

OCTOBER 27, 1998

## Mutant Zebrafish Provide Clues About Human Anemia

The names sauternes, chardonnay, chianti, weissherbst, and zinfandel usually connote varieties of fine wine. For HHMI researcher Leonard Zon, however, these names describe mutant zebrafish that represent one of the first fish models of human disease.

Like wine, these fish come in two varieties: red and white. The colors refer not to the shade of the fish's skin, which is transparent, but rather to the color of their blood cells. "The *sauternes* mutation gives the fish pale blood cells, so they're a white wine," says Zon, an HHMI investigator at Harvard Medical School and Children's Hospital in Boston.

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— Leonard I. Zon

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Zon isn't breeding fish to complement wines at the dinner table; he is using them to investigate the genetics of red blood cell diseases. In the November 1998 issue of the journal *Nature Genetics*, Zon's research group reports that they've cloned a gene that when mutated causes zebrafish to develop a disease that mimics congenital sideroblastic anemia (CSA) in humans. In the same issue of *Nature Genetics* another research group unveils a zebrafish model of another form of congenital anemia. These reports describe the first fish models of any human disease.

Zebrafish with the *sauternes* mutation develop a disease identical to CSA, a condition that reduces the number of red blood cells and lowers levels of hemoglobin in both fish and humans. Symptoms of CSA include fatigue, dizziness, and weakness. "CSA is a rare disease, but it can be very severe," says Zon.

In humans, CSA arises from a mutation in the gene *alas2*, which codes for an enzyme that helps produce heme, the oxygen-carrying component of hemoglobin. People who have the *alas2* mutation don't produce functional heme, which leads to hemoglobin deficiency. *Sauternes* mutants also have reduced levels of the protein beta-globin, another component of hemoglobin.

Fish may seem an unlikely model for human disease, but Zon had compelling reasons to study CSA in fish. "The amazing thing about the zebrafish is that they're completely transparent, so you can see the circulating blood cells," Zon says. Even though adult zebrafish are only one inch long, researchers can tell whether the fish's blood cells are making hemoglobin by examining the live fish under a microscope. Blood cells with a red hue carry hemoglobin, while pale blood cells lack the crimson molecule.

Zon's research group nailed down the fish equivalent of the *alas2* gene using a technique called positional cloning. First they mated *sauternes* mutants with a strain of zebrafish known to have high genetic variability. By tracking swatches of DNA shared by progeny carrying the *sauternes* mutation, the researchers mapped the gene to chromosome 8 in the zebrafish. Next, the scientists identified genetic markers that flanked the *sauternes* mutation and used a medley of genetic techniques to "walk" along the chromosome from the markers to the gene.

Like the human CSA gene, the *sauternes* gene encodes the enzyme ALAS2. Many researchers had hypothesized that if a mutation prevented proper expression of ALAS2, a similar enzyme called ALAS1 would be ramped up to compensate. But Zon's experiments showed that ALAS1 levels weren't boosted in the *sauternes* mutants. "That really threw out one of the models," Zon says.

Further experiments revealed that a transcription factor called GATA-1, normally abundant in early embryos and diminishing as blood cells begin to form, remains high in the *sauternes* mutants. "There's something wrong with the way these cells are maturing, and it may be that regulation of this GATA-1 is heme dependent," says Zon, who hopes future genetic studies on zebrafish will clarify the mechanisms behind the disease.

A few years ago, zebrafish were considered useful only in the realm of developmental studies. Now the future of zebrafish genetics studies looks bright. "When I first started working on the fish, there were concerns about whether this would be a relevant model system to study," says Zon. "By finding an instance where the fish and the human have the same disease, we've illuminated the relevance," he says.

While a lack of infrastructure had stalled zebrafish genetics studies, a recent \$5 million grant from the National Institutes of Health to fund the Zebrafish Genome Initiative should encourage more work in the field. "I really think more people are going to jump in," says Zon. "Soon every major institute and school will have a zebrafish project," he predicts.