Lawrence Berkeley National Laboratory

Recent Work

Title

The onset of homologous chromosome pairing during Drosophila melanogaster embryogenesis.

Permalink

https://escholarship.org/uc/item/7k67m108

Journal

The Journal of cell biology, 120(3)

ISSN

0021-9525

Authors

Hiraoka, Y
Dernburg, AF
Parmelee, SJ
et al.

Publication Date

1993-02-01

DOI

10.1083/jcb.120.3.591

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

The Onset of Homologous Chromosome Pairing during *Drosophila melanogaster* Embryogenesis

Yasushi Hiraoka, Abby F. Dernburg, Susan J. Parmelee, Mary C. Rykowski, David A. Agard, and John W. Sedat

Department of Biochemistry and Biophysics and The Howard Hughes Medical Institute, University of California, San Francisco, California 94143-0554

Abstract. We have determined the position within the nucleus of homologous sites of the histone gene cluster in *Drosophila melanogaster* using in situ hybridization and high-resolution, three-dimensional wide field fluorescence microscopy. A 4.8-kb biotinylated probe for the histone gene repeat, located approximately midway along the short arm of chromosome 2, was hybridized to whole-mount embryos in late syncytial and early cellular blastoderm stages. Our results show that the two homologous histone loci are distinct and separate through all stages of the cell cycle up to nuclear cycle 13. By dramatic contrast, the two homologous clusters were found to colocalize with high frequency during interphase of cycle 14. Concomitant with homolog pairing at cycle 14, both histone loci were also found to move from their position near the midline of the nucleus toward the apical side. This result suggests that coincident with the initiation of zygotic transcription, there is dramatic chromosome and nuclear reorganization between nuclear cycles 13 and 14.

For nearly a century, it has been debated whether interphase chromosomes follow ordered paths, whether there are special associations between the homologous chromosomes in diploid nuclei, and what roles such associations might play in regulating nuclear organization and function. Direct analysis of interphase nuclei is made difficult by the partially decondensed state of chromatin during this period of transcriptional activity.

The issue of homologous association has remained particularly significant in *Drosophila* biology because genetic evidence has shown that expression of certain alleles of several genes in *Drosophila* (such as *bx-c*, *dpp-c*, and *sgs-4*) can be affected by the allelic state of the homologous locus. These genetic effects, which appear to depend on trans interactions between homologous sequences, have been grouped as the phenomenon known as transvection (Lewis, 1954; Gelbart, 1982; Korge, 1977; Green, 1959; Jack and Judd, 1979; for recent reviews see Pirrota, 1990; and Wu and Goldberg, 1989). Other genetic effects, such as regulation of the *white* gene by the mutant *zeste*^{1} gene product and dominant position-effect variegation (Henikoff and Dreesen, 1989), also appear to depend on pairing in somatic cells. All of these effects are eliminated by large genetic rearrangements, such as translocations and inversions, which disrupt pairing of the expressed locus in the polytene chromosomes.

Pairing-dependent effects are probably not limited to *Drosophila*; at least one example of a transvection-like effect has been described in the snapdragon, *Antirrhinum majus* (Coen and Carpenter, 1988). The rarity of such effects in *Drosophila* makes it plausible that such interactions have eluded observation in other diploid systems because genetic analysis is less complete. In a recent review, homolog pairing–dependent phenomena were grouped under the term "trans-sensing effects" to emphasize their generality and importance (Tartof and Henikoff, 1991).

It has been assumed by many investigators that diploid, somatic tissues in *Drosophila* have their homologous chromosomes synapsed during interphase, although there is little direct cytological evidence to support this idea. A study by Metz in 1916 indicated that in Dipterans, homologous chromosomes in metaphase neuroblast spreads are usually found near each other (Metz, 1916), and this is often cited as evidence for diploid homolog pairing, although extrapolation from metaphase data to the interphase state may not be justified. Indirect evidence for somatic pairing comes from the genetic evidence for trans-sensing effects, and from direct visualization of nuclei in differentiated, postmitotic tissues containing giant polytene chromosomes. In nuclei of these tissues, bundles of chromatids derived from the two parental homologs are usually paired along their entire lengths. In mutants heterozygous for chromosomal rearrangements, homologs will undergo considerable contortions in order to maintain synapsis, which is often interrupted only in the immediate area of the breakpoint. It is not known when during development homologs of polytene chromosomes become

Dr. Hiraoka's present address is Kansai Advanced Research Center, Communications Research Laboratory, 588-2 Iwaoka, Iwaoka-cho, Nishi-ku, Kobe 651-24, Japan.

Dr. Rykowski's present address is Department of Anatomy, College of Medicine, University of Arizona, Tucson, AZ 85724.

© The Rockefeller University Press, 0021-9525/93/02/591/10 $2.00

synapsed, nor whether trans-sensing genetic effects in diploid tissues depend on the same close apposition of the two homologs.

We have looked for synapsis of homologous chromosomes in syncytial blastoderm embryos from *Drosophila melanogaster*. Early *Drosophila* embryos are useful for this study for several reasons. During the 10th to 13th nuclear division cycles, these embryos exist as syncytial blastoderms, with up to 5,000 diploid nuclei forming a single layer just beneath the embryo surface, dividing synchronously every 10–20 min (Zalokar and Erk, 1976). This allows us to examine a two-dimensional array of genetically identical nuclei at defined mitotic stages, facilitating the analysis of chromosome structure. We have previously analyzed the three-dimensional paths of embryonic chromosomes during mitosis from prophase through anaphase, when characteristic staining patterns of the condensed chromosomes allow them to be identified (Hiraoka et al., 1990*b*; Y. Hiraoka, unpublished results). Our results have revealed that chromosomes are not synapsed during the mitotic portion of the early embryonic nuclear cycles. However, they leave open the possibility that homolog pairing is dynamic, occuring only during interphase and breaking down for mitosis, or that synapsis begins at a time in development after the syncytial blastoderm. With cellularization in the 14th cycle, rapid, synchronous mitosis ceases, and patches of cells enter mitosis at different intervals (Foe, 1989). With the notable exceptions of imaginal and neural tissues, mitosis ceases altogether after cycle 16. Polytenization begins shortly after this point, as early as three hours after cycle 15 in salivary glands (Smith and Orr-Weaver, 1991). We reasoned that because few mitoses intervene between the blastoderm stages and terminal differentiation of polytene tissues, we might be able to detect the onset of synapsis.

To probe further the relationship between homologous chromosomes, we have analyzed the position of the homologous histone loci in diploid nuclei of *Drosophila* embryos. We used high resolution in situ hybridization and three-dimensional wide-field optical microscopy to obtain positional information about nuclei during interphase, a time when the chromosomes are decondensed and indistinguishable by other means. In this report, we examine the association state of homologous loci of the histone gene cluster by in situ hybridization to chromosomal DNA in a wild-type strain and a chromosomal translocation strain. Our results demonstrate that homologous loci of the histone genes are predominantly separated during nuclear cycles 11–13 and become associated at nuclear cycle 14. The frequency of homologous association of the histone loci is affected by their chromosomal position.

Materials and Methods

Drosophila Strains and DNA Clones

Drosophila melanogaster Oregon R strain was used as the wild type. A *Drosophila* mutant strain lt^{x13} (Wakimoto and Hearn, 1990) was obtained from Dr. Barbara Wakimoto (University of Washington, Seattle, WA). Heterozygous $lt^{x13}/+$ strain was constructed by mating lt^{x13}/lt^{x13} males with wild-type virgin females and vice versa. The plasmid bearing the 4.8-kb HindIII fragment of *Drosophila melanogaster* histone genes (Lifton et al., 1977) was a gift of Dr. Gary Karpen (Carnegie Institute of Washington, Baltimore, MD). The 4.8-kb HindIII fragment of the histone gene was re-cloned into pGEM2 (Promega Biotec, Madison, WI) by Dr. Tatsuya Hirano (University of California, San Francisco, CA). Either the original plasmid or the pGEM2 bearing the 4.8-kb HindIII fragment was used as a hybridization probe.

Embryo Preparation

Embryos of *Drosophila melanogaster* were prepared either by formaldehyde fixation or methanol/acetic acid fixation. In both procedures, chorions were removed by commercial bleach (5% sodium hypochlorite) as described previously (Mitchison and Sedat, 1983). In the formaldehyde fixation procedure, dechorionated embryos were fixed by shaking with 3.7% formaldehyde (freshly prepared from paraformaldehyde) in a mixture of heptane and buffer A (15 mM Pipes, pH 7.0, 80 mM KCl, 20 mM NaCl, 0.5 mM EGTA, 2 mM EDTA, 0.5 mM spermidine, 0.2 mM spermine, 0.1% 2-mercaptoethanol). After fixation, embryos were transferred to a 1:1 bilayer of heptane and methanol containing EGTA to remove the vitelline membrane as described previously (Mitchison and Sedat, 1983). In the methanol/acetic acid fixation procedure, dechorionated embryos were transferred to a 3:1 mixture of methanol/acetic acid layered with heptane. After brief shaking, devitellinized embryos were collected from the bottom. The embryos were transferred into a fresh solution of methanol/acetic acid. In both fixation procedures, fixed embryos were washed in a series of methanol/buffer A mixtures (75, 50, and 25% methanol) and then washed twice in buffer A. Embryos were stored in buffer A at 4°C typically for 1–3 d before in situ hybridization.

DNA Probes and Random Priming

Before random priming, plasmid DNA was fragmented by sonication or digestion with a combination of restriction enzymes, AluI, HaeIII, Sau3AI, RsaI, and MspI. To 1 μg of DNA fragments, 12.5 μg of random hexamer nucleotides (pd(N)6 50 U/ml; Pharmacia Fine Chemicals, Piscataway, NJ) was added as primer for the DNA synthesis reaction. The mixture was boiled for 5 min and then chilled in ice/ethanol bath to denature double-stranded DNA. The labeling reaction was carried out overnight at 16°C with 5 U of Klenow fragment (United States Biochemical, Cleveland, OH) in 25 μl of freshly prepared random priming buffer (100 mM Pipes, pH 7.0, 5 mM MgCl, 10 mM 2-mercaptoethanol) containing 0.03 mM each of dATP, dGTP, dCTP, and 0.02 mM biotin-16-dUTP (ENZO) or digoxigenin-dUTP (Boehringer Mannheim Biochemicals, Indianapolis, IN). The labeled DNA was purified and unincorporated nucleotides removed by spinning through a 1-ml G50 Sephadex column. For estimation of probe fragment size and efficiency of incorporation, labeled probe fragments were separated by alkaline agarose gel electrophoresis, and then transferred onto a nylon membrane. Digoxigenin-labeled probe fragments were detected using the Genius nucleic acid detection kit (Boehringer Mannheim Biochemicals). The same protocol was used for biotinylated probes by substituting streptavidin–alkaline phosphatase for the alkaline phosphatase–conjugated antibody. Under these conditions, probe fragment size was 200 to 300 nucleotides in length. This range of probe fragment size gave the most successful results for in situ hybridization to whole-mount embryos. With larger fragment sizes, probe fragments accumulated in cortical regions of embryos, yielding a high background in the cortex and no nuclear signal.

Hybridization to Whole-mount Embryos

Fixed embryos were rinsed twice in 2× SSC containing 0.1% Tween 20 (peroxide free; Pierce Chemical Co., Rockford, IL), washed for 10 min in 20% formamide in 4× SSC, 0.1% Tween 20, and in 50% formamide in 4× SSC, 0.1% Tween 20. Throughout the procedure, formamide, freshly deionized by mixing with ion-exchange resin (analytical grade mixed bed resin AG501-X8; BioRad Laboratories, Cambridge, MA), was used. Embryos were incubated in 50% formamide, 4× SSC, 0.1% Tween 20 for 1 h at 37°C. DNA probes in 25 μl of hybridization mixture (4× SSC, 50% formamide, and 0.1% Tween 20) were added to embryos. Double-stranded DNA probes were denatured immediately before use by heating at 90°C for 5 min and then chilling in ice water. Embryos in the hybridization mixture were heated to 70°C for 15 min to denature chromosomal DNA and then incubated at 37°C for 15–18 h. After hybridization, embryos were washed at a room temperature for 20 min sequentially in a series of 50%, 40%, 30%, 20%, 10% formamide in 4× SSC, 0.1% Tween 20, and washed twice in 4× SSC. In some experiments, 4× SSC was replaced by 2× SSC throughout the above procedures. Hybridization signals were then detected by incubating hybridized embryos with Texas red–conjugated avidin D (Vector Laboratories, Burlingame, CA) or rhodamine-conjugated anti-digoxigenin F(ab) fragments (Boehringer Mannheim Biochemicals) in 2× SSC containing 0.1% Tween 20. Embryos were washed at a room temperature

for 20 min twice in 2× SSC containing 0.1% Tween 20 and once in 2× SSC or PBS without Tween 20.

For microscopic observation, whole embryos were mounted in buffer A containing 0.1 μg/ml DAPI and covered with a coverslip (thickness No. 1.5) using two coverslips (thickness No. 1) as spacers to avoid flattening them; the edges were sealed with commercial nail enamel.

Optical Sectioning Microscopy of Embryos

To record images of hybridization signals at low levels of light, we used a cooled, scientific grade charge-coupled device (CCD)[1] as an image detector. A Peltier-cooled CCD camera (Photometrics Ltd., Tucson, Arizona), with a 1,340 × 1,037 pixel CCD chip (Kodak-Videk; Eastman Kodak Co., Rochester, NY) coated to improve short-wavelength sensitivity (Metachrome II coating; Photometrics Ltd., Tucson, AZ), is attatched to an Olympus inverted microscope IMT-2; microscope lamp shutter, focus movement, CCD data collection, and filter combinations are controlled by a MicroVax workstation (Hiraoka et al., 1991).

The doubly stained embryos were observed using an Olympus oil immersion objective lens (S Plan Apo 60/NA = 1.4). Each pixel represents 0.11 μm in the specimen plane. Optical section data were collected at 0.25-μm focus intervals by repeating the following sequence at each focal plane: two images were obtained sequentially for chromosomes (DAPI), and hybridization signals (Texas red), and then microscope focus was stepped by 0.25 μm. High-selectivity excitation and barrier filter combinations (Omega Optical, Brattleboro, Vermont) for DAPI and Texas red were used. For rapid wavelength switching during data collection, excitation, and barrier filters are mounted on revolving wheels controlled by the MicroVax workstation (Digital Equipment Corp., Maynard, MA). A single dichroic mirror with double-band pass properties designed for wavelengths of DAPI and Texas red (Omega Optical, Brattleboro, Vermont) was used to eliminate significant displacement of images during wavelength switching, and thus no further alignment was necessary (Hiraoka et al., 1991). The embryonic developmental stage was determined by the packing density of nuclei on the embryo surface as described previously (Foe and Alberts, 1983).

Plot of the Location of Hybridization Signals within the Nucleus

The three-dimensional position of hybridization signals was determined in a cylindrical coordinate system that was defined for each nucleus; the origin of the coordinate system was set at the center of each nuclear mass. Approximate positions of the nuclear center and the hybridization signals were determined using the interactive modeling option in the PRISM software package for image display and analysis (Chen et al., 1989). The center of mass was calculated for each nuclear mass around the approximate center. The position of hybridization signals was refined by using quadratic interpolation to find the local maximum. The position of hybridization signals was plotted in the r-z coordinate system with the center of nuclear mass as the origin, where the z axis is along the focal direction of the optical section data, i.e., perpendicular to the embryo surface, and r is the distance from the z axis. The depth z was measured as the physical movement of an objective lens and may be enlarged by a factor of up to 20% because of the "apparent depth" effect caused by a refractive index of specimens (Shaw et al., 1989). The plot is not corrected for the apparent depth effect, thus z should be taken as a relative distance, while r is an absolute one.

Results

In Situ Hybridization to Chromosomal DNA in Whole-mount Embryos

To determine the location of a specific chromosomal region in interphase nuclei, we hybridized a biotinylated probe for the histone repeat to whole-mount embryos of *Drosophila*. We subsequently detected the location of the hybridization by staining embryos with fluorescently tagged avidin and observing the embryos using three-dimensional wide-field fluorescence microscopy. Nuclear DNA is counterstained with the DNA-specific dye, DAPI. The hybridization signal and the nuclear DNA can be imaged independently using the appropriate filters. Optical sectioning microscopy reveals the three-dimensional location of the hybridization signals relative to each other and to other chromosomal structures.

We were concerned that our high-resolution analysis should allow us to preserve the native chromosome structure during hybridization procedures that necessarily involve drastic treatments in order to denature chromosomal DNA. In practice, fixation and denaturation conditions which produce strong in situ hybridization signals tend to do so at the expense of structural preservation. This work has emphasized structural preservation and utilized a highly sensitive, cooled CCD detector to partially compensate for the diminished signal. We have used two different fixation procedures in order to ensure that our results were independent of fixation conditions (see Materials and Methods). In the first protocol, we fixed embryos with 3.7% formaldehyde in buffer A (15 mM Pipes, pH 7.0, 80 mM KCl, 20 mM NaCl, 0.5 mM EGTA, 2 mM EDTA, 0.5 mM spermidine, 0.2 mM spermine, 0.1% 2-mercaptoethanol) which is known to preserve chromosome structure as judged by EM (Belmont et al., 1989). In most experiments, we examined embryos that were fixed with formaldehyde in buffer A without proteinase K digestion. We found that digestion with protease, which is essential to obtain signals when hybridizing to RNA (Hafen and Levine, 1986; Shermoen and O'Farrell, 1991), is not necessary for hybridization to chromosomal DNA and in fact does not affect our results. In the second protocol, we fixed embryos with acetic acid–methanol, a more traditional procedure for in situ hybridization to chromosomes (Pardue, 1986). Since interphase nuclei are decondensed and their fine detail is difficult to discern, we have evaluated our preservation of chromosome structure by comparing fixed, unhybridized mitotic nuclei to mitotic nuclei in embryos that have gone through the hybridization procedure (Fig. 1). We find that the chromosome structures in these nuclei look essentially unchanged by hybridization as seen by DAPI staining at the resolution of the light microscope.

We chose the histone gene cluster to probe the state of homologous chromosomes. The 5-kb cluster of histone genes tandemly repeats 100–150 times at a single locus, corresponding to polytene bands 39D-E, in *Drosophila melanogaster* (Lifton et al., 1977) and thus was expected to provide intense hybridization signals. Fig. 2 *B* shows an example of in situ hybridization in whole-mount embryos using this probe; each bright spot over the embryo surface represents the location of histone genes. This figure emphasizes that hybridization signals are observed in every nucleus throughout the entire embryo. Hybridization signals are resistant to digestion with RNase A or RNase H, indicating that the hybridization is to chromosomal DNA (data not shown).

Fig. 3 shows a higher magnification view of a portion of a hybridized embryo at a similar developmental stage displayed as a through-focus series (*A* and *B*) and as an edge view (*C* and *D*). The edge view shows that nuclei form a single layer near the embryo surface and that hybridization signals are situated within a narrow range of focal planes near the apical side of the nuclei. Using DNA probes for sequences near telomeres, signals were observed at the basal side of the nuclei (Hiraoka et al., 1990*a*). This indicates that the polarized arrangement of chromosomes with centromeres near the embryo surface and telomeres toward the em-

1. *Abbreviation used in this paper*: CCD, charge-coupled device.

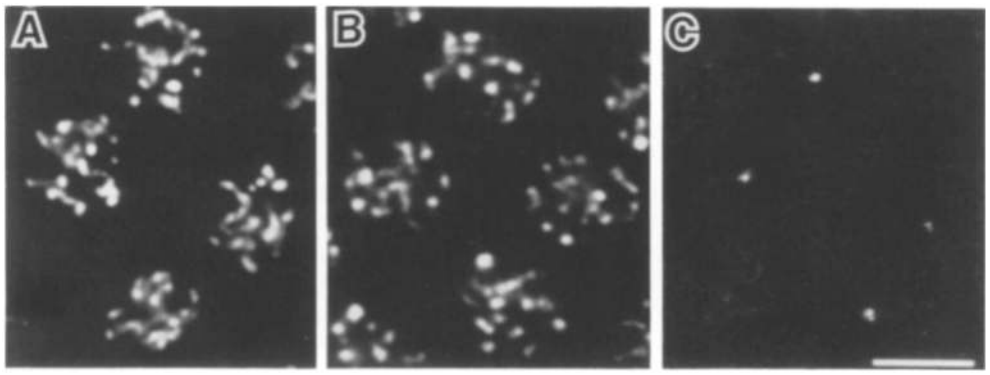

Figure 1. A comparison of the morphology of hybridized and non-hybridized prophase chromosomes. (*A*) DAPI-stained prophase chromosomes in a non-hybridized embryo. (*B*) DAPI-stained prophase chromosomes in a hybridized embryo. (*C*) In situ hybridization signals obtained in the same embryo as in *B*. Bar, 5 μm.

bryo interior (Foe and Alberts, 1985; Hiraoka et al., 1990*b*) persists in interphase and is preserved in hybridized nuclei.

Association of Homologous Sites of the Histone Gene Cluster

We analyzed the paired state and position of homologous chromosomes, as shown in Fig. 4. This whole-mount embryo was hybridized with the histone probe; hybridization signals (red) are superimposed on DAPI staining of nuclei (*blue*) displayed for a single focal plane (*left*). Examination of the entire three-dimensional nuclear volume showed that each nucleus had either one single or two distinct in situ hybridization signals, which we interpret to represent the paired/unpaired state of two homologous sets of the histone gene clusters (*right*; • and ○ represent nuclei having one and two spots, respectively). It is also evident in Fig. 4 that one fused spot is brighter than each of two individual spots. An example of a quantitative comparison of signal intensity between separated spots and fused spots is shown in Fig. 5; peak intensity of one fused spot is approximately twice that of each of two separated spots. In every case, in nuclei containing a single spot of hybridization signal, the intensity was twice that of double hybridization signals in nearby nuclei, consistent with the idea that one spot per nucleus represents the paired state of two homologous sites.

The paired state of the histone gene cluster was examined as a function of embryonic development. The developmental stage was determined solely by the packing density of nuclei on the embryo surface as described by Foe and Alberts (1983). Thus, cellularized and uncellularized cycle 14 embryos were not distinguished from each other. At nuclear cy-

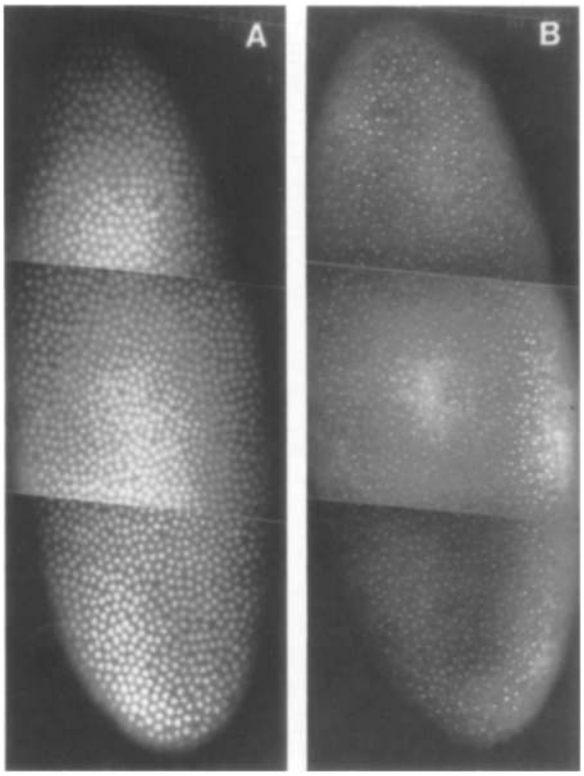

Figure 2. In situ hybridization to the histone locus in a whole-mount wild-type embryo. DAPI staining (*A*) and in situ hybridization (*B*) are shown for the same embryo at nuclear cycle 14.

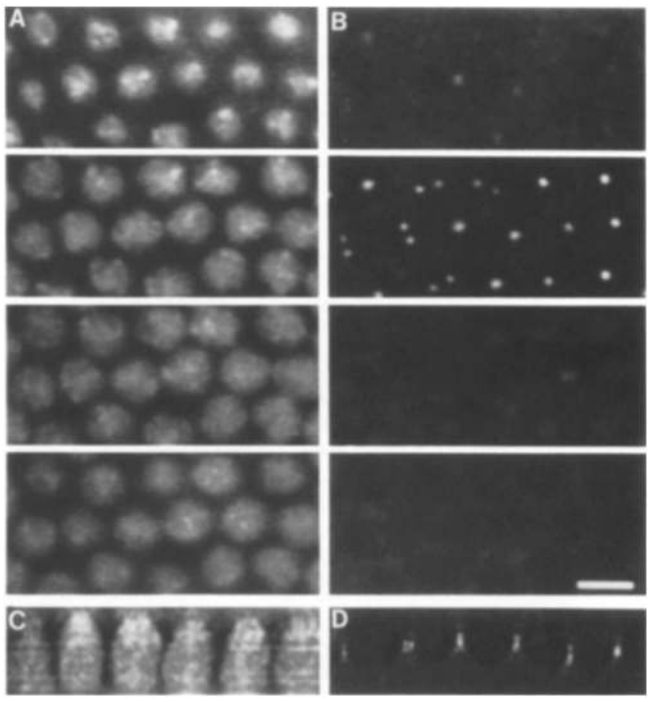

Figure 3. Optical section images of in situ hybridization signals. Optical section images of DAPI staining (*A*) and in situ hybridization (*B*) are shown together with the edge views of the corresponding optical section data set for DAPI staining (*C*) and in situ hybridization (*D*). In the edge views, the external side of embryo is on the upper side of the panel. The developmental stage of this embryo is the 14th nuclear cycle. Bar, 5 μm.

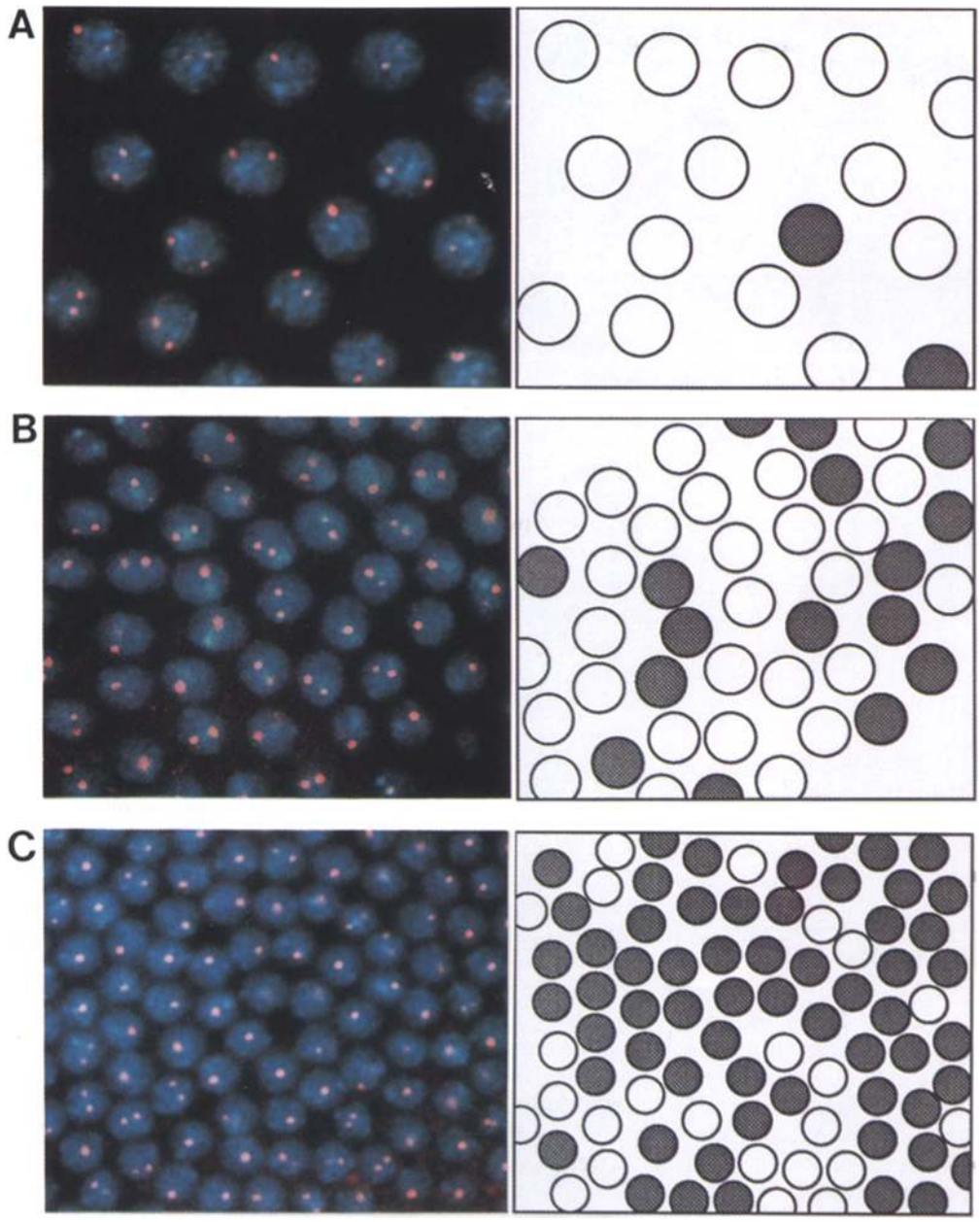

Figure 4. Paired and unpaired states of the histone gene loci. Hybridization signals (*red*) superimposed on DAPI staining (*blue*) for the nuclear cycles 12 (*A*), 13 (*B*), and 14 (*C*) are displayed for a single optical section in the left panel. In the right panel, the paired and unpaired states examined in the entire focus series are represented by hatched circles and open circles, respectively.

cle 12 (Fig. 4 *A*), the majority of nuclei were found to have two spots, indicating the separation of homologous sites. By dramatic contrast, at nuclear cycle 14 (Fig. 4 *C*), the majority of nuclei had only one spot, indicating the paired state of homologous chromosomes.

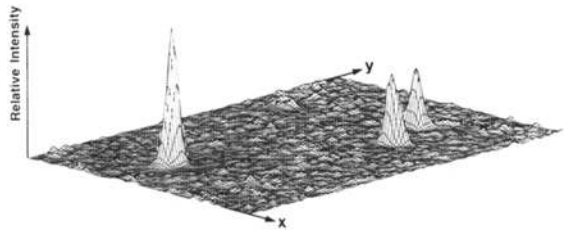

Figure 5. Comparison of the peak intensity of paired and unpaired signals. Intensity profile is shown for a fused signal (*left*) and separated signals (*right*) in neighboring nuclei in Fig. 4 *A*.

We saw the same results with a number of different fixation conditions, as summarized in Table I. This table shows that there is a clear trend toward homologous chromosome association at nuclear cycle 14. By the time of gastrulation, the proportion of nuclei paired at the histone gene cluster reached as high as 90–95%, but those embryos always had a small fraction of nuclei showing two distinct hybridization signals (data not shown).

We observed no simple pattern to the distribution of paired and unpaired nuclei on the embryos' surface. Quite frequently, paired or unpaired nuclei appear to form clusters (see Fig. 4), but these are highly variable in size and do not show consistent patterning from embryo to embryo. We compared the distribution of such clusters in several embryos at the same stage, as judged by the pattern of morphogenetic furrows, with the mitotic domains described by Foe (Foe, 1989) and saw no correlation. Given the number of nuclei we have examined, we cannot conclusively deter-

Table I. Pairing Frequency of the Homologous Histone Gene Loci

Nuclear cycle	Histone gene loci		Total number of nuclei examined	Fixation*	Proteinase K‡
	Paired	Unpaired			
12th	9.5	90.5%	21	MeOH	−
	16.7	83.3	30	FA	−
	17.4	83.6	23	FA	+
	20.0	80.0	20	MeOH	+
	20.8	79.2	24	FA	−
	29.3	60.7	28	FA	−
13th	14.8	85.2	27	FA	−
	22.9	77.1	70	FA	−
	32.5	67.5	40	FA	−
	35.3	64.7	34	FA	−
	37.5	62.5	64	FA	−
	39.1	60.9	69	FA	−
14th	62.9	37.1	116	FA	−
	63.5	36.5	104	MeOH	−
	71.6	28.4	88	FA	+
	71.9	28.1	121	FA	−
	81.8	18.2	99	FA	−
	86.5	13.5	104	MeOH	+

* FA, formaldehyde; MeOH, acetic acid-methanol.
‡ with (+) or without (−) proteinase K digestion of embryos prior to hybridization.

mine whether the distribution is purely random, but analysis of greater numbers of nuclei should allow this to be determined statistically in the future.

Histone Gene Localization and Pairing in a Strain Containing a Chromosomal Rearrangement

To test the effect of chromosomal position on the association of homologous chromosomes, we examined the nuclear location of the histone gene cluster in a chromosomal translocation strain, lt^{x13}. This strain contains a fusion of 3R and 2L such that the histone gene cluster is moved from the middle of the short arm of chromosome 2 to a position much more distant from the centromere. In the wild-type strain, the histone gene lies near the centromeric heterochromatin on chromosome arm 2L. The lt^{x13} strain, which is homozygous viable, bears a reciprocal translocation between the centromeric heterochromatin on chromosome arm 2L and a subtelomeric site on arm 3R (Wakimoto and Hearn, 1990). As a result, the histone gene cluster in these flies lies near the end of 3R on the translocated 3R/2L arm (see diagram in Fig. 7). The chromosomal arrangement for all combinations of wild-type and lt^{x13} chromosomes was assayed first in squashed preparations of salivary gland polytene chromosomes from the appropriate stocks of third-instar larvae. Homologous chromosomes were synapsed along their entire length both in the wild-type and the homozygous lt^{x13}/lt^{x13} strain, whereas the heterozygous $lt^{x13}/+$ strain showed homologous chromosomes that were typically synapsed at the distal portion of chromosome arm 2L and the proximal portion of 3R but were asynapsed over variable distances surrounding the break point of the translocation (data not shown).

The association frequency of the histone gene cluster in embryos of those translocation strains is summarized in Fig. 6. The histone loci are rarely paired (<10% of nuclei) in the $lt^{x13}/+$ strain. In the lt^{x13}/lt^{x13} strain, the frequency of paired loci at cycle 14 stayed at a level similar to that at cycle 13 (~30%), unlike the wild type case. Thus we conclude that the frequency of homologous association in embryos does not solely depend on homology between regions bearing the two histone loci but that chromosomal position can also play a role. This is in contrast to the synapsis observed in polytene nuclei. These results also indicate that the high frequency of homologous association observed in the wild-type nuclear cycle 14 is not simply a result of the decreasing size of nuclei as a function of the nuclear cycle.

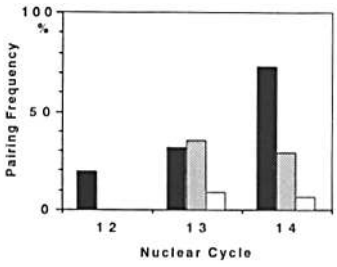

Figure 6. Frequency of the homologous association. Frequency of the paired state of the histone loci is shown for wild type (■), homozygous lt^{x13}/lt^{x13} (▨) and heterozygous $lt^{x13}/+$ (□). Total number of nuclei examined is as follows: 146 nuclei from six embryos (wild type, cycle 12); 304 nuclei from six embryos (wild type, cycle 13); 632 nuclei from six embryos (wild type, cycle 14); 40 nuclei from one embryo (lt^{x13}/lt^{x13}, cycle 13); 243 nuclei from three embryos (lt^{x13}/lt^{x13}, cycle 14); 54 nuclei from one embryo ($lt^{x13}/+$, cycle 13); 156 nuclei from two embryos ($lt^{x13}/+$, cycle 14).

Nuclear Location of the Histone Gene Cluster

Our ability to image and analyze three-dimensional data, together with the simple monolayer geometry of nuclear structures, has allowed us to examine the spatial organization of chromosomes relative to the polarized nuclear orientation. We used a cylindrical coordinate system for each nucleus simply by making the cylindrical axis perpendicular to the embryo surface. The three-dimensional position of the hybridized histone gene cluster was determined relative to the center of each nucleus (see Materials and Methods). Fig. 7 shows the nuclear location of the histone gene cluster plotted in the cylindrical coordinate system with radius (r) and depth (z). Filled and open symbols represent the paired and unpaired state, respectively; circles and squares represent the normal and translocated histone loci, respectively (see below). Our analysis showed that the wild-type histone gene locus is restricted to a small region or plane about halfway between the apical and basal sides of the nucleus at the 13th cycle and changes location toward the apical side of nuclei at the 14th cycle (Fig. 7 *A*). Pairing and nuclear location appear to be independent in the wild type.

Embryos homozygous or heterozygous for the lt^{x13} chromosomal translocation were analyzed at nuclear cycles 13 and 14 to determine the nuclear location of the histone gene cluster. In the lt^{x13} homozygotes, the translocated histone genes were distributed throughout the basal half of the nuclei at both the 13th and 14th cycles (Fig. 7 *B*). In the $lt^{x13}/+$ heterozygotes, one set of histone loci is localized at the nuclear midline as in the wild-type embryos and the other (presumably the rearrangement copy) is in the basal half of the nucleus (Fig. 7 *C*). The presumptive normal loci in the $lt^{x13}/+$ strain move toward the apical side at the 14th cycle despite the lack of homologous association, while the

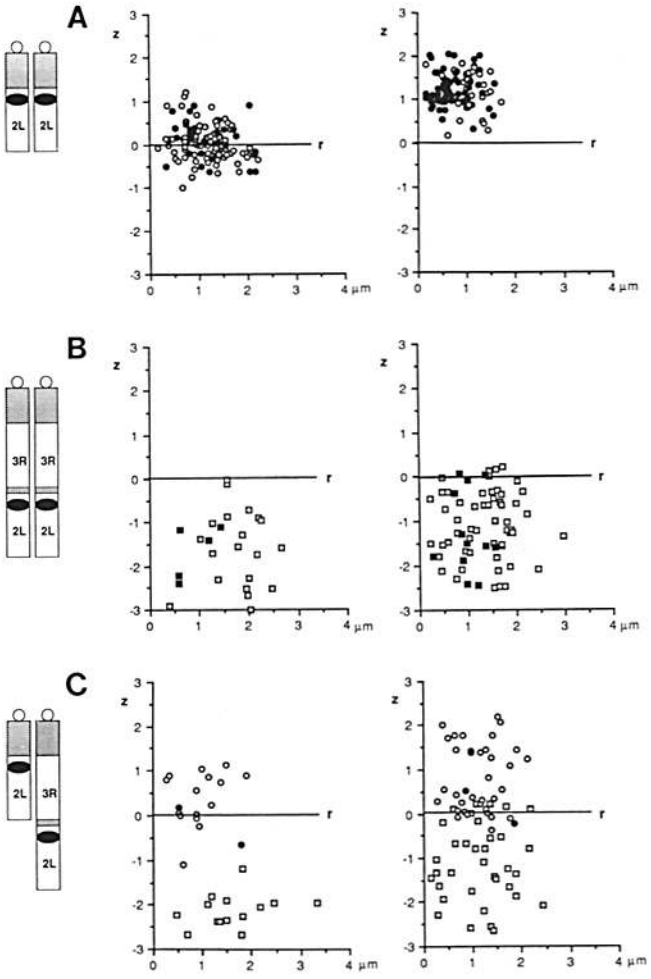

Figure 7. Nuclear positions of the histone gene loci. Nuclear positions of the histone loci are shown for wild type (*A*), homozygous lt^{xl3}/lt^{xl3} (*B*), and heterozygous $lt^{xl3}/+$ (*C*). Chromosome arrangement in each strain is diagrammed to the left of the plots of the positions; the histone locus is represented by a filled ellipse; hatched and open boxes represent heterochromatin and euchromatin, respectively; the open circle indicates the centromere. The positions are plotted for the nuclear cycles 13 (*left*) and 14 (*right*), where z is defined by the axis perpendicular to the embryo surface. Normal and translocated histone loci are represented by circle and square, respectively; the paired and unpaired states are represented by filled and open symbols, respectively. In heterozygous $lt^{xl3}/+$ (*C*), the identities of the normal and translocated loci were presumed by their depth in each nucleus; a filled circle in this case represents a paired circle and square. Total number of plotted hybridization spots and the number of nuclei are as follows: 154 spots in 90 nuclei from three embryos (wild type, cycle 13); 94 spots in 75 nuclei from two embryos (wild type, cycle 14); 26 spots in 16 nuclei from one embryo (lt^{xl3}/lt^{xl3}, cycle 13); 69 spots in 41 nuclei from two embryos (lt^{xl3}/lt^{xl3}, cycle 14); 32 spots in 17 nuclei from one embryo ($lt^{xl3}/+$, cycle 13); 73 spots in 38 nuclei from two embryos ($lt^{xl3}/+$, cycle 14).

presumptive translocated loci do not show significant change in depth (z).

Discussion

It has heretofore been unclear whether homologous chromosomes are associated with each other in diploid somatic cells. Contradictory results have been accumulated in a wide variety of organisms (reviewed in Avivi and Feldman, 1980; Hilliker and Appels, 1989). Genetic phenomena such as dominant position-effect variegation and transvection are interpreted to indicate that homologous chromosomes of *Drosophila melanogaster* are paired or synapsed, at least in some tissues (reviewed in Wu and Goldberg, 1989). Cytological analysis of squashed preparations of mitotic chromosomes from neuroblast cells of third-instar larvae also suggests some vestiges of pairing (Kaufmann, 1934). In addition, homologous association of the histone loci in larval ganglia cells and imaginal disc cells was demonstrated by in situ hybridization (Lifschytz and Hareven, 1982). On the other hand, cytological identification of embryonic cycle 12 and 13 chromosomes indicates that from prophase to anaphase, and late in interphase for the special case of anoxic nuclei, chromosomes are not synapsed (Foe and Alberts, 1985; Hiraoka et al., 1990*b*; Y. Hiraoka, unpublished results). This raises the questions of when and how homologous chromosomes become paired. Our three-dimensional light microscopy in conjunction with in situ hybridization techniques has allowed us to examine the nuclear position of the histone locus as a function of nuclear activities in diploid tissues during embryonic development, and has demonstrated that homologous pairing probably begins during cycle 14.

To address questions of nuclear organization, we had to give special consideration to preserving the structure and organization of chromosomes in our hybridization procedures. Two criteria were used to evaluate the success of our efforts. First, preservation of condensed mitotic chromosomes was tested by comparing hybridized chromosomes with samples which had simply been fixed and mounted without hybridization. The three-dimensional arrangement and structure of chromosomes was indistinguishable between hybridized and non-hybridized specimens. By contrast, using conventional in situ hybridization methods, the chromosomes were visibly damaged. Second, the preservation of the polarized orientation of chromosomes was examined. In *Drosophila* embryos at the syncytial blastoderm stage, chromosomes are aligned in a polarized orientation with centromeres near the embryo surface and telomeres toward the embryo interior (Foe and Alberts, 1985; Hiraoka et al., 1990*b*). Our results from in situ hybridization in interphase nuclei were consistent with this polarized chromosome arrangement. Thus, we are confident that chromosome structure and organization were well preserved during the hybridization procedures although we cannot completely rule out the possibility that the hybridization protocol may in some way affect the paired state of the histone locus. We have, however, ruled out the possibility of interference due to the avidin or antidigoxigenin antibodies used to detect hybridization by repeating these experiments using directly labeled fluorescent probes; our results were unaffected.

In situ hybridization to nascent RNA transcripts has been used to approximate the nuclear position of genes in some systems (e.g., Lawrence et al., 1989). In *Drosophila*, however, RNA transcription of most genes begins after the 13 syncytial mitoses. For this reason, hybridization to early embryonic RNA is not a useful way of approximating the postion of most genes in this system. We have probed the position of a gene by in situ hybridization directly to chromosomal DNA. Our hybridization signals are resistant to diges-

tion with RNase A or RNase H, and can be obtained at any stages of cell cycle or embryonic development without regard to levels of RNA transcription.

The Timing of Nuclear Reorganization

Our results show that there is a dramatic change in histone gene pairing and indicate a chromosome reorganization within the nucleus at the mitotic cycle 13/14 transition. This reorganization takes place concomitantly with vast changes in embryonic development: embryos become cellularized and dramatic changes in patterns of gene expression occur toward the onset of differentiation (Anderson and Lengyel, 1979; Edgar and Schubiger, 1986). It is suggestive that the timing of the structural reorganization coincides with an important temperature-sensitive period for position-effect variegation (Spofford, 1976), for the first time bringing together microscopic and genetic evidence for the reorganization of chromosomes at this developmental stage. It is tempting to speculate that the observed changes in chromosome organization are manifestations of or a prelude to the onset of zygotic transcription. Further experiments will be required to determine to what extent gene expression might either require, or be required for, the formation of homologous chromosome association.

Order in the nucleus is highly dependent on the particular tissue or cell type being studied (discussed in Billia and de Boni, 1991). For example, Arnoldus and co-workers have detected homologous association of human chromosome 1 in cells isolated from cerebellar tissue, but not in cerebral cells (Arnoldus et al., 1989). In general, in situ hybridization is carried out on isolated cells or nuclei, or on tissue sections preserved in paraffin. This report documents our success in applying these methods to a whole-mount multicellular organism, the developing *Drosophila* embryo. Our ability to analyze a large number of nuclei at well-defined developmental stages has enabled us to document a developmental transition in nuclear organization. We feel that this type of approach will allow us to ask other questions about nuclear organization in relevant tissues and as they relate to development. For example, our hybridization protocol has been successfully applied to other *Drosophila* tissues, including imaginal disks and oocytes (A. F. Dernburg, unpublished data). Another advantage to a whole-mount, three-dimensional approach is that the spatial relationships between daughter nuclei are preserved.

Nuclear Reorganization and Heterochromatin

Heterochromatin comprises ~30% of the total *Drosophila* genome and is mostly located at the pericentric regions and the Y chromosome (reviewed in Pimpinelli et al., 1986; Pardue and Hennig, 1990). During the process of polytenization, pericentric heterochromatin on different chromosomes becomes underrepresented, and fuses to form a relatively small "chromocenter" (reviewed in Pardue and Hennig, 1990) while the euchromatic portions endoreplicate to form polytene chromosomes, each of which consists of synapsed homologous chromosomes. Pericentric heterochromatin of a considerable length lies between the centromere and the histone gene locus. Thus, on mitotic chromosomes, the histone gene cluster lies in the middle of the chromosome arm, while on polytene chromosomes, it appears very close to the chromocenter at cytological locus 39 (for comparison of mitotic chromosomes and polytene chromosomes; see Hannah, 1951). This explains our observation of the position of the histone genes at the nuclear midline in early embryos. Movement of the histone loci to the apical side of the nucleus at the cycle 13/14 transition, described in our results, is likely to result from the condensation of heterochromatin, bringing the histone locus toward the centromere. This is consistent with observations that constitutive heterochromatin becomes more prominent at nuclear cycle 14 (V. E. Foe, personal communication). We emphasize that the histone loci move to the apical side whether or not homologous loci are associated; thus, the movement is not a result of the association.

Recognition and Association of Homologous Chromosomes

The demonstration of synapsis beginning in cycle 14 leads to the question of mechanism. It is possible that recognition between homologous chromosomes in somatic cells depends on mechanisms similar to those operating in meiosis I. Homologous chromosomes must find each other at some time prior to meiosis, after which they are held in place with a specialized structure, the synaptonemal complex. Many models for the recognition of meiotic homologous chromosomes have been proposed, including long-range attractive forces or extrachromosomal structures between a pair of homologous chromosomes, chance contact of randomly moving homologous chromosomes, and non-random arrangement of chromosomes which keep a homologous pair in proximity (reviewed in Ashley and Wagenaar, 1974; Maguire, 1984).

Our observations of chromosome organization in prophase and anaphase of early embryonic cell cycles have indicated that homologous chromosomes are not in proximity but rather are often separated by non-homologous chromosomes (Hiraoka et al., 1990*b*; Y. Hiraoka et al., unpublished results). Thus, homologous chromosomes must exclude other chromosomes in between them to make contact along the entire length. This exclusion process might be accomplished most easily by a unidirectional "zip-up" association from centromeres. The notion of homologous pairing occurring in a proximal-to-distal fashion was suggested by Smolik-Utlaut and Gelbart to explain genetic observations about transvection at the *bx-c* and *dpp-c* loci (Smolik-Utlaut and Gelbart, 1987).

Such a hypothetical mechanism would not explain all our observations. In polytene chromosomes from lt^{x13}/+ heterozygotes, the translocated chromosome 3R/2L arm pairs homologously with the normal 2L arm at telomeric regions in addition to the pairing of the normal 3R arm at centromeric regions. Therefore, it is unlikely that synapsis is directed from the centromeres alone; further association of homologous chromosome arms must take place at chromosomal sites other than centromeres, perhaps at later stages, once interfering chromosomes are excluded. The association of telomeres has been reported in a wide range of organisms, making them another possible site of initial association (reviewed in Ashley and Wagenaar, 1974; Dancis and Holmquist, 1979). In *Drosophila*, it has been reported that telomeres share DNA sequences with centromeric heterochromatin (Young et al., 1983); thus, those sequences may share similar functions. Alternatively, specific sites

along the chromosome arms may facilitate pairing; such sites have been implicated in meiotic pairing and can be genetically mapped (Hawley, 1979). This question of initiation sites for somatic homologous pairing can be addressed by examining the pairing behavior of additional rearrangements. It is possible that the molecular mechanism underlying the homologous associations seen here with diploid chromosomes also forms the basis for the assembly or maintenance of polytene chromosomes.

Two final points should be made. First, this paper documents pairing at cell cycle 14 at a single chromosomal locus. Obviously, to form a complete picture, it would be desirable to analyze the behavior of many additional loci spaced along the autosomes and the X chromosome. Such hybridization experiments are now underway, and probes for whole chromosomal arms to allow a detailed examination of the continuity of pairing are being developed (A. F. Dernburg, unpublished results). Secondly, at this time we cannot make a definitive statement as to the functional significance of diploid homolog pairing as evidenced by the histone locus. As dramatic as the pairing transition is in cell cycle 14 in wild-type embryos, no such transition is seen with the lt^{x13} chromosomal rearrangement. Furthermore, somatic pairing can be perturbed by chromosomal rearrangements with no detectable phenotype. As an extreme example, *Drosophila* stocks which are heterozygous for balancer chromosomes, specifically designed to disrupt homology (and thereby prevent meiotic recombination) between partner chromosomes, are viable, implying that somatic pairing must be nonessential at all loci, and/or that there may be compensatory mechanisms that deal with chromosomal and nuclear perturbations. In female meiosis, *Drosophila melanogaster* uses a distributive pathway to accurately segregate non-homologous partner chromosomes (reviewed in Hawley, 1989); it is possible that an analogous mechanism exists for managing non-homologous partners in somatic cells.

We have demonstrated that in situ hybridization can be used to probe three-dimensional nuclear organization in a whole-mount organism without gross disturbance of nuclear morphology. By studying the course of early embryonic development, we have identified a time period during which chromosomal reorganization occurs within the nucleus. There are many questions remaining to be asked about somatic association between homologous chromosomes. This technique has given us a new approach which promises to provide answers to these long-standing questions.

We thank Dr. Barbara Wakimoto for providing *Drosophila* lt^{x13} strain and Dr. Gary Karpen for providing the histone gene clone. We thank Dr. Jeremy Minshull and Jason Swedlow for critical reading of the manuscript. A. F. Dernburg is a Howard Hughes Medical Institute Predoctoral Fellow.

This work was supported by grants from the National Institutes of Health to J. W. Sedat (GM-25101) and D. A. Agard (GM-31627). J. W. Sedat and D. A. Agard are Howard Hughes Investigators.

Received for publication 25 August 1992 and in revised form 12 October 1992.

References

Anderson, K. V., and J. A. Lengyel. 1979. Rates of synthesis of major classes of RNA in Drosophila embryos. *Dev. Biol.* 70:217–231.

Arnoldus, E. P., A. C. Peters, G. T. Bots, A. K. Raap, and M. van der Ploeg. 1989. Somatic pairing of chromosome 1 centromeres in interphase nuclei of human cerebellum. *Hum. Genet.* 83:231–234.

Ashley, T., and E. B. Wagenaar. 1974. Telomeric association of gametic and somatic chromosomes in diploid and autotetraploid *Ornithogalum virens*. *Can. J. Genet. Cytol.* 16:61–76.

Avivi, L., and M. Feldman. 1980. Arrangement of chromosomes in the interphase nucleus of plants. *Hum. Genet.* 55:281–295.

Belmont, A. S., M. B. Braunfeld, J. W. Sedat, and D. A. Agard. 1989. Large-scale chromatin structural domains within mitotic and interphase chromosomes in vivo and in vitro. *Chromosoma.* 98:129–143.

Billia, F., and U. de Boni. 1991. Localization of centromeric satellite and telomeric DNA sequences in dorsal root ganglion neurons, in vitro. *J. Cell. Sci.* 100:219–226.

Chen, H., J. W. Sedat, and D. A. Agard. 1989. Manipulation, display, and analysis of three-dimensional biological images. *In* The Handbook of Biological Confocal Microscopy. J. Pawley, editor. IMP Press, Madison, WI. 127–135.

Coen, E. S., and R. Carpenter. 1988. A semi-dominant allele, *niv-525*, acts in trans to inhibit expression of its wild-type homologue in *Antirrhinum majus*. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:877–883.

Dancis, B. M., and G. P. Holmquist. 1979. Telomere replication and fusion in eukaryotes. *J. Theor. Biol.* 78:211–224.

Edgar, B. A., and G. Schubiger. 1986. Parameters controlling transcriptional activation during early Drosophila development. *Cell.* 44:871–877.

Foe, V. E. 1989. Mitotic domains reveal early commitment of cells in Drosophila embryos. *Development.* 107:1–22.

Foe, V. E., and B. M. Alberts. 1983. Studies of nuclear and cytoplasmic behavior during the five mitotic cycles that precede gastrulation in Drosophila embryogenesis. *J. Cell Sci.* 61:31–70.

Foe, V. E., and B. M. Alberts. 1985. Reversible chromosome condensation induced in Drosophila embryos by anoxia: visualization of interphase nuclear organization. *J. Cell Biol.* 100:1623–1636.

Gelbart, W. M. 1982. Synapsis-dependent allelic complementation at the decapentaplegic gene complex in Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA.* 79:2636–2640.

Green, M. M. 1959. Spatial and functional properties of pseudoalleles at the white locus in Drosophila melanogaster. *Heredity.* 13:303–315.

Hafen, E., and M. Levine. 1986. The localization of RNAs in Drosophila tissue sections by in situ hybridization. *In* Drosophila: A Practical Approach. D. B. Roberts, editor. IRL Press, Oxford, England. 139–157.

Hannah, A. 1951. Localization and function of heterochromatin in Drosophila melanogaster. *Adv. Genet.* 4:87–125.

Hawley, R. S. 1979. Chromosomal sites necessary for normal levels of meiotic recombination in Drosophila melanogaster. I. Evidence for and mapping of the sites. *Genetics.* 94:625–646.

Hawley, R. S. 1989. Genetic and molecular analysis of a simple disjunctional system in Drosophila melanogaster. *Prog. Clin. Biol. Res.* 311:277–302.

Henikoff, S., and T. D. Dreesen. 1989. Trans-inactivation of the Drosophila *brown* gene: evidence for transcriptional repression and somatic pairing dependence. *Proc. Natl. Acad. Sci. USA.* 86:6704–6708.

Hilliker, A. J., and R. Appels. 1989. The arrangement of interphase chromosomes: structural and functional aspects. *Exp. Cell Res.* 185:297–318.

Hiraoka, Y., M. C. Rykowski, J. Lefstin, D. A. Agard, and J. W. Sedat. 1990*a*. Three-dimensional organization of chromosomes studied by in situ hybridization and optical sectioning microscopy. *Proc. SPIE (Society of Photo-optical Instrumentation Engineers).* 1205:11–19.

Hiraoka, Y., D. A. Agard, and J. W. Sedat. 1990*b*. Temporal and spatial coordination of chromosome movement, spindle formation, and nuclear envelope breakdown during prometaphase in Drosophila melanogaster embryos. *J. Cell Biol.* 111:2815–2828.

Hiraoka, Y., J. R. Swedlow, M. R. Paddy, D. A. Agard, and J. W. Sedat. 1991. Three-dimensional multiple-wavelength fluorescence microscopy for the structural analysis of biological phenomena. *Semin. Cell. Biol.* 2: 153–165.

Jack, J. W., and B. H. Judd. 1979. Allelic pairing and gene regulation: a model for the zeste-white interaction in Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA.* 76:1368–1372.

Kaufmann, B. P. 1934. Somatic mitoses of Drosophila melanogaster. *J. Morphol.* 56:125–155.

Korge, G. 1977. Direct correlation between a chromosome puff and the synthesis of a larval saliva protein in Drosophila melanogaster. *Chromosoma.* 62:155–174.

Lawrence, J. B., R. H. Singer, and L. M. Marselle. 1989. Highly localized tracks of specific transcripts within interphase nuclei visualized by in situ hybridization. *Cell.* 57:493–502.

Lewis, E. B. 1954. The theory and application of a new method of detecting chromosomal rearrangements in Drosophila melanogaster. *Am. Nat.* 88: 225–239.

Lifschytz, E., and D. Hareven. 1982. Heterochromatin markers: arrangement of obligatory heterochromatin, histone genes and multisite gene families in the interphase nucleus of D. melanogaster. *Chromosoma.* 86:443–455.

Lifton, R. P., M. L. Goldberg, R. W. Karp, and D. S. Hogness. 1977. The organization of the histone genes in Drosophila melanogaster: function and evolutionary implications. *Cold Spring Harbor Symp. Quant. Biol.* 42: 1047–1051.

Maguire, M. P. 1984. The mechanism of meiotic homologue pairing. *J. Theor. Biol.* 106:605–615.

Metz, C. W. 1916. Chromosome studies on the Diptera. II. The paired association of chromosomes in the Diptera, and its significance. *J. Exp. Zool.* 21: 213–279.

Mitchison, T. J., and J. W. Sedat. 1983. Localization of antigenic determinants in whole Drosophila embryos. *Dev. Biol.* 99:261–264.

Pardue, M. L. 1986. In situ hybridization to DNA of chromosomes and nuclei. *In* Drosophila: A Practical Approach. D. B. Roberts, editor. IRL Press, Oxford, England. 111–137.

Pardue, M. L., and W. Hennig. 1990. Heterochromatin: junk or collectors item? *Chromosoma.* 100:3–7.

Pimpinelli, S., S. Bonaccorsi, M. Gatti, and L. Sandler. 1986. The peculiar genetic organization of Drosophila heterochromatin. *Trends Genet.* 2:17–20.

Pirrotta, V. 1990. Transvection and long-distance gene regulation. *Bioessays.* 12:409–414.

Shaw, P. J., D. A. Agard, Y. Hiraoka, and J. W. Sedat. 1989. Tilted view reconstruction in optical microscopy: three-dimensional reconstruction of Drosophila melanogaster embryo nuclei. *Biophys. J.* 55:101–110.

Shermoen, A. W., and P. H. O'Farrell. 1991. Progression of the cell cycle through mitosis leads to abortion of nascent transcripts. *Cell.* 67:303–310.

Smith, A. V., and T. L. Orr-Weaver. 1991. The regulation of the cell cycle during Drosophila embryogenesis: the transition to polyteny. *Development.* 112:997–1008.

Smolik-Utlaut, S. M., and W. M. Gelbart. 1987. The effects of chromosomal rearrangements on the zeste-white interaction in Drosophila melanogaster. *Genetics.* 116:285–298.

Spofford, J. B. 1976. Position-effect variegation in Drosophila. *In* Genetics and Biology of Drosophila. M. Ashburner, editor. Academic Press, London, England. Vol. 2*a*. 955–1019.

Tartof, K. T., and S. Henikoff. 1991. Trans-sensing effects from Drosophila to humans. *Cell.* 65:201–203.

Wakimoto, B. T., and M. G. Hearn. 1990. The effects of chromosome rearrangements on the expression of the heterochromatic genes in chromosome 2L of Drosophila melanogaster. *Genetics.* 125:141–154.

Wu, C. T., and M. L. Goldberg. 1989. The Drosophila zeste gene and transvection. *Trends Genet.* 5:189–194.

Young, B. S., A. Pession, K. L. Traverse, C. French, and M. L. Pardue. 1983. Telomere region in *Drosophila* share complex DNA sequences with pericentric heterochromatin. *Cell.* 34:85–94.

Zalokar, M., and I. Erk. 1976. Division and migration of nuclei during early embryogenesis of Drosophila melanogaster. *J. Micros. Biol. Cell.* 25: 97–106.