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Molecular deletion patterns in Duchenne and Becker type muscular dystrophy

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Summary. DNA from 80 Duchenne (DMD) and 15 Becker (BMD) index patients was analyzed with 12 genomic probes and the total cDNA. Deletions were detected in 24 DMD (30%) and 10 BMD patients (67%) by genomic probes alone, mostly p20, pXJ, and/or pERT87. All deletions were confirmed by cDNA probes, and an additional 29 DMD deletions were detected, resulting in a total of 63/95 deletions (66%). The majority of the deletions are localized between kb 6.7 and 9.7 of the cDNA; a smaller group, between kb 0.5 and 3.5. Of the deletions, 90% are detected by the three cDNA probes 1–2a, 7, and 8. This can be applied to strategies for carrier detection and prenatal diagnosis. The order of 13 exon-containing *Hin*dIII fragments in the region between probes 7 and 9–10, where most of the deletions are found, could be defined. The deletion patterns in DMD and BMD patients are different and well in accordance with the "reading frame theory" of Monaco and coworkers. Thus our findings indicate that a DMD or BMD phenotype may be predicted according to the breakpoint position and the number of deleted exons.

Introduction

X-linked Duchenne muscular dystrophy (DMD) is the most common hereditary myopathy in childhood. The mutation rate is high and accounts for the large proportion of sporadic cases. The first symptoms are observed at the age of about 2 to 3 years, and the patients are usually bound to a wheelchair around the age of 10 years. The average life expectancy is still below 20 years (Moser 1984). The less frequent Becker muscular dystrophy (BMD) shows similar, but less severe, clinical features and is probably allelic with DMD (Kingston et al. 1983; Kunkel et al. 1986).

X-autosomal translocations in females with the DMD or BMD phenotype (Verellen-Dumoulin et al. 1984; Lindenbaum et al. 1979; Canki et al. 1979; Greenstein et al. 1980; Jakobs et al. 1981; Boyd et al. 1986), genetic linkage analyses (Murray et al. 1982; Davies et al. 1983; Hofker et al. 1985; Brown et al. 1985), and DMD patients with cytogenetically detectable deletions and complex phenotypes including glycerol kinase deficiency, adrenal hypoplasia, retinitis pigmentosa, and chronic granulomatous disease (Francke et al. 1985; Bartley et al. 1986) independently led to the localization of the DMD/BMD locus in band Xp21. DNA cloning from deletion and translocation breakpoints resulted in the isolation of the well-known pERT (Kunkel et al. 1985) and pXJ probes (Ray et al. 1985), which detect intragenic restriction fragment length polymorphisms (RFLP) as well as molecular deletions in 7%–10% of DMD patients (Monaco et al. 1985; Kunkel et al. 1986; Thomas et al. 1986). These methods are now routinely in use for carrier detection and prenatal diagnosis (Darras et al. 1987; Junien et al. 1987; Akita et al. 1987).

Starting from cloned DNA sequences of the pERT87 (DXS164) locus, sequence conservation between mammals eventually led to the isolation of a 16-kb transcript and of a corresponding 1-kb human fetal muscle cDNA (Monaco et al. 1986). Likewise an adult muscle cDNA clone from the pXJ (DXS206) locus was isolated (Burghes et al. 1987). Recently the 14-kb human DMD cDNA corresponding to a complete representation of the fetal skeletal muscle transcript has been cloned (Koenig et al. 1987). The DMD transcript spans a gene locus of about 2000 kb with at least 60 exons on 65 different *Hin*dIII fragments. This paper presents the frequency and pattern of deletions in 80 DMD and 15 BMD patients investigated using 12 genomic probes and the complete cDNA. It was our aim to gather further information on the exact order of the exon-containing *Hin*dIII genomic fragments, to establish a deletion screening for family investigations (carrier detection, prenatal diagnosis), and to look for possible differences in the deletion patterns of DMD and BMD.

Patients and methods

Patients

Eighty DMD and 15 BMD index patients from the same number of families were examined. Most of them were seen during clinical follow-up examinations at pediatric outpatient departments of the university clinics in Bern and Zurich; from others, blood or extracted DNA was sent to us by different clinics and genetic departments in Switzerland, Germany, Austria, and Luxembourg. In each case the diagnosis of DMD and BMD was made on the basis of clinical findings and progression of the disease, serum CK levels, muscle histology, and family history. Special attention was given to the time at which a patient became bound to a wheelchair; e.g., all BMD patients are still able to walk, 14 at ages 14–52 years, and one BMD patient aged 7 years is still able to climb stairs at a run.

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Genomic probes

Twelve genomic probes were used: pERT84 (DXS142), J-MD, three subclones from locus DXS206 (pXJ5.1, 1.1, 10.1), five subclones from locus DXS164 (pERT87–42, –1, –8, 15, –30), J-Bir, and P20. Restriction enzyme digestions, fragment lengths, and references are summarized in Table 1.

cDNA

The 14-kb DMD cDNA, subdivided and subcloned at the *Eco*RI site of the bluescribe or bluescribt plasmid vector (Koenig et al. 1987), was amplified as described by Maniatis et al. (1984). The inserts were cut with *Eco*RI from all clones except for clone 1–2a, where the insert was cut with *Eco*RI and *Hin*dIII. The inserts of probes 5b–7 and 9–14 were additionally cut with the enzyme *Hin*cIII, resulting in two fragments (probes 5b–6 and 7) and four fragments (9–10, 11, 12a, 12b–14) respectively, which were used as hybridization probes. The cDNA probes and exon-containing genomic *Hin*dIII fragments are shown on the left in Fig. 2.

Table 1. Genomic probes used for deletion screening in DMD and BMD patients

Probe	Enzyme	Fragment(s) (kb)		References
pERT84	*Taq*I	3.4/1.75	RFLP	Monaco et al. (1987)
J-MD	*Xba*I	6.0		Monaco et al. (1987)
pXJ5.1	*Xba*I	3.0		
	*Sph*I	24.0/17.0	RFLP	
pXJ1.1	*Xba*I	10.1		Ray et al. (1985)
	*Taq*I	3.8/3.1	RFLP	
pXJ10.1	*Xba*I	4.7/2.4		
pERT87–42	*Xba*I	4.8		Kunkel et al. (1985)
pERT87–1	*Eco*RV	13.0/12.0	RFLP	Kunkel et al. (1985)
	Asp	8.7/7.5	RFLP	
pERT87–8	*Taq*I	3.8/2.7;1.1	RFLP	Kunkel et al. (1985)
pERT87–15	*Asp*	2.8/1.6;1.2	RFLP	Kunkel et al. (1985)
pERT87–30	*Xba*I	3.0/1.8		Kunkel et al. (1985)
J-Bir	*Bam*HI	21/18;5.0	RFLP	Bertelson et al. (1986)
P20	*Msp*I	6.8/3.5	RFLP	Van Ommen et al. (1987)

DNA analysis

Genomic DNA (2–3 µg) extracted from whole blood leukocytes anticoagulated in EDTA (Kunkel et al. 1977) was digested with appropriate restriction enzymes (*Hin*dIII for cDNA probes; for genomic probes see Table 1). For a 3- to 4-h digestion, 2–3 units of enzyme per microgram DNA were used. The resulting fragments were separated on 0.8% agarose gels. After denaturation and neutralization, the DNA was transferred overnight to nitrocellulose filters by Southern blotting (Southern 1975). Hybridization and filter washing followed Liechti-Gallati et al. (1987). cDNA and genomic probes were labeled either by nick translation (BRL kit) or by oligo-labeling (Boehringer kit). Autoradiographs with intensifying screens were processed at −70°C for 1–7 days.

Results

Deletions

The DNA of all patients was analyzed first with the 12 genomic probes listed in Table 1. Deletions were found in 10 BMD (67%) and 24 DMD patients (30%), as shown in Fig. 1. Half of these deletions (18) are detected by probe P20 alone and, with one exception (DMD: 102), are limited to this region. Of the remaining 16 deletions, 15 were detected by pXJ and/or pERT87 probes. In patient DMD: 80 the deletion was limited to the J-Bir region. These results confirm the patterns previously described by Kunkel et al. (1986), Lindlöf et al. (1988), and Bartlett et al. (1988). In a second step the DNA of all patients was examined by probes of the complete 14-kb DMD cDNA. The *Hin*dIII-digested genomic DNA of the patients was hybridized with the nine cDNA inserts as shown in Fig. 2.

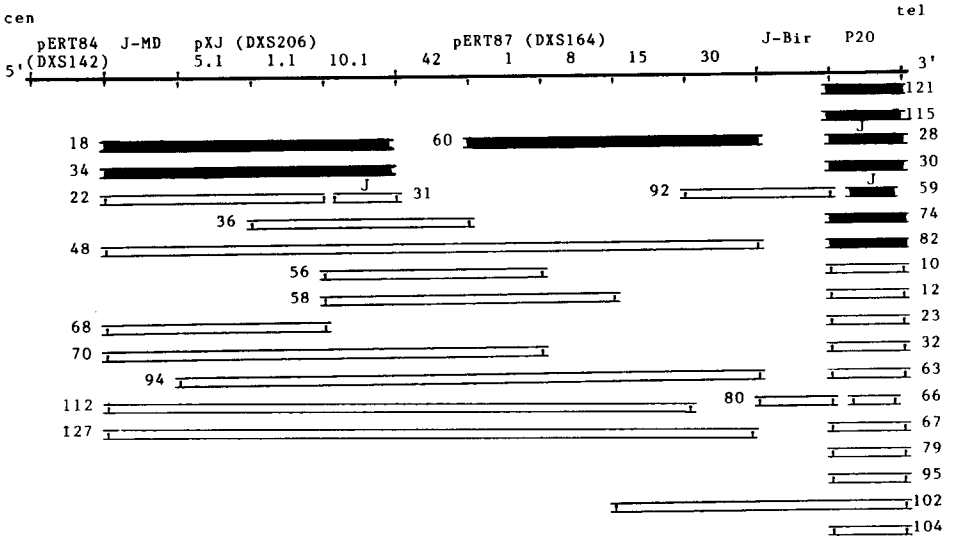

Fig. 1. Deletions in DMD and BMD patients detected by genomic probes. The probes are specified on the top of the figure according to their position along the DMD gene from centromere *(cen)* to telomere *(tel)*. *Black boxes* represent deleted fragments in BMD; *white boxes*, in DMD patients. *J* Junction fragment

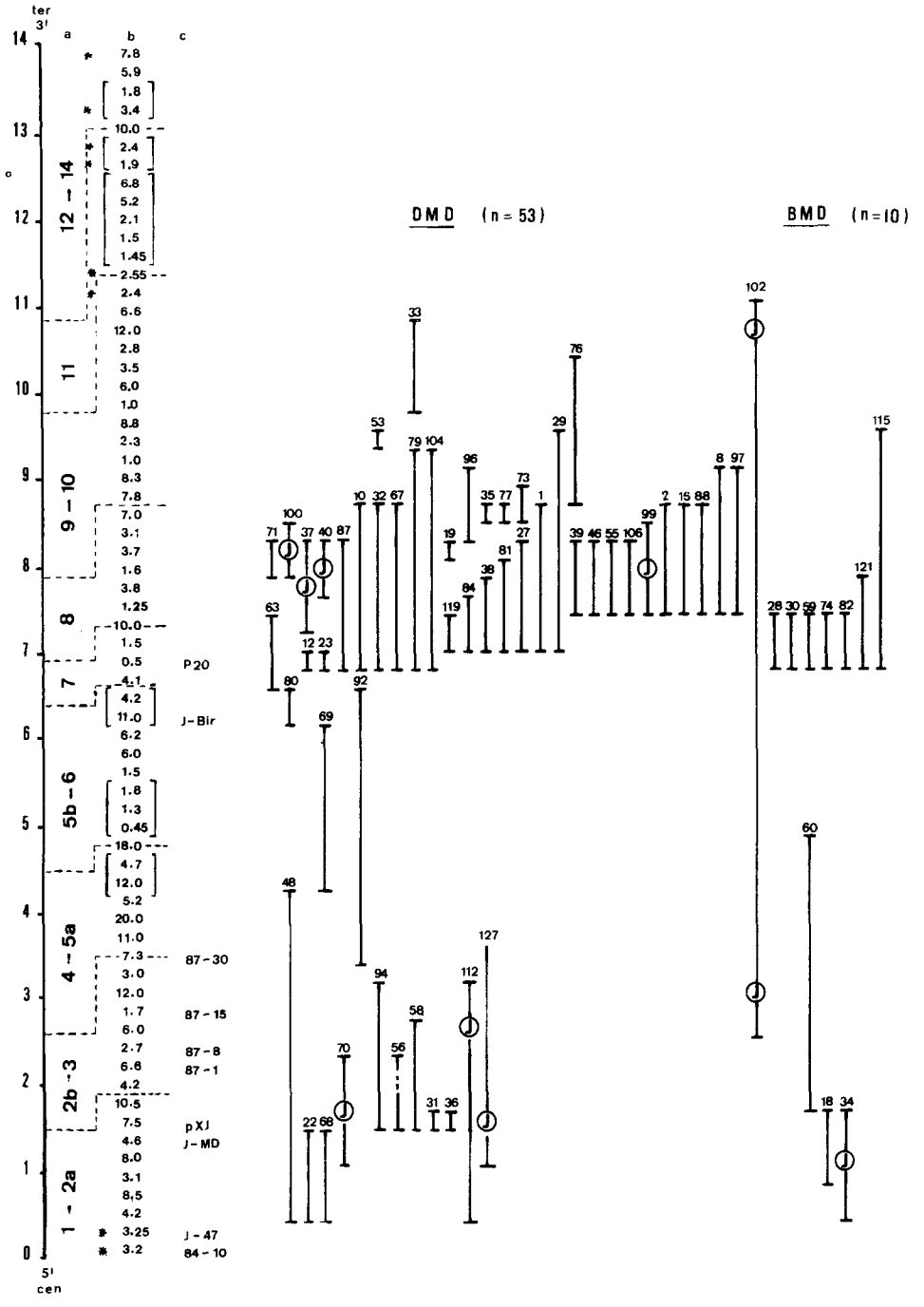

Fig. 2. DMD/BMD deletion pattern detected by the 14-kb DMD cDNA. The distribution of 10 BMD (on the *right*) and 53 DMD deletions relative to the cDNA map is shown. *Each line* represents one deletion marked by the patient's number. *Bars* indicate the beginning and end of the deletion; *dashed lines* represent hybridizing (nondeleted) exons within the deletion in patients 56. Ⓙ Junction fragment, *a* cDNA probes, *b* exon-containing *Hin*dIII cDNA fragments, *c* genomic probes positioned relative to their nearest exon-containing *Hin*dIII fragment. Size in kb and order are indicated according to our data (see Fig. 3). *Brackets* indicate order not yet defined; * order and additional fragments (3.4 kb/2.4 kb) according to McCabe et al. (1988) and Koenig et al. (1988)

The deletions mentioned above were confirmed by the cDNA probes, and an additional 29 deletions (all DMD) were detected, giving a total of 53 in 80 (66%) in DMD and 10 in 15 (67%) in BMD. These molecular defects vary widely in their position and their extent. It is very likely that most of the deletions start and/or end within an intron (Koenig et al. 1987; Smith et al. 1987; Monaco et al. 1988). Among our cases this can be demonstrated by the presence of a junction fragment (J) hybridizing either with genomic probes in three cases (DMD: 31; BMD: 28, 59; Fig. 1) or with cDNA probes in six additional cases (DMD: 37, 40, 70, 99, 101; BMD: 34, Fig. 2). Intragenically, the deletions seem to be localized preferentially to two regions: one containing exons of the first two kilobases of the cDNA (detected by probe 1–2a and comprising 22% of the deletions), and the second, larger one, associated with exons around the middle part of the cDNA (detected by probes 7 and 8). Within the latter, so-called hot spot region, there are three main locations for deletion breakpoints originating in introns: (1) between the exons of the genomic *Hin*dIII fragments 4.1 and 0.5, (2) between 0.5 and 1.5, and (3) between 10 and 1.25 kb. Of the deletions, 54% have one or both of their breakpoints within this region, with seven BMD deletions originating between fragments 4.1 and 0.5 kb.

The deletions involve from just 1 to up to 38 exons. This variation seems to be somewhat greater among DMD patients than among those with BMD, but this difference is not statistically significant. On the other hand, the breakpoints of the deletions and their corresponding length are obviously different; e.g., in the DMD patients the deletions with the same breakpoint locus covered 1, 7, 9, or 12 exons. On the other

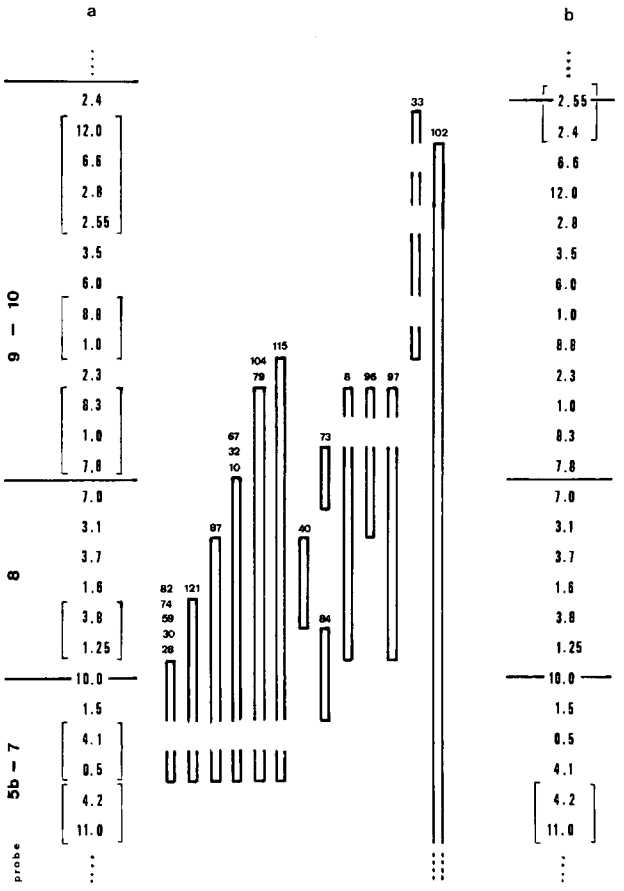

Fig. 3. Order of *Hin*dIII fragments based on DMD deletions. The *Hin*dIII genomic fragments spanning kb 6–10 of the DMD cDNA with arbitrary order for the fragments shown in parentheses are marked on the *left (a)* and the most likely rearranged order according to our results, on the *right (b)*. *Patient numbers* and *brackets* as in Fig. 2

hand, deletions of hot spot region with the same extension but different breakpoint locations may also result in different phenotypes (DMD: 81, BMD: 121; Fig. 2).

Mapping of probe P20

All deletions with one breakpoint between *Hin*dIII fragments 4.1 and 0.5 kb are also detected by the genomic probe P20 provided by Van Ommen et al. (1987), irrespective of their extent. These findings suggest that P20 is localized close to the 0.5-kb fragment detected by probe 7 of the cDNA, corresponding to detailed studies of Wapenaar et al. (1988).

Order of the genomic HindIII fragments

When the deletions are plotted according to an arbitrary order of fragments as indicated in parantheses by Koenig et al. (1987), many deletions appeared to be discontinuous, as shown in Fig. 3. For example, in the deletions of patients 28, 30, 59, 74, 82, 121, 87, 10, 32, 67, 79, 104, and 115 the 0.5-kb fragment would be localized distal to 4.1 and proximal to 1.5. Therefore a new order is suggested, on the right-hand side of Fig. 3, where 3′...10.0/1.25/3.8/1.6...5′ is implemented by the deletions in patients 40 and 84. Fragment 7.0 is followed by 7.8 according to patient 73, and the normal intensity of the 1.0-kb band in patients 8, 96, and 97, compared with half that of patients 79, 104, and 115, suggests the order 7.8/8.3/1.0/2.3 (there are two 1.0-kb fragments detected with probe 9–10). The deletion in patient 33 rearranges the following eight fragments: 8.8/1.0/6.0/3.5/2.8/12/6.6/2.55 (see Figs. 2, 3).

Finally, only the deletion in patient 56 remains discontinuous (Fig. 2). One explanation may be that the deletions occurred in two steps. However, DNA analyses in the patient's mother and her relatives with probes 1–2a and 2b–3 did not reveal any deletions. The findings could also be explained by a chromosomal rearrangement, placing fragment 4.2 kb distal to 6.6 kb.

Discussion

Our results of systematic screening for deletions in DMD and BMD patients with genomic probes and the complete cDNA largely confirm those of Koenig et al. (1987). In addition they point to some new aspects and conclusions concerning the order of *Hin*dIII fragments, the strategy for deletion analyses, and the differences in deletion patterns between DMD and BMD patients.

Koenig et al. (1987) reported that the number of deletions detected in DMD and BMD patients is considerably increased by using cDNA probes. With probes 8, 7 and 1–2a alone, 90% of the deletions were found, which makes them the most important tools for prenatal diagnosis in deletion families. For an optimum deletion analysis the following strategy is recommended: To avoid problems with overlapping bands, probes 5b–7 and 9–14 should be cut with *Hin*cIII into subprobes 5b–6, 7, 9–10, 11, 12a, and 12b–14. There is no need to use probe 12b–14, since it corresponds to the 3′ untranslated sequences of the transcript of the last exon, which is also detected by probe 12a (Koenig et al. 1988). In a first step, two hybridizations should be done: one with probes 7 and 8 and the other one with probe 1–2a. In a second step, probes 2b–3, 5b–6, and 9–10 should be used. With these five hybridization steps nearly, if not all, deletions will be detected, thus leading to highly reliable conditions for prenatal predictions in two-thirds of the families.

For carrier detection the same strategy can be used complemented with RFLPs of genomic probes: If the cDNA detects a junction fragment, carriers are easily diagnosed. If exons near genomic probes (Fig. 2) are deleted, RFLPs of these probes should also be examined. P20 is a very efficient probe detecting all mutations that are deleted for the 0.5-kb *Hin*dIII fragment. Deletions detected by probes 8 and 9–10 can be determined in carriers either by dosage analysis or in some cases by cDNA polymorphisms (Pst for probe 8), mentioned by Darras et al. (1988).

Based on our data we were able to define the order of 13 exon-containing *Hin*dIII fragments in the region between probes 7 and 9–10 (Fig. 3). The determination of the organization of this intragenic region highly prone to DMD and BMD deletions (hot spot) may be useful for further interpretation of deletions with molecular probes.

The most important finding of our investigations is the observation that within the hot spot the nature of a deletion may determine whether a patient gets DMD or BMD: If confirmed, this would allow a phenotype prediction from the breakpoint locus of an observed deletion and the number of

exons involved. Thus deletions with breakpoints between fragments 4.1 and 0.5 kb should result either in BMD phenotypes, if 3, 5, or 13 exons are missing, or in DMD phenotypes, if 1, 7, 9, or 12 are missing. DMD phenotypes may also arise from deletions with breakpoints between 0.5 and 1.5 kb covering 2, 3, 4, 5, 6, 8, or 12 exons, or with breakpoints between 10 and 1.25 kb covering 4, 5, 6, or 8 exons.

Our results for deletions in the hot spot region are well in agreement with the theory of Monaco et al. (1988): They demonstrated for deletions in the 5′ end genomic region that adjacent exons maintaining an open reading frame (ORF) in the spliced mRNA would give rise to the less severe BMD phenotype, whereas adjacent exons that cannot maintain an ORF because of a reading frame shift would result in DMD.

In two-thirds of the DMD and BMD patients the mutation is associated with a detectable molecular deletion. In the remaining third the phenotypes result either from point mutations or very small deletions that disrupt the splicing of introns (Monaco et al. 1988), and lead to either single exon deletions or the inclusion of introns. Intensive studies on patients with respect to the presence of deletions, the clinical course of the disease, synthesis and function of dystrophin (gene product, Hoffmann et al. 1987), and the DNA sequence of the hot spot region are needed to get more information on the nature of the normal and mutated DMD/BMD gene.

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