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Review article

Delta-Notch signalling in segmentation

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ABSTRACT

Modular body organization is found widely across multicellular organisms, and some of them form repetitive modular structures via the process of segmentation. It's vastly interesting to understand how these regularly repeated structures are robustly generated from the underlying noise in biomolecular interactions. Recent studies from arthropods reveal similarities in segmentation mechanisms with vertebrates, and raise the possibility that the three phylogenetic clades, annelids, arthropods and chordates, might share homology in this process from a bilaterian ancestor. Here, we discuss vertebrate segmentation with particular emphasis on the role of the Notch intercellular signalling pathway. We introduce vertebrate segmentation and Notch signalling, pointing out historical milestones, then describe existing models for the Notch pathway in the synchronization of noisy neighbouring oscillators, and a new role in the modulation of gene expression wave patterns. We ask what functions Notch signalling may have in arthropod segmentation and explore the relationship between Notch-mediated lateral inhibition and synchronization. Finally, we propose open questions and technical challenges to guide future investigations into Notch signalling in segmentation.

1. Introduction

Monitoring and predicting periodic events from the environment has evolutionary advantages. Therefore, cells and organisms have evolved various molecular machineries as biological clocks for determining time intervals. For example, circadian clocks contain entrainable oscillators with about 24-h rhythm for anticipating recurring daily activities. Even without modulatory signals from the environment, a circadian clock can still run by its endogenous machinery, but gradually loses synchrony with the external day–night rhythm (Golombek and Rosenstein, 2010).

Biological oscillators are not only used for coordinating with daily environmental factors, but have also been adopted to control periodicity of cellular or tissue events at higher frequency within organisms (Webb and Oates, 2016). Somitogenesis is a rhythmic process occurring in the presomitic mesoderm (PSM) in order to subdivide the undifferentiated tissue of the vertebrate embryo into the segments of the body axis, namely somites. The somites give rise to the segmented parts of the adult anatomy, namely the vertebrae, neural and hemal arches, ribs, and their associated muscles and overlying skin.

By studying avian embryos, Palmeirim et al. (1997) provided the first evidence that *c-hairy1*, an avian homologue of the *Drosophila hairy* gene, was expressed rhythmically in the PSM in a tissue-autonomous manner. Waves of rhythmic *c-hairy1* expression with 90-min period first appear in the posterior PSM, then travel anteriorly and finally arrest in the anterior PSM marking where each new somite boundary is defined (Palmeirim et al., 1997). Those findings provided the first evidence for the long-standing clock and wavefront hypothesis (Cooke and Zeeman, 1976) (see Section 2). The modern molecular version of this idea proposes that somitogenesis is driven by an oscillating multicellular genetic network termed the segmentation clock, and the term “cyclic gene” refers to those genes with expression resembling the *c-hairy1* wave patterns in PSM.

Several cyclic genes are found among the *hes*/*her* (*hairy* and *enhancer of split-related*) gene family in all species examined, suggesting that oscillation in this family is a conserved feature of the segmentation clock. In mouse, a single cyclic *hes*/*her* gene, *Hes7*, appears to play a central role in segmentation (Bessho et al., 2001a,

Abbreviations: PSM, pre-somitic mesoderm; Fgf, fibroblast growth factor; Wnt, wingless int-1; DAPT, N-[N-(3,5-difluorophenacetyl-L-alanyl)]-S-phenylglycine t-butyl ester; Nrarp, Notch-regulated ankyrin repeat protein; 3D, three dimensional; bHLH, basic helix-loop-helix; DSL, Delta/Serrate/lag-2.

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2003). In zebrafish the roster includes *her1* (Holley et al., 2000), *her7* (Oates and Ho, 2002), proposed as components of the segmentation clock core pacemaking circuit in this species (Schröter et al., 2012). *her11* (Gajewski et al., 2006), *her12* and *her15* (Shankaran et al., 2007), *her2* and *her4* (Krol et al., 2011) have been shown to oscillate as well, but their role in the clock is not yet known. In contrast *hes6* was found not to be cyclically expressed in the zebrafish PSM based on mRNA spatiotemporal patterns (Kawamura et al., 2005; Schröter and Oates, 2010), yet the protein dimerises with other Her proteins and functions as a core component (Schröter et al., 2012; Trofka et al., 2012; Hanisch et al., 2013).

The second cyclic gene identified was *lunatic Fringe* (*lFng*) from chick and mouse (Evrard et al., 1998; Mcgrew et al., 1998). The *Fringe* family of genes encode glycosyltransferase enzymes that can modify sugar residues on Notch receptors, altering their binding preferences – this link between the segmentation clock and Delta-Notch signalling emerged due to previous discoveries from fruit flies (*Drosophila melanogaster*) showing that *Fringe* could alter Notch signalling (Fleming et al., 1997; Panin et al., 1997; Aulehla and Johnson, 1999; Dale et al., 2003). In contrast to mouse and chick, genes of the *Fringe* family do not oscillate in zebrafish (Prince et al., 2001), but other members of the Notch pathway do (see below). Notch has a relatively long scientific history in biology (Fig. 1) since it was first discovered more than a century ago (Morgan and Bridges, 1916; Morgan, 1917). The first publication of a *Notch* mutant was by Dexter (1914), who characterized the "perfect notched" phenotype on the wing edges of *Drosophila*. Nowadays, Notch signalling is one the most-studied signalling pathways, because it is versatile in biological function and also evolutionarily conserved in most laboratory model animals. The role of Notch signalling in segmentation is the main theme of this review.

The standard picture of Notch signalling involves ligands of the DSL (*Delta*/*Serrate*/*lag-2*) family on the surface of the signal-sending cell binding to Notch receptors on the receiving cell's surface. Notch receptors in the signal-receiving cell are processed in ER and Golgi to produce non-covalent heterodimers between the Notch Extracellular Domain (NECD) and the Transmembrane Domain-Notch Intracellular Domain (TM-NICD) by Furin based cleavage (S1 cleavage) before delivery to the plasma membrane (Logeat et al., 1998). Upon ligand binding, endocytosis from the signal-sending cell (Wang and Struhl, 2004) provides a pulling force necessary to expose the S2 cleavage site for an extracellular protease, ADAM metalloprotease/TNF-α converting enzyme (Meloty-Kapella et al., 2012; Musse et al., 2012). After S2 cleavage, the signal-sending cell endocytoses the ligand with the remaining NECD, and the S3 cleavage in the signal-receiving cell by the intramembrane γ-secretase complex releases the NICD from the transmembrane domain (TM) (Levitan and Greenwald, 1995; De Strooper et al., 1999; Struhl and Greenwald, 1999). The NICD released from cell membrane translocates to the nucleus where it interacts with the CSL (*CBF1* in humans, *Suppressor of Hairless* in *Drosophila*, *Lag-1* in *Caenorhabditis elegans*) transcription factor complex, resulting in subsequent transcriptional regulation of target genes (Artavanis-Tsakonas et al., 1995).

Apart from the signals passing between neighbouring cells by *trans*-activation, the ligand-receptor interactions of Delta-Notch can occur within the same cell, resulting in functionally neutralized Notch receptors. This process is known as *cis*-inhibition (De Celis and Bray, 1997; Micchelli et al., 1997). Consequently, cells with high Delta levels turn into signal-sending cells and cannot receive signals via Notch (Sprinzak et al., 2010), giving rise to a unidirectional signalling mode during lateral inhibition termed the "walkie-talkie" model (Sprinzak et al., 2010, 2011). Cis-inhibition is also important for the mechanism of dorsal-ventral boundary formation in the *Drosophila* wing disc (Del Alamo et al., 2011).

The third major component unpacked out of the segmentation clock is the system of gradients extending along the anterior–posterior axis of the PSM and thought to provide positional information in the tissue. The first pathway identified was FGF signalling (Dubrulle et al., 2001; Sawada et al., 2001), which was joined shortly thereafter by Wnt (Aulehla et al., 2003; Aulehla and Herrmann, 2004) and Retinoic acid signalling (Diez Del Corral et al., 2003; Moreno and Kintner, 2004). Those findings provided an initial solution to where and when PSM cells are allocated to segments.

Modelling has played an important role in understanding vertebrate segmentation, partly because oscillators have a long history of study from a number of theoretical perspectives. A first,

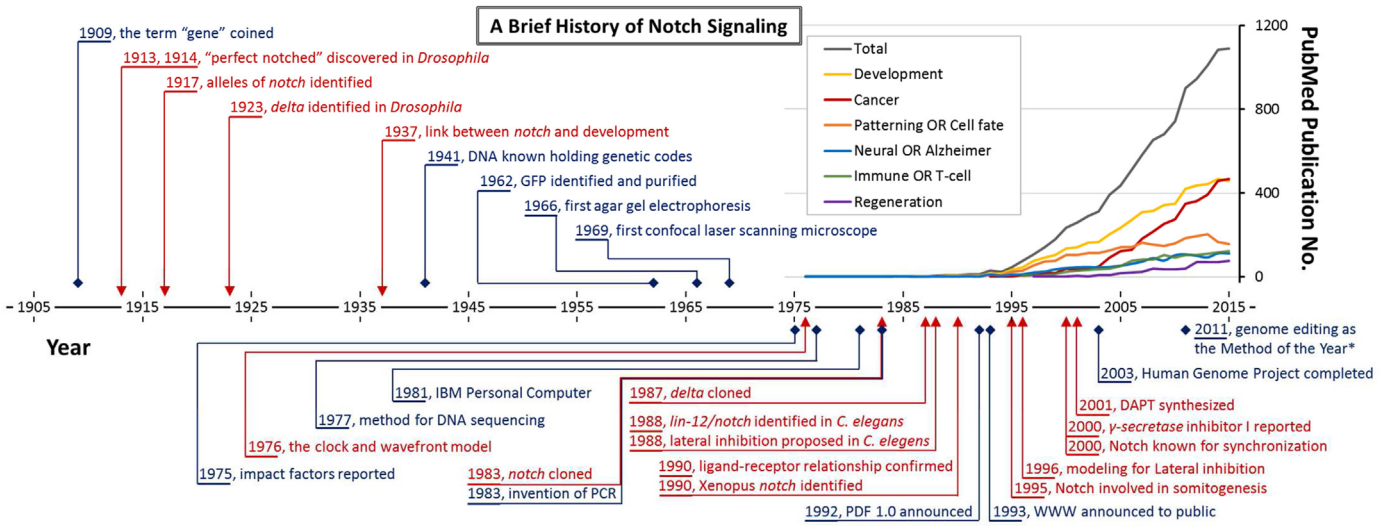

Fig. 1. A brief history of Notch signalling. A simplified chronicle of selected important events about Notch signalling (red text) and the appearance of modern scientific tools (blue text) is illustrated in honour of the pioneers in the field. The inset at top-right corner is the publication numbers of Notch signalling from 1976 to 2015 in the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) searched with related keywords. The total (grey line) is searched by "notch signalling" OR "notch pathway" OR "delta notch", and the sub-fields are AND search to the total with the keywords listed in the box. Two sub-fields have a different trend to the others; the cancer-related publications ascended faster than other others and the pattering/cell fate sub-field started to decline recently.

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