Instructive reconstruction: a new role for apoptosis in pattern formation

Subtitle:

Instructive Apoptotic Patterning establishes de novo tissue generation via the apoptosis linked production of morphogenic signals.

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Novel and canonical roles for apoptosis in developmental patterning events

An exciting new role for apoptosis in developmental pattern formation is emerging from recent studies. It has long been recognised that programmed cell death has an important function in the removal of tissue during developmental patterning events. Examples include the sculpting of digits from a paddle shaped mass of tissue during vertebrate limb development [1], and the removal of the larval tail during amphibian metamorphosis [2]. The removal of pre-existing tissue is the canonical form of apoptosis in pattern formation and I refer to it as **Destructive Apoptotic Patterning**. Although it leads to a positive patterning event tissue is destroyed during the process. However, it now appears that this is not the entire story. I report here a newly emerging role for apoptosis in instructing the site of de novo tissue generation via the apoptosis linked production of morphogenic signals, an event which I term **Instructive Apoptotic Patterning**. These new terms have been coined to help distinguish these distinct apoptotic processes. With our recent findings regarding the anti-apoptotic protein Hsc71 [3] a novel role for instructive apoptotic patterning is beginning to emerge during normal developmental patterning events, namely larval tail and polyp head specification. This new method of apoptosis mediated patterning could prove to have widespread repercussions for our traditional understanding of metazoan development.

Heat shock proteins, Wnt signalling and patterning

Our recently published research [3] has revealed an unexpected role for an anti-apoptotic heat shock protein (HSP) [4], Hsc71, in Cnidarian pattern formation. While our research also has implications for stem cell and cancer biology, this article focuses on the patterning aspect of our findings, which could have broader implications for human congenital defects (see original publication). We have shown that the anti-apoptotic Hsc71 gene (human homologue HSPA8) crosstalks with the Wnt mediated axial organiser in the Cnidarian *Hydractinia echinata*. Both heat induced and transgenic upregulation of Hsc71 expression prevented normal patterning of larval tails and polyp heads.

The Wnt organiser is active life long in *Hydractinia* and is responsible for patterning the larval tail and subsequently, after metamorphosis, patterning of the polyp head. The polyp head develops from the pole originally consisting of the larval tail [5,6]. The Wnt organiser remains active during metamorphosis and despite being located in the larval tail this organiser is omitted from the programmed apoptosis event that removes the rest of the tail tissue [3,7,8]. It is worth noting that planula-larva-like creatures were probably the ancestor of all metazoans [6,8,9]. With this in mind, Wnt specifying posterior patterning is a common theme throughout animal development [10,11].

Hsc71 is initially ubiquitously expressed throughout the animal during early stages of axial patterning events, first day of embryonic development and outgrowth stage of polyp budding. However, *Hsc71* expression is quickly restricted from the Wnt pole prior to larval tail or polyp head specification, becoming undetectable in the region around the Wnt organiser (Fig. 1A-C). This is the case for two types of head formation events: 1) post-metamorphosis and 2) subsequent polyp buddings, while the third type 3) head regeneration remains to be studied. The *Hsc71* expression pattern during larval tail and polyp head development suggests a selective pressure preventing *Hsc71* expression near the Wnt organiser. When we ectopically expressed *Hsc71* its expression extended to the area surrounding the Wnt organiser. This blocked both larval tail and polyp head formation. This inhibitory effect could be due to an apoptosis independent *Hsc71* function such as a role in the APC complex (see below). Alternatively, our results may indicate the need for some level of apoptosis during Wnt mediated head specification.

A Wnt-HSP-Apoptosis triad functions during de novo tissue patterning

Our results revealed novel HSP crosstalk in a variety of biological contexts. This crosstalk is likely to have a variety of important implications for human health and disease. Wnt signalling resulted in increased *Hsc71* mRNA expression in proliferating stem cells. However, overexpression of *Hsc71* deregulated the normally polar Wnt-organiser expression, ultimately resulting in patterning defects. While our research has not yet revealed to what extent *Hsc71's* anti-apoptotic ability blocks head formation, research performed by Chera et al. [12] in the closely related cnidarian *Hydra* has directly implicated apoptosis in a specific mode of head regeneration. Chera et al. showed that the initial phase of Wnt3 expression responsible for head regeneration was produced in apoptotic cells post injury and that blocking apoptosis using a pan-caspase inhibitor was sufficient to prevent head regeneration. While apoptosis has long been studied in *Hydra* [13], Chera et al. were the first to uncover a link between apoptosis and Wnt signalling during head regeneration. The 'instructive reconstruction' of this article's title refers to the instructive apoptotic patterning which occurs during cnidarian head regeneration.

The findings by Chera et al. initially received a sceptical reception due to the novelty of their results. However, mounting evidence including our recent research are lending further strength to their results and indeed expanding the potential role of apoptotic patterning. The identification of HSPs as likely components of Wnt-Apoptosis interactions (Fig. 1D) during development makes it tempting to

speculate that it is *Hsc71's* anti-apoptotic abilities that prevent head formation by interrupting apoptosis mediated Wnt expression. Further research focusing on this issue is required for a definitive answer as to whether 1) apoptosis is required in instigating head formation post-metamorphosis (a rich apoptotic event, particularly at the future head pole) or whether 2) this link has only evolved specifically for head regeneration after injury. If the former is the case, the widespread mechanism of 'apoptosis-induced compensatory proliferation and regeneration' [14-17] can be incorporated into a broader developmental mechanism of 'instructive apoptotic patterning'.

Evidence that suggests a link between apoptosis and post-metamorphosis head development in *Hydractinia* does indeed exist. Metamorphosis has been shown to be caspase-dependent; it can be prevented by blocking pan-caspase activity [18]. Additionally, knocking down caspase-3 alone prevented anterior larval metamorphosis. What is important, however, is the finding that despite regular larval tail removal by apoptosis, caspase-3 knock-down retarded polyp head development [19]. Intriguingly, Wittig et al. [19] also used Wnt5A as a control RNAi but unexpectedly found that down-regulation of Wnt5A induced increased caspase-3 expression, especially during metamorphosis, indicating further interactions between Wnt signalling and apoptosis. It is interesting to postulate whether the instructive role of apoptosis seen in *Hydra* head regeneration is a throwback to the colonial nature of *Hydra*'s ancestors, being a relic from head development after the metamorphosis of a larva stage. It would be highly informative to examine whether some apoptosis is also required in Wnt mediated patterning events not classically linked to large scale apoptosis e.g. *Hydractinia* larval tail formation or polyp budding.

Additional evidence linking HSPs and apoptosis during cnidarian development comes from a recent microarray based study of metamorphosis in the coral *Acropora* which identified alterations in the expression of several HSPs, although their function has yet to be investigated [20]. Our in situ hybridisation data showed that *Hsc71* expression is strongly upregulated following the induction of metamorphosis [3]. It has also recently been discovered that some coral HSPs exhibit transcriptional regulation linked to the day/night cycle, which the authors postulate is due to the diel pattern of stress experienced by this symbiotic coral [21]. That hypothesis by no means rules out the potential of these HSPs or indeed other HSP family members from performing functions in axial patterning. Indeed, our evidence (e.g. role of *Hsc71* in stem cell proliferation) and others demonstrates that HSPs have a large variety of functions and interact with numerous molecular pathways. The study by Levy et al. [21] only examined expression in adult corals. We have shown that the expression pattern of *Hsc71* in adult *Hydractinia* polyps differs greatly to that observed during polyp development.

While *Wnt3* transcripts are maternally deposited in the *Hydractinia* egg at the prospective (larva) posterior/(polyp) oral pole [5,22], apoptosis could act as a feedback mechanism to ensure that after massive proliferation (larval development and polyp budding) or large scale apoptosis (polyp development during metamorphosis) the Wnt pole remains situated in the correct position. A triangle of Wnt-HSP-Apoptosis interactions exists and its importance in head development/regeneration is being proposed (Fig. 1D). *Hsc71* expression seems to be abolished around the Wnt organiser to ensure normal crosstalk between Wnt-Apoptosis in head development. We have shown that HSP-Wnt interactions are involved in stem cell proliferation (guards against proliferative stress) and subsequent differentiation [23] with Wnt activating *Hsc71* expression. However, in axial patterning *Hsc71* expression is excluded from the Wnt organiser zone, possibly to allow a switch to Wnt-apoptosis interactions.

Beyond the Cnidaria: implications of instructive apoptotic patterning

Of broader interest is whether instructive apoptotic patterning exists in other metazoan phyla. This is likely to be the case as apoptosis-induced compensatory proliferation and regeneration is certainly conserved across numerous species [14-17]. Therefore, the link between apoptosis and developmental patterning should now be examined more closely in multiple phyla. There are also indications of a conserved Wnt-HSP link across metazoans. For instance, in the human colon cancer cell lines (primarily HT-29 cells) Hsc70 and Hsp70 proteins have been identified as binding to the Axin-APC complex which is known to degrade β-catenin, the cellular mediator of Wnt signalling [24,25]. New evidence continues to emerge of the importance of heat shock proteins for development, many of which could be mediated through Wnt-HSP interactions. In addition to examples given in our research article it has recently been shown that knocking down HSP90 prevents compound eye development in an insect [26]. Wnt signalling has long been associated with both vertebrate and invertebrate eye development [27].

While Wnt-Apoptosis links are known to exist in a variety of species [16,28] it is likely that other signalling pathways will also prove relevant to instructive apoptotic patterning. The sonic hedgehog signalling (Shh) pathway is a probable candidate. The Shh morphogen has known apoptosis interactions with an auto-regulatory process existing between Shh and cell death during chick limb development [29]. Another candidate is the related Hedgehog (Hh) morphogen which also functions in *Drosophila* apoptosis-induced compensatory proliferation [15]. In addition, caspases are known to

interact with a broad array of signalling pathways, including a Hh receptor [30] ,and given the known role of caspases in patterning events (see above) many of their interaction partners will also likely contribute to instructive apoptotic patterning. It is likely that HSPs crosstalk with other pathways involved in instructive apoptotic patterning and that the triad described here will be expanded upon in the future.

In our research paper we have proposed a model of Wnt-Hsc71 interactions. The next phase of our investigations will be validating the interactions of this model by identifying Hsc71-Wnt interacting proteins and elaborating on the place of apoptosis in the model. We are also endeavouring to reveal whether the interactions we identified occur in humans. We are beginning by studying Wnt-HSP interactions in cancer cell lines. However, it will also be important to study the Wnt-HSP-Apoptosis triad during vertebrate developmental patterning events.

Conclusion and outlook

Apoptosis has long been known to be involved in patterning by removal of tissue (destructive apoptotic patterning). However, it is now emerging that apoptosis also functions in signalling the site of de novo tissue generation (instructive apoptotic patterning) through morphogen release, as opposed to merely pruning pre-existing tissue. The key question is whether instructive apoptotic patterning occurs only in phyla displaying post-metamorphosis development or if it has been a hitherto hidden feature of metamorphosis-independent development. It will be intriguing to learn the extent of the occurrence of this new phenomenon throughout multicellular organisms.

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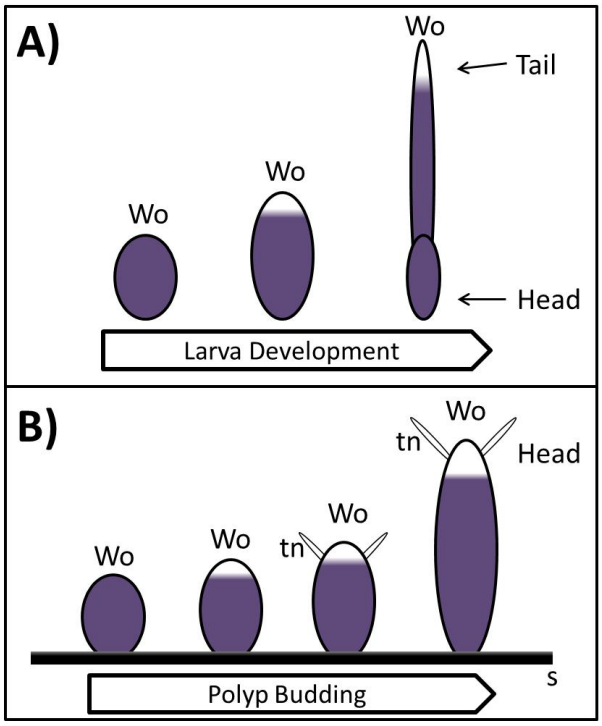
Figure Legend

Figure 1

Schematic of *Hsc71* expression during axial patterning. (A, B) Localisation of *Hsc71* expression is shown by purple shading. Wo indicates the pole of the Wnt organiser. Tn indicates tentacles. s represents the position of the stolons. **(A)** Progression of larval development from left to right. Embryos express *Hsc71* ubiquitously. By the 24hr gastrula stage *Hsc71* is no longer expressed in the region around the Wnt organiser. *Hsc71* continues to be restricted from the Wnt pole while the larval pole develops fully and the tail elongates. **(B)** Progression of secondary polyp budding from left to right. Early buds express *Hsc71* ubiquitously. Prior to head formation *Hsc71* becomes restricted from the region around the Wnt organiser. *Hsc71* expression continues to be repressed from the Wnt pole while the polyp head develops fully. *Hsc71* expression is never seen at the Wnt (oral) pole throughout the rest of the polyp's life. Conversely, Wnt is continuously expressed at the oral pole in adult polyps. **(C)** mRNA expression pattern of *Hsc71* (left) and *Wnt3* (right) in developing polyps. *Hsc71* is expressed throughout the polyp with the exception of the area around the Wnt organiser and the tentacles. *Wnt3* expression is restricted to the oral pole, being a primary component of the Wnt organiser. The expression pattern of these genes is mutually exclusive during head formation. **(D)** Schematic representation of Wnt-Hsc71-Apoptosis interactions (For a more comprehensive model of Wnt-Hsc71 interactions see original paper). Arrow head denotes an activating effect. Flat headed arrow denotes an inhibitory effect.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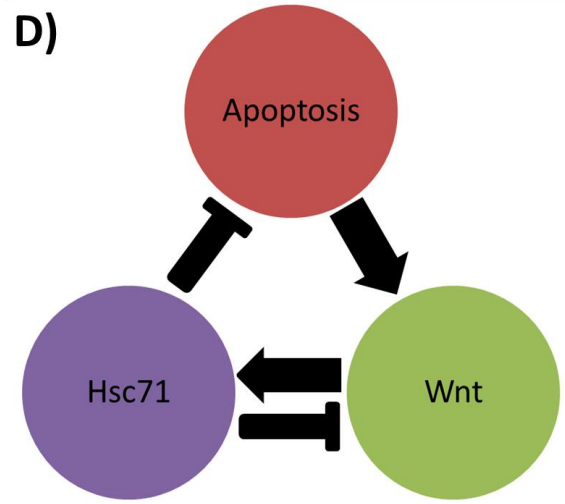
D)
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Hsc71
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