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Comparative chromosome analysis of human and rhesus macaque (*Macaca mulatta*) using human multicolor FISH probes

E. Nasonova*[1], N. Koshlan†[1], and I. Koshlan‡[1,2]

[1] *Joint Institute for Nuclear Research, 141980, Dubna, Russia*
[2] *Dubna State University, 141980, Dubna, Russia*

Abstract

We established chromosome homology between human and the rhesus macaque (*Macaca mulatta*) using a powerful modern molecular cytogenetic technique: multicolor fluorescence in situ hybridization (mFISH). mFISH allows the classification of mammalian chromosomes based on their DNA content rather than solely on morphological features such as banding patterns. The *Macaca mulatta* karyotype consists of 42 chromosomes: 20 pairs of autosomes and the X and Y sex chromosomes. We confirmed a pronounced similarity between the human (*Homo sapiens*, HSA) and macaque (*Macaca mulatta*, MMU) genomes: 16 out of 22 macaque chromosomes showed homology to a single human chromosome, and 13 of them have human homologs with the same numbers. Only three macaque chromosomes show hybridization signals from two human chromosomes, and a single human probe for chromosome 2 labels two pairs of acrocentric monkey chromosomes. Altogether, mFISH is expected to become an indispensable tool in comparative chromosome studies.

Keywords: chromosome homology, *Macaca mulatta*, monkey chromosomes, human chromosomes, multicolor fluorescence in situ hybridization (mFISH)

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1. Introduction

Knowledge of chromosome homology between humans and other primates is essential for understanding human genome evolution. Cytogenetic studies of the chromosome organization of the genome began in the 1960s, when it became possible to culture mammalian blood lymphocytes and prepare high-quality metaphase spreads [1]. In the 1970s, numerous banding techniques provided new insights into chromosome identification (Figure 1). The first comparative studies of human and other primate karyotypes were conducted at that time using cytogenetic karyotyping (i.e., the comparison of banding patterns) and gene mapping analysis.

*Corresponding author e-mail address: nasonova@jinr.ru
†Corresponding author e-mail address: nkoshlan@jinr.ru
‡Corresponding author e-mail address: koshlan@jinr.ru

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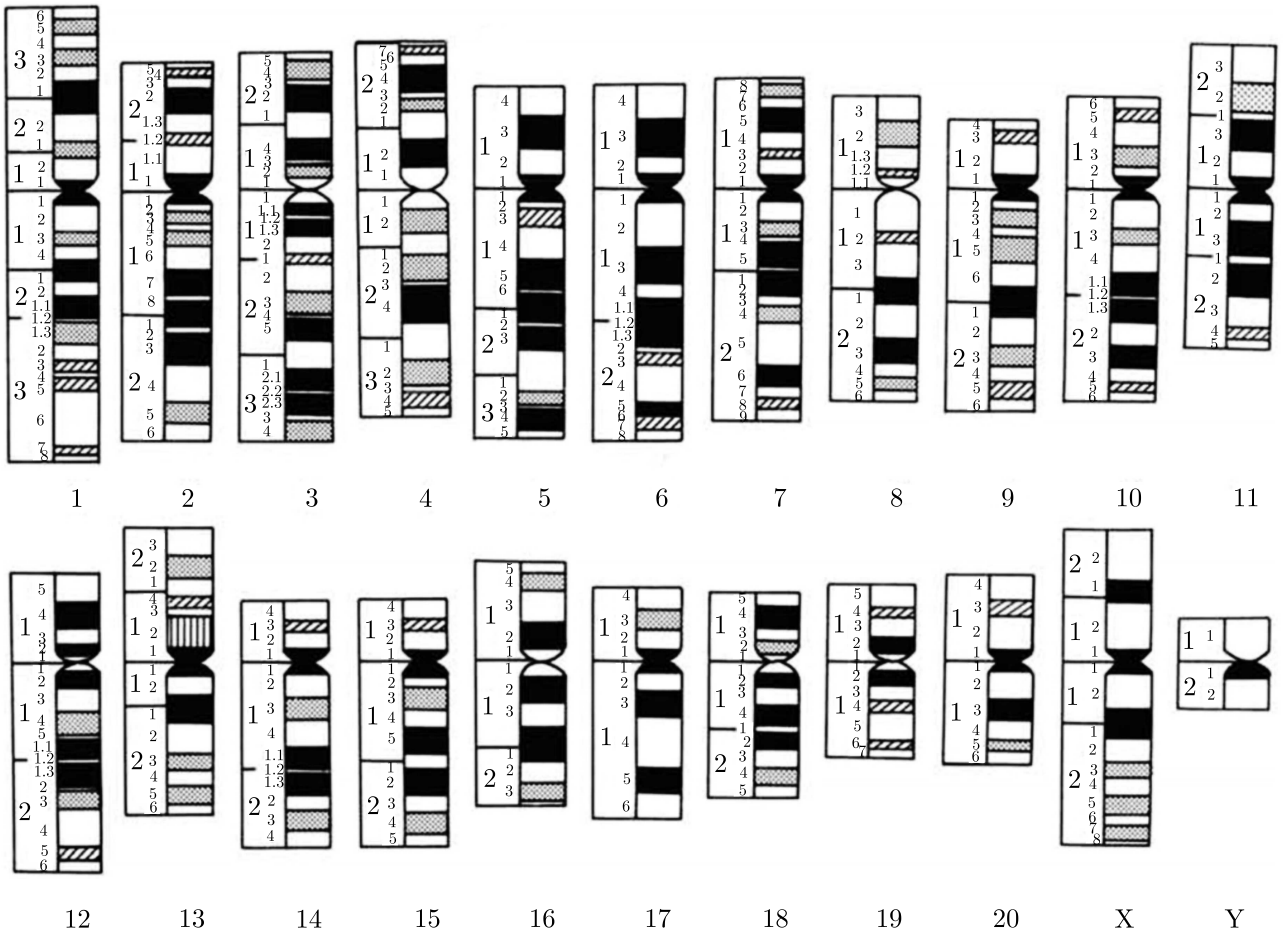

Figure 1. Idiogram of macaque metaphase chromosomes obtained using the G-banding method [8].

B. Dutrillaux and his colleagues, using all existing banding techniques, performed extensive studies of karyotype evolution in primates and reported a detailed analysis of chromosome similarities among them, detectable at a high-resolution banding level [2–5].

Another approach to chromosome homology research was gene mapping (see [6] and references therein), and it soon became clear that cytogenetic observations were not always supported by gene mapping data. These contradictions were resolved in 1978, when the karyotype drawn by A. Estop was recommended by the International Committee on Chromosome Nomenclature as the generally acceptable nomenclature for *Macaca mulatta* (Figure 1) [7, 8]. It was also demonstrated that all monkeys of the Papionini tribe (macaques, baboons, mandrills, and mangabeys) have a nearly identical karyotype [3–5, 8].

Fluorescence in situ hybridization (FISH) techniques became a completely new tool in cytogenetic investigations and, being based on sequence homology, solved many problems associated with chromosome similarities deduced only from visual inspection. The pioneering work of J. Wienberg's and T. Cremer's groups (the latter being the pioneer of the mFISH method) used a combination of banding techniques with chromosomal in situ suppression (CISS) hybridization using human chromosome-specific libraries (a prototype of mFISH), providing a more precise homology identification based on the superposition of two patterns [9].

In this study, we aimed to map the chromosome homology between humans (*Homo sapiens*, HSA) and the rhesus macaque (*Macaca mulatta*, MMU) using mFISH. This approach allows for

the precise classification of mammalian chromosomes based on their DNA content, overcoming the limitations of traditional morphological analysis such as banding patterns.

2. Materials and methods

Blood sample processing

Rhesus macaques (*Macaca mulatta*) served as subjects in large-scale experiments conducted by the Institute of Biomedical Problems of the Russian Academy of Sciences (IMBP RAS, Moscow, Russia) and the Institute of Primatology (Sochi, Russia) to investigate the combined effects of gravity and radiation factors simulating deep-space flights [10–12]. The ongoing study includes cytogenetic monitoring of chromosome damage induced in monkey blood by various radiation types. The Ethics Committees of the IMBP RAS and the Institute of Primatology approved the study in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. Blood samples were drawn at the IMBP RAS and the Institute of Primatology, and then transferred to the Laboratory of Radiation Biology of the Joint Institute for Nuclear Research (LRB JINR), where cell culturing, slide preparation, and chromosome aberration analysis were performed.

Blood samples were processed according to the standard protocols recommended by the IAEA with minor modifications [13, 14]. Briefly, 0.5 mL of blood was diluted in 4.5 mL of RPMI-1640 medium supplemented with 20% fetal calf serum (FCS), 4% phytohemagglutinin (PHA-P), 0.05% phorbol-12-myristate-13-acetate, 1% L-glutamine, and 2% antibiotics (penicillin/streptomycin), and then incubated for 48 h at 38°C in a humidified atmosphere containing 5% CO_2. Metaphases were accumulated by adding colcemid to the culture to a final concentration of 0.12 μg/mL two hours prior to the end of incubation. This was followed by hypotonic treatment (0.075 M KCl) and fixation with a methanol to acetic acid mixture (v/v 3:1). The cell suspension was dropped onto wet slides, air-dried, and stained with Giemsa solution for conventional cytogenetic analysis. For mFISH analysis, three slides prepared from the blood of three untreated control males were painted. Approximately 3000 cells were analyzed in total.

mFISH chromosome staining

Slides were stained using the 24XCyte whole-genome painting mFISH probe kit (MetaSystems, Altlussheim, Germany) according to the manufacturer's protocol. The chromosome-specific probes were labeled with five fluorochromes, and DAPI was used as a counterstain. mFISH images were captured using a 63× oil immersion objective on a Zeiss Axio Imager.Z2 microscope equipped with a set of six filters appropriate for the applied fluorochromes. The chromosome images were processed, identified, and analyzed using the ISIS/mFISH software (MetaSystems, Germany), which assigned "false colors" based on unique combinations of fluorochromes. This enabled the identification of 22 pairs of human autosomes, the X and Y sex chromosomes, and any rearrangements between them. Image processing concluded with the construction of a karyogram — a visual representation of the karyotype where chromosomes are ordered by size and centromere position.

3. Results

We performed mFISH painting using human chromosome-specific probes (24XCyte, MetaSystems, Germany) to precisely identify the rhesus macaque karyotype. The human karyotype

(Figure 2, left) contains 46 chromosomes: 22 pairs of autosomes and the X and Y sex chromosomes. The labeling scheme is shown below the line indicating the chromosome number. Circles on the left side represent the false colors generated by the software, while squares on the right side display the specific fluorochrome combinations used to paint each individual chromosome.

The macaque genome consists of 42 chromosomes: 20 pairs of autosomes and the X and Y sex chromosomes. Figure 2, right illustrates the automatic sorting of *Macaca mulatta* chromosomes performed by the ISIS software using the human mFISH painting template. Evidently, some chromosomes are arranged in their proper places, whereas others are clearly misclassified.

Figure 3 shows the reconstruction of the *Macaca mulatta* karyotype arranged according to the length and morphology of the chromosomes. Most macaque chromosomes (MMU) demonstrated homology to a single human chromosome (HSA). Specifically, 16 macaque chromosomes each hybridized to one human chromosome: MMU 1, 3, 4, 5, 6, 8, 9, 10, 11, 12, 14, 16, 17, 18, 19, and X. Thirteen of these, namely MMU 1, 3, 4, 5, 6, 8, 10, 11, 12, 17, 18, 19, and X, occupy corresponding positions on the karyogram, meaning they possess an HSA homolog with the same number (Figure 2, right).

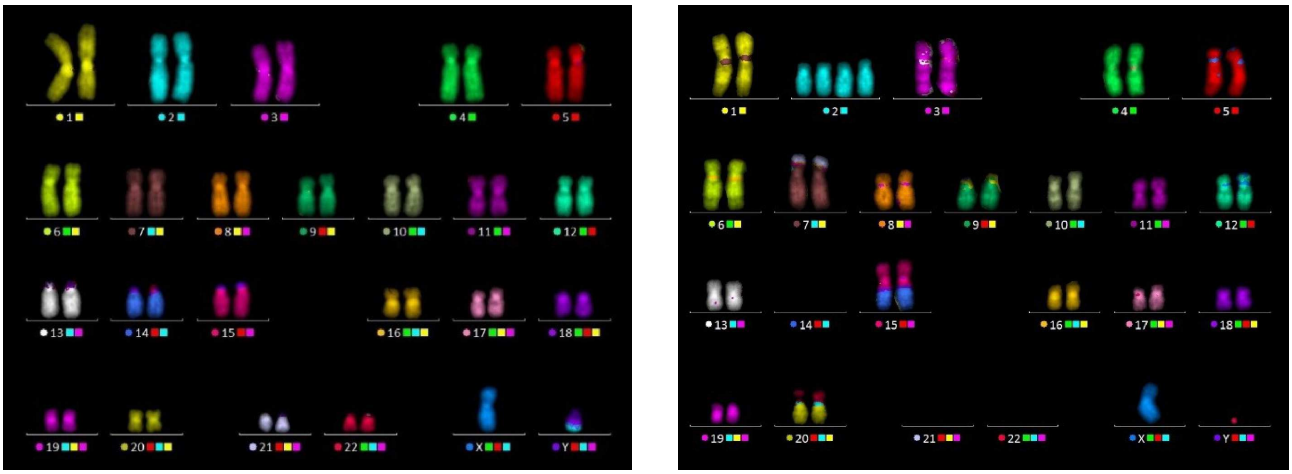

Figure 2. Left: Human karyotype, 46,XY (obtained from one of the healthy donors in [19]). Right: Automatic sorting of *Macaca mulatta* chromosomes according to the human mFISH painting template.

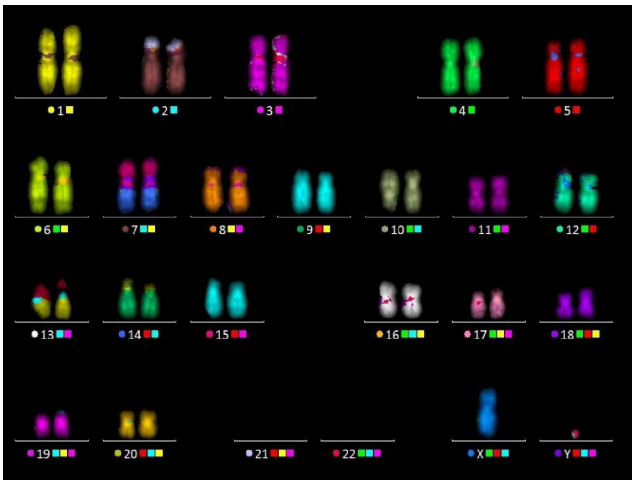

Figure 3. Our reconstruction of the *Macaca mulatta* karyotype arranged according to the length and morphology of the chromosomes.

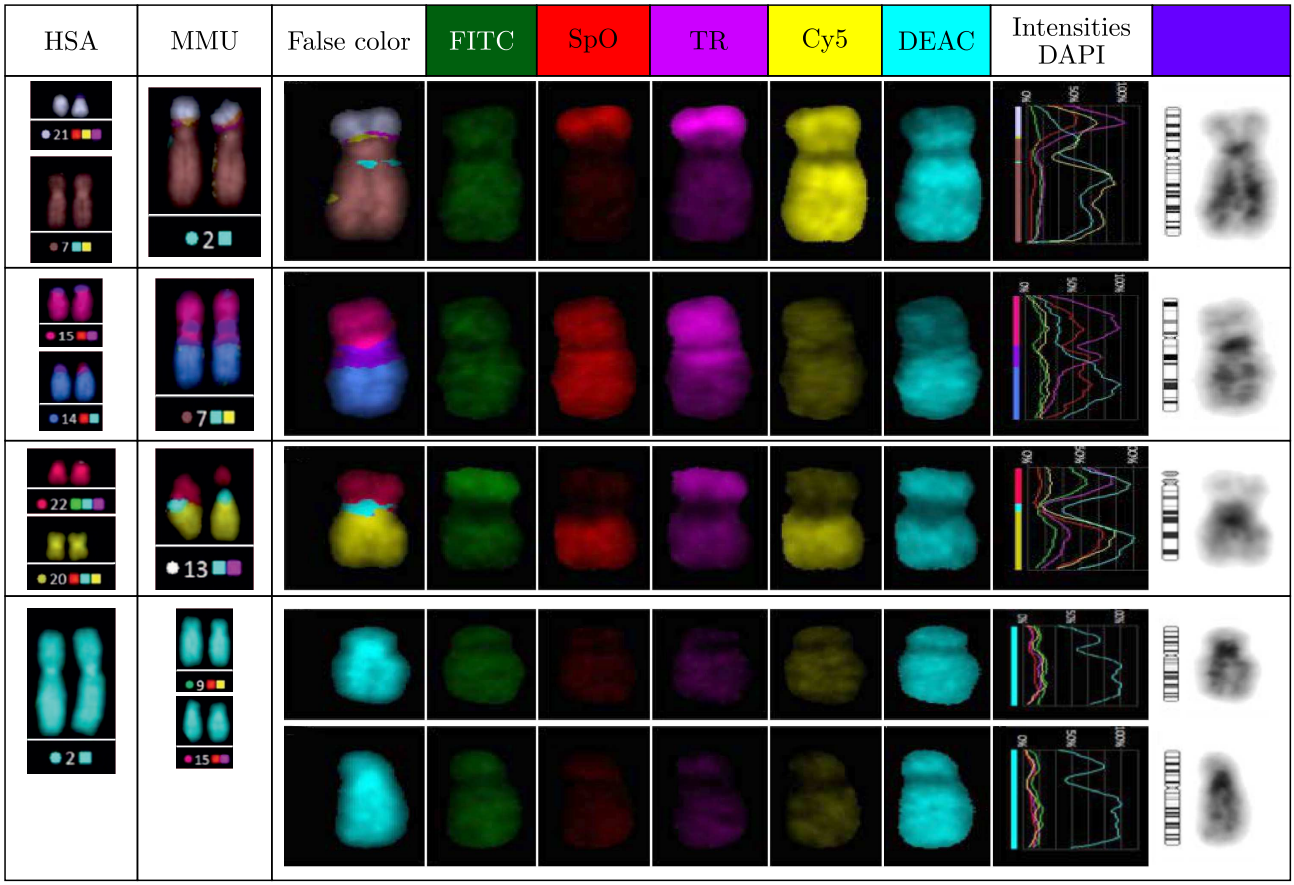

Figure 4. Profiles of three macaque chromosomes (MMU 2, 7, and 13) showing homology to two human chromosomes and two chromosomes (MMU 9 and 15) showing homology to one HSA 2.

Only three macaque chromosomes showed hybridization signals from two human chromosomes. First, rhesus macaque chromosome 2 (MMU 2) shares homology with two human chromosomes, HSA 7 and HSA 21: HSA 7 corresponds to the whole MMU 2q and proximal part of MMU 2p, and the distal part of MMU 2p is equivalent to HSA 21. Thus, it can be described as der(HSA7)t(HSA7,HSA21). MMU 7 is homologous to human chromosomes HSA 14 and HSA 15: MMU 7p and proximal part of MMU 7q correspond to HSA 15, and the distal part of MMU 7p corresponds to HSA 14. So, it could be described as der(HSA15)t(HSA15,HSA14). Finally, MMU 13 is homologous to human chromosomes HSA 20 and HSA 22, with HSA 20 being homologous to MMU 13q and HSA 22 — to MMU 13p, respectively (Figure 4).

In contrast, a single human probe, HSA 2, labeled two pairs of acrocentric monkey chromosomes, MMU 9 and MMU 15, which are homologous to HSA 2q and HSA 2p, respectively. Finally, the Y chromosome of macaque is very small; it has no specific colors and therefore definite place on karyogram. We put it manually on the Y place.

4. Discussion

To gain detailed insights into the differences between human and non-human primate chromosomes, banding techniques were widely used from the 1970s to the 1990s. At that time, it was already clear that the chromosomes of humans and other primates were very similar [2–5]. However, gene mapping and banding studies sometimes yielded contradictory results (see [6] and references therein). It remained unclear to what extent morphological resemblance cor-

responded to gene content — in other words, to what extent banding patterns reflected the genetic content of chromosomes.

The establishment of FISH techniques provided a new impetus for the study of human and primate genome evolution. The more advanced mFISH technique, which utilizes whole-genome painting, allows for the unequivocal detection of homology between human and macaque chromosomes based on both morphological similarities and gene content. Our reconstruction of the *Macaca mulatta* karyotype (Figure 3) is in good agreement with the description given by Wienberg et al. [9]. Their characterization was based on a combination of G-banding patterns and CISS hybridization experiments using flow-sorted human chromosomes (a prototype of mFISH), while also taking into account Estop's gene mapping data (see [6] and references therein) [9, 15].

We have demonstrated that most macaque chromosomes (16 out of 22) show homology to a single human chromosome, and 13 of them have an HSA homolog with the same number. Only three macaque chromosomes show homology to two human chromosomes: MMU 2 = HSA 7 + 21, MMU 7 = HSA 14 + 15, and MMU 13 = HSA 20 + 22. Notably, MMU 13 is known to segregate with a single rhesus nucleolar organizer region (NOR). Conversely, HSA 2 shows homology to two pairs of rhesus chromosomes, MMU 9 and MMU 15 (Figures 3, 4). The Y chromosome has no specific colors and therefore definite place on karyogram. Dutrillaux et al. [2] also mentioned the absence of specific bands on it.

Figure 5 illustrates our reconstruction of the macaque karyotype according to Dutrillaux et al. [2]. Obviously, several MMU chromosomes were misclassified by that group. Based solely on chromosomal morphological similarities (banding patterns obtained by nearly all possible banding techniques), they reported the homology of HSA 2 to MMU 13 (HSA 2q) and MMU 14 (HSA 2p). Even more serious errors concerned MMU 7 and MMU 9: according to their results, MMU 7 was a homolog of human chromosomes HSA 14 + 20 (where the MMU 7q arm corresponds to the whole HSA 14, and the MMU 7p arm — to the whole HSA 20). Furthermore, Dutrillaux et al. identified MMU 9 as a homolog of human chromosomes HSA 15 + 22, with HSA 15 being homologous to the MMU 9q arm and HSA 22 — to MMU 9p [2–5]. We must conclude, therefore, that chromosome comparison based solely on morphological similarities is insufficient for precise homology identification.

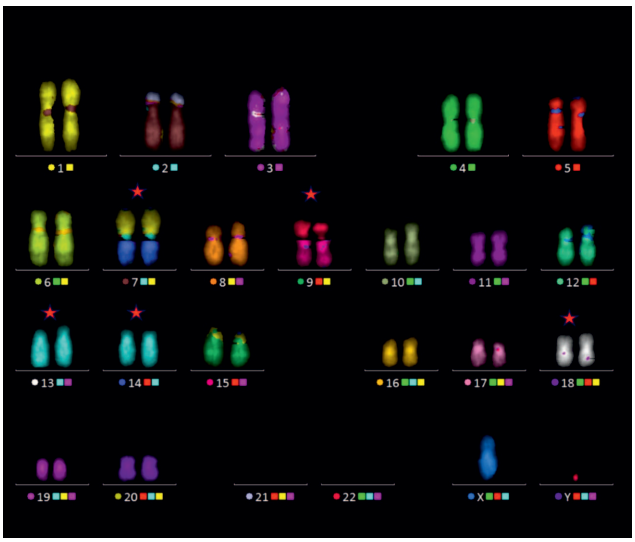

Figure 5. Our reconstruction of the *Macaca mulatta* karyotype based on the description by Dutrillaux et al. [2, 3]. Misclassified chromosomes are labeled with asterisks (⋆).

The most conflicting results in the literature concern the identification of the rhesus homolog for HSA 13: according to our data and Wienberg et al. [9], it is MMU 16; Kimura et al. [16] identified it as MMU 17; while Dutrillaux et al. [2] assigned it as MMU 18. Kimura's group is the only other team to have used human mFISH probes to date, specifically to study a trisomy of chromosome 17 in *Macaca fascicularis*. They claimed that the human chromosome 13 probe labeled three MMU 17 chromosomes.

To the best of our knowledge, we present here the first complete macaque karyotype obtained using 24XCyte mFISH probes. Although Kimura et al. utilized the same probe kit, no formal karyogram of *Macaca fascicularis* was presented in their paper — only a metaphase spread painted with the human probe.

Previous chromosomal studies of closely related primate species have shown that the karyotype does not evolve uniformly: some chromosomes remain highly conserved, while others have undergone gross morphological changes [3–5, 13, 15]. For example, the ancestral 14/15 association (MMU 7) is conserved in the rhesus macaque (*Macaca mulatta*, MMU). The evolutionary history of this ancestral 14/15 association is fascinating: a fission event, estimated to have occurred about 25 million years ago, gave rise to chromosomes HSA 14 and HSA 15. This event triggered the birth of two neocentromeres and the inactivation of the ancestral centromere. Similar fission events occurred within the ancestral associations 7/21 and 20/22. Interestingly, the human chromosome 2 (HSA 2) probe, which delineates two pairs of homologs in all non-human primates (including great apes), represents a key cytogenetic fusion event distinguishing the human genome from that of apes. The 14 chromosomes that possess an HSA homolog with the same number have been shown to be highly conserved across all primates and are assumed to be ancestral to the primate order [15, 17].

Altogether, mFISH, which is currently widely used at the Laboratory of Radiation Biology to study the cytogenetic effects of radiation [18, 19], is expected to become an indispensable tool in comparative chromosome studies and the most informative technique for investigating chromosome evolution.

Author contributions

E. Nasonova: Conceptualization, Methodology, Writing; N. Koshlan and I. Koshlan: Literature search, Reviewing and Editing.

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Conflicts of interest

The authors declare no conflicts of interest.

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