Gene expression suggests double-segmental and single-segmental patterning mechanisms during posterior segment addition in the beetle Tribolium castaneum

RALF JANSSEN*

Uppsala University, Department of Earth Sciences, Palaeobiology, Uppsala, Sweden

ABSTRACT In the model arthropod Drosophila, all segments are patterned simultaneously in the blastoderm. In most other arthropods, however, posterior segments are added sequentially from a posterior segment addition zone. Posterior addition of single segments likely represents the ancestral mode of arthropod segmentation, although in Drosophila, segments are patterned in pairs by the pair-rule genes. It has been shown that in the new model insect, the beetle Tribolium, a segmentation clock operates that apparently patterns all segments in pairs as well. Here, I report on the expression of the segment polarity gene H15/midline in Tribolium. In the anterior embryo, segmental stripes of H15 appear in pairs, but in the posterior of the embryo stripes appear in a single-segmental periodicity. This implies that either two completely different segmentation mechanisms may act in the germ band of Tribolium, that the segmentation clock changes its periodicity during development, or that the speed in which posterior segments are patterned changes. In any case, the data suggest the presence of another (or modified), yet undiscovered, mechanism of posterior segment addition in one of the best-understood arthropod models. The finding of a hitherto unrecognized segmentation mechanism in Tribolium may have major implications for the understanding of the origin of segmentation mechanisms, including the origin of pair rule patterning. It also calls for (re)-investigation of posterior segment addition in Tribolium and other previously studied arthropod models.

KEY WORDS: segmentation, arthropod development, arthropod evolution, segment polarity

Our understanding of arthropod segmentation comes primarily from studies on the model organism Drosophila melanogaster. Here, a hierarchic segmentation gene cascade operates to subdivide, in a stepwise fashion, a syncytial blastoderm that later develops without posterior segment addition into the complete adult body. Notably, one step of this segmentation mechanism comprises the temporal establishment of double-segmental units, as shown by the function (and expression) of the pair-rule genes. In most other arthropods, only anterior segments are formed from the blastoderm, and posterior segments are added from a posterior segment addition zone (Davis and Patel 2002). Posterior segment addition with a single-segmental periodicity likely represents the ancestral mechanism, as suggested by morphological observations and gene expression analysis (Schoppmeier and Damen 2005, Janssen 2011). Evidence for double-segmental patterning mechanisms in the blastoderm, superficially comparable to Drosophila pair-rule patterning, has, however, been found in distantly related arthropods (Dearden et al., 2002, Janssen et al., 2012).

Double-segmental patterning has also been found in tissue that is generated from the posterior segment addition zone in the beetle Tribolium castaneum (Choe et al., 2006) in addition to other insects (Davis et al., 2001, Mito et al., 2007, Erezyilmaz et al., 2009) and a distantly related arthropod, the centipede Strigamia maritima (Chipman et al., 2004). These findings support the idea that a double-segmental posterior patterning system may be a conserved component of arthropod (or at least mandibulate) segmentation. On the other hand, a vertebrate-like posterior segment addition mechanism was proposed for arthropods in which an oscillating clock mechanism would underlie posterior segment addition and patterning (Stollewerk et al., 2003, Chesebro et al., 2013). In vertebrates, posterior segments are strictly added and patterned as single segments (somites) (Gomez et al., 2008). Recent studies have revealed the presence of an oscillating vertebrate-like pat-

Abbreviations used in this paper: HH, hedgehog; SPG, segment polarity gene; wg, wingless.

*Address correspondence to: Ralf Janssen. Uppsala University, Department of Earth Sciences, Villavägen 16, 75236 Uppsala, Sweden. e-mail: ralf.janssen@geo.uu.se

Accepted: 16 July 2014. Final, author-corrected PDF published online: 30 September 2014.

ISSN: Online 1696-3547, Print 0214-6282
© 2014 UBC Press
Printed in Spain
patterning mechanism in *Tribolium*, and at the same time show that this mechanism acts in a two-segment periodicity (Sarrazin et al., 2013, El-Sherif et al., 2013).

I analyzed the expression pattern of the segment-polarity gene *H15* (aka *midline*) in *Tribolium* and found that this gene is likely regulated in a double-segmental pattern in the blastoderm and most of the posterior segments. However, in the later-developed segments, *H15* is apparently regulated in a single-segmental fashion. Thus, my data reveal the presence of a single-segmental patterning system in *Tribolium*, different from the previously described double-segmental mechanism. This single mechanism, which is likely ancestral, may then have evolved into the double-segmental patterning present in the anterior germ band of *Tribolium*. Most importantly, however, the new data suggest that an additional mechanism of posterior segment addition may have escaped scrutiny in previous studies in this emerging model organism.

**Results**

**Expression of Tribolium H15**

Expression first appears in the form of two segmental stripes that are associated with the primordia of the antennal and the mandibular segments (Fig. 1A). Note that the rudimentary intercalary segment will subsequently form between those stripes and express *H15* at a later developmental stage. Individuals with a single stripe of expression were never found. At the subsequent stage, two additional stripes of expression appear (associated with the maxillary and labial segments) (Fig. 1B). Embryos with three stripes were never found. At the next stage, six stripes are present, of which the posterior most two bands (in the first two thoracic segments) are of the same weakened intensity (compared to the more anterior stripes) (Fig. 1C). Embryos with five stripes were never observed. This periodicity of two additional stripes (and no intermediates) is repeated in three further events, resulting in embryos with eight, 10, or 12 stripes (Fig. 1 D-F). The next change in the expression pattern concerns the delayed appearance of the intercalary stripe between the antennal and the mandibular stripe (Fig. 1G). In the next stage, embryos with two additional posterior stripes (sixth and seventh abdominal segment) can be found (Fig. 1H). Notably, the pattern of posterior stripe-addition now changes towards a single-segmental mode, in which abdominal stripes eight, nine and ten form (Fig. 1 I-N). At later developmental stages *H15* is expressed along the ventral surface of the limbs (Fig. 1 K-O), the developing heart,
comes up slowly. In that way both stripes may appear at the same time the anterior stripe of H15 comes up quickly, while the posterior stripe is merely the ary remnant of an ancestral mandibulate patterning system that apparently comes with a general change of genetic regulation, and is not the result of a slower-ticking clock mechanism present. However, in other myriapods such as the centipede Strigamia (discussed in the following section) (Brena and Akam 2013). With our current knowledge we cannot decide whether the similarities found in Tribolium and Strigamia are the result of convergent evolution, or, alternatively, the evolutionary remnant of an ancestral mandibulate patterning system that involved single- and double-segmental patterning mechanisms.

Discussion

Unique regulation of H15 in Tribolium

In Drosophila, H15 acts as a segment-polarity gene (SPG) and its function is required to break symmetry of the otherwise bi-directional Hedgehog (Hh) signaling (Buescher et al., 2004). Since the overall expression pattern of H15 is conserved in all hitherto studied arthropods (Pric et al., 2003, Buescher et al., 2004, Janssen et al., 2008a, b, Svendsen et al., 2009), and since the SPG-network itself is also highly conserved in arthropods including Tribolium (Farzana and Brown 2008, Janssen et al., 2004, 2008a), this implies also that the function of H15 in the beetle is likely conserved. Notably, however, H15 appears to be the only SPG that is regulated in a double-segmental fashion. Other SPGs such as wingless (wg) (Nagy and Carroll 1994) (Fig. 2 A-C), engrailed (en) (Brown et al., 1994) and hedgehog (hh) (Farzana and Brown 2008) (Fig. 2 D-F) appear to be regulated in a single-segmental fashion. It has been shown, however, that the genetic interaction that leads to the activation of wg in odd and even numbered parasegments (i.e. in adjacent segments) differs considerably (Choe and Brown 2009). It may then be the case that the single-segmental (and strictly anterior to posterior) appearance of wg is merely the result of differences in the upstream regulatory network. This could lead to a temporal delay of wg expression in the posterior of two simultaneously established segmental units.

What is the cause of the double vs. single-segmental appearance of H15?

It is obvious that the regulation of H15 in the posterior abdomen in Tribolium is different when compared to the patterning of the more anterior segments. It is either the case that: two generally different patterning mechanisms function in Tribolium (like in the myriapod Strigamia (discussed in the following section) (Brena and Akam 2013)); or that the apparent regulation of H15 in pairs is just the result of upstream clock dynamics. This could be the case if the anterior stripe of H15 that is regulated by a dynamic wave of pair-rule gene expression comes up quickly, while the posterior stripe regulated by the previous wave of pair-rule gene expression comes up slowly. In that way both stripes may appear at the same time. This would be in line with the shifted appearance of H15 in adjacent segments compared to the waves of even-skipped (eve) expression (Choe and Brown 2007) and the fact that the last wave of eve-expression is delayed (El-Sherif et al., 2012). Slowing down of the first ‘tick’ of the clock towards the end of embryogenesis would then lead to the appearance of single stripes of H15 in the last formed segments (summarized in Fig. 3). An alternative scenario with paired (prd) being in control of H15 would not require slow and quick activation of H15 in adjacent segments because the double-segmental domains of prd are in register with the appearance of H15. It would, however, not explain the delayed appearance of H15 in A8 and A9 without further modification of H15-regulation.

A drastic alternative is that the segmentation-clock may change its periodicity from double- towards single-segmental in nature. If this is the case, then the question is what causes this switch? This may be a matter of available space. Firstly, it is known that the vertebrate segmentation clock “ticks” for as long as presomitic mesoderm is present (the amount of this tissue is consumed during the process of segment addition) (Gomez et al., 2008). It is therefore not unlikely that the arthropod segmentation clock requires comparable tissue (the segment addition zone), independent of whether vertebrate and arthropod segmentation clocks are homologous or analogous. Secondly, we find that in the centipede Strigamia (Geophilomorpha) a double-segmental patterning mechanism exists (likely clock-based as suggested by dynamic gene expression patterns in the saz) (Chipman et al., 2004, Brena and Akam 2013). In this species the saz is expansive. In other myriapods such as the centipede Lithobius forficus (Lithobiomorpha) the saz is much reduced and no evidence of a double-segmental patterning mechanism has been found. Although it is not unlikely that the double-segmental mechanism in Strigamia is the result of convergent evolution, the large saz may have provided the morphological prerequisite for the evolution of this patterning mechanism. For Tribolium, this could mean that the switch from double- to single-segmental patterning is caused by the shrinking of the saz towards the end of ontogenesis. In order to test this hypothesis it would be interesting to study gene expression of H15 (and other SPGs) in arthropod species with small, intermediate and large segment addition zones.

On the origin of pair-rule-like patterning mechanisms

The current study revealed the possible involvement of a single and a double-segmental patterning system in Tribolium. This is strikingly similar to what a very recent study has demonstrated to be the case for the centipede Strigamia (Brena and Akam 2013). However, in Strigamia the change from double- to single-segmental patterning apparently comes with a general change of genetic regulation, and is not the result of a slower-ticking clock mechanism (Brena and Akam 2013). With our current knowledge we cannot decide whether the similarities found in Tribolium and Strigamia are the result of convergent evolution or, alternatively, the evolutionary remnant of an ancestral mandibulate patterning system that involved single- and double-segmental patterning mechanisms.
This is because the unique patterning of the posterior-most abdominal segments has not been recognized until now, except for the statement of El-Sherif et al., (2012) that the appearance of the last stripe of even-skipped expression is significantly delayed.

**Future perspectives**

As a consequence of the current study, it will now be necessary to further investigate posterior (single) segment addition in Tribolium in order to find out if it underlies different regulatory mechanisms than double-segmental patterning, and if those are potentially similar to the mechanisms of single segment addition in Strigamia. We also will have to investigate posterior segment addition in other insects that pattern segments in pairs. The question is whether they pattern all segments by the same double-segmental mechanism, and if this is not the case, if single segmental posterior segment patterning underlies the same (or similar) genetic regulation system as in Strigamia and/or Tribolium. A first step must be to study the expression of known posterior segmentation genes, such as the pair-rule genes, in relation to the expression of H15, and to study functional aspects of H15 during anterior and posterior segmentation in Tribolium. The aim of this paper is to demonstrate that differences in anterior and posterior segmentation exist in the model arthropod Tribolium, and to highlight the urgent need for further detailed investigation of Tribolium segmentation mechanism(s). If both, single and double-segmental patterning mechanisms were present in the last common ancestor of arthropods (or at least mandibulates), this would explain the widespread appearance of pair rule-like expression patterns throughout Arthropoda.

**Materials and Methods**

Gene cloning before expression of Tribolium H15 in the developing heart has been described before (Janssen and Damen 2008). Fragments of wingless (wg) and hedgehog (hh) were amplified with the degenerate primers described by Damen (2002) and Janssen et al., (2004). Expression of wg was described by Nagy and Carroll (1994) and expression of hh has been described by Farzana and Brown (2008). In situ hybridization of embryos was performed as described by Tautz and Pfeifle (1989). Flat-mounted embryos were analyzed under a Leica MZFLIII dissection microscope and an Axiovert 135 with a Zeiss 100WHS epifluorescence microscope equipped with a Leica DFC490 digital camera, or a Nikon ECLIPSE E400 microscope equipped with a Nikon D70 portable digital camera. Brightness, contrast and color values were adjusted in all images using the image processing software Adobe Photoshop CS2 (Version 9.0.1 for Apple Macintosh).

**Acknowledgements**

I would like to thank native English speakers Aodhán D. Butler, Illiam Jackson and Stephen Poropat for proofreading of the final version of the manuscript.

**References**


Further Related Reading, published previously in the *Int. J. Dev. Biol.*

**Genetic control of morphogenesis - Hox induced organogenesis of the posterior spiracles**
James Castelli-Gair Hombría, María Luisa Rivas and Sol Sotillos
http://dx.doi.org/10.1387/ijdb.072421jc

**Head-tail patterning of the vertebrate embryo: one, two or many unresolved problems?**
Claudio D. Stern, Jeroen Charité, Jacqueline Deschamps, Denis Duboué, Anthony J. Durst, Marie Knita, Jean-François Nicolas, Isabel Palmeirim, Jim C. Smith and Lewis Wolpert
Int. J. Dev. Biol. (2006) 50: 3-15
http://dx.doi.org/10.1387/ijdb.052095cs

**Transcriptional regulation and the evolution of development**
Gregory A Wray
http://www.intjdevbiol.com/web/paper/14756343

**Segmentation: mono- or polyphyletic?**
Elaine C Seaver
http://www.intjdevbiol.com/web/paper/14756334

**Cell lineage analysis of pattern formation in the Tubifex embryo. I. Segmentation in the mesoderm**
A Goto, K Kitamura and T Shimizu
http://www.intjdevbiol.com/web/paper/10470648

*5 yr ISI Impact Factor (2011) = 2.959*