The non-random repositioning of whole chromosomes and individual gene loci in interphase nuclei and its relevance in disease, infection, aging and cancer

Joanna M. Bridger; Halime D. Arican-Goktas; Helen A. Foster; Lauren S. Godwin; Amanda Harvey; Ian R. Kill; Matty Knight; Ishita S. Mehta; Mai Hassan Ahmed

Correspondence to:

Joanna M. Bridger

Centre for Cell and Chromosome Biology, Biosciences

Brunel University

Kingston Lane

Uxbridge, UB8 3PH. UK

joanna.bridger@brunel.ac.uk

Miss Halime D. Arican-Gotkas

Laboratory of Nuclear and Genomic Health

Centre for Cell and Chromosome Biology, Biosciences

Brunel University

Kingston Lane

Uxbridge, UB8 3PH. UK

Halime.Arican@brunel.ac.uk

Dr Helen A. Foster

Biosciences

Brunel University

Kingston Lane

Uxbridge, UB8 3PH. UK

Helen.foster@brunel.ac.uk

Dr Lauren S. Godwin

Molecular Cell Biology Group

Biomedical Sciences Research Centre

Jenner Wing (box J2A)

St. George's, University of London

Cranmer Terrace

London SW17 0RE, UK

laurengodwin08@googlemail.com

Dr Ian Kill

Biosciences

Brunel University

Kingston Lane

Uxbridge, UB8 3PH. UK

Ian.kill@brunel.ac.uk

Dr Matty Knight

Dr Amanda Harvey

Brunel Institute for Cancer Genetics and Pharmacogenomics

Biosciences

Brunel University

Kingston Lane

Uxbridge, UB8 3PH. UK

Amanda.harvey@brunel.ac.uk

Dr Mai Hassan Ahmed

Laboratory of Nuclear and Genomic Health

Centre for Cell and Chromosome Biology,

Biosciences

Brunel University

Kingston Lane

Uxbridge, UB8 3PH. UK

maieljeboury@hotmail.com

Dr Ishita S. Mehta

Tata Institute of Fundamental Research,

Homi Bhabha Road,

Mumbai - 400005, India

mehtaishi@gmail.com

Abstract

The genomes of a wide range of different organisms are non-randomly organized within interphase nuclei. Chromosomes and genes can be moved rapidly, with direction, to new non-random locations within nuclei upon a stimulus such as a signal to initiate differentiation, quiescence or senescence, or also the application of heat or an infection with a pathogen. It is now becoming increasingly obvious that chromosome and gene position can be altered in diseases such as cancer and other syndromes that are affected by changes to nuclear architecture such as the laminopathies. This repositioning seems to affect gene expression in these cells and may play a role in progression of the disease. We have some evidence in breast cancer cells and in the premature ageing disease Hutchinson-Gilford Progeria that an aberrant nuclear envelope may lead to genome repositioning and correction of these nuclear envelope defects can restore proper gene positioning and expression in both disease situations.

Although spatial positioning of the genome probably does not entirely control expression of genes, it appears that spatio-epigenetics may enhance the control over gene expression globally and/or is deeply involved in regulating specific sets of genes. A deviation from normal spatial positioning of the genome for a particular cell type could lead to changes that affect the future health of the cell or even an individual.

Abbreviations: 2-dimensional, 2D; 3-dimensional, 3D; chronic myeloid leukaemia, CML; fluorescence in situ hybridization, FISH; green fluorescent protein, GFP; Hutchinson-Gilford progeria syndrome, HGPS.

Introduction

The development of the technique of fluorescence in situ hybridization (FISH) and suitable probes to reveal whole chromosomes and individual genes for diagnostic purposes on mitotic chromosomes concomitantly allowed interphase nuclei to be analyzed by scientists interested in how the nucleus behaved functionally. The painting of whole chromosomes and individual gene loci led to the recognition that chromosomes and genes sit in individual locations within interphase nuclei. Indeed, chromosomes are found within their own nuclear territories and gene loci housed upon those chromosomes often sit at the edges of those chromosome territories [1], but can also be nearer the interior of the territories or at a distance away from the core individual chromosome territories, distended on chromatin loops (see Figure 1) [2].

The ability of FISH to reveal whole chromosomes and genes soon led to mapping endeavours whereby it was discovered that chromosomes reside in non-random radial locations within interphase nuclei with more gene-poor chromosomes such as 4, 13, 18, and X found at the nuclear periphery whereas gene-rich chromosomes such as 17 and 19 were found towards the nuclear interior [3, 4]. It should be noted that this correlation with gene density was found in proliferating lymphoblasts and young proliferating primary fibroblasts [5, 6].

A non-random distribution of the genome that is maintained throughout interphase with all the dynamic processes that occur during this time *e.g.* replication and transcription, must require energy and significant anchorage points that are dynamic in response to external stimuli. Indeed, when one looks further into cells that are no longer young and proliferating, diseased or subjected to an external stimulus, specific chromosomes and genes change nuclear location. The Bridger laboratory has put tremendous effort into finding situations where specific chromosomes and gene loci change location. This is so that we can ask

questions about how and why the genome is spatially organized and then how and why individual genes and chromosomes become reorganized within the nuclear space. We have found that specific chromosomes and genes change nuclear position in aged senescent cells [7] in laminopathy patient cells [8, 9], cancer cells [10; Hassan Ahmed, Harvey, Karteris and Bridger unpublished data], cells exposed to parasites [11; Arican, Bridger, Knight unpublished data], cells subjected to nutritional alterations [6; 12] and temperature change [Arican, Knight and Bridger unpublished data]. All the changes in position that we have revealed have been shown to be non-random and even in some experiments reversible when the situation/treatment is removed/reversed.

The reason why the cell invests energy in the relocation of chromosomes and genes to new positions in the nucleus is being answered by determining what happens to them at their new location with respect to gene up- or down-regulation. This is either done by techniques such as reverse transcriptase-PCR, quantitative-Real Time-PCR, micro array analysis, RNA FISH or by ChIP-seq and in many cases the repositioning correlates with changes in expression. However, how the chromosomes and genes move and why they are targeted/directed to areas of the nucleus at a distance from their initial environment is not yet clear and requires much more investigation. These questions are what stimulates our laboratory and we use a number of different situations, external stimuli and organisms to ask the questions where, how and why are chromosomes and genes relocated. Here we will describe several different experimental systems where such changes in spatial genome organization have been observed, ending with similar types of changes that we and others have observed in cancer cells that may be able to be taken advantage of for new therapies.

How we map genes and chromosomes in interphase nuclei

Our laboratory has mapped many chromosomes and genes in many different cell types and organisms but we always use the same two ways of mapping for all situations for consistency and reproducibility.

We always employ both 2-dimensional (2D) mapping that allows us to do lots of mapping relatively fast and 3-dimensional mapping that takes longer but is important to confirm the 2D data. For the mapping to work it is critical that the 2D sample is properly flattened, since we normalize and extrapolate out to 3-dimensional (3D) with our findings and it is critical that the 3D sample has not undergone any structural changes since we take precise size and distance measurements from these samples.

For 2D samples imaging is performed, capturing fifty images of each chromosome/gene in each cell type. These images are run through a bespoke script that was devised by Dr Paul Perry in Prof Wendy Bickmore's group in the MRC Human Genetics Unit in Edinburgh [5]. This script outlines the entire nucleus based on a DNA dye (such as DAPI) and erodes this mask, creating five shells of equal area. Within these five shells the intensity of the fluorescent signal from the DNA dye and the FISH probe is measured and recorded for each nucleus. In order to normalize for more DNA being in the interior shells when a spherical object is flattened, the probe signal is divided by the DNA signal. The data are plotted as a bar chart. This method does under record the signals that are at the periphery since they may appear interior if on the top or bottom of the nuclei but interiorly located signals always appear interior and since it is always used in a comparative way with other chromosomes, other cell types etc., it works exceptionally well as a method for mapping chromosomes and genes.

The 3D FISH method is based on one developed by Profs. Lichter and Cremer in Heidelberg to preserve the 3-dimensionality of a nucleus while still allowing good

penetration of the FISH probe [13]. We then use a confocal laser scanning microscope to collect optical sections and then the position of a chromosome or gene is measured in these images from the geometric center of its signal to the nearest nuclear edge, whether that be in the x,y or z axis. The results can be normalized to a measurement for the size of the nucleus but this does not often change the final outcome. The data are plotted as a frequency distribution. This method gives accurate measurements and we find that there are virtually no differences in general position when compared to the 2D method.

Live cell imaging for chromosome and gene movement is something we are presently working on. It is made complicated because we are often working in primary cells where transfection and selection of clones would make it impossible to collect proliferating cells at the end of the selection *i.e.* they would have become senescent through the number of passages it would need to collect a colony of cells from a single cell. We also need to know what genes and chromosomes we are assessing – this is imperative since some chromosomes do not move at all and some move considerably. This can however be done using the GFP-lac repressor system [14' 15] stably transfected, but it is important when such sequences are added into a chromosome that they do not change its behavior, which could happen if the large number of repeats created a region of heterochromatin within a chromosome.

Alterations to gene and chromosome position using growth factor addition and removal

Addition of specific growth factors to cells in culture can induce cellular differentiation and removal of growth factors induces quiescence, a period of reversible growth arrest in cells. Both of these situations are controllable windows in which the cells have dramatic changes in

their gene expression profiles. Thus, we have developed systems that can be controlled easily by the addition or removal of growth factors that have allowed us to analyze changes to genome organization in nuclei.

Porcine mesenchymal stem cells were isolated from fresh pig bone marrow and grown until there were copious numbers of cells and the culture was purely mesenchymal stem cells [16]. By adding human adipogenic growth factors to the medium the pig stem cells differentiated into adipocytes over a two-week period, giving committed pre-adipocytes at 7 days. We were interested in what would happen from a spatial organizational perspective to genes involved in the adipogenic process during this *in vitro* differentiation. We studied seven genes involved in the adipogenesis pathway and six of them had moved to a more interior location after 14 days of treatment with growth factors, which was correlated with an up-regulation in gene expression in all these genes. The seventh gene was *GATA2*, a gene involved in pre-adipogenesis and this gene like the others was more peripheral at day 0 and then was found to be more interiorly located on day 7, but it had moved back to its original location towards the nuclear periphery by day 14. The movement of this gene to the interior also correlated with its up-regulation in expression at day 7 and its down-regulation by day 14 [17]. These were quite broad time points and we cannot determine from these experiments how fast genes respond after a stimulus. Other experiments in other experimental systems (see below) address this better.

During the induced adipogenesis of the mesenchymal stem cells the nuclear lamina was altered and the longer in adipogenesis induction medium the more cells became negative for A-type lamins, with the majority of cells being negative by day 11 (Foster and Bridger unpublished data). This is a major change in nuclear architecture and may be involved in allowing various regions of the genome to be freer to move into the interior of the nuclei, but as yet there is no direct evidence.

11

A follow-on study allowed us to ask the question – "where are these genes going?". In agreement with some other studies [18] we found that the gene loci were co-localized in significantly high numbers with SC35 splicing speckles [19]. Others have also found genes moving to transcription factories [20]; however, it is possible that these transcription factories were very close to splicing speckles and this is why both structures have been reported as a gene's destination. By analyzing three genes concomitantly that were each from different areas of the genome, it became clear that all six loci (in diploid cells) were found in the same splicing speckle in an individual nucleus much more often than could be considered a random occurrence. These data add to building body of evidence that genes from the same pathway may be transcribed together at common transcription factories or other nuclear structures [21]. This implies that some genes may have to travel large distances across the nucleus, avoiding the transcriptional structures in their locale and be directed to a specified nuclear location. We know from our studies inducing adipogenesis in porcine mesenchymal stem cells that whole chromosomes do not tend to move but we have seen genes loop out away from the core chromosomal territories on peninsulas that reach into the nucleoplasm.

In another series of experiments we reduced growth factors by placing cells into low serum. This makes proliferating primary cells, and some immortalized cells, enter a state called quiescence, a reversible growth arrest. As with differentiation this comes with a lot of gene expression changes and so makes an interesting inducible biological system in which to study changes to genome organization through the spatial positioning of chromosomes and genes. We have also been able to use this system to measure very precisely when the genome first responds to an external stimulus. We knew from proliferation marker staining that cells are not thought to be quiescent for at least 3 days after the removal of serum. We also knew that at 7 days after serum removal some whole chromosomes have a different nuclear location, such as chromosomes 13, 18 and 10 [6, 12]. A number of other chromosomes do not

change their nuclear location, although some individual genes could, as in the adipogenesis system, still change their location through looping out. Most interestingly, when we investigated the specific timing of the chromosome shifts we found the response to the removal of serum to be much more rapid than 7 days or even 3 days. Indeed, after starting our time course at 7 days post-serum removal and working backwards, we found that whole chromosomes had become relocated within just 15 minutes after serum removal. This implies a directed repositioning requiring energy. In subsequent experiments where ATP and GTP were inhibited, the chromosomes would no longer relocate after serum removal. These data then begged the question what structures/entities that require energy could move chromosomes so rapidly? We followed a controversial line of thought that was based upon a small amount of evidence that in the nucleus there existed both actin and myosin isoforms that could work in concert to create a nuclear motor capable of moving chromatin around the nucleus [22-24]. Using immunofluorescence, some nuclear myosin 1β was observed in nuclei throughout the nucleoplasm, at the nuclear periphery and at nucleoli [12]. This distribution changed dramatically when serum was removed from the cells. The myosin 1β became located only in aggregates within the nucleoplasm. Using chemical inhibitors of both nuclear actin and myosin we also blocked the movement of the chromosomes upon serum withdrawal. Nuclear myosin 1β was also found to be a major player in chromosome relocation when we used short interference RNA protocols to remove it in >95% of the cells. This study provides strong evidence to support that certain specific chromosomes are moved within the nucleus to new non-random locations by a nuclear motor (see Figure 1) [see 23, 24].

When quiescence is induced in young proliferating primary fibroblasts chromosome 10 moves from an intermediate location to a peripheral location. If the hypothesis is absolute that the nuclear interior is for gene expression and the nuclear periphery is a region for gene

silencing and down-regulation, then the movement of a whole chromosome should simultaneously alter the expression of many genes. We found that out of 10 genes on chromosome 10 only two were significantly down-regulated whereas five where up-regulated when the chromosome moved to the periphery. Although this type of question requires global analysis, this small gene set already indicates that the nuclear periphery is not purely about gene silencing and down-regulation and the effects of repositioning depend on either individual characteristics or the local environment of specific genes.

Although many of the genes found associated with the nuclear lamina at the nuclear periphery are silenced or down-regulated, active genes can be moved towards the nuclear periphery on a chromosome and remain up-regulated [25]. A lot of interest is being focused on genes that tether to nuclear pore complex proteins as an area of the envelope that is associated with active genes [26]. This further shows that spatial positioning within nuclei is involved at some level with the regulation of gene expression, but it is much more complicated than a gene just being at the edge of the nucleus or the interior.

Alterations to gene and chromosome position in aging and premature aging

As chromosome and gene position was found to change specifically and reproducibly in primary human dermal fibroblasts as they become reversibly arrested, it was pertinent to also assess what happens to genome organization in human dermal fibroblasts that become silenced at the end of their replicative lives and become senescent. Cellular senescence does not mean cells die through apoptosis or necrosis. They sit within our tissues and probably send out signals that affect others cells around them in a negative capacity. We have mapped

all chromosomes in senescent cells and have found for the most part their nuclear locations resemble those in quiescent human dermal fibroblasts [7]. For example, chromosome X remains at the nuclear periphery and chromosomes 13 and 18 move to the nuclear interior, becoming associated (or more intensely associated) with the nucleolus. We also have some data that shows that chromosome 18 becomes embedded within and tightly attached to nucleoli in senescent human dermal fibroblasts (Bridger, unpublished data).

Most interestingly there were two chromosomes that were found to be in different compartments in senescent cells when compared to quiescent human dermal fibroblasts. These were chromosome 15 and chromosome 10. Chromosome 10 was the most dramatic with it being at the nuclear periphery in quiescent cells and deeply in the nuclear interior in senescent cells — in fact, radially, at two opposing locations within the nuclei. This we postulate is so that different levels of control can be maintained over the chromosomes. When we looked at the same ten genes as we did for the quiescent human dermal fibroblasts, six of them were down-regulated, two did not change expression and two were up-regulated. Although this is again a small number of genes, it does show that there are measureable differences between the two arrested states. Quiescence and senescence are thus maybe not as similar as people believe and it is possible that the changes in gene expression are controlled by other means rather than just location. Loss of lamin B1 has been implicated in controlling genome behavior in senescence i.e. allowing the creation of senescence associated heterochromatic foci and the relocation of genomic regions away from the nuclear periphery [27]. This also shows us that gene down-regulation does happen deep within the nucleus. This maybe a different type of silencing than is seen for genes at the periphery and this needs to be further investigated since any event that prevents proper silencing of genes at senescence could lead to re-expression and transformation of normal cells to cancer cells.

The chromosome and gene mappers are frequently asked how relevant is looking at chromosome positioning in tissue culture cells compared to real situations. When we map chromosomes in sections of tissue preserved for their 3-dimensionality we find similar locations for chromosomes to the *in vitro* observations — this is has been seen both in the pig for a number of tissues from different cell sources in the pig [16] and in human skin (Mehta, Kill, Bridger, unpublished data). Indeed, in skin we have found chromosome 10 in three different locations depending on cell state – in proliferating nuclei at an intermediate location as it is in tissue culture cells and in non-proliferating cells at two opposing locations, at the nuclear periphery and in the nuclear interior, as was also seen *in vitro*. The cellular senescence field has been searching for a long time for a suitable biomarker that can properly differentiate between senescent and quiescent cells *in vivo* and perhaps the nuclear location of chromosome 10 could be this biomarker if exploited in the right way.

Chromosome repositioning in patient cells with mutations in the lamin A gene

We believe that the nuclear envelope and the proteins found there are responsible for organizing the genome in interphase nuclei: thus we postulate that chromosomes and genes might reposition in cells from patients where proteins of the nuclear envelope are affected. We started with the nuclear lamina and the A-type lamins and used primary fibroblasts from a group of patients that have a laminopathy. These patients had different mutations along the *LMNA* gene, which encodes for nuclear lamins A and C. These laminopathies ranged from muscular dystrophies such as autosomal and X-linked Emery-Dreifuss muscular dystrophies to lipodystrophies such as Dunnigan's partial familial lipodystrophy to premature ageing syndromes such as Mandibuloacral dysplasia and Hutchinson-Gilford Progeria Syndrome

(HGPS). What we were expecting to do was to map the genome organizing regions of lamin A by mapping chromosome location in the different disease cells that were proliferating. Interestingly, we found that all the diseases had a completely altered chromosome location from the wild-type lamin A that was nonetheless similar between the cells from the different diseases [8]. Initially, with the chromosomes we were assessing this reorganization resembled the chromosome distribution of all non-proliferating cells, which would fit the premature ageing aspect of some mutations in lamin A since chromosomes 13 and 18 were found in the nuclear interior. This was also seen by another group that also saw changes in gene expression associated with the chromosome repositioning [28]. It was not until we assessed chromosome 10 that we found, particularly in the proliferating HGPS cells, that it was not a senescent type pattern of chromosome positioning. Instead, it was a quiescent one, since territories of chromosome 10 were found at the nuclear periphery in the HGPS cells [9]. The effect that *LMNA* mutations have on these cells seems to uncouple the control over the chromosomes position in proliferating cells and allows them to take a resting state. This links lamin A to genome organization as has been shown recently by Solovei and colleagues [29] and others [30]. In HGPS cells chromosome position can be rescued by treating cells with a farnesyl transferase inhibitor that does not permit mutant lamin A with a uncleavable farnesyl group to be produced [9]. Thus, the toxic lamin A that retains a farnesyl group and associates with the nuclear membrane affects genome positioning with cells appearing as in a quiescent-like state when they display proliferating markers.

A genome-wide study of the sequences associated with the mutant toxic lamin A at the nuclear periphery in mouse confirms that A-type lamins are involved in chromatin and genome organization in nuclei, such that some genes have changed their location and are away from the nuclear periphery. On the other hand some genes have an enhanced association with the nuclear envelope in the mouse Progeria model [31]. In HGPS patient

cells we have also shown that fewer telomeres are bound to the nuclear architecture which will inevitably affect genome stability and regulation (Godwin and Bridger unpublished).

It is interesting that nuclear myosin 1β, the myosin we believe is involved in moving some chromosomes around nuclei, is distributed quite differently in non-proliferating cells and in HGPS cells – as large aggregates in the nucleoplasm and absent at the nuclear periphery and nucleoli. Thus, we would predict that chromosomes and maybe even genes are not transposable around the nucleus in a resting state or a diseased state such as HGPS. This hypothesis is yet to be tested in normal cells, but we do know that chromosomes are not repositioned in restimulated quiescent human dermal fibroblasts until the cells have been through mitosis [6,12], which takes more than 24 hours from when the serum is re-added. These timescales are similar to those seen for chromosome movement when specific nuclear envelope transmembrane proteins (NETs) as opposed to lamins are removed [32].

Alterations to gene position in cells exposed to a pathogen

Pathogens are known to use their hosts for their own benefit and this may go as far as manipulating the hosts' genomes to alter host gene expression. We have been studying the effects on genes within the secondary host of the tropical disease schistosomiasis (bilharzia) which eventually leads to liver cancer in the human host. The host is the fresh water snail *Biomphalaria glabrata.* The snail is infected by miracidia found in water polluted with human feces. The miracidia burrow into the snail and develop into the next stage of their life-cycle. We have been looking at genes that are up-regulated in the snail after an infection with schistosoma. Two systems have been employed: an *in vitro* cell system whereby miracidia are only placed in the media with a snail cell line [11] and actual infected whole organisms.

The schistosoma miracidia can be irradiated so that they are still alive and can burrow into the snails but are attenuated and do not progress further to an infection. In both these systems we have determined that exposure to the fully functioning parasite induces the genes that are up-regulated in these infections to be relocalized within the nuclear environment (Arican, Bridger and Knight unpublished). By doing close time studies we have been able to show that the gene moves slightly prior to its being switched on and expressed. This helps answer an important question in the field as to whether genes and chromosomes move before they alter their transcriptional status as some have proposed that transcriptional activation can actually drive a gene away from the nuclear periphery.

The experiments with the attenuated parasite have been an important control since in the co-culture *in vitro* system the normally up-regulated genes were not relocated in interphase nuclei and remained stationary [11]. Further, in the whole organism experiments two genes did not move with attenuated parasite, remaining in their non-random location. However, one gene moved in the same direction under the same early time point in both attenuated and unirradiated miracidia samples. This shows that this gene is expressed due to the infiltration of the parasite into the host rather than any control or influence over the host genome. This species of snail also has a resistant laboratory bred strain in which the two genes that move in the susceptible strain are not expressed and remain stationary.

In order to work towards understanding where these genes are travelling to and what takes them to their new location upon a parasitic infection, we went back to the snail cells in culture and established a heat-shock stimulus, where cells were moved from 27°C to 32°C for 1 hour. This allowed the gene *hsp70* to be expressed. From the literature we know that genes can move to PML bodies [33], SC35 speckles and transcription factories when they become active. Using 3D fixation and immuno-FISH in the snail we were able to see these structures with the gene loci of interest. Upon heat shock we found that there was a significantly

19

increased number of gene loci associated with transcription factories as revealed by anti-RNA polymerase II antibodies. This association correlated with the increased gene expression of the *hsp70* gene.

Whilst staining the snail cells with antibodies that may have crossed the species we found that anti-nuclear myosin 1β that recognizes a nuclear myosin in human cells stained very strongly around the nuclear envelope and had foci throughout the nucleoplasm of the snail cells. We are already advocates of a nuclear motor system in cells moving chromosomes and possibly genes around functionally in the nucleoplasm. A drug that inhibits nuclear myosin polymerization was used and it removed all the internal foci of nuclear myosin 1β staining within the nucleoplasm. It also inhibited the genes moving to their new internal location and a produced much reduced expression of the *hsp70* gene. Thus, we believe, even in organisms such as molluscs they use the same system of moving around specific genes to regulate their expression and they do this via a nuclear motor system as has been shown in human cells.

We believe that the system we have found whereby a pathogen will influence genome reorganisation in a host is a general mechanism benefitting the pathogen. This has been seen with a viral infection that elicited specific chromosome repositioning [34]. In a long term infection it may difficult for a host to regain control of its genome and cells may change their behavior through instability and become transformed, leading to either cellular premature senescence, death or immortalisation.

Unlike other studies in the mammalian cells where we have shown genes moving more to the nuclear interior when they get up-regulated, in the snail cells we see genes that become activated move towards the nuclear periphery. This may have to do with the snail nuclear envelope being different to higher organisms and not such an area for down-

regulation and silencing. Indeed, *B. glabrata* seems to have nuclear lamins, but they are more akin to *Drosophila* lamins than mammalian lamins (Town and Bridger, unpublished) and may instigate a different type of genome organization within this species/ Genus.

Alterations to genome organization in cancer

Genome organization is altered in cancer cells as has been shown nicely many years ago by the distribution of centromeres and telomeres being altered in bladder carcinoma cells [35]. A number of more recent studies now show abnormal chromosome positioning in cancer cells. Abnormal relocation of chromosome 18 from the nuclear periphery to the interior has been reported in several types of tumor cell lines, including those derived from melanoma, cervix carcinoma, colon carcinoma, Hodgkin's lymphoma, and metastasizing cells from a colon carcinoma [36]. Moreover, several reports support the idea of a functional correlation between non-random chromosome positioning and formation of specific chromosome translocations, for example human chromosomes 9 and 22 in chronic myeloid leukaemia (CML) [37] as well as the correlation between tissue specific spatial organization and tissue specific translocations [38]. These findings are particularly compelling because chromosomes that tend to be adjacent to one another are much more likely to form particular fusion proteins from translocations that are prevalent in a particular tumor type. That this would be observed in large numbers of patients at the level of a specific fusion protein underscores that patterns of spatial genome organization are very highly conserved. Furthermore, the nuclear positions of chromosomes 10, 18 and 19 were assessed in normal thyroid tissue and compared to several types of thyroid cancers including adenomatous goiters, papillary carcinomas and undifferentiated carcinomas. There was no difference in chromosome position in the normal and goiter tissue with chromosomes 10 and 18 positioned towards the nuclear periphery and

chromosome 19 in a central location. However, in the papillary carcinoma tissue chromosome 19 was located more centrally. Furthermore, in undifferentiated carcinomas all the chromosomes assessed were mislocalised [37]. Marella *et al.*, in 2009 [40], used normal human WI38 lung fibroblast and MCF10A epithelial breast cells and identified that similar levels of association were found in WI38 and MCF10A (both are non-tumorigenic) for chromosomes 1, 4, 11, 12, 14, and 16 whereas a nearly 2-fold increase in chromosomes 4 and 16 associations was found in a malignant breast cancer cell line (MCFCA1a) compared to the related normal epithelial cell line (MCF10A). This demonstrates that chromosome associations are cell-type specific and undergo alterations in cancer cells [40]. Furthermore, Wiech *et al.*, 2005 analyzed chromosome 8 positions in wax embedded pancreatic cancer tissue samples. Their results obtained from non-neoplastic pancreatic cells indicated that the radial arrangement of the chromosome 8 territories did not significantly differ between normal individuals. However, in pancreatic tumours, the radial distance changes indicated the repositioning of chromosome 8 to the nuclear periphery. Positioning changes were also observed in breast cancer. In non-neoplastic ductal epithelium of the breast there was a large distance between the position of the centromere 17 and *HER2* domains among individuals. In neoplastic epithelial breast cells, the distances between centromere and gene domains were smaller than in non-neoplastic cells. The centromere and the gene encoding *HER2* on chromosome 17 were shown to reposition to a more internal location [41,42]. A later study by Wiech *et al.*, in 2009 looked at cervical carcinomas. They reported repositioning of chromosome 18 during cell differentiation of cervical squamous epithelium towards the nuclear center whereas, cervical squamous carcinomas showed a repositioning of chromosome 18 towards the nuclear periphery [43].

Therefore, changes in the radial position of specific gene loci in cancer cells could contribute to tumorigenesis, but further investigation is still needed. These observations

strongly support the idea that the genomic regions influenced by states of gene activity and cell-type specific genome architecture are predisposed towards translocations that are characteristic to specific cell types and cancers.

All the aforementioned studies did not assess the status of the nuclear structure, especially those proteins involved in genome organization. Other studies only look at nuclear structure changes with respect to cancer and do not look at any genome behavioral changes [44]. Changing the nuclear architecture will have a direct effect on the genomes' stability and may then lead to cancer.

Alterations to gene and chromosome position in breast cancer cell lines can possibly be manipulated to reduce cancer phenotypes

A number of studies have shown that in cancer cells whole chromosomes and specific gene loci can change nuclear location away from the norm. Indeed translocations prevalent in cancer cells can place genes in new locations in interphase nuclei that affect their behavior and expression profiles as has been shown with *HLXB9* in pediatric leukaemia [45].

One of the best studies for looking at gene repositioning in cancer is that of Meaburn and Misteli for loci of genes that are involved in some of the important changes in breast cancer. These authors showed a number of genes were non-randomly located at new locations in a 3D culture model system and in tumor tissue sections. They showed altered positioning of cancer-associated genes such as *AKT1*, *BCL2*, *ERBB2*, and *VEGF* loci, although no correlation was found between this radial redistribution and gene activity levels [46].

Meaburn *et al.*, in 2009 expanded on their study of the repositioning of genes that are involved in breast cancer. From 11 normal human breast and 14 invasive breast cancer tissue specimens, they identified eight genes (*HES5, ERBB2, MYC, FOSL2, HSP90AA1, AKT1, TGFB3, and CSF1R*) that had altered their position in breast cancer [47] Excitingly, the position of a specific gene, *HES5*, a transcription repressor that regulates cell differentiation, could distinguish between a cancerous tissue and a healthy one with almost 100 percent accuracy. Alteration or repositioning of this gene has been associated with tumorigenesis and was observed in several types of breast cancer so that it could prove a useful diagnostic tool [47].

The studies by Meaburn and Misteli did not link any actual nuclear or chromosomal event or aberration in nuclear structure to this change in location. However, in a study using a panel of breast tumor epithelial cell lines we found that whole chromosomes were mis-positioned as well as individual genes. Most interestingly, when genes were mislocalized without repositioning of the whole chromosome they were found out on loops at some distance from the chromosomes. Interestingly, we also found that a number of the cells lacked or had reduced levels of lamin A and lamin B receptor that have been implicated in gene/chromosome/chromatin position by us and others. The cells also had large accumulations of B-type lamins in the centre of their nuclei. The cell lines with the most pronounced loss or changes in nuclear envelope proteins had the most changes with respect to breast cancer gene relocation. In fact the three genes that were focused upon, *HER2*, *HSP90AA* and *AKT1*, were found towards the nuclear periphery in these aberrant cells. When the cells were treated with a drug that restored lamin B receptor to the nuclear periphery and placed lamin B back at the nuclear periphery, one cell line had the genes *HER2*, *HSP90AA* and *AKT1* become more internal with a corresponding up-regulation of all three genes. However, another cell line pulled the same genes more towards the nuclear periphery after

24

treatment which correlated with a down-regulation of expression in *AKT1* and *HSP90AA* (Hassan Ahmed, Harvey, Karteris and Bridger, unpublished data).

This is a very important finding because it links nuclear envelope aberrations with genome mislocalization in cancer. Though the reasons for the differences between cell lines need to be determined, our ability in this study to correct to a certain extent the nuclear envelope abnormalities and correspondingly restore proper gene location and expression may open the way for novel therapeutic treatments.

Summary

The non-random spatial positioning of the genome within nuclei appears to be highly relevant to controlling gene expression and silencing [49]. The gene-density distribution of chromosomes in proliferating cells requires energy and highly organised tethering to nuclear structures to be maintained. This organization is changed dramatically when cells become non-proliferating, perhaps some of the positioning is more relaxed requiring less energy to maintain. However, there are some noticeable differences between quiescent and senescent cells and we believe this is due differences in gene expression profiles but also the absolute need to silence irreversibly in senescent cells to prevent reactivation and these cells becoming cancerous. This silencing, we postulate, will be deep within nucleus.

It is not only chromosomes that move around nuclei after a stimulus but individual genes. These genes can move to new areas around the nucleus without the whole chromosome moving. We predict that these genes translocate across the nucleus in a directed manner, to structural entities such as transcription factories or splicing speckles for example

using nuclear motor activity (see Figure 1). We believe that the nuclear motors require nuclear envelope proteins such as emerin and the lamins to function correctly [48]. These genes will meet other genes at the transcription factories and if this movement, co-occupation, transcription and the return of the gene to its original location is not functioning correctly then it is possible that chromosomal translocations are formed that are a hallmark of cancer [44].

Pathogen-led spatial reorganization of the genome is a newly discovered process and we need to discover how the pathogen controls specific selected gene expression but further we need to determine what irreversible alterations have been elicited in the cell that will affect its future.

Data from breast cancer cells and the premature ageing disease HGPS demonstrates that the nuclear envelope is involved in chromosome and gene positioning, especially proteins such as A-type lamins, lamin B receptor and lamin B. Finally, and perhaps most importantly, these studies on gene repositioning and its consequences in cancer cells may pave the way for novel therapeutic interventions or combination treatments to inhibit tumor cell proliferation and restablise the genome in cancer cells.

Acknowledgements

Figure 1. The translocation of genes to transcription factories via nuclear motor activity. This cartoon shows how genes may be relocated to transcription factories at some distance from the main body of the chromosome territory that houses the gene. The nuclear myosin moves along actin filaments that polymerise where they are needed. There must be a signal from the chromatin to be moved and this must unravel due to changes in chromatin modification i.e. the histone code. If this process does not proceed correctly then genome stability may be affected, genes may be over or under expressed or become translocated with other chromosomes.

References

1. Cremer T, Kurz A, Zirbel R, Dietzel S, Rinke B, Schröck E, Speicher MR, Mathieu U, Jauch A, Emmerich P, Scherthan H, Ried T, Cremer C, Lichter P. (1993) Role of chromosome territories in the functional compartmentalization of the cell nucleus. Cold Spring Harb Symp Quant Biol. 58:777-792

2. Foster HA, Bridger JM (2005) The genome and the nucleus: a marriage made by evolution. Genome organisation and nuclear architecture. Chromosoma 114: 212-229.

3. Bridger, J.M. and Bickmore W.A. Putting the genome on the map (1998). Trends Genet. 14 403-410.

4. Boyle S, Gilchrist S, Bridger JM, Mahy NL, Ellis JA, Bickmore WA (2001) The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells. Hum. Mol. Genet. 10: 211-219.

5. Croft JA, Bridger JM, Boyle S, Perry P, Teague P and Bickmore WA (1999). Differences in the localization and morphology of chromosomes in the human nucleus. J. Cell Biology 145 1119-1131

6. Bridger JM, Boyle S, Kill IR, Bickmore WA (2000) Re-modelling of nuclear architecture in quiescent and senescent human fibroblasts. Curr Biol 10: 149-152.

7. Mehta IS, Figgitt M, Clements CS, Kill IR, Bridger JM (2007) Alterations to nuclear architecture and genome behavior in senescent cells. Ann N Y Acad Sci 1100:250-263.

8. Meaburn KJ, Cabuy E, Bonne G, Levy N, Morris GE, Novelli G, Kill IR, Bridger JM (2007) Primary laminopathy fibroblasts display altered genome organization and apoptosis. Aging Cell 6: 139-153.

9. Mehta IS, Eskiw CH, Arican HD, Kill IR, Bridger JM (2011) Farnesyltransferase inhibitor treatment restores chromosome territory positions and active chromosomes dynamics in Hutchinson-Gilford progeria syndrome cells. Genome Biology 12: R74.

10. Wazir U, Hassan Ahmed M, Bridger JM, Harvey A, Jiang WG, Sharma AK, Mokbel K (2013) A study of Lamin A mRNA expression in human breast cancer and potential implications regarding the role of nuclear stability in breast carcinogenesis. European Journal of Surgical Oncology In press.

11. Knight M, Ittiprasert W, Odoemelam EC, Adema CM, Miller A, Raghavan N, Bridger JM (2011) Non-random organization of the Biomphalaria glabrata genome in interphase Bge cells and the spatial repositioning of activated genes in cells co-cultured with Schistosoma mansoni. Int J Parasitol 41:61-70.

12. Mehta IS, Amira M, Harvey AJ, Bridger JM (2010) Rapid chromosome territory relocation by nuclear motor activity in response to serum removal in primary human fibroblasts. Genome Biol 11:R5.

13. Bridger, JM; Lichter, P. (1999). Analysis of mammalian interphase chromosomes by FISH and immunofluorescence. In Chromosome Structural Analysis: a practical approach. Edited by Dr. Wendy Bickmore IRL Press.

14. Chuang CH, Carpenter AE, Fuchsova B, Johnson T, de Lanerolle P, Belmont AS (2006) Long-range directional movement of an interphase chromosome site. Curr Biol 16:825-831.

15. Chubb JR, Boyle S, Perry P, Bickmore WA.(2002) Chromatin motion is constrained by association with nuclear compartments in human cells. Curr Biol. 12:439-445.

16. Foster HA, Griffin DK & Bridger JM (2012). Interphase chromosome positioning in in vitro porcine cells and ex vivo porcine tissues. BMC Cell Biology 13:30

17. Szczerbal I, Foster HA, Bridger JM (2009) The Spatial Repositioning of Adipogenesis Genes Is Correlated with Their Expression Status in a Porcine Mesenchymal Stem Cell Adipogenesis Model System. Chromosoma 118: 647–663.

18. Brown JM, Green J, das Neves RP, Wallace HA, Smith AJ, Hughes J, Gray N, Taylor S, Wood WG, Higgs DR, Iborra FJ, Buckle VJ (2008) Association between active genes occurs at nuclear speckles and is modulated by chromatin environment. J Cell Biol 182:1083-1097

19. Szczerbal I, Bridger JM (2010) Association of adipogenic genes with SC-35 domains during porcine adipogenesis. Chromosome Res 18:887-895

20. Osborne CS, Chakalova L, Mitchell JA, Horton A, Wood AL, Bolland DJ, Corcoran AE, Fraser P (2007) Myc dynamically and preferentially relocates to a transcription factory occupied by Igh. PLoS Biol 5:e192.

21. Xu M, Cook PR. (2008) The role of specialized transcription factories in chromosome pairing. Biochim Biophys Acta. 1783(11):2155-2160.

22. Hofmann WA, Johnson T, Klapczynski M, Fan JL, de Lanerolle P. (2006) From transcription to transport: emerging roles for nuclear myosin I. Biochem Cell Biol. 84:418-426.

23. Bridger JM. (2011) Chromobility: the rapid movement of chromosomes in interphase nuclei. Biochem Soc Trans. 39:1747-1751

24. Bridger JM and Mehta IS. (2011) Nuclear Molecular Motors for Active, Directed Chromatin Movement in Interphase Nuclei. Advances in Nuclear Architecture. Eds Niall Adams and Paul Freemont. Springer

25. Finlan LE, Sproul D, Thomson I, Boyle S, Kerr E, Perry P, Ylstra B, Chubb JR, Bickmore WA. (2008) Recruitment to the nuclear periphery can alter expression of genes in human cells. PLoS Genet. 4(3):e1000039.

26. Arib G, Akhtar A. (2011) Multiple facets of nuclear periphery in gene expression control. Curr Opin Cell Biol. 23(3):346-353.

27. Sadaie M, Salama R, Carroll T, Tomimatsu K, Chandra T, Young AR, Narita M, Pérez-Mancera PA, Bennett DC, Chong H, Kimura H, Narita M. (2013) Redistribution of the Lamin B1 genomic binding profile affects rearrangement of heterochromatic domains and SAHF formation during senescence. Genes Dev. 27:1800-1808.

28. Mewborn SK, Puckelwartz MJ, Abuisneineh F, Fahrenbach JP, Zhang Y, MacLeod H, Dellefave L, Pytel P, Selig S, Labno CM, Reddy K, Singh H, McNally E (2010) Altered chromosomal positioning, compaction, and gene expression with a lamin A/C gene mutation. PLoS ONE 5:e14342.

29. Solovei I, Wang AS, Thanisch K, Schmidt CS, Krebs S, Zwerger M, Cohen TV, Devys D, Foisner R, Peichl L, Herrmann H, Blum H, Engelkamp D, Stewart CL, Leonhardt H, Joffe B. (2013) LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation.Cell. 152(3):584-98.

30. Taimen P, Pfleghaar K, Shimi T, Moller D, Ben-Harush K, Erdos MR, Adam SA, Herrmann H, Medalia O, Collins FS, Goldman AE, Goldman RD (2009) A progeria mutation reveals functions for lamin A in nuclear assembly, architecture, and chromosome organization. Proc Natl Acad Sci U S A 106:20788-20793.

31. Kubben N, Adriaens M, Meuleman W, Voncken J, van Steensel B, Misteli T (2012) Mapping of lamin A- and progerin-interacting genome regions. Chromosoma 121: 447-464.

32. Zuleger N, Boyle S, Kelly DA, de Las Heras JI, Lazou V, Korfali N, Batrakou DG, Randles KN, Morris GE, Harrison DJ, Bickmore WA, Schirmer EC. (2013) Specific

nuclear envelope transmembrane proteins can promote the location of chromosomes to and from the nuclear periphery.Genome Biol. 14(2):R14.

33. Dundr M, Ospina JK, Sung MH, John S, Upender M, Ried T, Hager GL, Matera AG (2007) Actin-dependent Intranuclear Repositioning of an Active Gene Locus in Vivo. J Cell Biol 179: 1095–1103.

34. Li C, Shi Z, Zhang L, Huang Y, Liu A, Jin Y, Yu Y, Bai J, Chen D, Gendron C, Liu X, Fu S.(2010) Dynamic changes of territories 17 and 18 during EBV-infection of human lymphocytes. Mol Biol Rep. 37(5):2347-2354.

35. Poddighe PJ, Ramaekers FC, Smeets AW, Vooijs GP, Hopman AH.(1992) Structural chromosome 1 aberrations in transitional cell carcinoma of the bladder: interphase cytogenetics combining a centromeric, telomeric, and library DNA probe. Cancer Res. 52(18):4929-4934.

36. Cremer M, Kupper K, Wagler B, Wizelman L, von Hase J, Weiland Y, Kreja L, Diebold J, Speicher MR, Cremer T (2003) Inheritance of gene density-related higher order chromatin arrangements in normal and tumor cell nuclei. J Cell Biol 162:809-820.

37. Lukasova E, Kozubek S, Kozubek M, Kjeronska J, Ryznar L, Horakova J, Krahulcova E, Horneck G (1997) Localisation and distance between ABL and BCR genes in interphase nuclei of bone marrow cells of control donors and patients with chronic myeloid leukaemia. Hum Genet 100:525-535

38. Parada LA, McQueen PG, Misteli T (2004) Tissue-specific spatial organization of genomes. Genome Biol 5:R44

39. Murata S, Nakazawa T, Ohno N, Terada N, Iwashina M, Mochizuki K, Kondo T, Nakamura N, Yamane T, Iwasa S, Ohno S, Katoh R (2007) Conservation and

alteration of chromosome territory arrangements in thyroid carcinoma cell nuclei. Thyroid 17:489-496

40. Marella NV, Bhattacharya S, Mukherjee L, Xu J, Berezney R (2009) Cell type specific chromosome territory organization in the interphase nucleus of normal and cancer cells. J Cell Physiol 221:130-138.

41. Wiech T, Timme S, Riede F, Stein S, Schuricke M, Cremer C, Werner M, Hausmann M, Walch A (2005) Human archival tissues provide a valuable source for the analysis of spatial genome organization. Histochem Cell Biol 123:229-238

42. Timme S, Schmitt E, Stein S, Schwarz-Finsterle J, Wagner J, Walch A, Werner M, Hausmann M, Wiech T (2011) Nuclear position and shape deformation of chromosome 8 territories in pancreatic ductal adenocarcinoma. Anal Cell Pathol (Amst) 34:21-33

43. Wiech T, Stein S, Lachenmaier V, Schmitt E, Schwarz-Finsterle J, Wiech E, Hildenbrand G, Werner M, Hausmann M (2009) Spatial allelic imbalance of BCL2 genes and chromosome 18 territories in nonneoplastic and neoplastic cervical squamous epithelium. Eur Biophys J 38:793-806.

44. Bourne G, Moir C, Bikkul U, Hassan Ahmed M, Kill I R; Eskiw C H., Tosi S and Bridger JM. (2013). Interphase chromosomes in disease. Human Interphase Chromosomes: the Biomedical Aspects Ed Yuri Yurov. Springer.

45. Ballabio E, Cantarella C D, Federico C, Di Mare P, Hall G, Harbott J, Hughes J, Saccone S, Tosi S (2009) Ectopic Expression of the HLXB9 Gene Is Associated with an Altered Nuclear Position in T(7;12) Leukaemias. Leukemia 23: 1179–1182

46. Meaburn KJ, Misteli T (2008) Locus-specific and activity-independent gene repositioning during early tumorigenesis. J Cell Biol 180:39-50.

47. Meaburn KJ, Gudla PR, Khan S, Lockett SJ, Misteli T (2009) Disease-specific gene repositioning in breast cancer. J Cell Biol 187:801-812.

48. Mehta IS; Amira M; Kill IR; Bridger JM. (2008) Nuclear motors and nuclear structures containing A-type lamins and emerin: is there a functional link? Biochem Soc Transactions 36:1384-1388

49. Elcock LS, Bridger JM. (2010) Exploring the relationship between interphase gene positioning, transcriptional regulation and the nuclear matrix. Biochem Soc Trans. 38:263-267.