A novel neural beta tubulin from the antarctic fish *Notothenia coriiceps neglecta*

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From the standpoint of cold adaptation, the ectothermic fishes of antarctic coastal waters, which now experience body temperatures as low as the freezing point of sea water (–1.86°C), present biochemists with ideal experimental systems. Subjected to an increasingly severe thermal environment as the southern ocean began cooling approximately 40 million years ago, antarctic fishes diverged from temperate fishes (DeWitt 1971) and evolved cellular and biochemical adaptations that maintain metabolic efficiency and preserve macromolecular structure at their now chronically low body temperatures (–1.86 to +2°C). Recent work from my laboratory has been focused on the molecular adaptations that enable the cytoplasmic microtubules of antarctic fishes to assemble from their component proteins, tubulin alpha–beta dimers and microtubule-associated proteins (MAPs), in such an extreme thermal regime (Detrich and Overton 1986; Detrich, Prasad, and Ludueña 1987; Detrich, Johnson, and Marchese-Ragona 1989; Detrich et al. 1990, 1992; Skoufias, Wilson, and Detrich 1992). Together, these studies indicate that the major locus of functional adaptation is the tubulin dimer, not the MAPs. To understand the nature of these adaptations, we have initiated efforts to determine the primary sequences (that is, the order of amino acid subunits) of the alpha and beta tubulins of antarctic fishes by molecular–biological methods. In this report, we describe the sequence of a neural beta tubulin from the antarctic rockcod, *Notothenia coriiceps neglecta* (Detrich and Parker 1993).

Microtubules are a major component of the eukaryotic cytoskeleton, and they play critical roles in many cellular processes, including mitosis, intracellular transport, and the determination of cell shape. In vertebrates, the alpha- and beta-tubulin subunits of microtubules are encoded by 12–14 genes (6–7 alpha and 6–7 beta), each of which yields a distinct polypeptide, or "isotype" (Sullivan 1988). Because brain tissues express multiple alpha- and beta-tubulin isotypes (Sullivan 1988), we have focused our adaptational analysis on the neural tubulins of *N. coriiceps neglecta*. We hypothesize that the cold-adapted properties of these tubulins result, at least in part, from primary sequence changes located in the inter-dimer contact domains of the alpha and/or beta subunits.

To facilitate determination of the primary sequences of tubulins, we purified messenger ribonucleic acid (mRNA) from brain tissues of *N. coriiceps neglecta* and generated a complementary deoxyribonucleic acid (cDNA) library in the bacteriophage lambda gt10 (Detrich and Parker 1993). From this library, we isolated a 1.8-kilobase neural beta-tubulin cDNA, Ncn*beta1*. This cDNA contains an open reading frame of 446 codons, 67 nucleotides of 5' untranslated sequence, and 425 nucleotides of 3' untranslated sequence beyond the stop codonTAG (figure 1). The tubulin encoded by Ncn*beta1* (figure 1) is almost equally related to neural beta chains of classes II (95.0–95.7 percent sequence homology) and IV (95.1–96.2 percent homology). However, joint consideration of protein and nucleotide (not shown) sequence homologies suggests that the Ncn*beta1* polypeptide is most parsimoniously assigned to the beta-II isotypic class.

The carboxyl terminus of the Ncn*beta1* polypeptide (residues 431–446) is noteworthy because it has diverged substantially from those of other vertebrate neural beta isotypes (figure 2). In higher vertebrates, the carboxy-terminal region largely defines the highly conserved beta-chain isotypic classes (Sullivan 1988). Thus, strong conservation of beta-chain isotypes across higher vertebrate taxa, which is characteristic of mammals and birds, may not extend to the more distantly related fishes.

To identify potentially adaptive residue changes in Ncn*beta1*, we have compared its primary sequence with those of other vertebrate beta tubulins (Detrich and Parker 1993). As shown in the table, the Ncn*beta1* polypeptide contains several unique amino acid substitutions and an unusual carboxy-terminal residue insertion. Three conservative replacements (isoleucine for methionine at position 267, glycine for serine at 278, and serine for alanine or glycine at 283) are clustered near a region of beta tubulin that is thought to form contacts between tubulin dimers (Arévalo et al. 1990) in the microtubule. Furthermore, replacement of tyrosine by phenylalanine at position 200 is unusual in vertebrate beta chains, where it occurs only in the erythroid-specific class VI. Substitution of alanine for a bulky aliphatic amino acid (valine or isoleucine) at position 333 is unique and nonconservative, and the tyrosine inserted at position 442 is novel. We conclude that the Ncn*beta1* chain is a class-II beta isotype that contains several sequence changes that are likely to contribute to its unique functional properties at low temperature. Currently, we are pursuing cDNAs for all neural alpha and beta tubulins so that we may catalog the repertoire of primary sequence alterations of potential adaptive importance.

Experiments performed at Palmer Station during the 1992–1993 field season addressed four other project objectives. As part of our effort to characterize the functional properties of antarctic fish tubulin isotypes, Laura Camardella purified tubulins from nucleated erythrocytes of two nototheniids (*N. coriiceps neglecta* and *Gobionotothen gibberifrons*) and examined their assembly reactions *in vitro*. Anthony Frankfurter analyzed the brain, egg, erythrocyte, and sperm tubulins of antarctic fishes for carboxy-terminal post-translational modifications that may influence microtubule assembly and stability *in vivo*. Stephen King purified and

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                                                -67   CTGCTTCGTTCGCAGCTGAATCACTGCAGTCCAACAACTTAGCCTTTTTCGGTCTTTGTTCACCAAA

  1  ATG AGG GAA ATC GTG CAC CTT CAG GCT GGC  CAG TGT GGA AAC CAA ATT GGA TCC AAG TTT  TGG GAA GTC ATT AGC GAC GAG CAT GGC ATC
  1  Met Arg Glu Ile Val His Leu Gln Ala Gly  Gln Cys Gly Asn Gln Ile Gly Ser Lys Phe  Trp Glu Val Ile Ser Asp Glu His Gly Ile

 91  GAC CCA ACC GGG TCT TAC CAT GGG GAC AGC  GAC CTG CAG CTG GAT CGC ATC AAC GTG TAT  TAC AAC GAG GCT TCA GGC GGA AAG TAT GTC
 31  Asp Pro Thr Gly Ser Tyr His Gly Asp Ser  Asp Leu Gln Leu Asp Arg Ile Asn Val Tyr  Tyr Asn Glu Ala Ser Gly Gly Lys Tyr Val

181  CCC CGG GCA GTG CTG GTG GAC TTG GAG CCC  GGC ACC ATG GAC TCA GTG AGG TCC GGT CCC  TTT GGC CAG ATT TTT AGA CCA GAC AAC TTT
 61  Pro Arg Ala Val Leu Val Asp Leu Glu Pro  Gly Thr Met Asp Ser Val Arg Ser Gly Pro  Phe Gly Gln Ile Phe Arg Pro Asp Asn Phe

271  GTC TTT GGC CAG AGC GGA GCT GGT AAT AAC  TGG GCT AAA GGT CAC TAC ACT GAG GGA GCC  GAG CTG GTG GAC TCA GTC CTG GAT GTG GTG
 91  Val Phe Gly Gln Ser Gly Ala Gly Asn Asn  Trp Ala Lys Gly His Tyr Thr Glu Gly Ala  Glu Leu Val Asp Ser Val Leu Asp Val Val

361  AGG AAG GAG GCG GAG GGA TGC GAC TGC CTG  CAG GGC TTC CAG CTC ACA CAC TCC CTG GGT  GGA GGG ACT GGC TCG GGC ATG GGC ACG CTG
121  Arg Lys Glu Ala Glu Gly Cys Asp Cys Leu  Gln Gly Phe Gln Leu Thr His Ser Leu Gly  Gly Gly Thr Gly Ser Gly Met Gly Thr Leu

451  CTC ATC AGC AAA ATC AGA GAG GAG TAT CCA  GAC CGC ATC ATG AAC ACT TTC AGC GTG GTG  CCT TCG CCT AAG GTT TCA GAC ACA GTG GTG
151  Leu Ile Ser Lys Ile Arg Glu Glu Tyr Pro  Asp Arg Ile Met Asn Thr Phe Ser Val Val  Pro Ser Pro Lys Val Ser Asp Thr Val Val

541  GAG CCA TAC AAC GCC ACC CTC TCG GTC CAC  CAG CTG GTG GAG AAC ACA GAT GAG ACC TTC  TGC ATT GAT AAT GAG GCG CTG TAT GAC ATC
181  Glu Pro Tyr Asn Ala Thr Leu Ser Val His  Gln Leu Val Glu Asn Thr Asp Glu Thr Phe  Cys Ile Asp Asn Glu Ala Leu Tyr Asp Ile

631  TGT TTC CGC ACG CTG AAG CTC ACC ACC CCC  ACC TAT GGA GAC CTC AAC CAC CTC GTC TCA  GCC ACC ATG AGC GGG GTG ACC ACA TGT CTG
211  Cys Phe Arg Thr Leu Lys Leu Thr Thr Pro  Thr Tyr Gly Asp Leu Asn His Leu Val Ser  Ala Thr Met Ser Gly Val Thr Thr Cys Leu

721  CGC TTC CCC GGC CAG CTC AAT GCT GAT CTG  AGG AAA CTG GCC GTC AAC ATG GTG CCC TTC  CCC AGA CTG CAC TTC TTC ATT CCG GGC TTT
241  Arg Phe Pro Gly Gln Leu Asn Ala Asp Leu  Arg Lys Leu Ala Val Asn Met Val Pro Phe  Pro Arg Leu His Phe Phe Ile Pro Gly Phe

811  GCC CCG CTG ACC AGT CGT GGC GGC CAG CAG  TAC AGG TCG TTG ACT GTT CCT GAG CTC ACC  CAG CAG ATG TTC GAC TCC AAG AAC ATG ATG
271  Ala Pro Leu Thr Ser Arg Gly Gly Gln Gln  Tyr Arg Ser Leu Thr Val Pro Glu Leu Thr  Gln Gln Met Phe Asp Ser Lys Asn Met Met

901  GCA GCC TGT GAC CCG CGC CAC GGC CGC TAC  CTC ACG GTA GCC GCC ATC TTC AGA GGC CGC  ATG TCC ATG AAG GAA GTG GAT GAG CAG ATG
301  Ala Ala Cys Asp Pro Arg His Gly Arg Tyr  Leu Thr Val Ala Ala Ile Phe Arg Gly Arg  Met Ser Met Lys Glu Val Asp Glu Gln Met

991  TTG AAT GCA CAG AAC AAA AAC AGC AGC TAC  TTC GTT GAG TGG ATC CCA AAC AAC GTG AAG  ACT GCC GTC TGC GAC ATT CCT CCC CGT GGC
331  Leu Asn Ala Gln Asn Lys Asn Ser Ser Tyr  Phe Val Glu Trp Ile Pro Asn Asn Val Lys  Thr Ala Val Cys Asp Ile Pro Pro Arg Gly

1081  CTC AAG ATG GCC GCC ACC TTC ATC GGC AAC  AGC ACG GCC ATT CAG GAG CTG TTC AAG CGC  ATC TCA GAG CAA TTC ACT GCC ATG TTC CGC
 361  Leu Lys Met Ala Ala Thr Phe Ile Gly Asn  Ser Thr Ala Ile Gln Glu Leu Phe Lys Arg  Ile Ser Glu Gln Phe Thr Ala Met Phe Arg

1171  CGC AAG GCC TTC CTC CAC TGG TAC ACT GGC  GAG GGC ATG GAT GAG ATG GAG TTC ACA GAG  GCT GAG AGC AAC ATG AAC GAC CTG GTG TCT
 391  Arg Lys Ala Phe Leu His Trp Tyr Thr Gly  Glu Gly Met Asp Glu Met Glu Phe Thr Glu  Ala Glu Ser Asn Met Asn Asp Leu Val Ser

1261  GAG TAC CAG CAG TAC CAG GAC GCC ACT GCT  GAG GAG GAG GGC GAG TTT GAA GAG GAG GGC  GAA TAT GAA GAT GGA GCC     TAG ATGCCCA
 421  Glu Tyr Gln Gln Tyr Gln Asp Ala Thr Ala  Glu Glu Glu Gly Glu Phe Glu Glu Glu Gly  Glu Tyr Glu Asp Gly Ala     Amb

1349  TAACAACCTTTTTCTCTCCCAATGCAACAGTTAATTAAAGAAGTCATATTATGCTTAGTTCAAAGGACCGATTTGCATTTTGTGCCTCTTCTGTGACATGTTTACATGGCTTAATGT
1466  TCAAAACGTGCTTTATTTTTCTCATTCTGCTGCTGTATTCATTTGTGATAATGTAACAGTGACCAGACCAGGGGACTAATCACTGAATGTGACAATTACTGTCTGATGTGCAGTGTT
1583  ATTCTTTTGCCAAAAAAAATCATAGTTTACACTAAAAGCTACACATAGTGTCAGGAACTTGTCTCTGTTATAATATTCCTGATGTGCTTGTAGTGGTAAATTGGGATCAAATGTGAT
1700  TTTAATAAAGATGTATTATTTGGTTCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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Figure 1.Nucleotide sequence of the Ncn*beta1* cDNA and deduced primary sequence of the encoded beta tubulin. Eight amino acid residues that differentiate Ncn*beta1* from other vertebrate neural tubulins (classes I–IV) are shown in outline font. Nucleotide positions and amino acid residues (three-letter code) are numbered on the left. Amb indicates the amber translation termination codon, and the probable polyadenylation signal is underlined. The nucleotide sequence of the Ncn*beta1* cDNA (GenBank accession number L08013) was established by use of the dideoxynucleotide chain-termination procedure (Sanger, Nicklen, and Coulson 1977) as described elsewhere (Detrich and Parker 1993). [Reprinted from Detrich and Parker (1993) with permission. Copyright 1993 Wiley-Liss, Inc.]

Class	Organism	Tubulin	431	%Identity
I	mouse	Mβ5	E E E E D F G E E A E * E * E A	56
	chicken	cβ7	E E E E D F G E E A E * E * E A	56
II	mouse	Mβ2	D E Q G E F E E E E G * E D E A	62
	chicken	cβ1/2	D E Q G E F E E E G E * E D E A	75
	N. coriiceps	Ncnβ1	E E E G E F E E E G E Y E D G A	
IVb	mouse	Mβ3	E E E G E F E E E A E * E E V A	75
	chicken	cβ3	E E E G E F E E E A E * E E A E	69
IVa	mouse	Mβ4	E E * G E F E E E A E * E E V A	69
III	chicken	cβ4	E E E G E M Y E D D E E E S E Q G A K	58

Figure 2.The unique carboxyl terminus of Ncn*beta1* tubulin. Presented for comparison with the Ncn*beta1* peptide are the isotype-defining sequences (beginning at position 431) of neural beta chains (classes I–IV) from higher vertebrates. Asterisks indicate single amino acid gaps introduced to establish maximal sequence homology. For each pairwise comparison with the Ncn*beta1* carboxyl terminus, sequence homology was calculated as percentage residue identity with respect to the longer sequence. The Ncn*beta1*/class-III alignment is shown in suboptimal register, but its homology value (11 matches/19 residues × 100 percent = 58 percent) is calculated for the optimal alignment (single amino acid gaps positioned after Ncn*beta1* residues 435 and 437). Beta-isotypic sequences for mouse and chicken are from Wang et al. (1986) and from Monteiro and Cleveland (1988), respectively. [Reprinted from Detrich and Parker (1993) with permission. Copyright 1993 Wiley-Liss, Inc.]

Comparative analysis of the Ncnbeta1 primary sequence[a]

Position	Ncn*beta1*	Neural beta-tubulin isotypes II	IVa/b	I	III
18	Ser	Ala	Ala	Ala	Ala
126	Gly	Ser	Ser	Ser	Asn
200	Phe	Tyr	Tyr	Tyr	Tyr
267	Ile	Met	Met	Met	Met
278	Gly	Ser	Ser	Ser	Ser
283	Ser	Ala	Ala/Gly	Ala	Ala
333	Ala	Val	Val	Val	Ile
442	Tyr	—	—	—	—

[a]The table includes those sequence positions that uniquely differentiate the Ncn*beta1* polypeptide from vertebrate neural beta tubulins of isotypic classes I, II, and/or IV (the isotypes most closely related to Ncn*beta1*). Classes I–IV are ordered from left to right by sequence homology (high to low) to Ncn*beta1*. Because the tyrosine at position 442 in Ncn*beta1* represents a sequence insertion, vertebrate classes I–IV lack a corresponding residue (dashes). Reference vertebrate beta-tubulin sequences (GenBank database) are from chicken (classes I–IV), mouse (I, II, and IV), human (I, IV), pig (II), rat (II), and the amphibian *Xenopus laevis* (II). [Reprinted from Detrich and Parker (1993) with permission. Copyright 1993 Wiley-Liss, Inc.]

characterized inner- and outer-arm dyneins from sperm flagella of *N. coriiceps neglecta*; these specimens will support future research on cold adaptation of these important mechanochemical MAPs. Finally, graduate student Martin Billger developed methods for culturing primary explants of notothenid and channichthyid skin cells and for immunofluorescent staining of their microtubule cytoskeletons.

To support our research, we obtained specimens of two notothenids (*N. coriiceps neglecta* and *G. gibberifrons*), three icefishes (*Chaenocephalus aceratus, Champsocephalus gunnari*, and *Chionodraco rastrospinosus*), a bathydraconid (*Parachaenichthys charcoti*), and two bathyrajids (*Bathyraja maccaini* and *B. griseocauda*) by bottom trawling from R/V *Polar Duke* south of Low Island [Western Bransfield Strait Marine Site of Special Scientific Interest (MSSSI) Number 35] and near Brabant Island (East Dallmann Bay MSSSI Number 36). Several deep-water benthic notothenids, bathydraconids, artedidraconids, and zoarcids were collected at 900 meters in Crystal Sound east of Lavoisier Island. Fishes were transported to Palmer Station, where they were maintained in sea water aquaria at –1 to +1°C.

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