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# Refined localization of the asparagine synthetase gene (ASNS) to chromosome 7, region q21.3, and characterization of the somatic cell hybrid line 4AF/106/KO15

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**Abstract.** We have mapped the asparagine synthetase gene (ASNS) to 7q21.3 by fluorescence in situ hybridization. While this study refined the localization of the gene, it also revealed a rearrangement in a somatic cell hybrid line which was used in previous ASNS mapping. Using additional probes from other regions of human chromosome 7, we showed that this cell line (4AF/106/KO15) contained a rearranged chromosome 7 in which a segment of the long arm was apparently duplicated and inserted into the short arm. Caution should be used therefore when interpreting data obtained from this cell line for gene mapping studies.

Asparagine synthetase (AS) is a house-keeping enzyme responsible for the biosynthesis of asparagine in mammalian cells. Cells lacking AS activity require an exogenous supply of this amino acid. The gene encoding AS (ASNS) was mapped to human chromosome 7 through karyotype analysis of somatic cell hybrids made from human B lymphocytes and a AS-deficient Chinese hamster ovary cell line following growth in asparagine-free medium (Arfin et al., 1983). With additional data from somatic cell hybrids containing reduced amounts of chromosome 7 material, a tentative regional assignment for ASNS was made to include the region around the centromere of chromosome 7 (Arfin et al., 1983). The investigators noted, however, that three of five subclones of the original fusion experiment contained rearranged or deleted chromosomes 7. It was further noted that rearrangements of donor chromosomes could be particularly high in a CHO background (Fournier and Moran, 1983).

A nearly full-length cDNA for ASNS was isolated by means of expression cloning (Andrulis et al., 1987) and the molecular structure of the gene was determined (Zhang et al., 1989). Regional localization of ASNS was only performed, however, following the isolation of the ts11 gene, which complemented a temperature sensitive mutation that blocks the G1 phase of the cell cycle (Greco et al., 1989). DNA sequence analysis showed that ts11 corresponded to the previously reported ASNS gene. A 1.7-kb genomic DNA segment containing a portion of the ts11 gene was used to probe a panel of rodent × human somatic cell hybrids to regionally localize ASNS to 7cen→q35 and then used with in situ hybridization to refine the location to 7q21→q31 (Greco et al., 1989). To obtain an independent regional assignment for ASNS, we performed FISH on banded human chromosomes using a genomic DNA clone containing exons 4–11 of the gene (Zhang et al., 1989). We also analyzed one of the somatic cell hybrid lines which were used in the previous pericentric localization of ASNS (Arfin et al., 1983).

## Materials and methods

*Probes*

Phage clone M114 contained a 12-kb genomic DNA fragment spanning exons 4–11 of the gene (Zhang et al., 1989). Cosmid clone cW10-20 contained a 36-kb genomic DNA fragment containing exons 4–7 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene (Rommens et

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al., 1989). The α-satellite probe specific to D7Z1 located at the human chromosome 7 centromere region was purchased from Oncor. The YAC clone HSC7E662 (280 kb) containing the ASNS locus was isolated from a chromosome 7-specific YAC library with the AS-2 probe (Scherer et al., 1993). HSC7E43 (280 kb) was a YAC clone isolated with the Lam4-917 probe (D7S15) from the 7q21 region (Scherer et al., 1993).

Cosmid DNA was prepared from *E. coli* hosts and purified using the QIAGEN column according to the manufacturer's instructions. Phage DNA was prepared by standard techniques (Grossberger, 1987). Total yeast DNA was isolated with the glassbead method as described (Scherer and Tsui, 1991). The purified DNA was labeled with biotinylated dATP using the BioNick kit (BRL) for 1 h at 15 °C (Heng et al., 1992). Longer labeling time was used for the YAC clones (2 hr at 15 °C). The labeled probes were purified with the use of Nick Columns (Pharmacia).

*Chromosome preparation*

Lymphocytes were prepared from human cord blood as described (Heng et al., 1993). Chromosomes were prepared by BrdU incorporation, hypotonic treatment, fix and air dry method (Lin, et al., 1985; Heng and Tsui, 1993). For propagation of somatic cell hybrid line 4AF/106/KO15, selective medium αMEM minus asparagine supplemented with 10% fetal calf serum was used. After growing in the selective medium for 2–3 days, the semiconfluent culture was trypsinized and split 1:2. The next day, Colcemid (0.1 μg/ml) was added for 30 min and the cells were harvested. The slides were made after hypotonic treatment (0.4% KCl for 10 min at 37 °C) and routine fixing in methanol:acetic acid (3:1).

*In situ hybridization and detection*

For each slide, 50 ng of biotin-labeled phage, or cosmid probes, or 200 ng of labeled total yeast DNA including the YAC clone were used. The probes were mixed with human Cot1 DNA (2 μg) in 13 μl of hybridization buffer (50% formamide, 2 × SSC and 10% dextran sulphate), denatured at 75 °C for 5 min, and then prehybridized at 37 °C for 15 min before transfer to the slides (Heng et al., 1992). Total yeast DNA (2 μg sonicated into 100–500 bp fragments) was added before probe hybridization when YAC probes were used. To prepare the slides for hybridization, they were submerged in 70% formamide at 70 °C for 1 min, dipped in ethanol and air dried. Hybridization was carried out for 16–36 hr. The slides were then washed with 50% formamide in 2 × SSC and 2 × SSC alone at 46 °C. The probes were detected by incubation with FITC-avidin. The FISH signal was further amplified by incubation with biotinylated goat-antiavidin followed by a second round of FITC-avidin as described (Heng and Tsui, 1993a and 1993b).

*Microscopy*

A Leitz-Aristoplan epifluorescent microscope with a DAPI filter and FITC filter was used for slide examination and photography. Kodak Ectachrome P800/1600 film was used with the exposure pushed to 3200 ASA (exposure times: DAPI 0.1–1 s, PI and/or FITC 4–7 s). The FISH signals were localized to the appropriate chromosome band by superimposing the FITC-PI image and the DAPI image.

## Results and discussion

We examined 108 mitotic figures for positive hybridization signals after hybridizing with the ASNS genomic DNA probe (M114). Seventy-two (67%) showed fluorescent spots on both sister chromatids of chromosome 7. Pictures of 25 mitotic figures were taken and the regional localization of the hybridization signals was determined by superimposing the image with DAPI-banding patterns (Fig. 1A, B). Chromosomes showing hybridization with only one of the two sister chromatids and those with unclear DAPI-banding patterns were not scored. As illustrated in Fig. 2, our data showed that ASNS is most likely located in band 7q21.3. This localization was also confirmed by data obtained with HSC7E662, a YAC clone containing ASNS (Fig. 3A, B). In contrast to the previous report (Greco et al., 1989), we did not observe any specific hybridization signals elsewhere in the genome but this discrepancy could be explained by differences in the genomic DNA probes used.

Our mapping data, therefore, confirmed the assignment of ASNS to 7q and improved its regional localization to band 7q21.3. This conclusion obviously raised concern about the previous implication of a centromeric localization for ASNS (Arfin et al., 1983), especially since subclones of the somatic cell hybrid lines generated from that study had subsequently been used in mapping of many other DNA sequences on chromosome 7 (Zengerling et al., 1987; Rommens et al., 1988).

To investigate the latter problem, we prepared metaphase chromosome spreads for one of the subclones of the hamster-human cell hybrid 4AF (Arfin et al., 1983; 4AF/106/KO15 provided to us by Leon Carlock) and probed them with M114. The karyotype of the cell line has always been suspicious but the extent of rearrangement was thought to be small. As shown in Fig. 3D, we noticed that the only human chromosome detected in the mitotic figure contained a clearly discernable chromosome 7 long arm (indicated by arrow a). The short arm was cytogenetically unrecognizable, indicating a possible rearrangement of 7p. We were surprised by the presence of two locations of fluorescent signals on the hybrid human chromosome 7 (Fig. 3C). One signal was located on the long arm at 7q21, as expected for hybridization of the probe to the ASNS locus, but the second signal was found near the centromere on the short arm. The same hybridization pattern was observed with the use of YAC clone HSC7E662 (not shown).

Since the two sets of hybridization signals in 4AF/106/KO15 showed similar intensities with both phage and YAC probes, we concluded that the extra sequence was derived from duplication of ASNS and its surrounding sequences. To determine the extent of this duplication, additional genomic DNA clones derived from 7q were then used as probes for FISH analysis. One of the probes was a cosmid clone (cW10-20) containing exons 4–7 of the CFTR gene located at 7q31 (Heng et al., 1993). Another was a YAC clone (HSC7E43) also mapped to 7q21 in this study. As shown in Fig. 3 (panels E, F, G and H), both probes showed hybridization signals in two regions of the hybrid's human chromosome. The pattern for HSC7E43 was similar to that of ASNS with the extra signal on the short arm near the centromere, as could have been predicted by the close proximity of the two loci on 7q. However, the location of the extra FISH signal for cW10–20 was near the center of the rearranged short arm. The distance between the two sets of signals, from ASNS and HSC7E43 to cW10-20 was approximately equal, suggesting that the entire chromosome segment between these loci had been duplicated and inserted into the short arm.

Since the duplicated HSC7E43 and ASNS loci appear to be extremely close to the centromere of the rearranged chromosome in 4AF/106/KO15, we next examined an α-satellite repeat sequence (D7Z1) located at the centromeric region using a probe specific for human chromosome 7. While strong and uniform FISH signals were observed at the 7cen region for lymphocytes from six unrelated donor individuals, a relatively weak signal intensity was consistently noted for the hybrid (Fig. 3I, J). Since only a small number of individuals were

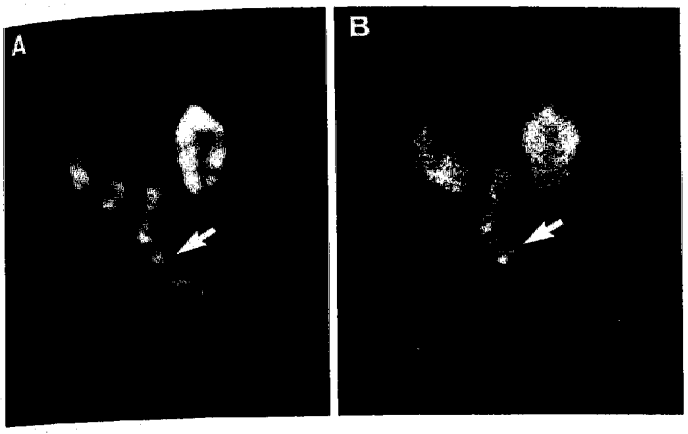


**Fig. 1.** Hybridization of ASNS probe M114 to banded human chromosome 7. (**A**) DAPI banded chromosome 7; (**B**) FITC signals on each sister chromatid.

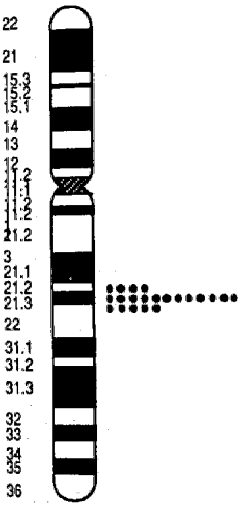


**Fig. 2.** FISH analysis of ASNS (probe M114) to human chromosome 7. Each dot represents a double fluorescent signal detected on chromosome 7.

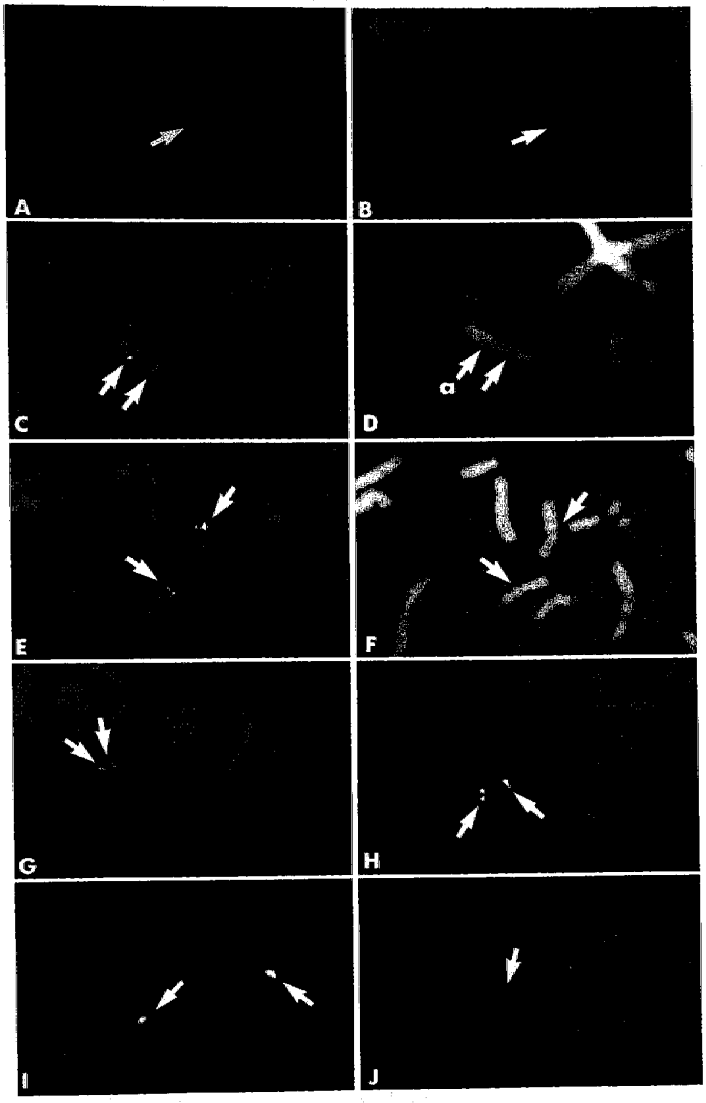


**Fig. 3.** FISH analysis of hybrid cell line 4AF/106/KO15 with various genomic DNA probes. (**A, B**) Localization of an ASNS-containing YAC clone (HSC7E662) to the 7q21→22 region of a human lymphocyte chromosome 7; (**C**) hybridization of the ASNS probe (M114) to the hybrid's human chromosome 7 (note the presence of two FISH signals, one at the expected 7q21 region and the other in the short arm near centromere); (**D**) DAPI-staining pattern of the mitotic figure in (**C**), arrow (a) also indicates the long arm of the hybrid chromosome 7; (**E, F**) localization of YAC clone HSC7E43 on normal human lymphocyte chromosome 7; (**G**) hybridization pattern of HSC7E43 to the hybrid's human chromosome 7; (**H**) hybridization pattern of the CFTR cosmid probe cW10-20 to the hybrid's human chromosome; (**I**) localization of the α-satellite probe for D7Z1 on normal human lymphocyte chromosome 7; and (**J**) hybridization pattern of the α-satellite probe in the hybrid (note the weak signal intensity).

examined, it remained unclear if the rearrangement affected the D7Z1 locus or the weak intensity merely represented a variation of the sequence organization of the original human B cell line (8866) used for the construction of the cell hybrid. It should be noted that the relatively short array of α-satellite repeat sequences at the D7Z1 locus in this hybrid cell line was also observed in a long range physical mapping study (Wevrick and Willard, 1991).

Taken together, the simplest interpretation we could offer to explain the FISH results for 4AF/106/KO15 is shown in Fig. 4. A segment of 7q spanning the genomic DNA sequence for HSC7E43, ASNS and CFTR had apparently been inserted into the short arm of chromosome 7 contained in this cell line. One boundary of this duplicated sequence appeared to be closely proximal to the HSC7E43 sequence and the other distal to CFTR. The insertion of a portion of 7q into the short arm was accompanied by a partial loss of 7p material. It was noted that several known short arm markers were absent from 4AF/106/KO15 and that short arm sequences were generally under-represented in a YAC library made from this cell line (Scherer et al., 1991; data not shown). The extent of the deletion was not determined but the deletion might extend into the

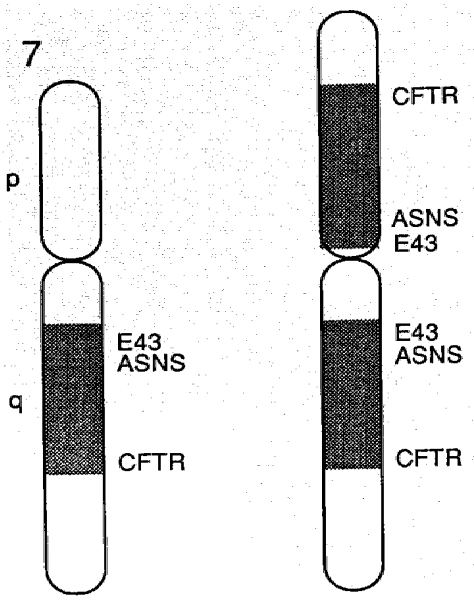


**Fig. 4.** Schematic diagram explaining the chromosome 7 rearrangement detected in human-hamster hybrid 4AF/106/KO15.

centromeric region. The loss of 7p material had also been reported for another subline of the somatic cell hybrid 4AF (Arthur Beaudet, personal communication).

It is of interest to note that 4AF/106 was isolated at the same time as two other somatic cell hybrid lines, 1CF which appeared to retain 7cen→qter and 1EF which contained a centric chromosome fragment (Arfin et al., 1983). The tentative assignment of ASNS to the centromeric region of chromosome 7 was primarily based on the persistence of AS activity in these hybrid lines. Since all three cell lines have been under biochemical selection for ASNS activity for many generations, it is possible that rearrangement has occurred and selected for the retention of both ASNS and the centromere. While this and other assumptions may be tested with the use of the 1CF and 1EF hybrid cell lines as well as the original human B cell line (8866), caution is raised for mapping studies involving the use of cell lines derived from 4AF, 1CF and 1EF.

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## References

Andrulis IL, Chen J, Ray PN: Isolation of human cDNAs for asparagine synthetase and expression in Jensen rat sarcoma cells. Mol cell Biol 7:2435–2443 (1987).

Arfin SM, Cirullo RE, Arredondd-Vega FX, Smith M: Assignment of structural gene for asparagine synthetase to human chromosome 7. Somat Cell Genet 9:517–531 (1983).

Fournier RE, Moran RG: Complementation mapping in microcell hybrids: localization of *Fpgs* and *Ak-1* on *Mus musculus* chromosome 2. Somat Cell Genet 9:69–84 (1983).

Greco A, Ittmann M, Barletta C, Basilico C, Croce CM, Cannizzaro LA, Huebner K: Chromosomal localization of human genes required for G1 progression in mammalian cells. Genomics 4:240–245 (1989).

Grossberger D: Minipreps of DNA from bacteriophage lambda. Nucl Acids Res 15:6737 (1987).

Heng HHQ, Squire J, Tsui L-C: High-resolution mapping of mammalian genes by in situ hybridization to free chromatin. Proc natl Acad Sci, USA 89: 9509–9513 (1992).

Heng HHQ, Shi X-M, Tsui L-C: Fluorescence in situ hybridization mapping of the cystic fibrosis transmembrane conductance regulator (CFTR) gene to 7q31.3. Cytogenet Cell Genet 62:108–109 (1993).

Heng HHQ, Tsui L-C: Modes of DAPI banding and simultaneous in situ hybridization. Chromosoma 102:325–332 (1993).

Heng HHQ, Tsui L-C: FISH detection on DAPI banded chromosomes, in Choo KHA. (ed): Methods of molecular biology: in situ hybridization protocols, Vol 10 (Humana Press, Clifton, New Jersey 1993, in press).

Lin CC, Draper PN, Braekeleer M: High resolution chromosomal location of β gene of the human β globin gene complex by in situ hybridization. Cytogenet Cell Genet 39:269–274 (1985).

Rommens J, Iannuzzi MC, Kerem BS, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N, Zsiga M, Buchwald M, Riordan JR, Tsui L-C, Collins FS: Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 245:1059–1065 (1989).

Scherer SW, Tsui L-C: Cloning and analysis of large DNA molecules, in Adolph K (ed): Advanced Techniques in Chromosome Research, pp 33–72 (Marcel Dekker, New York 1991).

Scherer SW, Tompkins BJ, Tsui L-C: A human chromosome 7–specific genomic DNA library in yeast artificial chromosomes. Mammal Genome 3:179–181 (1991).

Scherer SW, Rommens JM, Soder S, Plavsic N, Tompkins B, Wong E, Beattie A, Kim J, Tsui L-C: Refined localization and yeast artificial chromosome (YAC) contig-mapping of genes and DNA segments in the 7q21→q32 region. Hum molec Genet 2:751–760 (1993).

Wevrick R, Willard HF: Physical map of the centromeric region of human chromosome 7: relationship between two distinct alpha satellite arrays. Nucleic Acids Res 19:2295–2301 (1991).

Zengerling S, Tsui L-C, Grzeschik KH, Olek K, Riordan JR, Buchwald M: Mapping of DNA markers linked to the cystic fibrosis locus on the long arm of chromosome 7. Am J hum Genet 40:228–236 (1987).

Zhang YP, Lambert MA, Cairney AEL, Wills D, Ray PN, Andrulis IL: Molecular structure of the human asparagine synthetase gene. Genomics 4:259–265 (1989).