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Cyclin-Dependent Kinase Sensor Transgenic Zebrafish Lines for Improved Cell Cycle State Visualization in Live Animals

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Adikes *et al.*[1] described a novel zebrafish cell cycle sensor that delineates all phases of the cell cycle based on levels of cyclin-dependent kinase (CDK) activity. The CDK sensor consists of a fragment of human DNA Helicase B (DHB) fused to a fluorescent protein.[2] DHB contains a dominant nuclear localization sequence (NLS) and a nuclear export sequence (NES) flanked by CDK-specific phosphorylation sites. Cells that contain low levels of CDK activity have an exposed NLS that localizes the CDK sensor to the nucleus. As the cell cycle progresses, CDK activity increases causing phosphorylation of DHB, which occludes the NLS and allows the NES to promote export of the sensor into the cytoplasm (Fig. 1). The authors showed that quantitative analysis of this ratiometric sensor can distinguish CDK low (CDK^{low}), quiescent G0 arrested cells from CDK increasing (CDK^{inc}), cycling G1 phase cells, as well as identify S, G2, and M phases of the cell cycle.

References

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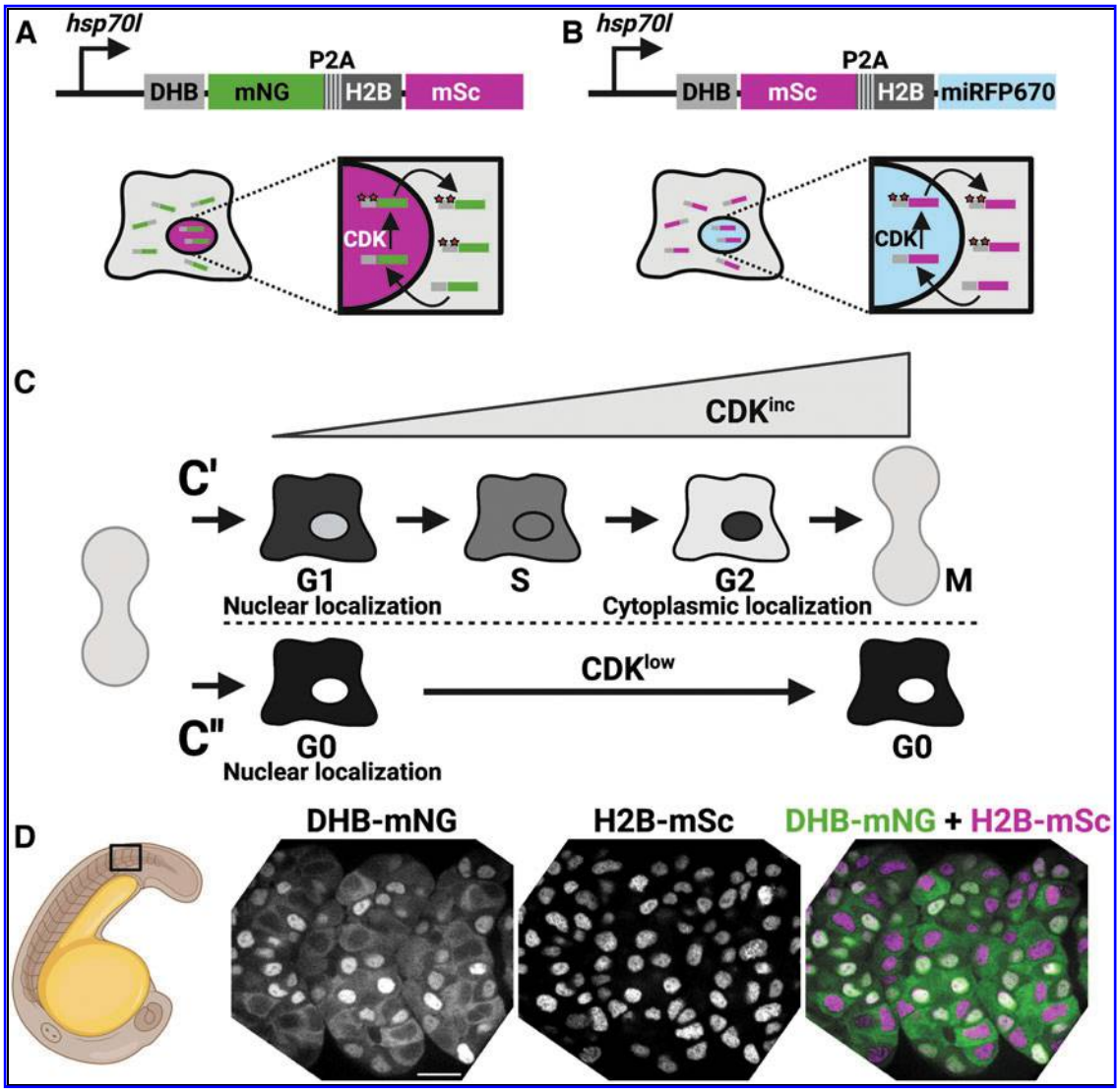

FIG. 1. CDK sensor transgenic zebrafish lines. **(A, B)** Two transgenic CDK sensor lines with different fluorophores for compatibility with other transgenic lines were generated using the hsp70l promoter for robust expression after heat-shock induction. The P2A viral peptide sequence allows for the formation of two independent proteins, the CDK sensor (DHB fluorophore fusion) and a nuclear marker (H2B fluorophore fusion). The fragment of DHB is phosphorylated by CDKs causing export out of the nucleus (phosphorylation sites designated by stars). **(C)** The nuclear-to-cytoplasmic ratio of the sensor indicates cell cycle state with increasing CDK (CDK^{inc}) activity causing nuclear export (C′, *lighter shading* indicates DHB localization to match the DHB micrograph in **D**). The sensor can also distinguish between cells in G1 versus G0 based on a lower cytoplasmic-to-nuclear ratio (C", CDK^{low}). **(D)** Cells in different stages of the cell cycle are observed in the posterior-most somites of a 24 hours postfertilization *hsp70l:DHB.mNG-p2a-H2B.mSc* transgenic embryo. Scale bar = 20 μm. Figure created with BioRender.com. CDK, cyclin-dependent kinase; DHB, DNA Helicase B. Color images are available online.