The *C-MYB* story—is it definitive?

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The *MYB* gene family has provided examples that bear on two interesting questions in evolutionary biology. Why do duplicated genes persist in the face of purifying selection? How do new organs arise? All vertebrate animal species are believed to have three closely related *MYB* genes (*C-MYB*/*MYB*, *A-MYB*/*MYBL1*, and *B-MYB*/*MYBL2*) (1). Most invertebrate animal species examined thus far have a single *MYB* gene, an exception being the nematode *Caenorhabditis elegans*. Genetic studies in the laboratory mouse and in *Drosophila* have provided important clues about the functional evolution of the *MYB* gene family (Fig. 1) (2–6). However, two recent reports of *C-MYB* mutants in two species of teleosts (bony fish) have raised new questions (7, 8).

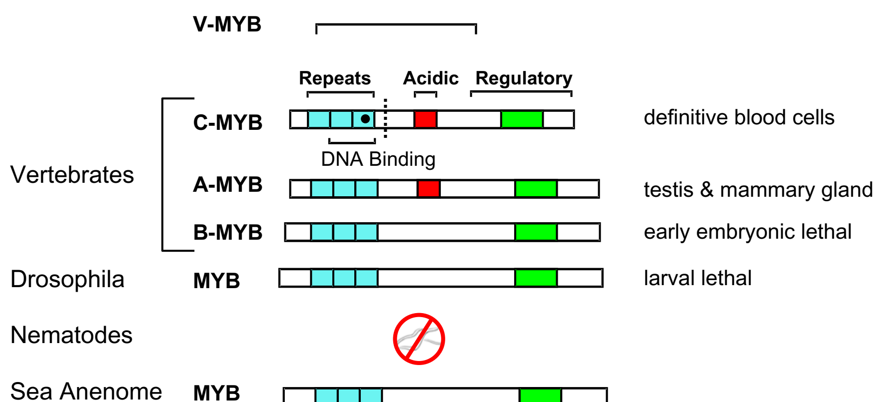

Fig. 1. Animal MYB proteins. Colors indicate various domains of the MYB proteins of animals (blue, tandem repeats that comprise the DNA-binding domain; red, acidic transcriptional activation domain; green, conserved negative regulatory domain). Note that the central transcriptional activation domain of C-MYB and A-MYB is lacking in B-MYB and in MYB proteins of invertebrates. The bracket above C-MYB indicates the extent of the truncated protein encoded by the *V-MYB* oncogene of the avian myeloblastosis virus. The notations to the right of the protein bars indicate the loss-of-function phenotypes of germ-line mutants in laboratory mice and in *Drosophila*. The black dot within C-MYB indicates the position of the amino acid substitution in the zebrafish mutant. The dotted line indicates the position of the nonsense mutation that is predicted to cause truncation of the C-MYB protein in the medaka mutant.

C-MYB

C-MYB was discovered as the cellular proto-oncogene from which the *V-MYB* oncogene arose via retroviral transduction in the avian myeloblastosis virus (1). This virus causes rapidly fatal myelomonocytic leukemia in chicken. Myeloid and lymphoid malignancies in both mice and chicken were shown to be caused by insertional mutagenesis of *C-MYB* by retroviruses that do not themselves contain an oncogene. Recently, a subset of human T-cell leukemias was found to contain a somatic duplication of *C-MYB* that appears to be a cause of this disease (9). Together, these observations imply that gain-of-function mutations of *C-MYB* are a common road to hematopoietic malignancies in vertebrates.

The development of knockout genetics in the laboratory mouse permitted the isolation and characterization of mice lacking a functional *C-MYB* gene (2). Homozygous null mutants died on day 15 of embryogenesis with severe anemia. Interestingly, the early primitive hematopoiesis that originates in the embryonic yolk sac appeared to develop normally in the absence of *C-MYB*. In contrast, the definitive hematopoiesis that occurs in fetal liver, and then in bone marrow, was completely dependent upon the presence of *C-MYB*. The ability of *C-MYB* null embryonic stem cells to differentiate into primitive hematopoietic cells in vitro is consistent with these results in vivo (10). Subsequent studies with conditional knockout mice revealed that *C-MYB* is also required subsequent to the initiation of definitive hematopoiesis at multiple steps in the proper development of T and B lymphocytes and for the maintenance of adult hematopoietic stem cells (11–13). In general, these experiments have shown that loss-of-function mutants of *C-MYB* cause failures of definitive hematopoiesis. These findings therefore provided a satisfying counterpart to previous observations that gain-of-function mutants of *C-MYB* caused hematopoietic malignancies.

Primitive vs. Definitive Hematopoiesis

A distinction between primitive and definitive hematopoiesis was first made in mammals based on the observation that the early erythrocytes arising from the yolk sac are relatively large and retain their nuclei, in contrast to the smaller enucleated erythrocytes that arise from fetal liver and adult bone marrow (14). Because the erythrocytes of adult fish, amphibians, reptiles, and birds are relatively large and retain their nuclei, it was hypothesized that the early yolk sac hematopoiesis of mammals was therefore analogous to this "primitive" hematopoiesis of non-mammalian vertebrates. The sequential differentiation of primitive and definitive hematopoietic cells in mammals was believed to be a continuous process that required the migration of extraembryonic yolk sac cells into the embryo proper. However, the careful analysis of chicken-quail chimeras led to the conclusion that yolk sac hematopoiesis was a process distinct from and independent of definitive intraembryonic hematopoiesis in birds (15). Eventually, similar conclusions were reached about mammalian hematopoiesis (14). In birds and mammals both, primitive yolk sac hematopoiesis generates transient populations of cells of the erythroid, megakaryocyte-thrombocyte, and macrophage-granulocyte lineages that can migrate into the embryo, but are not self-sustaining. In contrast, the production of adult hematopoietic stem cells, which can also generate cells of the lymphoid lineages, appears to be restricted to the definitive hematopoiesis that arises within the embryo itself in birds and mammals. Together, these findings suggest that evolution of distinct primitive extraembryonic and definitive intraembryonic hematopoietic systems predated the divergence of birds and mammals.

Two Fish Stories

The PNAS paper by Soza–Ried et al. (8) identifies a *C-MYB* mutant in zebrafish, which provides an opportunity to examine this question further. This mutant was isolated in a forward genetic screen for failure of thymopoiesis. Consistent with previous findings in the laboratory mouse,

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this recessive loss-of-function allele of *C-MYB* caused a failure of definitive hematopoiesis, which begins in the aorta-gonad-mesonephros and then migrates into the kidney marrow (16). As in the laboratory mouse, this *C-MYB* mutant had little or no effect on primitive hematopoiesis, which normally occurs in the intermediate cell mass and rostral blood island. In contrast to the embryonic lethality of *C-MYB* null mice, these mutant fish survive for months as adult animals in the complete absence of erythrocytes, presumably due to diffusion of oxygen from their aqueous environment. As expected in the absence of definitive hematopoiesis, no lymphoid cells were observed, and thymic development failed. A similar phenotype was caused by treatment with morpholinos (stable antisense oligonucleotides) directed against *C-MYB*. In summary, it appears that, as is the case in the laboratory mouse, *C-MYB* is required for definitive but not primitive hematopoiesis in the zebrafish.

Contemporaneously with these studies in zebrafish, a *C-MYB* mutant was isolated in medaka, another teleost used as a model organism for studies of vertebrate development (7). A forward genetic screen for a reduced number of erythrocytes led to the isolation of a mutant known as *beni fuji* (*bef*), which turned out to be a loss-of-function mutant of *C-MYB*. The development of primitive and definitive hematopoiesis in medaka appears to be morphologically very similar to that in zebrafish. However, *C-MYB* mutant animals displayed a significant reduction in the number of primitive erythrocytes and a delay in the appearance of primitive macrophage, in addition to a failure of definitive hematopoiesis. These findings suggest that, in medaka, the *C-MYB* gene is required for both primitive and definitive hematopoiesis.

The Fish or the Fouls?

How might one explain the rather different phenotypes in zebrafish and medaka mutants of *C-MYB*? One possibility is that the genetic control of primitive hematopoiesis may differ, with that of zebrafish being more similar to that of the laboratory mouse than to that of medaka ("the fish"). Another possibility is that the genetic control of primitive hematopoiesis is similar in all three species, but the nature of the mutant *C-MYB* alleles leads to different phenotypes in zebrafish and medaka ("the fouls").

The *C-MYB* mutant isolated in zebrafish causes an amino acid substitution (Ile181Asn) within the highly conserved amino-terminal DNA-binding domain of the C-MYB transcription factor (8). A recombinant protein with this substitution failed to bind DNA in vitro. These findings suggest that this mutation is a functional null, consistent with the ability of morpholino inhibitors to mimic the mutant phenotype in vivo.

The *C-MYB* mutant isolated in medaka is a nonsense mutation predicted to encode a truncated protein that would retain a functional DNA-binding domain but lack the central transcriptional activation domain and carboxyl-terminal negative regulatory domain (7). No biochemical studies were performed with this mutant protein. However, previous studies with analogous mutations in *C-MYB* imply that such a protein would bind DNA in vitro and may function as a dominant loss-of-function (dominant negative) allele in vivo (17, 18). In addition to inhibiting the function of the C-MYB protein, such an allele might also inhibit the function of A-MYB and B-MYB.

A Family Affair

Germ-line loss-of-function mutants in the laboratory mouse have shown that loss of *C-MYB* causes embryonic lethality due to a failure of definitive intraembryonic hematopoiesis (2). Loss of *A-MYB* results in viable adults, but the males are sterile due to a failure of spermatogenesis (3). The females are viable and fertile, but cannot nurse their pups due to a failure of mammary gland proliferation. Loss of *B-MYB* results in a very early embryonic lethality, consistent with the expression of *B-MYB* in all dividing cells (4). Interestingly, many of the same genes that control the development of the macrophage/granulocyte-like hematopoietic system of *Drosophila* also control hematopoiesis in vertebrates (19). *Drosophila MYB*-null mutants are deficient in hematopoietic cells, and vertebrate *B-MYB* (but neither *A-MYB* nor *C-MYB*) can rescue this phenotype (20). *A-MYB* and *C-MYB* are lethal when expressed in *Drosophila*. These findings suggest that *A-MYB* and *C-MYB* survived purifying selection via neofunctionalization following an initial gene duplication. An interesting question is whether this *A-MYB*/*C-MYB*-like gene was then selected for its ability to regulate the development of a definitive self-renewing hematopoietic system. However, *C-MYB* has recently been shown to play an important role in the development and homeostasis of various epithelia in the laboratory mouse (9). One therefore wonders whether it might play a similar role in all vertebrates, including teleosts. One also wonders whether in various tissues there might be a functional redundancy between *C-MYB* and *A-MYB*. The analysis of appropriate combinations of mutants and transgenic rescues both in mice and fish might be highly informative in attempting to reach definitive conclusions.*

*For simplicity, I have refrained from using the idiosyncratic nomenclature for wild-type and mutant genes in different species.

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