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Dynamic instability of microtubules from antarctic fishes

H. WILLIAM DETRICH, III, *Department of Biology, Northeastern University, Boston, Massachusetts 02115*
ROBLEY C. WILLIAMS, JR., *Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235*

Microtubules are dynamic cellular polymers that undergo alternating periods of growth and shortening, both *in vivo* and *in vitro*, through the end-dependent addition and loss of tubulin alpha-beta dimers. Although this "dynamic instability" has been analyzed extensively using microtubules from mammalian brain tissues (Mitchison and Kirschner 1984; Walker et al. 1988; Gildersleeve et al. 1992), the dynamic properties of microtubules from cold-living poikilotherms are only partially understood (Himes and Detrich 1989; Simon, Parsons, and Salmon 1992). In this context, microtubules from the ectothermic fishes of antarctic coastal waters are particularly interesting. As the southern ocean began to cool approximately 40 million years ago, the antarctic fishes diverged from temperate fishes (DeWitt 1971) and evolved molecular, cellular, and physiological adaptations that preserve metabolic efficiency and macromolecular function at their now chronically low body temperatures (–1.86°C to + 2°C). Recent work from my laboratory has shown that the molecular adaptations that enable the microtubules of antarctic fishes to assemble and to remain stable in this extreme thermal regime reside in their tubulin subunits (Detrich, Johnson, and Marchese-Ragona 1989; Detrich et al. 1990, 1992; Detrich and Parker 1993). Here we describe the dynamic instability of brain microtubules from two antarctic rockcods, *Gobionotothen gibberifrons* and *Notothenia coriiceps*. Our results suggest that functional adaptation of tubulins to low temperatures yields microtubules characterized, at both physiological and nonphysiological temperatures, by unusually slow subunit exchange. For a more detailed analysis, see Billger et al. (1994).

Tubulin free of microtubule-associated proteins (MAPs) was purified from brain tissues of the two rockcods by methods previously described (Detrich and Overton 1986; Detrich et al. 1989). To assess the dynamic instability of microtubules assembled from these tubulins, we measured *in vitro* the rates of growth and shortening of individual antarctic fish microtubules (nucleated from sea urchin flagellar axonemes), and the frequencies of interconversion between these states, by video-enhanced differential interference contrast microscopy (cf. Walker et al. 1988; Gildersleeve et al. 1992) at temperatures between 5°C and 25°C. The table shows that microtubules from *G. gibberifrons* (*Gg*) or from *N. coriiceps* (*Nc*) display dynamic instability at the near-physiological temperature of 5°C. Their rates of growth and shortening, however, as well as their frequencies of catastrophe (transition to shortening) and rescue (resumption of growth), are an order of magnitude (or more) smaller than those observed for cow (*Bos taurus; Bt*) brain microtubules at 37°C. Thus, the fish microtubules exhibit dynamic instability but at slower rates and with smaller transition frequencies than their mammalian counterparts. Attempts to assemble microtubules from bovine tubulin at 5°C were unsuccessful. Therefore, no direct, isothermal comparison of piscine and bovine microtubule dynamics was possible.

Antarctic fish brain microtubules also grow slowly at 25°C, and two rate classes, presumably corresponding to the distinct plus (+) and minus (–) ends of the polar microtubule polymer, are observed (*see* table). Disassembly rates also

Kinetic parameters for microtubule dynamic instability

Species	Temperature (°C)	Assembly rate (μm/min)	Disassembly rate (μm/min)	Catastrophe frequency (per minute)	Rescue frequency (per minute)
Gg/Nc[a]	5–8	0.21±0.16	–1.9±0.6	0.008	<0.0004
Gg/Nc[a]	25	(+) 0.53±0.08 (–) 0.12±0.07	–0.24±0.11	ND	ND
Bt (cow)[b]	37	2.5±0.58	–37±28	0.06–0.3	1.2–3.0

[a]Measurements were made as described in Billger et al. (1994).
[b]Assembly and disassembly rates for bovine tubulin are from Gildersleeve et al. (1992), and catastrophe and rescue frequencies (+ ends) are from Walker et al. (1988).
ND, not determined.

remain small at this temperature. Thus, it is unlikely that the large differences in the dynamics of antarctic fish microtubules at low temperatures and bovine microtubules at high temperatures are due to the temperature difference alone. Rather, some difference in tubulin structure must account for them.

In summary, our results show that microtubules from the cold-adapted antarctic fishes are dynamic polymers at low temperatures but much less so than are the microtubules of homeotherms. The greater stability of antarctic fish microtubules, relative to the mammalian polymer, appears to result not from the alteration of a single aspect of dynamic instability but from alteration of them all.

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RACER: Feeding incidence and yolk resorption in three species of antarctic larval fishes

EDWARD A. LAMAN *and* VALERIE J. LOEB, *Moss Landing Marine Laboratories, Moss Landing, California 95039*

Fishes of the perciform suborder *Notothenioidei* show numerous adaptations to the extreme and variable environment in the southern oceans. Yolk reserves of spring-hatched larvae may allow survival for extended periods in the plankton if the breakup of the pack ice and subsequent spring bloom are delayed. Notothenioid larvae over a wide range of length and development have been observed with yolk reserves, indicating that yolk resorption may be related to early feeding success and food availability (North and Kellermann 1989; Kellermann 1990). Exogenous feeding can commence prior to complete yolk resorption for some species (e.g., *Chionodraco rastrospinosus* and *Lepidonotothen larseni*; Kellermann 1986, 1990) and is possibly characteristic of all notothenioids. Hatching periods for each species show little variation between years (North and Kellermann 1989) and may be linked to the highly seasonal secondary production cycle (North and White 1987, pp. 381–390).

This study examined the relationship between feeding incidence and yolk utilization by three species of larval fishes collected in Gerlache Strait by the Research on Antarctic Coastal Ecosystem Rates (RACER) program during spring (October and November) 1989. Feeding incidence and yolk utilization were also examined with respect to possibly different feeding conditions within regional hydrographic regimes in Gerlache Strait. The three species, *Lepidonotothen larseni, Trematomus lepidorhinus,* and *T. newnesi,* numerically dominated the 1989 samples. All three had variable amounts of yolk reserves present over the whole size range sampled (Laman and Loeb 1993). Yolk reserves were measured using a specific surface area (SSA) index with the aid of an image analysis system (*see* Laman and Loeb 1993). Feeding incidence for these fishes was calculated as the percentage of guts containing food out of all guts sampled. Yolk utilization rates were estimated as the slope from a regression of SSA on larval length. Using reported larval growth rates, length was converted to units of time to yield an estimate of resorption; indicated by a decreasing SSA.

During spring of 1989, the hydrography of Gerlache Strait was characterized by a swift axial current (ranging from 15 to 60 centimeters per second) flowing from southwest to north-