Loss of *Drosophila borealin* causes polyploidy, delayed apoptosis and abnormal tissue development

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Summary

The chromosomal passenger complex (CPC) is a key regulator of mitosis in many organisms, including yeast and mammals. Its components co-localise at the equator of the mitotic spindle and function interdependently to control multiple mitotic events such as assembly and stability of bipolar spindles, and faithful chromosome segregation into daughter cells. Here, we report the first detailed characterisation of a CPC mutation in *Drosophila*, using a loss-of-function allele of *borealin* (*borr*). Like its mammalian counterpart, Borr colocalises with the CPC components Aurora B kinase and Incenp in mitotic *Drosophila* cells, and is required for their localisation to the mitotic spindle. *borr* mutant cells show multiple mitotic defects that are consistent with loss of CPC function. These include a drastic reduction of histone H3 phosphorylation at serine 10 (a target of Aurora B kinase), a pronounced attenuation at prometaphase and multipolar spindles. Our evidence suggests that *borr* mutant cells undergo multiple consecutive abnormal mitoses, producing large cells with giant nuclei and high ploidy that eventually apoptose. The delayed apoptosis of *borr* mutant cells in the developing wing disc appears to cause non-autonomous repair responses in the neighbouring wild-type epithelium that involve Wingless signalling, which ultimately perturb the tissue architecture of adult flies. Unexpectedly, during late larval development, cells survive loss of *borr* and develop giant bristles that may reflect their high degree of ploidy.

Key words: Chromosomal passenger complex, Mitotic spindle, Polyploidy, Borealin/Dasra, Tissue repair

Introduction

The chromosomal passenger complex (CPC) is conserved from yeast to humans, and consists of at least three components that regulate multiple mitotic events. Its name stems from the observation that CPC proteins colocalise on condensing chromosomes during prophase, and are carried along to centromeres and to the equator of the mitotic spindle during metaphase (Earnshaw and Bernat, 1991). After metaphase, the CPC components re-localise to the midzone and midbody of the spindle, where they remain until the completion of cytokinesis (Andrews et al., 2003; Carmena and Earnshaw, 2003). The CPC components include Aurora B kinase, inner centromere protein (Incenp) and Survivin, an inhibitor of apoptosis-like protein (Bolton et al., 2002), as well as the recently discovered Borealin/Dasra protein (Gassmann et al., 2004; Sampath et al., 2004).

Borealin/Dasra was identified in human cell lines and in *Xenopus* extracts, respectively, and found to colocalise with other CPC proteins throughout mitosis (Gassmann et al., 2004; Sampath et al., 2004). The correct localisation of human Borealin in mitotic cells depends on the function of the other CPC components; conversely, RNAi-mediated depletion of Borealin in HeLa cells causes mislocalisation of Aurora B, Incenp and Survivin (Gassmann et al., 2004). Human Borealin binds directly to Incenp and Survivin in vitro, and forms a complex with the other CPC components in vivo. Its loss of function, like that of other CPC components, causes multiple mitotic defects, including failures in chromosome attachment to the spindle, multifocal spindles and uneven chromosome segregation. This typically results in multinucleate cells, aneuploidy and polyploidy, as well as, ultimately, apoptosis (Gassmann et al., 2004; Sampath et al., 2004). However, cells that lack CPC function can also occasionally escape apoptosis as they appear to be defective for their spindle attachment checkpoint (Lens and Medema, 2003; Yang et al., 2004).

Little is known about the role of the CPC during development, except for its function in the early *C. elegans* embryo (Kaitna et al., 2000; Kaitna et al., 2002; Romano et al., 2003). Here, we present the first detailed characterisation of a CPC mutation in *Drosophila*, using a loss-of-function allele of *borealin*. This gene was identified independently in a recent RNAi screen for cytokinesis defects in cultured *Drosophila* cells, and was named *borr* (*borealin-related*) (Eggert et al., 2004). We provide evidence, based on its subcellular localisation and function during the cell cycle, that Borr is the functional counterpart of vertebrate Borealin/Dasra. We show that *borr* is an essential gene, and that loss of *borr* function causes mitotic defects, including multipolar spindles that result in large polyploid cells and often in delayed apoptosis. The developmental consequences of these defects include striking cell-autonomous and non-autonomous defects in cell-type specification and tissue architecture.

Materials and methods

Isolation, mapping, and identification of the *borr* mutant allele

E133 was isolated fortuitously in an EMS screen for genes interacting with activated Armadillo (Thompson et al., 2002). E133 turned out to be a non-interacting 'passenger' hit, and its lethality was mapped to 30A on chromosome 2L. RNA interference experiments (see below) identified CG4454 as the gene affected by E133. Double-stranded sequencing of genomic DNA identified a single base-pair deletion in the CG4454 coding region.

Plasmids, cell culture and transfection

Borr was tagged N-terminally with green fluorescent protein (GFP) by Gateway cloning (Invitrogen), using the *borr* cDNA from clone LD36125 and the pAGW vector (Terence Murphy, Carnegie Institution of Washington). The resulting construct was confirmed by sequencing.

Kc167 cells were cultured at 25°C in Schneider's medium supplemented with 10% heat-inactivated foetal bovine serum and antibiotics. DmD8 cells were obtained from the Drosophila Genome Resource Center, and cultured similarly, with 10 μg/ml insulin (Sigma) added to the medium. They were transfected with the FuGene transfection reagent (Roche) according to the manufacturers instructions, with a ratio of 4 μg DNA:1 μl FuGene. Cells were processed for analysis 24 hours after transfection.

RNA interference

To identify the gene responsible for the embryonic phenotype of E133, an RNA interference screen of candidate open reading frames within the genomic region 30A was performed, as follows. Genomic DNA was isolated from *yw* flies, and amplified by PCR with primer pairs containing a T7 promoter sequence at the 5′ end designed to amplify a large uninterrupted stretch of coding DNA. PCR products were used as templates in transcription reactions using the MegaScript RNAi kit (Ambion), which resulted, in the case of CG4454, in a dsRNA of 264 bp. The predicted size of the dsRNA products was verified by agarose gel electrophoresis, and their concentrations were determined by comparison with a known standard.

Injection of dsRNA into embryos was carried out as described (Desbordes and Sanson, 2003), except that the dsRNA was delivered in water. All preparation and injection steps were carried out at room temperature, and the embryos were aged for ~24 hours at 18°C before fixation.

RNAi of Kc167 cells was carried out basically as described (Clemens et al., 2000), except that 500 μl of cells were plated at a concentration of 10^6 per well of a 24-well plate. Control cells were treated identically, but without dsRNA.

Estimation of nuclear volumes and mitotic indices

To estimate nuclear volumes, individual wild-type and *borr* mutant ventral nerve cord (VNC) nuclei stained with Hoechst were outlined using ImageJ, and their maximal circumference was measured. From these measurements, the volumes of the corresponding spheres were calculated, providing estimates of nuclear volumes. This modelling of nuclear volumes by spheres was validated as a best approximation by 3D reconstructions of individual nuclei. To estimate mitotic indices, the mitotic cells were identified on the basis of chromatin morphology, Hoechst and serine-10 phosphorylated histone H3 (P-H3) staining, and their numbers were determined per hemineuromere for abdominal segments 4, 5 and 6 (see also Results and Fig. 4 legend).

Clonal analysis

FRT/FLP mediated recombination (Xu and Rubin, 1993) was used to induce homozygous mutant *borr* clones. Flies of the genotypes *borr^{E133} FRT40A/SM6a-TM6b* and *yw hsflp; Ub-NLS-GFP FRT40A/CyO* or *f yw hsflp; ck, f+FRT40A/Cyo* (kindly provided by K. Basler) were crossed. Embryos were collected for 24 hours, aged at 25°C, and heat-shocked after a further 36 or 84 hours. Mutant phenotypes were analysed in dissected larval imaginal discs, dissected pupal wings or in adult tissues.

Antibody staining and fluorescence microscopy

Embryos were immunostained as previously described (Cliffe et al., 2003). Imaginal discs and pupal wings were stained using standard methods. Briefly, tissues were fixed with 4% formaldehyde (30 minutes at room temperature for imaginal discs, overnight at 4°C for pupal wings), washed, blocked and incubated overnight at 4°C with primary antibodies in PBS+0.1% Triton-X-100+1% BSA (BBT). Tissues were then washed several times in BBT and incubated with secondary antibodies (Molecular Probes) for 2-3 hours at room temperature. The following primary antibodies were used: mouse E7 anti-β-tubulin (1:100; Developmental Studies Hybridoma Bank, DHSB); rabbit anti-P-H3 (1:500; Abcam); rabbit anti-activated human caspase 3 (1:700; BD Biosciences), which has been shown to cross-react with the *Drosophila* ortholog (Yu et al., 2002); mouse anti-Wg (1:100; DHSB); mouse anti-Cut (1:100; DHSB); rabbit anti-GFP (1:2000; gift from R. Arkowitz); guinea pig anti-Senseless (1:1000) (Barbosa et al., 2000); rabbit anti-Aurora B (Giet and Glover, 2001) (1:200); and rabbit anti-Incenp (Adams et al., 2001) (1:500). DNA was stained with Hoechst dye or DAPI (Fig. 5). Images were collected on a BioRad 1024 confocal microscope or a Zeiss Axiovert 200M (Fig. 5).

Results

E133 is a loss-of-function allele of CG4454, with a single base pair deletion at position 290 in the first exon of its coding region. The resulting frameshift introduces a stop codon immediately after this deletion into the predicted protein, truncating it after serine 98 (Fig. 1A). The CG4454 locus consists of three exons that encode a protein of 319 amino acids, without recognisable domains or known sequence motifs. Stringent Psi-Blast searches revealed a significant similarity between CG4454 and Borealin/Dasra, the only protein with any detectable sequence relationship to CG4454 (see also Gassmann et al., 2004). This suggests that CG4454 may be the *Drosophila* ortholog of Borealin/Dasra.

borr is an essential gene required for embryonic mitoses

Zygotic homozygosity for the *borr* mutation results in late embryonic lethality, but the mutant embryos lack overt morphological defects, probably owing to rescue by maternal gene product. Consistent with this, *borr* is ubiquitously expressed in the early *Drosophila* embryo, although it appears to be restricted to the VNC and brain during later embryonic stages (Berkeley Drosophila Genome Project in situ data).

Given its high expression levels in the embryonic nervous system, we scrutinised this tissue more carefully after staining embryos with Hoechst dye. Indeed, by stage 12, we detected cells in the VNC and brain with abnormally large nuclei (Fig. 1B,C). We estimate that the volumes of the *borr* mutant VNC nuclei are on average ~3 times larger than those of wild-type VNC nuclei (Fig. 1D). This implies an increased DNA content (>2N) of the mutant cells, and suggests that *borr* loss affects the divisions of VNC cells. We also observed similarly oversized nuclei in other tissues (in addition to severe morphological defects such as failure of germ band retraction), after injection of *borr* dsRNA into wild-type embryos, which potentially also depletes maternal gene product (not shown).

Thus, *borr* loss appears to affect many, if not all, dividing cells in the embryo.

To monitor the mitotic events that are affected in the *borr* mutant embryos, we stained these embryos with an antibody against serine 10 phosphorylated histone H3 (P-H3), a histone modification specifically found in mitotic cells that has been ascribed to Aurora B kinase activity in several organisms, including *Drosophila* (see below) (Giet and Glover, 2001; Hsu et al., 2000). Counting the mitotic cells per hemi-neuromere in wild-type and *borr* mutant embryos, we found that these numbers were reduced significantly in the mutants, to ~50% of the wild type at stage 12, and to ~20% at stage 14 (Fig. 2A; see also Fig. 4). Our estimates suggest that, in mutant embryos, the overall number of cells per hemi-neuromere is also lower than normal (although it is technically difficult to obtain accurate counts of total cell numbers). Nevertheless, these counts suggest that the fraction of mitotic cells (i.e. the mitotic index) in the VNC of *borr* mutant embryos may be reduced compared with the wild type.

To see whether the *borr* mutation affects a specific mitotic stage, we classified each P-H3-positive cell as one of four different mitotic stages (based on the shapes of their chromatin masses; see below), and we determined the frequencies of these stages as a percentage of the total of mitotic cells. This revealed that the percentages of prophase and prometaphase cells were higher in *borr* mutants compared with the wild type, whereas anaphases and telophases were underrepresented in the mutants (Fig. 2B,C). This profile shift of the mitotic stages appears to be progressive during embryonic development, and becomes more pronounced by stage 14 when telophases have become exceedingly rare (Fig. 2C), maybe as a result of cumulative defects during consecutive abnormal cell divisions. This profile shift suggests that *borr* loss causes a severe attenuation, or block, prior to metaphase.

Two further features were noticeable in the P-H3 staining patterns of the *borr* mutant VNC cells. First, many of the rare anaphases detected at stage 12 appeared abnormal, showing evidence of uneven segregation of chromatin (Fig. 2D; see also Figs 3, 5). Second, the P-H3 staining intensity was reduced markedly, which is particularly noticeable during metaphase, but also during telophase when P-H3 staining normally fades away (Fig. 2D; see also Fig. 4). These observations are consistent with the profile shift of the mitotic stages in *borr* mutant embryos (Fig. 2B,C), and they underscore the notion that the first major defect during the mutant cell cycle occurs prior to metaphase. A similar prometaphase block has been reported for human Borealin (Gassmann et al., 2004) and for other CPC components in *Drosophila* cells (Adams et al., 2001; Giet and Glover, 2001).

Borr colocalises with CPC components

In order to observe the subcellular localisation of Borr, *Drosophila* DmD8 cells were transfected with a construct encoding GFP-tagged full-length Borr. As expected, GFP-Borr is associated with chromatin during prometaphase (Eggert et al., 2004) (not shown), and is subsequently concentrated at the central spindle midbody and at the cell cortex in the cleavage furrow during telophase and cytokinesis (Fig. 3A-C,E,F). We shall refer to this pattern as 'localisation to the mitotic spindle'. Significantly, GFP-Borr colocalises with both endogenous Aurora B and Incenp (Fig. 3B-G), in agreement with the results

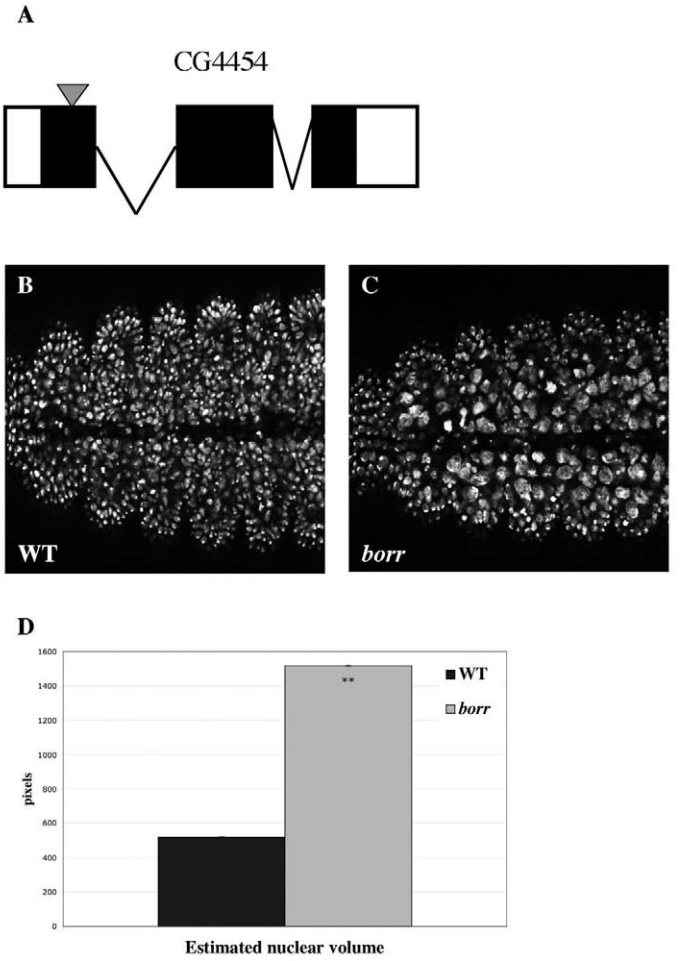

Fig. 1. *borr* is required for mitosis in embryonic VNC cells. (A) Schematic representation of the *borr* locus (black, coding; white, non-coding). The position of the E133 mutation is indicated by an arrowhead. (B,C) Ventral views of stage 12 (B) wild-type and (C) *borr* mutant embryos; nuclei are stained with Hoechst dye to visualise DNA. (D) Average volumes of wild-type and *borr* mutant VNC nuclei; numbers of pixels were calculated for each genotype based on outline tracings of 50 nuclei from five different embryos (see Materials and methods).

by Eggert et al. (Eggert et al., 2004), who also observed co-localisation of Borr and Aurora B throughout mitosis. These results are consistent with Borr being a CPC component, like its vertebrate counterparts.

RNAi-mediated depletion of Borr causes mitotic defects in *Drosophila* Kc cells

To further study the function of *borr* during mitosis, we used dsRNA interference in *Drosophila* Kc167 tissue culture cells. Indeed, 72 hours after addition of *borr*-specific dsRNA, Kc167 cells displayed a range of mitotic defects when compared with their controls (Fig. 3H-M). Most notably, highly abnormal multipolar spindles were observed in mitotic cells (Fig. 3I,J), and interphase cells often showed single large nuclei – reminiscent of the VNC nuclei in *borr* mutant embryos (Fig. 1C) – or became multi-nucleate (Fig. 3K-M). Some of these cells appear to have up to eight distinct nuclei, in addition to DNA fragments strewn around the cytoplasm (Fig. 3M,

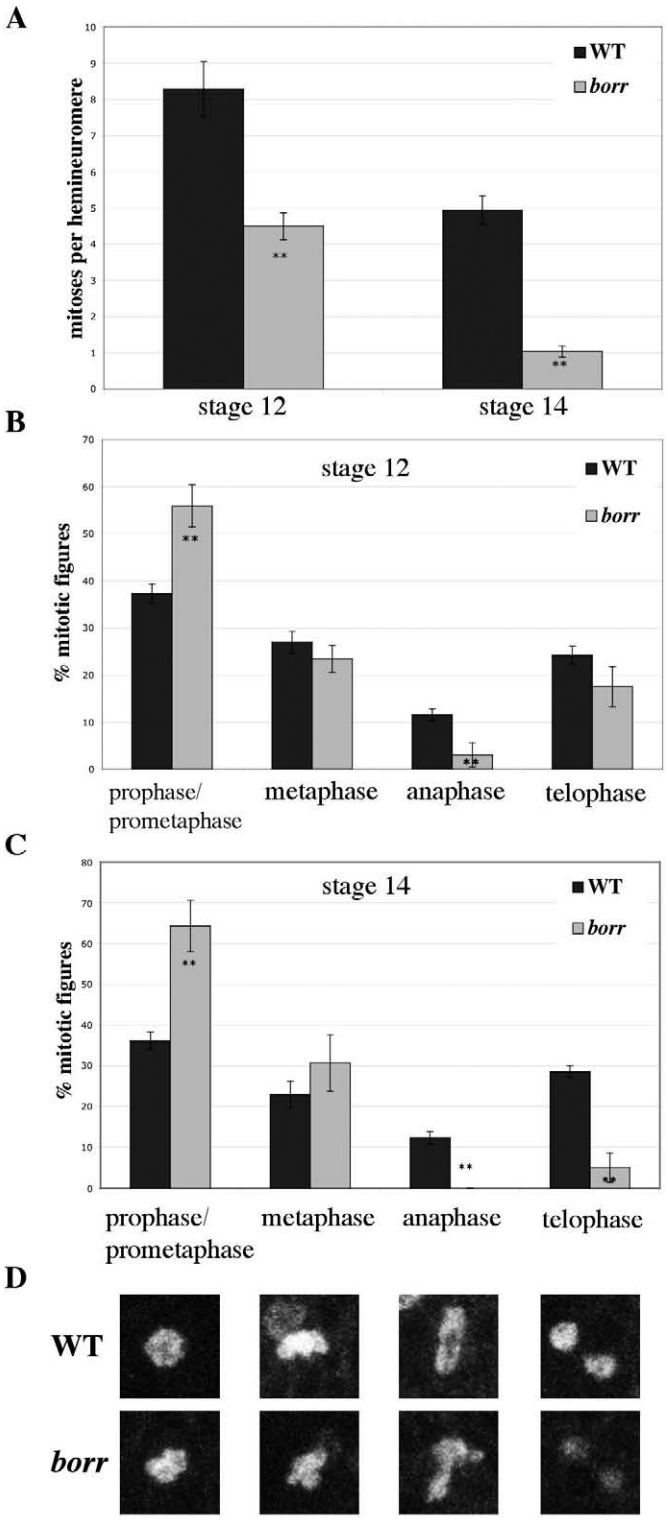

Fig. 2. Mitotic progression is affected in the VNC of *borr* mutant embryos. (A) Counts of mitotic cells, as judged by P-H3 staining and chromatin morphology (see also Fig. 4B,D), in the VNC of wild-type or *borr* mutant embryos at stage 12 (n=6 or 7, respectively), and at stage 14 (n=12 or 10, respectively). (B,C) Relative frequencies of the four main mitotic stages (see D), expressed in percentages at stage 12 (B) and stage 14 (C). Asterisks in A-C indicate statistical significance (**P<0.0005) of the observed differences. (D) Examples of mitotic cells in which chromatin has been visualised by P-H3 staining, selected to illustrate the four main mitotic stages of wild-type and *borr* mutant VNC cells. There is abnormal chromatin segregation in the mutant anaphase.

arrows). Similar phenotypes were observed in HeLa cells after RNAi-mediated depletion of Borealin, and also after RNAi-mediated depletion of CPC components in *Drosophila* cells (Adams et al., 2001; Eggert et al., 2004; Gassmann et al., 2004; Giet and Glover, 2001; Sampath et al., 2004). They support the notion that Borr is a functional ortholog of human Borealin. Furthermore, the multi-nucleate cells and the multipolar spindles suggest that Borr is required for faithful segregation of chromosomes during mitosis, and that its loss can cause polyploidy and/or aneuploidy (for simplicity, we shall refer to this as 'polyploidy').

borr is required for high levels of histone H3 phosphorylation at serine 10

One crucial role of the CPC during mitosis is to mediate the H3 phosphorylation of serine 10 (P-H3) by Aurora B, as has been demonstrated in budding yeast, *C. elegans* and *Drosophila* (Adams et al., 2001; Giet and Glover, 2001; Hsu et al., 2000). As already mentioned (Fig. 2), the numbers of P-H3-positive (dividing) cells are reduced in the VNC of *borr* mutant embryos (Fig. 4A-D). Furthermore, the P-H3 levels of individual *borr* mitotic nuclei are typically reduced compared with those of wild-type nuclei (Fig. 4E-J; see also Fig. 2D). Often, they exhibit blotchy P-H3 staining (Fig. 4H,J) rather than the more 'structured' staining outlining condensed chromosomes as observed in the wild type (Fig. 4E,G). A similar loss of P-H3 staining has also been observed in *borr* RNAi-depleted Kc167 cells (Eggert et al., 2004). This reduction of the P-H3 levels in *borr* mutant cells is consistent with a loss of Aurora B kinase activity and, thus, with a disruption of CPC function.

Despite the strong reduction of the P-H3 levels in mitotic VNC cells of *borr* mutant embryos, these cells display only a slight undercondensation of their chromatin (Fig. 4I, arrow, compare with mitotic cell in F), although the degree of undercondensation is somewhat variable from cell to cell (Fig. 4I, and not shown). These results suggest that *borr* may not be essential for chromatin condensation.

borr is required for the localisation of Aurora B and Incenp to mitotic spindles

To examine the effects of *borr* loss on actively dividing epithelial cells, we used FRT-FLP-mediated recombination (Xu and Rubin, 1993) to generate *borr* mutant clones in imaginal discs whose cells undergo cell divisions throughout larval development. If *borr* mutant clones are induced during early larval stages and examined in fully grown larval discs, these clones are rare and are much smaller than the corresponding wild-type twin spots, suggesting that a large fraction of the mutant cells die (see below). Hoechst staining revealed that many of the surviving *borr* mutant cells are large, with giant but well-formed nuclei that appear healthy, and well integrated into the epithelial tissue (see Movie 1 in the supplementary material).

We stained imaginal discs bearing *borr* mutant clones with

antibodies against Incenp and Aurora B, to assess the effect of *borr* loss on these CPC components during mitosis. Wild-type cells in metaphase show characteristic well-ordered mitotic spindles, with distinct staining of Aurora B and Incenp at specific sites along condensed chromatin (Fig. 5A,F,B′-J′). By contrast, *borr* mutant cells invariably show abnormal mitotic spindles, including multipolar ones (Fig. 5A-J). Most of these mutant spindles do not show any chromatin-associated Incenp or Aurora B staining (Fig. 5C,H), although occasionally patches of Incenp staining can still be observed, but they do not seem to be associated with any of the spindle components (not shown). These staining patterns suggest that these CPC components fail to localise properly to mitotic spindles in the absence of *borr* (and their levels may also be reduced, though the low frequency of surviving *borr* mutant cells does not allow us to assess this quantitatively). Therefore, as in mammalian cells, the correct localisation of Incenp and Aurora B to mitotic spindles of dividing imaginal disc cells depends on Borr. This is further evidence that Borr is a CPC protein, and that it interacts functionally with other known CPC components.

Borr loss causes delayed apoptosis of imaginal disc cells

As already mentioned, early-induced *borr* mutant clones are rare, and are much smaller than their twin spots (Fig. 6A, blue arrow). Indeed, many twin spots do not appear to have mutant cells associated with them (Fig. 6A, red arrow), indicating that the mutant cells have all died. The frequency of surviving *borr* mutant clones is increased if they are induced in a *Minute* background, which provides the mutant cells with a proliferative advantage. They can thus occupy a significant fraction of imaginal disc territories in third instar larvae (Fig. 6B). All discs are equally affected, and they tend to be smaller than wild-type discs of an equivalent stage. Larvae with these clones do not survive pupariation.

Closer examination of the *borr* mutant cells revealed essentially two distinct phenotypes: large cells with giant well-formed nuclei, as described above (Fig. 6B,C, grey arrow), and cells that appear to be undergoing apoptosis. The clearest examples of the latter show compacted almost perfectly spherical nuclei that are found at the basal-most level of the disc epithelium, well separated from the healthy nuclei of the wing pouch (Fig. 6C, white arrow). We also observed *borr* mutant cells that may be at an earlier step in the apoptotic process: their nuclei are less compacted, and they are just beginning to drop basally within the epithelium (Fig. 6D, red arrow). Antibody staining against active caspase 3 confirmed that the *borr* mutant cells with compacted DNA are indeed undergoing apoptosis (Fig. 6E, white arrows), in contrast to the *borr* mutant cells that are well-integrated into the epithelium and display only background levels of active caspase 3 staining (Fig. 6E, grey arrow). Cells with low caspase staining can also be observed (Fig. 6E, red arrow): these show apparently fragmented but not yet compacted DNA, and may thus represent an intermediate stage similar to that shown in Fig. 6C.

These results, together with our observations in Borr-depleted embryos and tissue culture cells, suggest that *borr* mutant cells can undergo several consecutive abnormal mitoses, which results in large polyploid cells that eventually undergo apoptosis. Apoptotic cells appear to be cleared by basal extrusion from the epithelium.

Early *borr* mutant clones have non-autonomous effects on tissue architecture

To assess the consequences of Borr loss on the development of the imaginal discs, we induced *borr* mutant clones in first or early second instar larvae, and we examined the resulting adult

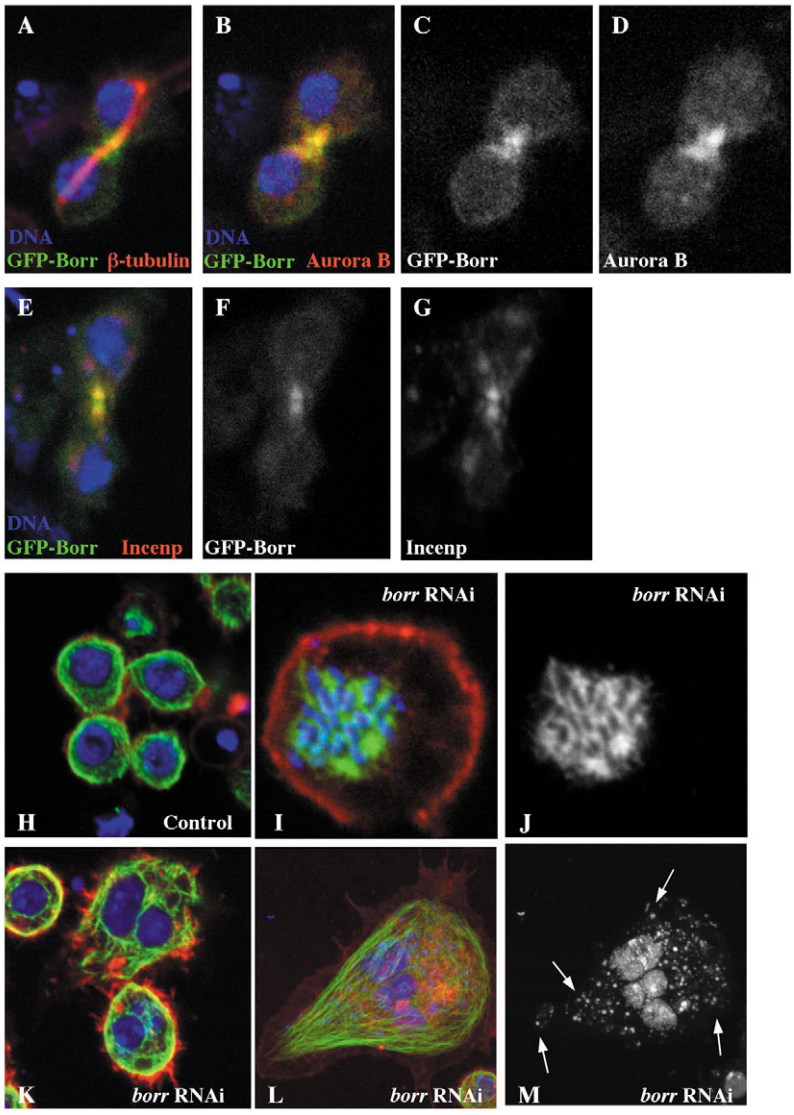

Fig. 3. Subcellular localisation and function of Borr in cultured *Drosophila* cells. (A-G) Mitotic DmD8 cells transiently expressing GFP-Borr, stained with Hoechst dye and antibodies against β-tubulin, Aurora B or Incenp, as indicated. (H-M) Kc167 cells, (H) mock-treated or (I-M) treated with *borr* dsRNA. Staining is with Hoechst dye (blue) and phalloidin (red), and with antibody against β-tubulin (green). Arrows indicate DNA fragments in the cytoplasm of a multi-nucleate Borr-depleted cell (M).

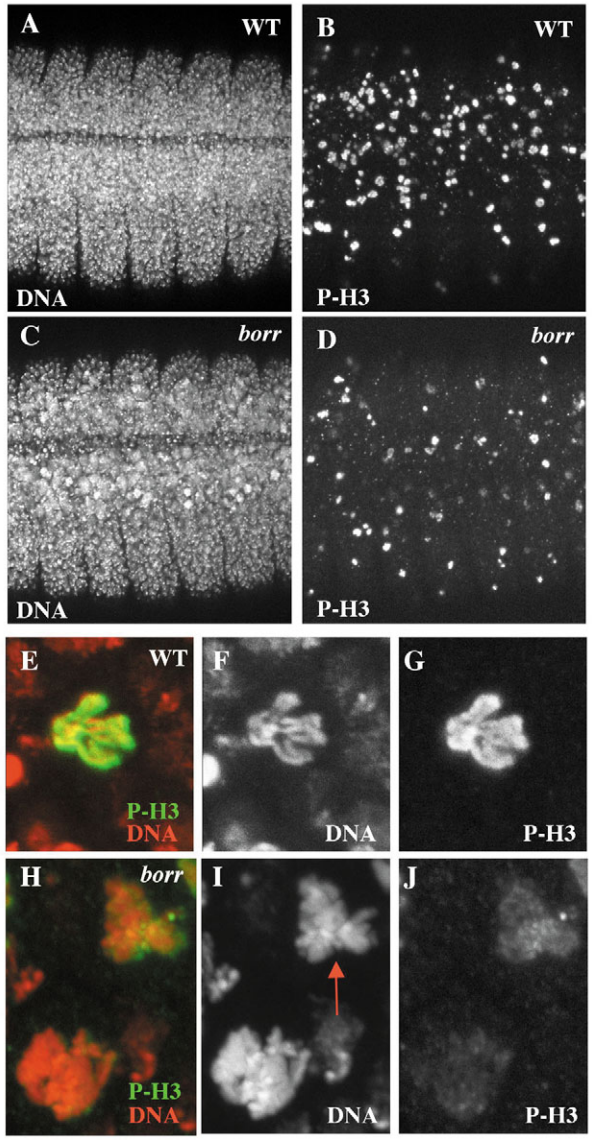

Fig. 4. Reduced P-H3 levels in VNC cells of *borr* mutant embryos. (A-D) Ventral views of stage 13 (A,B) wild-type or (C,D) *borr* mutant embryos, stained with Hoechst dye and antibody against P-H3; projections of *z*-stacks of confocal sections with identical settings are shown, revealing lower numbers of mitotic cells in the mutant and reduced P-H3 levels (see also Fig. 2A,D). (E-J) Individual mitotic VNC nuclei prior to anaphase from (E-G) wild-type or (H-J) *borr* mutant stage 13 embryos; arrow in I indicates a mutant nucleus with a degree of chromatin condensation similar to that in wild type (F). There are reduced levels of P-H3 staining in both mutant nuclei (J, compare with G).

flies. The most common defects in these flies are abnormal legs and rough eyes (see Fig. S1 in the supplementary material). In addition, they often show other striking defects in tissue architecture, e.g. large wing nicks (Fig. 7A,B). In all these cases, a twin spot is apparent (e.g. Fig. 7C, outlined in white), but no mutant tissue is detectable. This indicates that, by the adult stage, each of these early-induced *borr* mutant cells has undergone apoptosis. The nature and extent of the adult defects also suggests that they may be due partly to non-autonomous effects of the *borr* mutant clones on their neighbouring wild-type tissue.

To gain more direct evidence for these putative non-autonomous effects, we examined the expression of Wingless (Wg) in wing discs bearing *borr* mutant clones, a secreted morphogen that is expressed in a thin stripe along the developing margin of the wild-type disc (Fig. 7D) and controls its formation (Couso et al., 1994; Neumann and Cohen, 1996). As expected from the adult phenotypes, Wg expression is perturbed in various ways by *borr* mutant clones. Some of the surviving giant *borr* mutant cells within the Wg-expressing territory cause a significant lateral expansion of Wg staining by virtue of their sheer size (Fig. 7E-G, red arrows). Other cases of expanded Wg staining are not detectably associated with mutant cells (Fig. 7E,G, blue arrows), and thus appear to be cell non-autonomous consequences of *borr* loss.

We also observe clear non-autonomous effects of *borr* mutant cells if we examine the expression of *cut* and *senseless*, two of the ultimate target genes responding to the Wg morphogen in the marginal region (Neumann and Cohen, 1996; Parker et al., 2002). For example, a single surviving giant *borr* mutant cell expressing high levels of Cut can cause suppression of Cut and Senseless expression in neighbouring wild-type cells (Fig. 7H-J). A similarly striking example is the introduction of a V shape into the patterns of Cut and Senseless expression caused by a *borr* mutant clone (Fig. 7K-M). The presence of a large twin spot associated with this abnormality indicates that the causative *borr* mutant clone arose early when the disc contained only a small number of cells. Again, the *borr* mutant cells have disappeared in this case, most likely through apoptosis (see above). The kink introduced into the expression domains of both proteins appears to coincide with a rearrangement of cells in this region (Fig. 7L, arrow). Indeed, it appears that a single giant *borr* mutant cell (see Movie 1 in the supplementary material), in the process of basal displacement, might drag along normal epithelial cells. Thus, apoptosis and basal extrusion of a giant cell may exert sufficient disruption of the epithelium to induce compensatory cell rearrangements aimed at repairing epithelial integrity, which in the event compromise patterning.

Late *borr* mutant clones are viable, but affect external sensory organ development

If *borr* mutant clones are induced late (from the early third larval instar onwards), the resulting flies are viable and display no gross patterning defects. Indeed, analysis of marked clones and twin spots in adult wings suggests that all *borr* mutant clones are fully viable, given that they occupy roughly the same amount of territory as their twin spots (Fig. 8A). This is somewhat unexpected in the light of our results with earlier-induced clones whose survival was severely compromised (Figs 6, 7) owing to abnormal mitoses (Fig. 5). Indeed, the size of the late-induced *borr* mutant clone in Fig. 8A indicates that the mutant cells have survived three or four consecutive (abnormal) mitoses without entering the apoptotic pathway.

Closer examination of the flies bearing late-induced *borr* mutant clones revealed that their wing blades contain clusters of hairs (trichomes) surrounded by large clearings, rather than the usual regularly spaced single hairs (Fig. 8A). The number of hairs per cluster varies, with the largest cluster observed consisting of 12 hairs. All these hair clusters are produced by *borr* mutant cells (as judged by their trichome marker), so this phenotype is strictly cell-autonomous. The *borr* mutant clones

do not significantly affect the planar polarity in the wing blade as mutant and surrounding wild-type hairs appear normally oriented (Fig. 8A).

Examination of *borr* mutant clones in pupal wing discs supports our notion that all late-induced *borr* mutant clones occupy roughly the same amount of territory as their twin spots, confirming that the mutant cells are fully viable at this stage (Fig. 8B-D). In support of this, we did not observe any nuclei with compacted DNA (that would indicate imminent apoptosis; see Fig. 6E). As in the larval discs, the surviving *borr* mutant cells in the pupal discs are much larger than their neighbours, often with giant nuclei (Fig. 8B, arrows), indicating a high degree of ploidy. These giant *borr* mutant cells appear healthy and are well integrated within the epithelial tissue (Fig. 8B-D). Their large size provides an explanation for the observed adult phenotype, and are consistent with a single *borr* mutant cell producing multiple hairs: other conditions that produce large cells – for example, *cdc2*, *UltA* or *UltB* mutant clones, or wounding – result in similar cell-autonomous clusters of trichomes, albeit in some cases with fewer hairs per cluster (Adler et al., 2000; Weigmann et al., 1997) (data not shown).

We also observe abnormal giant bristles in the wing margins of flies bearing late-induced *borr* mutant clones; these giant bristles invariably lack sockets (Fig. 8E). As we could not determine whether these abnormal bristles are derived from mutant cells (owing to the weak phenotype of their bristle marker), we visualised incipient bristles in the pupal wing by β-tubulin antibody staining. This revealed large *borr* mutant trichogen cells (identifiable by their lack of GFP) that generate bristles twice the normal size (Fig. 8F,G). In addition, unlike wild-type bristles, these giant bristles do not exhibit any β-tubulin accumulation at their bases (Fig. 8H), confirming that the developing socket is absent around the *borr* mutant bristles.

Bristles are part of sensory organs, which are composed of four cells – the trichogen (bristle-producing cell), tormogen (socket-producing cell), neuron and thecogen (sheath cell); these are the progeny of a single sensory organ precursor cell produced by consecutive invariant lineage divisions (Lai and Orgogozo, 2004). Evidently, loss of *borr* compromises the lineage-generating divisions, and the single polyploid mutant cell seems to develop invariably as a trichogen at the expense of the tormogen and, possibly, of the other two sensory organ cells.

Discussion

Evidence that Borr is a CPC component

Four independent lines of evidence argue that Borr is the functional ortholog of vertebrate Borealin/Dasra. First, based on stringent database searches, we found that *borr* is the only gene in the *Drosophila* genome with significant sequence similarity to *Borealin/Dasra*, and vice versa. Second, like vertebrate Borealin/Dasra and other CPC components (Andrews et al., 2003; Carmena and Earnshaw, 2003; Gassmann et al., 2004; Sampath et al., 2004), Borr colocalises with endogenous Incenp and Aurora B in transfected mitotic *Drosophila* DmD8 cells. Third, like its vertebrate counterpart (Gassmann et al., 2004), *borr* is required for the correct subcellular localisation of Incenp and Aurora B in dividing cells. Fourth, Borr loss causes similar mutant phenotypes in mitotic Kc cells and in developing embryonic and larval cells as does depletion of other *Drosophila* CPC components in tissue culture, or depletion of Borealin/Dasra and other CPC components in mammalian cell lines. These phenotypes include abnormal spindles and uneven chromosome segregation, leading to giant multi-nucleate

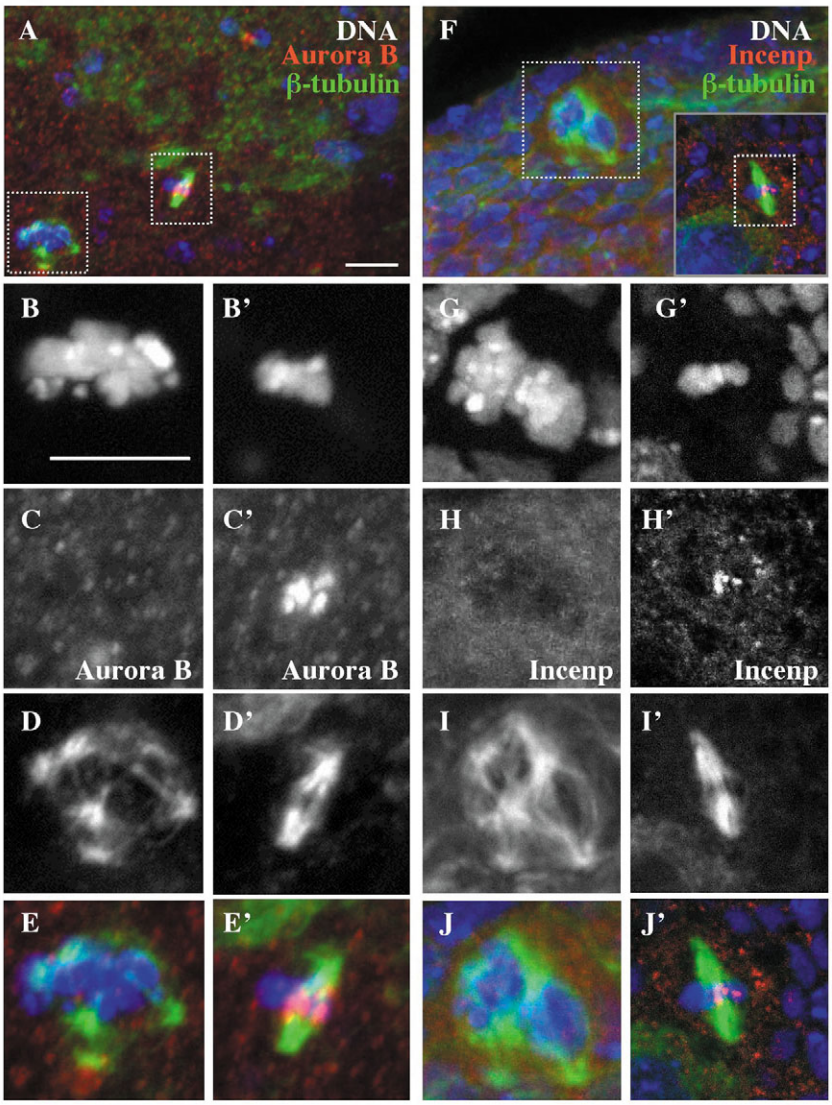

Fig. 5. Aurora B and Incenp fail to localise to mitotic spindles in *borr* mutant imaginal disc cells. Wing discs of late third instar larvae bearing *borr* mutant clones, triple-stained with Hoechst dye and antibodies against β-tubulin and (A-E′) Aurora B or (F-J′) Incenp. Areas containing wild-type or mutant mitotic cells are boxed, and the corresponding individual channels are shown below (B′-E′,G′-J′, wild-type cells; B-E,G-J, *borr* mutant cells). Localised Aurora B (C) and Incenp (H) staining is missing in the abnormal *borr* mutant spindles. Scale bars: in A, 5 μm for A,F; in B, 5 μm for B-E′,G-J′.

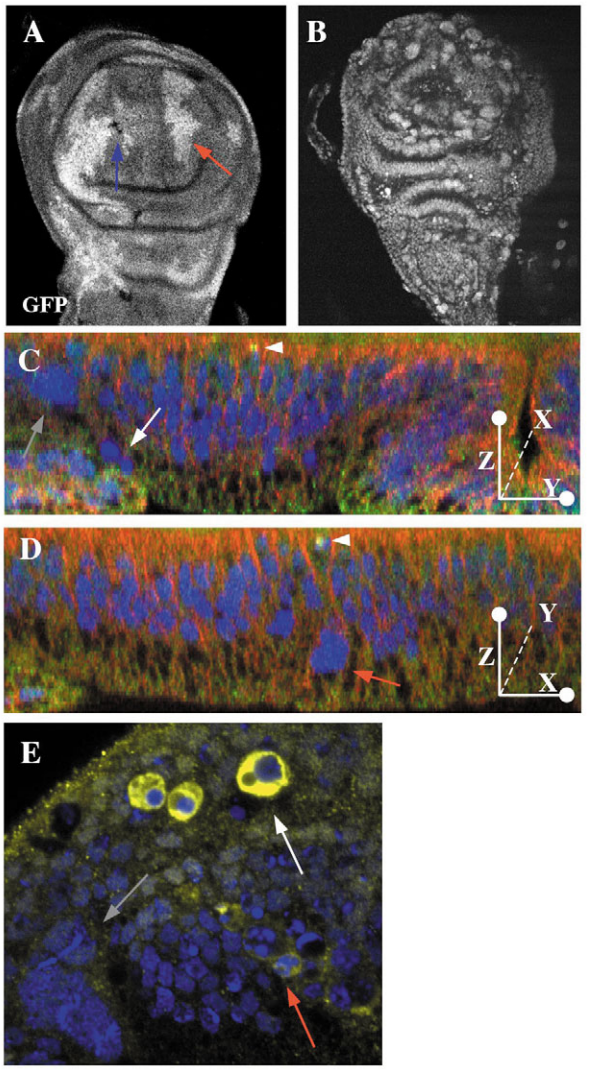

Fig. 6. Delayed apoptosis of large *borr* mutant cells. (A) Third larval instar wing disc, bearing early-induced *borr* mutant clones (marked by absence of GFP, indicated by blue arrow) that are invariably small; red arrow indicates large twin spot (showing strong GFP fluorescence), which lacks an associated *borr* mutant clone. (B) Third instar larval *Minute*/+ wing disc, bearing early-induced *borr* mutant clones (that are *Minute*$^+$ and thus have a proliferative advantage), stained with Hoechst dye, showing numerous large *borr* mutant cells that have apparently survived multiple abnormal mitoses. (C,D) Wing disc as in A, showing DNA (blue), β-tubulin (red, to mark cellular outlines) and Aurora B staining (green, to mark normal mitotic cells within the apical plane, indicated by arrowheads). Dying mutant cells are extruded basally (bottom). Both images are optical 3D reconstructions through the pouch regions of the disc along the *y* (C) or *x* (D) axis, generated from a *z*-stack of 0.25 μm confocal sections. (E) Wing disc as in A, stained with Hoechst dye and antibody against active caspase. Arrows in C-E indicate large *borr* mutant cells that are healthy (grey), apoptotic (white) or intermediate (red).

and/or polyploid cells and, usually, to apoptosis (see also below). A noticeable molecular consequence of Borr loss is also the reduction in the P-H3 levels – given that this phosphorylation event is mediated by Aurora B (Adams et al., 2001; Giet and Glover, 2001; Hsu et al., 2000), this links Borr function specifically to the activity of this CPC component. We note that the *C. elegans* protein CSC-1 appears to be another functional ortholog of Borealin/Dasra, despite showing very limited sequence similarity to these proteins, based on its mutant phenotypes in the embryo and on its functional interactions with other CPC components (Romano et al., 2003).

borr is required for high P-H3 levels during mitosis

One striking mutant phenotype of mitotic *borr* mutant VNC cells is a significant reduction of their P-H3 levels (Fig. 2D; Fig. 4). Normally, this phosphorylation appears during prophase and spreads throughout the chromosomes, with peak levels during metaphase, followed by dephosphorylation during anaphase and telophase (Hans and Dimitrov, 2001; Nowak and Corces, 2004; Wei et al., 1998). Although the function of P-H3 is not known, correlations have been noted in many species between the P-H3 levels and the degree of DNA condensation, and a *T. thermophila* strain with a non-phosphorylatable version of H3 showed perturbed chromosome condensation and abnormal chromosome segregation (Wei et al., 1998). This led to the hypothesis that S10 phosphorylation of H3 may be necessary for chromosome condensation.

However, in *borr* mutant embryos, the condensation of the chromosomes in mitotic VNC cells is barely affected, yet their P-H3 staining is often strongly reduced (Fig. 2D; Fig. 4D,J). This argues that H3 phosphorylation occurs in parallel or subsequent to chromosome condensation, rather than driving it. Consistent with this, others have also reported a lack of correlation between chromosome condensation and P-H3 levels (Adams et al., 2001), including Yu et al. (Yu et al., 2004) who have observed normal levels of P-H3 on undercondensed chromosomes in *greatwall* mutants of *Drosophila*. Indeed, it has been suggested that P-H3 may be a sort of licensing factor, namely a mark placed on mitotic chromosomes to indicate their readiness to undergo separation during the subsequent stages of the cell cycle (Hans and Dimitrov, 2001).

The striking reduction of the P-H3 levels in *borr* mutant embryonic cells, and in Borr-depleted cultured *Drosophila* cells (Eggert et al., 2004), is in contrast to the situation in HeLa cells in which RNAi-mediated depletion of Borealin did not affect their P-H3 levels (Gassmann et al., 2004). These authors suggested that, in these cells, H3 phosphorylation may be mediated by a Borealin-independent subcomplex of Aurora B and Incenp (Gassmann et al., 2004). More work is required to determine whether this apparent discrepancy between human and *Drosophila* Borealin function in mediating phosphorylation of H3 is genuine and cell type- or species-specific, or whether it is simply due to methodological differences in the analyses.

borr loss causes polyploidy and delayed apoptosis in developing tissues

We have shown that *borr* is an essential gene in *Drosophila*, and that *borr* loss results in multiple successive defects during mitosis, including a reduction of P-H3, a severe attenuation prior to metaphase, multipolar spindles and uneven chromosome segregation. These defects may all reflect a function of Borr in the attachment of kinetochores to the mitotic spindle, given that this process often fails in Borealin-

depleted HeLa cells (Gassmann et al., 2004). However, it is also possible that they reflect additional underlying activities of the CPC during the progression of mitosis. However, all of these mitotic defects are probably due, ultimately, to the observed failure of other CPC components such as Aurora B to localise correctly to the mitotic spindle (Fig. 5).

Multifocal spindles as observed in *borr* mutant cells (Fig. 2D; Fig. 3J; Fig. 5D,I) are expected to cause aneuploidy, and may trigger checkpoint function. They should thus be cleared from the developing tissue by apoptosis. Our observation of apoptotic *borr* mutant cells in larval imaginal discs (Fig. 6E) provide direct support that cell death is often the ultimate consequence of *borr* loss at the cellular level. However, *borr* mutant cells can also clearly evade apoptosis, and can undergo several consecutive abnormal divisions, given that the surviving (and dying) *borr* mutant cells in imaginal disc epithelia are typically large, with giant nuclei and greatly increased ploidy. Consistent with this, mammalian cells lacking CPC function appear to be defective for their spindle attachment checkpoint and can thus escape apoptosis (Lens and Medema, 2003; Yang et al., 2004). A similar defect in the checkpoint function of *borr* mutant epithelial cells would explain why these cells can survive multiple abnormal mitoses, instead of entering apoptosis in response to the uneven chromosome segregation of a single abnormal mitosis. However, the survival capacity of the mutant cells is clearly limited, and most of them die ultimately – except in late larval and pupal discs in which they survive, possibly because of the slowing down of mitotic activity and/or growth at these stages, which perhaps provides a more permissive environment for the abnormally dividing *borr* mutant cells.

Non-autonomous effects of *borr* loss appear to involve Wg signalling

We found that *borr* mutant epithelial cells can cause major non-autonomous disruptions of the patterning of adjacent wild-type cells. This is unusual as imaginal discs can tolerate considerable cell death without compromising the development of normal adult tissues (see Perez-Garijo et al., 2004; Ryoo et al., 2004). The reason for this appears to be that apoptotic imaginal disc cells activate transient bursts of extracellular signalling by Wg and Dpp, to induce compensatory cell divisions in their wild-type neighbours. However, if apoptosis is suppressed through inhibition of caspase activity (which creates 'undead' cells) (Perez-Garijo et al., 2004; Ryoo et al., 2004), this produces more sustained signalling, which in turn causes gross pattern abnormalities in the resulting adult tissue. It thus appears that interfering with, or suspending, the apoptotic pathway leads to over-compensatory responses.

We propose that a similar situation arises in the case of *borr* mutant imaginal disc cells: given that these can survive multiple abnormal divisions, they may be doomed – i.e. on a suspended apoptosis path – for an extended period of time and thus mimic some characteristics of 'undead' cells. Like the latter (Perez-Garijo et al., 2004; Ryoo et al., 2004), doomed

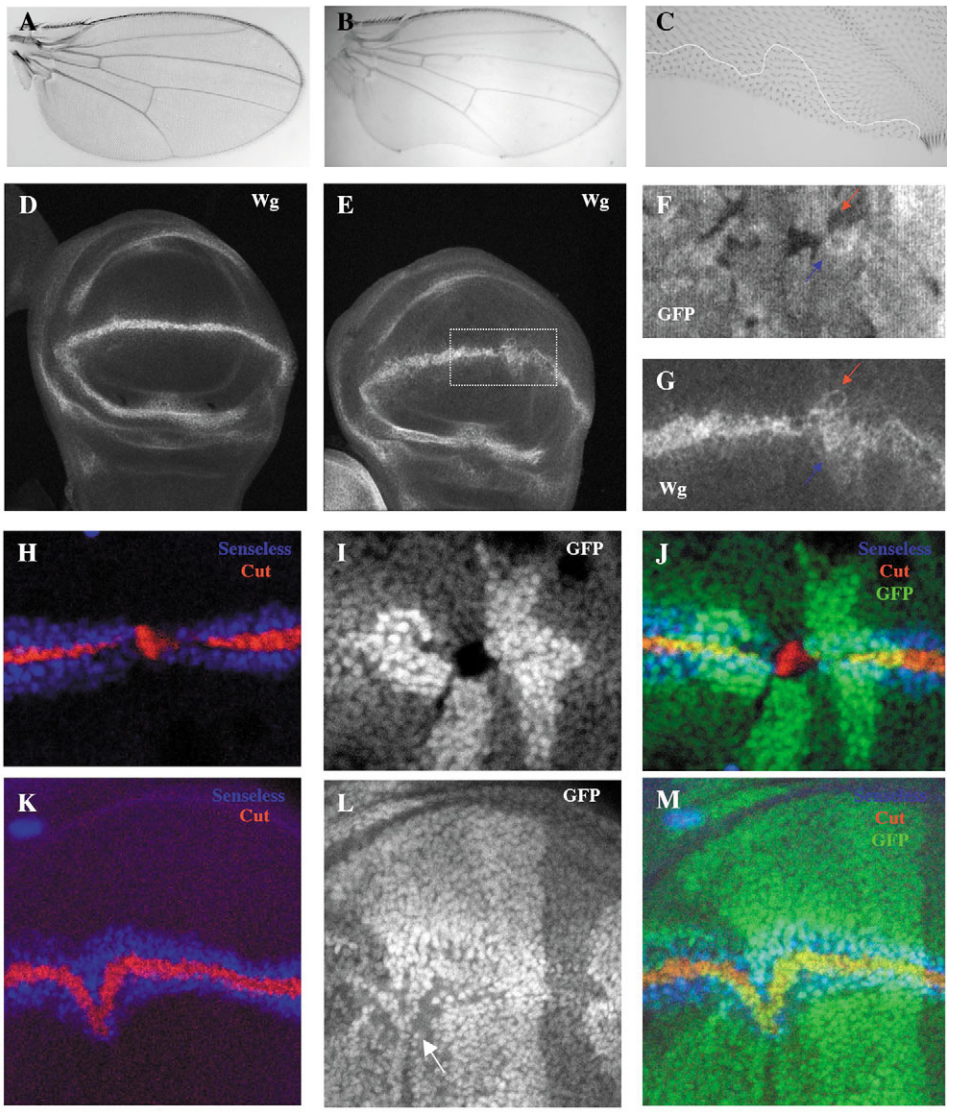

Fig. 7. Early-induced *borr* mutant clones cause non-autonomous defects. (A-C) Adult wings (A) without or (B,C) with *borr* mutant clones; (C) enlargement of wing nick in B, with twin spot (marked with *ck*) outlined in white. (D-G) Third instar wing discs (D) without or (E-G) with *borr* mutant clones (marked by absence of GFP in F), stained with antibody against Wg; (F,G) magnifications of the boxed region in E. Red arrows indicate giant *borr* mutant cell expressing Wg, blue arrows indicate Wg expansion in wild-type cells that are not associated with a *borr* mutant clone. (H-M) Marginal regions of wing discs with *borr* mutant clones, stained as indicated; (LA) arrow indicates cell rearrangement that may have resulted from a dying *borr* mutant clone.

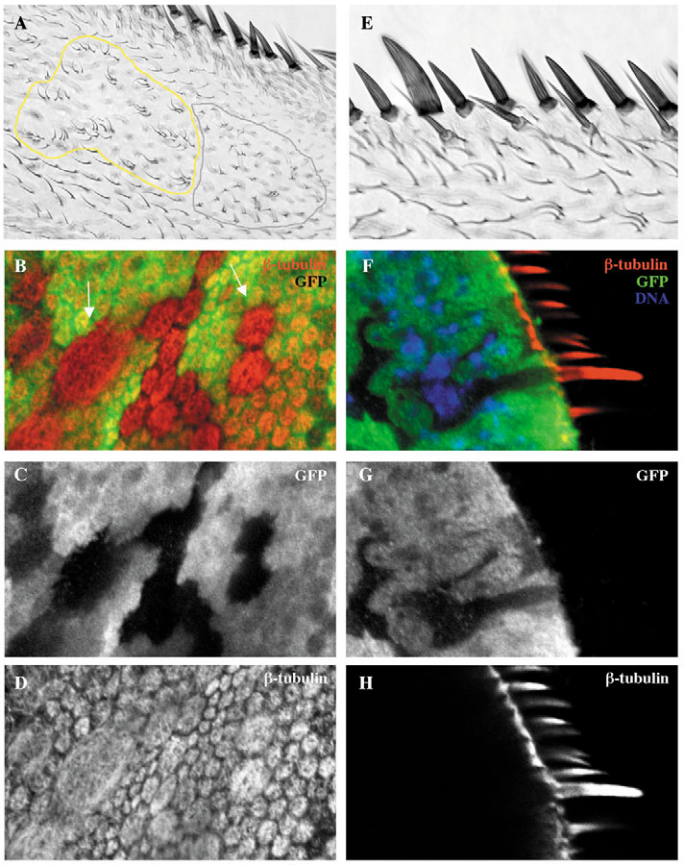

Fig. 8. Survival and cell-autonomous defects of late-induced *borr* mutant clones. (A) Adult wing blade, with *borr* mutant clone (marked by *f*, outlined in yellow) and associated twin spot (marked by *ck*, outlined in grey). (B-D) Single confocal section through the epithelial sheet of a pupal wing blade with *borr* mutant clones (marked by absence of GFP), grazing the bases of the cell nuclei within the epithelial plane, stained as indicated in the panels; arrows indicate individual giant *borr* mutant cells. (E) Margin area from a wing bearing *borr* mutant clones with a giant bristle. (F-H) Single confocal section through a pupal wing, stained as in B-D and also with Hoechst dye. An individual large *borr* mutant cell (lacking GFP) gives rise to a giant bristle. β-Tubulin staining is absent at the base of the bristle, indicating the lack of a socket.

borr mutant cells may induce a burst of compensatory responses in their neighbours by stimulating the expression of extracellular signals such as Wg. This is suggested by our analysis of larval discs bearing early-induced mutant clones (Fig. 7), which revealed examples of overexpressed Wg in giant *borr* mutant cells, and also lateral expansion of Wg in twin spot areas whose associated *borr* mutant cells have died. Doomed *borr* mutant cells may also affect signalling by other pathways, e.g. the Notch pathway, given some of the *borr* mutant phenotypes (Fig. 7H-J; see Fig. S1 in the supplementary material) (e.g. Neumann and Cohen, 1996), but we have not examined this directly.

Our analysis further suggests that cell rearrangements can take place as a result of dying, or dead, *borr* mutant cells (Fig. 7L). These could be a consequence of compensatory signalling, and they may be aimed at repairing the substantial gaps in epithelial integrity expected to arise after the death of a giant *borr* mutant cell.

Polyploidy caused by *borr* loss may be instructive for bristle development

We have shown that *borr* loss also affects the lineage divisions of the external sensory organs: our evidence from late-induced *borr* mutant clones indicates that surviving giant *borr* mutant cells develop large bristles without sockets (Fig. 8). This phenotype suggests a defect or block in the division of the pIIa precursor cell that normally gives rise to the trichogen and tormogen (Lai and Orgogozo, 2004). It is less likely that the division of pI (the initial sensory organ precursor cell) is blocked by *borr* loss in these instances, as evidence from the analysis of embryonic sensory organs suggests that blockage of the first lineage division should result in the precursor cell adopting a neural fate (Hartenstein and Posakony, 1990).

Why a *borr* mutant cell should adopt the bristle fate at the expense of the socket fate is not immediately obvious. One possibility is that the determining factor is its increased DNA content and large size. Notably, the trichogen cells that produce the stout bristles of the wing margin undergo at least one round of endoreplication during their differentiation (Hartenstein and Posakony, 1989; Hartenstein and Posakony, 1990) (though in other external sensory organs the tormogen does as well) (Lai and Orgogozo, 2004). Thus, *borr* loss could mimic an aspect of normal trichogen development, and could actively promote the acquisition of the bristle fate. It is thus conceivable that endoreplication is instructive during the process of sensory organ development.

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Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/132/21/4777/DC1

References

Adams, R. R., Maiato, H., Earnshaw, W. C. and Carmena, M. (2001). Essential roles of Drosophila inner centromere protein (INCENP) and aurora B in histone H3 phosphorylation, metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation. *J. Cell. Biol.* **153**, 865-880.

Adler, P. N., Liu, J. and Charlton, J. (2000). Cell size and the morphogenesis of wing hairs in Drosophila. *Genesis* **28**, 82-91.

Andrews, P. D., Knatko, E., Moore, W. J. and Swedlow, J. R. (2003). Mitotic mechanics: the auroras come into view. *Curr. Opin. Cell Biol.* **15**, 672-683.

Barbosa, V., Yamamoto, R. R., Henderson, D. S. and Glover, D. M. (2000). Mutation of a Drosophila gamma tubulin ring complex subunit encoded by discs degenerate-4 differentially disrupts centrosomal protein localization. *Genes Dev.* **14**, 3126-3139.

Bolton, M. A., Lan, W., Powers, S. E., McCleland, M. L., Kuang, J. and Stukenberg, P. T. (2002). Aurora B kinase exists in a complex with survivin and INCENP and its kinase activity is stimulated by survivin binding and phosphorylation. *Mol. Biol. Cell* **13**, 3064-3077.

Carmena, M. and Earnshaw, W. C. (2003). The cellular geography of aurora kinases. *Nat. Rev. Mol. Cell. Biol.* **4**, 842-854.

Clemens, J. C., Worby, C. A., Simonson-Leff, N., Muda, M., Maehama, T., Hemmings, B. A. and Dixon, J. E. (2000). Use of double-stranded RNA

interference in Drosophila cell lines to dissect signal transduction pathways. *Proc. Natl. Acad. Sci. USA* **97**, 6499-6503.

Cliffe, A., Hamada, F. and Bienz, M. (2003). A role of Dishevelled in relocating Axin to the plasma membrane during wingless signaling. *Curr. Biol.* **13**, 960-966.

Couso, J. P., Bishop, S. A. and Martinez Arias, A. (1994). The wingless signalling pathway and the patterning of the wing margin in Drosophila. *Development* **120**, 621-636.

Desbordes, S. C. and Sanson, B. (2003). The glypican Dally-like is required for Hedgehog signalling in the embryonic epidermis of Drosophila. *Development* **130**, 6245-6255.

Earnshaw, W. C. and Bernat, R. L. (1991). Chromosomal passengers: toward an integrated view of mitosis. *Chromosoma* **100**, 139-146.

Eggert, U. S., Kiger, A. A., Richter, C., Perlman, Z. E., Perrimon, N., Mitchison, T. J. and Field, C. M. (2004). Parallel chemical genetic and genome-wide RNAi screens identify cytokinesis inhibitors and targets. *PLoS Biol.* **2**, E379.

Gassmann, R., Carvalho, A., Henzing, A. J., Ruchaud, S., Hudson, D. F., Honda, R., Nigg, E. A., Gerloff, D. L. and Earnshaw, W. C. (2004). Borealin: a novel chromosomal passenger required for stability of the bipolar mitotic spindle. *J. Cell Biol.* **166**, 179-191.

Giet, R. and Glover, D. M. (2001). Drosophila aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis. *J. Cell Biol.* **152**, 669-682.

Hans, F. and Dimitrov, S. (2001). Histone H3 phosphorylation and cell division. *Oncogene* **20**, 3021-3027.

Hartenstein, V. and Posakony, J. W. (1989). Development of adult sensilla on the wing and notum of Drosophila melanogaster. *Development* **107**, 389-405.

Hartenstein, V. and Posakony, J. W. (1990). Sensillum development in the absence of cell division: the sensillum phenotype of the Drosophila mutant string. *Dev. Biol.* **138**, 147-158.

Hsu, J. Y., Sun, Z. W., Li, X., Reuben, M., Tatchell, K., Bishop, D. K., Grushcow, J. M., Brame, C. J., Caldwell, J. A., Hunt, D. F. et al. (2000). Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell* **102**, 279-291.

Kaitna, S., Mendoza, M., Jantsch-Plunger, V. and Glotzer, M. (2000). Incenp and an aurora-like kinase form a complex essential for chromosome segregation and efficient completion of cytokinesis. *Curr. Biol.* **10**, 1172-1181.

Kaitna, S., Pasierbek, P., Jantsch, M., Loidl, J. and Glotzer, M. (2002). The aurora B kinase AIR-2 regulates kinetochores during mitosis and is required for separation of homologous Chromosomes during meiosis. *Curr. Biol.* **12**, 798-812.

Lai, E. C. and Orgogozo, V. (2004). A hidden program in Drosophila peripheral neurogenesis revealed: fundamental principles underlying sensory organ diversity. *Dev. Biol.* **269**, 1-17.

Lens, S. M. and Medema, R. H. (2003). The survivin/Aurora B complex: its role in coordinating tension and attachment. *Cell Cycle* **2**, 507-510.

Neumann, C. J. and Cohen, S. M. (1996). A hierarchy of cross-regulation involving Notch, wingless, vestigial and cut organizes the dorsal/ventral axis of the Drosophila wing. *Development* **122**, 3477-3485.

Nowak, S. J. and Corces, V. G. (2004). Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. *Trends Genet.* **20**, 214-220.

Parker, D. S., Jemison, J. and Cadigan, K. M. (2002). Pygopus, a nuclear PHD-finger protein required for Wingless signaling in Drosophila. *Development* **129**, 2565-2576.

Perez-Garijo, A., Martin, F. A. and Morata, G. (2004). Caspase inhibition during apoptosis causes abnormal signalling and developmental aberrations in Drosophila. *Development* **131**, 5591-5598.

Romano, A., Guse, A., Krascenicova, I., Schnabel, H., Schnabel, R. and Glotzer, M. (2003). CSC-1: a subunit of the Aurora B kinase complex that binds to the survivin-like protein BIR-1 and the incenp-like protein ICP-1. *J. Cell Biol.* **161**, 229-236.

Ryoo, H. D., Gorenc, T. and Steller, H. (2004). Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. *Dev. Cell* **7**, 491-501.

Sampath, S. C., Ohi, R., Leismann, O., Salic, A., Pozniakovski, A. and Funabiki, H. (2004). The chromosomal passenger complex is required for chromatin-induced microtubule stabilization and spindle assembly. *Cell* **118**, 187-202.

Thompson, B., Townsley, F., Rosin-Arbesfeld, R., Musisi, H. and Bienz, M. (2002). A new nuclear component of the Wnt signalling pathway. *Nat. Cell Biol.* **4**, 367-373.

Wei, Y., Mizzen, C. A., Cook, R. G., Gorovsky, M. A. and Allis, C. D. (1998). Phosphorylation of histone H3 at serine 10 is correlated with chromosome condensation during mitosis and meiosis in Tetrahymena. *Proc. Natl. Acad. Sci. USA* **95**, 7480-7484.

Weigmann, K., Cohen, S. M. and Lehner, C. F. (1997). Cell cycle progression, growth and patterning in imaginal discs despite inhibition of cell division after inactivation of Drosophila Cdc2 kinase. *Development* **124**, 3555-3563.

Xu, T. and Rubin, G. M. (1993). Analysis of genetic mosaics in developing and adult Drosophila tissues. *Development* **117**, 1223-1237.

Yang, D., Welm, A. and Bishop, J. M. (2004). Cell division and cell survival in the absence of survivin. *Proc. Natl. Acad. Sci. USA* **101**, 15100-15105.

Yu, S. Y., Yoo, S. J., Yang, L., Zapata, C., Srinivasan, A., Hay, B. A. and Baker, N. E. (2002). A pathway of signals regulating effector and initiator caspases in the developing Drosophila eye. *Development* **129**, 3269-3278.