Supplementary Discussion:

Relationship between intron spot measurements and transcriptional activity

In our measurements, we obtain both the probability of finding an intron spot as well as the intensity of that spot. Here, we present a simple model of intron dynamics that relates transcriptional dynamics to these two measurements.

We assume that transcription occurs in bursts, which is supported by several studies in higher eukaryotes as noted in the main text. We assume that the transcription of a gene as a function of time is given by the function $\mu(t) = \mu_0 f(t)$, where μ_0 is a constant and $f(t)$ is a stochastic process that randomly fluctuates between having value 0 and value 1 (corresponding to the gene being active or inactive, respectively). We do not assume any form for $f(t)$ other than that the time in the active state or the inactive state is on average considerably longer than the time to degrade introns, although several groups model the dwell times in the active or inactive state as being exponentially distributed, and there is experimental support using time-lapse imaging for this view (Golding et al. Cell 2005; Chubb et al. Curr. Biol. 2006; Suter et al. Science 2011). We assume that the fraction of time the gene is in the active state is given by a. The (continuous) equation governing the intron dynamics is:

$$dI/dt = \mu(t) - \delta I$$

where I is the number of intron molecules and δ is the rate of intron degradation. The steady state of this equation when the $\mu = 0$ or $\mu = 1$ is 0 or μ_0/δ, respectively. The degradation rate, δ, is what determines how rapidly I heads to steady state. Based on our Actinomycin D experiments (Supplementary fig. 3), we believe the intron half-lives of the genes we examined to be less than 5 minutes. In this case, where δ is considerably larger than the rates of the gene switching on or off, then

$$I(t) \sim= \mu(t)/\delta$$

i.e., I(t) is non-zero only when the gene is actively transcribing, and zero when the gene is inactive. The time average of I(t) is then

$$\langle I(t) \rangle = a\mu_0/\delta$$

while the time averaged rate of transcription is given by

$$\langle txn \rangle = a\mu_0$$

By measuring the percentage of the time we observe the gene actively transcribing, we can estimate a, the probability of the gene being active, in absolute terms. When the gene is active, the rate of transcription is μ_0, but we can only measure the intron spot intensity, which is proportional to the rate of transcription. Thus, we cannot measure the rate of transcription when

the gene is active up to a constant of proportionality that is 1/δ, which in principle may vary from one gene to another. Nevertheless, we can compare the relative changes in the rate of transcription of the same gene from one chromosome to another by comparing our measurements of both a and μ_0/δ. In our experiments, we found that in virtually all situations, the spot intensity (μ_0/δ) did not change (Supplementary fig. 14), but we did observe changes in the probability of finding an intron spot (a), which implies a proportional change in the overall time-averaged rate of transcription. We interpret this to mean that whatever causes the changes in transcription on the hyperactivated t(13;19) chromosome in HeLa cells (as compared with the intact chromosome 19s in HeLa cells), it is most likely not something that is changing the rate of transcription when the gene is active, but rather is changing the probability that the gene is active itself. We note that this is not necessarily the same as saying that the transcriptional burst frequency has changed while the transcriptional burst size remains the same: if transcriptional bursts lasted for longer, then both the burst size and the probability of finding a spot would increase, even if the burst frequency remained constant.

Comparison of the distance between active DNA loci to previous experiments

We were somewhat surprised to find that the distance we observed between transcriptionally active loci was quite large even for relatively short genomic separations; for instance, we observed a mean physical displacement of 1.7μm for genes separated by only 0.36 kilobases. We suspected that these large distances were due to the relatively decondensed chromatin thought to accompany actively transcribed genes. To check whether such a hypothesis was consistent with the published literature, we examined the data from the excellent study by Mateos-Langerak et al. PNAS 2009, in which the authors measured the relationship between the physical and genetic separation of DNA loci. In particular, they examined the distances genes in transcriptionally active regions of DNA (ridges) and transcriptionally inert regions of DNA (anti-ridges), finding that the transcriptionally active regions were considerably more physically spread out than the transcriptionally inert regions.

We posit, given that transcription is fundamentally pulsatile, that the mean physical separation between two loci in a transcriptionally active region fluctuates between a short distance when the genes are inactive and a long distance distance when the genes are active (consistent with the findings of Tumbar et al. JCB 1999). From this perspective, the observations by Mateos-Langerak et al. correspond to measuring the mean inactive gene separation (DNA FISH between transcriptionally inert regions) and the weighted average of the mean inactive and active separation (DNA FISH between transcriptionally active regions), weighted by (1-a) and a, respectively, where a is the probability of the gene being active. Our measurements of interpair separations correspond to the mean active gene separation.

We checked for consistency between these different sorts of measurements when comparing our data to that of Fig. 2B, left panel from Mateos-Langerak et al. At a genetic distance scale of roughly 490 kilobases, Mateos-Langerak report an mean square distance of around $0.23\mu m^2$ for inactive loci and $0.84\mu m^2$ for “ridges”, the latter of which we believe corresponds to the weighted average of active and inactive loci as described above. Our measurements of a mean square distance of $3.57\mu m^2$ between active loci at this genetic distance scale would imply a weighting

factor of 0.18, which falls squarely within our observed variation in probabilities of genes transcribing. Thus, we conclude that our data are at least consistent with the previous DNA FISH observations of Mateos-Langerak et al. with this simple model for the distance between active and inactive loci. Further studies may elucidate whether such a model is indeed an accurate description of conformational dynamics.