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Intrachromosomal telomere-like DNA sequences in Chinese hamster

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The simple repetitive sequence (TTAGGG)n constitutes the telomeres of vertebrate chromosomes. In several species, telomeric repeats have been observed at intrachromosomal sites (Meyne et al. 1990). It has been proposed that interstitial (TTAGGG)n sequences may be prone to fragility and recombination and that breakage within such repetitions may produce telomere-like structures that stabilize the ends of rearranged chromosomes (Ijdo et al. 1991; Meyne et al. 1990). This hypothesis is supported by the cytogenetic correlation between induced breaks and intrachromosomal telomeric sites in Chinese hamster cell lines (Alvarez et al. 1993; Balajee et al. 1994). Furthermore, it has been shown that the recombination events that accompanied *CAD* gene amplification frequently involved (TTAGGG) repetitions (Bertoni et al. 1994) and that the introduction of human telomeres into rodent or human cells could induce chromosome fragmentation (Farr et al. 1991; Barnett et al. 1993).

In Chinese hamster the precise localization of (TTAGGG)n sequences has not been analyzed. In this paper we report the chromosomal localization of these sequences in primary diploid fibroblasts, and we show that they map at telomeric, centromeric and interstitial sites. The interstitial sequences, which have not been described before in Chinese hamster, contain a relatively small number of repeats and may derive from telomere fusion or centromere inactivation of ancestral chromosomes. Moreover, we have observed that spontaneous breaks, occurring during the senescence crisis of primary fibroblasts, are located preferentially within chromosome bands containing (TTAGGG)n repeats. We then mapped the (TTAGGG)n sequences in a CHO-K1 derived cell line and showed that the chromosomes can be identified without banding. New information about the origin of some marker chromosomes has been obtained.

Metaphase chromosomes from a Chinese hamster (*Cricetulus griseus*) primary fibroblast cell line (CHL) were hybridized *in situ* with a (TTAGGG)n probe and G-banded after hybridization according to the methods previously described (Bertoni et al. 1994). The probe was a mixture of biotinylated synthetic (TTAGGG)n fragments with a size range between 1 and 20 kb. The localization of hybridization signals has been analyzed on 37 metaphases, which were obtained from cells passaged 8 times. In Fig. 1a two examples from each chromosome pair are shown. The karyotype of CHL cells is identical to the normal Chinese hamster karyotype, which has been described (Ray and Mohandas 1976; Simi et al. 1988). Briefly, 11 chromosome pairs are present: 9 metacentric or submetacentric (1, 2, 3, 4, 8, 9, 10, X, and Y) and 3 acrocentric pairs (5, 6, and 7).

All chromosomes show hybridization signals at both ends, indicating that long stretches of (TTAGGG)n repetitions are present at the telomeres. A subterminal localization of the telomeric signal, which is observed in some CHL chromosomes of Fig. 1a, has been previously described by others and seems to be due to chromosome condensation (Moyzis et al. 1988).

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Strong hybridization signals were always present at the centromere of Chromosomes (Chrs) 3, 4, 5, 6, 7, 8, 10, and X (Fig. 1a). These signals were very intense, covering the pericentromeric regions of the metacentric and submetacentric chromosomes or the entire short arm of the acrocentric chromosomes. The centromere of Chr 9 was also always labeled, but the signal was consistently less intense, indicating that a reduced number of repeats is present in this location or that homology with the probe is reduced. The centromere of Chr 2 showed specific hybridization signals (double spots) on only 27 of the 74 analyzed chromosomes (Fig. 2) suggesting that the number of repeats is greatly reduced compared to the heavily labelled centromeres. The observation of double spots on the centromere of two Chrs 1 only (Fig. 2) suggests that a small number of repetitions may be localized in this region. The centromere of Chr Y lacks detectable telomere-like repetitions. Several observations have suggested that repetitive sequences may be involved in centromere function (Tyler-Smith and Willard 1993). It remains to be established whether in the Chinese hamster TTAGGG repeats play a role in the functional organization of centromeric DNA.

Specific signals have been observed at several interstitial (non-telomeric, noncentromeric) locations (Fig. 1); the intensity of these signals is greatly reduced compared with those located around the heavily labeled centromeres. The distribution of double spots in the 37 metaphases is shown in Fig. 2. The frequency of hybridization at the different sites is variable. Six or more specific signals have been observed on seven locations (1q17, 1q22, 1q26, 1q36, 3p21, 4q16, Yq13), and the most frequent sites are on 1q17 and 1q26. Some sites have hybridized specifically with the probe four to five times (1p24, 1p15, 1q32, 2p31, 2p16, 2q24, 3q31, Xp21), and other sites showed one to three specific signals only. As the signal to background ratio was very good, we can propose that even the rare sites probably contain telomere-like repeats. In conclusion, Chinese hamster chromosomes are characterized by a high number of intrachromosomal (TTAGGG)n sites. In addition, it is possible that short stretches of telomeric repeats, which would be undetectable with *in situ* hybridization, are present in the genome.

Non-telomeric (TTAGGG)n sites may derive from chromosome fusions at the telomeres. A well-characterized example is human Chr 2, where the telomeric sequences in 2q13 mark the fusion point of two ancestral ape chromosomes (Ijdo et al. 1991). The analysis of G-banded chromosomes from several hamster species has suggested that Chinese hamster Chr 1 was derived from telomere fusion of ancentral submetacentric chromosomes (Gamperl et al. 1976; Yerganian 1985), followed by inactivation of one of the centromeres. The fusion point and the inactivated centromere are localized in 1q17 and in 1q26 respectively and correspond to frequent sites of hybridization with the (TTAGGG)n probe (Fig. 2). This observation is in agreement with the hypothesis that some interstitial sites were derived from telomere fusions or from inactivated centromeres.

(TTAGGG)n sequences were also localized on the chromosomes of a CHO-K1-derived cell line (CHO-PV). The karyotype of these cells has been previously described (Bertoni et al. 1993)

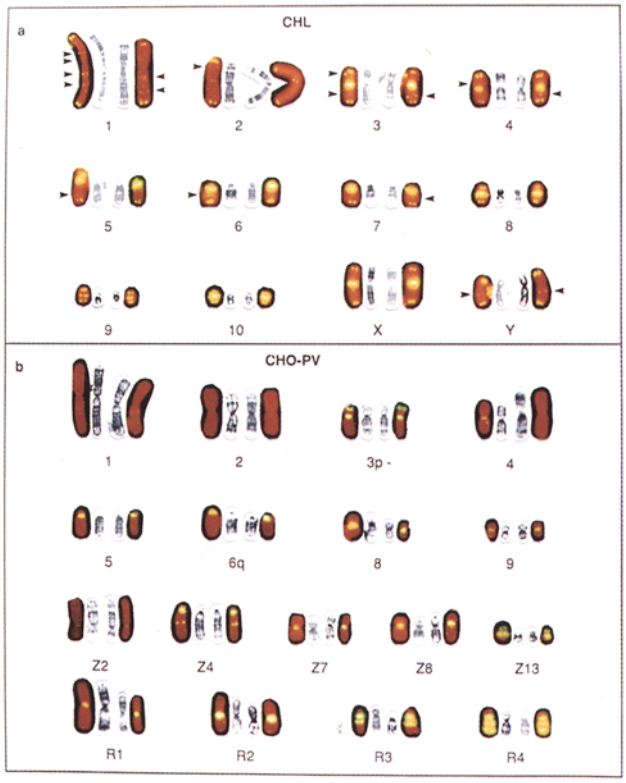

Fig. 1. Chromosomal localization by fluorescence *in situ* hybridization of (TTAGGG)n sequences on the chromosomes of the primary fibroblast cell line CHL **(a)** and of the CHO-PV cell line **(b).** Two examples of each chromosome, derived from two different metaphases, are shown after *in situ* hybridization and G-banding. Arrows point to some intrachromosomal sites of hybridization with CHL chromosomes.

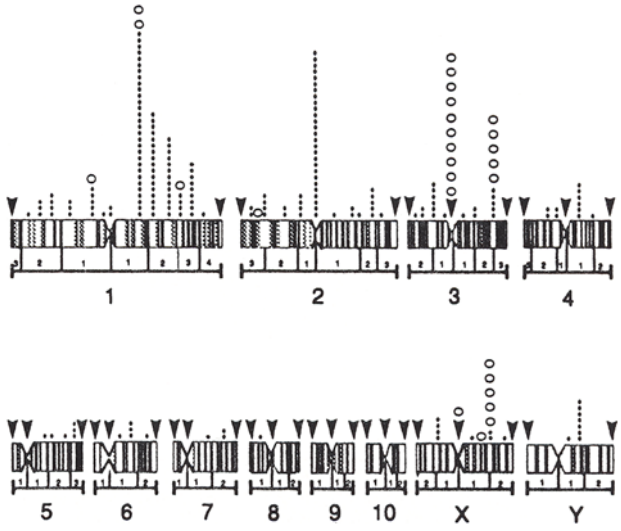

Fig. 2. Distribution of hybridization signals and of break points on G-banded CHL chromosomes. The localization of (TTAGGG)n hybridization signals has been analyzed in 37 metaphases obtained from low-passage cells. Arrows indicate telomeric and centromeric sites showing hybridization signals on all chromosomes. Each dot represents one positive hybridization signal on both chromatids (double spot). The localization of break points has been performed on 140 high-passage cells and derives from the analysis of breaks, translocations, deletions, triradials, and dicentrics. Each open circle represents one break point.

and includes eight unrearranged chromosomes (one pair of Chr 1, one pair of Chrs 5, one Chr 2, one 4, one 8, and one 9), two chromosomes with simple rearrangements (3p- and 6q), five rearranged chromosomes of the Z set, which have been already described in CHO-K1 cells (Siciliano et al. 1985), and four chromosomes of the R set, which are typical of this cell line.

Sixty-four CHO-PV metaphases were analyzed, and in Fig. 1b two examples of each chromosome are shown. The ends of most chromosomes do not show any hybridization signal, and a weak telomeric signal could be observed on a small random fraction of the chromosomes. This observation confirms that the telomeric repeats are shorter in the CHO-PV than in the CHL chromosomes (Bertoni et al. 1994; Balajee et al. 1994). However, three marker chromosomes always show a strong specific signal on one end. Chr 3p- was labeled at the short arm end; the intensity of this signal was similar to that observed at the heavily labeled centromeres. Chrs R4 and Z13 showed a strong signal at the long and short arm telomere respectively.

Similarly to CHL cells, the strongest hybridization signals were found in the pericentromeric regions of most chromosomes. The centromeres of Chrs 3p-, 5, 6q, 8, Z4, Z7, Z8, Z13, R1, R2, R3, and R4 were intensely labeled in all metaphases, while a weaker signal was always present on the centromere of Chrs 9 and 4. A specific signal at the centromere of Chr Z2 was found in 17 of the 64 metaphases, while the centromere of Chrs 1 and 2 was labeled in two and three metaphases respectively.

In CHO-PV, as in CHL cells (Fig. 2), weak interstitial signals were present in a fraction of the chromosomes (data not shown). Furthermore, in CHO-PV cells three marker chromosomes (Z4, R3, and R4) showed intense interstitial signals in all metaphases (Fig. 1b).

The localization of telomere-like sequences has provided new information on the origin of some marker chromosomes. It had been previously hypothesized (Deaven and Petersen 1973; Siciliano et al. 1985) that chromosome Z4 was derived from a pericentric inversion of Chr 3. While confirming this hypothesis, we now propose that the interstitial TTAGGG repeats within the long arm of Chr Z4 (Fig. 1b) are located at the site of breakage and fusion and that these repetitions were probably derived from the original telomere of Chr 3. On the basis of G-banding it had been proposed that Chr 3p- was derived from a terminal deletion of Chinese hamster Chr 3 (Bertoni et al. 1993). However, the presence of a strong hybridization signal at the short arm telomere suggests an alternative hypothesis: the short arm of Chr 3p- could have arisen from a complex rearrangement involving centromeric sequences. A precise molecular characterization will be necessary to define the origin of the short arm of Chr 3p-. The telomeric ends of R4q and of Z13p are also heavily labeled, suggesting that they were derived from breakage within pericentric (TTAGGG)n DNA. Simple addition of telomeric repeats to terminally deleted chromosomes has been described (Wilkie et al. 1990). However, it seems unlikely that large blocks of repeats such as those observed at the end of 3p-, R4q, and Z13p would have been added by a telomerase activity that, on the other hand, maintains very short telomeres at all other termini.

Combining the analysis of chromosome morphology and of (TTAGGG)n hybridization patterns, it is possible to identify each chromosome of the CHO-PV cell line (Fig. 1b). Small metacentric chromosomes such as 8, 9, and Z13 can be easily recognized because Chr 8 shows an intense centromeric signal; a consistently fainter signal is present at the centromere of Chr 9, while Chr Z13 is characterized by two regions of hybridization (centromere and short arm telomere). The middle-sized metacentrics 4 and R2 can be identified on the basis of signal intensity in the centromeric region, which is always particularly strong on Chr R2. The small submetacentric Chrs R3 and R4 have similar morphology and show two and three strong hybridization signals respectively, which make them easily identifiable.

The chromosomal localization of break points and TTAGGG

repeats was analyzed in 140 metaphases of CHL fibroblasts. The chromosome preparation was obtained from high passage cells, which were entering a senescence crisis. In these cells chromosomal aberrations were more frequent than in nonsenescent cells and included chromatid and chromosome breaks, translocations, deletions, triradials, and dicentrics. Twenty-five break points out of 27 were localized within bands containing TTAGGG repeats (Fig. 2). The most frequent break site was the centromeric region of Chr 3, where a fragile site has been mapped (Simi et al. 1990). The break generates a 3q Chr. In all 3q Chrs observed, a large amount of (TTAGGG)n sequences of centromeric origin has been retained at the new end. Given the resolution limits of FISH and G-banding, it is possible to conclude that spontaneous chromosomal breaks may preferentially occur within interstitial telomeric DNA or adjacent sequences. A similar correlation has been previously observed with radiation and with restriction enzyme-induced breaks (Alvarez et al. 1993; Balajee et al. 1994). Ten of the 11 fragile sites, which have been mapped so far in Chinese hamster (Simi et al. 1990; Kuo et al. 1994; Slijepcevic and Natarajan 1995), are localized within bands where we have observed interstitial repeats. However, it must be pointed out that the correlation between chromosomal fragility and (TTAGGG)n sites is only based on cytogenetic data and needs to be proved at the molecular level. Chromosomes derived from centric fissions may be particularly frequent because large numbers of TTAGGG repeats at the site of breakage could contribute to stabilization of the end by providing a template for synthesis of a new telomere.

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