation is not limited by the Bcd physical parameters, but more probably by the assembly of the transcription machinery after decondensation of mitotic chromosomes and the delay of transcribing sufficient numbers of MS2 stem loops for signal detection. Mechanistically, as shown by the Bcd-dependent lengthening of activity events in the anterior, as a transcription activator, Bcd may be critical to maintain the flux of polymerases initiating transcription. Second, transcriptional repression in the posterior initiates mildly during interphase 11 and progresses over cycles 12 and 13. In the posterior, as the number of active loci decreases from one cycle to the next, initiation times of activity events become more variable, suggesting a posterior repressor becoming stronger. This putative repressor does not necessarily require Bcd as the overall repression exhibits the same feature in an embryo lacking Bcd. Third, as early as interphase 10, a "silencing" mechanism prevents the erratic posterior expression of the canonical *hb* promoter observed upon insertion as a reporter transgene in the genome. As endogenous *hb* is not expressed in the posterior, this third regulation mechanism must be encoded in the genomic DNA outside of the canonical promoter and could involve the newly identified distal shadow or stripe enhancers of *hb* [17, 19]. This last silencing mechanism together with the Bcd induced activation of transcription are likely responsible for the sharp border observed for endogenous *hb* as early as cycle 11 [3]. In absence of this early silencing in the posterior, as exemplified by the *hb-MS2* reporter, a second unidentified mechanism of repression (discussed in the second point) can rescue the formation of the sharp boundary by cycle 13.

Our ability to observe the early transcription of developmental genes in live embryos opens news perspectives for the understanding of the patterning processes. Despite not recapitulating all the features of the endogenous regulation, access to new quantitative measurements sheds light on this critical biological process. At the mechanistic level, this approach indicates how the Bcd transcription factor could activate transcription: it is not absolutely required for transcription initiation at the promoter and it does not allow faster initiation at the promoter after mitosis, but it is essential for the maintenance of the activity event once the latter has been initiated.

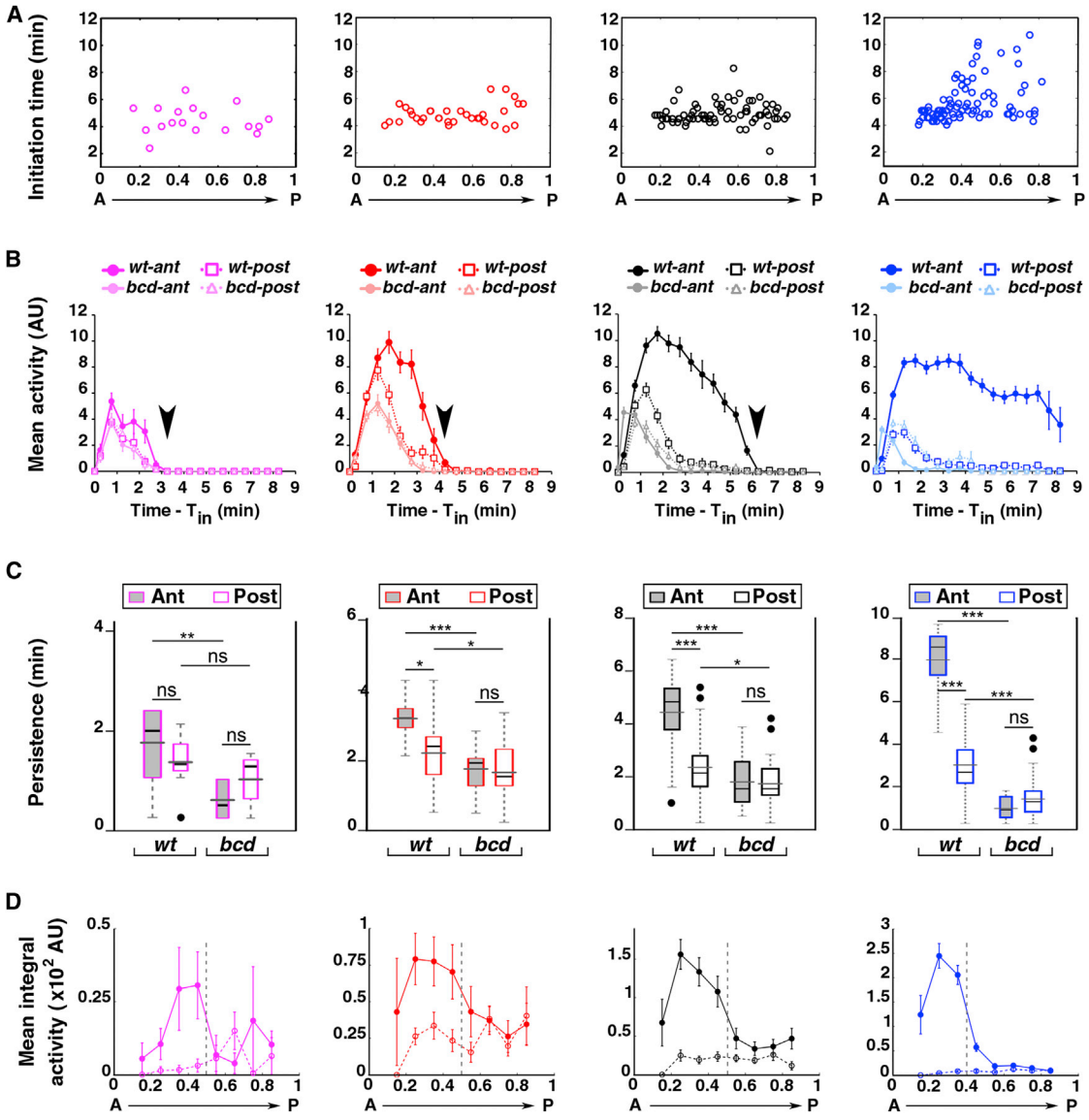

Figure 3. Transcriptional Activity at the Single Locus Resolution

Data were extracted from Movie S1 (clip 1) and shown for interphase 10 in purple (left), interphase 11 in red (middle left), interphase 12 in black (middle right), and interphase 13 in blue (right).

(A) For each expressing locus, the initiation time of the activity period (T_{in}) is indicated by a circle as a function of position along the AP axis.

(B) Mean of activity as a function of time for anterior (plain) or posterior (open) nuclei in a wild-type embryo (dark) or in an embryo from *bcd* females (light) according to the cartoon legend on the top. Mean activities per active locus quickly rise in less than 2 min, fluctuate around a plateau, then rapidly decrease and disappear. Maximal levels of activity in the anterior of the embryo are higher than in the posterior and they are similar during interphases 11, 12, and 13, showing that the system has reached its maximal efficiency. Mean activities were computed by binning the time axis in temporal steps of two frames, corresponding to ~0.5 min each. Error bars represent the SEM. All the nuclei positioned below 45% egg length (EL) were considered anterior, the ones above 55% EL posterior. Arrowhead, onset of mitosis. 0% EL is anterior.

(C) Persistence of activity periods was calculated at each expressing locus (Figure S1), and their distribution in the anterior domain (gray, x < 0.5 EL) were compared using a classical boxplot representation, to those of the posterior domain (white, x > 0.5 EL), except for interphase 13, where the distribution in gray was for x < 0.4 EL and in white was for x > 0.4 EL. In the anterior, the mean persistence values increase progressively from ~2 min in interphase 10 to ~8 min in interphase 13 and tend to be limited by the duration of the interphase. In contrast, in the posterior, mean persistence remains at ~2 min over the cycles. The median value is indicated in black and the mean value is in gray. Statistical tests were performed on R [18] using a nonparametric test (Mann-Whitney-Wilcoxon test, two-tailed). ns, $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

(D) Mean integrated activities per nucleus as a function of position along the AP axis in a wild-type embryo (plain) and in an embryo from a *bcd* mutant female (open). The border separating roughly the two groups of nuclei is indicated by a dashed gray line. It is positioned in the middle of the biggest decrease of the integral activity and corresponds to the highest derivative. For each nucleus with or without a period of activity, the integral activity was defined as the sum of the activity over all the time points of an interphase. The means were obtained by binning of the AP axis in ten equal bins, and the error bars represent the SEM.

See also Figure S3 and Movie S2.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, three figures, and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.08.053.

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