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Estrogen fueled, nuclear kiss

Did it move for you?

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and
Kocanova S, Kerr EA, Rafique S, Boyle S, Katz E, Caze-Subra S, et al. Activation of estrogen-responsive genes does not require their nuclear co-localization. PLoS Genet 2010; 6:1000922. PMID: 20421946.

A paper appearing in late 2008,[1] attracted considerable attention with its description of a dramatic juxtaposition of two estrogen responsive genes on different chromosomes within 15–60 minutes of adding estradiol. These results challenged a growing consensus of limited chromosome mobility within interphase nuclei, while raising questions of whether a hitherto unknown molecular mechanism might exist to move chromosomes long distances within the nucleus. These results also raised the fascinating question of how two genes on widely separated chromosomes might find each other over such a short time span. Now, a more recent paper[2] reports no such long-range interaction or chromosome movements in the same cell types under what appear to be well replicated conditions, forcing a reexamination of the prior results.

A steadily increasing number of experimental spin-offs from the original chromosome conformation capture (3C) method introduced by Job Dekker have revealed a dazzling plethora of long-range, intrachromosomal and interchromosomal interactions, as reviewed recently.[3,4] Besides pointing to a new "chromatin-hub" model of gene regulation in which promoters, enhancers, LCRs and boundary elements all interact through DNA looping, these new methods are forcing us to consider new possibilities of genome "interactomes", whereby co-regulation of both small and large gene sets occurs through long-range interactions mediated by common sets of transcription factors and cofactors.

All of these methods start with formaldehyde cross-linking, followed typically by sonication to break apart nuclei and release what are assumed to be local DNA-protein complexes, followed by restriction enzyme digest, dilution and DNA ligation of DNA fragments contained within the same cross-linked complex. Because many of these methods use sensitive, PCR based detection schemes, obvious questions of what percentage of the cells in a population will have a specific long-range interaction and for how long in time will this interaction exist are typically addressed by combinations of 3C methods with microscopy and even live cell imaging.[5] There is also the less obvious question of the validity of the underlying assumption of these methodologies-namely that the released complexes actually correspond to specific DNA-DNA looping interactions mediated by particular proteins mediating this DNA looping as opposed to larger protein-DNA networks, for instance produced by cross-linking of pieces of nuclear bodies (Uli Laemmli, personal communication). Indeed a number of these long-range interactions, when validated by FISH, in fact appear to correspond to adjacent, non-overlapping cytological interactions, for instance through interactions with nuclear speckles or "transcription factories", as opposed to exact co-localization mediated by molecular scale interactions.

Regardless of the actual molecular explanation for these interactions, there are now a number of well-documented examples of long-range, "kissing" interactions in which distant DNA sequences come together at cytologically close distances of less than 1 micron, as verified

by DNA FISH. These observations therefore raise fundamental questions related to how such interactions are first established. It is now well documented that in most species and cell types examined, interphase chromosomes fold locally into spatially distinct chromosome territories.[6] Moreover, live cell imaging has led to a growing consensus, at least for somatic mammalian cells, of fast, "constrained diffusion" (i.e., trajectories consistent with classical Brownian motion but within a confinement volume) of chromosome loci on the order of an ~0.5 μm radius, but not longer range movements exceeding ~1 μm.[7,8] These experiments examined interphase chromosome diffusion in growing cells in a physiological steady-state and did not rule out the existence of long-range movements. But these studies did establish that if long-range movements of gene loci exist, they are the exception rather than the rule, at least in the mammalian somatic cell types examined so far by live cell imaging.[7,8]

In fact, prior to the papers being discussed here,[1,2] some examples of longer range chromosomal movements in somatic mammalian cells had been described. One was an apparently directed movement of a multi-copy plasmid transgene array from the nuclear periphery towards the nuclear interior after tethering of a transcription factor activation domain; this movement was shown to be directly or indirectly dependent on actin and nuclear myosin I.[9] Movements of transgenes towards nuclear bodies, for instance nuclear speckles[10] and coiled bodies,[11] have also been observed although the details of these movements have been less well characterized. However, even in these few examples of "long-range" movements, the actual trajectory length of the movements was typically limited to ~1–2 μm.

Given the high compaction of chromatin in typical mammalian nuclei, on the order of one to several Mbp per μm length of interphase chromatids,[10,12] it is easy to imagine how quickly "constrained diffusion" type movements might facilitate pairing interactions over Mbps of DNA. Together with the compact folding of interphase chromatids into chromosome territories, it is also easy to imagine how such fast, constrained movements might facilitate pairing interactions between widely separated sites on the same chromosome, at least in a fraction of the cell population. These rapid but limited movements, together with more infrequent, long-range movements of 1–2 μm, could also easily explain how specific chromosome sites might rapidly associate or dissociate from nuclear bodies, such as nuclear speckles (interchromatin granule clusters, a.k.a. SC35 domains) or transcription factories, found at high numbers within interphase nuclei. It is also easy to imagine how two chromosome loci on different chromosomes, each independently associating with the same type of nuclear body, might be found together at low frequency based on their association with the same nuclear body in a small fraction of cells. However, it becomes much harder to explain how two loci on different chromosomes would become co-localized at high frequency within a cell population, unless these chromosomes themselves are nonrandomly distributed within the nucleus.[7,13]

It is with this foregoing context that the Hu et al. paper[1] attracted so much attention and interest. The authors first used a 3C-like molecular assay to demonstrate interactions between the enhancer of the TFF1 gene on chromosome 21 with the enhancer and promoter of the GREB1 on chromosome 2. Both genes are responsive to estrogen receptor alpha (ERα), and these interactions were assayed in the breast cancer cell line MCF7 60 minutes after 17β-estradiol (E2) treatment. Follow-up experiments validated these interactions using DNA FISH in the original MCF7 cells as well as primary cultures of human mammary epithelial cells (HMECs). In both cell types this interaction was estradiol dependent.

What was most dramatic and surprising, though, was the extent of this interaction, with the percentage of colocalizing TFF1 and GREB1 genes increasing from a few percent to more than 60% within 60 minutes of E2 addition. With median gene separations of ~8 μm prior to hormone addition, this implied gene movements spanning a large fraction of the total nuclear diameter in the majority of cells. Moreover, the percentage of nuclei with co-localization of TFF1 and GREB1 increased from the baseline ~5% to ~20% in just 15 minutes, implying not just long-range chromosome movements but fast long-range movements, approaching,in a significant number of nuclei, the ~1 μm/min speeds typical of the machinery that propels chromosome separation during mitosis. Finally, whole chromosome FISH "paints" showed that these co-localization events occurred through movements of the two entire chromosomes, with the juxtapositioning of the TFF1 and GREB1 genes accompanied by "kissing" of their respective chromosomes. The >5-fold increase in the reported frequency of these chromosome kissing events after estradiol treatment was less than half the fold increase in TFF1-GREB colocalization. Therefore the authors reasoned that colocalization of genes in trans occurred through both long-range gene looping interactions plus long-range whole chromosome movements. No quantitative measurements of distances between chromosomes 2 and 21 were shown before or after estradiol treatment; therefore the degree to which these chromosomes might have been in relatively close proximity prior to estradiol treatment was not described.

The authors also showed that estradiol led to association of the TFF1 and GREB genes with nuclear speckles. As described previously, independent colocalization to a common nuclear body would be one mechanism to explain an increased association of the TFF1 and GREB genes. However, because of the large number of nuclear speckles per nucleus, this does not explain the very high fraction of nuclei with colocalizing TFF1 and GREB alleles at the same speckle. Moreover, speckle association was only observed for TFF1-GREB1 interacting pairs; noninteracting TFF1 and GREB1 alleles were not found associated with speckles. Therefore this still leaves open the question of how the TFF1 and GREB1 genes find each other so rapidly.

Further experiments using siRNA appoaches revealed a dependence of this E2 induced, TFF1-GREB1 co-localization on ERα and its coactivators, CBP/p300 and SRC1, but not the histone demethylase, LSD1, essential for E2 dependent gene activation. A role for nuclear actin

and myosin in this TFF1-GREB1 interaction was suggested through a combination of drug inhibitor, siRNA, antibody microinjection and siRNA plasmid rescue experiments. Additional factors, including the BAF53 chromatin remodeling subunit and dynein light chain-1, were implicated through similar experiments. A functional role for this TFF1-GREB1 interaction was suggested by RNA FISH experiments revealing larger volume signals for gene copies associating in a monoallelic interaction. However, this larger volume signal might also be related to the entry of nascent RNA from these genes into the nuclear speckle[14] and then accumulation and distribution of the RNA throughout the speckle volume since this speckle interaction was specific to colocalized TFF1 and GREB1 alleles.

Thus, this paper[1] impressively revealed not only a rapidly induced, long-range chromosome pairing interaction, but presented a degree of molecular dissection, linking the induced TFF1-GREB1 pairing to nuclear actin and myosin I, association with nuclear speckles, and possibly enhanced transcription. Moreover, these results raised the fascinating question of how such long-range movements on different chromosomes might result in specific pairing events.

It was therefore unsurprising that other investigators would seek to confirm these surprising new findings. A recent publication in PLoS Genetics[2] in fact describes exactly such an attempt. However, this new publication reports dramatically different findings. Despite making a serious attempt to use similar protocols in the same cell types, the authors did not detect any statistically significant colocalization of the TFF1-GREB1 genes before or after estradiol addition in HMECs or MCF7 cells, as well as in additional cell lines. The nuclear separation between these two genes, and their chromosomes, was unchanged by hormone addition despite observed hormone dependent gene activation in MCF7 cells. Moreover, no detectable ERα was observed either by immunostaining or western blots in HMECs. A final discrepancy is that in the more recent paper[2] MCF7 cells were found to be highly aneuploid, with both TFF1 and GREB1 genes each showing 4–6 signals per nucleus and karyotyping revealing more than two copies of chromosome 2. In contrast, the first paper[1] discussed the results and employed a data analysis procedure assuming a diploid karyotype for MCF7 cells (see for example, Fig. 2B,[1] which showed 4 chromosome territories but, inexplicably, both one TIFF1 and one GREB1 signal, or a merged TIFF1-GREB1 signal, in each of these territories).

In light of the profound implications of the Hu et al. paper[1] with regard to basic questions about chromosome movements and long-range chromosomal interactions in mammalian nuclei, the discordance between these two studies is disappointing. It is clearly important to the field of nuclear structure and dynamics that this disagreement is eventually resolved, yet the path forward is not obvious.

Both research groups used what were nominally the same cell types with the same cell culture protocols. In a previous example of long-range chromosome movement, the observed long-range mobility was completely inhibited at light exposure levels that did not have any effect on the rapid, locally constrained chromosome mobility and which allowed cell cycle progression. It is therefore at least conceivable that subtle differences in growth conditions, for example serum lots, might have a disproportionate effect on the described estradiol induced chromosome pairing. Moreover, the two groups used different sources for their HMECs. The second group observed no detectable ERα expression in these cells. For cell lines that are not dependent on ERα for growth, loss of ERα is common and rapid,[15] and this rapid loss has been reported for HMECs[16] specifically. Although this seems to be a common problem for the field, it is conceivable that the isolation and culture conditions followed by the first group prevents such loss of ERα. Finally, while both groups used MCF7 cells, the source and culture pedigree of these cells might have differed as well. Various published studies and the American Type Culture Collection report an aneuploid state for MCF7 cells, which can vary among sources.[2] It is possible that early freezes of this cell line might have retained a more diploid state and a different physiological response to E2. In this case the first group could do a great service to the research community by making their MCF7 cells available to the research community, which is the policy on sharing of materials of the journal in which that study was published.

The question of long-range chromosomal interactions and movements has garnered increasing attention and this estradiol induced motion of the TIFF1 and GREB1 gene loci appears to be potentially the best example to date for investigating this question. Therefore, on behalf of the entire community of scientists working on nuclear structure and dynamics I urge both research groups to cooperate to resolve their conflicting results. A simple path forward would begin by an exchange of cell stocks, sera, media and detailed cell passage conditions.

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