Does CDKL1 regulate the development of the cardiovasculature? Developing and testing a hypothesis.

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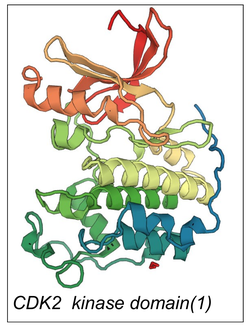
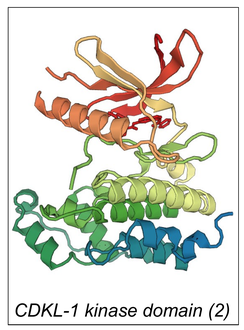
Abstract and Hypothesis.

The **cyclin dependent protein kinase** family regulates a wide range of cellular functions such as cell cycle progression, differentiation, and apoptosis. They have two functional domains, a cyclin binding domain and a kinase domain. Cyclin binding activates these proteins mediating a cellular process via a signalling cascade. CDKL1 has been classed as cyclin dependent "like" as it contains the conserved domain for binding cyclin as well as a kinase domain. The structure of the kinase domain has been determined and is very similar to the well characterised human CDK2 (1,2).

No function has been assigned to CDKL1. Our study identified an elevated expression in response to heart attack and *In Situ* hybridisation analysis of Zebrafish indicates that CDKL1 may signal blood vessel formation. Through *Genome Expression Omnibus* searches we propose that **CDKL1** mediates this through the **Sonic Hedgehog, VEGF, NOTCH** signalling pathway. Inhibition of NOTCH in the mindbomb mutant however reduces CDKL-1 expression and this hypothesis requires further study.



CDK2 kinase domain(1)



CDKL-1 kinase domain (2)

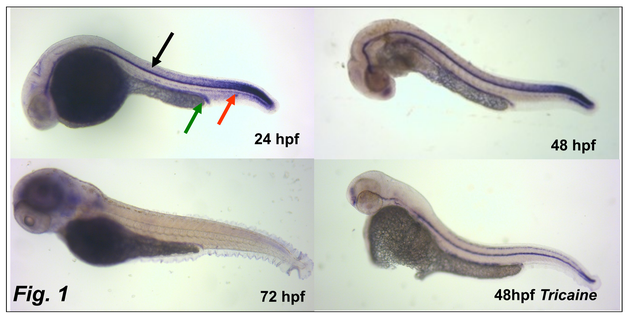
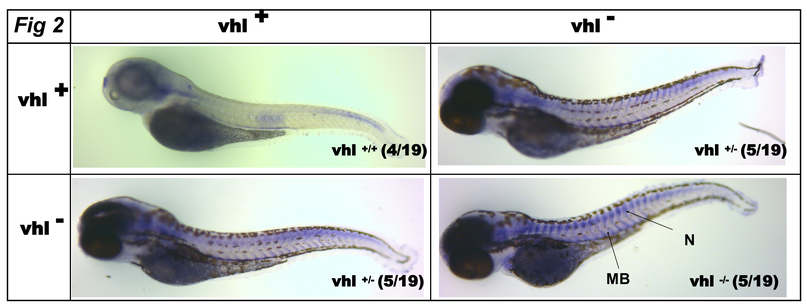
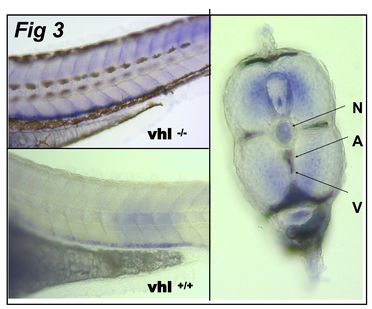


Fig. 1

CDKL1 expressed in the Zebrafish hypochord and elevated expression in hypoxic embryos.

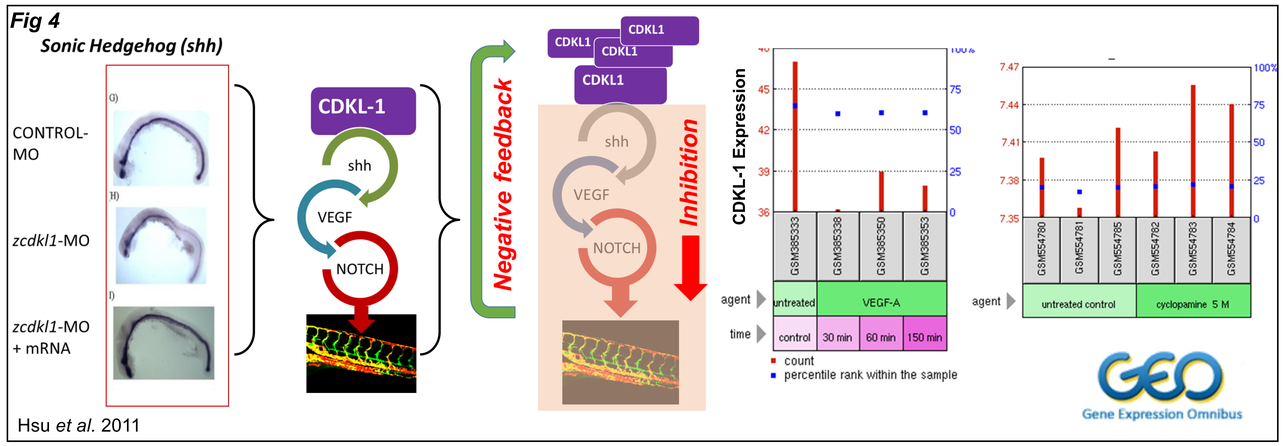
CDKL1 expression normally decreases from 24-72hpf but it persists in the notochord (black arrow), hypochord (red arrow) and the prenephric duct (green arrow) (Figure 1). Localisation of CDKL1 to the hypochord is significant because the hypochord plays a role in positioning and development of the dorsal aorta (3), which develops in an anterior to posterior direction. Figure 1 shows CDKL1 expression along the hypochord at 24hpf, an anterior regression at 48hpf and absence of labelling at 72hpf. We propose that CDKL1 is involved in signalling the development of the dorsal aorta. The effect of blood flow on CDKL1 expression was studied by stopping the heart in 48hpf embryos with the anaesthetic Tricaine. The label for CDKL1 was similar to that in untreated embryos. Embryos that develop in oxygen-deficient conditions (hypoxia) grow more blood vessels. Our preliminary data suggests that CDKL1 expression is upregulated in VHL mutant (hypoxic) embryos (3dpf). These embryos were generated from a heterozygous cross (vhl+/- x vhl +/-), noting that homozygous m tants are not viable beyond the embryo stage. Figure 2 shows the segregation of 19 offspring with the expected phenotypic ratios of a monohybrid cross (the vhl gene exhibits codominance). The bottom right panel shows higher expression of CDKL-1 between the muscle blocks (MB) and in the notochord (N). Figure 3, higher magnification as well as a transverse section showing CDKL-1 expressed in Notochord and axial aorta (A) and vein (V).





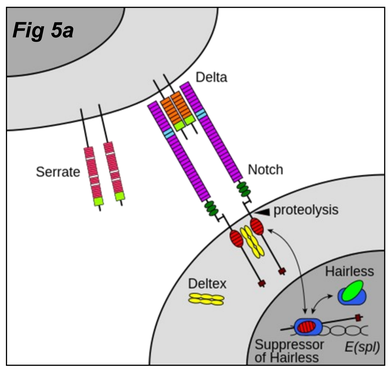
Proposed mechanism: Sonic Hedgehog Regulation.

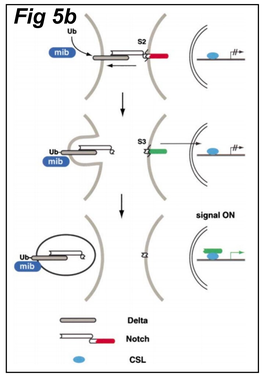
Hsu *et al.* 2011 (4) characterised CDKL1 expression in Zebrafish, they performed morpholino reverse genetics analysis on CDKL1. Removal of CDKL1 resulted in a reduced expression of Sonic Hedgehog (SHH), rescue with CDKL1 mRNA elevated SHH relative to the control, Figure 4. This data suggests that CDKL1 has a regulatory role over the gene expression of SHH. Sonic Hedgehog is a morphogenic protein that is involved in embryonic development and the regulation of adult stem cell differentiation. Importantly, its role in **Arterial Endothelial Differentiation** has been widely reported. (5)



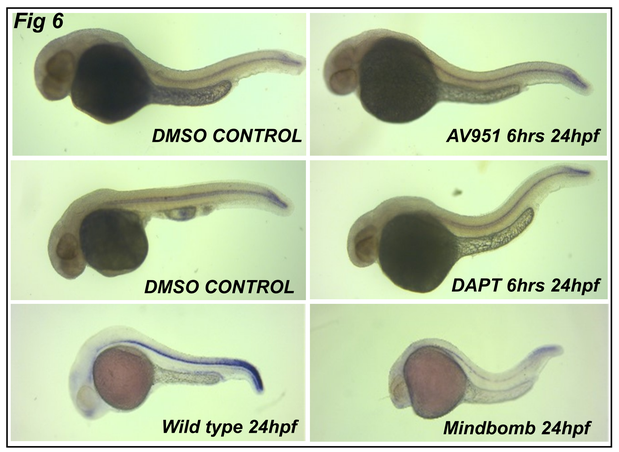
Genome expression Omnibus data corroborates the negative feedback hypothesis.

The Gene Expression Omnibus (GEO) is a public repository for high-throughput gene expression data and allows the retrieval of heterogeneous data sets from high-throughput gene expression experiments. In Figure 4 the two studies presented are of CDKL1 expression levels in Human Endothelial Cells in response to inhibition of SHH with cyclopamine and the addition of VEGF. The elevated expression of CDKL1 in response to an inhibition of SHH can be explained via a negative feedback mechanism, whilst the addition of VEGF, which acts downstream of both SHH and CDKL1, demonstrates a reduced level of CDKL1 expression.





Testing the hypothesis experimentally



Inhibition of the proposed downstream effectors of CDKL-1 (*SHH, VEGF and NOTCH*) was performed pharmacologically as well as genetically Figure 6. Drug treatments for 6 hours at 24hpf of embryos with AV951 and DAPT were performed to inhibit VEGF and DAPT respectively, when compared to the control no difference in CDKL-1 expression in the hypochord was observed.

NOTCH signalling as summarised in Figure 5a/b, is dependent on interaction of extracellular domains of the membrane spanning proteins Delta and NOTCH, such interactions result in a cleavage of the intracellular domain of NOTCH *(inhibited by DAPT)* which translocates to the nucleus to propagate a signalling pathway. As we observed no expected difference in CDKL-1 expression with DAPT treatment Mindbomb mutants were analysed for CDKL-1 expression. Surprisingly these showed a reduced expression level of CDKL-1, Mindbomb reduces NOTCH signalling as without Mindbomb's ubiquitin ligase activity and subsequent degradation of NOTCH extracellular domains, the cell-cell contact signalling mediated by NOTCH is inhibited.

It was expected that CDKL-1 levels would be elevated in this instance. As CDKL-1 expression was significantly reduced in Mindbomb mutants our hypothesis needs further examination. Despite this we remain confident in the role of CDKL-1 in the development of the cardiovasculature.

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References

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