Direct evidence for interphase chromosome movement during the mid-blastula transition in *Drosophila*

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In *Drosophila*, several genetic phenomena are most easily explained by a model in which homologous chromosomes pair, at least transiently, and use regulatory information present on only one homolog to pattern expression from both homologs [1–3]. To accomplish pairing of sites on different chromosomes, there must be a mechanism by which communication between homologs is facilitated. However, except in the case of meiotic prophase, directed, rapid chromosomal movement has not yet been observed. Some studies suggest that chromosomes are relatively immobile during interphase [4,5], while others suggest that chromatin can reposition during interphase [6–8] and may be free to undergo substantial Brownian motion [9]. Using high-resolution, three-dimensional imaging techniques, we determined directly the structure and nuclear location of eleven different loci, both active and inactive, in embryos at cycle 14, the mid-blastula transition. We show that during a single interphase, portions of chromosomes moved in a cell-cycle-specific, directed fashion, independently and over long distances. All eleven regions showed long distances (5–10 μm), whereas those nearer the telomere expanded in the same place and became oriented along the nuclear axis. Gene motion was independent of replication, transcription and changes in nuclear shape. Because individual genes on the same chromosome move independently, the movement is unlikely to be mediated by centromeres, Brownian motion or random drift and must be caused by an active mechanism.

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Received: **4 August 1999**
Revised: **23 November 1999**
Accepted: **13 January 2000**

Published: **25 February 2000**

Current Biology 2000, 10:285–288

0960-9822/00/$ – see front matter

Results and discussion

To discern subtle gene movements using high-resolution imaging techniques, it is essential to localize the smallest possible regions within a large population of synchronous nuclei whose cell cycle phase is known and whose internal structure is as predictable as possible. By these criteria, the cycle 14 embryo of *Drosophila melanogaster* is ideal. Chromosomes are not only highly ordered in the Rabl orientation (centromeres and telomeres clustered at opposite poles), but the nuclei themselves are polarized relative to the embryo surface [10]. Thus, a gene lies at a predictable distance below the embryo surface in all the nuclei [11]. Cell cycle phase is determined by measuring the length of the invaginating cell membrane (Figure 1a).

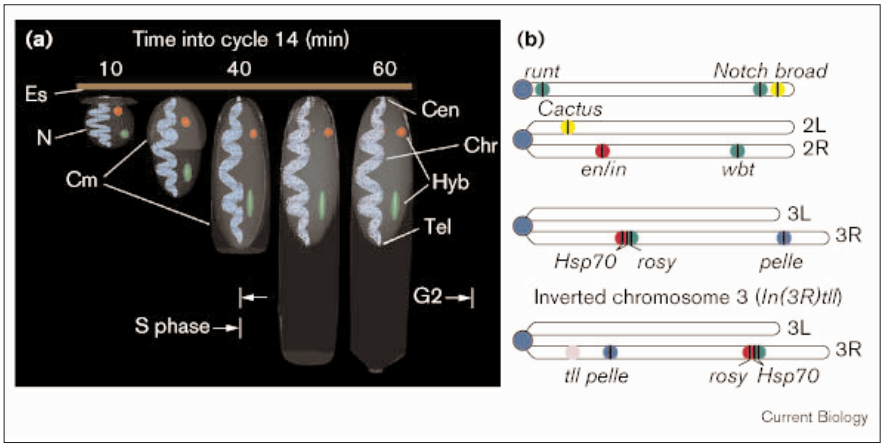
We grouped genes into two classes: genes located centromere-proximal (*Hsp70*, *rosy*, *cactus*, *engrailed/invected* and *runt* on wild-type chromosomes and *pelle* on the chromosome *In(3R)tll*, containing an inversion on 3R; see later), and those located near the telomere (*windbeutel*, *pelle*, *Notch* and *broad* on wild-type chromosomes and *Hsp70* on chromosome *In(3R)tll*; Figure 1b). We predicted on the basis

25% (1.7 ± 0.5 μm) below the nuclear apex at the beginning of cycle 14 and would end the cell cycle at about the same relative depth (5.1 ± 1.5 μm) below the nuclear apex (Figure 1a). We found instead a striking movement pattern as development proceeds.

The *rosy* and *Hsp70* loci (on chromosome 3R) showed the most dramatic pattern of movement, migrating with the greatest speed over long distances (Figure 2a). The *rosy* gene started at about 30% nuclear depth, migrated towards the nuclear interior with a speed of 0.2 μm/minute (Table 1), to about 70% nuclear depth, and then changed direction, moving an average of 7.5 μm between 45 minutes to cellularization (a speed of 0.77 μm/minute).

Similarly, the *Hsp70* loci moved from a position at about 50% nuclear depth at 34 minutes to a position of about 10% nuclear depth at cellularization, with a speed of about 0.5 μm/minute (Figure 2a). The pattern of movement of *Hsp70* and *rosy* were distinct, indicating that the motion of individual loci is independent. Because the *Hsp70* gene is transcriptionally inactive [12] and *rosy* is active, we conclude that movement does not depend on the transcriptional state of the locus. Two other centromere-proximal genes, *cactus* and *runt* located on chromosome 2R and X, both migrated (Figure 2a,e), but at slower speeds and at

Figure 1



(a) Diagram of nuclear structure at the cellular blastoderm stage. The embryo surface (es) is at the top: nuclei (n), illustrated in cross-section, extend toward the embryo interior. A single chromosome arm (chr) is indicated by the blue structure, centromere (cen) near the apex and telomere (tel) near the base of the nucleus. The cell membrane (cm) invagination begins at 10 min after mitosis 13 and continues to grow towards the embryo interior until about 55 min into cycle 14. S phase begins immediately after mitosis 13 and continues for 40 min followed by a short G2 phase. The location of genes is determined by the positions of hybridization signals (hyb). Based on earlier work [10], we assumed that the chromosomes would merely expand to fill the available space as the nuclei expand, and the hybridization spots might elongate slightly in the direction of nuclear expansion. **(b)** Map locations of the genes used in this study; *runt*, *Notch*, *broad* on chromosome X, *cactus*, *engrailed/invected* (*en/in*) and *windbeutel* (*wbt*) on chromosome 2 and heat shock protein 70 (*Hsp70*), *rosy* and *pelle* on chromosome 3. The euchromatic portions of the three large chromosomes and their approximate relative sizes are indicated; the heterochromatin portions are highly condensed in cycle 14 and contribute relatively little to the length. The arrangement of genes on chromosome 3R containing the inversion is also shown. Note that *Hsp70* is divided into two loci separated by an estimated 1 Mb. The *tailless* mutant strains contain a centromere–telomere inversion at 85F10–86A1;100A5,6–B1,2, near the *tailless* gene. The *pelle* gene is located centromere-proximal on chromosome *In(3R)tll* and *Hsp70* and *rosy* are near the telomere.

different times of inflection (Table 1), indicating that the centromere-proximal genes located on other chromosomes also move, although independently.

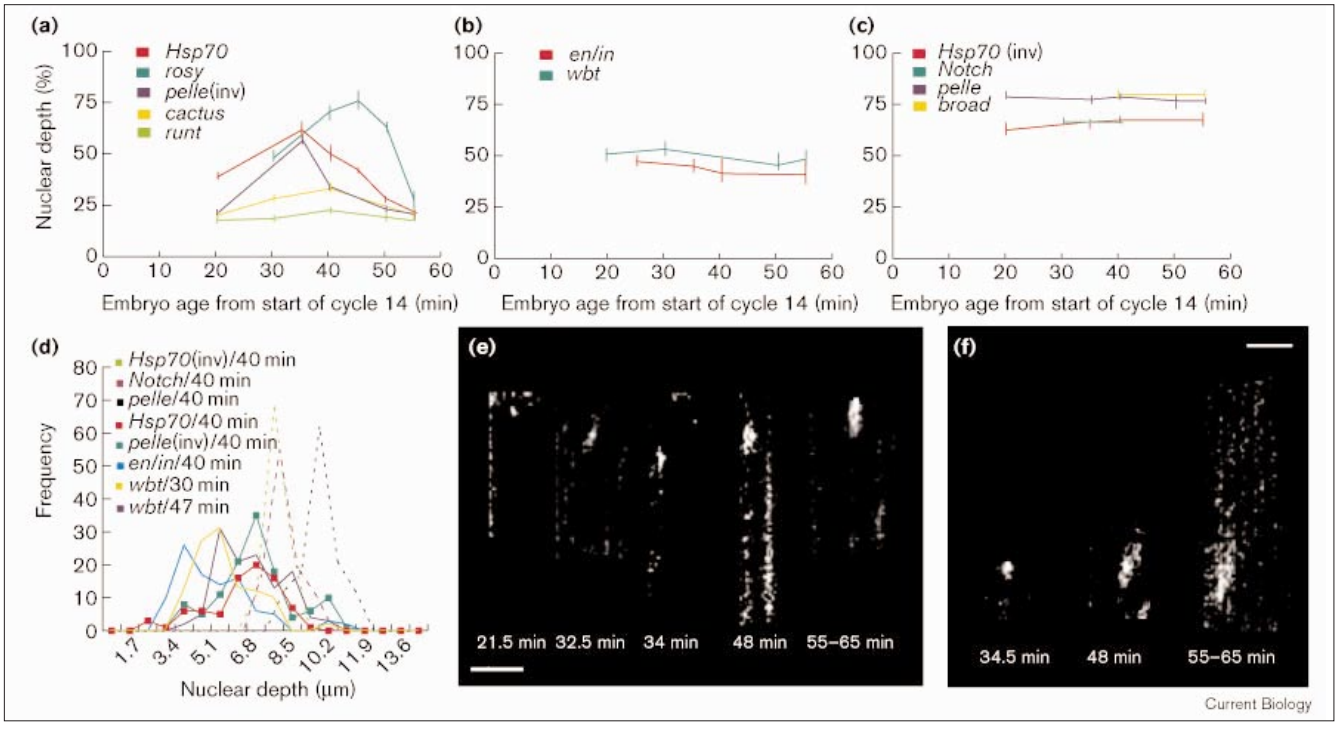
Genes near the telomere did not move within the nucleus (Figure 2c) but became extended along the nuclear axis as the cell cycle progressed (Figure 2f). All three telomere genes, *pelle* on chromosome 3 and *Notch* and *broad* on the X chromosome, failed to migrate as a population towards the nuclear apex during the first 55 minutes of cycle 14. Consistent with their Rabl location, all three genes were located at about 60–70% nuclear depth, with no apparent movement within the nucleus (Figure 2c). However, as developmental age progressed, the frequency distributions of the distance from the nuclear apex for all three loci became broader and more complex than expected (data not shown), and the length of individual hybridization signals increased substantially (Figure 2e), even after nuclear elongation had ceased (S.G., E. Heddle and M.C.R., unpublished observations), indicating that these loci undergo intragenic motion.

Engrailed/invected (*en/in*) and *windbeutel* (*wbt*) present an intermediate case. Though they lie far apart on chromosome 2R, they never assumed their Rabl-directed positions but rather remained at about 50% nuclear depth through the cell cycle (Figure 2b). The population of both *en/in* and *wbt* loci changed nuclear position at much slower speeds and with a less pronounced pattern of movement than *rosy* and *Hsp70*. However, the broad and complex distributions of the nuclear depth for *en/int* and *wbt* were like those of the proximal genes and unlike those of the distal genes (Figure 2d). This indicates that both genes were moving but that the pattern of motion was quite different from that experienced by the more centromere-proximal genes.

To test whether chromosomal location directs the amount and pattern of gene movement, we used a mutant strain containing a centromere–telomere inversion on chromosome 3R called *In(3R)tll* (85F10–86A1;100A5,6–B1,2; Figure 1b). This inversion effectively interchanges the locations of the *Hsp70* and *pelle* genes. We predicted that the movement pattern of these two genes would likewise interchange. Indeed, we found that the *pelle* gene on chromosome *In(3R)tll* migrated much like the *Hsp70* gene in the wild type, moving from the apex at 20 minutes to 50% nuclear depth at 34 minutes, to a position about 10–15% nuclear depth at cellularization (Table 1 and Figure 2a). Conversely, the *Hsp70* gene cluster did not migrate within the nucleus on chromosome *In(3R)tll*, but rather behaved like the other distal genes (Figure 2c). The *Hsp70* loci lay at about 50–60% nuclear depth on chromosome *In(3R)tll*, a depth distribution similar to that observed for *Notch*, *broad* and *pelle* in wild-type embryos. These results indicate a strong correlation between the position of the gene on the chromosome and the amount of gene migration within the nucleus.

We tested whether homolog pairing was a possible mechanism for gene migration. Synapsis of homologs is

Figure 2



Genes migrate within the interphase nucleus during the mid-blastula transition. The migration pattern of eleven genes are plotted as percentage nuclear depth versus embryo age from the start of cycle 14. Each time point represents a single synchronous embryo. The percentage nuclear depth of each gene was calculated from a single data set containing 95–135 nuclei and measured from the lateral midline of the embryo [10]. Vertical bars indicate the standard error of mean for each time point. Inv indicates the situation on chromosome *In(3R)tll*. **(a)** Centromere-proximal genes; **(b)** intermediate genes; **(c)** genes near the telomere. Note that the genes near the telomere are in their Rabl directed positions. **(d)** The frequency distributions of the distance from the nuclear apex for embryos 30–47 min into cycle 14. **(e)** Data sets taken at different developmental ages are computationally rotated and projected to reveal the three-dimensional structure of the hybridization signals of the *runt* gene. The view is a cross-section through the nucleus, with the embryo surface at the top, and the embryo interior at bottom. Note the discrete pattern of movement. **(f)** Three-dimensional images of *pelle* gene hybridization. Note that the *pelle* gene does not move within the nucleus but instead expands along the nuclear axis as the embryo ages. The scale bar represents 5 µm.

incomplete but significant during this time [13], suggesting that the machinery responsible for homolog pairing is active. As further evidence that homolog pairing had already begun, we found that the homologous copies of *rosy*, *broad* and *Notch* maintained a more similar spatial relationship than could be explained by chance ($p > 0.01$; see Supplementary material). Further, we found that the length of the *Notch* gene hybridization signal was more similar between homologs than expected at random ($p > 0.01$; see Supplementary material). We reasoned that if homolog pairing were responsible for gene elongation, loci with no pairing partners should be less extended than those in which two homologs were beginning synapsis. We compared the extent of *Notch* gene elongation in two female embryos to that of male embryos previously studied (S.G., E. Heddle and M.C.R., unpublished observations). We found that the extent of intragenic movement in females was about the same as that predicted from male embryos (see Supplementary material). These observations suggest that the ability to pair does not profoundly affect gene movement. However, we cannot exclude the possibility that intragenic movement is caused by the attempt to pair, rather than the actual ability to do so.

What can be responsible for gene movement during interphase? Our unpublished studies show no movement or interchromosomal changes in centromere regions during the mid-blastula stage, indicating that gene movement is restricted to euchromatin (S.G., K.A. Maggert, G.H. Karpen and M.C.R., unpublished observations). The movement we observe is too rapid to result from Brownian motion [9], bulk flow [4] or random diffusion [7]. We find movement of genes averaging 2–5 times faster (0.77 µm/minute) and over longer distances (5–10 µm) than Marshall *et al.* [9], who conclude that chromatin should take roughly 1–10 minutes to diffuse 1 µm in a random direction. Further, the movement of genes we observe is 5–6 times faster than that observed by Shelby *et al.* [4], who find that a small minority of centromeres within an interphase HeLa cell nucleus moved on the

Table 1

The rate of gene movement.

Gene	Inward movement (μm/min)	Outward movement (μm/min)
runt	0.24	0.05
rosy	0.2	0.77
Hsp70	–	0.5
cactus	0.04	0.13
pelle (inv)	–	0.2
en/in	–	–
wbt	–	0.1
Notch	–	–
Hsp70 (inv)	–	–
broad	–	–
pelle	–	–

Inward denotes towards the interior of the nucleus and outward denotes towards the nuclear apex. Genes are ordered centromere-proximal to distal. Inv, on the *In(3R)tll* chromosome.

order of 7–10 μm/hour. Because of the timing, extent and directionality of movement, we propose that the interphase chromatin movements we observe in *Drosophila* embryos are mediated by an active mechanism.

No traditional motors localize to interphase nuclei, but both DNA and RNA polymerases may fill that role. The replication complex acts to bring about DNA transposition [14], and RNA polymerase is a motor more forceful than any of the traditional motors known [15]. Added to the energy required to distribute the contents of the compact nucleus early in cycle 14 to one roughly three times the volume within about 30 minutes, and the constriction caused by cell membrane invagination, a great deal of gene distortion may occur. Although all this happens during S phase, before a great deal of gene motion has occurred, we cannot exclude the possibility that gene movement is delayed by high nuclear viscosity.

On the other hand, it is possible that specific proteins, through a series of ordered contractions, are responsible for gene motion [16]. Specific gene colocalizations occur [3,7,17,18], but the question is, do they occur rapidly enough to require an active mechanism? For example, homologous sites containing two imprinted loci, the Prader–Willi/Angelman loci on chromosome 15 and the *H19* locus on chromosome 11, associate preferentially only during late S phase in human cells [8]. Because homolog association is of limited duration, it may result from an active mechanism. Chromosomal mobility in early development and in later stages of differentiation may be a general and necessary prerequisite to normal chromosomal patterning in all organisms. If so, we must investigate the extent to which gene movements are required for normal gene expression, and to what extent the failure to traverse critical periods of chromatin and nuclear development will prevent normal expression of transfected genes.

Supplementary material

Supplementary material including a Figure with graphs of the distance between homologs is available at http://current-biology.com/supmat/supmatin.htm.

Acknowledgements

We thank Carl Wu, Robert Levis, Cynthia Baker, Simon Kidd, Peter Gergen, Lynn Manseau, Mary Konsolaki, Steven Wasserman and Steven Poole for generous gifts of plasmids; Judith Lengyel for providing the *tll2* strains; Robert Erickson, Lisa Nagy, and Carol Gregorio for critical reading of the manuscript; Eleanor Calma for technical assistance and David Goldberg for helpful conversations. This work was supported by grants NIH-GM-44553 and NSF-MCB-9507091 (to M.C.R.) and a Genetics Training Grant from the Flinn Foundation (to S.G.).

References

1. Lu BY, Bishop CP, Eissenberg JC: **Developmental timing and tissue specificity of heterochromatin-mediated silencing.** *EMBO J* 1996, **15:**1323-1332.
2. Orlando V, Jane EP, Chinwalla V, Harte PJ, Paro R: **Binding of trithorax and polycomb proteins to the bithorax complex: dynamic changes during early *Drosophila* embryogenesis.** *EMBO J* 1998, **17:**5141-5150.
3. Gemkow MJ, Verveer PJ, Arndt-Jovin DJ: **Homologous association of the Bithorax-Complex during embryogenesis: consequences for transvection in *Drosophila melanogaster*.** *Development* 1998, **125:**4541-4552.
4. Shelby RD, Hahn KM, Sullivan KF: **Dynamic elastic behavior of alpha-satellite DNA domains visualized in situ in living human cells.** *J Cell Biol* 1996, **135:**545-557.
5. Abney JR, Cutler B, Fillbach ML, Axelrod D, Scalettar BA: **Chromatin dynamics in interphase nuclei and its implications for nuclear structure.** *J Cell Biol* 1997, **137:**1459-1468.
6. Manuelidis L: **Individual interphase chromosome domains revealed by *in situ* hybridization.** *Hum Genet* 1985, **71:**288-293.
7. Buchenau P, Saumweber H, Arndt-Jovin DJ: **The dynamic nuclear redistribution of an hnRNP K-homologous protein during *Drosophila* embryo development and heat shock. Flexibility of transcription sites *in vivo*.** *J Cell Biol* 1997, **137:**291-303.
8. LaSalle JM, Lalande M: **Homologous association of oppositely imprinted chromosomal domains.** *Science* 1996, **272:**725-728.
9. Marshall WF, Straight A, Marko JF, Swedlow J, Dernberg A, Belmont A, *et al.*: **Interphase chromosomes undergo constrained diffusional motion in living cells.** *Curr Biol* 1997, **7:**930-939.
10. Gunawardena S, Heddle E, Rykowski M: **"Chromosomal puffing" in diploid nuclei of *Drosophila melanogaster*.** *J Cell Sci* 1995, **108:**1863-1872.
11. Marshall WF, Dernberg AF, Harmon B, Agard DA, Sedat JW: **Specific interactions of chromatin with the nuclear envelope: positional determination within the nucleus in *Drosophila melanogaster*.** *Mol Biol Cell* 1996, **7:**825-842.
12. Elgin SCR, Hood LE: **Chromosomal proteins of *Drosophila* embryos.** *J Biol Chem* 1973, **12:**4984-4991.
13. Hiraoka Y, Dernberg AF, Parmelee SJ, Rykowski MC, Agard DA, Sedat JW: **The onset of homologous chromosome pairing during *Drosophila melanogaster* embryogenesis.** *J Cell Biol* 1993, **120:**591-600.
14. Hozak P, Hassan AB, Jackson DA, Cook PR: **Visualization of replication factories attached to a nucleoskeleton.** *Cell* 1993, **73:**361-373.
15. Yin H, Wang MD, Svoboda K, Landick R, Block SM, Gelles J: **Transcription against an applied force.** *Science* 1995, **270:**1653-1657.
16. Heck MMS: **Condensins, cohesins and chromosome architecture: How to make and break a mitotic chromosome.** *Cell* 1997, **91:**5-8.
17. Dernburg AF, Broman KW, Fung JC, Marshall WF, Philips J, Agard DA, Sedat JW: **Perturbation of nuclear architecture by long-distance chromosome interactions.** *Cell* 1996, **85:**745-759.
18. Fung JC, Marshall WF, Dernberg A, Agard DA, Sedat JW: **Homologous chromosome pairing in *Drosophila melanogaster* proceeds through multiple independent initiations.** *J Cell Biol* 1998, **141:**5-20.