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NEWS

Regulatory giants join forces

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Numerous disease states are characterized by ectopic expression of genes. Recent findings suggest that cross-talk between systems that regulate gene expression during animal development ensures responsiveness to environmental cues.

Animals regulate their crucial processes, such as developmental timing, through temporal- and spatial-specific expression of regulatory factors. Aberrant expression of such factors can result in morphological defects and developmental retardation. Bethke and colleagues (Bethke et al., 2009) have described the interaction of two key pathways that are involved in animal developmental regulation: the steroidal hormone and microRNA-directed regulatory pathways. Through specific cross-talk, these systems regulate protein expression with exquisite responsiveness to the organisms' surrounding environmental conditions.

Both hormones and microRNAs play key roles in animal development (Bourguet et al., 2000; Mangelsdorf et al., 1995). Hormones are produced in response to stimuli that indicate whether environmental conditions — such as temperature and nutrient levels — are favourable for development (Rougvie, 2005). These hormones bind specific proteins, known as nuclear receptors, triggering developmental progression (Weinholds and Plasterk, 2005). In the absence of hormones the cell is arrested in development. A markedly different regulatory system from the hormone-receptor interaction, microRNAs are small RNA species that function by binding to sites on specific messenger RNA (mRNA) targets (Bartel, 2004). In animals, this interaction results in the attenuation of protein output from target mRNA.

Previous studies have shown that the nematode, *Caenorhabditis elegans*, exhibits a similar aberrant developmental phenotype whether it is lacking a nuclear receptor (DAF-12) or microRNAs *mir-48*, *mir-241* and *mir-84* (collectively known as *let-7* microRNAs) (Antebi et al., 2000; Abbott et al., 2005). This observation led Bethke and colleagues (Bethke et al., 2009) to probe the interaction between DAF-12 and the microRNA regulatory pathway. By fusing the promoter of one of the *let-7* microRNAs with a reporter gene, the authors showed that DAF-12 with bound hormone specifically activates the expression of the reporter gene construct in cultured human cells. Removal of the *let-7* microRNA promoter results in the loss of reporter gene expression. These results provide solid evidence of cross-talk between the microRNA and steroidal hormone regulatory pathways.

Bethke *et al*. confirmed the interaction between the DAF-12 nuclear receptor and the *let-7* microRNAs by generating *C. elegans* containing a *let-7* microRNA promoter fused to the green fluorescent protein (GFP) gene. While GFP was broadly expressed in wild-type, fluorescence from GFP was diminished in mutants lacking DAF-12. The authors showed that there was direct regulation of the *let-7* microRNAs by the steroidal hormone-DAF-12 interaction. Importantly, DAF-12 without a bound steroidal hormone was found to negatively regulate *let-7* microRNAs. Hence the interaction of the nuclear receptor with the microRNA functions as a molecular switch: input of steroidal hormone determines whether the output is microRNA repression or activation (Fig. 1). As steroidal hormones are synthesised in response to environmental cues, *let-7* microRNA regulation is therefore ultimately dependent on environmental conditions. A nuance to this model lies in the finding that *let-7* promoter-GFP construct expression levels varied across

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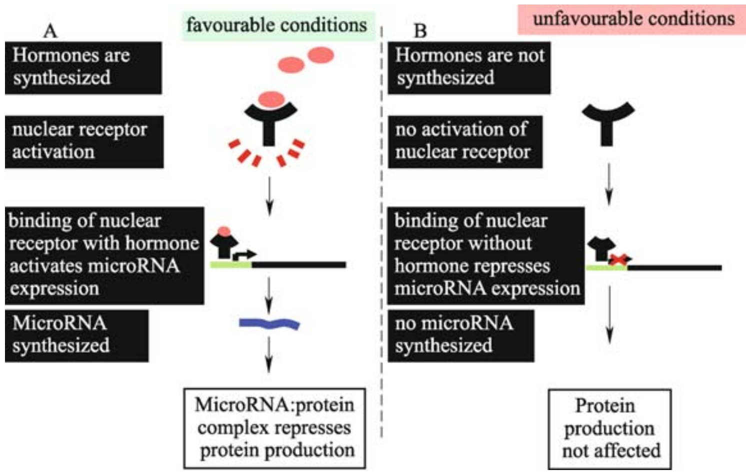

Fig. 1 Cross-talk between microRNAs and steroidal hormones allows microRNAs to be in-tune with the environment. Adapted from (Bethke et al., 2009). **(A)** In response to stimuli indicating a favorable environment, such as suitable temperature, steroidal hormones are produced by the cell. These bind to specific proteins known as nuclear receptors, which become activated. This complex binds to the promoter of microRNA genes resulting in their expression. Once incorporated into a protein complex, the microRNA directs the silencing of the target messenger RNA attenuating protein production. **(B)** When the cell senses unfavourable environmental conditions, no steroidal hormones are produced. The empty nuclear receptor binds to the promoter of the microRNA, preventing its expression. In the absence of the microRNA, the specific targets of the microRNA are unaffected.

tissues, indicating that steroidal hormone regulation of microRNAs is highly tissue-specific. Dependence on the presence of multiple factors enables the cell to fine-tune the protein profile of a cell.

Finding a direct link between environmental stimuli and microRNA regulation is consistent with microRNAs' key role as developmental regulators. They need to sense the favorability of external conditions to ensure that their impact on a cell's protein expression spectrum is favourable to the whole organism. However, the utility of microRNAs as developmental regulators does not simply lie in their ability to be in-tune with surrounding conditions. What makes microRNAs so adept to regulating developmental progression is their capacity to rapidly diminish the production of specific proteins — rather than be dependent on the rate of mRNA degradation, the cell actively manages its protein expression profile. In fact, a high level of resistance to degradation is important if the mRNA encodes proteins involved at a prior stage of development (Farh et al., 2005). By silencing stage-specific genes, microRNAs prepare the cell for the next stage of development, which is fully active once a new cohort of proteins is produced by the cell (Bartel and Chen, 2004; Fig. 2). Essentially, microRNAs clear the stage of prior important factors to facilitate developmental progression.

It remains to be seen whether this hormone-based regulation of *let-7* microRNAs is common to other microRNAs and, indeed, whether microRNAs are involved in other hormonal pathways. As to the former, a corresponding system may already have been identified

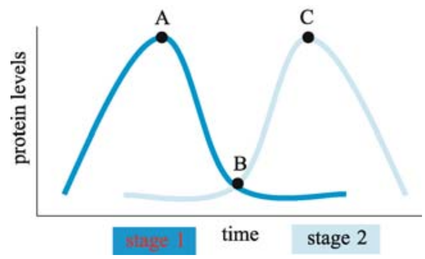

Fig. 2 MicroRNAs facilitate developmental progression. Each stage of development is characterized by the expression of specific proteins. At points A and C, stage 1 and stage 2 specific proteins, respectively, are expressed. At point B, transcripts encoding proteins from the prior developmental stage are cleared by microRNAs, preventing further protein expression. When this is coupled with the expression of proteins required for the next stage of development, the development of the organism progresses. Failure to clear the proteins involved in a prior stage of development can result in aberrant development.

in *Drosophila* (Varghese and Cohen, 2007). One possibility is that this cross-talk is a feature of specific microRNAs that need to be responsive to the environment, such as stress-responsive microRNAs found in animals (Bhattacharyya, 2006) and plants (Jones-Rhoades and Bartel, 2004). By contrast, microRNAs that are expressed throughout an organism are unlikely to benefit from "environmentally-aware" regulation.

If this model is broadly applicable, Bethke *et al*. suggest that it may present a paradigm explaining developmental timing and stem cell differentiation in animals. The conserved features of these crucial processes are the input of environmental stimuli and the output of stringent regulation of protein expression — features that are met by the proposed mechanism (Fig. 1). Furthermore, an understanding of how the cell regulates gene expression under normal conditions greatly benefits research on the many human diseases characterized by the failure of these regulatory mechanisms. In particular, this model may have repercussions for cancer research, a disease characterized by aberrant gene regulation, including microRNA genes (Hanahan and Weinberg, 2000).

The findings of Bethke *et al*. serve as a timely reminder of the importance of taking a holistic approach to research. A given pathway does not operate in isolation from its regulatory neighbours; indeed, greater understanding of developmental regulation will likely see the norm established as cross-talk between conceptually distinct pathways. Already, there is increased appreciation of the importance of feedback loops to microRNA expression (Martinez, 2008). It is unsurprising that the emerging picture of animal gene regulation is complex: misregulation can have a severe impact on cell integrity. The animal cell has evolved the ability to modulate the expression of regulatory species such as microRNAs in response to changes in environmental conditions. Only with such cross-talk can development and other cellular processes be completed with high fidelity.

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