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Transcribed genes are localized according to chromosomal position within polarized *Drosophila* embryonic nuclei

Gavin S. Wilkie[*], **Antony W. Shermoen**[†], **Patrick H. O'Farrell**[†], and **Ilan Davis**[*]

[*]Institute of Cell and Molecular Biology, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, Scotland, UK

[†]Department of Biochemistry and Biophysics, Box 0448, University of California San Francisco, San Francisco, California 94143, USA

Abstract

When some genes are silenced, their positions within the nucleus can change dramatically [1,2]. It is unclear, however, whether genes move to new positions when they are activated [3]. The chromosomes within the polarized nuclei of the fruit fly *Drosophila* have a well-characterized apical–basal orientation (the Rabl configuration [4]). Using a high-resolution *in situ* hybridization method [5], we found that each of 15 transcribed genes was localized as predicted by their chromosomal position and by the known polarized organization of the chromosomes. We also found that, within their specific apical–basal plane, most nascent transcript foci could occupy any radial position. There was no correlation between the apical–basal position of the transcribed locus and the final cytoplasmic site of localization of the RNA along the apical–basal axis of the cell. There was also no relationship between the distance of loci from the nuclear periphery and the amount of nascent mRNA decorating the gene. Our results are consistent with the view that effective transcription can occur without major re-localization of the genes themselves.

Results and discussion

Nascent transcript foci are consistently positioned in the apical–basal nuclear axis

It is unclear to what extent the intranuclear position of genes changes according to their differing transcriptional states in various parts of the *Drosophila* embryo. Such potential variation in nuclear localization would not necessarily be apparent in studies describing the average intranuclear position of genes throughout the entire embryo [6]. We used a recently developed high-resolution RNA *in situ* hybridization method [5] to examine the localization of 15 different transcribed genes. In addition to cytoplasmic mRNA, nascent transcripts are visible as one to four small fluorescent foci within each nucleus that expresses a gene, depending on the state of pairing and DNA replication. The positions of 19–52 individual nascent transcript foci from each gene were mapped in nuclei from optical sections through the middle of two to three embryos of similar age (Figure 1b). The apical–basal distribution of the nascent transcript foci on the X chromosome and chromosome arms 2R and 3R were determined by plotting all the data onto the outline of a single representative nucleus (Figure 1c and see Supplementary material).

Correspondence: Ilan Davis, ilan.davis@ed.ac.uk.

Supplementary material: Supplementary material including additional methodological details and results is available at http://current-biology.com/supmat/supmatin.htm.

We found that the position of each transcribed gene was restricted to a particular plane along the apical–basal nuclear axis as predicted by the cytological position of the gene and the apical–basal, Rabl conformation of chromosomes. Genes near the centromeres were located in the apical hemisphere and those near the telomeres localized towards the basal pole of the nucleus. Genes in other parts of the chromosomes lay in intermediate positions in the apical–basal axis (Figure 1c and Supplementary material). All of the genes we studied occupied distinct locations, including *fushi tarazu* (*ftz*) and *hunchback* (*hb*), which are separated by only 10 centimorgans (one-tenth of a chromosome arm) and have overlapping but distinguishable nuclear distributions (Figure 1c). Our results also show conclusively that the intranuclear position of transcribed genes is not related to the cytoplasmic distribution of the mRNA. These results complement and extend previous findings [7].

Within their constrained apical–basal position, transcribed genes do not adopt a consistent radial position

We also investigated whether nascent transcript foci are consistently positioned with respect to nuclear axes perpendicular to the apical–basal axis. Nascent transcript foci from 11 endogenous genes were examined within a cross section through many nuclei (Figure 2b–m). For each of these genes, we imaged 23–103 nascent transcript foci distributed among two to three embryos of similar age and plotted their positions onto a profile of a typical nucleus. In all but one case, we detected no restriction of the foci to particular radial positions nor any consistent preference or exclusion from the nuclear envelope (Figure 2b–m). The genes studied included *ftz* (Figure 2f), of which the intranuclear position is in agreement with previous studies of a large genomic region containing *ftz* [6]. In contrast, *paired* (*prd*) nascent transcript foci were found to localize mostly nearer the periphery (Figure 2e), in agreement with studies of a genomic region containing the *prd* gene, which is located near nuclear envelope attachment sites [6]. In conclusion, the intranuclear distribution of genes, within sub-regions of the embryo where they are expressed, simply follows the expected average position of their chromosomal sites over the entire embryo.

The intranuclear location of transcribed genes is independent of their level of expression

The perinuclear localization of chromatin helps to establish transcriptional silencing of some genes [8], and entire chromosomes can vary in their proximity to the nuclear periphery [9]. We tested whether the level of transcriptional activity of individual genes, in *Drosophila*, is related in any way to their proximity to the nuclear periphery. We first determined whether the intensity of fluorescence of a nascent transcript focus is a measure of the transcriptional activity of the gene. We measured the intensity of *run* nascent transcript foci in different parts of an embryo known to express *run* at different levels [10] (Figure 3a–c). Our results show that the intensity of the fluorescent signal is related to the level of expression of a gene (see Supplementary material). We then plotted, for a single embryo, the intensity of individual *run* nascent transcript foci against their distance from the nuclear periphery. Such plots were generated for a number of different transcribed genes and were reproducible from one embryo to another. In all cases that we studied, including different domains of *run* expression, a visual inspection of the plots showed that the intensity of nascent transcript foci was independent of their distance from the nuclear periphery (Figure 3d and Supplementary material). In all cases, linear regression analysis showed that the slope of the best-fit line was not significantly different from 0 (data not shown), confirming that the amount of nascent mRNA and the distance from the nuclear periphery were independent.

The intranuclear position of genes is not affected by abnormally abundant nascent mRNA

To test whether very large genes with abundant nascent mRNA are localized differently from the 11 shorter genes described above, we investigated the intranuclear distribution of *Ubx*, a

77 kb gene requiring 55 minutes to be transcribed completely [11]. *Ubx* nascent transcript foci were not specifically localized with respect to the nuclear periphery (Figure 4b), in agreement with studies that used DNA probes to the region surrounding *Ubx* [12].

To further test whether a large increase in the amount of nascent mRNA could influence the intranuclear position of a gene, we used transgenic flies expressing constructs lacking a polyadenylation signal. In these constructs, transcription continues past the end of the gene and into flanking sequences, increasing the number of nascent transcripts decorating the DNA. We first examined the nascent transcripts of *Xho25*, a *lacZ* fusion inserted in the *en* locus and lacking a polyadenylation signal ([13]; see Supplementary material). We found that *lacZ* and *en* genes have a similar distribution with respect to the nuclear envelope (Figure 4c,d), showing that increasing the number of nascent transcripts decorating the DNA does not alter its intranuclear localization. To test other cytological locations, in addition to *en*, we constructed *X44*, a fusion construct that lacks a polyadenylation signal. Transcription of *X44* is driven by the *hb* promoter and it contains 44 copies of a non-coding neutral sequence without a polyadenylation signal (see Supplementary material), leading to unusually bright nascent transcript foci (Figure 4a). The intranuclear distribution of nascent transcripts from the five most highly expressed lines showed no specific localization in cross sections of nuclei (Figure 4a,e–i). As in the case of smaller genes, the intensity of *X44* foci was unrelated to their distance from the nuclear periphery (see Supplementary material).

In summary, our results show that transcribed genes are distributed within the nucleus according to the known Rabl configuration of chromosomes and proximity of certain chromosomal sites to the nuclear periphery. We found that different transcribed genes have distinct intranuclear distributions and their level of transcription is independent of their distance to the nuclear envelope. We interpret these results to mean that activation of transcription does not cause a major change in the intranuclear location of genes. If activated genes do move to new positions, then the distance moved must be small, and the sites to which they move must be numerous and evenly distributed. At least in *Drosophila* blastoderm nuclei, the distribution of actively transcribed genes is consistent with the possibility that transcriptional components are recruited to the site of the activated gene. These conclusions are likely to be general. Fundamental mechanisms such as recruitment of transcriptional components to genes and intranuclear transport of mRNA are unlikely to have evolved for a unique specialized role in the early *Drosophila* embryo. Indeed, there is evidence for a similar nuclear organization, and intranuclear transport of RNA in other systems [4].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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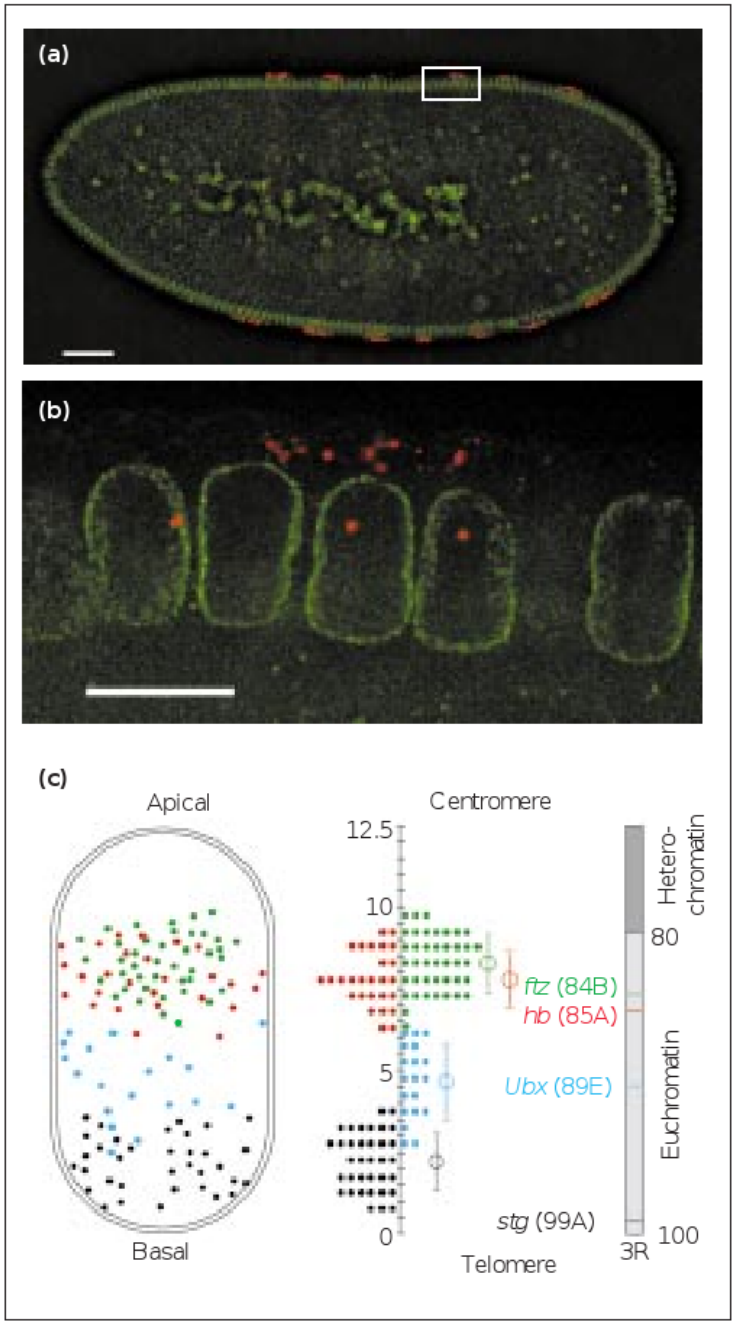

Figure 1.

The position of nascent transcript foci along the apical–basal axis of embryonic nuclei. **(a,b)** Optical sections through the middle of a mid-interphase 14, blastoderm embryo expressing *ftz* mRNA (red) in seven stripes. AlexaFluor488-conjugated wheat germ agglutinin (AlexaWGA) marks the nuclear envelope (green). (a) A low-magnification view of the embryo. (b) A high-magnification view of the boxed area in (a). Note that *ftz* mRNA is exclusively localised in the apical cytoplasm above the nuclei and as nascent transcript foci within expressing nuclei [14]. The scale bar represents 50 μm in (a) and 10 μm in (b). **(c)** A map of the intranuclear positions of four transcribed genes *fushi tarazu* (*ftz*, green), hunchback (*hb*, red), *Ultrabithorax* (*Ubx*, blue) and *string* (*stg*, black) from the right arm of chromosome 3

(3R). Left, the observed nuclear positions plotted onto an average nucleus, 12.5 μm in length. Right, the cytological positions of the genes on a metaphase chromosome arm drawn to scale (based on [15]). Middle, a histogram drawn from the distribution of the nascent transcript foci that lie within 0.5 μm intervals. The average position is indicated by an open circle and the error bar represents the standard deviation.

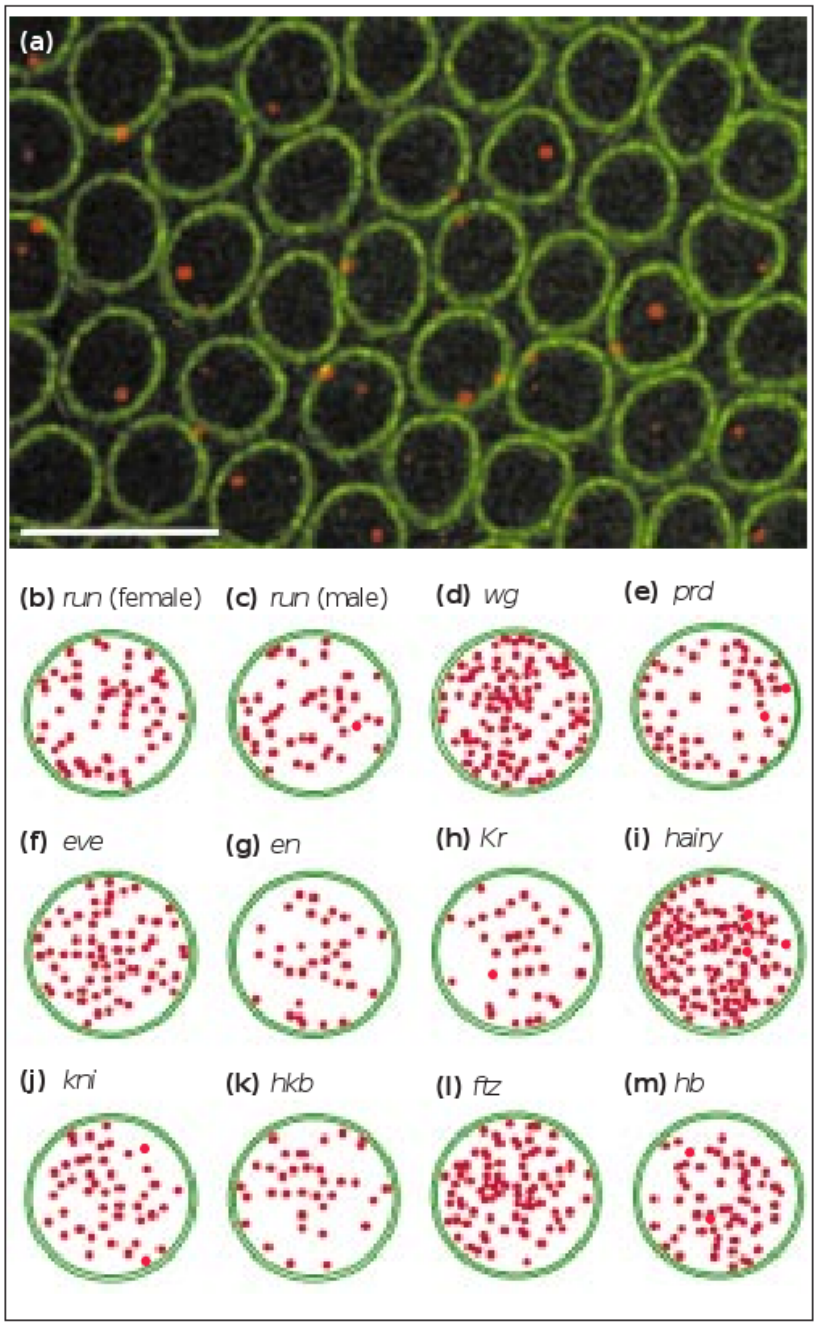

Figure 2.

The position of transcribed genes in the plane perpendicular to the apical–basal axis of the nucleus. **(a)** Optical section through the layer of nuclei at the surface of the embryo, showing *hairy* (*h*) nascent transcript foci (red). AlexaWGA marks the nuclear envelope in green. The scale bar represents 10 μm. **(b–m)** Distribution of the nascent transcript foci of 11 genes plotted within average nuclear outlines. The distance to the nuclear envelope on the diagram is the same as the actual measured distance. Of the 11 genes, 10 had nascent transcript foci that could localize to any part of the nucleoplasm; *prd* showed some preference for the nuclear envelope, in accordance with the proximity of the gene to nuclear envelope attachment sites. No difference was observed between (b) female and (c) male embryos expressing *runt* (*run*),

despite the different number of copies of the gene in the two sexes. Other gene abbreviations are: *en, engrailed; eve, even-skipped; hkb, huckebein; kni, knirps; Kr, Krüppel; wg, wingless*.

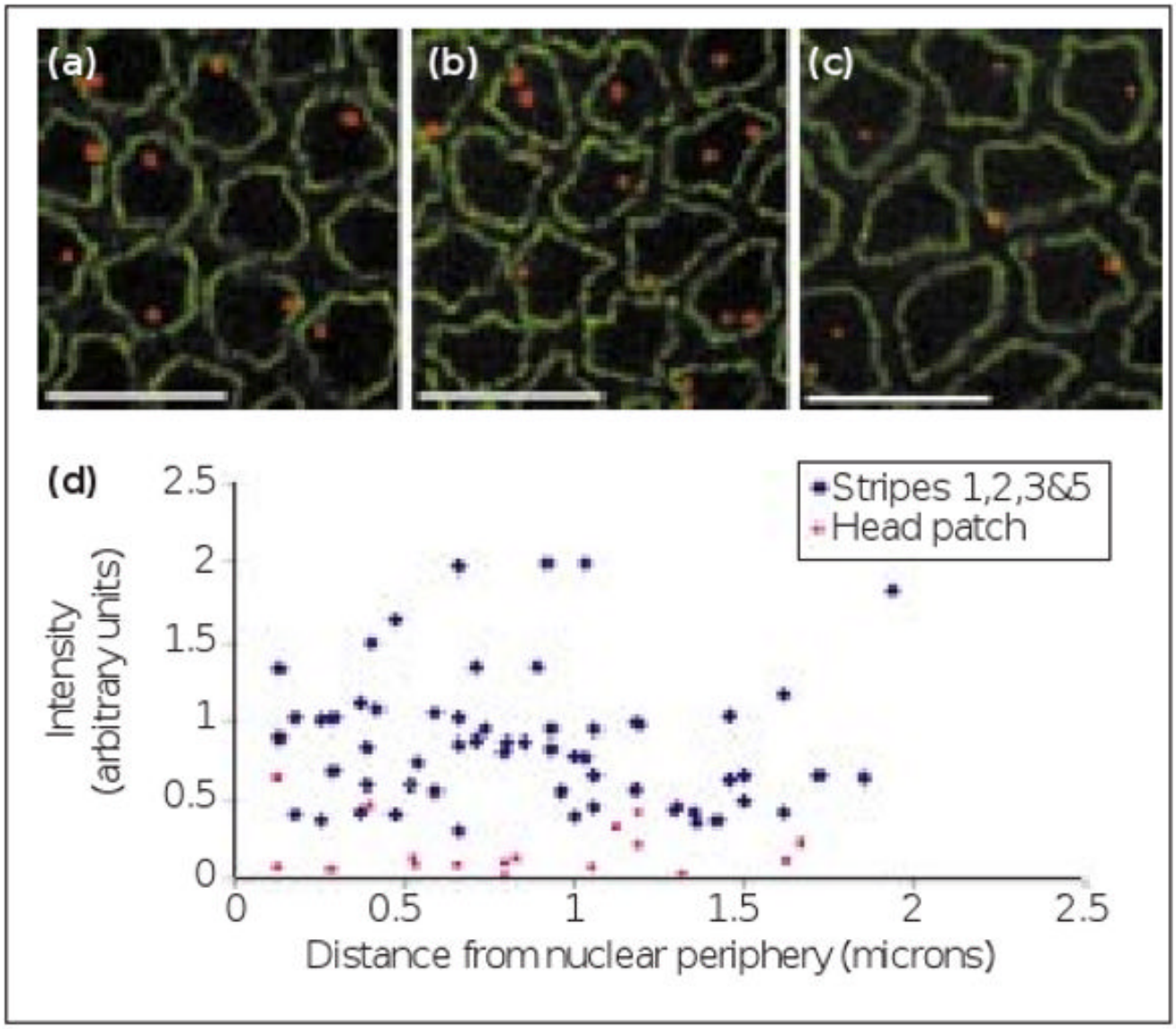

Figure 3.

The intranuclear position and intensity of nascent transcript foci in different *run* expression domains. Representative optical sections through blastoderm nuclei used to quantify the relative fluorescence of nascent transcript foci in different *run* expression domains (red). AlexaWGA marks the nuclear envelope (green). The scale bars represent 10 μm. **(a–c)** The nascent transcript foci from stripe 1 of *run*, an X-linked gene, in (a) a male embryo, (b) a female embryo and (c) in the weakly expressed head patch of the embryo shown in (b). Each nucleus in (a) has only a single nascent transcript focus, but in (b) many nuclei contain a pair of nascent transcript foci, although some nuclei have a single nascent transcript focus due to pairing of homologous chromosomes. **(d)** A plot of the relative fluorescence intensity and distance from the nuclear periphery of individual *run* nascent transcript foci from different expression domains in a single embryo. The intensity and distance are unrelated whether in strongly expressing stripes (blue diamonds) or in the weaker head patch (pink squares).

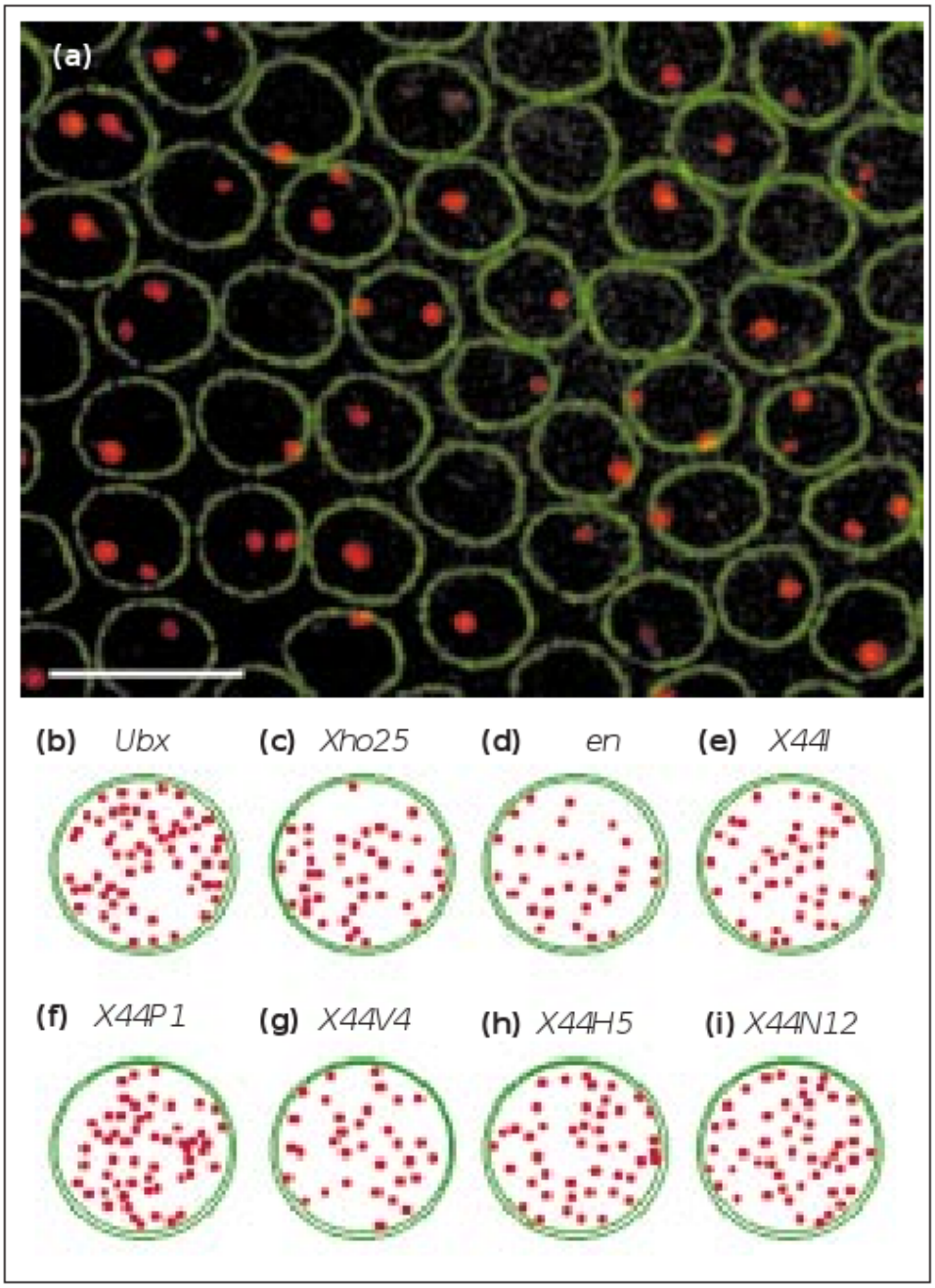

Figure 4.

The intranuclear distribution of unusually abundant nascent transcript foci. **(a)** Nascent transcript foci (red) of the *X44* transgene, which lacks a polyadenylation signal (see Supplementary material). AlexaWGA marks the nuclear envelope (green). The scale bar represents 10 μm. **(b–i)** The intranuclear distribution of genes containing large nascent transcript foci viewed within a plane perpendicular to the apical–basal axis of the nucleus, showing no restrictions on their intranuclear position. (b) *Ubx*. (c,d) *Xho25* embryos stained with (c) a *lacZ* probe and (d) an *en* probe. (e–i) *X44* embryos from five of the strongest expressing transgenic lines stained with probes against the untranslated sequences in the construct.