Note

In situ hybridization analysis of gibbon chromosomes suggests that amplification of alpha satellite DNA in the telomere region is confined to two of the four genera

Sudarath Baicharoen,[1, 2] Visit Arsaithamkul,[3] Yuriko Hirai,[1] Toru Hara,[1] Akihiko Koga [1] and Hirohisa Hirai [1]

[1] Primate Research Institute, Kyoto University, Inuyama City 484-8506, Japan

[2] Bureau of Conservation Research and Education, Zoological Park Organization, Bangkok 10300, Thailand

Running title: Alpha satellite DNA in the telomere region

Corresponding author: Hirohisa Hirai

E-mail: hirai.hirohisa.7w@kyoto-u.ac.jp

Phone: +81 568 63 0528

Fax: +81 568 63 0085

Abstract: The siamang *Symphalangus syndactylus*, a species of the family Hylobatidae (gibbons), carries large blocks of constitutive heterochromatin in the telomere region of chromosomes. We recently found that alpha satellite DNA constitutes these heterochromatin blocks as a main component. Alpha satellite DNA, tandem repeat sequences of 171-bp repeat units, is a major component of centromeres in primates. In addition to siamang, the white-cheeked gibbon *Nomascus leucogenys* was previously found to carry the alpha satellite DNA in the telomere region, although not as large a scale as the siamang. Gibbons comprise 4 genera: *Hoolock*, *Hylobates*, *Nomascus* and *Symphalangus*. Here we report that the amplification of alpha satellite DNA in the telomere region is probably confined to 2 genera: *Nomascus* and *Symphalangus*. We examined 1 species of *Hoolock* and 4 species of *Hylobates*, and obtained evidence against such an amplification event in these species. The phylogenetic relationship of the 4 gibbon genera remains unclear. One simple explanation for the current distribution of the telomere region alpha satellite DNA would be that *Nomascus* and *Symphalangus* are relatively closely related and the amplification occurred in their common ancestor.
Key words: heterochromatin, telomere, alpha satellite DNA, gibbon, hominoids

Some primate species carry large blocks of constitutive heterochromatin in the telomere regions of chromosomes. A prominent example is the telomere region heterochromatin of the chimpanzee *Pan troglodytes* and the bonobo *Pan paniscus* (Royle et al. 1994; Hirai et al. 2005; Koga et al. 2011; Ventura et al. 2012), which are the closest species to humans in extant primates. The main DNA component of their telomere region heterochromatin is tandem repeat sequences consisting of 32-bp long, AT-rich repeat units, which were named subterminal satellite (StSat) repeats (Royle et al. 1994). Heterochromatin blocks containing StSat repeats are also present in the gorilla *Gorilla gorilla* but are not found in humans. We demonstrated by statistical analysis of their sequence

variation that the StSat repeats were lost in the human lineage after the divergence of the chimpanzee/bonobo and humans (Koga et al. 2011). Another clear example of the telomere region heterochromatin is that of the siamang *Symphalangus syndactylus* (Wijayanto et al. 2005), a species belonging to the family Hylobatidae (gibbons). We have recently cloned and sequenced DNA fragments of the siamang heterochromatin and found that the main component is alpha satellite DNA (Koga et al. 2012). Alpha satellite DNA is a repetitive sequence known to be the primary component of centromeres in primates. The presence of alpha satellite DNA in telomere regions has also been reported in the white-cheeked gibbon *Nomascus leucogenys* (Cellamare et al. 2009), although not on such a large scale as to form visible heterochromatin blocks as C-bands.

For the StSat repeats of the great apes (chimpanzee, bonobo and gorilla), it is not known by what mechanisms these repeats were formed in a common ancestor and subsequently swept out of the human genome. The telomere region alpha satellite DNA of the gibbons (siamang and white-cheeked gibbon) is in an earlier stage of analysis; how they are distributed among the species or genera in the family Hylobatidae is not known. This family comprises 4 genera, which are *Hoolock*, *Hylobates*, *Nomascus* and *Symphalangus* (Wilson and Reeder 2005; Mootnick 2006). Of these, *Nomascus* and *Symphalangus* include species that carry telomere region alpha satellite DNA, as described above. In the present study, we focused on the other 2 genera. We conducted fluorescent *in situ* hybridization (FISH) analysis of chromosomes for the presence/absence of telomere region alpha satellite DNA, using as many available species as possible. All cell samples used in the present study were collected from animals bred at Primate Research Institute of Kyoto University or zoos administrated by the Zoological Park Organization of Thailand.

The probe used was the fosmid clone pFosSia1 that had been used in our previous analysis (Koga et al. 2012). The vector of this clone was pCC1FOS and its insert portion was a 38-kb DNA fragment that had been cloned from a

siamang genomic library and contained telomere region alpha satellite DNA. A nucleotide sequence of part of this clone was previously deposited in GenBank (accession number AB678729). The conditions of the FISH analysis were the same as those in our previous analysis, except that only the pFosSia1 clone was used as a probe, whereas 2 other clones for other purposes had been used together previously. Figure 1 shows the results of the FISH analysis of 8 gibbon species. Some of these species had been examined in our previous analysis by using other chromosome preparations, but we included these species in the present study for confirmation. We examined the hybridization patterns of more than 10 complete metaphase spreads for each species and confirmed that all these spreads showed identical patterns. *S. syndactylus* exhibited strong signals in the telomere regions of all chromosomes. *N. leucogenys* and *N. gabriellae* showed relatively weak signals in the telomere regions of most chromosomes. In contrast, species of the genus *Hoolock* and *Hylobates* did not exhibit detectable signals in the telomere regions. In all the samples we examined, signals were observed at the centromeres of some or most of the chromosomes. This is due to the ubiquitous presence of alpha satellite DNA in the centromeres, and these signals can be regarded as evidence of successful FISH reactions. The reason for the appearance in limited number of centromeres but not in all centromeres is considered to be a stronger specificity of the probe DNA for the telomere region alpha satellite DNA than for the centromere region alpha satellite DNA, as suggested in previous studies (Cellamare et al. 2009; Koga et al. 2012). In addition to the signals in terminal regions and at centromeres, interstitial signals were also observed on 2 chromosomes of *S. syndactylus* (panel a), 8 chromosomes of *N. leucogenys* (panel b), and 8 chromosomes of *N. gabriellae* (panel c). These interstitial signals are indicated by arrowheads and vary in intensity, including some strong signals.

We combined the results of our previous and present analyses, and listed those results of the terminal regions in Table 1. There was no contradiction

between the results of the 2 analyses. A commonly accepted classification of gibbons places 2 species in the genus *Hoolock* and 7 species in the genus *Hylobates* (Wilson and Reeder 2005; Mootnick 2006). Thus, the species we examined in the present study are about half the number of species of these genera. It is likely that the amplification of alpha satellite DNA in the telomere region is confined to *Nomascus* and *Symphalangus*. Gibbons are endangered species, and cell samples of the other species were not available for us to examine at the time of the study.

The mechanisms for the amplification of alpha satellite DNA in the telomere region and preceding migration from the centromere or possibly another source are unknown. One question to be solved to clarify the mechanisms is whether the telomere region alpha satellite DNA found in the 2 genera originate from a single amplification event. One approach may be a statistical analysis of sequence variation, similar to the one we conducted with the StSat repeats of great apes (Koga et al. 2011). This is, however, not directly applicable to the telomere region alpha satellite DNA because sequence information collected from genome sequence databases also contains the sequences of canonical alpha satellite DNA in the centromeres.

The phylogenetic relationship of the 4 gibbon genera remains unclear. One simple explanation for the presence of telomere region alpha satellite DNA in *Nomascus* and *Symphalangus* and the probable absence in *Hoolock* and *Hylobates* would be that the former 2 genera are relatively closely related and the amplification occurred in their common ancestor.

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Table 1. FISH results at terminal regions.

Genus	Species [a]	Koga et al. (2012) [b]	Present study [b]
Hoolock	*hoolock*	-	0
	[1]	-	-
Hylobates	*agillis*	0	0
	lar	0 *	0
	muelleri	-	0
	pileatus	-	0
	[3]	-	-
Nomascus	*gabriellae*	-	+
	leucogenys	+ *	+
	[3]	-	-
Symphalangus	*syndactylus*	++	++

[a] Numbers in brackets indicate those of the other species within each genus according to the classification by Wilson and Reeder (2005) and Mootnick (2006).

[b] 0, +, and ++ indicate no, weak, and strong signals in the telomere region, respectively. The 2 asterisks indicate that a hybrid animal of these 2 species was examined.

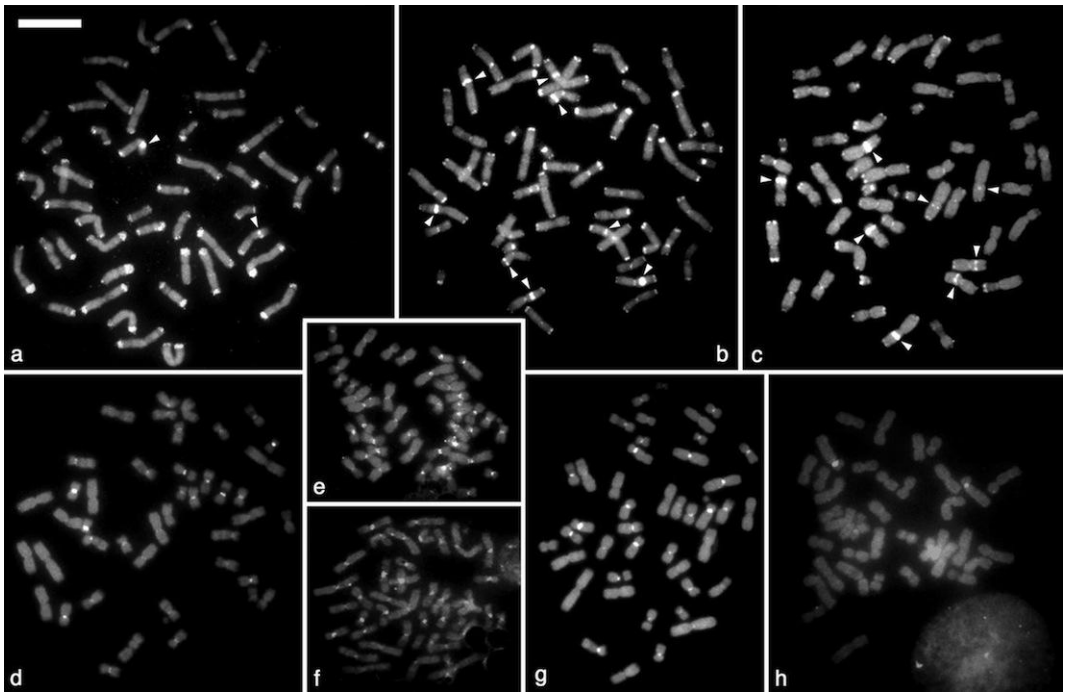

Fig. 1. Photographs of FISH analysis. The probe was labeled with biotin and bright regions are positive for the probe. The contrast was enhanced by using PhotoShop. The bar represents 10 μm. The arrowheads indicate signals detected in interstitial regions. **a**. *Symphalangus syndactylus*, female. **b**. *Nomascus leucogenys*, female. **c**. *Nomascus gabriellae*, male. **d**. *Hylobates lar*, male. **e**. *Hylobates agilis*, male. **f**. *Hylobates muelleri*, male. **g**. *Hylobates pileatus*, male. **h**. *Hoolock hoolock*, female.