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Quantitative dynamics and increased variability of segmentation gene expression in the *Drosophila Krüppel* and *knirps* mutants

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ABSTRACT

Here we characterize the response of the *Drosophila* segmentation system to mutations in two gap genes, *Kr* and *kni*, in the form of single or double homozygotes and single heterozygotes. Segmentation gene expression in these genotypes was quantitatively monitored with cellular resolution in space and 6.5 to 13 min resolution in time. As is the case with wild type, we found that gene expression domains in the posterior portion of the embryo shift to the anterior over time. In certain cases, such as the *gt* posterior domain in *Kr* mutants, the shifts are significantly larger than is seen in wild type embryos. We also investigated the effects of *Kr* and *kni* on the variability of gene expression. Mutations often produce variable phenotypes, and it is well known that the cuticular phenotype of *Kr* mutants is variable. We sought to understand the molecular basis of this effect. We find that throughout cycle 14A the relative levels of *eve* and *ftz* expression in stripes 2 and 3 are variable among individual embryos. Moreover, in *Kr* and *kni* mutants, unlike wild type, the variability in positioning of the posterior Hb domain and *eve* stripe 7 is not decreased or filtered with time. The posterior Gt domain in *Kr* mutants is highly variable at early times, but this variability decreases when this domain shifts in the anterior direction to the position of the neighboring Kni domain. In contrast to these findings, positional variability throughout the embryo does not decrease over time in double *Kr*;*kni* mutants. In heterozygotes the early expression patterns of segmentation genes resemble patterns seen in homozygous mutants but by the onset of gastrulation they become similar to the wild type patterns. Finally, we note that gene expression levels are reduced in *Kr* and *kni* mutant embryos and have a tendency to decrease over time. This is a surprising result in view of the role that mutual repression is thought to play in the gap gene system.

Introduction

The segmented body plan in *Drosophila* is determined by a rapid cascade of maternal and zygotic gene expression that operates at the early stages of embryonic development. Gap genes are the most upstream zygotic component of the segmentation system in regulatory terms. They are also the first zygotic genes to establish discrete territories of gene expression, being expressed in broad and partially overlapping domains along the anteroposterior (A–P) axis of the *Drosophila* blastoderm embryo (Jaeger, 2011). Regulation of pair-rule genes by gap genes creates the characteristic pair-rule pattern of seven transverse stripes. The concerted action of the pair-rule system in turn gives rise to the initial expression of segment polarity genes at cellular resolution at the onset of gastrulation (Ingham, 1988; Nüsslein-Volhard et al., 1987; DiNardo et al., 1988). This is the time when the segmental pattern is determined (Simcox and Sang, 1983).

Over the last decade, quantitative studies have yielded significant progress in understanding the genetic network controlling segmentation in *Drosophila* (Houchmandzadeh et al., 2002; Spirov and Holloway, 2003; Gregor et al., 2007a,b; He et al., 2008; Surkova et al., 2008a; Manu et al., 2009b). In wild type, these quantitative studies have led to two new discoveries. First, in the posterior of the embryo expression domains shift towards the anterior over time. Second, the variability of gene expression decreases over time. In this paper, we quantitatively characterize the molecular phenotypes of mutations in two trunk gap genes,

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Krüppel (*Kr*) and *knirps* (*kni*). A closely related study (Janssens et al., this issue) concerns the molecular phenotype of the terminal gap gene *tailless* (*tll*). A central issue for both studies is the role of variability and shifts in the mutant genotypes.

Recently it was shown that the morphogenetic field controlling segmentation in wild type embryos exhibits error-correcting properties. For example, high spatial variability inherent to long-range gradients of Bicoid (Bcd) and Caudal (Cad) is decreased or filtered at a level of zygotic expression of gap and pair-rule genes (Houchmandzadeh et al., 2002; Spirov and Holloway, 2003; Surkova et al., 2008a). Moreover, this filtration is a dynamic process as the early zygotic segmentation gene expression is also spatially variable and this variability decreases when gastrulation approaches. In general, all kinds of variability in gene expression are decreased in individual wild type embryos by the onset of gastrulation, which is the time of segment determination (Surkova et al., 2008a). This is an illustration of the phenomenon of canalization proposed by C.H. Waddington. Waddington correctly predicted the observed reduction in variability over 60 yr ago (Waddington, 1942) and his prediction was based on the fact that phenotypic variability in mutants was greater than in wild type. Thus, it is important to check whether his observation remains correct at the molecular level.

Null mutations in *Kr* and *kni* result in significant defects in the *Drosophila* larval cuticle, which often vary from embryo to embryo. *Kr* null mutants show deletions of thoracic and anterior abdominal segments (A1–A5). Mutant embryos may have three denticle bands or four, and one band may or may not have reverse polarity (Nüsslein-Volhard and Wieschaus, 1980; Wieschaus et al., 1984). In *kni* mutant embryos there are only two rather than eight abdominal denticle bands. Segments A1–A7 are fused into a single field and the eighth abdominal segment is normal (Nüsslein-Volhard and Wieschaus, 1980; Lehmann, 1988).

Our goal was to investigate to what extent the variability in cuticular phenotypes is determined by the variability in gene expression at the time when segments are determined. A limited amount of information on variability of gene expression in mutants has been previously published. In *Kr* mutants the positional variability of the posterior border of anterior Hunchback (Hb) domain remains unchanged with respect to wild type (Houchmandzadeh et al., 2002), but doubles in *Kr;kni* double mutants, which also cause an increase in variability of the posterior border of the anterior Giant (Gt) domain (Manu et al., 2009b). To get a more comprehensive view of this problem, we decided to examine both single *Kr* and *kni* mutants and double *Kr*;*kni* mutants.

We also sought to compare dynamic movements of expression domains in wild type and mutant embryos. In wild type, the posterior domains of gap and pair-rule genes dynamically shift their positions in an anterior direction during cycle 14A (Surkova et al., 2008a; Jaeger et al., 2004b). In *Kr* mutants this phenomenon is known to be enhanced, as it has been reported from qualitative data that in late cycle 14 the posterior Gt domain is shifted into the central region of an embryo (Eldon and Pirrotta, 1991; Kraut and Levine, 1991b). The shifted position of Gt presumably causes significant reduction in the amplitude of the *kni* domain observed in these mutants (Pankratz et al., 1989; Capovilla et al., 1992). In this paper we demonstrate that the posterior Gt domain shows complex dynamic behavior which is not consistent with the previously published results.

A further issue is that the literature contains contradictory statements concerning domain positions in mutants. For example, it has been reported that in *Kr* mutants the posterior border of the anterior Hb domain is shifted posteriorly into the territory of the absent Kr domain (Jäckle et al., 1986; Warrior and Levine, 1990; Hülskamp et al., 1994), but an early quantitative study reported that the posterior Hb boundary in *Kr* mutants has the same position as in wild type embryos (Houchmandzadeh et al., 2002).

In this paper we present a detailed analysis of the spatial variability of gap gene and *even skipped* (*eve*) expression domains in *Kr*, *kni* and *Kr*;*kni* mutant embryos. We study how the level of positional error and the variability in the shape of the expression profile depends on anterior–posterior (A–P) position and developmental time within cycle 14A. We characterize the averaged expression of *Kr*, *kni*, *hb*, *gt* and *eve* throughout cycle 14A and compare the dynamic changes in position and amplitude of expression domains in mutants and wild type.

Materials and methods

We obtained *Kr*− embryos from the *Drosophila melanogaster* Kr^1 amorphic allele (Wieschaus et al., 1984). *kni*− embryos were collected either from Df(3L)ri-79c or Df(3L)ri-XT1, ru[1] st[1] e[1] ca[1] stocks. *Kr*;*kni* double mutant embryos were made by crossing Kr^1 and Df(3L)ri-79c flies. 3–4 h old embryos were fixed and stained as described elsewhere (Kosman et al., 1998; Janssens et al., 2005). We used primary antibodies against Bcd, Cad, Kr, Kni, Gt, Hb, Tll, Eve and Fushi tarazu (Ftz) (Kosman et al., 1998; Azpiazu and Frasch, 1993) and secondary antibodies conjugated to Alexa Fluor 488, 555, 647, and 700 (Invitrogen). Each embryo was also stained with either anti-histone H1.4 antibody (Chemicon) or Hoechst 34 580 (Invitrogen) to mark the nuclei.

Laterally oriented embryos were scanned using a Leica TCS SP2 or Leica TCS SP5 confocal microscope as described (Janssens et al., 2005). Null mutants had no expression in *Kr* or/and *kni* channels, and in heterozygotes expression level was nearly halved.

For each experiment, the microscope gain and offset parameters were set to the maximum expression level of a given gene in wild type embryos and then the same settings were applied for mutants. The 8-bit 1024×1024 digital images of gene expression in mutants were acquired from embryos belonging to cleavage cycle 14A. Embryos from the earlier cleavage cycles had very low levels of *Kr* and *kni* expression that did not allow us to distinguish null mutants from heterozygotes. Each embryo was additionally scanned in the differential interference contrast (DIC) mode to estimate the degree of membrane invagination (Surkova et al., 2008a).

Quantitative gene expression data and averaged (integrated) patterns were acquired as previously described (Janssens et al., 2005; Myasnikova et al., 2005; Surkova et al., 2008a, 2011) using the recently developed tools ProStack and BREReA (Kozlov, 2008; Kozlov et al., 2009). For spatial registration and data integration, embryos from cleavage cycle 14A were distributed into 8 time classes about 6.5 min each on the basis of measurement of degree of membrane invagination, as well as characteristic features of the *eve* gene expression pattern (Surkova et al., 2008a). In order to increase sample sizes for statistical analysis of both positional shifts and the spatial variability of expression domain in mutants, we merged each of two neighboring temporal classes into four time groups with a time resolution of about 13 min each (Fig. 1). These time groups roughly correspond to four phases of blastoderm cellularization (Lecuit and Wieschaus, 2000; Schroeder et al., 2011).

Our dataset includes quantitative data on expression of *hb*, *kni*, *gt*, *eve*, *ftz*, *bcd*, *cad* and *tll* in 270 *Kr*− embryos, as well as data on expression of *hb*, *Kr* and *eve* in 68 *kni*- embryos. Data on the expression of *hb* and *gt* in 29 *Kr*;*kni* double mutant embryos were obtained for time classes 4–7 of cycle 14A. We analyzed *ftz* expression in *Kr*− embryos and *eve* expression in *kni*+/*kni*− heterozygotes only in individual embryos because of the absence of sufficient amount of data for averaging. Processed data on expression of the same genes in wild type embryos were taken