

Cloning and Sequencing of HSP70 in Antarctic Fish

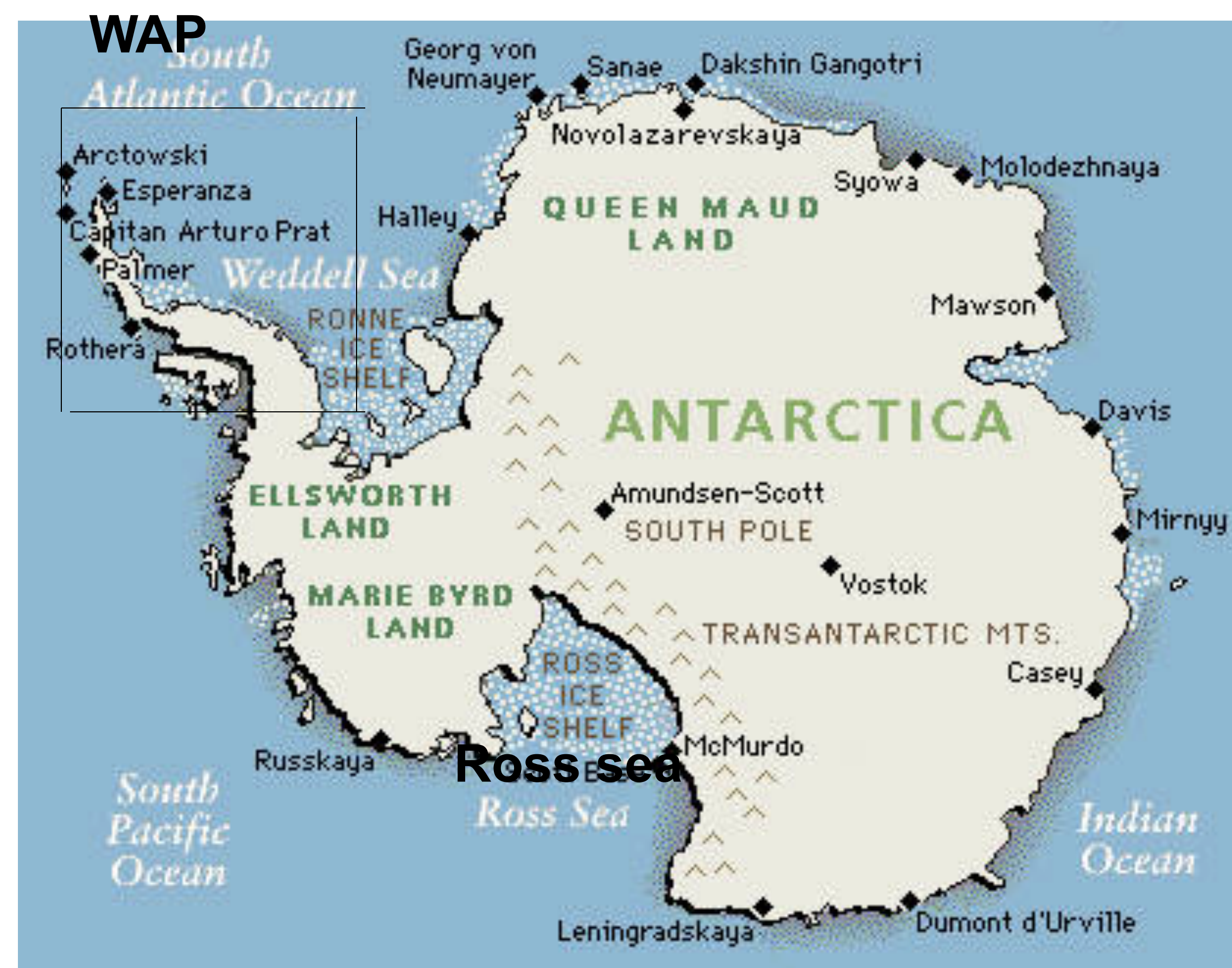
Sarah Marshall



Introduction and Background

The cold water shelves surrounding the Antarctic are characterized by constant subzero temperatures. These waters are home to fish of the suborder Notothernioidei. Within this suborder there are red-blooded fish, which have retained hemoglobin expression and white-blooded fish, which have lost hemoglobin expression. The white-blooded Notothenioids belong to the family Channichthyids, commonly referred to as icefish.

Because of the cold stable environment in which these fish live, evolutionary pressure has not selected for a Heat Shock Response (HSR). The HSR is characterized by an elevated level of heat shock proteins in response to thermal stress. Heat shock proteins (HSPs) are molecular chaperones responsible for folding newly synthesized proteins, as well as those that have been denatured as a result of stress, especially heat. Although the HSR is highly conserved among species, studies have shown that certain Notothenioids lack a response (Hoffman et al. 2000).



Previous research on Hsps focused on *Trematomus bernacchii*, a fish native to the Ross Sea. The Ross Sea is both the coldest and most stable region of the Southern Ocean, with temperatures at a constant -1.8C year round. It was determined that these fish do not have a HSR (Hoffman et al. 2000). My undergraduate cell biology lab continued research on the HSR in two species of Notothenioids, the red-blooded *Notothenia coriiceps* and the white-blooded *Chaenocephalus aceratus*, that are native to the Western Antarctic Peninsula (WAP). The WAP is warmer and more

variable than the Ross Sea, with temperatures ranging from -1.8C to 2C. Although there was a difference in HSP mRNA levels between the two fish, neither exhibited a HSR. HSP70 mRNA levels tended to be higher in the white-blooded fish.

The aim of this study is to determine if levels of HSP 70 increase in response to prolonged thermal stress, 4C for one week, and if these levels differ between the red and white-blooded species. We will determine this through quantitative real time PCR. First, we will need to clone and sequence HSP70 from various Notothenioids to ensure that our gene specific primers are accurate for all of the species that will be studied.

I hypothesize that HSP70 will be highly conserved among notothenioids, and that our sequence specific primers will work for all species studied.

Results

Through RNA isolation, PCR, molecular cloning, and sequencing we have acquired the sequence of HSP70 for various additional antarctic fish. Through sequence analysis we can see that our gene specific primers will be accurate for all species of Notothenioids in this study.

MAC_HSP:atatctgcagaattcgcccttctg**ttcactgacaccgagaggctcatc**
RAS_HSP:atatctgcagaattcgcccttctg**ttcactgacaccgagaggctcatc**

ggggacg**cagcaaagaaccaggtggct**ttgaaccctagcaacacgggtgtttgatg
ggggacg**cagcaaagaaccaggtggct**ttgaaccctagcaacacgggtgtttgatg

Figure 1: HSP70 sequences in Antarctic Fish. These are the first portion of sequences from *E. maclovinus* (top), a red-blooded Notothenioid, and *C. rastrispinosus* (bottom), a white blooded Notothenioid. Both sequences were obtained through a big dye reaction and sequencing. The portion of the sequence highlighted in green indicates where the forward gene specific primers would bind during qPCR. The portion of the sequence highlighted in blue indicates where the reverse gene specific primers would bind during qPCR.

Conclusion

- ◆ HSP70 sequences for all species studied were identical in the primer binding region
- ◆ Our gene specific primers are accurate for all our species of interest

Discussion

Now that we have ensured that our gene specific primers are accurate for all of the Notothenioid species we studied, we can move on to the next phase of the project, which is a qrtPCR. This will give us the relative expression of HSP70 for fish that were held at 4C for one week. The ability of these Antarctic fish to exhibit a heat response may be important as the waters surrounding Antarctica are warming with the climate change.

