Deletion in the Z-line region of the titin gene in a baby hamster kidney cell line, BHK-21-Bi

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Abstract The gene for titin, a 4MDa myofibrillar protein, was analysed in golden hamster DNAs from different sources, using human cDNA probes and PCR. In the DNA from the BHK-21-Bi subline of baby hamster kidney cells, extended sequences coding for Z-line associated domains were missing, indicating a deletion that renders titin non-functional. These sequences were present in the original BHK-21 line and in hamster DNAs. Our finding shows that, due to the absence of selective pressure on a gene's function, genomic deterioration can occur in a permanent cell line and can lead to a loss of overlapping DNA stretches in both autosomes.

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Key words: Cytoskeleton; Sarcomere; Permanent cell line; Genetic deterioration

1. Introduction

The structures of large genes, like those for dystrophin [1] or genes coding for very large polypeptides, like titin, may be subject to a particularly high mutation pressure and are probably maintained by a counteracting selection against mutated individuals. In the case of the dystrophin gene, functional deficits shorten survival due to muscle fibre necrosis as in the case of dystrophin-deficient Duchenne patients. In permanent cell lines, selective pressure is relieved for genes coding for non-housekeeping proteins or proteins whose function is only required in special stress situations. Thus, a spontaneous deletion within the X-linked dystrophin gene has been observed in a myogenic rat cell line, L8 (Nudel and Yaffe, pers. commun.); in cell culture, dystrophin's function is only demonstrable in cells subjected to stress [2,3], and there is probably no selective pressure to maintain its function under standard cell culture conditions.

Here we report on a deletion in the subline BHK-21-Bi of the commonly used baby hamster kidney cell line, BHK-21, comprising essential domains of the gene for titin, a giant polypeptide thought to provide elasticity to, and to protect the sarcomere against irreversible damage during the stretch phase of the sarcomere [4,5].

2. Materials and methods

2.1. Cells, tissues, DNA preparation and Southern blot analysis

The following cell lines and tissues were used as sources for DNA: the Golden Hamster cell line 'baby hamster kidney cells', BHK-21/C13 [6], as obtained (catalogue number 'CCL-10') in 1995 from the American Type Culture Collection, Rockville, MD, USA, termed 'BHK-21-ATCC', and a subline derived during the years 1990–1995 from the ATCC line, termed 'BHK-21-Bi'. Morphologically and in their growth properties these lines were indistinguishable. In addition, the murine fibroblast cell line C3H 10 $T_{1/2}$ clones (fibroblastic; from ATCC) and C2C12 (myogenic; from ATCC) [7,8] were used as controls.

For reference purposes, DNAs from kidneys of the Golden Hamster (*Mesocricetus auratus*; Waterhouse, 1839) and the laboratory mouse (*Mus musculus* strain C3H) were used. DNAs were extracted from tissues and from cell cultures by standard procedures. About 20 μg digested DNA was separated on a 0.8% agarose gel, blotted onto nylon membrane and hybridised with ^{32}P-labeled probes [9].

2.2. Hybridisation probes

For titin, partial cDNAs from the full-length human titin cDNA [10] were used as probes. HH2, HH3b, HH4, HH5 are from the Z-disc region of the titin gene, HH3 from the C-zone (for sequence description, see http://www.embl-heidelberg.de/ExternalInfo/Titin/). For nebulin, the partial cDNA N14 was used as a probe [11]. A 1500 bp mouse desmin cDNA *Eco*RI fragment [12] was a gift from Dr. H. Herrmann (DKFZ Heidelberg).

2.3. Primer pairs

A panel of primer pairs was used, covering the principal domains of the full length 100 kb coding sequence of titin expressed in human soleus muscle. Primer pairs seq 5/9 and iso 11/12 match to the Z-disc region and five primer pairs like iso 13/14 to iso 21/22 cover the I-band domain, whereas the primer pair OL 278/279 binds to the A-band of the titin gene. The pair Bk 21/116 probes the M-line. Primer pair sequences have been deposited under http://www.embl-heidelberg.de/ExternalInfo/Titin/.

Polymerase chain reaction was performed using 300 ng genomic DNA in a volume of 25 μl containing 10 pmol of both primers with 2 U Taq-DNA-polymerase under the following conditions: 4 min 94°C, 35 cycles with 1.5 min 55°C, 3 min 72°C, 1 min 90°C (Trio-Thermoblock from Biometra, Göttingen, Germany). The resulting products were separated in a 1% agarose gel and visualised by ethidium bromide staining.

3. Results

In the course of a comparative analysis of the titin gene in rodents [13], the BHK-21-Bi cell line (a subline of the well-known baby hamster kidney cell line, ATCC/CCL-10) was

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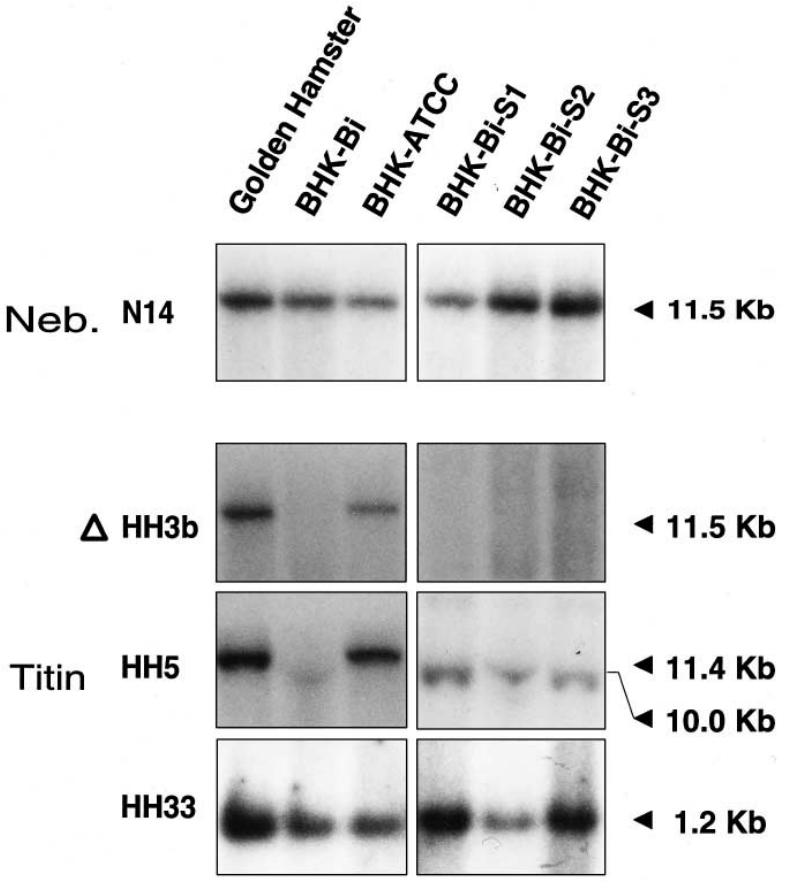


Fig. 1. Autoradiographs of a Southern blot with *Eco*RI digested DNA (20 μg per lane) from Golden Hamster, the cell lines BHK-Bi, BHK-ATCC, and BHK-Bi sublines 1, 2 and 3. The blots were hybridised consecutively with titin probes from the Z-disc region, HH2, HH3b, and HH5, from the C-zone of the A-band, HH33, and with the nebulin probe N14. The lengths of the *Eco*RI fragments are given on the right. Δ, lack of signal in BHK-21-Bi lines, indicating deletion.

used to represent the species Golden Hamster, *Mesocricetus auratus*. Unexpectedly, certain titin probes gave no signal on Southern blotting although signals were seen with control probes like those for the desmin and nebulin genes and in another rodent species, the laboratory mouse (strain C3H) and murine permanent cell lines, C_2C_{12} and 10 $T_{1/2}$ (not shown). It turned out that the lack of signals representing the Z-disc domain of titin was a specific property of the BHK-21-Bi cell line: the DNA of the species of origin, *Mesocricetus auratus*, did give a specific signal, and so did the cell line of origin BHK-21-ATCC (Fig. 1). Thus, we suspected that a deletion had happened during the last years of subculturing of the BHK-21 cell line. To further characterise this deletion a number of hybridisation probes covering the major domains of the titin molecule were tested on DNAs from the Golden Hamster, the cell lines BHK-21-ATCC and BHK-21-Bi, as well as 10 sub-sublines (BHK-Bi S1 to S10) derived from the latter, in order to test for a possible heterogeneity within the cell population of BHK-21-Bi (Fig. 1). Abnormalities were found with the cDNA probes derived from the Z-line region of the human cardiac titin gene: HH3b from the center of the Z-line region gave a signal with hamster DNA and in the original line BHK-21-ATCC, and no signal in BHK-21-Bi and its sublines 1 trough 10, whereas HH5 also derived from this region gave weak bands of reduced size (Fig. 1). Probes from other regions of the titin gene as well as a nebulin probe used as a control gave positive signals in all tested cell lines and with hamster DNA.

To confirm this result by an independent method, genomic amplification by PCR of representative titin gene regions was performed: seq 5/9 and iso 11/12 for the Z-disc, iso 21/22 for I-band, OL 278/279 for C-zones within the A-band, and Bk21/116 for the M-line. Representative examples are shown in Fig. 2. As is evident from the amplification products, regions defined by the primers are present in both BHK-21-ATCC and BHK-21-Bi cell lines, whereas a region in the Z-zone defined by primer pair seq 5/9 is present in BHK-21-ATCC and absent in BHK-21-Bi DNA, thus confirming the result obtained by Southern blotting. The length of the hamster amplification product, 0.87 kb, is much smaller than that expected from the known human sequence (3.2 kb). On comparing hamster, mouse and human DNAs, a high species variability was evident in this particular region of the Z-line domain.

Taken together, the Southern and PCR results define 3′ and 5′ limits for the extent of the BHK-21-Bi deletion, as shown in Fig. 3. The 5′ limit of the deletion is probably in the region of the HH5 probe mice bands with reduced lengths were observed in Bi and all of its 10 tested sublines (Fig. 1). However, preliminary analysis of additional sublines of BHK-21-Bi indicated that other regions of the titin gene (HH33 probe; C-zone, central A-band titin) may also be subject to spontaneous mutations.

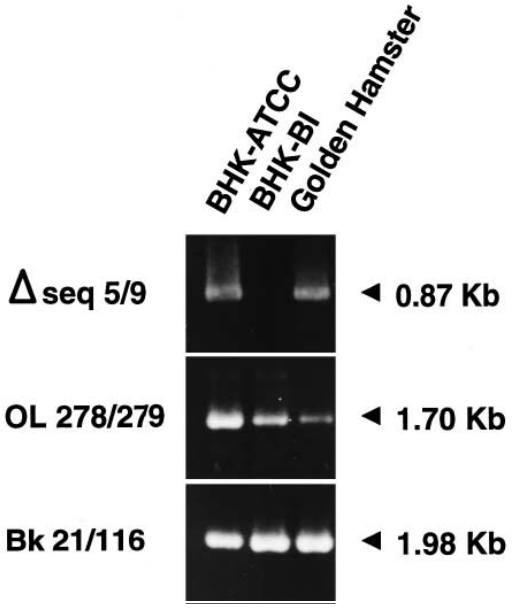


Fig. 2. Ethidium bromide stained agarose gel of PCR products obtained with primer pairs seq. 5/9 (from the Z-disc region), OL 278/279 (C-zone of A-band), and Bk-21/116 (M-line) of the titin gene. Comparisons of DNAs from the cell lines BHK-ATCC, BHK-21 and the Golden Hamster. Δ marks the PCR-primer pair which shows the deletion in the DNA from cell line BHK-21-Bi.

4. Discussion

The chance discovery of the spontaneous occurrence of the BHK-21-Bi deletion is an example of 'evolution in the test tube' in the sense that genes not essential for the proliferation of cells in culture may undergo a stepwise elimination in the absence of selective pressure on their function. Presumably, a large number of such cases goes unnoticed. The loss of the dystrophin gene in the L8 rat cell line was apparently a singular event, as no repetition of this mutation was found in subsequent screens (D. Yaffe and U. Nudel, pers. commun.). The opposite situation, gene duplication upon passaging of cells under selective pressure, is a phenomenon long known to cell biologists. Thus, neither gene dosage nor the detailed structure of genes in permanent cell lines can be taken for granted to represent the genomic situation in the species from which they are derived.

For X- and Y-chromosomal genes in male cell lines, a single mutational event is sufficient for a deficiency to become manifest. The L8 rat myogenic cell line has been derived from L118, and this from a male neonatal rat (Nudel and Yaffe, pers. commun.), i.e. prior to the mutation, the gene was present in only one copy in the cell line. In mouse [14] and man [15], the titin gene is located close to the nebulin gene, on Chr2 and 2q, respectively. A close linkage of the titin and nebulin loci may therefore be assumed to hold for the hamster as well, based on conserved synteny. In mitotically multiplying cells, the manifestation of a deletion of a gene present in two copies requires at least two events: either two deletion events in which the lost chromosomal segments overlap (compound heterozygote) or a deletion with a subsequent somatic recombination that would lead to daughter cells either homozygous for one and the same deletion or homozygous wild type. A more detailed analysis of the exact borders of the deletion reported here would be required to distinguish between the two mechanisms in the case of titin gene deletions in BHK-21-Bi. The heterogeneity at the borders of the BHK-21-Bi sublines could represent the ongoing loss of sequences following the first deletion event. Thus, once the intrinsic stability of the gene is lost, further deterioration may accelerate.

The BHK-21-Bi cell line may be viewed as an 'accidental knock-out' of the titin gene which has hit a domain, the Z-line anchoring structure, which due to its high degree of evolutionary conservation appears to be essential for titin's function [10]. By RT-PCR we have found that BHK-21-ATCC and BHK-21-Bi cells contain titin mRNA (data not shown).

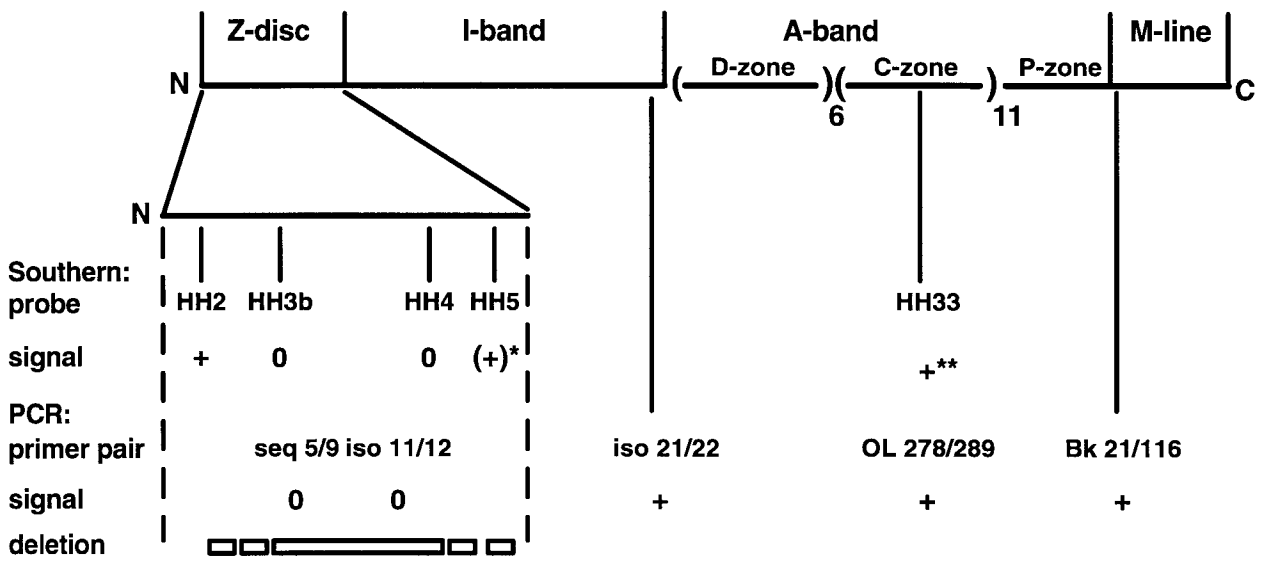


Fig. 3. Schematic representation of the titin gene with location of Southern probes and PCR products from primer pairs and results obtained with DNA from the BHK-21-Bi cell line ('+', signal; '0', no signal; * reduced fragment size in BHK-21-Bi and all of its sublines; ** altered fragment sizes, heterozygous or homozygous, in some of the sublines. The enlarged Z-disc region shows the approximate extent of the deletion inferred from Southern and PCR results shown in Figs. 1 and 2.

Under certain medium conditions, the BHK-21 cell line has been reported to produce immunologically detectable titin protein [16]. It might be possible therefore to convert BHK cells into myogenic cells and to study the effect the deletion of the Z-line domain of titin on the formation of myofibrils.

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References

[1] M. König, E.P. Hoffman, C.J. Bertelson, A.P. Monaco, C.A. Feener, L.M. Kunkel, Cell 50 (1987) 509–517.
[2] A. Menke, H. Jockusch, Nature 349 (1991) 69–71.
[3] A. Menke, H. Jockusch, J. Cell Sci. 108 (1995) 727–733.
[4] K. Maruyama, Biophys. Chem. 50 (1994) 73–85.
[5] S. Labeit, B. Kolmerer, W.A. Linke, Circ. Res. 80 (1997) 290–294.
[6] M.P.G. Stoker, J.A. Macpherson, Virology 14 (1961) 359–370.
[7] D. Yaffe, O. Saxel, Nature 270 (1977) 725–727.
[8] H.M. Blau, C.P. Chin, C. Webster, Cell 32 (1983) 1171–1180.
[9] A.P. Feinberg, B.C. Vogelstein, Anal. Biochem. 132 (1983) 6–13.
[10] S. Labeit, B. Kolmerer, Science 270 (1995) 293–296.
[11] S. Labeit, B. Kolmerer, J. Mol. Biol. 248 (1995) 308–315.
[12] H. Li, et al. J. Cell Biol. 124 (1994) 827–841.
[13] Witt, C. (1995) Thesis. University of Bielefeld.
[14] M. Müller-Seitz, K. Kaupmann, S. Labeit, H. Jockusch, Genomics 18 (1993) 559–561.
[15] S. Labeit, D.P. Barlow, M. Gautel, T. Gibson, J. Holt, C.-L. Hsieh, U. Francke, K. Leonard, J. Wardale, A. Whiting, J. Trinick, Nature 345 (1990) 273–274.
[16] G. Schaart, F.R. Pieper, H.J.H. Kuijpers, H. Bloemendal, F.C.S. Ramaekers, Differentiation 46 (1991) 105–115.