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l(2)41Aa, a heterochromatic gene of *Drosophila melanogaster*, is required for mitotic and meiotic chromosome condensation

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Summary

Genetic and cytological approaches have yielded significant insight into the mapping and organization of genes located in the heterochromatin of *Drosophila melanogaster*. To date, only a few of these genes have been molecularly characterized in detail, and their function unveiled. As a further step towards the identification of heterochromatic gene functions, we have carried out a cytological analysis of mitotic and meiotic cell divisions in mutants carrying different allelic combinations of *l(2)41Aa*, a gene located in the proximal heterochromatin of chromosome 2. Our results showed that larval brains of *l(2)41Aa* mutants display a high frequency of cells with irregularly condensed chromosomes. In addition, defective chromosome condensation was detected in male meiosis, consequently affecting chromosome segregation and giving rise to irregular spermatids. Taken together, these findings indicate that *l(2)41Aa* is a novel cell cycle gene required for proper chromosome condensation in both somatic and germ line cells.

1. Introduction

Constitutive heterochromatin is a ubiquitous component of eukaryotic chromosomes and exhibits similar cytological and molecular properties in animals and plants (John, 1988). These properties include: (i) compact state throughout most of the cell cycle; (ii) late replication during S-phase; (iii) pericentromeric and/or peritelomeric location; (iv) low gene density; (v) low genetic recombination; (vi) enrichment in repetitive sequences.

Constitutive heterochromatin has generally been thought to be genetically inactive. In the last two decades, however, cytological and molecular studies in *Drosophila melanogaster* have shown that this peculiar component of the eukaryotic genome may perform important cellular functions, such as gene regulation, centromere and telomere function, and meiotic chromosome transmission (Gatti & Pimpinelli, 1992; Weiler & Wakimoto, 1995; Elgin, 1996; McKee, 1998; Eissenberg & Hilliker, 2000; Henikoff *et al.*, 2000). Moreover, heterochromatin of chromosomes 2 and 3 contains at least 28 essential genes required for viability and fertility (Hilliker, 1976; Dimitri, 1991; Schulze *et al.*, 2001; Koryakov *et al.*, 2002; Dimitri *et al.*, 2002). These genes were originally identified by recessive lethal mutations and have been suggested to correspond to single-copy sequences (Hilliker, 1976). Thus far, molecular analyses have confirmed their prediction: the genes *light*, *concertina*, *rolled* and *Nipped-B* on chromosome 2 and *l(3)80Fh*, *l(3)80Fi* and *l(3)80Fj* on chromosome 3 all consist of single-copy exons (Devlin *et al.*, 1990; Parks & Wieschaus, 1991; Schulze *et al.*, 2001). In addition *light*, *concertina*, *rolled* and *Nipped-B* exhibit a high density of TE-homologous sequences within their intronic and flanking regions (Devlin *et al.*, 1990; Dimitri *et al.*, 2003). These latter results concur with fluorescence *in situ* hybridization (FISH) data showing that most of those genes map to regions which harbour several clusters of TE-homologous sequences (Pimpinelli *et al.*, 1995; Berghella & Dimitri, 1996) and lack highly repetitive satellite DNAs (Lohe *et al.*, 1993).

Single-copy heterochromatic genes on chromosome *2* require a heterochromatic environment to function (Weiler & Wakimoto, 1995) and are involved in a variety of important cellular processes. The *light* gene controls vacuole organization and biogenesis

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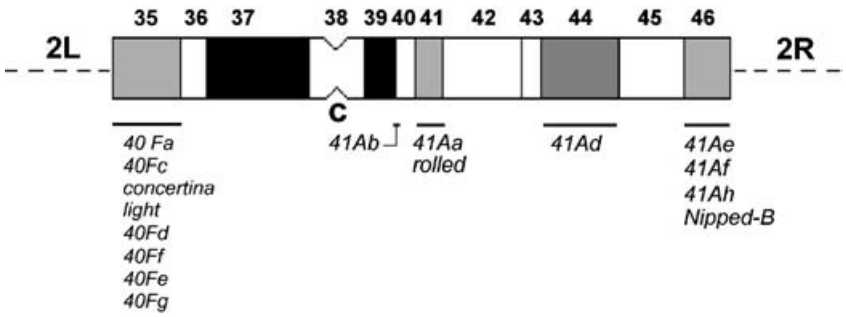

Fig. 1. Cytological mapping of vital genes in the heterochromatin of chromosome 2. The heterochromatin of chromosome 2 has been subdivided into 13 regions (numbered h35 to h46), on the basis of its cytological pattern after banding techniques (Dimitri, 1991). Filled areas represent the Hoechst 33258 or DAPI-bright regions, the shaded boxes represent regions of intermediate fluorescence and the open boxes are regions of dull fluorescence. 2L, left arm of chromosome; 2R, right arm of chromosome; 2C, centromeric region. Mapping of the vital genes (Dimitri, 1991; Dimitri *et al*., 2002) is shown below. The *l(2)41Aa* gene maps to the h41 region and is genetically separate from *rolled* by the breakpoint of *Df(2Rh)Rsp*^{1}.

(Warner *et al*., 1998); *concertina* encodes a maternal α-like subunit of a G protein essential for gastrulation (Parks & Wieschaus, 1991); the *rolled* product is a mitogen-activated protein (MAP) kinase required in the sevenless signal transduction pathway (Biggs *et al*., 1994) and may also play a role in mediating the spindle integrity checkpoint (Inoue & Glover, 1998). Finally, *Nipped-B* protein is homologous to a family of chromosomal adherins and performs an architectural role in enhancer-promoter communication and possibly in chromosome structure (Rollins *et al*., 1999).

In the most recent release of the *Drosophila melanogaster* genome sequence, only limited portions of the 60 Mb of heterochromatin have been sequenced (Adams *et al*., 2000). Thus, the structure and function of most of the heterochromatic genes detected to date by conventional genetic analysis remains unknown. In particular, amongst the heterochromatic genes of chromosome 2 that have yet to be cloned, *l(2)41Aa* exhibits interesting features. This gene is deeply embedded within the *2Rh*, in that it maps to the region h41, proximal to the *rolled* gene (Fig. 1; Hilliker, 1976; Dimitri, 1991). Animals hemizygous for *EMS31*, a lethal allele of *l(2)41Aa*, die at the third instar larval stage and have severely defective imaginal discs (Hilliker, 1976; Dimitri, 1991). Both these phenotypes are suggestive of a disruption within an essential cell cycle gene (Gatti & Baker, 1989). In this paper, we have asked whether *l(2)41Aa* identifies a new function required for proper cell cycle behaviour in dividing cells. To this end, we have performed cytological analyses of both mitotic and meiotic cell divisions in mutants carrying different lethal alleles of *l(2)41Aa*. Our results show that mutations in *l(2)41Aa* strongly interfere with proper chromosome condensation in both kinds of divisions. Mitotic brain cells of *l(2)41Aa* mutants display a high proportion of cells (30–60%) with irregularly condensed and poorly condensed metaphases. Similarly, we see irregular chromosome condensation in spermatocytes. Interestingly, postmeiotic cells containing abnormal associations between nuclei and mitochondrial derivatives are observed in testes, a phenotype caused by a failure of cytokinesis. Taken together, our results indicate that *l(2)41Aa* encodes a product that is required for proper chromosome condensation in both somatic and germ line cells.

2. Materials and methods

(i) *Fly stocks*

The chromosome 2 heterochromatic deletions *Df(2Rh)B* and *Df(2Rh)Rsp*^{31} have been characterized previously at both genetic and cytological levels by Hilliker (1976), Ganetzky (1977) and Dimitri (1991). *EMS-31* is an EMS-induced lethal allele of *41Aa* (Hilliker, 1976), while *IR18* and *Df(2Rh)IR7* were induced by I-R dysgenesis (Dimitri *et al*., 1997). Fly stocks and genetic crosses were maintained on standard *Drosophila* medium at 25 °C ($\pm$1 °C). Other mutations, genetic markers and special chromosomes used in this work were described by Lindsley & Zimm (1992).

(ii) *Chromosome preparations*

The heterochromatic lethal mutations in *41Aa* were all balanced over the *T(2;3) TSTL*, *Cy Tb*, a translocation involving the *In(2LR)O* and *TM6b* balancer chromosomes which carry the *Curly* and *Tubby* dominant markers, respectively. Trans-heterozygous or hemizygous larvae for a combination of two *41Aa* alleles were selected as *Tubby*$^{+}$ and dissected. Mitotic chromosomes from larval neuroblasts were prepared as described by Gatti & Goldberg (1991). Meiotic chromosome preparations from larval testes were carried out according to Cenci *et al*. (1994).

(iii) *Microphotography*

Chromosome preparations were analysed using a computer-controlled Zeiss Axioplan epifluorescence

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microscope equipped with a cooled CCD camera (Photometrics). Fluorescence was visualized using the Pinkel No. 1 filter set combination (Chroma Technology). The fluorescent signals were recorded by IP Spectrum Lab Software and edited with Adobe PhotoShop 5.0.

3. Results

(i) *Mitotic defects in l(2)41Aa mutants*

It has previously been shown that hemizygous larvae for a mutant allele of *l(2)41Aa* survive until the third instar and exhibit poorly developed imaginal discs (Hilliker, 1976; Dimitri, 1991). Given that late lethality is characteristic of many cell cycle mutants (Gatti & Baker, 1989), we were interested in examining whether mitotic cell division was affected in animals carrying different mutant allele combinations of *l(2)41Aa*. For this purpose, two lethal alleles of this gene, *l(2)IR18* and *l(2)EMS-31*, and three heterochromatic deletions. *Df(2Rh)B*, *Df(2Rh)Rsp*^{31} and *Df(2Rh)IR7* (hereinafter indicated as *DfB*, *DfRsp31* and *DfIR7*), that uncover the *l(2)41Aa*, were analysed. Transheterozygous and hemizygous mutant larvae developed very slowly in comparison with heterozygote larvae, and exhibited severely reduced imaginal discs (Fig. 2).

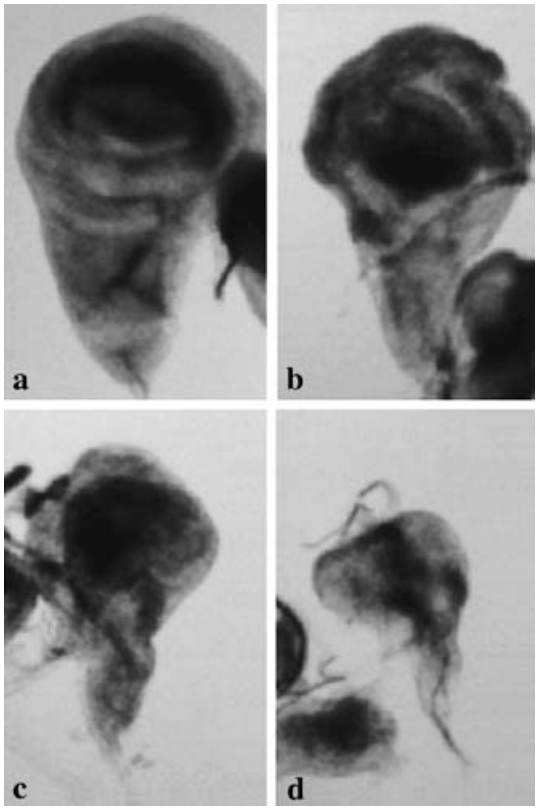

Fig. 2. Imaginal wing disc in *l(2)41Aa* mutants. (*a*) Normal-sized wing disc in *DfB*/*Cy* larvae; (*b*) abnormal wing disc in *DfB*/*IR7* mutant larvae; (*c*) reduced wing disc in *DfB*/*IR18* mutant larvae; (*d*) strongly reduced wing disc in *DfRsp31*/*IR18* mutant larvae.

Examination of colchicine-treated brain squashes from larvae bearing various *l(2)41Aa* mutant allele combinations revealed a common cytological phenotype. Both aceto-orcein- and Hoechst 33258-stained metaphases from all combinations showed defective chromosome condensation. In particular, a high proportion of cells (44–63%; Table 1) displayed swollen and unevenly condensed chromosomes (Fig. 3); this was accompanied, but only rarely, by the presence of cells with low levels of chromosome breakage (approximately 1–2%). An extremely high proportion of abnormally condensed chromosome figures was observed in brains from *DfB*/*DfB* larvae (83% of total metaphases; Table 1). *DfB* also failed to complement *l(2)41Ab*, another vital heterochromatic gene of chromosome 2 (Hilliker, 1976; Dimitri, 1991; Fig. 1) However, an involvement of *l(2)41Ab* in chromosome condensation can be excluded, as hemizygous larvae for lethal alleles of this gene exhibit cells with regularly condensed chromosomes (data not shown). In general, undercondensation of chromosomes was not restricted to the euchromatic regions, as the pericentromeric heterochromatin was also slightly elongated and understained in mutant cells with respect to control (Fig. 3*f*).

Other aspects of mitosis were also taken into consideration. The mitotic index, a parameter measuring the proportion of cells engaged in mitosis, was clearly reduced in different *l(2)41Aa* allelic combinations (Table 1). We believe that this decrease is caused by a delay in progressing through interphase, and may explain the observed reduction in imaginal disc size. The ratio of anaphase to metaphase figures in all but one *l(2)41Aa* mutant did not significantly differ from wild-type (Table 1). The only exception was *DfB*/*DfIR7*, which exhibited a higher anaphase/metaphase ratio with respect to wild-type. This single inconsistency is likely to be due to the genetic background of this particular combination. Finally, in all *l(2)41Aa* mutant genotypes analysed, the anaphases were normal and chromosome segregation defects were not observed.

(ii) *Mutations in l(2)41Aa also affect male meiosis*

The analysis of onion stage spermatids provides a reliable method for the detection of an irregular execution of male meiotic processes (Gonzalez *et al*., 1989; Fuller, 1993). Normally, *Drosophila* spermatids at the onion stage consist of a round, phase-light nucleus associated with a single, phase-dark mitochondria conglomerate called the Nebenkern. The nucleus and Nebenkern display similar sizes in wild-type spermatids at the onion stage (Fig. 4*a*; reviewed by

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Table 1. *Quantification of the mitotic defects in* l(2)41Aa *mutants*

Genotype	Total metaphases scored	Irregular metaphases (%)	MI	Metaphases	Anaphases	A/M
Wild-type	842	1·8	0·81	310	38	0·12
DfB/Irh18	711	57	0·37	188	21	0·11
DfB/EMS31	450	50	0·20	202	23	0·11
DfB/DfIR7	545	63	0·41	121	28	0·27
DfRsp31/IR18	151	48·3	0·39	185	29	0·15
EMS31/IR18	735	60	0·50	275	24	0·09
DfB/DfB	341	83	0·20	150	16	0·11

MI, mitotic index; A/M, anaphases/metaphases ratio.

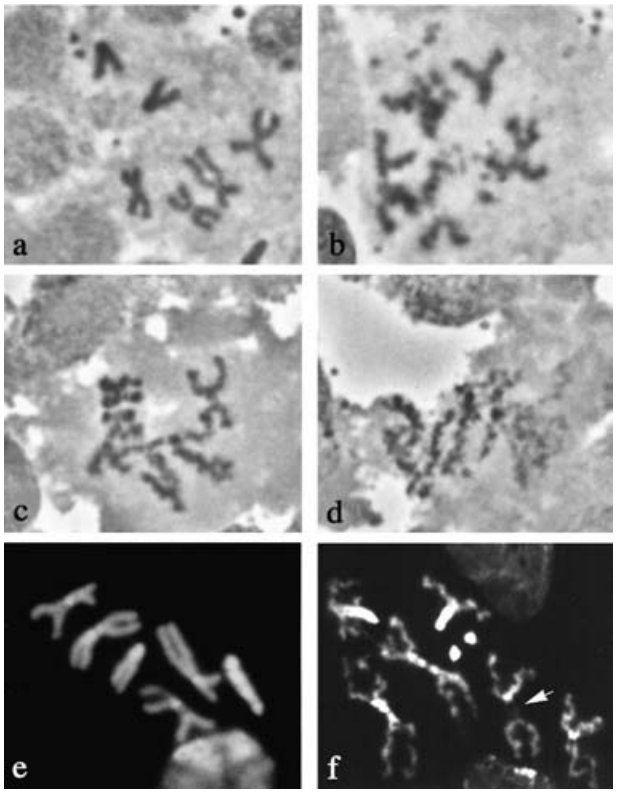

Fig. 3. Mitotic defects in *l(2)41Aa* neuroblast cells. (*a*) Orcein-stained metaphases from the *DfB/TSTL* control strain; (*b*) orcein-stained metaphase from *DfB/EMS31* mutant; (*c*) orcein-stained metaphase from *DfB/IR18* mutant; (*d*) orcein-stained metaphase from *DfRsp31/IR18* mutant; (*e*) Hoechst 33258-stained metaphase from the *DfB/TSTL* strain; (*f*) Hoechst 33258-stained metaphase from *DfRsp31/IR18* mutant. Chromosomes in mutant genotypes clearly exhibit the uncoiled chromatin phenotype. Note that some undercondensation also occurs at pericentromeric regions (white arrow).

Fuller, 1993). In contrast, different-sized nuclei and variable-sized Nebenkerns are diagnostic of defects in chromosome segregation and in cytokinesis (partition of mitochondria) (Gonzalez *et al*., 1989; Fuller, 1993).

To determine whether *l(2)41Aa* mutations may also affect male meiotic divisions, we first analysed onion stage spermatids from larval testes of various *l(2)41Aa* mutant allele combinations *in vivo*. Most combinations had very small larval testes, consistent with the general reduction in cell proliferation in mutant larvae. However, three allelic combinations – *DfB/IR18*, *DfB/DfIR7* and *EMS31/IR18* – were suitable for our cytological analysis in that they exhibited normal-sized larval testes. As shown in Fig. 3 and Table 2, mutant spermatids displayed two types of defects. Firstly, a high proportion of mutant spermatids (62% in *DfB/IR18*, 25% in *DfB/DfIR7* and 10% in *EMS31/IR18*) carried micronuclei. Secondly, a fraction of spermatids had large Nebenkerns associated with either two or four nuclei or micronuclei (44% in *DfB/IR18*, 12% in *DfB/DfIR7* and 4% in *EMS31/IR18*; Table 2). Taken together, these abnormalities suggest that *l(2)41Aa* mutations affect both chromosome segregation and cytokinesis during male meiosis. Intriguingly, the *DfB/DfIR7* combination involves two deficiencies and its meiotic phenotype is weaker than that exhibited by *DfB/IR18*, which is a deficiency/mutant combination. This inconsistency may be due to the genetic background of this particular combination. Alternatively, this suggests that *DfIR7* and or *DfB* are not cleancut deficiencies but more complex rearrangements and that their combination is not null for the *l(2)41Aa* gene.

In order to define the primary defects which lead to irregular spermatid formation, mutant testes from *DfB/IR18*, *DfB/DfIR7* and *EMS31/IR18* combinations were fixed and stained with anti-α-tubulin and Hoechst 33258. This procedure allowed us simultaneously to follow the behaviour of spindle structures and chromosomes throughout spermatogenesis (Cenci *et al*., 1994; Bonaccorsi *et al*., 2000). An effect of *l(2)41Aa* mutations was observed in primary spermatocytes at the prometaphase stage. During this stage, bivalents normally begin to condense and appear as three major dots located just under the nuclear envelope (Cenci *et al*., 1994). In a significant fraction of *l(2)41Aa* mutant primary spermatocytes, bivalents did not condense properly and chromatin filaments

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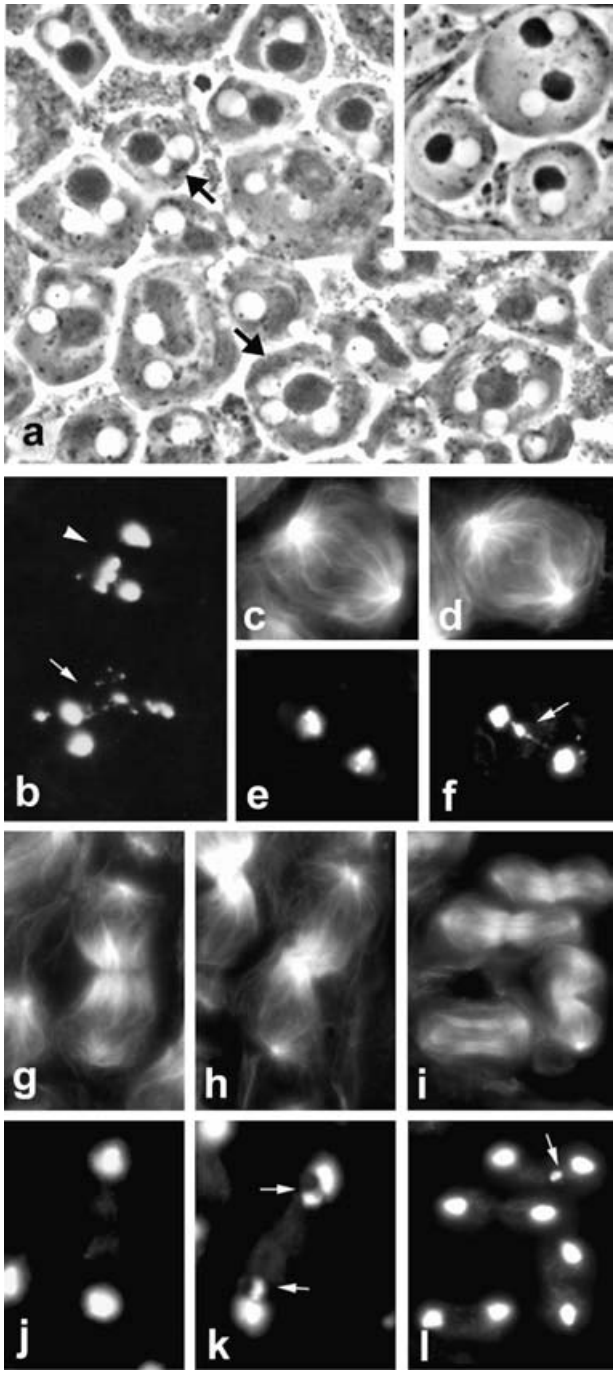

Fig. 4. Meiotic defects in *l(2)41Aa* spermatids. (*a*) Onion stage spermatids in *DfB*/*IR18* larval testes. The insert shows regular onion stage spermatids with nuclei and Nebenkerns of similar sizes from the control. Arrows show irregular spermatids with micronuclei and large Nebenkerns. Anti α-tubulin staining (*c*, *d*, *g*, *h*, *i*) and Hoechst staining (*b*, *e*, *f*, *j*, *k*, *l*) of male meiotic divisions. (*b*) *DfB*/*IR18* primary spermatocyte bivalents showing chromatin filaments along the three major dots of chromatin (arrow) which are not visible in regular nuclei (arrowhead). (*c*, *e*) Wild-type anaphase I. (*d*, *f*) *DfB*/*IR18* anaphase I showing a chromatin bridge (arrow). (*g*, *j*) Wild-type ana-telophase I. (*h*, *k*) *DfB*/*IR18* ana-telophase I showing laggards (arrows). (*i*, *l*) *DfB*/*IR18* ana-telophases II; the panel shows three regular divisions and one with lagging chromosomes (arrow). Note that in all cases the spindle structures look normal.

were visible along the three major dots of chromatin. This abnormal phenotype was seen in 60% of *DfB*/*IR18*, 30% of *DfB*/*DfIR7* and 10% of *EMS31*/*IR18* primary spermatocytes (Fig. 4*b*, Table 2). The under-condensation pattern is clearly reminiscent of the condensation defects observed in *l(2)41Aa* neuroblast metaphase chromosomes. Furthermore, chromatin bridges connecting the two sets of chromosomes were clearly visible as the chromosomes moved to opposite poles during anaphase I (Fig. 4*e*, *f*). These bridges are likely to alter chromosome segregation as 62%, 28% and 15% of lagging chromosomes were seen during the anaphase/telophase I stage in *DfB*/*IR18* (62%; $n=70$), *DfB*/*DfIR7* (28%; $n=40$) and *EMS31*/*IR18* (15%; $n=33$) mutant allele combinations (Fig. 4*i*, Table 2). In prometaphase II cells we were unable to detect clear evidence of chromosome condensation defects, perhaps because of the small size of chromosomes. However, chromatin bridge-like filaments leading to lagging chromosomes were also visible in second meiotic divisions with a frequency of 54% ($n=56$), 21% ($n=42$) and 14% ($n=28$) in *DfB*/*IR18*, *DfB*/*DfIR7* and *EMS31*/*IR18*, respectively (Fig. 4*k*; Table 2). It is worth noting that in both meiotic divisions the spindles were always regular (Figs. 4*c*, *g*, *j*), suggesting that chromosome missegregation in *l(2)41Aa* mutants is not due to spindle defects.

(iii) *Hoechst 33258-treated testes exhibit cytokinesis defects in onion stage spermatids*

To assess whether chromosome condensation defects in meiosis are primarily responsible for anaphase bridges, micronuclei and failure in cytokinesis, additional experiments were performed. While the presence of micronuclei in *l(2)41Aa* onion stage spermatids appear to be a direct consequence of defective chromosome migration during meiotic divisions, the correlation between the *l(2)41Aa* primary lesion and the presence of irregular Nebenkern is less obvious. We thus treated whole wild-type testes with Hoechst 33258, a compound which has been shown to cause decondensation of AT-rich stretches of chromosomes that are abundant in the heterochromatin (Gatti *et al.*, 1976). We incubated wild-type larval testes in solutions containing two different Hoechst 33258 concentrations (10 μg/ml and 100 μg/ml) for two different treatment times (1 h and 5 h). We found that, after 5 h, testes squashes analysed *in vivo* displayed irregular spermatids with Nebenkerns of different sizes and micronuclei (Fig. 5*b*). In particular, 30% of 125 spermatids observed from treated testes exhibited either 2 : 1 (22%) or 4 : 1 (8%) nuclei–Nebenkern irregular association. In addition, a significant fraction (12%) of irregular spermatids also displayed micronuclei, while only 1·4% (3 of 210 spermatids) irregular spermatids were found in testes treated for 5 h with phosphate buffer

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Table 2. *Meiotic abnormalities in larval testes of* l(2)41Aa *mutant alleles*

Genotype	Prometaphase I		Anaphase I		Anaphase II		Spermatids		
	T	I (%)[a]	T	I (%)[b]	T	I (%)[b]	T	I (%)	
								[c]	[d]
Wild-type[e]	40	0	50	0	50	0	120	0	0
DfB/Cy	30	0	40	0	40	0	150	0	0
DfB/IR18	70	60	70	62	56	54	88	63	44
DfB/DfIR7	60	30	40	28	42	21	110	25	12
EMS31/IR18	40	10	33	15	28	14	85	10	4

T, total of cells scored; I, percentage of cells showing meiotic defects.
[a] Percentage of prometaphases I with undercondensed chromatin.
[b] Percentage of anaphases with lagging chromosomes.
[c] Percentage of spermatids with micronuclei.
[d] Percentage of spermatids with large Nebenkerns.
[e] Control.

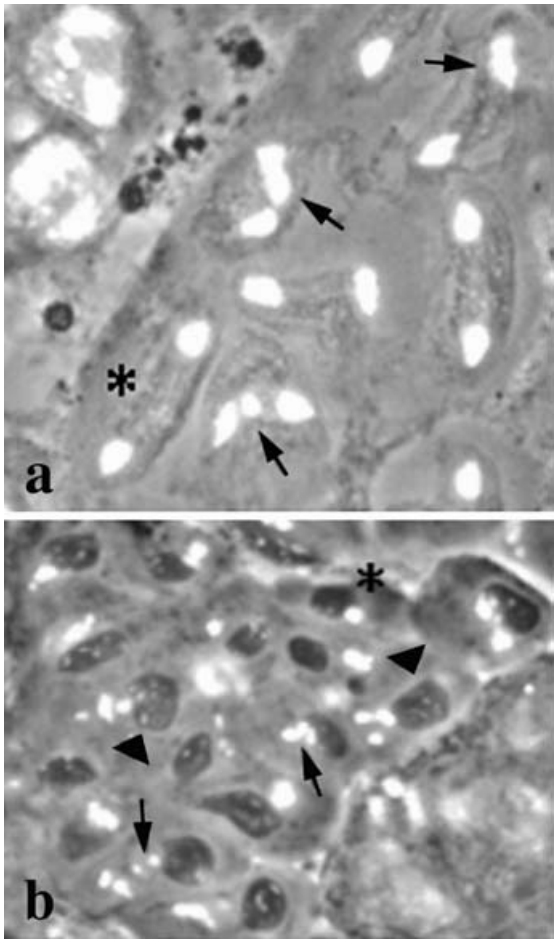

Fig. 5. Effects of Hoechst 33258 on male meiosis. (*a*) *In vivo* second meiotic divisions and (*b*) onion stage spermatids in larval testes treated with 100 μg/ml Hoechst 33258 after 5 h of incubation. Note lagging chromosomes (arrows) in anaphases II (*a*) and micronuclei (arrows) together with large Nebenkerns (arrowheads) in spermatids (*b*). Asterisks indicate a regular anaphase II in (*a*) and a regular onion stage spermatid in (*b*). Both phase-contrast images were taken under a fluorescent light microscope which allowed simultaneous visualization of bright chromatin.

only (negative control). Moreover, lagging chromosomes in both first and second meiotic divisions were also observed in treated testes with a frequency of 15% in both cases (total figures analysed: 25 and 20, respectively; Fig. 5*a*).

Thus, it appears that the Hoechst treatment mimics the meiotic phenotype of *l(2)41Aa* mutants. Taken together, these observations suggest that defects in chromatin condensation, while associated with specific portions of chromosomes, disrupt chromosome segregation as well as cytokinesis in both male meiotic divisions. It is possible that chromosome missegregation gives rise to spermatids with micronuclei as a result of a Hoechst-induced dysfunction of centromere behaviour and not just a general consequence of chromatin undercondensation. This should not be the case, however, as cytokinesis in male meiosis has been shown to occur successfully in mutants where proper centromere behaviour is affected (Basu *et al.*, 1999). Rather, it is conceivable that, in male meiosis, chromosome missegregation caused by defective chromatin condensation interferes with the completion of cytokinesis, resulting in the formation of large Nebenkerns (see also Section 4).

4. Discussion

Genetic dissection of cell division in higher eukaryotes has been successfully carried out in *Saccharomyces cerevisiae* (Hartwell, 1974; Pringle & Hartwell, 1981; Moir & Botstein, 1982), *Schizosaccharomyces pombe* (Fantes, 1984) and *D. melanogaster* (Glover, 1991), and has led to the identification of several genes controlling different steps of mitosis. In *D. melanogaster*, the cytological characterization of late lethal mutations has allowed the identification of genes whose wild-type functions are important for progression through interphase, chromosome condensation, maintainance of chromosome integrity, spindle formation

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and/or function, and completion of chromosome segregation or cytokinesis (Gatti & Baker, 1989). In this work we found that mutations in the *Drosophila melanogaster l(2)41Aa* gene, which is located in the proximal heterochromatin of chromosome 2, affect condensation of both mitotic and meiotic chromosomes. In this respect, the *l(2)41Aa* gene is unique in that it represents the first example of a heterochromatic gene whose mutations affect chromosome condensation in somatic and germline cells.

Mutations causing aberrant chromosome condensation have been isolated in *Drosophila* (Gatti & Baker, 1989; Gatti & Goldberg, 1991) and most of the genes identified by these alterations have been cloned. Some of them encode factors involved in DNA replication (ORC, PCNA, MCM4; Landis *et al*., 1997; Pflumm & Botchan, 2001) and cell cycle checkpoints (Rfc4; Krause *et al*., 2001), whereas others encode proteins required for chromatid cohesion and chromosome segregation (BARREN: Bhat *et al*., 1996; GLUON: Steffensen *et al*., 2001). Mutants affecting DNA replication have been shown to display defective metaphase arrest with abnormally condensed chromosomes (Loupart *et al*., 2000; Pflumm & Botchan, 2001). It has been postulated that cells resulting from incomplete DNA replication may be arrested in mitosis by checkpoints sensitive to chromosome integrity. In contrast, mutations in genes encoding the condensation complex (i.e. both non-SMC and SMC proteins) exhibit aberrant chromosome condensation which does not hinder the cells in proceeding through the metaphase–anaphase transition. In addition, these cells show chromosome segregation defects suggesting that proper chromosome condensation is essential for normal sister chromatid separation in *Drosophila* (Bhat *et al*., 1996; Steffensen *et al*., 2001). This is consistent with the finding that depletion of Aurora B Kinase by RNAi causes both defective chromosome condensation and abnormal segregation in *Drosophila* S2 cells (Giet & Glover, 2001). Nevertheless, none of the genes required for proper mitotic chromosome condensation have been seen to play a role in male meiosis. However, genetic analysis of the peculiar *Stellate (Ste)* elements of *Drosophila melanogaster* reveals that *Stellate* copy number is responsible for abnormality in chromosome condensation only during male meiosis and spermatogenesis, giving rise to very frequent non-disjunction events (Palumbo *et al*., 1994).

The low mitotic index observed in brain cells of all combinations of *l(2)41Aa* alleles suggests that cells defective in chromosome condensation exhibit a checkpoint-induced delay in passing through interphase. Moreover, the behaviour of *l(2)41Aa* mutants in mitosis is peculiar, given that, unlike other *Drosophila* condensation mutants, anaphases are normal and neither lagging chromatids nor chromatin bridging are observed. One can argue that normal anaphases result only from regularly condensed metaphases. Consequently, if this is true, the anaphase frequency of *l(2)41Aa* mutants should be reduced with respect to control. However, we did not observe significant differences in the anaphase/metaphase ratio of mutant and control larvae. This suggests that most irregular cells are likely to arrest at prophase/prometaphase, whereas those with less severe defects may be able to proceed to anaphase. On the basis of our results, nonetheless, we were not able to ascertain whether the abnormal chromosome condensation observed in mitotic cell divisions of *l(2)41Aa* mutants is caused by a defect in a component of the condensation complex or is due to, for example, alteration of DNA replication.

The cytological characterization of *l(2)41Aa* mutant larval testes has revealed that chromosome condensation defects also occur during male meiosis. Undercondensed bivalents are present in primary spermatocyte prometaphases from different allelic combinations. In contrast with mitotic metaphases, these meiotic figures display an uncoiled chromosome pattern which is associated with chromosome breaks and chromatin bridging during anaphase I, suggesting that chromosome bridging is a consequence of chromosome condensation defects in *l(2)41Aa* mutants. This is consistent with the behaviour of other *Drosophila* mutants affecting both chromosome condensation and chromatin cohesion, and exhibiting, in addition, high levels of chromosome missegregation (Bhat *et al*., 1996; Steffensen *et al*., 2001). In the second meiotic divisions, chromosome breaks and chromatin bridging at anaphase were observed. Lagging and/or fragmented chromosomes were recovered as micronuclei in spermatids, which also exhibited Nebenkerns 2 and 4 times larger than normal. We were not able to detect the uncoiled chromosome phenotype in secondary spermatocytes. However, given that chromatin bridges and lagging chromosomes were also present during anaphase II, it is conceivable that chromosome undercondensation may also take place in these types of cells, but is not easily detectable. Moreover, the results of the Hoechst 33258 treatment are also consistent with a dysfunction in chromosome condensation in secondary spermatocytes. This compound is known to induce decondensation of a large heterochromatic portion of chromosomes. It mimics the meiotic effect of *l(2)41Aa*, manifesting both the presence of anaphase bridges in meiosis II and spermatids with 2:1 and 4:1 nuclei–Nebenkern irregular associations, the latter being the consequence of failure of cytokinesis in both meiosis I and II.

From these results it appears that both primary and secondary spermatocytes can proceed through cell division despite the presence of undercondensed chromatin and chromosome missegregation. In addition, the finding that secondary spermatocytes are

also affected by the mutations leads us to hypothesize that the *l(2)41Aa* mutant undercondensation phenotype is due to an alteration of the chromosome condensation complex rather than to defective DNA replication, which does not take place in secondary spermatocytes. To date, we are unable to explain why chromosome segregation is affected in meiosis but not in mitotic cells. It is plausible to assume that mitotic and meiotic cells may differ in the mechanics or speed of anaphase chromosome movement. This is also suggested by the fact that the meiotic spindle is much larger than the mitotic spindle (Cenci *et al*., 1997). These differences can be taken in account to explain how the same chromosome condensation defect has a different outcome, depending on the type of cell division. It is possible that a degree of chromatin bridging may occur during the onset of anaphase in mitosis and that these mitotic cells, unlike meiotic cells, have more time to resolve a putative underwinding, so that no breakage occurs. Alternatively, the *l(2)41Aa* gene product may be bifunctional, in that it may play a role in chromatin condensation and may also be required for the regulation of chromosome movement in meiotic anaphases. However, Hoechst treatment in testes strongly suggests a direct link between chromosome decondensation, segregation defects and failure in cytokinesis during male meiosis.

We have shown that onion stage spermatids from different mutant combinations consist of different-sized nuclei and Nebenkerns 2 or 4 times larger than normal. As discussed above, Nebenkerns of different sizes are derived from a failure in completion of cytokinesis during both meiotic divisions. Our findings do not address how cytokinesis completion is influenced by chromosome condensation in cells where spindle organization is normal. In animal systems, the execution of normal cytokinesis depends upon several factors (Fishkind & Wang, 1995; Glotzer, 1997). In general, signals that stimulate contractile ring formation and cytokinesis may be provided by either metaphase chromosomes (Earnshaw *et al*., 1991), asters (Hiramoto, 1971; Rappaport, 1971, 1986) or the central spindle (Rappaport & Rappaport, 1974; Fishkind *et al*., 1996). However, recent data have proved that neither asters nor the congression of chromosomes in metaphase are required for a cytokinetic signal in *Drosophila* male meiosis (Bonaccorsi *et al*., 1998). Thus, in this system only the central spindle appears to play a crucial role during cytokinesis (Bonaccorsi *et al*., 1998; Giansanti *et al*., 1998). In *l(2)41Aa* mutant meiotic cells, failure of cytokinesis is not due to defects in central spindle formation as it does not appear to be affected in both meiotic divisions. Although we cannot rule out the possibility that the contractile ring is altered, we believe that chromatin bridge-like structures, resulting from chromosome undercondensation during the meiotic divisions, might interfere with proper assembly of the cleavage furrow, giving rise to abnormal cytokinesis. This conclusion is consistent with results obtained with other meiotic mutants. In flies deleted for the *crystal (cry)* element of Y and bearing different *Ste* copies on the X chromosome, undercondensed chromosomes segregate irregularly at meiosis and are associated with anaphase bridges and apparently normal spindles (Palumbo *et al*., 1994). However, onion stage spermatids present irregular Nebenkerns, denoting a defect in cytokinesis (Palumbo & Bonaccorsi, unpublished). Moreover, *UbcD1* telomeric mutants which exhibit chromatin bridges do not affect spindle structures (Cenci *et al*., 1997), but display defective cytokinesis (Cenci & Gatti, unpublished). Finally, other mutations causing extensive chromatin bridging during meiotic divisions have been shown to generate onion stage spermatids with irregular Nebenkerns (Bonaccorsi & Giansanti, personal communication). Together, these data suggest that chromatin bridging caused by irregular condensation of chromosomes and/or other abnormal events such as telomeric attachments may indeed interfere with proper execution of cytokinesis at least during male meiosis.

The molecular characterization of the *l(2)41Aa* gene will allow us to define its function in mitotic and meiotic chromosome organization. Recently, the computational analysis of the *D. melanogaster* genome sequence (Adams *et al*., 2000) has identified approximately a hundred of the predicted heterochromatic genes. However, cytogenetic mapping suggests that *l(2)41Aa* does not correspond to these genes (Dimitri *et al*., 2002; in preparation). We have currently undertaken mutagenesis experiments with single *P*-elements located in the proximal heterochromatin of chromosome 2 aimed at isolating *l(2)41Aa* insertional alleles.

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