Frame-Shift Deletions in Patients with Duchenne and Becker Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) and its less severe form Becker muscular dystrophy (BMD) are allelic disorders. It has been suggested that in the mutations involving BMD, the translational reading frame of messenger RNA is maintained and a smaller, though partially functional, protein is produced. In order to test this, the exon-intron boundaries of the first ten exons of the DMD gene were determined, and 29 patients were analyzed. In a number of BMD patients (mild and severe BMD), the reading frame of messenger RNA was not maintained. On the basis of these findings, a model for reinitiation from an internal start codon is suggested.

DUCHENNE AND BECKER MUSCULAR dystrophies are X-linked, recessive, neuromuscular disorders. Both disorders map to band p21 of the X chromosome and have been shown by linkage analysis and deletion studies to arise from mutations in the same gene (*1*). Duchenne muscular dystrophy is characterized by progressive muscular weakness with the majority of the DMD patients becoming wheelchair-bound before the age of 12 (*2*). Few patients survive much beyond their early 20s. Becker muscular dystrophy is distinguished from DMD by its milder phenotype and slower rate of progression. Patients with BMD are generally defined as those who remain ambulatory until the age of 16. Some patients with mild BMD have near normal life to the age of 40 or 50 (*3*). However, there is a range of severity in BMD patients, and those who lose ambulatory capacity before the age of 16 are often referred to as severe Beckers. In this report, we have used the terms mild, intermediate, and severe as these mutations are allelic. The intermediate category includes mild DMD, also known as outliers (*4*), and severe BMD. Our criteria used for classification are the age of becoming wheelchair-bound (Table 1) and clinical progression. It should be realized that there is a continual spectrum of disease severity, and the definitions are somewhat arbitrary.

Cloned sequences from the BMD/DMD locus have been obtained by two methods: (i) cloning of a DNA fragment spanning a translocation junction in an affected female having a t(X;21) translocation (DXS206 locus) (*5*), and (ii) enrichment of sequences from within a large deletion of band Xp21 in a male patient with DMD and three other X-linked disorders (DXS164 locus) (*6*). Expressed sequences from the BMD/DMD gene have been isolated from the DXS164 (*7*) and DXS206 (*8*) loci and complete cloning of the 14-kb mRNA has been reported (*9*). The cDNA and genomic clones have been used to probe DNA blots from BMD and DMD patients, revealing that over 50% have deletions in the Xp21 region (*9, 10*). Lindlof *et al.* (*11*) and Hart *et al.* (*12*) have shown that no correlation exists between the site or extent of deletions and the severity of phenotype, since very small deletions can give rise to DMD phenotype and relatively large deletions can give rise to a BMD phenotype. One possible explanation for the different severity of phenotypes is that in the Becker mutations the translational reading frame is maintained, and some functional protein is produced. In the intermediate cases, one might speculate that the

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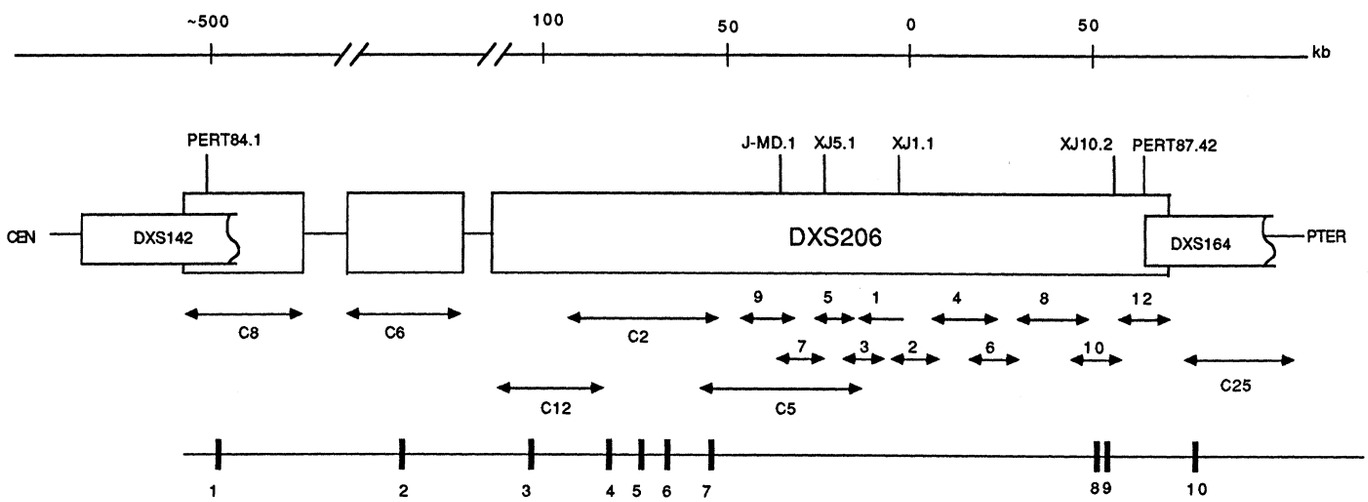

Fig. 1. A schematic map of the 5′ end of the DMD gene. The DXS206 locus was expanded by chromosome walking from the t(X;21) junction clone XJ1. Phage clones XJ2 to XJ12 (designated 1, 2, ..., 12 in the figure) were obtained by successive isolation of overlapping clones from an XXXXY DNA library in Charon 35. A 2.0-kb cDNA clone isolated by homology to phage clone XJ10 was used as a probe on a partial Hind III or Sau 3A library of XXXXY DNA in cosmid vector Lorist B to isolate cosmid clones XJ C5, C2, C12, C6, and C8 (*33*). The cosmid clone C25 was isolated by X. Hu in our laboratory by homology with a genomic probe from the region between PERT 87.42 and PERT 87.1. This genomic probe was derived from the cloned junction of a patient with a duplication of exons 8 and 9 (*34*). The details of chromosome walking will be reported separately (*16*). The restriction endonuclease fragments of cosmid clones containing exon sequences were isolated and subcloned into the Bluescript vector. Ten of the 16 exons detected by the 2.0-kb cDNA probe are represented by the blocks underneath and are numbered from the 5′ end. Their positions, according to their known genome locations, are indicated. The end points of the cosmids have not yet been mapped.

frame is maintained, but the deletion removes a functionally important region or regions. In DMD cases the model assumes that reading frame is disrupted by the deletion, leading to misreading of the mRNA and premature termination or the production of completely inactive protein. Studies of a limited number of patients—two mild, one intermediate (severe BMD), and three severe (DMD)—have supported this hypothesis (*13*).

In a large collaborative study, 511 patients have been examined with cDNA probes covering the first 23 exons of the gene (*14*). This has resulted in the precise ordering of these exons and has also provided a collection of well-characterized deletion patients. In the present study, we have precisely determined the exon/intron boundaries for the first ten exons of the BMD/DMD gene and used the information to determine whether or not the translational frame is maintained in the group of patients found to have deletions at the 5′ end of the BMD/DMD gene. As exons do not necessarily contain an integral number of triplet codons, deletion of a DNA segment consisting of one or more exons with a nonintegral number of triplet codons would cause a shift in the reading frame, whereas deletion of an exon or exons containing an integral number of triplet codons would maintain the reading frame. In our study of 29 patients, we have found 6 BMD and 7 severe BMD patients whose deletions disrupt the translational frame, indicating that other mechanisms must exist that compensate for the frame-shift mutation and result in the mild Becker phenotype.

The first ten exons of the BMD/DMD gene were isolated in phage or cosmid clones. All were sequenced and, by comparison to cDNA sequence, their exon-intron boundaries have been established (Table 1). Exons 8 and 9 are localized to the DSX206 locus within phage walk clone XJ10 (*8*), telomeric to the t(X;21) translocation clone, XJ1 (Fig. 1). Genomic clones containing exons 1 to 7 were isolated by screening a partial Hind III or Sau 3A genome library in the cosmid vector lorist B (*15*), with a 2.0-kb cDNA subclone from the 5′ end of the gene (*8, 16*). The genomic region surrounding exon 10 was isolated by screening the cosmid library with a genomic clone derived from the region between PERT 87.42 and PERT 87.1 in the DXS164 locus (*17*). The cloned genomic region and the position of exons are shown schematically in Fig. 1. Restriction fragments containing exon sequences were subcloned into the Bluescript vector (Strategene) for sequencing. Our original 2.0-kb cDNA was sequenced on both strands and matched with that described by Koenig *et al.* (*9*). The sequences flanking each exon were determined by dideoxynucleotide sequencing (*18*) on both strands of the genomic clones with oligonucleotide primers complementary to the cDNA and the T7 and T3 primers of the vector.

The nucleotide sequences at the exon-intron boundaries were established by DNA sequence comparison of cDNA and genomic subclones and the exon boundaries are consistent with those determined or inferred by Koenig *et al.* (*19*). The 5′ donor and the 3′ acceptor splice sites in each of the introns conform to the GT..AG rule, and the adjacent sequences agree well with consensus sequences compiled for the exon-intron boundaries of other genes (Table 1) (*20*). There is a significant homology of the 5′ end of BMD/DMD gene to the actin binding domain of α-actinin (*21, 22*). This homology extends from exons 3 to 9. The amino acids encoded by exons 4 and 5 of the BMD/DMD gene are, respectively, 50% and 61% identical to the actin binding domain of α-actinin (*21*). This highly conserved protein sequence might be expected to have functional significance, and deletion of this region might therefore be expected to produce a severe phenotype.

Of the 511 patients analyzed in the collaborative study, 29 patients were found to

Table 1. Exon-intron organization of the first ten exons of the BMD/DMD gene.

Exon	Exon size (bp)	Sequence of intron-exon junction*
1	≥238	5′-ATG CTT TGG TGG GAA GAA GTA GAG GAC TGT T/*gt*aagtacaaagtaactaaaa
2	62	ttttttatttgcattttt*ag*/AT GAA.....TCT AAG/*gt*aagaatggtttgttacttt
3	93	gtgtatttttttaatttc*ag*/TTT GGG.....AAA CTG/*gt*atgtgacttatttttaaga
4	78	actcttttgttttgttctc*ag*/CCA AAA.....AAT AAT/*gt*aagtagtaccctggacaag
5	93	ttttaggggtttctttaac*ag*/GTT GAT.....TGG CAG/*gt*aagaatcctgatgaatggt
6	173	aaaatttatttccacatg*tag*/GTC AAA.....CAT AG/*gt*aagaagattactgagacat
7	119	gtgtatgtgtatgtgtttt*ag*/G CCA.....GAA G/*gt*tggtaaatttctggactac
8	182	gtagtgttaatgtgcttac*ag*/AT GTT.....CAA CAG/*gt*aaagtgtgtaaag
9	129	actcccgaacctctctcgc*ag*/ATC ACG.....TCA CAG/*gt*ctgtcaacatttactctct
10	189	ccaatttattttattgtgc*ag*/CAT TTG.....CAT GAG/*gt*aaactaaaacgttaattta - 3′

*Exon sequences are in capital letters, intron sequences are in lowercase letters. The 5′ donor and the 3′ acceptor splice sites are italic.

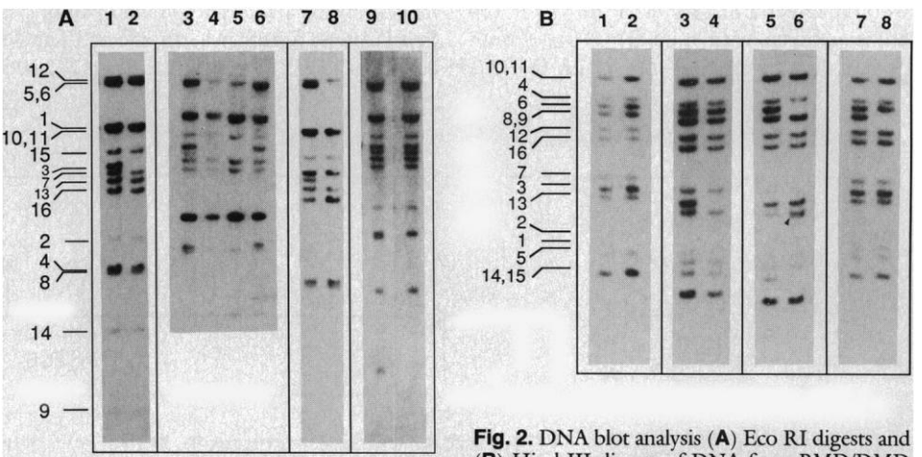

Fig. 2. DNA blot analysis (**A**) Eco RI digests and (**B**) Hind III digests of DNA from BMD/DMD patients probed with a 2.0-kb cDNA clone. (A) Lanes 1, 3, 7, and 9, genomic DNA from normal individuals; lane 2, intermediate patient 785 with a deletion of exon 3; lanes 4 and 5, BMD patient 490 and intermediate patient 1323 with deletions of exons 3 to 7; lane 6, a DMD patient MB with deletion of exon 7; lane 8, intermediate patient 704 with deletion of exons 5 to 7; and lane 10, DMD patient, 483 with deletion of exons 8 and 9. In lanes 3 to 6 exon 9 is not shown; however, exon 9 was confirmed by Hind III digests as well as by shorter runs of Eco RI gels. (B) Lanes 1, 3, 5, and 7, genomic DNA from normal individuals; lane 2, DMD patient 353 with deletion of exon 1; lane 4, intermediate patient 785 with deletion of exon 3; lane 6, intermediate patient 704 with deletion of exons 5 to 7; and lane 8, DMD patient 483 with a deletion of exons 8 and 9. Arrowhead indicates a junction fragment. DNA, digested with Eco RI or Hind III, was separated by electrophoresis on agarose gels and transferred to Hybond (Amersham). The filters were probed overnight at 42°C with a 2.0-kb cDNA subclone labeled by random priming (*35*) in 40% formamide, 5× saline sodium citrate (SSC), and 10% dextran sulfate. In (A), lanes 3 to 6 were probed with various subclones of 2.0-kb cDNA such that a very strong signal for exon 2 was obtained. Filters were washed in 2× SSC at room temperature for 15 min and in 0.2× SSC at 60°C for 30 min twice. The exon numbers indicated on the left side were deduced originally by deletion mapping (*8, 14*) and later confirmed by sequencing. The exons are numbered from the 5′ end.

have a deletion confined to the first ten exons of the gene. DNA blots digested with Eco RI and Hind III were probed with a 2.0-kb subclone of the cDNA containing the first 16 exons. The restriction fragment patterns of DNA from normal individuals and BMD/DMD patients are shown in Fig. 2. As the cDNA probe does not contain any Eco RI or Hind III sites, each band in the normal individual represents a minimum of one exon, and the sequence data have confirmed the number of exons on each restriction fragment. Exon order and the corresponding genomic regions are shown in Fig. 1.

The characteristics of the deletions in all 29 patients are presented in Table 2. In the majority of cases missing bands are assumed to correspond to deleted exons. The absence of an altered restriction fragment was taken as good evidence that the exon-containing fragments were intact and that the deletion edges were in the introns. In the case of DMD patient AB, a break within an exon cannot be ruled out, as an altered band was observed in the Hind III digest, but insufficient DNA was available to allow testing with Eco RI. In the case of patient 704, deletion of exons 5 to 7 was observed in both Eco RI and Hind III digests. However, an extra band was observed only in a Hind III digest and not in an Eco RI digest (Fig. 2). This suggests that the break was not in an exon, although the possibility remains that the Eco RI fragment was too small to be retained on the gel. Finally, in another patient (MB), exon 7 was deleted (Hind III and Eco RI digest), and the Hind III fragment containing exon 6 was altered in size. Since the exon 6 Eco RI fragment was unaltered, the break cannot be within the exon.

The number of codons deleted from the transcript for each of the patients examined is shown in Table 2. All the patients who are ambulatory beyond 16 years of age (BMD) showed a frame-shift mutation resulting from deletion of exons 3 to 7. Two of these patients have a cousin who is classified in Table 2 as intermediate as he lost ambulatory capacity at the age of 14; the clinical progression of all three individuals suggested classification as severe BMD. This emphasizes the arbitrary nature of the classification and shows that similar deletion patients can have somewhat different clinical progression. In the intermediate category eight of ten patients had a frame-shift deletion. Five patients with exons 3 to 7 deletion were classified clinically as severe Beckers. An additional patient having a deletion of exons 8 and 9 was also classified as a severe Becker. Two patients with deletion of exons 5 to 7 were initially diagnosed as having DMD but appear to show a milder progression. Two patients in this category had in-frame deletions of exons 5 to 9 and exon 3 alone. The patient having the exon 3 deletion had been originally diagnosed as having DMD. The patient with the in-frame deletion of exons 5 to 9 (JB), a severe BMD, is discussed later. All 13 severe (DMD) patients were found to carry frame-shift deletions.

The hypothesis that BMD results from a translational in-frame mutation and that DMD is the result of a frame-shift mutation is attractive for its simplicity and its amenability to detailed testing. Monaco *et al.* (*13*) have studied three BMD and three DMD patients in detail and presented evidence to support the hypothesis. One of those BMD patients was a severe case (intermediate), and the other two were mild according to our classification scheme. One of the patients studied was JB whom we confirm to have an in-frame mutation, but whom we classified as an intermediate phenotype. In our more extensive study we do not find a simple correlation between severity of phenotype and type of deletion. Furthermore, in the patients studied here as well as in two of those studied by Monaco *et al.* (*13*) deletions occurring over the α-actinin homologous region (putative actin binding domain) may result in a mild phenotype, indicating that despite its evolutionary conservation, this region might not be essential to the function of the protein.

A major unexpected result of the study is the number of BMD patients having deletions of exons 3 to 7, resulting in the disruption of the translational reading

Table 2. Deletions at the 5′ end of the BMD/DMD gene. The patients were classified as mild, intermediate, and severe. Patients wheelchair-bound by age 12 are classified as severe, those wheelchair-bound at age 16 or later are classified as mild, and those wheelchair-bound at age 12 to 15 are classified as intermediates. Clinical details of all of the patients will be published elsewhere (*14*). All patients are unrelated, unless otherwise stated.

Patient	Exons deleted	Codons missing from transcript	Frame-shift	New nucleotide constitution
		Mild (BMD)		
Guys 484[a]	3–7	185⅓	Yes	T AAG.....AT GTT
Guys 490[a]	3–7	185⅓	Yes	T AAG.....AT GTT
Guys 1456[b]	3–7	185⅓	Yes	T AAG.....AT GTT
Card 1118[c]	3–7	185⅓	Yes	T AAG.....AT GTT
Card 1216[c]	3–7	185⅓	Yes	T AAG.....AT GTT
Card 26-5[d]	3–7	185⅓	Yes	T AAG.....AT GTT
		Intermediate		
Guys 829[a]	3–7	185⅓	Yes	T AAG.....AT GTT
Guys 991[e]	3–7	185⅓	Yes	T AAG.....AT GTT
Guys 470[f]	3–7	185⅓	Yes	T AAG.....AT GTT
Guys 1587[g]	3–7	185⅓	Yes	T AAG.....AT GTT
HSC 1323[h]	3–7	185⅓	Yes	T AAG.....AT GTT
Guys 1707[i]	8–9	103⅔	Yes	GAA G.....CAT TT
Glas JB[j]	5–9	232	No	T AAT.....CAT TT
Guys 1642[g]	5–7	128⅓	Yes	T AAT.....AT GTT
HSC 704[k]	5–7[l]	128⅓	Yes	T AAT.....AT GTT
HSC 785[m]	3	31	No	T AAG.....CCA AA
		Severe (DMD)		
Glas MB[n]	7	39⅔	Yes	CAT AG...AT GTT
Guys 164[p]	8–9	103⅔	Yes	GAA G.....CAT TT
Guys 1403[p]	8–9	103⅔	Yes	GAA G.....CAT TT
HSC 483[n]	8–9	103⅔	Yes	GAA G.....CAT TT
Glas AK[n]	8–9	103⅔	Yes	GAA G.....CAT TT
Card 1113[p]	8–9	103⅔	Yes	GAA G.....CAT TT
Glas AB[n]	3–7[l]	185⅓	Yes	T AAG.....AT GTT
Card 1319[n]	3–7	185⅓	Yes	T AAG.....AT GTT
Card 1301[n]	3–7	185⅓	Yes	T AAG.....AT GTT
Card 184[n]	5–7	128⅓	Yes	TAAT .....AT GTT
Card 634[d]	3–6	112⅓	Yes	T AAG.....G CCA
Card 10[n]	1	10⅓	q	.....AT GAA
HSC 353[n]	1	10⅓	q	.....AT GAA

[a]Guys 484, 490, and 829 are cousins and presumably carry the same deletion. They all are clinically diagnosed as severe Beckers. Patients 484 and 490 became wheelchair-bound at age 16 or later. Patient 829 became wheelchair-bound at age 14. [b]Wheelchair-bound at age 24, and is now 34 years old. [c]Ambulatory. Card 1118 is 22 years old; card 1216 is 15 years old and diagnosed as a mild BMD. [d]Clinical details not available. [e]Wheelchair-bound at age 13, and is now 21 years old. [f]Patient is 11 years old now and diagnosed as a severe BMD. [g]Patients are 12 years old and diagnosed as atypical BMD. [h]Patient is 10 years old now and the disease progression is similar to that of severe BMD. [i]Wheelchair-bound at age 14.5 and diagnosed as a severe BMD. [j]Wheelchair-bound at age 13 and is now 19 years old. [k]Ambulatory at age 13, was diagnosed as DMD. [l]Junction bands visualized by Hind III restriction enzyme. [m]Originally considered as a limb-girdle patient, wheelchair-bound at age 14. [n]Wheelchair-bound before age 12. [p]Under 12 years old and ambulatory; disease progression is similar to that of DMD. [q]Both patients are deleted for the initiation codon in exon 1 and therefore are not expected to produce any protein.

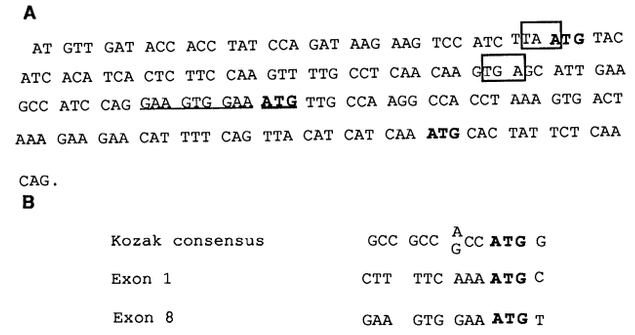

Fig. 3. Sequence of exon 8. (**A**) The sequence of exon 8 is shown. All in-frame ATG codons are shown in boldface. The most probable alternate initiation ATG and its flanking sequence is underlined. The stop codons in the 3 to 7 frame-shift deletion are boxed. (**B**) The Kozak consensus and the corresponding sequences in exon 1 and exon 8 are presented for comparison. Kozak consensus indicates the consensus sequence for initiation of translation in vertebrates (*23*). According to Kozak, 12 nucleotides preceding the ATG initiator codon are deficient in thymine residues, especially in positions −1 to −4. In positions −6 and −9, guanine is preferred. At position −3, 61% of vertebrate mRNAs have an adenine, and 36% of vertebrate mRNAs have guanine.

frame. The mild phenotype in these patients must be due to either the production of a partially functional protein or some other secondary factors that counteract the absence of dystrophin. One possibility may be that differential splicing masks the frameshift mutations, creating an in-frame mutation and resulting in the production of a partially functional protein. If, for example, exon 8 were spliced out of the message in the exons 3 to 7 deletion patients, then the mature message would be missing exons 3 to 8 (246 codons), and the remainder of the protein could be produced in the correct reading frame. There is no evidence to date, however, that indicates that the DMD/BMD gene is differentially spliced.

A second mechanism that could generate a partially functional protein in these patients is the occurrence of a new in-frame translational start site immediately downstream from the deletion. In this situation, a shortened protein could be produced from the deleted message initiated from the normal promoter. This possibility is supported by the fact that there are three in-frame ATG codons in exon 8, and the sequences flanking one of these ATGs conform to Kozak's consensus sequence for an initiation start (Fig. 3) (*23*). In the patients with a deletion of exons 3 to 7 the in-frame translational start site in exon 8 would be situated nearer to the promoter than in the normal case, and since the start site conforms to the Kozak consensus sequence even better than the start codon in exon 1, initiation in exon 8 may occur (Fig. 3). In this situation it is likely that translation would initiate at exon 1, read through exon 2, encounter an out-of-frame stop signal in exon 8, and then reinitiate translation at the next potential initiation codon. Even though reinitiation from an internal ATG codon has never been unambiguously observed in eukaryotic mRNAs, it has been demonstrated in vivo with synthetic constructs (*24*).

A third possibility for the exons 3 to 7 deletion cases is the presence of a second promoter possibly downstream from the deletion in the 110-kb intron (*8*) between exons 7 and 8, since that region is very large. There are examples of such dual promoters in muscle protein genes, for example, the myosin light chain gene. The two different isotypes for alkali myosin light chains, $MLC1_F$ and $MLC3_F$, are encoded by a single locus, which has two distinct promoters and gives rise to two different transcripts (*25*). While it is possible that a second promoter exists in the DMD/BMD gene there is no evidence of it so far. If it does exist, however, the ATG initiation codon in exon 8 could act as the primary initiation site for the shortened message. Analysis of the dystrophin produced in these patients, coupled with ribonuclease protection studies (*26*), should make it possible to determine whether protein is produced by these patients and, if so, to distinguish among the three alternatives presented above.

Secondary factors, such as genetic background, would appear also to play an important role in producing different phenotypes. This is evidenced by the fact that the exon 3 to 7 deletion occurs in several BMD patients, a number of intermediate cases, and two severe cases. An example of a factor that could be implicated is the level of growth hormone as DMD patients with growth deficiency show a milder progression (*27*) and severity has recently been reported to correlate with height (*27*). Duchenne and Becker cases, presumably carrying the same mutation, can also exist in the same family (*3, 29, 30*). In one family, for example, an affected boy became wheelchair-bound at age 10 (classical DMD), whereas an affected second cousin, presumably carrying the same mutation, became wheelchair-bound at age 19 (BMD) (*29*). In another family described by Forrest *et al.* (*30*) an apparently identical deletion in one family produced classical BMD in two brothers but produced only very mild muscle weakness in their great-great-uncle. This again illustrates that the disease phenotype shows a continuous spectrum. In a similar situation a deletion in the DXS206 locus appears to cause DMD in one boy, but to have no phenotype effect in his normal brother (*31*).

In conclusion, the definitive exon-intron boundaries for the first ten exons of the BMD/DMD gene have been established. Sequences flanking each of the exons are provided and should be of value for the application of the polymerase chain reaction (PCR) technique (*32*) to diagnostic studies aimed at identifying missing exons, substitutions, small deletions, or duplications in the 5′ end of the gene. The finding that frameshift deletions occur in patients with a Becker phenotype indicates that an alternative mechanism might exist to generate a protein with partial function. It is possible that an in-frame ATG codon in exon 8 may be used as an alternate initiation signal in these deletions. Analysis of a large number of BMD and DMD patients carrying deletions more toward the 3′ end of the gene, coupled with the establishment of exon-intron boundaries throughout the gene, will be important to further test the concept of non–frame shifting deletions being a common cause of the milder phenotype of Becker muscular dystrophy.

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The Effect of Histone Gene Deletions on Chromatin Structure in *Saccharomyces cerevisiae*

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As a way of studying nucleosome assembly and maintenance in *Saccharomyces cerevisiae*, mutants bearing deletions or duplications of the genes encoding histones H2A and H2B were analyzed. Previous genetic analysis had shown that only one of these mutants exhibited dramatic and pleiotropic phenotypes. This mutant was also the only one that contained disrupted chromatin, suggesting that the original phenotypes were attributable to alterations in chromosome structure. The chromatin disruption in the mutant, however, did not extend over the entire genome, but rather was localized to specific regions. Thus, while the arrangement of nucleosomes over the *HIS4* and *GAL1* genes, the telomeres, and the long terminal repeats (δ sequences) of Ty retrotransposons appeared essentially normal, nucleosomes over the *CYH2* and *UBI4* genes and the centromere of chromosome III were dramatically disrupted. The observation that the mutant exhibited localized chromatin disruptions implies that the assembly or maintenance of nucleosomes differs over different parts of the yeast genome.

THE YEAST *SACCHAROMYCES CEREVISIAE* contains two genes for histone H2A (designated *HTA1* and *HTA2*) and two genes for histone H2B (designated *HTB1* and *HTB2*) (*1*). These genes are located in two divergently transcribed gene pairs, an *HTA1-HTB1* gene pair on chromosome IV and an *HTA2-HTB2* gene pair on chromosome II (*1, 2*). The regulation of these two histone gene loci has been characterized in considerable detail. At one level, the timing of their transcription is precisely controlled so that histone genes are transcribed only during the late G_1 and early S phases of the cell cycle (*3, 4*). At a second level, the amounts of transcripts from the two loci are controlled. Surprisingly, the latter regulation, which probably ensures that H2A and H2B remain in stoichiometric balance with H3 and H4 (*2, 5, 6*), is mediated solely through the *HTA1-HTB1* gene pair (*2, 7*). Thus, in an *HTA1-HTB1* Δ*hta2-htb2* mutant, the intracellular level of transcripts from the *HTA1-HTB1* gene pair increases to compensate for the Δ*hta2-htb2* mutation; in a Δ*hta1-htb1*, *HTA2-HTB2* mutant, however, the intracellular level of transcripts from the *HTA2-HTB2* gene pair does not change (*2*).

As a result of this differential regulation, strains bearing deletions of the individual gene pairs exhibit different phenotypes. Thus, whereas an *HTA1-HTB1*, Δ*hta2-htb2* mutant is essentially wild type, a Δ*hta1-htb1*, *HTA2-HTB2* mutant is altered in the mitotic and meiotic cycles, the heat-shock response, germination, exit from stationary phase, and transcription (*2, 6*). The latter phenotypes were postulated to be caused by general changes in chromatin structure which, in turn, were caused by the inability of the remaining *HTA2-HTB2* locus to compensate for the Δ*hta1-htb1* mutation (*2, 6*). In this report, we directly tested this hypothesis by analyzing the arrangement of nucleosomes in isogenic mutants bearing various deletions and duplications of the two *HTA-HTB* gene pairs.

In the simplest model, decreased histone mRNA levels in the Δ*hta1-htb1*, *HTA2-HTB2* mutant would lead to a decreased intracellular concentration of H2A-H2B dimers. If this were to lead to a decreased number of nucleosomes and if nucleosomes were uniformly distributed along chromosomes, then the length of the linker DNA separating nucleosome cores should increase in the Δ*hta1-htb1*, *HTA2-HTB2* mutant. However, nucleosome ladders, which were produced by treating purified nuclei with micrococcal nuclease and separating the resulting DNA fragments on a 1% agarose gel, were essentially identical in all of the strains (Fig. 1). To rule out the possibility that the putative change in linker length was very small and hence indistinguishable on a 1% agarose gel, we also analyzed the digested DNA on a 5% acrylamide gel. Again, no difference in nucleosomal repeat length was detected (*8*). Therefore, the decreased level of histone gene transcripts in the Δ*hta1-htb1*, *HTA2-HTB2* mutant did not result in an increase in the length of the linker DNA between nucleosome cores, at least not in bulk chromatin.

Nonetheless, on closer examination, the nucleosome ladder in the Δ*hta1-htb1*, *HTA2-HTB2* mutant appeared to be superimposed on a slightly diffuse or smeared background (Fig. 1). This suggested that some chromosomal regions in the mutant were particularly sensitive to nuclease digestion. To test this possibility, we transferred DNA from the gel to nylon filters and performed DNA blot analysis (*9*), using radioactively labeled probes from a number of different regions of the yeast genome.

We first analyzed the structure of chromatin around various genes. Two of the genes, *HIS4* (*10*), which is involved in histidine biosynthesis (Fig. 1 and Table 1), and *GAL1* (*11*), which is involved in galactose catabolism (Table 1), exhibited essentially wild-type nucleosome ladders in all of the experimental strains, although a slight smearing of the two ladders was evident in the Δ*hta1-htb1*, *HTA2-HTB2* mutant. We therefore conclude that the structure of chromatin over *HIS4* and *GAL1* is essentially unaffected in any of the histone mutants. Two other genes, however, *UBI4* (*12*), which encodes ubiquitin (Fig. 1 and Table

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