cape digestion and either remain within the food vacuole or emigrate into the parasite cytoplasm. These results serve to emphasize the complexity and intricacy of parasite dependence on host resources. Knowledge of such dependence may, in the future, suggest alternative approaches to the control of parasitic diseases.

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Multigene Family for Sarcomeric Myosin Heavy Chain in Mouse and Human DNA: Localization on a Single Chromosome

Abstract. *Cloned myosin heavy chain DNA probes from rat and human were hybridized to restriction endonuclease digests of genomic DNA from somatic cell hybrids and their parental cells. The mouse myosin heavy chain genes detectable by this assay were located on chromosome 11, and three different human sarcomeric myosin heavy chain genes were mapped to the short arm of chromosome 17. A synteny between myosin heavy chain and two unrelated markers, thymidine kinase and galactokinase, was found to be preserved in the rodent and human genomes.*

Myosin heavy chain (MHC) is a major protein of the contractile apparatus. There are many forms of MHC, and their expression is developmentally regulated and tissue-specific (*1*). Analyses of complementary DNA (cDNA) and genomic clones indicate that sarcomeric MHC is encoded by a conserved, multigene family consisting of at least 13 different members (*2–6*). It is important to determine whether the genes of such a multigene family are in tandem arrays, are clustered in an interspersed pattern on one or several chromosomes, or are individual genes at multiple loci. Mammalian globin genes exist in small clusters that are dispersed on two chromosomes (*7*), whereas *Drosophila* actin genes and chicken tubulin genes are located at multiple loci (*8, 9*). Two other multigene families, the histocompatibility genes and the major urinary protein genes of the mouse exist in large complexes on one chromosome each (*10–12*).

Because MHC proteins are functionally related and the genes encoding them exhibit finely tuned differential expression, knowledge about the organization of these genes may help to distinguish molecular aspects of their control. Also, knowledge about the genomic organization of multigene families will ultimately shed light on molecular evolution. The nucleotide sequence of the messenger RNA (mRNA) for sarcomeric MHC is not related to the sequence of mRNA for nonsarcomeric MHC (*2*). It will be interesting to determine whether sarcomeric and nonsarcomeric MHC genes are located on the same chromosome.

We found that, in the human, at least three different skeletal MHC genes are located on one chromosome, and in the mouse, all detectable MHC genes are located on one chromosome. Furthermore, a syntenic relation between MHC genes and two unrelated markers has been preserved in the mouse and human genomes. The chromosomal localization was determined by hybridization of cloned MHC DNA probes to restriction enzyme digests of genomic DNA from somatic cell hybrids and their parental cells. The human MHC genes were assigned to the short arm of chromosome 17 and the mouse MHC genes to chromosome 11.

In these chromosomal localization studies, two types of somatic cell hybrids were used: mouse × rat and human × mouse. Somatic cell hybrids formed between primary mouse cells and the thymidine kinase–deficient (TK^-) rat cell line FT-1 retain a complete set of rat chromosomes together with smaller numbers of mouse chromosomes. Likewise, cell hybrids between primary human cells and a permanent mouse cell line segregate human chromosomes, retaining the full mouse complement. Mouse × hamster and human × mouse hybrids and their use in making gene assignments have been described (*13–16*).

A cDNA clone of rat MHC was used as a hybridization probe in the assignment of the mouse MHC gene complex. This clone corresponds to embryonic skeletal MHC mRNA and represents 630 nucleotides at the 3′ end of the mRNA (*17*). Vertebrate sarcomeric MHC genes are highly conserved (*2*). Because of cross-hybridization, an enzyme that distinguished restriction fragment patterns of mouse and rat MHC genes was needed. Hind III resulted in the most polymorphism between these species (data not shown).

Analysis of a series of mouse × hamster somatic cell hybrids had eliminated all but mouse chromosome 11 as the chromosome containing the MHC genes (data not shown). However, we were unable to make a positive assignment with these hybrid cells since mouse × hamster hybrids preferentially lose mouse chromosome 11 (*18*). Analyses of mouse × rat hybrids resulted in the assignment of the mouse MHC gene complex to chromosome 11. This assignment was based on positive hybridization to mouse MHC genes rather than on elimination of the other 19 mouse chromosomes by their failure to hybridize.

The mouse gene for TK, a selectable marker, is located on chromosome 11 (*19*). A rat TK^- cell line fused with primary mouse cells can be used with hypoxanthine - aminopterin - thymidine (HAT) selection (*20*) to isolate mouse × rat cells that contain mouse chromosome 11. This cell line grows in HAT medium and therefore expresses TK and also expresses the mouse and rat forms of galactokinase, an enzyme whose gene is located on mouse chromosome 11. Figure 1A shows the pattern obtained when the rat MHC cDNA probe was hybridized to Hind III digests of DNA from rat, mouse, and a hybrid rat × mouse cell. The DNA in lane 3 (Fig. 1A) contains MHC-specific bands from both rat and mouse. Two of the mouse-specific bands are obvious in lane 3, and two are faint. Karyotypic analysis indicates that the only mouse chromosome in this hybrid cell line is chromosome 11. These results concur with a recent report which assigned three muscle-specific mouse gene complexes (of which one was MHC) to three different chromosomes (*21*).

Three cloned human MHC probes were used to assign the human genes (*22*). Two of the probes represented subcloned fragments from two different adult skeletal muscle MHC genes, designated MYHSA1 and MYHSA2, whereas the third probe represented a subcloned fragment of an embryonic skeletal MHC gene, designated MYHSE1. Probes p2-3 and p8-3 directly flank the 3′ proximal ends of MYHSE1 and MYHSA1, respectively, and p10-3 is a coding-region probe from MYHSA2. These cloned human DNA probes exhibit a much less complex pattern of hybridization with genomic DNA than does the rat MHC cDNA clone (see lane 1 of Fig. 1A). Because cDNA is composed of exons spliced together, it represents many more genomic DNA fragments than does a single cloned genomic fragment. Two of the human DNA probes represent single-copy sequences that flank two MHC genes. They are less likely than coding-region probes to hybridize to other regions of the genome. Thirty-two hybrid cell lines were examined for hybridization to human MHC probes and isozyme markers (data not shown). There were no examples of discordance in these analyses with human MHC genes and chromosome 17, whereas all other chromosomes showed over 30 percent discordance in segregation. Twenty-three hybrids were examined for human MHC gene sequences, chromosomes, and isozymes (Table 1 and Fig. 1B); this resulted in the assignment of two of the genes to the short arm of human chromosome 17. Figure 1B shows the pattern obtained when p10-3 and p2-3 were hybridized together to human, mouse, and eight hybrid cell DNA's digested with Hind III. For example, lane 5 contains DNA from a hybrid cell line, ITW, that contains only human chromosome 17 on a mouse background (Table 1). Similar results were obtained when the third cloned human

Table 1. Segregation of MHC with human chromosomes in cell hybrids. Human myosin heavy chain genes were scored as plus (+) or minus (−) in cell hybrids as described (legend to Fig. 1B). Human-mouse cell hybrids were constructed by fusing four different mouse cell lines to ten different human fibroblast lines and selecting for hybrids in HAT medium (*24*). Chromosomes of hybrid cells were karyotyped and banded by Giemsa-trypsin staining (*15*). Enzyme markers assigned to each chromosome except the Y have been tested on each hybrid (*24*) confirming the chromosome analysis. The hybrids were WIL (WI-38 × LTP), REW (WI-38 × RAG), JWR (JoVa × RAG), XTR (GM194 × RAG), ATR (A1Tr × RAG), XER (GM2859 × RAG), JSR (JoSt × RAG), DUA (DUV × A9), TSL (GM2808 × LM/TK^-), ITW (IT22 × Weri) (obtained from R. Godbout, R. Phillips, and A. Bernstein), and NSL (GM2836 × LM/TK^-) (*14, 24, 31, 32*). WIL, REW, and ITW hybrids were derived from karyotypically normal parental cells; the others were derived from parental cells with well-defined translocation chromosomes for regional chromosome mapping. The translocation chromosomes are described in the references cited above. The 17/9 translocation retained in the NSL-5 hybrid contained only the p11 → qter region of human chromosome 17 (*14*) (see text). The presence or absence of chromosomes is indicated by + or −, respectively.

Hybrid	MHC	Chromosomes																							Translocations for regional mapping												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	2/1	1/2	X/3	3/X	10q⁻	5/X	11/X	X/11	7/9	X/15	17/3	17/9
WIL-7	+	−	+	+	−	+	+	−	+	−	+	+	−	+	+	−	−	+	+	−	−	+	−	+												
WIL-8X	+	−	−	+	+	+	−	+	+	−	+	+	+	−	+	−	−	+	+	+	+	+	−	+												
WIL-13	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+	−												
WIL-15	+	−	+	+	+	−	−	+	−	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+												
REW-5	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−	+	+	+												
REW-7	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+												
REW-10	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+												
REW-11	−	−	−	−	+	−	−	−	−	−	−	+	+	+	−	−	+	−	−	−	+	+	−	+												
REW-15	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+												
JWR-22H	+	−	−	−	+	−	+	−	−	−	+	+	−	−	+	−	−	+	+	−	+	+	−	−	+												
JWR-26C	−	−	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	−	+	−	+	+	−	+		+											
XTR-22	−	−	+	−	+	+	+	−	+	−	+	+	−	−	−	+	−	−	+	+	+	+	+	−			+										
XTR-3 BSAgB	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−				+	+								
ATR-13	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	−	−	−	−						+							
XER-7	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	−	−	−	+							+						
XER-11	+	+	−	+	+	−	+	+	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	−							+	+					
JSR-17S	+	+	−	+	−	+	−	−	+	+	+	+	+	+	+	+	−	+	+	−	+	+	+	−									+				
DUA-1A	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−										+			
EXR-5CSAZ	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−								+					
TSL-2	+	−	+	−	−	+	+	−	−	−	+	−	+	−	−	−	−	−	+	−	+	+	−	+											+		
NSL-9	+	−	−	−	−	+	−	−	+	−	+	−	+	+	+	+	+	+	−	−	+	+	+	−													+
NSL-5	−	+	−	−	−	−	−	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	−	−													+
ITW	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−													

DNA, p8-3, was used as a probe (data not shown). These results show that three different human skeletal MHC genes are located on chromosome 17. A hybrid line, NSL-5, containing the translocation, (17qter → 17p11::9q12 → 9qter) does not contain the human MHC bands (lane 8), regionally assigning the genes to the 17p11 → pter short arm region of chromosome 17.

Our results show (i) that the MHC sarcomeric multigene family is localized on one chromosome in both mouse and human and (ii) that there is a preserved synteny between MHC and two nonrelated markers, TK and galactokinase (*20, 23, 24*), in both mouse and human genomes. This finding might suggest that this locus is under evolutionary constraints since even unrelated markers remain linked through rodent and human evolution. Such a preserved linkage has also been found between the histocompatibility multigene family and an unrelated marker, glyoxylase I (*11, 25*). Our results suggest that chromosomal duplication such as that observed in the globin genes has not played a role in the generation of the sarcomeric MHC multigene family.

The rat cDNA probe pMHC25, which was used to assign the mouse genes to chromosome 11, represents 630 of 7100 nucleotides of the 3′ end of MHC mRNA. Since this clone represents less than 10 percent of the coding capacity of any one of the MHC genes, each band probably corresponds to a different gene. Genomic cloning studies have demonstrated that seven of eight bands hybridized to pMHC25 in a Hind III digest of rat genomic DNA represent seven distinct genes and that some bands contain more than one gene-specific sequence (*26*). Many of those bands have been isolated in genomic clones and identified as adult and embryonic, cardiac and skeletal, MHC genes (*6*). Therefore, it is likely that most, if not all, mouse sarcomeric MHC genes are located on one chromosome.

The assignment of three specific human MHC genes (two adult skeletal and one embryonic skeletal) to the short arm of chromosome 17 does not exclude the possibility that other human MHC genes are located on other chromosomes. However, since all of the detectable mouse MHC genes are clustered and syntenic with the genes for galactokinase and thymidine kinase, it seems likely that all human MHC genes, rather than just the three we examined, are clustered.

Of particular interest is whether the chromosomal location and the genomic organization of the sarcomeric MHC multigene family is directly involved in the tissue-specific and developmental regulation of each individual member, as seems to be the case for the globin (*27*) and immunoglobulin (*28*) genes.

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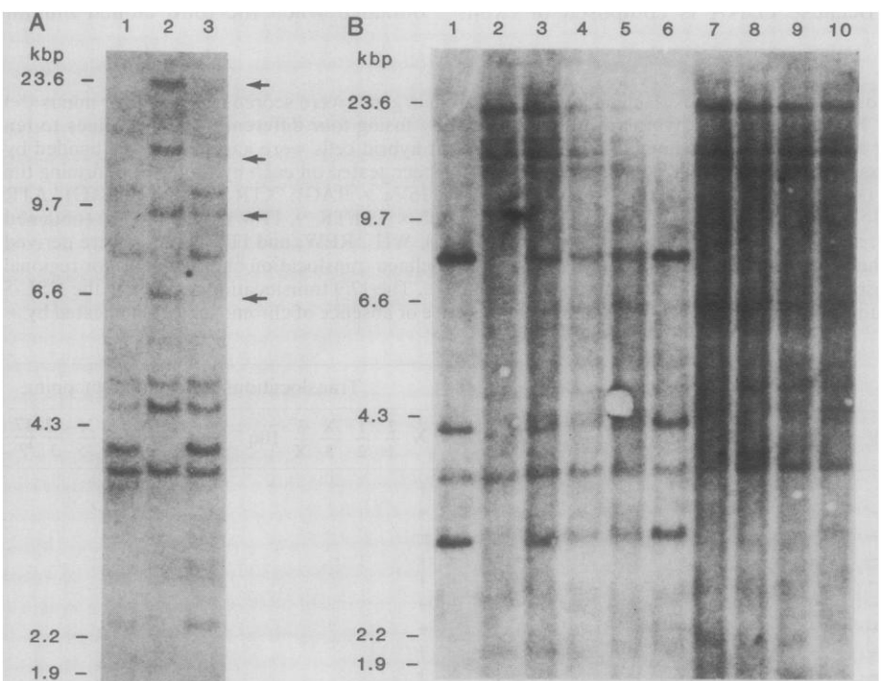

Fig. 1. Hybridization patterns of MHC clones to genomic digests of parental and hybrid cell DNA's. Genomic DNA was prepared as described (*15*), digested with Hind III restriction endonuclease, subjected to electrophoresis on agarose gels, transferred to nitrocellulose (*29*), and hybridized to probes as described (*15*). Cloned DNA was radioactively labeled according to (*30*). Each lane contains 10 μg of DNA. Size markers are indicated along the vertical axis in kilobase pairs (kbp). For the karyotypic analysis of each human × mouse hybrid cell line, see Table 1. (A) Hybridization of rat MHC clone, pMHC 25 (*19*), to rat, mouse, and hybrid cell DNA. (Lane 1) rat, (lane 2) mouse, and (lane 3) F7 (a rat × mouse hybrid). Mouse MHC restriction fragments that are distinguishable from those of the rat are indicated by arrows. (B) Hybridization of p10-3 and p2-3 to human, mouse, and hybrid cell DNA. Human MHC clones have been described (*22*). (Lane 1) human, (lane 2) mouse, (lane 3) JSR 17S, (lane 4) ATR13, (lane 5) ITW, (lane 6) WIL13, (lane 7) XER7, (lane 8) NSL5, (lane 9) XTR22, and (lane 10) ATR11. The positive hybrids are in lanes 3, 4, 5, and 6.

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Nitrite Inhibition of *Clostridium botulinum*: Electron Spin Resonance Detection of Iron–Nitric Oxide Complexes

Abstract. *Vegetative cells of* Clostridium botulinum *were shown to contain iron-sulfur proteins that react with added nitrite to form iron–nitric oxide complexes, with resultant destruction of the iron-sulfur cluster. Inactivation of iron-sulfur enzymes (especially ferredoxin) by binding of nitric oxide would almost certainly inhibit growth, and thus is probably the mechanism of botulinal inhibition by nitrite in foods.*

Sodium nitrite is widely used as an additive in cured meats, where it has important antimicrobial (*1, 2*) and antibotulinal (*3, 4*) properties. The addition of nitrite to cured meat products has been a source of controversy, however, since nitrite may be a precursor to the carcinogenic nitrosamines, especially in fried, cured meats such as bacon (*5, 6*). A number of compounds have been tested as nitrite substitutes, but no compound having all the desirable properties of nitrite (including color, antioxidant, and antibotulinal properties) has been found (*7*). The search for an effective nitrite replacement has been complicated by the fact that the antibotulinal mechanism of nitrite remains unknown, although several mechanisms have been proposed (*8, 9*).

Mirna (*10*) and van Roon and Olsman (*11*) demonstrated that nitrite via nitrous acid may react with sulfur-containing amino acids. Tompkin *et al.* (*12*) suggested that nitrite may react with ferredoxin, an iron-containing enzyme necessary for energy production in some clostridial vegetative cells (*13*), thus inhibiting growth. Woods and Wood (*14*) showed that nitrite does inhibit the phosphoroclastic reaction, presumably catalyzed by ferredoxin, in *Clostridium sporogenes* and *Clostridium botulinum*, although *C. botulinum* has not been shown to contain ferredoxin. We report here the presence of iron-sulfur centers in *C. botulinum* and the disappearance of these centers with simultaneous formation of nondialyzable iron sulfur–nitric oxide complexes on treatment of vegetative botulinal cells with sodium nitrite and ascorbate.

Untreated, air-oxidized samples of *C. botulinum* exhibited an electron spin resonance (ESR) signal characteristic of a "HiPiP-type" iron-sulfur center (Fig. 1A) (*15*). This axial signal at $g = 2.02$ was not appreciably saturated by 20-mW power at 6.5 K and disappeared when the temperature was increased to 30 K, properties indicative of the presence of Fe_3-S_3* or Fe_4-S_4* centers. A crude sonicated cell suspension of *C. botulinum* would probably have other metal-protein complexes, but these would not exhibit a fast-relaxing axial signal at $g = 2.02$ at liquid helium temperature, which is characteristic for iron-sulfur centers.

Addition of sodium dithionite as a reductant to the preparation resulted in the disappearance of the signal at $g = 2.02$ and the appearance of features characteristic of "$g = 1.94$–type" iron-sulfur centers (Fig. 1B). The feature at $g = 1.94$ also relaxed very rapidly at 7 K and broadened significantly (although it was still present) at 30 K, further confirming that the signal was due to the presence of reduced iron-sulfur centers (*16*). These results demonstrate the presence of iron-sulfur centers in *C. botulinum*.

Neither untreated (Fig. 1) nor nitrite-treated samples of *C. botulinum* exhibited an ESR signal characteristic of heme centers (high or low spin), consistent with the reported absence of cytochromes in Clostridia (*17, 18*). Figure 2A shows the ESR signal that appeared on addition of 200 ppm sodium nitrite to cell suspensions. This axial signal has a principal upward feature at $g = 2.035$ and is characteristic for nitrosyl complexes of a variety of iron-containing proteins, peptides, and chelate complexes (*19*), including purified succinate dehydrogenase (*20*). Thus, addition of nitrite to preparations of *C. botulinum* vegetative cells results in the formation of iron-nitrosyl complexes.

Figure 2B shows a spectrum for a preparation that was exposed to 500 ppm ascorbic acid in addition to nitrite. The signal is identical in shape to that in Fig. 2A but is approximately eight times more intense (note the difference in instrument gain). This signal did not disappear or decrease in intensity on dialysis with 0.075*M* EDTA in 0.1*M* phosphate buffer (*p*H 7.0). The increase in signal intensity in the presence of ascorbate is in agreement with the observation that nitrite is more inhibitory to microorganisms in the presence of reducing agents (*21*). Thus ascorbate enhances the formation of iron-nitrosyl complexes, probably by accelerating the reduction of nitrite to nitric oxide.

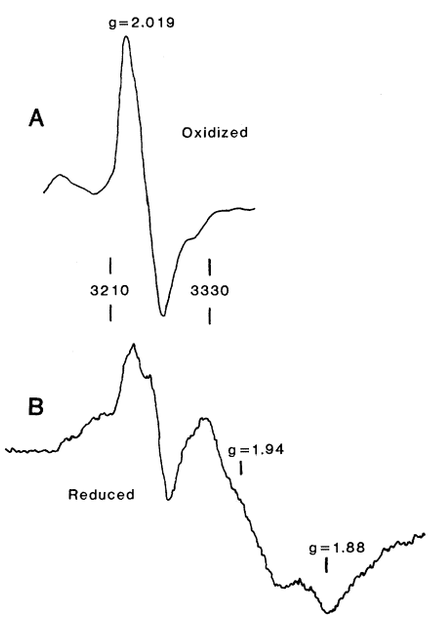

Fig. 1. Electron spin resonance spectra for untreated preparations of *C. botulinum* Type A (American Type Culture Collection). The cells were grown in chopped liver broth (*25*) and then propagated in anaerobic growth medium (*26*) for 1 week at 37°C. Actively growing vegetative cells were centrifuged at 7000*g* for 20 minutes, resuspended in 0.1 percent peptone in water, and centrifuged and suspended twice again to obtain washed, packed cells. Next the cells were sonicated with a microtip probe (Heat Systems–Ultrasonics, Inc.), transferred to ESR tubes, frozen in liquid nitrogen, and stored at 77 K. Instrument settings: microwave frequency, 9.136 GHz; modulation frequency, 100 kHz; and modulation amplitude, 12.5 G (6.5 K). The instrument gain is 5×10^2 in (A) (oxidized) and 8×10^2 in (B) (reduced with excess sodium dithionite). The *x*-axis represents magnetic field in gauss.