

Extra View

# Minichromosome Maintenance Proteins as Markers for Proliferation Zones during Embryogenesis

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## KEY WORDS

MCM, zebrafish, proliferation zones, stem cells, embryogenesis

## ABBREVIATIONS

MCM minichromosome maintenance  
pre-RC pre-replicative complex  
dpf days post fertilization  
hpf hours post fertilization  
cmz ciliary marginal zone  
PCNA proliferating cell nuclear antigen

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## ABSTRACT

Regulation of cell proliferation is of fundamental importance for growth and prevention of cancer. An obligatory step in the regulation of cell proliferation is the control of the initiation of DNA synthesis. The minichromosome maintenance (MCM) proteins are essential DNA replication factors crucial for initiating DNA synthesis once every cell cycle. Recent studies show that the level of MCM proteins is stringently regulated to correlate with cell proliferation and carcinogenesis. Here we discuss recent data, which highlights the usefulness of minichromosome maintenance (MCM) gene expression for detecting proliferating cells as well as zones containing proliferative/stem cells during embryogenesis in a whole organism.

## MCM PROTEINS AND DNA REPLICATION LICENSING

Proper duplication of the genome during the cell cycle is of paramount importance for the life of all organisms. Deregulation of this process prevents normal embryonic development, and could lead to cancer. In eukaryotes, "replication licensing" denotes a cellular mechanism that ensures the complete duplication of the genome once and only once per cell cycle.<sup>1</sup> Accordingly, each replication origin is "licensed" to fire once per cell cycle through the cell cycle dependent formation and destruction of a prereplication complex (preRC).<sup>2-4</sup> A key component of this preRC is a family of six structurally related proteins, MCM2 through MCM7, which are evolutionarily conserved in all eukaryotes. The MCM proteins were originally identified as proteins required for the minichromosome maintenance in *Saccharomyces cerevisiae*.<sup>5</sup> MCM2-7 belong to a distinct subgroup of the large AAA+ ATPase family and share a conserved central region of approximately 200 amino acids (MCM box).<sup>6</sup> Biochemical studies in *Xenopus* have initially established the role of MCM2-7 as replication licensing factors.<sup>7-9</sup> Subsequent studies illustrate that proper orchestration of the functional interactions between MCM2-7 proteins and other components of the preRC by cell cycle dependent protein kinases results in initiation of DNA synthesis once every cell cycle.<sup>10,11</sup> The MCM2-7 proteins appear to form hetero-hexamers and play important roles in initiation and elongation during DNA replication.<sup>5,12,13</sup> These data suggest that MCM2-7 might be the replicative helicase for eukaryotic DNA replication.<sup>10</sup> Interestingly, in many organisms, MCM2-7 are highly abundant proteins whose copy number far exceeds the number of origin of replication sites. Further, the majority of MCM2-7 chromosomal binding sites do not correlate with the replication origin locations. This suggests that MCM2-7 might have other functions within the cell. Indeed, recent evidence supports involvement of MCMs in many other chromosome transactions including transcription, chromatin remodeling, and genome stability.<sup>14</sup>

## MCM PROTEIN EXPRESSION AS A MARKER FOR PROLIFERATING CELLS

The initiation of DNA synthesis is a critical step in growth control. Since removal of licensing is tightly coupled with the replication initiation, the level of MCM proteins is stringently regulated to correlate with proliferation and growth. In yeast, the expression of *mcm3* is abundant in logarithmically growing cells but is completely repressed in stationary, starved, or quiescent cells.<sup>15</sup> Similarly, the expression of *Drosophila mcm2* and *Arabidopsis mcm7* during development follows a pattern that corresponds to rapidly dividing cells.<sup>16,17</sup>

Furthermore in human cells, MCM proteins have recently emerged as an important biomarker for growth and carcinogenesis. A strong correlation has been observed between entry into quiescent, differentiated or senescent state and downregulation of MCM

proteins.<sup>18,19</sup> In fact, deregulation of MCM2-7 appears to be an early event in tumorigenesis in a range of different tumor types and has led to the recent development of human MCM antibodies as diagnostic tools for common carcinomas.<sup>20-24</sup>

## MCM EXPRESSION MARKS PROLIFERATION ZONES IN ZEBRAFISH

In order to explore the usefulness of *mcm* expression as a marker for proliferation zones in a whole organism, we analyzed the expression pattern of *mcm5* using whole-mount in situ hybridization in developing zebrafish embryos (see also Thisse et al., www.zfin.org). From fertilization to early somitogenesis, when cells in the whole embryo divide rapidly, *mcm5* expression is ubiquitous (data not shown). During mid-somitogenesis stage, *mcm5* expression is broad but is stronger in the anterior part of the embryo (10 somite stage; Fig. 1A). Expression in the tail bud is maintained throughout tail elongation during somitogenesis. By the 21-somite stage, *mcm5* expression has further decreased in the trunk region while remaining high in the cephalic and branchial regions of the embryo (Figs. 1B and C). By 3 days post fertilization (dpf), *mcm5* expression has been further refined to include the retinal ciliary marginal zone (CMZ), the pallial proliferation zone, the tectal proliferation zones, the branchial arches, and endodermal tissues (Fig. 1E and F-K). These tissues represent the most prominent proliferation zones of zebrafish embryos at this stage, as detected by the expression of Proliferating Cell Nuclear Antigen (PCNA), an auxiliary protein of the DNA polymerase delta and a reliable marker for cycling versus postmitotic cells.<sup>25</sup> Further, we analyzed the expression patterns of *mcm2*, *mcm3*, and *mcm4* and found them to be indistinguishable to that of *mcm5* (Ryu S, Driever W, unpublished data and Thisse et al., www.zfin.org). Side by side comparison of the *pcna* and *mcm5* transcript patterns showed that *pcna* and *mcm5* were expressed in virtually identical regions with a slight difference in signal intensity (Fig. 1F-K). This may be due to the difference in the efficiency of the probes or in transcript levels. Taken together, our data suggests that the expression of *mcm* genes can be used to detect the location of proliferation zones throughout the developing zebrafish embryo.

## MCM5 EXPRESSION IN ZEBRAFISH RETINA IS AN INDICATOR FOR THE DIFFERENTIATION STATE OF CELLS

To better compare the pattern of *mcm5* expression with cell proliferation, we recently analyzed the expression of *mcm5* in the zebrafish retina.<sup>26</sup> The zebrafish retina offers an ideal system due to the existence of several well characterized cell types whose proliferation and differentiation pattern have been well described.<sup>27</sup> Furthermore, the zebrafish retina contains a population of stem cells in an area called ciliary marginal zone (CMZ), where proliferative cells are maintained, even in adult fish.<sup>28,29</sup> Exploiting these features, we compared the expression of *mcm5* in three populations of cells; proliferative cells present in CMZ, early retinoblast cells, and differentiated neurons. We labeled proliferating cells based on expression of PCNA, early retinoblast cells based on expression of *ath5*, a bHLH transcription factor, and differentiated ganglion cells and the majority of amacrine cells based on expression of an RNA

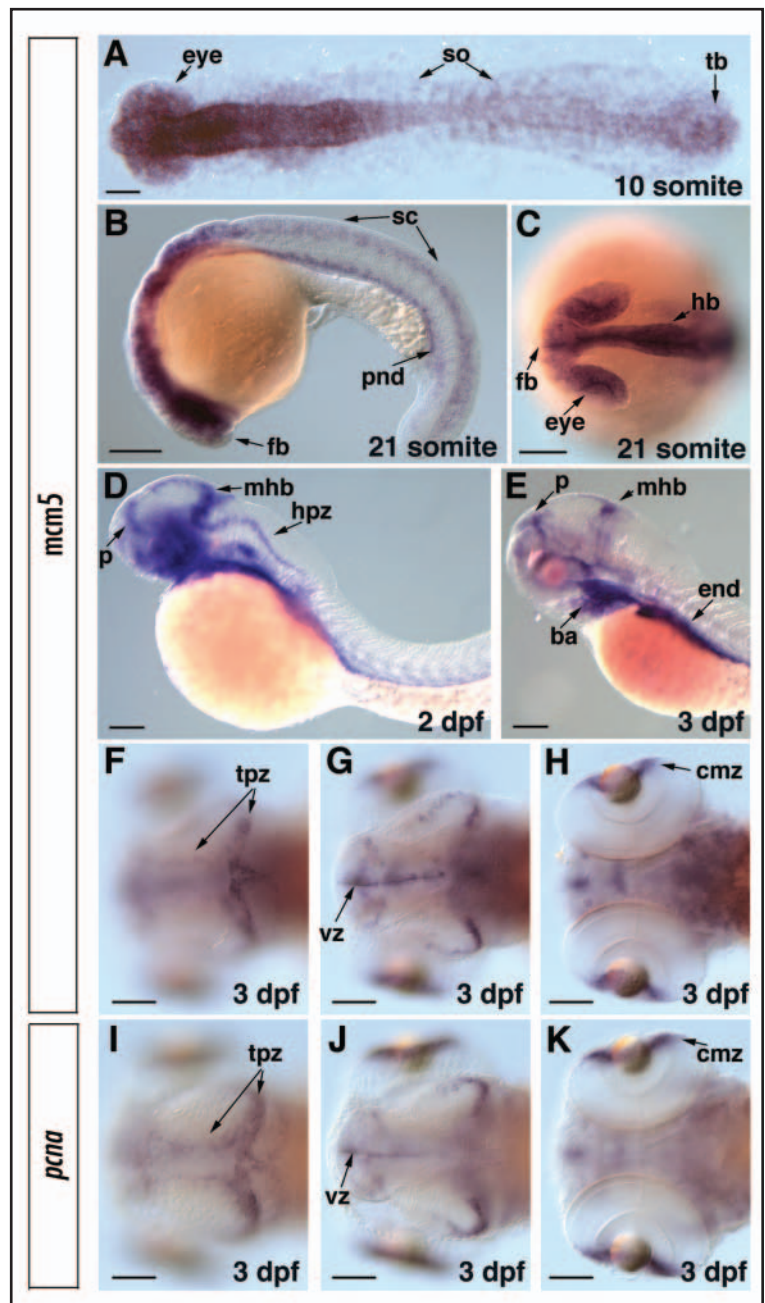


Figure 1. *mcm5* expression correlates with the pattern of cell proliferation throughout embryogenesis. (A) Flat-mounted 10-somite stage embryo showing broad expression of *mcm5*. (B and C) At the 21-somite stage, *mcm5* is expressed in the head region, spinal cord and pronephric duct. At 2 dpf (D) and at 3 dpf (E and F-H), *mcm5* is expressed in the major proliferation zones in the brain, which include the pallial and tectal proliferation zones, the ventricular zone, and the midbrain-hindbrain boundary. Additionally, it is expressed in the branchial arches and in endodermal tissues, which also proliferate rapidly at these stages. At 3 dpf (I-K), *proliferating cell nuclear antigen (pcna)* is expressed in the same regions as *mcm5*. (A-K) Anterior towards the left. (A, C and F-K) Dorsal views; (B, D and E) lateral views. Whole mount in situ hybridization was performed as described in reference X. Size bar indicates 100  $\mu$ m. ba, branchial arches; cmz, ciliary marginal zone; end, endoderm; fb, forebrain; hb, hindbrain; hpz, hindbrain proliferation zone; mhb, midbrain-hindbrain boundary; p, pallial proliferation zone; pnd, pronephric duct; sc, spinal cord; so, somites; tb, tail bud; tpz, tectal proliferation zones; vz, ventricular zone.

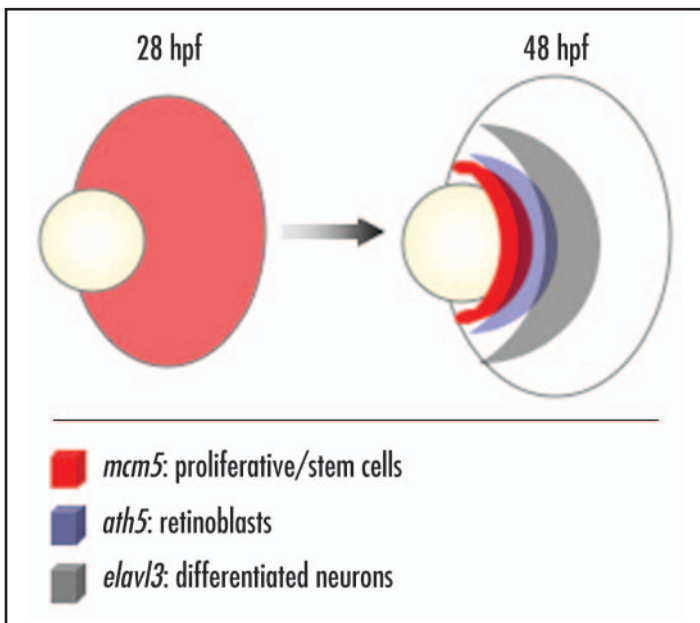


Figure 2. Schematic diagram of progressive restriction of *mcm5* expression during zebrafish retina development. The pattern of *mcm5* expression follows closely the pattern of proliferation in the zebrafish retina. Left: a cartoon representing zebrafish eye at 28 hours post fertilization (hpf) when *mcm5* is ubiquitously expressed in the entire retina. Right: a cartoon representing zebrafish eye at 48 hpf, when *mcm5* expression has mostly receded from the maturing central retina. However, *mcm5* expression remains detectable in the CMZ, and its expression domain still overlaps partially with that of early stage neurogenesis marker (*ath5*), but is excluded from areas of the retina expressing the late stage neurogenesis marker (*elavl3*). These findings suggest that *mcm5* is rapidly downregulated as differentiation proceeds.

binding protein, *elavl3*, which is a marker for differentiating neurons.<sup>30,31</sup>

Our analysis showed that *mcm5* and *pcna* are expressed in virtually identical populations of proliferative cells in the CMZ. *mcm5* expression overlaps partially with that of *ath5*, but does not overlap with that of *elavl3*, suggesting that *mcm5* is rapidly downregulated as cells become post-mitotic and differentiation proceeds. Our analysis corroborates the MCM expression studies in human cells where MCM expression is shown to be coupled to the differentiation status of tumors.<sup>32</sup> In these tumor cells, MCM2-7 are shown to be expressed in both proliferating cells and differentiating cells, while being downregulated in terminally differentiated cells. In contrast, other replication licensing factors such as Cdc6, Cdt1, and geminin are only expressed in proliferating cells and not in early differentiating cells. Thus our expression analysis in the retina suggests that *mcm5* expression provides an useful indicator for the proliferation and earliest differentiation stage of cells.

## SUMMARY

In conclusion, the expression of *mcm* genes serves as a sensitive marker to localize proliferation zones during embryogenesis. *mcm* expression appears to be temporally and spatially restricted to correlate with the proliferation and differentiation state of the cell. This feature makes *mcm* genes a highly useful marker for identifying progenitor cells at early stage of differentiation as well as those cells with a capacity for further proliferation.

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