Genomics 102 (2013) 288–295

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

The interplay between genome organization and nuclear architecture of primate evolutionary neo-centromeres

Mariana Lomiento [a,c,*], Florian Grasser [b], Mariano Rocchi [c], Stefan Müller [b,d]

[a] Department of Biomedical Sciences, University of Modena and Reggio Emilia, via Campi 287, 41100 Modena, Italy
[b] Department of Biology II, Human Genetics, Ludwig-Maximilians University Munich, Großhaderner Straße 4, 82152 Planegg-Martinsreid, Germany
[c] Department of Biology, University of Bari, Via Orabona 4, 70125 Bari, Italy
[d] Institut für Humangenetik, Ludwig-Maximilians University Munich, Goethestrasse 29, 80336 München, Germany

ARTICLE INFO

Article history:
Received 28 September 2012
Accepted 25 April 2013
Available online 3 May 2013

Keywords:
Evolutionary Neo-Centromere
Nuclear architecture
3D FISH

ABSTRACT

An Evolutionary Neo-Centromere (ENC) is a centromere that emerged in an ectopic region of a chromosome during evolution. It is thought that the old centromere must be inactivated because dicentric chromosomes are not viable. The aim of the present study was to investigate whether 3D arrangement in the interphase nucleus of the novel and old centromeric domains was affected by the repositioning event. The data we present here strongly indicate that the ENC phenomenon does not affect the 3D location of either novel or old centromeres. Very likely, other features, such as gene density, rather than the newly acquired or lost functions, define positioning in the nucleus.

1. Introduction

In the last decades the 3D arrangement of interphase nuclei was extensively studied [1–3]. Nevertheless, how the unpacked metaphase chromosomes are organized in interphase nucleus is far from fully understood. Several studies reported that chromosomes appear as distinct entities, known as Chromosome Territories (CTs). CTs exhibit internal polarity, with gene-poor and late replicating chromatin facing the nuclear border [4–6], while gene-dense or early-replicating chromatin is localized in the nuclear interior [4,5,7]. This spatial distribution pattern appears to be evolutionarily highly conserved [8–10]. The interior nuclear compartment may thus provide an evolutionarily conserved genomic environment enhancing the possibility of efficiently transcribing genomic loci. In contrast, the nuclear periphery and, in particular, the association of genes with peripherally located constitutive pericentromeric heterochromatin, were suggested to represent an epigenetic mechanism resulting in their down-regulation [11].

Studies on the evolutionary history of chromosomes have shown that a centromere can reposition along the chromosome without marker order variation. These Evolutionary Neo-Centromeres (ENCs) have no phenotypic consequences and are relatively frequent. They have been reported in primates [12–16], non-primate placental mammals [17–19], marsupials [19], birds [20], and plants [21]. The fixation of the novel centromere is usually accompanied by the acquisition of the complex organization typical of a mature centromere, featuring a large core of satellite DNA surrounded by intra- and interchromosomal segmental duplications [22]. Concomitantly, the inactivated centromere loses this complexity [22]. We wanted to determine if the 3D nuclear arrangement of the novel centromere and of the old inactivated centromere are affected by these events. Accordingly, we surveyed the literature and found four ENC examples appropriate for investigation, three in Old World Monkeys (OWMs) and one in New World Monkeys (NWMs). In each case, a homologous chromosome, representing the reference ancestral situation, was available in another primate species. Lastly, we compared two chromosomes harboring distinct ENCs which also differed for a pericentric inversion, in order to better understand the consequences, on 3D arrangement, of these two types of chromosomal rearrangements. Our results indicate that the ENC occurrence has only marginal consequences both on the 3D arrangement of the ENC domain and on the domain harboring the inactivated centromere. On the contrary, the consequences of the inversion were significant.

2. Results

We first surveyed the literature for species with “mature” ENCs. Mature ENCs, have large arrays of satellite DNA accompanied by inactivated centromeric domain which have completely lost their satellite arrays. We found that macaque chromosomes, possess nine distinct mature ENCs [22] and matched our requirements. All members of the genus share identical karyotypes and all centromeres, including the ENCs, possess large blocks of alpha satellite DNA, while the arrays of satellite DNA, very thought to be previously present in the old centromeres, have been completely lost [22]. This feature is probably the consequence of their relatively old age. All these ENCs were seeded, in fact, before Cercopithecinae/Colobinae divergence [22], that is at least

* Corresponding author at: Department of Biomedical Sciences, University of Modena and Reggio Emilia, via Campi 287, 41100 Modena, Italy. Fax: +39 0592055410.
E-mail address: mariana.lomiento@unimore.it (M. Lomiento).

0888-7543/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygeno.2013.04.017

about 18 My ago [23]. These chromosomes represented an ideal model for our investigation. From the genus *Macaque*, we chose fibroblast cultures from *Macaca nemestrina* (MNE) for our 3D experiments. Then we extensively surveyed the literature in search for primate chromosomes (i) orthologous to one of the nine macaque chromosomes bearing an ENC; (ii) with the centromere maintaining the ancestral position; and (iii) showing an identical marker order arrangement. The latter feature was required to exclude 3D variations due to chromosomal structural changes such as inversions or translocations. Some species-specific changes of the radial nuclear distribution patterns of orthologous DNA sequences in primate fibroblast nuclei have in fact been observed, for instance, when evolutionary fusions or fissions resulted in larger or smaller chromosomes [10]. We identified three such reference chromosomes in gorilla (*Gorilla gorilla*, GGO). The three couples of corresponding chromosomes (macaque/gorilla) were: MNE13/GGO12 (human 2p), MNE17/GGO14 (human 13), and MNE18/GGO16 (human 18) (Fig. 1a). In the survey we took advantage of the reconstruction of the of OWM and primate ancestral karyotypes reported by Stanyon et al. [24].

Our literature search included the New World Monkeys. However, NWM species have, on average, a more reshuffled karyotype with respect to OWMs [24]. The best example was found in woolly monkey (*Lagothrix lagotricha*, LLA). LLA7 harbors an ENC and the orangutan (*Pongo pygmaeus*, PPY) chromosome 6 can be used as orthologous reference chromosome of LLA7. The example, however, only partially met the three requirements listed above. LLA7 constitutes only a part of the ancestral chromosome represented by the PPY6 (human 8). In LLA ancestor, a rearrangement(s) generated LLA4 and LLA7. LLA4 retained the short arm and the centromere of the ancestral chromosome, while LLA7 retained the acentric long arm (Fig. 1b) whose viability was rescued by an ENC.

Lastly, we considered two orthologous chromosomes, MNE4 and PPY5 (human 6), both harboring an ENC, but in distinct positions with respect to the primate ancestor [25], and also differing for an inversion of about 50 Mb, encompassing the centromere, present in macaque (http://www.biologia.uniba.it/macaque/MMU/MMU_04.html) (Fig. 1c). This case allowed us to compare the impact of the two distinct kinds of chromosomal changes, centromere repositioning and inversion, on 3D arrangement.

In summary, we focused our 3D investigations on the following chromosome couples: MNE13/GGO12, MNE17/GGO14, MNE18/GGO16, LLA7/PPY6, and MNE4/PPY5. To this aim we selected appropriate human BAC clones closely flanking, on both sides, the old and the newly formed centromeres. Centromeres are usually surrounded by segmental duplications. We excluded BACs yielding duplicate signals because they could make the analysis ambiguous. Their precise mapping, derived from the UCSC genome browser (http://genome.ucsc.edu/; hg19), is reported in Table 1. All these BACs were first validated by FISH experiments on metaphase chromosomes (data not shown).

In all multicolor 3D-FISH experiments each set of BAC clones was co-hybridized with an appropriate human Whole Chromosome Painting probe (WCP). The only exceptions were the case of chromosomes MNE13/GGO12 (human 2p) for which we used the chimpanzee (*Pan troglodytes*) WCP 12 (human 2p), and LLA7/PPY6 for which we used the WCP of SOE16 (*Saimiri oerstedii*), orthologous to human chromosome 8q.

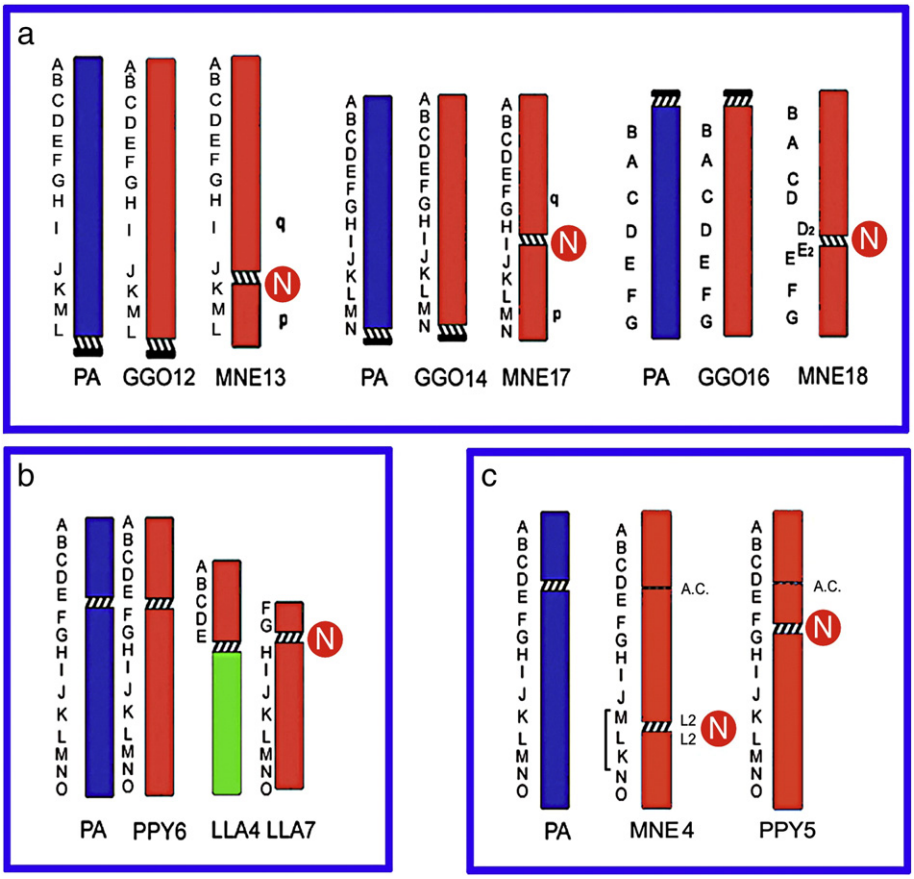

Fig. 1. The figure illustrates the organization (a) of the macaque chromosomes MNE13, MNE17, and MNE18. On the left are reported the gorilla (GGO) reference chromosomes and the Primate Ancestral (PA) chromosomes, according to Ventura et al. [22]; (b) woolly monkey chromosomes LL4 and LLA7, as reported by Lomiento et al. [33], along with the orangutan (PPY) and PA reference chromosomes; (c) the macaque MNE4 the orangutan PPY5 along with the PA chromosomes. A square bracket encompasses the macaque inversion. A.C. is for ancestral centromere. The letter N in a circle represents the ENC. For detail see text.