1. FINAL PUBLISHABLE SUMMARY REPORT

Tile : Role of canonical Wnt signaling during somite formation in the zebrafish embryo

Vertebrae, ribs and most skeletal muscles derive from the somites, which are segmented embryonic structures. They form periodically at the anterior extremity of the unsegmented presomitic mesoderm (PSM) and this can be seen by the formation of a new somitic boundary (See Figure1). In the meantime, new cells are being added in the PSM by posterior growth of the embryo. It has been shown that the rhythm of this process is set up by oscillation of cyclic gene expression, and it has been proposed that the position where the future boundary forms is defined by a determination front. This front would regress posteriorly with the growth of the embryo, thereby shifting posteriorly the position of the next boundary after one period of cyclic gene expression. The canonical Wnt pathway has been suggested in the mouse to control the position of the determination front and to regulate some cyclic genes (*1-4*). However, it remains difficult to specifically address its function in the segmentation of the PSM because this pathway plays an important role earlier in development for the formation of posterior structures (*5, 6*). In particular, the role of Wnt in somite formation is unknown in zebrafish embryo.

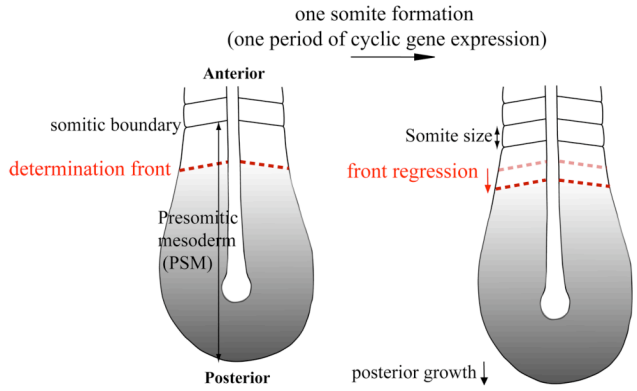

Figure 1 : Schematic representation of a dorsal view of the posterior end of a zebrafish embryo before and after one somite formation.

The first objective of this project was to transiently inhibit or activate the canonical Wnt pathway during somite formation and bypass the earlier effects of this pathway on the formation of the PSM. For that purpose we used transgenic fishes in which we can induce overexpression of either *dkk (7)*, an inhibitor of the pathway, or *Wnt8* (*8*), one of the ligands activating the pathway, by delivering a heat-shock to the embryo.
I observed a strong increase of *dkk* or *Wnt8* transcripts already 30 minutes after the heat-shock.
I then addressed how induction of these genes affects the Wnt pathway using βcatenin immunostaining. This protein translocates into the nucleus in response to the activation of the canonical Wnt pathway. In normal embryos, I could show that the level of nuclear βcatenin is higher in the posterior extremity of the embryo, also called tail bud, and gradually decreases more anterior in the PSM. Preliminary results suggest that the level of nuclear βcatenin is reduced in the tail bud when *dkk* is overexpressed and increased in the anterior PSM when *Wnt8* is ectopically expressed. These results are consistent with a gradient of Wnt signaling across the PSM that can be modulated with the heat-shock transgenes.

The second objective was to provide an exhaustive (temporal and spatial) analysis of the effects of this transient modulation of the Wnt pathway on somite formation. The first aspect of this analysis consisted in performing multiple time-lapse movies of developing heat-shocked transgenic embryos. This technique allows quantitative measurements of both period of somite formation and of somite size (*9, 10*). I showed that inhibition of the pathway results in the formation of bigger somites (up to 20%), whereas ectopic activation of the pathway results in the formation of smaller somites (up to 15%, see Figure2).

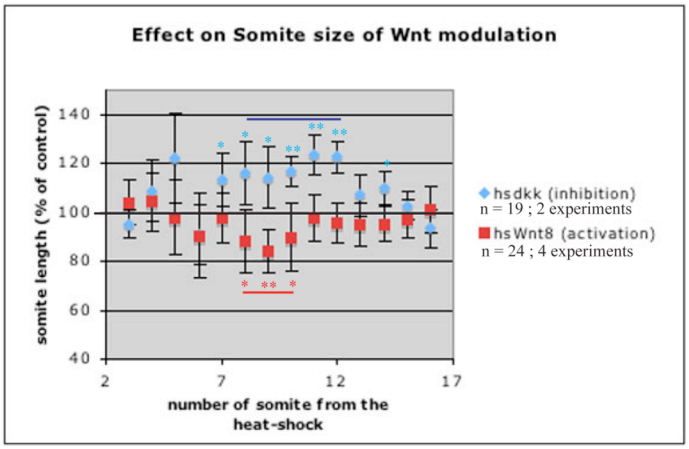

Figure 2: Size of the somites forming after heat-shock induction of *dkk* (blue) or *wnt8* (red). Somite size (distance between two consecutive boundaries) is measured from time-lapse movies and average to somite size in control embryos. Inhibtion of Wnt results in formation of bigger somites (blue line), whereas activation results in formation of smaller somites (red line). * $p<0.01$, ** $p<0.001$

The modifications of somite size were confirmed by measuring the length of the presumptive somite in the anterior PSM. I showed that presumptive somite size is significantly increased after inhibition of Wnt (133 +/- 11% of the control, $p<0.001$). According to the current model, somite size results from the distance crossed by the determination front during one period of somite formation (see Figure 1). Therefore, a change in somite size could be due to a change in either the position of the front or in period, or both. No significant change in the period of somite formation was observed at the time when bigger somites form after Wnt inhibition. Consistent with this, I showed that the key cyclic genes *her1*, *her7* and *deltaC* are still expressed and still cyclic after Wnt inhibition or activation.
In contrast, using a panel of appropriate gene expression markers, I showed that the relative position of the determination front in the PSM is significantly more posterior after Wnt inhibition. Interestingly, I also observed a posterior shift of *tbx24*, which is essential for boundary formation (*11*). These results indicate that the larger somites observed after reduction of Wnt signaling are caused by a shift of the determination front within the PSM.

To conclude, this study demonstrates for the first time that the canonical Wnt pathway regulates somite formation in the zebrafish embryo, suggesting a conservation of this regulatory path among vertebrates. Importantly, I show that the main role of the Wnt pathway is to set the position where the future somitic boundary will form, without strikingly affecting the rhythm of the process.

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